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Ecotoxicological evaluation of industrial port of Venice (Italy) sediment samples after a decontamination treatment

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A sediment washing technique was assessed for port contaminated sediment remediation and reuse, indicating its reduced efficiency and the need for further improvements.

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ABSTRACT

This work assesses the ecotoxicological effects of polluted sediment after a decontamination treatment process using a new sediment washing technique. Sediment samples were collected from four sites in Marghera Port industrial channels (Venice, Italy). Ecotoxicological evaluations were performed with *Vibrio fischeri* and *Crassostrea gigas* bioassays. Whole sediment and elutriate were deemed as the most suitable environmental matrices for this study. Toxicity scores developed in the Lagoon of Venice for *V. fischeri* on whole sediment and for *C. gigas* on elutriate were considered for the final ranking of samples. Ecotoxicological results showed that the treated sediment samples presented both acute and sub-chronic toxicities, which were mainly attributed to the presence of some remaining chemicals such as metals and polyaromatic hydrocarbons. The acute toxicity ranged from low to medium, while the sub-chronic one from absent to very high, suggesting that treated sediments could not be reused in direct contact with seawater.

1. Introduction

In Venice, there is a need to manage large amounts of dredged materials and maximize, whenever possible, their reuse in the lagoon for morphological restoration (Apitz et al., 2007). It was recently estimated that 20 million m^3 of material with various levels of contamination will be produced by the dredging activities for the construction of the mobile gates to protect Venice from high tides, for navigational and environmental purposes of the Porto Marghera industrial channels and the enlargement of the Port of Chioggia (Apitz et al., 2007). It is evident that the traditional studies based only on chemical analyses are not sufficient to support sediment management, since these measurements provide no estimation of deleterious effects on living organisms (His et al., 1997). Toxicity bioassays, responding to the bioavailable fraction of toxicants and thus providing ecologically relevant information, are currently used as a rapid and cost-effective screening tool to evaluate sediment pollution (Beiras et al., 2003).

The aim of this work was to assess the potential ecotoxicological effects of contaminated sediment (i.e. metals and organics) treated by the Venice Port Authority (VPA) with a sediment washing technique. Sediment was sampled from four sites in Marghera Port industrial channels. The sediment washing process itself generated two sub-samples per sampling site: the coarser fraction was produced by the hydrocyclone stage and the finer one by the centrifuge phase. Unfortunately, this research study was commissioned only at the end of the VPA project, therefore just the treated (i.e. washed) sediment samples were available. Due to this fact, no information could be provided about the toxicity removal performance achieved by the treatment process. Anyway, previous ecotoxicological information from the same study area evidenced high and very high toxicity levels in elutriates with *Crassostrea gigas* (Losso et al., 2007a) and very high toxicity levels on whole sediment with Microtox® Solid Phase (SP) test (unpublished data).

The same two toxicity tests were selected for this study: (a) the Microtox® SP test on whole sediment, based on the bioluminescent bacterium *Vibrio fischeri*, which is a widely accepted toxicity test in environmental monitoring and screening activities all over the world for its ecological relevance, sensitivity, reproducibility,

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standardisation and simple execution (Volpi Ghirardini et al., 1999; Losso et al., 2004); (b) the embryotoxicity test with the oyster *C. gigas* on elutriate (i.e. representative for water-extractable components), which is internationally recognised as a very sensitive, simple, inexpensive and rapid method (Dame, 1996; His et al., 1997, 1999; OSPAR, 2000).

2. Materials and methods

2.1. Sediment management and sample collection

Sediment samples were collected from four sites in the Marghera industrial port as shown in Fig. 1. Sites 1 and 2 were located in the lower and upper reach of the Canale Industriale Ovest, respectively, site 3 was located in the Canale Industriale Nord and site 4 near the convergence of the Molo Sali and the Canale Industriale Nord.

Sediment samples were treated for decontamination using a pilot sediment decontamination technology, the testing of which was supported the VPA during winter 2003–2004. The decontamination process involved two liquid/solid separation stages among a six treatment steps procedure including dredged material preparation, pre-processing, application of collision impact forces, primary solid/liquid separation, organic contaminant oxidation and secondary solid/liquid separation on a five runs basis.

The dredged material preparation involved the initial screening of the sediment down to a size smaller than 6 mm in order to remove shells, trash and stones. Screened sediment was pumped to a set of four interconnected holding tanks where it was diluted with clean water to approximately 32% dry solids content. After mixing, the homogenised slurry was transferred in volumes of 15–30 m^3 to a temporary building in preparation for each test run. The pre-processing step consisted of the addition of surfactants, chelating agents and defoamers to prepare the sediment for decontamination. The sediment was then pumped to the pre-processor unit where physical action from high-pressure water jets disaggregated sediment particles and separated any loosely associated biomass-coated particles, suspending them in the slurry. The application of collision impact forces isolated the particles to strip the biofilm layer from the solid particles and transfer it into the aqueous phase and away from the sediment particle surfaces. Pollutants adsorbed on solid particles, as well as the biomass, were transferred into the aqueous phase. During the primary solid/liquid separation, the sediment was pumped to a tank and fed the hydrocyclone (H) coupled with a wet screen. The processed separated sediment was discharged from the hydrocyclone in a thick slurry onto a vibrating screen. The treated sediment that was captured on the screen was collected, weighed and stockpiled for sampling and removal. The water leaving the hydrocyclone and passing the screen was then pumped to the next process unit. Organic contaminant oxidation (cavitation and oxidation) involving partial wastewater treatment was used to destroy the organic contaminants and biomass that had been segregated from the sediment particles. Hydrogen peroxide was added to the sediment slurry upstream of the cavitation system. During the secondary solid/liquid separation, the slurry was processed through a centrifuge (Cen) to recover additional decontaminated solids from the liquid fraction containing the inorganic contaminants and residual organic contaminants. The centrifuge removed sediment particles down to approximately 10 μm in size. Some sediment solids were discharged in the overflow water from the centrifuge based on the cut-off point of the centrifuge.

