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Is the 1:4 elutriation ratio reliable? Ecotoxicological comparison of four different sediment:water proportions

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Abstract

Methodological research was carried out to evaluate the discriminatory capability of three toxicity bioassays toward different elutriation ratios (1:4, 1:20, 1:50, and 1:200 sediment:water ratios). Samples from six sampling stations of the Lagoon of Venice have been investigated. The toxicity bioassay results (sea urchin *Paracentrotus lividus* Lmk sperm cell and embryo toxicity bioassays and bivalve mollusk *Crassostrea gigas* Thunberg embryo toxicity bioassays) have shown that elutriates generated from the widely used 1:4 ratio were less toxic than those from intermediate ratios (1:20 and 1:50).

Keywords: Sediment; Elutriate; Elutriation ratios; Toxicity bioassays; Sea urchin; *Paracentrotus lividus*; Oyster; *Crassostrea gigas*; Confounding factors

1. Introduction

Most contaminants originating from human activities and discharged into surface water are eventually deposited and concentrated in sediment. Due to its propensity to sequester both organic and inorganic contaminants, sediment can be defined as the main sink and source of pollutants. The sediments in estuaries and coastal areas thus constitute important reserves of contaminants and represents potential sources of pollution. In particular, marine disposal of dredged material, dumping of wastes, and shipping and fishing activities can play major roles in sediment particulate resuspension, a possible source of pollution (USEPA, 1977; Forstner and Wittmann, 1983). In sediment, in addition to independent actions of contaminants, the presence of complex mixtures could produce additive, synergic, and antagonistic interactions. Their concentrations in sediment may be several orders of magnitude higher than that in overlying water; however, bulk sediment concentrations are not highly correlated to bioavailability (Burton, 1992).

Elutriate is an environmental matrix that enables the replication of sediment mobilization phenomena (Shuba et al., 1978) and the prediction of the release of contaminants from the sediment to the water column (ASTM, 1990). It was first developed for evaluating the potential effects of disposing of dredged material in open water and is nowadays also applied to the quality evaluation of in situ sediment (Beiras et al., 2001; Lazorchak et al., 2003).

Briefly, the elutriation procedure consists of the vigorous shaking of a predetermined part of sediment with parts of water to release sorbed pollutants. This mixture is allowed to settle and the liquid phase is centrifuged (ASTM, 1991; Burton and MacPherson, 1995). Analyses of elutriate samples provide information on the water-soluble constituents potentially released from the sediment to the water column. The method has been proved suitable for detecting altered and toxic sediment, supplying information on water-bioavailable components (Williams et al., 1986).

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Several methods of elutriate preparation have been proposed in the literature. The main differences consist of sediment:water ratios, sediment shaking techniques, times, and temperatures, and supernatant centrifuging techniques. The 1:4 sediment:water ratio, suggested by USEPA (1991), is the most commonly employed sediment: water proportion (USACE, 1978; Burton et al., 1989; Daniels et al., 1989; Munawar et al., 1989; Long et al., 1990; Ankley et al., 1991; USEPA, 1991; Vashchenko and Zhada, 1993; Andreatta et al., 1994; Hurkey et al., 1994; Bridges et al., 1996; Schuytema et al., 1996; Da Ros et al., 1997; Sibley et al., 1997). Nonetheless, a wide series of alternative ratios have flourished: the 1:2 ratio (Meador et al., 1990; Vashchenko and Zhada, 1993), 1:3 ratio (Matthiessen et al., 1998), 1:5 ratio (Lee et al., 1978; Da Ros et al., 1997), 1:8 ratio (McFadzen, 2000), 1:10 ratio (Daniels et al., 1989; Da Ros et al., 1997), 1:20 ratio (Lee et al., 1978; Daniels et al., 1989), 1:25 (Lee et al., 1978), 1:50 ratio (Long et al., 1990; Van den Hurk, 1994; Van den Hurk et al., 1997; Da Ros et al., 1997), 1:200 ratio (Da Ros et al., 1997), 2:1 ratio (Williams et al., 1986), and 2:5 ratio (Thain et al., 1996). Moreover, Daniels et al. (1989) reported that the choice of the dry or wet weight of sediment to achieve the sediment:water proportions is questionable.

Different sediment shaking devices are also employed: rotary shaker tables (Williams et al., 1986; Ankley et al., 1991; Matthiessen et al., 1998), rotary tumblers (Daniels et al., 1989; Munawar et al., 1989), air systems (USACE, 1978; Daniels et al., 1989; McFadzen, 2000), shakers (Burton et al., 1989; Daniels et al., 1989; Long et al., 1990; Van den Hurk, 1994; Matthiessen et al., 1998; His et al., 1999), magnetic stirrers (Bridges et al., 1996), and ultrasonic baths (Andreatta et al., 1994) have all been proposed. Shaking times varied from about 30 min to 24 h (Ankley et al., 1991; ASTM, 1991; Burton and MacPherson, 1995; Liß and Ahlf, 1997).

Despite all these methodological differences, only limited studies have been conducted to highlight the procedure best able to simulate natural sediment resuspension phenomena or to render pollutants more bioavailable (Daniels et al., 1989; Da Ros et al., 1997).

The aim of this work was to assess the discriminatory capability of three toxicity bioassays toward four different elutriation ratios (1:4, 1:20, 1:50, and 1:200) to evaluate which ratio can produce greater effects for water-column bioindicators. Moreover, this research aimed to determine whether the widely used 1:4 sediment:water ratio represents the best method for a quality control sediment diagnosis.

Static nonrenewal bioassays using sea urchin sperm cells, embryos, and larvae, and oyster embryos and larvae, widely used in biomonitoring programs (ASTM 1998a, b), have been employed to evaluate elutriates toxicity. To evaluate the reliability of the results, the toxicity data are discussed in relation to possible confounding factors (ammonia and sulfide).

2. Materials and methods

2.1. Sampling sites

The sediments were sampled in late summer (August–September 1998) at six sites in the Lagoon of Venice, northeast Italy, as part of the "Orizzonte 2023 Project." The stations were located along a pollution gradient: two presumably unpolluted sites (CE(a) and CE(b)), two oil-refinery and industrial sites (BR and SA), and two estuarine sites (DE and OS) close to agricultural areas.

