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Liquid versus solid phase bioassays for dredged material toxicity assessment

M.C. Casado-Martínez *, N. Fernández, J.M. Forja, T.A. DelValls

Departamento de Química-Física, Universidad de Cádiz, Polígono Río San Pedro, S/N., C.P. 11510 Puerto Real, Cádiz, Spain

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Abstract

Since 1994 the results of the analyses of key chemical compounds (trace metals, polychlorinated biphenyls and polycyclic aromatic hydrocarbons) and the comparison with the corresponding sediment quality guidelines (SQGs) are used in decision-making for dredged material management in Spain. Nonetheless in the last decades a tiered testing approach is promoted for assessing the physical and chemical characteristics of dredged sediments and their potential biological effects in the environment. Bioassays have been used for sediment toxicity assessment in Spain but few or no experiences are reported on harbour sediments. We studied the incidence of toxicity in the 7d bioassay using rotifers (*Brachionus plicatilis*) and the 48h bioassay using sea urchin (*Paracentrotus lividus*) embryos over a series of experiments employing 22 different elutriates. The relative performance of this exposure phase was not comparable to data on the 10-d acute toxicity test using the burrowing amphipod *Corophium volutator* and the polychaete *Arenicola marina*, carried out on the whole sediments. These results evidence the importance of the exposure route and the test selected in decision-making, as the toxicity registered for the undiluted elutriates was largely due to the different solubility of sediment-bound contaminants. This work and other studies indicate that for many sediments, a complete battery of test is recommended together with physico-chemical analyses to decide whether dredged sediments are suitable for open water disposal or not.

Keywords: Ecotoxicity; Contaminated sediments; Elutriates; Dredged material characterisation

1. Introduction

Ports, rivers and water ways often need regular dredging to keep them open for navigation. Environmental concerns arise when dredged sediments are anoxic and particularly if they come from harbours or industrialized estuaries, since these sediments can be contaminated with different substances due to poor environmental policies in the past. When these dredged materials are excavated and relocated the contaminants can be transferred to the disposal grounds, where they can affect the local benthic community. Moreover during these operations sediments are oxygenated and dispersed and the contaminants may change their chemical speciation, cease to be adsorbed on to silt particles, and then enter food chains and do harm. Several countries are already applying laboratory bioassays for sediment quality assessment and/or dredged material management. One of the issues addressed by several regulatory bodies is the development of standard and sensitive methods since effects-based testing is still under development (den Besten et al., 2003; Peters et al., 2002). This study summarises the results of two different liquid phase tests for elutriate toxicity assessment: the sea-urchin embryo-larval bioassay, that is widely applied for sediment toxicity assessment including sediment elutriate and interstitial water (Beiras et al., 2001; Carr et al., 1996) and the 7-d bioassay using a population of the rotifer *Brachionus plicatilis*, previously used on sediment pore water and elutriates in Spain (DelValls et al., 1998; Riba et al., 2004a). The results are compared with standard 10-day static toxicity tests carried out on the whole sediments: the bioassay using the burrowing amphipod *Corophium volutator* (ASTM, 1991) and the bioassay using the polychaete *Arenicola marina* (Thain and Bifield, 2001). This design allows making direct intertest comparisons and, together with the physico-chemical characterisation of the sediments, to study the performance of

* Corresponding author. Departamento de Química-Física - Edifico CASEM, Facultad de Ciencias del Mar y Ambientales, Universidad de Cádiz, Polígono Río San Pedro, s/n., 11510 Puerto Real Cádiz, Spain. Tel.: +34 956016794; fax: +34 956016040.
E-mail address: mcarmen.casado@uca.es (M.C. Casado-Martínez).

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elutriate tests for sediment toxicity assessment in the context of navigational dredging.