All considered sediment samples were subjected to four consecutive treatment runs. The last run, that was the fourth one, was chosen for this study. Treated sediment samples were collected during the primary and secondary solid/liquid separation. Coarser (sand and fine sand) from the hydrocyclone and finer (silt and some clay) from the centrifuge specimens were stored in 2 l hermetically sealed glass pots at room temperature. Eight sub-samples from four sampling sites were finally obtained for ecotoxicological analyses. Samples were named by pairing the collection site number and their H- or Cen-origin.

Sediment sub-samples were transported to the laboratory and homogenised at room temperature. Part of these was used for chemical analyses, part for elutriate preparation and the remainder was directly tested via Microtox® SP.

2.2. Elutriate preparation

Elutriate samples were prepared according to Arizzi Novelli et al. (2006) using three different sediment:water ratios, 1:4, 1:20 and 1:50, as suggested by various authors (van den Hurk, 1994; Da Ros et al., 1997; Arizzi Novelli et al., 2006). Briefly, the elutriation steps were as follows:

- addition of artificial seawater (ASTM, 2004) to sediment samples considering a sediment:water volume ratio of 1:4, 1:20 and 1:50 w/v (w = sediment dry weight at 105 °C; v = dilution water volume);
- sediment:water mixture stirring at 230 rpm for 24 h at 4 °C using a Jar test (ISCO, Vittadini, Milan, Italy);
- mixture settling for 60 min at 4 °C;
- supernatant centrifugation at 7700*g* for 15 min at 4 °C using a refrigerated ultra-centrifuge (L7-35, Beckmann, Milan, Italy); and
- supernatant storage in 100 ml polyethylene containers via freezing at −18 °C for later ecotoxicological analyses (ASTM, 1992; Carr and Chapman, 1995).

Elutriate samples were not filtered (Carr and Chapman, 1995; Da Ros et al., 1997), except for total ammonia (N-NH_4) and sulphide (S^{2-}) analyses (Whatman GF/F 0.7 μm filters).

2.3. Chemical analyses

Total ammonia and sulphide concentrations were measured in elutriates with a spectrophotometer (DR/2010, HACH, Loveland, CO, USA) using the salicylate (Reardon et al., 1966) and methylene blue methods (USEPA SM 4500-S2 D), in that order. The pH was measured using a pH-meter (perpHecT LogR meter, model 330, Orion, Beverly, MA, USA).

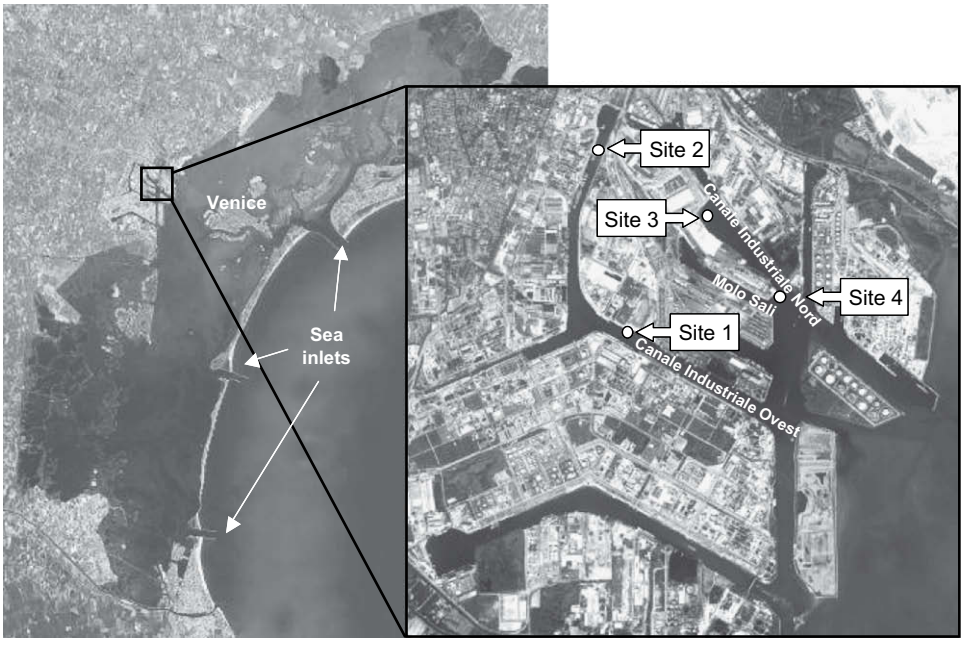

Fig. 1. Location of sediment sites.