At Centrega Marsh (CE), in the northern Lagoon, CE(a) and CE(b) were chosen as two possible reference sites, intertidal and subtidal, respectively, because of their location characterized by a fast water renewal time (due to the vicinity of the Lido sea inlet), morphological diversity, high biodiversity, and expected minimal contaminant levels. Previous investigations in this area had reported very low levels of some heavy metals (Volpi Ghirardini et al., 1998, 1999) and organic micropollutants (Di Domenico et al., 1998).

The South Industrial Canal (SA) and Lusore-Brentelle Canal (BR) are located within the Porto Marghera industrial zone. The Lusore-Brentelle Canal samples can be expected to be extremely polluted according to Spoladori (2004). Some information on sediment contamination of both industrial canals is already available (Di Domenico et al., 1998; Volpi Ghirardini et al., 1998, 1999; MAV, 2004).

The DE and OS sampling stations are at the mouths of the Dese (DE) and Marzenego (Osellino Canal, OS) rivers, respectively. Both stations are influenced by freshwater carrying agricultural runoff. In addition, OS is affected by multifactorial contamination due to urban (treated and untreated wastewaters from the town of Mestre) and industrial (vicinity of Porto Marghera industrial zone) sources and to an uncontrolled landfill of the Campalto saltmarshes, just behind the Osellino Canal (i.e., possible release of leachates), where confining works (CVN, 1999) were started after the end of this project.

Detailed data on particle size fractions (percentage of sand, silt, and clay), total organic carbon (TOC) (percentage of dry weight), organic matter (OM) (loss on ignition at 450 °C overnight, percentage dry weight), and water content (W) (105 °C overnight; percentage of water content) (ASTM, 1990), chemical analyses of metals (in the acid extracts determined by ICPM according to USEPA 1998) and organic micropollutants concentrations (extraction at 4 °C with n-hexane/dichloromethane 50/05 at 150 °C and 1500 psi; analyses conducted using a HP 6890 plus gas chromatograph coupled to a Micromass Autospec Ultima mass spectrometer; determinations of PCDDs/PCDFs and PCBs-HCB performed according to the USEPA, 1994, 1999), and the geographical coordinates of the sampling stations (Volpi Ghirardini et al., 2005) are reported in Table 1.

2.2. Sediment collection, characterization, and storage

Samples were collected at low tide by a 5-cm-diameter Plexiglas corer. At each site, the area (a circle of approx. 30 m diameter, with a central point fixed by geographical coordinates) and sample sizes (eight sediment cores at depths of 15–20 cm) were defined, and integrated sampling (with cores equally distributed over the area) was done to take any spatial microvariability in pollution/bioavailability into account, according to the scheme reported in Volpi Ghirardini et al. (2005).

Cores from each station were pooled in 2-L glass jars and stored at 4 °C. At 1 to 2 days after sampling, each integrated sediment sample was homogenized and sieved (1 mm mesh size), and subsamples were prepared for sediment characterization and chemical analyses and processed to obtain elutriates for toxicity bioassays.

2.3. Elutriation procedure

In preparing elutriates, the USEPA (1991) standard procedure was used as reference, but some modifications based on the more recent

Table 1
Results of particle size fractions (sand, silt, and clay, %), TOC (%), OM (%), water content (*W*, %), and chemical analyses of metals and organic micropollutants with geographical coordinates of the sampling stations

Stations	Coordinates		Particle size fractions			TOC	OM	*W*	Metals								Organic micropollutants					
	Latitude	Longitude	Sand	Silt	Clay				As	Cd	Cr	Cu	Hg	Ni	Pb	Zn	PCBs Sum	POCs	HCB	PAHs	PCDDs	PCDFs
CE(a)	45°28′39″	12°26′42″	10.8	65.7	23.5				7	<5.0	15	13	0.6	14	19	80	0.23	0.5	<0.1	124	24.9	48.2
CE(b)	45°28′37″	12°26′41″	42.0	53.0	5.0				7	0.12	12	10	0.5	12	18	52	0.38	<5.0	<50.0	127	15.1	22.7
OS	45°28′45″	12°17′09″	3.6	55.0	41.4	22	3.2	38	22	3.2	38	105	0.8	33	100	605	48.18	47.2	2.7	2077	676.8	724.8
DE	45°32′16″	12°23′14″	1.1	64.1	34.8	16	0.5	30	16	0.5	30	41	0.3	26	42	180	3.21	6	0.2	206	115.1	217.8
BR	45°27′07″	12°12′55″	27.8	52.9	19.3	20	0.8	35	20	0.8	35	82	0.7	30	64	235	38.78	22.5	36.1	6436	231.7	269.1
SA	45°26′26″	12°14′21″	8.4	69.6	22.0	22	3.2	35	22	3.2	35	38	0.5	31	93	54	18.46	2.6	19.4	2103	560.0	6061.2

The values of metals are expressed in mg/kg; PCBs, POCs, HCB, and PAHs are expressed in mg/kg, and Σ2,3,7,8-PCDDs/PCDFs are expressed in ng/kg. Data are elaborated from Volpi Ghirardini et al. (2005).

scientific literature (Daniels et al., 1989; Vashchenko and Zhada, 1993; Da Ros et al., 1997) and on the first calibration of the methodology for lagoon sediments (Volpi Ghirardini et al., 2005), were introduced. The 1:4, 1:20, 1:50, and 1:200 sediment:water ratios were performed using the following steps: determination of the dry weight/volume (w') to obtain the wet weight/volume of sediment samples (w' = sediment dry weight) (SETAC, 1993); addition of artificial seawater (v) (Ocean Fish, Prodac International, Cittadella, PD, Italy) to sediment samples (v = dilution water volume); stirring of the sediment–water mixture (w'/v) at 230 rpm for 24 h at 4 °C using a Jar test (Mod. ISCO, Vittadini, Milan, Italy); settlement of the mixture for 60 min at 4 °C and centrifugation of the supernatant at 7700*g* for 15 min at 4 °C using a refrigerated ultracentrifuge (Mod. L7-35, Beckmann, Milan, Italy); and storage of the supernatant without filtering in 100-mL polyethylene containers and then freezing at −18 °C for subsequent toxicological analyses; only 100 mL of each elutriate sample was filtered, using Whatman GF/F 0.7-mm filters, for total ammonia and sulfide analyses.

