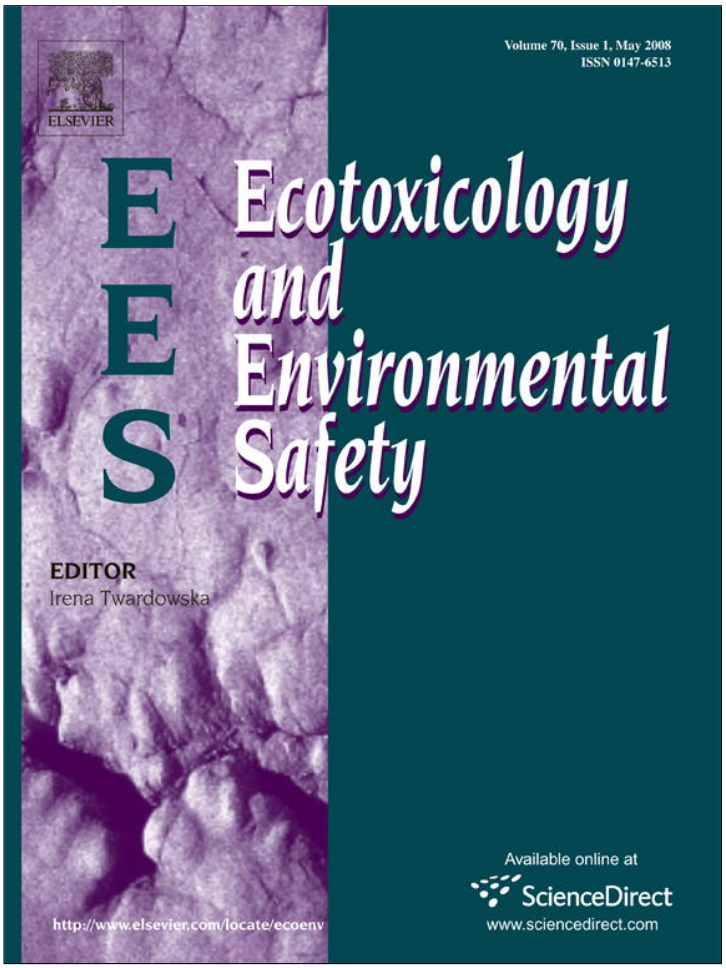

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Evaluation of *Corophium orientale* as bioindicator for Venice Lagoon: Sensitivity assessment and toxicity-score proposal ☆

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Abstract

The 96-h water-only exposure and 10-d sediment toxicity tests with the amphipod *Corophium orientale* were performed in order to enhance the knowledge about its overall sensitivity and its applicability to Venice Lagoon sediments. The values obtained with cadmium as reference toxicant demonstrated a certain variability of the LC_{50}; the higher value was found in spring and the lower in late summer. Tests with other pure chemicals (Ni, Total Ammonia, Sodium Dodecyl-Sulphate) showed good discriminatory power; the toxicity gradient observed was: Cd (LC_{50} of 3.3 mg/L) > SDS (LC_{50} of 8.7 mg/L) > total ammonia (LC_{50} of 126 mg/L) > Ni (LC_{50} of 352 mg/L). Sediment toxicity test results were used to obtain information on non-treatment factors (grain-size, TOC content) that could act as confounding factors, and to develop a site-specific toxicity-score based on minimum significant difference approach. Confounding factors seem not to affect test results. The procedure to develop the toxicity score took into account the relatively lower sensitivity of *C. orientale* with respect to other amphipods commonly used in toxicity tests (*Ampelisca abdita* and *Rhepoxynius abronius*).

Keywords: Amphipods; Toxicity-score; Sediment toxicity test; Toxicity classes

1. Introduction

When using a battery of bioassays for sediment quality assessment, the use of sediment reworker species is recommended for the high ecological relevance of whole sediment testing. Sediment reworkers live in microhabitats beneath the sediment–water interface and are exposed to sediment-bound contaminants as well as pore-water and overlying water contaminants (USEPA, 1994; Ingersoll, 1995). The most suitable and reliable *taxa* for marine and estuarine sediments assessment are amphipods, echinoids, polychaetous annelids and bivalves (see Nendza, 2002, for an exhaustive list of species and standard methods available). Amphipods are the more widely and frequently used test organisms for whole sediment testing, not only due to their worldwide distribution but also their: (a) short life cycle, which makes them suitable for chronic tests development (USEPA, 1992, 2001); (b) good discriminatory ability towards contaminated sediments and laboratory spiked-sediments with different organics and heavy metals (Erdem and Meadows, 1980; Bryant et al., 1985; Ciarelli et al., 1997; Bat and Raffaelli, 1998; Bat et al., 1998); (c) ease of maintaining a laboratory-culture.

Whole sediment tests with the amphipods *Rhepoxynius abronius*, *Eohaustorius estuarius*, *Leptocheirus plumulosus*, and *Grandidierella japonica* were reported by Ingersoll (1995) as being among the most widespread methods for assessing marine and estuarine sediment contamination. ICES (2003) suggests use of the amphipods *Corophium* sp., *Ampelisca* sp., *Rhepoxynius* sp., *Leptocheirus* sp., and *Grandidierella* sp. in monitoring programs.

Several standard methods and guidelines have been published for the 10-d mortality test with Atlantic and Pacific amphipod species, both in North America and

☆All the assays carried out with amphipods during the experimental period were performed in accordance with national and institutional guidelines for animal welfare and the protection of wildlife.

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Europe (Environment Canada, 1992, 1998; USEPA, 1994; PARCOM, 1995; RIKZ, 1999; ASTM, 2003). A number of test protocols, derived from standard guidelines and differing by some experimental conditions, were developed and applied by different authors (Bryant et al., 1985; Bat and Raffaelli, 1998; Bat et al., 1998). Recently ISO (2005) proposed an international standard for the determination of acute toxicity with Atlantic, Pacific and Mediterranean amphipod species.