For chemical analyses on whole sediment (in duplicate), samples were homogenised and sieved to eliminate large stones, shells, fauna and plant debris with size >2 mm. At first, the dry matter was determined according to the method ISO 11465 (1993) considering a sub-sample of 10 g in triplicate and dried at 105 ± 5 °C (Binder FED 53, Tuttlingen, Germany) per 24 h. All chemical data were expressed after normalisation to sediment dry mass.

The determination of Heavy Metals (HM) such as arsenic (As), cadmium (Cd), total chromium (Cr), copper (Cu), nickel (Ni), lead (Pb) and zinc (Zn) was performed according to the method DIN EN ISO 17294-2 (2005) using an Inductively Coupled Plasma Mass Spectrometry (ICP-MS Agilent 7500c, Santa Clara, California, USA). The pre-treatment procedure consisted in an acid microwave-assisted digestion (Milestone 1200 Mega unit, Milestone Ethos plus, Rome, Italy). An ICP-MS multi-element standard solution (Merck 10580) was considered for calibration and Quality Assurance/Quality Control (QA/QC) procedures.

Mercury (Hg) was determined on the basis of ISO 5666 (1999) method after aqua regia digestion via Cold Vapour Atomic Absorption Spectrometry (CVAAS SIMAA 6000, Perkin–Elmer, Darmstadt, Germany). A Baker standard solution for trace metal analysis (1000 μg Hg ml^{-1}) was considered for calibration and QA/QC procedures.

The determination of PolyAromatic Hydrocarbons (PAH, sum 18–26) required the treatment of 20 g sediment sub-samples using a mixture of solvents as described in ISO 13877 (1998) method. The extract was cleaned using Al_2O_3 and concentrated via a nitrogen stream. The PAHs were determined via a High-Pressure Liquid Chromatography (HPLC, Hewlett–Packard, Agilent HP 1100, Waldbronn, Germany) equipped with fluorescence detector (FLD). A standard solution (PAH Mix, EPA 610) was considered for QA/QC procedures.

The ISO 10382 (2002) method was used for quantitative determination of some selected organochlorine pesticides (aldrin, atrazine, α-hexachlorohexane, β-hexachlorohexane, γ-hexachlorohexane, chlordane, 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane (DDD), 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT), 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene (DDE), dieldrin and endrin) and 11 Polychlorinated biphenyls (PCBs, sum of PCB 18, 31, 28, 52, 44, 101, 118, 153, 138, 180, 194) in sediments. The separation of analytes was performed using a Perkin–Elmer Autosystem gas chromatograph (Shelton, USA) equipped with an Electron Capture Detector (ECD) using a Supelco PTE-5 30 m capillary column with a film thickness of 0.25 μm and an internal diameter of 0.25 mm (Bellefonte, PA, USA) for both organochlorine pesticides (except for atrazine) and PCBs. A Supelco CLP Organochlorine Pesticide Mix and a Supelco CEN PCB Congener Mix-1 were taken into account for QA/QC procedures.

The atrazine content was checked via the ISO 11264 (2005) method. It required an initial extraction procedure on a 10 g sediment sub-sample using a mixture of acetone and water (2:1). After 6 h of mechanical horizontal agitation (IKA Labortechnik, HS 501 digital, Staufen, Germany) NaCl and dichloromethane were added to the solution to isolate the organic phase that was concentrated and transferred to an acetonitrile–water mixture and analyzed without any further clean-up. The analysis was performed using a HPLC (Hewlett–Packard, Agilent HP 1100, Waldbronn, Germany) with photoDiode-Array Detection (DAD). A Supelco Triazine Pesticide Standard Mix was used for QA/QC procedures.

The mineral oil content determination was based on the standard method ISO/TR 11046 (1994). Sediment sub-samples of 15 g were air dried and extracted using trichlorofluoromethane (25 ml). Eventually extracted polar substances were then separated from hydrocarbons by a clean-up step with Na_2SO_4 and Florisil®. The mineral oils concentrations were assessed via a Fourier Transform Infrared (FT-IR) Spectrometry (Perkin–Elmer Spectrum BX, Shelton, USA) that enabled both natural and anthropogenic mineral oils determination (molecules with more than 12 carbon atoms, C > 12). A Fluka® squalane standard solution (>99%) was used for QA/QC procedures.

2.4. Embryotoxicity test with C. gigas on elutriate samples

The test with *C. gigas* is a sub-chronic test for saline aqueous matrices based on embryo-larval development abnormalities. Conditioned oyster adults were purchased from the Guernsey Sea Farm Ltd. hatchery (Guernsey, UK). The embryotoxicity test was performed according to the method proposed by His et al. (1997), modified to use gametes pools. Adults were induced to spawn by thermal stimulation. Good quality gametes from the best males and females were selected and filtered at 32 μm (sperm) and 100 μm (eggs) to remove impurities. A pool of eggs from at least three females (1000 ml) were fertilised by injecting 10 ml of sperm suspension; fertilisation was verified by microscopy. Egg density was determined by counting four sub-samples of known volume. Fertilised eggs, added to test solutions in order to obtain a density of 10–20 eggs ml^{-1}, were incubated for 24 h at 24 °C in 3 ml volume dilutions. At the end of the test, samples were fixed with buffered formalin and 100 larvae were counted, distinguishing between normal larvae (D-shape) and abnormalities (malformed larvae and pre-larval stages). Acceptability of test results was based on: (a) negative control for a percentage of normal D-shape larvae ≥80% (His et al., 1999); (b) Effect Concentration where 50% of the population exhibited a response (EC50) with reference toxicant (Cu as $Cu(NO_3)_2$) falling within the intra-laboratory acceptability range, 8.36 μg Cu l^{-1}–18.51 μg Cu l^{-1}, determined as the average EC50 value ($n = 11$) for Cu ± 2σ (CV = 18.9%). Toxicity tests were performed in triplicate using at least four geometrically scaled dilutions for each elutriate sample.