2.4. Chemical analyses

Concentrations of sulfides and total ammonia in elutriates were measured with a spectrophotometer (Mod. DR/2010, HACH, Loveland, CO, USA) using the methylene blue method (USEPA SM 4500-S2 D) for sulfides and the salicylate method (Reardon et al., 1966) for total ammonia. These analyses were performed to evaluate the possible influence of these chemical compounds on elutriate toxicity. Elutriate pH was measured using a pH meter (perpHecT LogR meter; Mod. 330, Orion, Beverly, MA, USA).

2.5. Paracentrotus lividus sperm cell and embryo toxicity bioassays

Adults of *P. lividus*, collected from unpolluted sites in the northern Adriatic (Italy), south of the island of Pellestrina (Ca' Roman 50°16′51″N, 23°08′75″E) and north of the island of Lido (S. Nicolò 45°25′14″N, 12°25′49″E), were stored according to the procedures reported in Volpi Ghirardini and Arizzi Novelli (2001), at mean temperature, salinity, and pH of 18±1 °C, 35±1‰, and 7.8±0.2, respectively (March 2003–June 2003). The fertilization (sperm cell toxicity) test was performed according to the protocol reported by Volpi Ghirardini and Arizzi Novelli (2001). Sea urchins were induced to spawn by injecting 1 mL of 0.5–1 M KCl solution. Pools of male and female gametes (minimum three males and three females) were prepared. A volume of 0.1 mL of adjusted suspension of 4×10^7 sperm (thermostated at 18 °C) was exposed to 10-mL aliquots of test solution and incubated in a thermostatic bath at 18 °C for 60 min. After exposure, 1 mL of 2000 standardized egg suspension (thermostated at 18 °C) was added to the sperm suspension and left for 20 min. Samples were preserved in 1 mL of concentrated buffered formalin, and the fertilization percentage in each treatment was determined by counting 200 eggs.

The embryo toxicity test was performed using the procedure reported in detail in Arizzi Novelli et al. (2002). It consists of the same steps as before, up to sperm and egg density determination, after which the gametes were put together at sperm:egg ratio of 10:1. A period of 20 min was allowed for fertilization.

The test was performed by adding 1 mL of 2000 fertilized egg suspension to 10-mL aliquots of test solution, and the aliquots were then incubated in a dark room at 18 °C for 72 h. At the end of the experiment, samples were preserved in 1 mL of concentrated buffered formalin, and the percentage of plutei with normal development in each treatment was determined by observing 100 larvae.

Male gametes and zygotes were exposed for 60 min and 72 h, respectively, to different elutriate dilutions (6%, 12%, 25%, 50%, 75%, and 100% for each elutriate sample and elutriation ratio) using sterile polystyrene six-well microplates with lids (Iwaki Brand Asahi Techno Glass Corp., Tokyo, Japan) as test chambers. Dilution water (for test solutions and gametes) for both toxicity tests was artificial seawater reconstituted according to ASTM (1998a) at 34 ppt salinity. Three experimental replicates were used for each dilution and for control tests, and both toxicity tests were executed contemporarily to use the same pool of gametes. At the same time, tests were carried out on positive controls (with copper as reference toxicant).

Acceptable test results were a fertilization rate and a percentage of normal plutei ⩾70% in all control tests using artificial seawater and the EC_{50} using the reference toxicant (copper) within previously defined acceptability ranges for both tests (Volpi Ghirardini and Arizzi Novelli, 2001; Arizzi Novelli et al., 2002).

2.6. Crassostrea gigas embryo toxicity bioassays

Conditioned oysters (*C. gigas*) were purchased from an English hatchery (Guernsey Sea Farm Ltd.). The embryo toxicity test was performed according to the method proposed by His et al. (1997), based on the standard USEPA (1995) protocol. Adults were induced to spawn by thermal stimulation (temperature cycles at 18 and 28 °C). Gametes of good quality derived from the best males and females were selected (sperm with high motility and eggs with homogeneous dimensions and regular shape) and filtered at 32 μm (sperm) and 100 μm (eggs) to remove impurities. Eggs (1000 mL) were fertilized by injecting 10 mL of sperm; fecundation was verified by microscope, controlling the presence of the fertilization membrane and the number of sperm cells (10–20) around each eggs (His et al., 1997).

Egg density was determined by counting four subsamples of known volume. Fertilized eggs, added to test solutions to obtain a density of 60 eggs mL^{-1}, were incubated for 24 h at 24 °C and fixed with buffered

formalin. One hundred larvae were counted, distinguishing between normal larvae (D-shape) and abnormalities (malformed larvae and prelarval stages). The acceptability of test results was based on negative control for a percentage of normal D-shape larvae $\geqslant$80% (His et al., 1999). Three replicates for each sample (nominal concentration for both copper and elutriates) were tested.

Zygotes were exposed for 24 h to different elutriate dilutions (6%, 12%, 25%, 50%, 75%, and 100%) using sterile polystyrene 24-well microplates with lids (Iwaki Brand; Asahi Techno Glass Corp.) as test chambers. Dilution water (for test solutions and gametes) was artificial seawater reconstituted according to ASTM (1998a) at 34 ppt salinity.

2.7. Toxicological data analysis

Data have been expressed as EC_{50} values and percentage of effect based on the percentages of "nonfertilized eggs" (sperm cell toxicity test with sea urchin) and "abnormalities" (embryo toxicity test with sea urchin and oyster). The percentage of effect discriminated less toxic samples (Volpi Ghirardini et al., 2003): in this case the responses to each treatment (% of fertilized eggs and % of abnormalities) were corrected for effects in control tests by applying Abbott's formula (Finney, 1971). For the more toxic samples, the EC_{50} values with 95% confidence limits were calculated by trimmed Spearman–Karber statistical methods (Hamilton et al., 1978). The data expressed as EC_{50} were also transformed into toxicity units ($TU_{50} = 1/EC_{50} \times 100$) to reveal any direct relationship between toxic effects and measurement system used.