2. Material and methods

2.1. Sediment sampling and chemical characterization

Sediments were sampled in the ports of Huelva, Cádiz, Barcelona, Cartagena, Bilbao and Pasajes with a 0.025 m^2 Van Veen grab from approximately the top 20 cm of the sediment (Fig. 1). Sediments were pooled until enough volume was sampled (around 40 L) and were brought to the laboratory, where they were homogenized, sieved through a 2 mm mesh to eliminate debris and stored at 4 °C, darkness and closed hermetically no longer than two weeks prior to tests. Afterwards the sediments were subsampled for sediment chemical characterization, which followed Spanish recommendations for dredged materials (CEDEX, 1994). The analyses consisted of grain size distribution, organic matter content measured as loss of ignition and the concentration of As, Cd, Cr, Cu, Hg, Ni, Pb, Zn, the sum of 7 polychlorinated biphenyls and 12 polycyclic aromatic hydrocarbons. All metals were quantified using flame or furnace atomic absorption spectrometry except As and Hg, measured by hydride generation and cold vapor technique respectively. PCBs were determined by gas chromatography with electron capture detection (EPA 8080) and PAHs by HPLC with fluorescence detection (EPA 8310). Detailed information of the sediment characterization has been recently reported in Casado-Martínez et al. (2006a).

2.2. Liquid phase bioassays

2.2.1. Sediment elutriates

Sediment elutriates were obtained using a modification of the US EPA method (1998). Sediments were homogenized and mixed with clean seawater in a proportion 1:4 v/v (sediment:water) for 30 min at approximately 20 °C. The mixture was left to settle overnight and then the supernatant was siphoned. The sediment elutriates were kept at 4 °C and darkness until they were used in the toxicity tests but no longer than one week. The day the tests were initiated elutriates were transferred to the test chambers manually and they were left to reach the test temperature without additional aeration before the addition of the test organisms.

2.2.2. Rotifer population decay bioassay

Test parameters and conditions followed the protocol developed by DelValls et al. (1996) and are summarized in Table 1. This test evaluates the decrease in a population of the rotifer *B. plicatilis* exposed to the sediment elutriates for 7 days. The test organisms were maintained for 48 h on starving conditions prior to tests to empty the guts and the population decrease was registered throughout the test duration counting 100 organisms under an optical loupe three times a day. The number of surviving organisms was used to calculate the time needed for a decrease of 50% of the initial population under starving conditions (LT50) using a modification of the probit method (DelValls et al., 1996). A negative toxicity control was included on each batch of samples consisting on the same seawater used for culturing the test organisms and to obtain the sediment elutriates. The results were corrected for the corresponding control to compare different batch of experiments.