Sterile polystyrene micro-plates (3 ml 24 wells) with lids (Iwaki Brand, Asahi Techno Glass Corporation, Tokyo, Japan) were used as test chambers for toxicity tests. Dilution water (for test solutions and gametes) was artificial sea water reconstituted according to ASTM (2004) at a salinity of 34‰.

2.5. Test with V. fischeri (Microtox® SP)

The Microtox® SP test is an acute toxicity test for solid disaggregated samples based on the natural bioluminescence inhibition of marine bacteria *V. fischeri*. The test was performed on the basis of Volpi Ghirardini and Pantani (1996) and Volpi Ghirardini et al. (in press) protocols, which introduced some modifications (i.e. adaptation to lagoon environments) to the Azur Environmental (1998) standard protocol that was repeatedly applied to various monitoring surveys in the Venice Lagoon (Volpi Ghirardini et al., 1998, 1999, 2005). The Microtox® SP was performed only on the silt–clay fraction resulting from the ultra-centrifuge treatment since the finer fraction can be considered as the sink of toxicity due to the greater specific surface area increasing the bacteria exposure to toxicants (Bulich et al., 1992).

Lyophilised bacteria, reconstitution solution, cuvettes, filters and tubes for the SP test, were in accordance with Strategic Diagnostics (Pencader Drive, Newark DE, USA). A Microtox® analyzer M500 (Azur Environmental) was used to read light emissions.

A fixed quantity of wet sediment (10.0 g) was resuspended in 100 ml of diluent solution (buffered K_2HPO_4–KH_2PO_4 0.1 M in 20‰ NaCl) with magnetic stirring at 1000 rpm for 10 min and 400 rpm for 3 min. Sub-samples from this suspension were used for serial dilutions (1.5 ml, five replicates) and determination of sediment dry weight. Dilutions were equilibrated for 10 min in a thermostatic bath at 15.0 ± 0.2 °C. Afterwards, 20 μl of revitalized bacteria were added to each tube, gently mixed with a pipette and left in the thermostatic bath to incubate at the same temperature for 20 min with shaking and 5 min without shaking. The bacteria were then separated from the sediment by filtration and a 500 μl sub-sample of the liquid phase was transferred into the glass cuvettes in the Microtox® analyzer and allowed to equilibrate for 5 min before reading. Light emission was recorded and the output data analyzed using MicrotoxOmni® software Version 1.18 (Azur Environmental). EC50 was then recalculated on the basis of sediment dry weight.

Sediment dry weight was determined by gravimetric measures via drying 1.5 ml of the initial suspension at 105 ± 5 °C for 24 h (five replicates). The salt content correction was also applied. All tests were performed with two controls and six dilutions in two replicates.

Reference toxicant ($ZnSO_4 \cdot 7H_2O$) was used to control *V. fischeri* batch quality according to the Basic Test procedure (Azur Environmental, 1998).

2.6. Data analysis

Toxicity data from oysters were expressed as EC50 values based on the percentages of abnormalities. EC50 values with 95% confidence limits were calculated by Trimmed Spearman-Karber statistical method (ASTM, 2004). Toxic Unit at 50% of the population exhibiting a response (TU50) was determined as 100/EC50 to provide values directly correlated to the toxicity magnitude. The responses for each treatment (percentage of abnormalities) were corrected for effects in control tests by applying Abbott's formula (ASTM, 2004), obtaining the Percentage of Effect (PE). Moreover, in order to test the null hypothesis that the different treatments had no effect on larval development, the percentages of normal larvae at each concentration were compared to the controls using a one-way ANOVA after conducting Cochran's test for homogeneity of variance. If the data failed this test, an arcsin $P^{1/2}$ transformation was applied to the data to achieve homoschedasticity.

Microtox® EC50 values were obtained by linear regression between sample concentration and the fraction of light loss to light remaining (I') in a logarithmic scale where the EC50 corresponds to the sample concentration matching $I' = 1$ with 95% confidence limits.

Principal component analysis (PCA) was considered to summarise the relationships between variables and account for the variation present in the data set matrix via biplotting both the ordination component scores and the variable loading coefficients. Particularly, chemical data were weighed for toxicity effects in both *C. gigas* and Microtox® SP. Normality of data and homogeneity of variance were previously checked. Correlation coefficients were calculated by the method of parametric linear correlation (Pearson). XLSTAT software, version 2008.4.01, that is a data analysis and statistical application available for Microsoft Excel®, was used for data elaboration.