Sulfide and ammonia concentrations were extrapolated to the sample concentrations able to produce EC_{50} values, if any.

3. Results

3.1. Quality assurance/quality control for toxicity tests

In the tests performed, controls showed 83±5% of fertilized eggs, 70±3% of normally developed plutei of *P. lividus*, and 83±4% of normally developed embryos of *C. gigas*, according to each methodology's limits. Experiments using copper as reference toxicant confirmed the good repeatability of all assays. The sperm cell test had a mean EC_{50}±standard deviation (SD) of 49±3 μg/L (CV = 6%, $n = 3$). These data are well within the EC_{50} acceptability range (39–71 μg/L) (Volpi Ghirardini and Arizzi Novelli, 2001). The sea urchin embryo development assay also had good repeatability, with a mean EC_{50}±SD of 52±2 μg/L, and are within the acceptability limits (51–87 μg/L) (Arizzi Novelli et al. 2002). The embryo development assay to the bivalve mollusk showed a mean EC_{50}±SD of 14.3±0.7 μg/L (CV = 5%, $n = 2$), comparable to the mean EC_{50}±SD values of 18.2±7.8 μg/L ($n = 6$) obtained from previous tests with the same reference toxicant.

3.2. Elutriate toxicity results

Elutriate toxicity results are presented in Figs. 1–3 as percentage of effect and as TU_{50}. In the sperm cell test with *P. lividus* (Fig. 1), CE(a) and CE(b) toxicities increase with increasing sediment:water ratio, while OS, DE, BR, and SA toxicities tend to increase until one of the intermediate sediment:water ratios (1:20 or 1:50), and fall at the highest ratio (1:200). In the embryo test with *P. lividus* (Fig. 2), CE(a) toxicity increases with increasing sediment:water ratio, OS toxicity tends to decrease with increasing sediment:water ratio, while CE(b), DE, BR, and SA toxicities increase until one of the intermediate sediment:water ratios (1:20 or 1:50) and fall at the highest ratio (1:200); DE, BR, and SA maintained the same trend as that found in the sperm cell test. In the embryo test with *C. gigas* (Fig. 3), OS toxicity tends to decrease with the increasing sediment:water ratio as in the *P. lividus* embryo test, while CE(a), CE(b), DE, BR, and SA toxicities increase until one of the intermediate sediment:water ratios (1:20 or 1:50) and fall at the highest ratio (1:200); DE, BR, and SA maintained the same trend as that found in the *P. lividus* sperm cell and embryo tests.

3.3. Confounding factors on elutriates

The comparison between ammonia/sulfide extrapolated concentrations in elutriates, elutriate toxicity, and sensitivity limits of methods are reported in Fig. 4. Sulfide sensitivity limits are NOEC = 0.11 mg/L for the sperm cell test with *P. lividus* (Losso et al., 2004), NOEC = 0.10 mg/L for the embryo toxicity test with *P. lividus* (Losso et al., 2004), NOEC = 0.10 mg/L and for the embryotoxicity test with *C. gigas* (ASTM, 1998b). Ammonia sensitivity

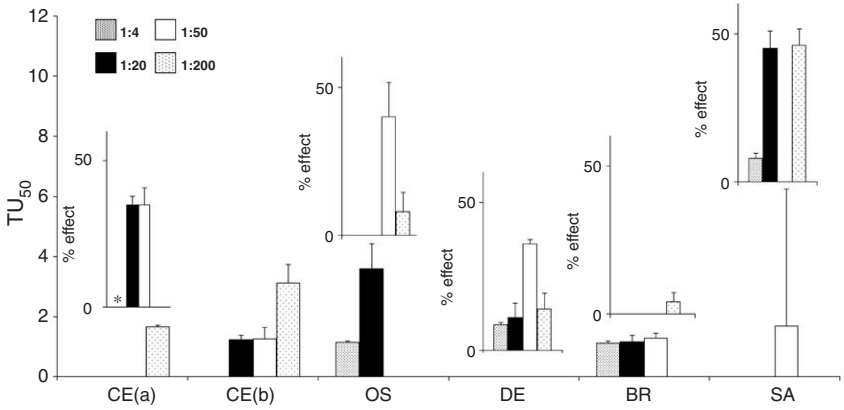

Fig. 1. Sperm cell toxicity bioassay with *P. lividus*. Trend of toxicity (TU_{50} when calculable and % of effect when EC_{50} value cannot be determined) according to the increasing sediment:water elutriation ratios. Asterisk (*) indicates that sample is not toxic.

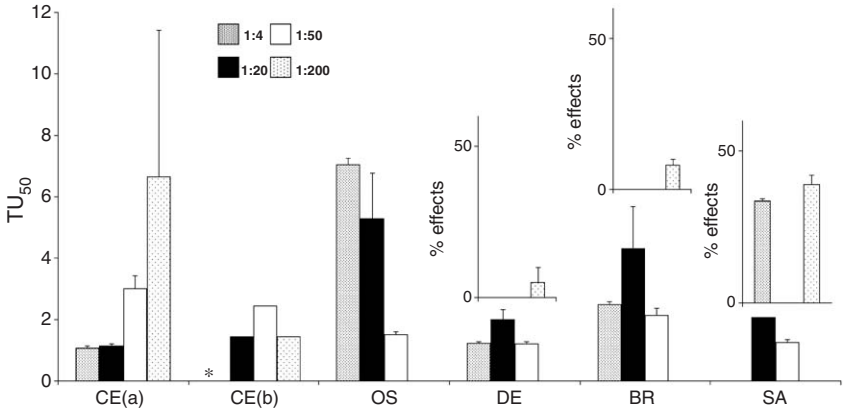

Fig. 2. Embryo toxicity bioassay with *P. lividus*. Trend of toxicity (TU_{50} when calculable and % of effect when EC_{50} value cannot be determined) according to the increasing sediment:water elutriation ratios. Asterisk (*) indicates that sample is not toxic.