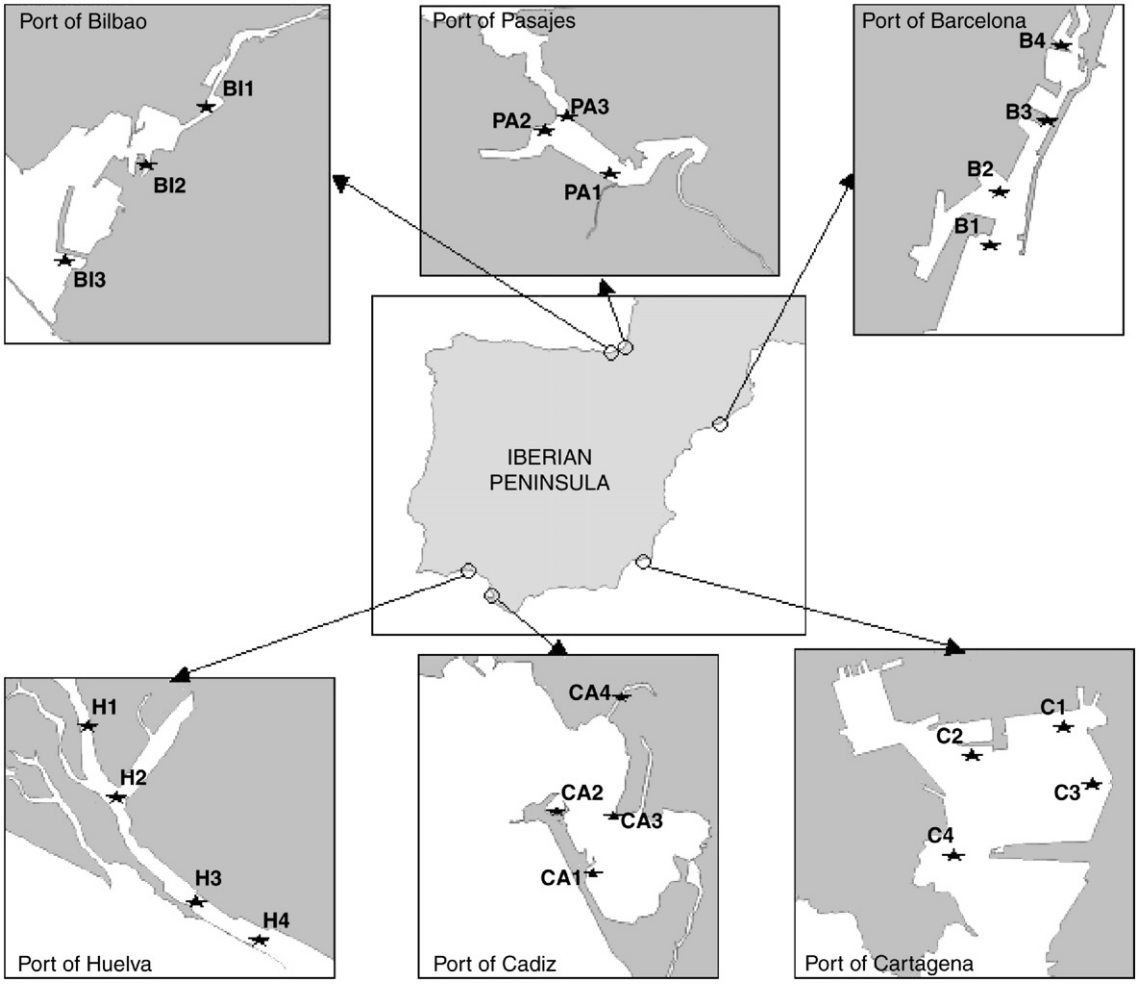

Fig. 1.

Table 1
Test parameters and conditions for the test using a population of rotifers of the species B.plicatilis and the sea-urchin embryo-larval bioassay

Parameter	Rotifer population decay	Sea-urchin embryo-larval
Type of test	Static. On liquid phase	Static. On liquid phase
Temperature	25 °C	20 °C
Salinity	38±2	38±2
Photoperiod	24 h light	No
Test chambers	2 L chambers for acclimation 50 mL glass chambers for bioassay	25 mL glass chambers
Sample volume	50 mL	20 mL
Water renewal	None	None
State of the organisms and number per test chamber	A whole population under normal growth conditions; 200 organisms/ mL	20–30 embryos/mL
Replicates	3	5
Feeding regime	None	None
Aeration	None	None
Water quality	Temperature, salinity, pH and dissolved oxygen	Temperature, salinity, pH and dissolved oxygen
Test duration	7 days	48 h
Endpoints	Survival, TL50	Embryogenesis success (percentage of normal pluteus larvae)
Test acceptability	Decrease to 50% of initial population in clean seawater between 48 and 72 h (LT50)	90% normal larvae in controls

2.2.3. Sea urchin embryo-larval bioassay

Fecundation and test conditions followed the protocol developed by Fernández (2002) and are summarized in Table 1. Gametes were obtained from a single male and female sea urchin by direct extraction with a pipette and, once the fecundation was successfully completed, embryos were introduced in 25 mL vials with the sediment elutriates at 20 °C to a density of 20–30 embryos per mL. Five replicates were used per sample and a negative toxicity control consisting of clean seawater was tested in parallel with the samples. After 48 h at 20 °C and darkness the samples were fixed with two drops of 40% formaldehyde. The measured endpoint was embryogenesis success measured in 100 organisms per replicate after exposure to the undiluted sediment elutriates. The results are expressed as percentage of normal pluteus (defined as those with four well developed arms) normalized to the corresponding control.