3. Results and discussion

3.1. Chemical data

Chemical analyses (Table 1) evidenced that all sediment samples were contaminated by both heavy metals and organic compounds. The main differences among the four sites were as follows:

Table 1
Chemical data and classification of sediment samples (H from Hydrocyclone, Cen from Centrifuge) according to the current quality criteria used to classify dredged sediments in the Venice Lagoon (Italian Ministry of the Environment, 1993)

Parameter	1 H (mg/kg)	2 H (mg/kg)	3 H (mg/kg)	4 H (mg/kg)	1 Cen (mg/kg)	2 Cen (mg/kg)	3 Cen (mg/kg)	4 Cen (mg/kg)	Classification[a]							
									1 H	2 H	3 H	4 H	1 Cen	2 Cen	3 Cen	4 Cen
Arsenic	29.1	52.1	44.9	12.9	35.0	47.6	67.0	14.2	C	C	C	A	>C	C	>C	A
Cadmium	10.5	3.31	28.8	6.09	9.47	2.75	34.4	5.93	C	B	>C	C	C	B	>C	C
Chromium (total)	31.1	44.3	28.7	31.6	34.1	41.0	47.6	29.2	B	B	B	B	B	B	B	B
Copper	51.9	66.9	114	26.5	68.2	61.1	162	30.8	C	C	C	A	C	C	C	A
Lead	132	104	404	50.7	150	90.9	523	50.3	C	C	C	B	C	B	>C	B
Mercury	4.45	4.31	4.99	2.19	5.19	3.71	6.62	2.54	C	C	C	C	C	C	C	C
Nickel	21.3	24.0	20.9	19.1	22.7	21.7	25.9	21.8	A	A	A	A	A	A	A	A
Zinc	807	450	2330	625	804	411	3150	604	C	C	C	C	C	C	>C	C
Total metals									C	C	>C	C	>C	C	>C	C
Sum of PAH (18-26)	14.76	3.94	48.21	7.16	13.73	3.04	42.9	4.36	C	B	>C	B	C	B	>C	B
Total chlorinated pesticides	<0.001	0.040	<0.001	<0.000	0.005	0.06	<0.001	<0.001	A	C	A	A	B	C	A	A
PCBs	0.17	0.71	0.21	0.05	0.14	1.05	0.35	0.03	B	C	C	B	B	C	C	B
Hydrocarbons (Mineral oils)	200	398	216	142	68	94	45	57	B	B	B	B	B	B	B	B
Organic compounds									C	C	>C	B	C	C	>C	B

Cen, Centrifuge; H, Hydrocyclone.
[a] Italian Ministry of the Environment (1993).

- sediment samples from site 4 evidenced a lower quantity of As and PCBs; and
- sediment samples from site 3 showed higher levels of Cu, Cd and Pb, but, more particularly, of PAHs and Zn.

There was no great difference between the coarser and finer fractions, with the exception of the hydrocarbons level that was higher in all coarser sediments. After several test runs, a general reduction of metals, PAHs and hydrocarbons was observed (VPA, unpublished data).

As shown in Table 1, sediment samples were also classified according to the current quality criteria for dredged sediments in the Venice Lagoon (Italian Ministry of the Environment, 1993), reported in detail in Apitz et al. (2007), which are focused on sediment reuse and disposal: class A sediment can be used for restoration of lagoon morphology, i.e. in morphological structures directly in contact with water; class B sediment can be used to restore lagoon islands, avoiding contaminants runoff from sediments; class C sediment can be used to raise islands, but avoiding any sediment contact with water. In addition, the "$>$C" class was introduced for when the contamination level was greater than C class limits. These sediments must be disposed of outside the lagoon and permanently confined in a controlled dump. For total chlorinated pesticides, no information was available about heptachlor and heptachlor epossid as required by the quality criteria, so mineral oils were considered in place of total hydrocarbons. As shown in Table 1, this classification highlighted that:

- for total metals, sediments from sites 2 and 4 and the coarser fraction of site 1 were classified as C, site 3 as $>$C; more specifically, all samples are in class A for Ni, in class B for Cr and in class C for Hg; for As and Cu the sites were in class C, with the exception of site 4 that was in class A; all sediments were in classes B or C for Cd and Pb and in class C or $>$C for Zn; and
- for organic compounds, site 4 was in class B, sites 1 and 2 in class C, site 3 in $>$C. In details, for PAHs, sites 2 and 4 were in class B, site 1 in class C and site 3 in class $>$C; for pesticides, samples were in class A with the exception of site 2 that belonged to class C and the finer fraction of site 1 that was in class B; for PCBs, sites 1 and 4 were in class B, sites 2 and 3 in class C; all sediment samples were in class B for mineral oils.

The final conclusion is that sediment samples after the hydrocyclone treatment were classified as C or $>$C for HMs and B, C or $>$C for organic compounds. The same classification was substantially maintained after centrifugation, only sample 1 for HMs showed a worse classification ($>$C) after centrifugation.

3.2. *Embryotoxicity test with* C. gigas

The negative control showed $86 \pm 6\%$ of normal larvae, whereas the positive control registered an EC50 value equal to 11.81 (10.45–13.35) $\mu g\ Cu\ l^{-1}$ in accordance with the above mentioned intra-laboratory acceptability range.

Elutriates toxicity results are shown on the basis of each sediment:water ratio in Fig. 2. The toxicity range was wide, from 0 PE to 14.29 TU50.

Elutriates at 1:4 sediment:water ratio evidenced that finer sediment fractions were generally more toxic than coarser ones: for site 1 from 5.6 PE (1 Cen) to 11.3 PE (1 H), for site 2 from 10.00 TU50 (2 Cen) to 36.8 PE (2 H), for site 4 from 7.63 TU50 (4 Cen) to 34.2 PE (4 H). The finer fraction was less toxic (3 Cen = 2.26 TU50) than the coarser one (3 H = 14.29 TU50) only for site 3.