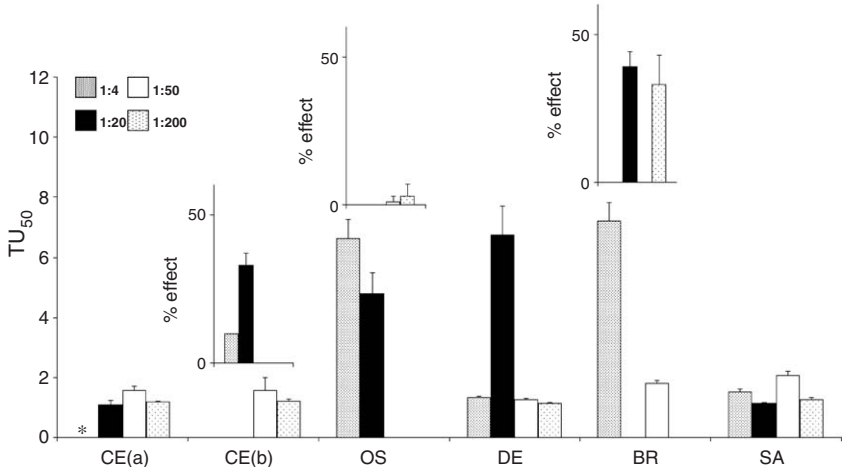

Fig. 3. Embryo toxicity bioassay with *C. gigas*. Trend of toxicity (TU_{50} when calculable and % of effect when EC_{50} value cannot be determined) according to the increasing sediment:water elutriation ratios. Asterisk (*) indicates that sample is not toxic.

limits are $EC_5 = 15.3$ (11.3–17.7) mg/L and $EC_{50} = 25.4$ (22.8–26.8) mg/L for the sperm cell test with *P. lividus* (Arizzi Novelli et al., 2004), and LOEC = 1 mg/L $EC_{50} = 4.2$ (3.9–4.6) mg/L for the embryotoxicity test with *P. lividus* (Arizzi Novelli et al., 2004), NOEC = 4.7 mg/L and for the embryo toxicity test with *C. gigas* (ASTM, 1998b).

All extrapolated sulfide concentrations (Figs. 4 A–C) were lower than each methodology's sensitivity limits. Ammonia concentrations in some samples presented a greater concentration than one or more of the sensitivity limits (Fig. 4 D–F), so ammonia could be considered a confounding factor for those samples.

4. Discussion

Toxicity results showed that contaminant bioavailability seemed to be sampling station based and highlighted that the commonly employed 1:4 sediment:water ratio (USEPA, 1991) has not always been useful in detecting sediment toxicity, generally demonstrating a lower discriminatory capability than the intermediate ratios (1:20 and 1:50) and, sometimes, than the 1:200 ratio. A particularly interesting result is that the two possible reference sites, CE(a) and CE(b), which present low concentrations of metal and organic pollutants (Table 1) with respect to other sampling stations, showed low toxicity levels for the 1:4 ratio, while toxicity increased for other ratios according to all bioassays.

A possible explanation for the higher toxicities of intermediate ratios is that larger volumes of water might facilitate the toxicants passing from the solid to liquid phase. On the other hand, it should be stressed that, generally, excessive volumes of water could produce dilution effects, inhibiting the recognition of sediment toxicity, as presumably occurred in the case of the 1:200 ratio for OS and BR samples.

Daniels et al. (1989) suggested that the 1:4 ratio, rather than the 1:10 and 1:20 ratios, is the better way to make contaminants bioavailable. Our results are not in