2.3. Solid phase bioassays

The battery of solid phase tests included the 10-day static sediment toxicity test using the crustacean amphipod *C. volutator* and the 10-day static sediment toxicity test using the polychaete *A. marina*. The results of these bioassays have been previously reported in Casado-Martínez et al. (in press).

Table 2
Sediment quality guidelines (Action Levels) used in Spain for dredged material management (CEDEX, 1994)

Compound	AL1	AL2
As	80	200
Cd	1.0	5.0
Cr	200	1000
Cu	100	400
Hg	0.6	3.0
Ni	100	400
Pb	120	600
Zn	500	3000
Σ_7-PCB	30	100

All values are expressed as $mg \cdot kg^{-1}$ except Σ_7-PCB expressed as $\mu g \cdot kg^{-1}$.

2.4. Data analysis

The non-parametric Fisher Exact test was performed on the toxicity tests results to identify significantly different responses ($P=0.05$) related to control. The algorithm and the method are included in the Simple Interactive Statistical Analysis (SISA) available on-line at (http://home.clara.net/sisa/). The toxicity results were interpreted in terms of management categories established by the limit values used in Spain for dredged material (Table 2): Category I for not contaminated sediments, Category II for moderate contaminated sediments and Category III for heavily contaminated sediments. Spearman rank correlations were used to link sediment contamination and toxicity. Correlation coefficients were developed using the statistical program STATISTICA® 6.0.

3. Results

3.1. Sediment chemical characterization

Three sediment samples (CA1, H4 and BI3) fell within Category I, with all chemical concentrations lower than the corresponding AL1 (Table 2). Samples classified in Category II included CA3, CA4, B1 and B3 while the rest of samples fell within category III according to the high concentrations reported for several contaminants. Sediments were affected by a mixture of compounds depending on the sources and activities at each port. The sediments from Huelva and Cartagena reported high metallic concentrations although Cartagena was also affected by high concentrations of PCBs. The ports of Bilbao and

Table 3
Spearman rank correlation coefficients for the contaminants of concern and the liquid and solid phase bioassays

	Spearman rho			
	Rotifer population decay	Embryo-larval bioassay	Amphipod	Polychaete
% fines	−0.29	0.06	0.36	0.09
% TOC	−0.31	−0.12	0.46*	0.11
Metallic compounds				
As	0.08	−0.16	0.63**	0.56*
Cd	0.24	−0.23	0.54**	0.35
Cr	0.01	−0.53	0.22	0.22
Cu	0.21	0.25	0.65**	0.35
Hg	0.04	−0.18	0.55**	0.22
Ni	−0.07	−0.29	0.55**	0.57*
Pb	0.02	−0.29	0.58**	0.37
Zn	0.08	−0.19	0.62**	0.47*
Organic compounds				
PCBs	−0.45*	−0.27	0.41	0.04
PAHs	−0.19	−0.59**	0.38	0.25
Bioassays				
Rotifer population decay		−0.03	−0.17	0.13
Embryo-larval bioassay			−0.05	−0.12
Amphipod				0.65**

*Significant at 0.05; **Significant at 0.01.
Results of bioassays were expressed so as to obtain higher toxicities for high contamination.

Pasajes reported high organic contamination with some metals exceeding the corresponding ALs.

3.2. Liquid phase bioassays

3.2.1. Rotifer population decay bioassay

The results of the sediment toxicity tests are summarised in Fig. 2. The negative toxicity controls for each batch of experiments were in the range for test acceptability (DelValls et al., 1996). This bioassay identified slight toxic effects for elutriates CA1, CA4, H1, H2, H3 and BI2, that reported LT50 values lower than the corresponding control, while the population of rotifers needed a longer time to decay when exposed to the rest of elutriates (Fig. 2). If we use the SQG developed by DelValls et al. (1996) to consider sediment toxicity (LT50s <48 h) all sediments would be considered not toxic to the population of rotifers under the described test conditions.