Elutriates at 1:20 sediment:water ratio highlighted lower toxicity values than 1:4 elutriates for sites 1, 2 and 4, while TU50 values for site 3 were comparable for 1:4 and 1:20 ratios. In the case of site 3, it can be supposed that larger volumes of water might facilitate the toxicants passing from the solid to liquid phase, as reported in Arizzi Novelli et al. (2006).

Elutriate toxicity greatly decreased at 1:50 with percentages of effect lower than 5 PE for sites 1 and 2 and lower than 15 PE for site 4. Conversely, for site 3, even if toxicity values were lower than 1:20 rate, the coarser fraction presented 4.20 TU50 (3 H).

The possible contribution of ammonia and sulphide, as confounding factors, was investigated according to Losso et al. (2007b). Elutriates total sulphide concentrations ranged between 2 and 7 $\mu g\ l^{-1}$. All sulphide concentrations were under the no observed effect concentration (NOEC) of 100 $\mu g\ l^{-1}$ (ASTM, 2004), which is considered as the sensitivity threshold limit value of the bivalves embryotoxicity test towards sulphide. They were therefore not considered as confounding factors.

Elutriates total ammonia concentration was in the range 0.03–37.50 $mg\ l^{-1}$. Fig. 3 shows the relationship between elutriates ammonia concentration, that was extrapolated for the dilution of samples causing 50% of effect, and elutriates toxicity: only two samples, 4 Cen 1:20 and 4 Cen 1:50, exceeded the NOEC limit for total ammonia (4.7 $mg\ l^{-1}$) (ASTM, 2004), indicating that their toxicity could have been influenced by total ammonia

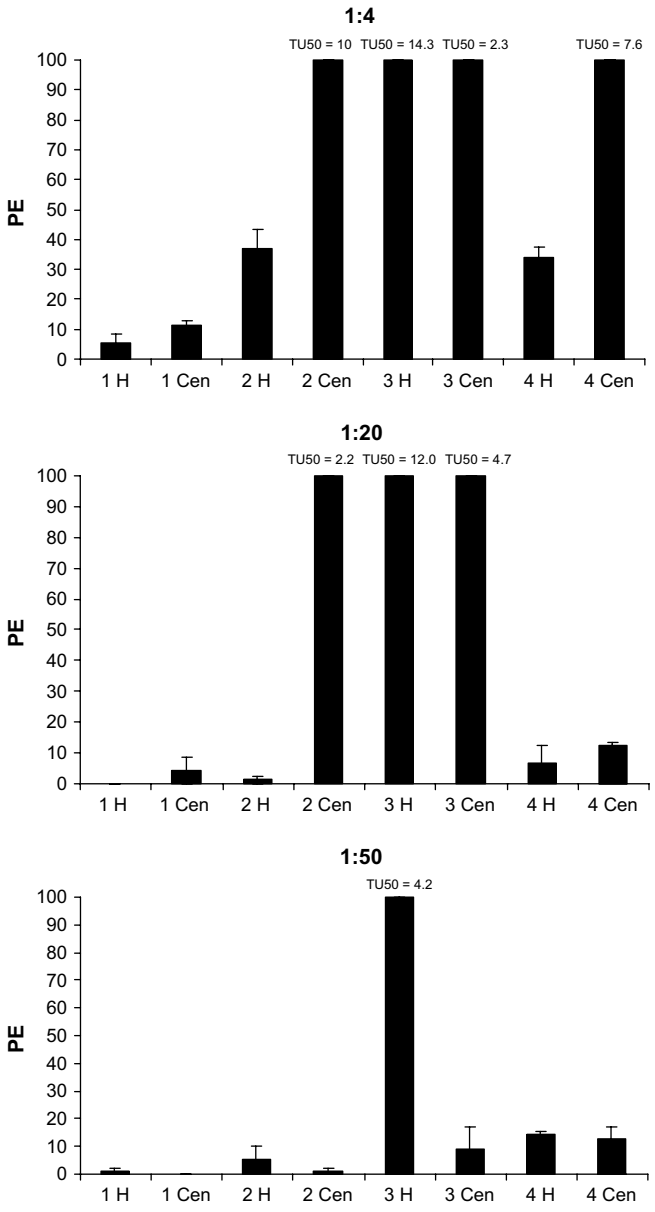

Fig. 2. Toxicity data expressed as percentage of effect with *C. gigas* for each sediment:water ratio of elutriate samples.

concentration. Indeed, their toxicity was quite low: 12.56 PE and 12.99 PE, respectively.

In order to make a concise judgement and provide a practical usefulness for the toxicity data, it was decided to interpret the 1:4 elutriates toxicity results on the basis of the toxicity score developed with *C. gigas* to check the quality of Venice Lagoon sediments (Losso et al., 2007a) as shown in Table 2. The toxicity score was based on five toxicity classes: *absent*, *low*, *medium*, *high* and *very high*. Site 1 evidenced absence of toxicity for both coarser and finer fractions (1 H and 1 Cen 1:4 elutriates). Site 2 showed low toxicity for the coarser fraction (2 H 1:4) and very high toxicity for the finer fraction (2 Cen 1:4). Site 3 is the most toxic, with elutriates at high toxicity from coarser (3 H 1:4) and very high toxicity from finer sediment (3 Cen 1:4). Site 4 highlighted low toxicity for coarser (4 H 1:4) and very high for finer sediment (4 Cen 1:4).