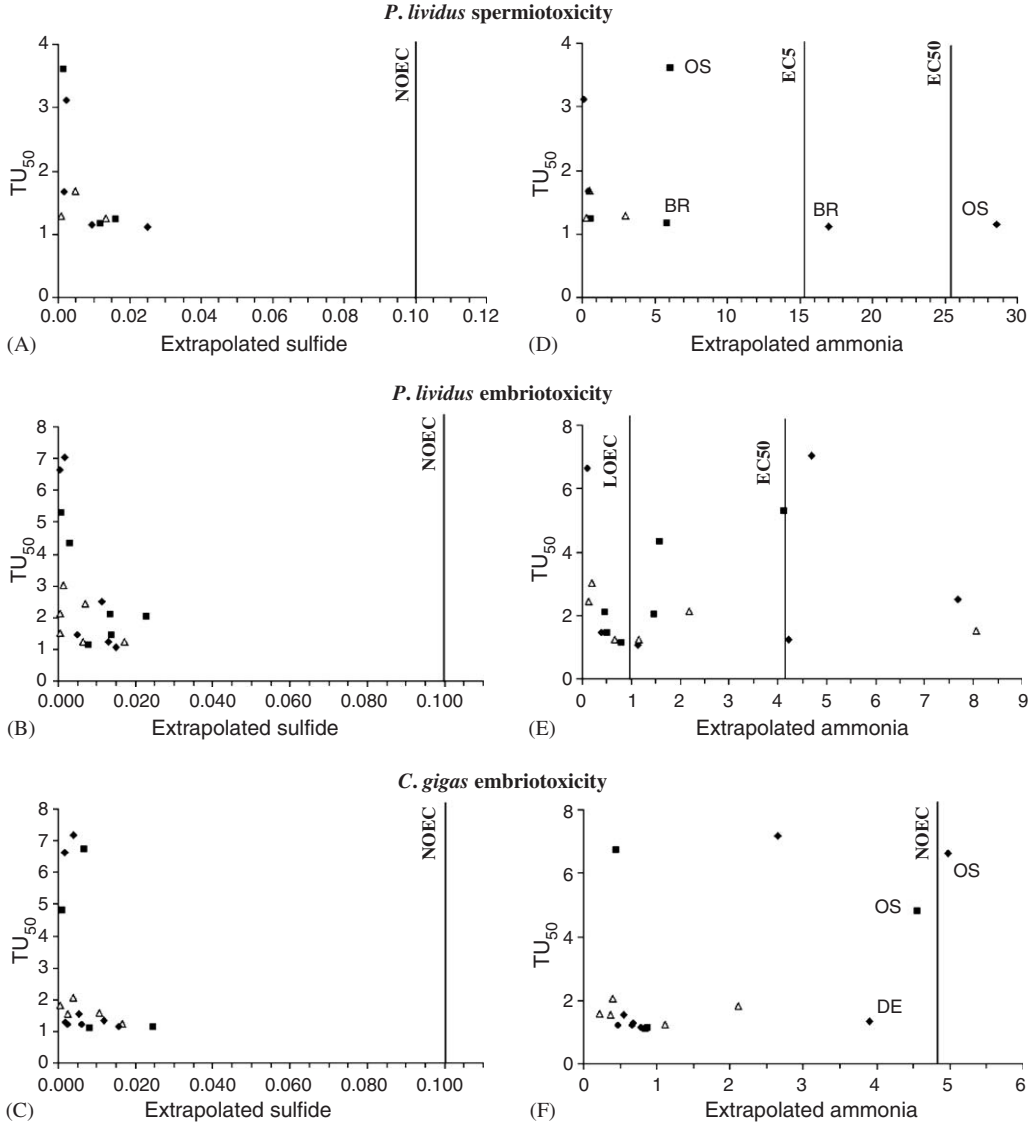

Fig. 4. (A, B, and C) Toxicity (TU_{50}) at four elutriation ratios (◆ 1:4; ■ 1:20; △ 1:50; and ● 1:200) against extrapolated sulfide concentrations (mg/L). (D, E, and F) Toxicity (TU_{50}) at four elutriation ratios against extrapolated ammonia concentrations (mg/L).

agreement with this, probably due to the fact that the authors used a very different elutriation method (rotary tumbling, 4-rpm agitation cycles, and 1-h agitation time).

On the contrary, Da Ros et al. (1997) reported that the 1:50 ratio seemed to provide better information on sediment toxicity than the 1:5 and 1:200 proportions and that, according to chemical analyses, there was no direct proportionality between biological response and sediment volume.

Possible false positives in toxicity data were investigated, comparing toxicological results with ammonia and sulfide concentrations. Sulfide seemed to play no part in toxicity definition. Ammonia levels, obtained from sediment of industrial and estuarine sites, appeared to influence some elutriates toxicity toward sea urchins bioassays. Whether the ammonia concentrations found could be due to natural background levels (ammonia as a confounding factor) or to anthropogenic contamination phenomena (ammonia as a pollutant) is a matter for discussion, but previous studies indicated the latter as the most feasible scenario (MAV-CVN, 2001). In particular in Figs. 4 D–F, it can be noted that some samples with different ammonia concentrations have demonstrated similar toxicity levels, while others with comparable ammonia concentrations, even lower than each methodology's sensitivity limits, have shown considerably different toxicity levels, suggesting the absence of a direct relationship between the ammonia concentration and the toxicity of samples.

5. Conclusions

A preliminary understanding of the discriminatory capabilities of the four different elutriation ratios chosen for this study (1:4, 1:20, 1:50, and 1:200) has been reached.

It has been highlighted that there is no best sediment:water proportion, but rather that the different ratios can contribute in specific manners to the sediment quality assessment procedure. In general, the samples generated from the widely used 1:4 ratio have shown to be less toxic than those from intermediate ratios, 1:20 and/or 1:50, and sometimes the 1:200 ratio. This suggested that intermediate sediment:water proportions made contaminants more water-soluble and, presumably, more available toward the biota.

It appears to be ascertained that, in biomonitoring programs, the evaluation of sediment quality through elutriation should be anticipated by a preliminary study to establish the most representative elutriation proportions for the environment of interest. In any case, the use of more than one sediment:water proportion could assure better discriminatory capabilities of biological toxicity tests.

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References

Andreatta, A., Scotto, S., Fabris, R., Manente, S., Perin, G., 1994. Applicazione della tecnica di elutriazione come metodo avanzato per la valutazione della tossicità di un sedimento su alghe unicellulari. Confronto dei dati tossicologici con il contenuto di inquinanti organici e inorganici. In: Dalla Tossicologia alla Ecotossicologia, Seminario di Studi CISBA, Pordenone 16–17 settembre 1994, pp. 261–265.

Ankley, G.T., Shubauer-Berigan, M.K., Dierkes, J.R., 1991. Predicting the toxicity of bulk sediments to aquatic organisms with aqueous test fractions: pore water vs elutriate. Environ. Toxicol. Chem. 10, 1359–1366.

Arizzi Novelli, A., Argese, E., Tagliapietra, D., Bettiol, C., Volpi Ghirardini, A., 2002. Toxicity of tributyltin and triphenyltin to early life stages of *Paracentrotus lividus* (Echinodermata: Echinoidea). Environ. Toxicol. Chem. 21, 859–864.

Arizzi Novelli, A., Picone, M., Losso, C., Volpi Ghirardini, A., 2004. Ammonia as confounding factor in toxicity bioassays with the sea urchin *Paracentrotus lividus* (Lmk). Toxicol. Environ. Chem. 85 (4–6), 183–191.

ASTM, American Society for Testing and Material, 1990. Standard guide for collection, storage, characterisation and manipulation of sediments for toxicological testing. E1390–E1391.

ASTM, 1991. Standard Guide for Collection, Storage, Characterization, and Manipulation of Sediments for Toxicological Testing and for Selection of Samplers Used to Collect Benthic Invertebrates. E1391-03.

ASTM, 1998a. Standard Guide for Conducting Static Acute Toxicity Tests with Echinoid Embryos E1563-98.

ASTM, 1998b. Standard Guide for Conducting Static Acute Toxicity Tests Starting with Embryos of Four Species of Saltwater Bivalve Molluscs. E724-98.

Beiras, R., Vàzquez, E., Bellas, J., Lorenzo, J.I., Fernandez, N., Macho, G., Marino, J.C., Casas, L., 2001. Sea-urchin embryo bioassay for in situ evaluation of the biological quality of coastal seawater. Estuarine Coastal Shelf Sci. 52, 29–32.