3.2.2. Sea-urchin embryo-larval bioassay

Even if the mean percentage of normal pluteus and the individual values for all replicates were in the range for test acceptability (>90%), the embryogenesis success endpoint was a very variable endpoint among replicates for the treatment sediments. The highest toxic effects were reported after exposure to elutriates from Cádiz (CA#), H2, H3 and B2, with percentages of normal pluteus lower than 25% (Fig. 2). All the elutriates causing a percentage of abnormal pluteus higher than 25% were statistically different from controls (CA1, CA2, CA3, CA4, H2, H3 and B2) although samples H1, H4, BI2, B3 and BI3, that reported percentages lower than 25%, were also statistically different from controls. In general the elutriates that caused slight toxic effects to rotifers were also toxic to sea urchin embryos.

3.3. Linking sediment contamination and toxicity

The biological endpoints were correlated with the chemical concentrations and other parameters included in the sediment characterisation ($n=16$–22; Table 3). Taking into consideration that the correlation coefficients should be positive (high chemical concentrations related to high toxic effects); toxicity to sea-urchin embryos was not correlated neither with the chemical compounds nor with the variables used to describe the sediment properties. The high concentrations reported in Cartagena and the absence of toxic effects may be responsible for the low correlation between contamination and this endpoint. Similarly toxicity to rotifers was not correlated neither with the concentration of metals nor with PAHs in sediments although the correlation coefficients were positive. Negative correlation coefficients, that evidence an inverse relationship between sediment contamination and toxicity, were reported between embryogenesis success and the sum of PAHs (-0.56; $p<0.01$). Rotifer population decay was inversely correlated with the proportion of fines and organic matter content (-0.29 and -0.31, respectively) but the