Previous data from the same study area, but not exactly from the same sampling sites, indicated toxicity levels ranging from high in 2003 (DRA-s03) to very high in 2002 and 2004 (BA1-w02, BA2-w02 and DRA-w04) (Losso et al., 2007a). Considering these values as a starting background levels for sediment toxicity, it could be stated that the toxicity was reduced only for site 1, while for sites 2, 3 and 4 its removal was relatively limited or absent.

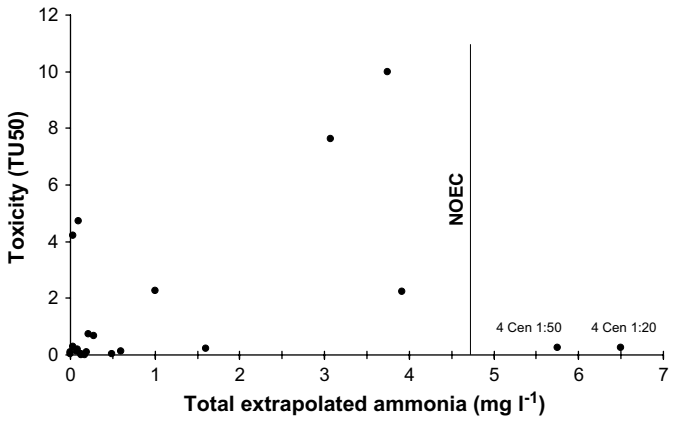

Fig. 3. Total ammonia concentration in elutriates (extrapolated for the dilution of samples causing 50% of effect), related to elutriate toxicity (expressed as TU50) tested with *C. gigas*. The black bar highlights NOEC for total ammonia (ASTM, 2004).

3.3. Microtox® test

Toxicity data from the Microtox® SP test on the finer sediment fractions are reported in Fig. 4. The most toxic sample was from site 1 (TU50 = 3297), followed by site 3 (TU50 = 2081), site 2 (TU50 = 869) and site 4 (TU50 = 548).

As for *C. gigas*, Microtox® SP toxicity data were compared to the toxicity score developed on the basis of a data set including 1994–2006 toxicity results from Venice Lagoon sediment samples (unpublished data). Therefore, finer sediment samples from sites 1 and 3 were classified as medium toxic, while from sites 1 and 2 low toxic.

Previous data from the same study area, but not exactly from the same sampling sites, indicated very high toxicity levels in 2002 (unpublished data). Considering this state-of-the art as background level for sediment toxicity, it could be stated that the toxicity seemed to be reduced for all sites.

3.4. Data integration

Some considerations can be made about the toxicity data for the finer fractions of sediment. The sub-chronic toxicity by the oyster embryotoxicity test for the 1:4 elutriates in increasing order is 1 Cen < 4 Cen < 2 Cen < 3 Cen, while the Microtox® SP acute toxicity for the whole sediment is 4 Cen < 2 Cen < 3 Cen < 1 Cen. These data suggested that sediment contamination was characterised by a component strictly linked to the sediment particles, therefore detectable via the Microtox® SP test, and by a hydrophilic one that

Table 2
Toxicity class for each sediment sample

Samples	Microtox® SP test	*C. gigas*–1:4 elutriate	Worst case scenario
1 H	–	Absent	–
2 H	–	Low	–
3 H	–	Very high	–
4 H	–	Low	–
1 Cen	Medium	Absent	Medium
2 Cen	Low	Very high	Very high
3 Cen	Medium	High	High
4 Cen	Low	Very high	Very high

The worst case scenarios are reported only for finer sediments.

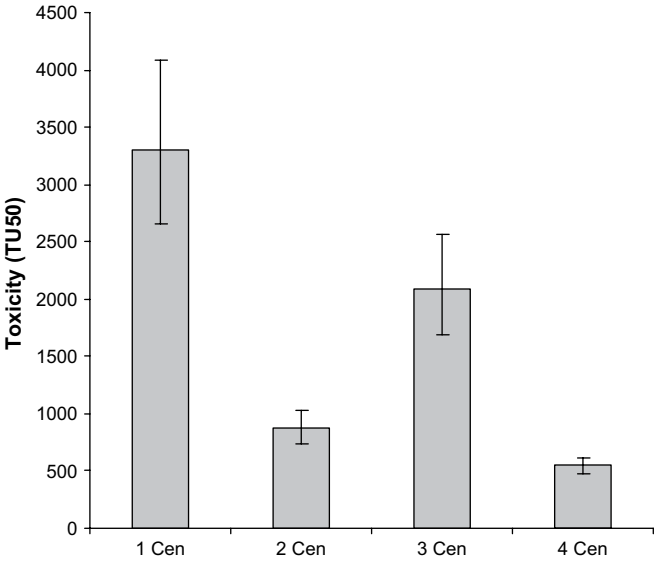

Fig. 4. Toxicity of finer sediment fractions according to Microtox® SP test.