Bridges, T.S., Wright, R.B., Gray, B.R., Gibson, A.B., Dillon, T.M., 1996. Chronic Toxicity of Great Lakes sediments to *Daphnia magna*: elutriate effects on survival, reproduction and population growth. Ecotoxicology 5, 83–102.

Burton Jr., G.A., 1992. Sediment Toxicity Assessment. Lewis Publishers, Boca Raton FL.

Burton Jr., G.A., MacPherson, C., 1995. Sediment toxicity testing issues and methods. In: Hoffman, D.J., Rattner, B.A., Allen Burton, Jr., G., Cairns, Jr., J. (Eds.), Handbook of Ecotoxicology. CRC Press, Inc., Lewis Publishers, pp. 71–103.

Burton, G.A., Stemmer, B.L., Winks, K.L., Ross, P.E., Burnett, L.C., 1989. A multitrophic level evaluation of sediment toxicity in Waukegan and Indiana Harbors. Environ. Toxicol. Chem. 8, 1057–1066.

CVN-Consorzio Venezia Nuova, 1999. Interventi per la messa in sicurezza delle barene del Passo Campalto. In Quaderni Trimestrali Consorzio Venezia Nuova 1, 64–67.

Da Ros, L., Marin, M.G., Fossato, V.U., Campesan, G., 1997. Sedimenti lagunari: prove di tossicità su embrioni di riccio di mare, *Paracentrotus lividus*. Biol. Marina Mediterranea 4 (1), 632–636.

Daniels, S.A., Munawar, M., Mayfield, C.I., 1989. An improved elutriation technique for the bioassessment of sediment contaminants. Hydrobiologia 188/189, 619–631.

Di Domenico, A., Baldassarri, L.T., Ziemacki, G., De Felip, E., La Rocca, C., Ferrari, G., Cardelli, M., Volpi, F., Ferri, F., Iacovella, N., Lupi, C., Rodriguez, F., D'Agostino, O., Sansoni, R., Settimo, G., 1998. Priority microcontaminants in sediment samples from the Venice lagoon: a selection of concentration data and predominant analytical features. Organohalogen Compounds 39, 205–210.

Finney, D.J., 1971. Probit Analysis. Cambridge University Press, London, UK.

Forstner, U., Wittmann, G.T.W., 1983. Metal Pollution in the Aquatic Environment, second ed. Springer, Berlin, pp. 197–270 (Chap. E).

Hamilton, M.A., Russo, R.C., Thurston, R.V., 1978. Trimmed Spearman–Karber method for estimating median lethal concentrations in toxicity bioassays. Environ Sci. Technol. 12, 714–720.

His, E., Seaman, M.N.L., Beiras, R., 1997. A simplification: the bivalve embryogenesis and larval development bioassay method for water quality assessment. Water Res. 31 (2), 351–355.

His, E., Beiras, R., Seaman, M.N.L., 1999. The assessment of marine pollution bioassays with bivalve embryos and larvae. Adv. Mar. Biol. 37, 1–178.

Hurkey, G.A., Landrum, P.F., Klaine, S., 1994. Comparison of whole sediment, elutriate and pore-water exposures for use in assessing sediment-associated organic contaminants in bioassays. Environ. Toxico. Chem. 13 (8), 1315–1329.

Lazorchak, J.M.M., Smith, E., Crocker, P., Euresti, A., Haring, H., Bennett, M., 2003. A comparison of bulk sediment toxicity testing methods and sediment elutriate toxicity. Presented at Biological Advisory Committee Meeting, Newport, RI, October 14–16.

Lee, F.G, Jones R.A., Saleh F.Y., Mariani G.M., Homer D.H., Butler J.S., Bandyopadhyay P., 1978. Evaluation of the elutriate test as a method of predicting contaminant release during open-water disposal of dredged sediments and environmental impact of open-water dredged material disposal. US Army Engineer Waterways Experiment Station, Technical Report D-78-45.

Liß, W., Ahlf, W., 1997. Evidence from whole-sediment, porewater and elutriate testing in toxicity assessment of contaminated sediments. Ecotoxicol. Environ. Saf. 36, 140–147.

Long, E.R., Buchman, M.F., Bay, S.M., Breteler, R.J., Carr, R.S., Chapman, P.M., Hose, J.E., Lissner, A.L., Scott, J., Wolfe, D.A., 1990. Comparative evaluation of five toxicity tests with the sediments from San Francisco Bay and Tomales Bay, California. Environ. Toxicol. Chem. 9, 1193–1214.

Losso, C., Arizzi Novelli, A., Picone, M., Volpi Ghirardini, A., Rudello, D., Ugo, P., 2004. Sulphide as confounding factor for toxicity

bioassays (sperm cell toxicity test and embryo toxicity test) with the Mediterranean sea urchin *Paracentrotus lividus*. Environ. Toxicol. Chem. 23 (2), 396–401.

Matthiessen, P., Bifield, S., Jarret, F., Kirby, M.F., Law, J., McMinn, W.R., Sheahan, D.A., Thain, J.E., Whale, G.F., 1998. An assessment of sediment toxicity in the River Tyne estuary, UK by means of bioassays. Mar. Environ. Res. 45 (1), 1–15.

MAV-CVN, 2001. Drain project. In: Collavini, F., Zonta, R., Bettiol, C., Fagarazzi, O., Zaggia, L. (Eds.), Trasferimento di acqua dolce e inquinanti dal bacino scolante alla Laguna di Venezia. Documento di progetto 15A.

MAV-Magistrato alle Acque di Venezia, 2004. Qualità delle acque e degli scarichi idrici dell'area di Porto Marghera, dati relativi al 2001–2002. Servizio Antinquinamento del Magistrato alle Acque (SAMA), Venice, Italy, p. 184.

McFadzen, I.R.B., 2000. Determination of water quality in the Venice lagoon utilizing the early life stages of a fish (*Sparus aurata*) and an echinoderm (*Paracentrotus lividus*). Man and the Biosphere Series. In: Lasserre, P., Marzollo, A. (Eds.), The Venice Lagoon Ecosystem: Inputs and Interactions Between Land and Sea, vol. 25. UNESCO Paris and The Parthenon Publishing Group, pp. 339–355.