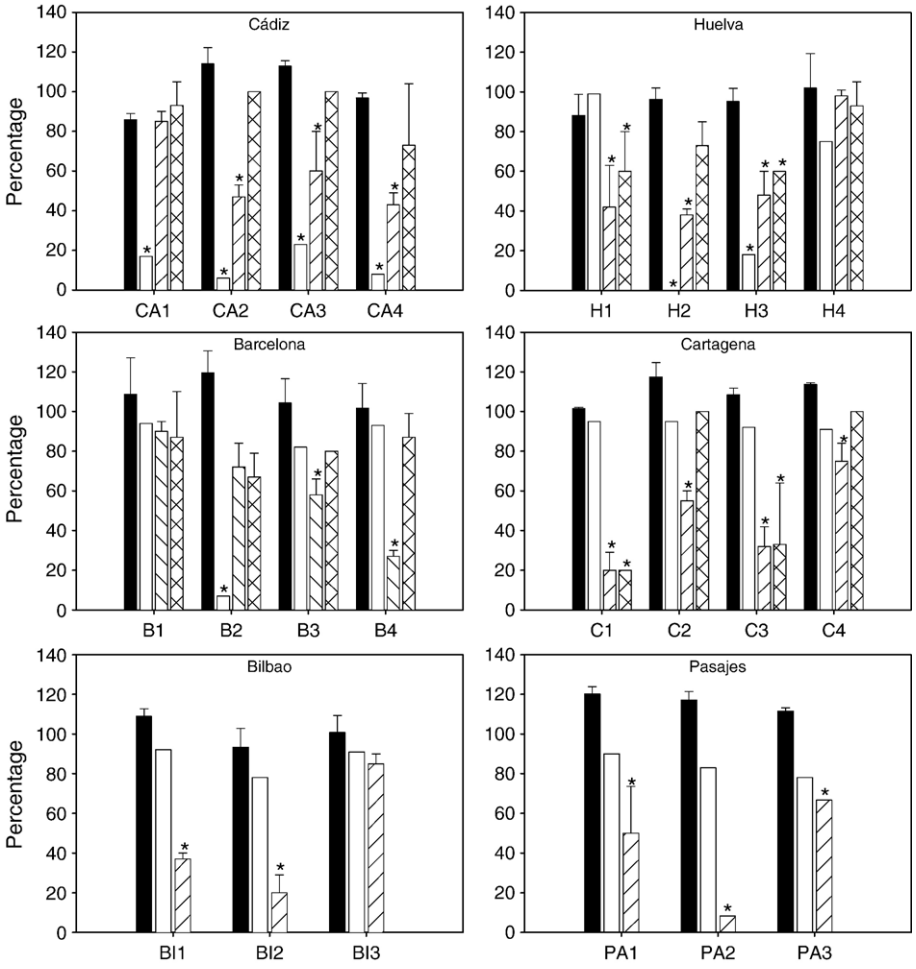

Fig. 2.

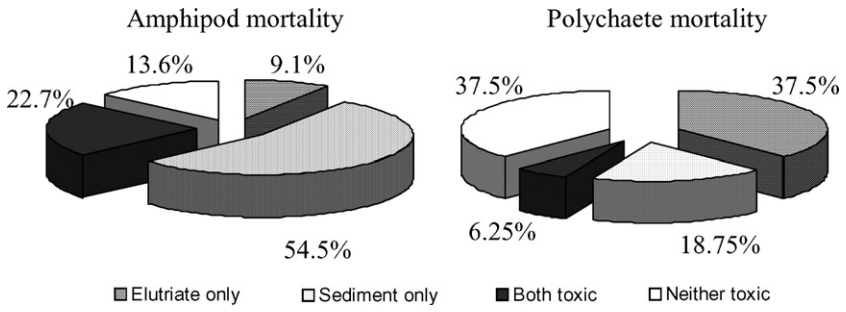

Fig. 3.

correlation was only significant for PCBs (−0.45; $p<0.05$). On the contrary both whole sediment toxicity tests were correlated with contaminant concentrations measured in sediments (Casado-Martínez et al., in press). Toxicity to amphipods was highly correlated with all metals analysed ($p<0.01$) except with Cr while the lethal effects in polychaetes were only correlated with As, Ni and Zn ($p<0.05$). Both solid-phase bioassays were slightly correlated with the organic contaminants (PAHs and PCBs) even though these correlations were not significant ($p<0.05$). As expected, the endpoints measured after exposure to the whole sediments were better correlated with the sediment properties. The two variables included in this study, total organic matter content and proportion of fines, are two important confounding factors for whole-sediment toxicity. On the contrary these two confounding factors seem to have a lower effect when testing sediment elutriates according to the lower correlation coefficients observed.

4. Discussion

Two different tests have been used to evaluate dredged material toxicity through exposure to undiluted sediment elutriates: the bioassay using a population of the rotifer *B. plicatilis* and the bioassay using sea urchin embryos. Both test organisms are pelagic and would be exposed to contamination throughout the water column. Nonetheless substantial differences have been reported by these two measurement endpoints. Our results showed no toxicity or slight toxicity to the population of rotifers after exposure to the elutriates and even for 72% of the total number of samples the LT50 values were higher than that reported for the negative control. The dredged sediments tested fell within the three management categories (CEDEX, 1994), with different chemical compounds failing the corresponding AL2s-defined by the London Convention as the numeric value for chemical concentrations above which open water disposal is prohibited (IMO, 1998). Embryogenesis success was a more sensitive endpoint than rotifer population decay, nonetheless the highest toxic effects were reported for the lower sediment chemical concentrations while the elutriates corresponding to the port of Cartagena, highly contaminated with metallic and organic contaminants, did not cause significant toxicities. The only contaminant of concern identified in sediments considered into category II (CA3 and CA4) was Cd, which in turn has reported lower toxicity to sea urchin embryos than other metals (Fernández and Beiras, 2001; Radenac et al., 2001). The high toxicity caused by sample CA1, that fell into category I, suggests that other toxicant not measured may contribute in elutriate toxicity. The high toxic effects could be related to the presence of hydrogen sulfide according to previous concentrations reported in the area (unpublished data). This substance, which is a natural component of highly reduced sediments, greatly increases during sediment storage under anoxic conditions (Lapota et al., 2000) and results in a very toxic effect on the sea urchin embryos (Knezovich et al., 1996). Moreover toxicity to sea urchin embryos was reduced considerably after a brief aeration of the sediment elutriates from site CA1 (Casado-Martínez et al., 2006b), which supports this hypothesis. Ammonia is another naturally-occurring toxicant that may be produced in sediments extremely rich in organic matter and results in high toxicities to different species of rotifers (Lahr et al., 2003) and sea-urchin embryos (Losso et al., 2004a,b) but no measurements were included in this study.