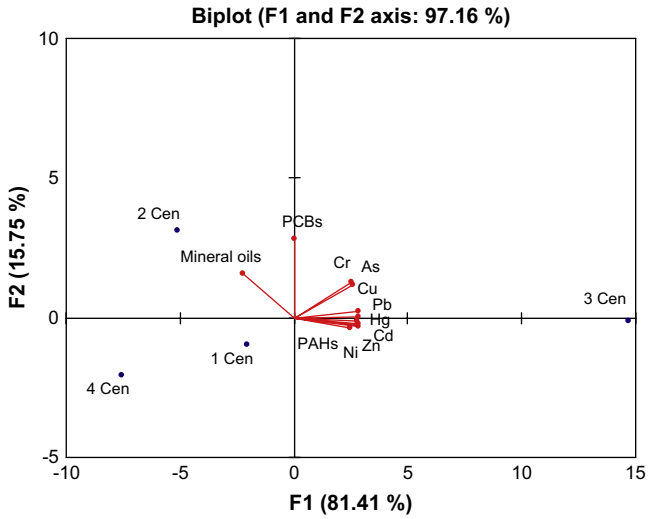

Fig. 6. Principal component analysis biplot of chemical data with loadings and scores in the coordinates of the first two principal components (F1 and F2) weighed on Microtox® SP toxicity.

has been released from the sediment to the water column as indicated by the elutriate toxicity results.

Considering the toxicity classes for both toxicity tests (Table 2), the worst case scenario according to Harremoës (2000) suggested that all finer sediments were toxic: the sediment from site 1 showed medium toxicity, site 3 high toxicity and sites 2 and 4 very high toxicity.

A biplot summarising PCA results concerning chemical data weighed on *C. gigas* toxicity values is shown in Fig. 5. The first two principal components accounted for 59.61 and 26.36% of the variation, respectively. Thus the 85.97% of the variation can be depicted by a two-axis ordination diagram. The biplot regarding components loadings suggested that the first component (F1) scores are strongly influenced by high values of Hg, Cu, PAHs, Cd, Zn and Pb in this increasing order, which are clustered together and have high positive loadings on the first axis. In addition, the loading of total Cr and PCBs on the second component (F2) suggested that the second component scores could reflect the concentrations of these compounds in the samples.

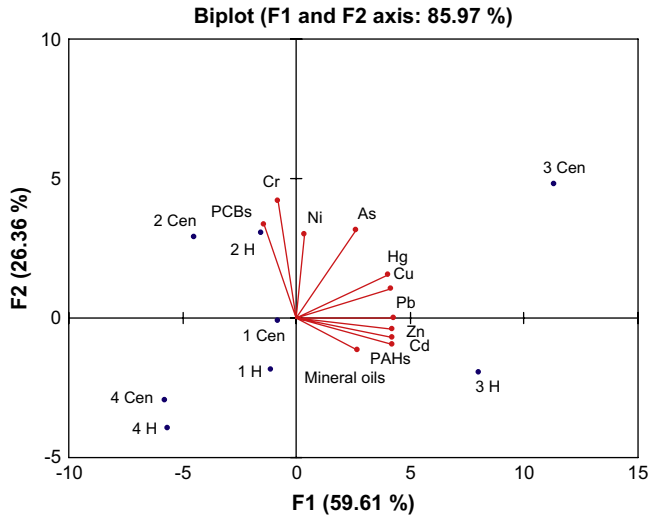

Fig. 5. Principal component analysis biplot of chemical data with loadings and scores in the coordinates of the first two principal components (F1 and F2) weighed on *C. gigas* toxicity.

Looking at the ordination plot of component scores present in the F1–F2 biplot, it was found that the 3 H toxicity could be explained by HMs and PAHs, whereas 3 Cen was more related to HMs contamination. Conversely, 2 Cen toxicity was negatively correlated to total Cr and PCBs and 4 Cen toxicity was not directly related to the considered contaminant concentrations, suspecting the presence, e.g. of potential mixture effects.

A biplot summarising PCA results concerning chemical data weighed on Microtox® SP test results was shown in Fig. 6 for samples 1 Cen, 2 Cen, 3 Cen and 4 Cen. The first two principal components accounted for 81.41 and 15.75% of the variation, respectively. Thus the 97.16% of the variation can be depicted by a two-axis ordination diagram. The biplot regarding components loadings suggested that the first component (F1) scores are strongly influenced by all HMs and PAHs, while PCBs are independent. Sample 3 Cen was more influenced by HMs and PAHs, while 1 Cen, 2 Cen and 4 Cen were uncorrelated to the assessed parameters, except for 2 Cen sample that resulted negatively correlated to mineral oils. Thus, the observed toxicity could not be clearly explained suggesting the presence of undetected contaminants or mixture effects.

Indeed, it could not be excluded that samples toxicity could be due to residual concentration of surfactants, chelating agents and defoamers that, occasionally, had not be totally removed from the treated sediment samples after H- and Cen-treatment.

4. Conclusions

This research work evidenced that sediment samples, treated via hydrocyclone and centrifuge technologies, continued generating both acute and sub-chronic toxicity effects, which were mainly attributed to the presence of residual chemicals such as metals and PAHs. Thus the decontamination procedure was able to remove only a part of the original sediment contamination, increasing, potentially, the mobilisation of the remaining one. In particular, it was evidenced that the coarser fraction was relatively easier to clean rather than the finer one, that continued presenting from medium to very high toxicity levels. Treated sediment samples showed no homogeneous characteristics, suggesting that the treatment efficiency could be directly related to the sediment initial contamination level.

In conclusion, both ecotoxicological and chemical results evidenced that VPA treated sediments could not be reused in direct contact with brackish and salt water. The present decontamination technique should be improved considering, for example, the possibility to perform more treatment runs on the same polluted sediment batch and, perhaps, improving the formula of cleaning additives.

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