Meador, J.P., Ross, B.D., Dinnel, P.A., Picquelle, S.J., 1990. An analysis of the relationship between a sand-dollar embryo elutriate assay and sediment contaminants from station in a urban embayment of Puget sound, Washington. Mar. Environ. Res. 30, 251–272.

Munawar, M., Norwood, W.P., McCarthy, L.H., Mayfield, C.I., 1989. In situ bioassessment of dredging and disposal activities in a contaminated ecosystem: Toronto Harbour. Hydrobiologia 188/189, 601–618.

Reardon, J., Foreman, J.A., Searcy, R.L., 1966. New reactants for the colorimetric determination of ammonia. Clin. Chim. Acta 14 (3), 203–205.

Schuytema, G.S., Nebeker, A.V., Cairns, M.A., 1996. Comparison of recirculating, static, and elutriate aquatic sediment bioassay procedures. Bull. Environ. Contamin. Toxicol. 56 (5), 742–749.

SETAC, 1993. Guidance Document on sediments toxicity tests and bioassays for freshwater and marine environments. In: Hill, I.R., Matthiessen, P., Heimback, F. (Eds.), Workshop on Sediment Toxicity Assessment, 8–10 November 1993, Renesse (NL).

Shuba, P.J., Tatem, H.E., Carroll, J.H., 1978. Biological assessment methods to predict the impact of open-water disposal of dredged material. Technical Report: D-78-5Q. US COE, Washington, DC.

Sibley, P.K., Legler, J., Dixon, D.G., Burton, D.R., 1997. Environmental health assessment of benthic habitat adjacent to a pulp mill discharge. Acute and chronic toxicity of sediments to benthic macroinvertebrates. Arch. Environ. Contamin. Toxicol. 32 (3), 274–284.

Spoladori, A., 2004. Dumps of toxic industrial waste in the drainage area of the Lagoon of Venice. In: Guerzoni, S., Raccanelli, S. (Eds.), The sick lagoon. Dioxin and other persistent organic pollutants (POPs) in the Lagoon of Venice. Libreria Editrice Cafoscarina, Venice, Italy, pp. 61–70.

Thain, J.E., Allen, Y., Matthiessen, P., 1996. Monitoring sediment toxicity in UK estuaries and offshore waters from 1990–1993. Development and Progress in Sediment Quality Assessment: Rationale, Challenges, Techniques & Strategies, pp. 127–142.

USACE, 1978. Ecological evaluation of proposed discharge of dredged material into ocean water; implementation manual for section 103 of PL 92-532. Environmental Laboratory, US Army Corps Engineer, Waterways Experiment Station, Vicksburg, MS.

USEPA, 1977. Ecological Evaluation of Proposed Discharge of Dredged Material into Ocean Waters. ES Army COE Waterways Experiment Station, Vicksburg, MS.

USEPA, 1991. Evaluation of dredged material proposed for ocean disposal testing manual. EPA 503/8-91/001.

USEPA, 1994. Tetra-through octa-chlorinated dioxins and furans by isotope dilution HRGC/HRMS. Method 1613B, 1994.

USEPA, 1995. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to west coast marine and estuarine organisms. USEPA, 600/R-95/136, Cincinnati, Ohio.

USEPA, 1998. Inductively coupled plasma-mass spectrometry. Method 1998; 818 6020.

USEPA, 1999. Chlorinated biphenil congeners in water, soil, sediment, and tissue by HRGC/HRMS. Method 1668A, 1999.

Van den Hurk, P., 1994. Effects of natural sediment properties on test results in bioassays with oyster larvae (*Crassostrea gigas*) on sediment elutriates. J. Aquat. Ecosyst. Health 3, 185–191.

Van den Hurk, P., Eertman, R.H.M., Stronkhorst, J., 1997. Toxicity of harbour canal sediments before dredging and after off-shore disposal. Mar. Pollut. Bull. 34 (4), 244–249.

Vashchenko, M.A., Zhada, P.M., 1993. Bioassay of bottom sediments of Petr velikiy Gulf (Sea of Japan) with sexual cells, embryos and larvae of sea urchins. Oceanology 33 (1), 102–106.

Volpi Ghirardini, A., Arizzi Novelli, A., 2001. A sperm cell toxicity test procedure for the Mediterranean species *Paracentrotus lividus* (Echinodermata: Echinoidea). Environ. Technol. 22, 439–445.

Volpi Ghirardini, A., Delaney, E., Cavallini, L., Bettiol, C., Tagliapietra, D., Ghetti, P.F., 1998. *H. diversicolor*, *N. succinea* e *P. cultrifera* come indicatori di bioaccumulo di metalli pesanti dai sedimenti: indagine preliminare in Laguna di Venezia. S.It.E. Atti 18, 519–522.

Volpi Ghirardini, A., Cavallini, L., Delaney, E., Tagliapietra, D., Ghetti, P.F., Argese, E., Bettiol, C., 1999. *H. diversicolor*, *N. succinea* and *P. cultrifera* (Polychaeta: Nereididae) as bioaccumulators of cadmium and zinc from sediments: preliminary results in the Venetian lagoon (Italy). Toxicol. Environ. Chem. 71, 457–474.

Volpi Ghirardini, A., Arizzi Novelli, A., Losso, C., Ghetti, P.F., 2003. Sea urchin toxicity bioassays for sediment quality assessment in the Lagoon of Venice (Italy). Chem. Ecol. 19 (2–3), 99–111.

Volpi Ghirardini, A., Arizzi Novelli, A., Tagliapietra, D., 2005. Sediment toxicity assessment in the Lagoon of Venice (Italy) using *Paracentrotus lividus* (Echinodermata: Echinoidea) fertilization and embryo bioassays. Environ. Int. 31, 1065–1077.

Williams, L.G., Chapman, P.M., Ginn, T.C., 1986. A comparative evaluation of marine sediment toxicity using bacterial luminescence, oyster embryo and amphipod sediment bio-assays. Mar. Environ. Res. 19, 225–249.