Further studies on chemical concentrations in the sediment elutriates would address this misfit between contamination and toxicity and would improve significantly the results of elutriate tests on behalf of dredged material management strategies. The results of the correlation study are especially interesting for the organic micropollutants, that reported an inverse cause–effect relationship with the elutriate toxicity tests. This inverse relationship was especially significant for the sea urchin embryo-larval bioassay and PAHs ($p<0.01$) and between the rotifer population decay and PCBs ($p<0.05$). Low elutriate:bulk concentrations ratios have been reported in the literature (Thompson et al., 1999) and there are numerous results on harbour elutriates showing absence of metal release from sediments (McDonald, 2005; Pedersen et al., 1998). Even positive effects on invertebrate larval metamorphosis at low metal concentrations have been documented (Beiras and His, 1994). Sediment geochemical properties determine the type of metal bindings and its trend to desorb, while factors such as pH and salinity can also determine the bioavailability of chemicals bound to sediments (Riba et al., 2004b). Also organic matter affects metal speciation (Lorenzo et al., 2002) and plays a major role binding different contaminants and may be the responsible of the negative correlation coefficients between organic matter content, concentrations of organic contaminants and elutriate toxicity.

To elucidate the relative performance of elutriate tests on a whole battery of bioassays a comparative study on solid-phase and elutriate toxicity was performed on the results through the percentage of agreement between methodologies (Fig. 3). The toxicity endpoint to sea urchin embryos was in agreement with

toxicity to amphipods for 36% of samples ($n=22$) and this percentage increases to 46 when compared with the results of the test using polychaetes ($n=16$). The test using amphipods was more sensitive than the test using sea urchin embryos (77.2% to 31.8% respectively) but, on the contrary, embryotoxicity seems a more sensitive endpoint than the bioassay using polychaetes (42% and 25% respectively). The percentages of agreement reported in this study are lower than the percentages of agreement reported by Bay et al. (2003) for pore water toxicity tests (54%) even if the use of this aqueous extract can report higher toxicities than sediment elutriates according to previous results on urban harbour sediments and uncontaminated reference locations (McDonald, 2005). When addressing sediments falling into category II, which are needed of further biological assessment to decide whether they are suitable for open water disposal or not, elutriate tests reported a considerably lower incidence of toxicity than the amphipod bioassay (14.3 and 88.5% respectively) and only a 42.9% of agreement (sediments considered similarly independently of the measurement endpoint considered). This means that the selection of one or another exposure route can influence critically the classification of dredged sediments as toxic or not toxic. Bay et al. (2003) pointed out that pore water toxicity tests are much more likely than solid phase tests to detect toxicity due to 1) the different sensitivity to contaminants among species, 2) variations in contaminant exposure or bioavailability related to the test method, and 3) the influence of naturally occurring toxicants. According to our results solid phase bioassays are more likely than elutriate tests to detect toxicity and evidence the importance of the variations in contaminant exposure and bioavailability related to this extraction procedure. The solid phase bioassays were better correlated with the sediment contamination and this may indicate that empirically-derived SQGs are better predictors of toxic effects in some benthic organisms (Long et al., 2000). Nonetheless the suitability of such guidelines for predicting elutriate toxicity seems compromised thus elutriate tests are further justified in the context of navigational dredging to mimic sediment resuspension scenarios and to study direct water column effects.

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