In Italy, the most commonly used species in sediment quality assessment is *Corophium orientale*, a tube-building infaunal amphipod living in muddy or muddy-sand sediments of brackish and estuarine areas. Although it has often been used for the assessment of harbour and marine sediments toxicity (Onorati et al., 1999; Bigongiari et al., 2001), there is a lack of methodological studies concerning its sensitivity towards different classes of chemicals in water-only exposure systems and, to the best of our knowledge, no data are available for spiked-sediments.

Within the framework of the ICSEL-Project (Integrazione delle Conoscenze sull'Ecosistema Lagunare), promoted by the Venice water authority (Magistrato alle Acque di Venezia) through its concessionaire Consorzio Venezia Nuova, the 10-d sediment toxicity test with the endemic Mediterranean amphipod *C. orientale* is one of the bioassays chosen for toxicity assessment of sediments in the Venice Lagoon. *C. orientale* whole-sediment test was successfully applied to harbour sediments from the northern Tyrrhenian Sea (Onorati et al., 1999) and from harbour and deep sediments (10 and 150 m) from the Ligurian, Tyrrhenian and Adriatic Seas (Bigongiari et al., 2001). This test is also routinely used for the assessment of coastal and marine sediments by different Italian Regional/Local Environmental Protection Agencies. This study was therefore done to improve knowledge on the overall sensitivity of *C. orientale*, in order to achieve the best possible accuracy in understanding sediment bioassays responses, taking into account (a) the relative sensitivity of this species with respect to other amphipods; (b) the possible effects of non-treatment factors. Water-only exposure tests were performed in order to elucidate the sensitivity of *C. orientale* towards chemicals characterised by different toxicity mechanisms: two heavy metals (cadmium, used as reference toxicant, and nickel), an organic (sodium-dodecyl-sulphate—SDS) and an inorganic compound (total ammonia, expressed as sum of ammonium ion and unionised ammonia). Cadmium is widely used as reference toxicant in water phase tests with amphipods (McGee et al., 1998; Onorati et al., 1999; Kater et al., 2000; Bigongiari et al., 2001) and its stability during 96-h exposure is well documented (Kater et al., 2000). Nickel was chosen because it is the metal first released into interstitial water when there is a surplus of metals over acid volatile sulphide (AVS) after oxidation that could occur in situ or during laboratory sediment tests (Di Toro et al., 1990; Berry et al., 1996). SDS is an anionic surfactant widely used as organic reference toxicant in water phase tests with amphipods and sea-urchins (Carr et al., 1996; Lera et al., 2004) due to its high water solubility. Ammonia is a well-known inorganic compound that is naturally present in sediments as by-product of nitrogen-rich organic matter and could also act as a confounding factor in sediment toxicity testing, causing "false positive" responses (Postma et al., 2002). Ammonia is proposed as reference toxicant by RIKZ (1999). In order to take into account the possible joint toxicity of unionised ammonia and ammonium ion proposed by several authors (Tabata, 1962; Armstrong et al., 1978; Arizzi Novelli et al., 2003), the data are reported as the sum of both compounds and referred to in the text as total ammonia.

Whole sediment toxicity tests were performed on shallow and channel sediments of the Venice Lagoon. All sampling sites were selected along a chemical pollution gradient and characterised by different grain size, TOC (total organic carbon) content, heavy metals and organic micropollutants concentrations on the basis of previous studies (MAV-CVN, 1999; Bocci et al., 2005; Carrer et al., 2005). Results from the whole sediment test were used to assess possible effects of non-treatment factors (grain-size and TOC), to evaluate applicability and discriminatory ability of the test protocol. The data were then used to develop a site specific toxicity-score for Venice Lagoon sediments, on the basis of the more recent and suitable statistical methods proposed in the literature to establish the threshold between presence and absence of toxicity (Thursby et al., 1997; Phillips et al., 2001). The score was conceived as a tool not only to distinguish between toxic and non-toxic samples, but also to classify test responses into different toxicity classes (absent, low, medium, high and extremely high toxicity). Following this design, the score could be a reliable tool to support decision-makers in regulatory and management frameworks, as the different toxicity classes could be associated with different remediation and/or management options. Very few examples of toxicity-scores appear to be available in the literature, with most of the proposed methods being based on the authors' experience and use of simple statistical tests, as the *t*-test (Arizzi Novelli et al., 2001; Bigongiari et al., 2001; Onorati and Volpi Ghirardini, 2001). Instead, Bombardier and Bermingham (1999) proposed a classification system based on "toxicity incremental factors" (TIF), calculated by normalisation of the toxicity measured in toxicity units (TU) to a detectable limit for the method used.

2. Materials and methods

2.1. Amphipods collection and holding

Amphipods were purchased from CIBM (Consorzio Interuniversitario Biologia Marina) (Livorno, Italy). *C. orientale* specimens were sampled in the Magra River estuary (La Spezia, Italy) during the period from August 2003 to March 2004, following the procedure reported by Onorati et al. (1999). The sampling site is characterised by fine sediments and salinity generally <20‰. Juveniles and young adults, passed through 1000-μm and retained by 710-μm mesh sieve, were selected for overnight shipment to the laboratory by express courier. During transport, the amphipods

were maintained in a sealed plastic container filled with native sediment and sampling site water. In the laboratory, amphipods were gradually acclimatised to test conditions ($T = 16 \pm 2\,^{\circ}C$, $S = 35‰$), never increasing temperature and salinity by more than 2 °C and 5‰ per day, respectively (ISO, 2005). The culture was maintained under continuous light (>100 lux) and aeration for a period not exceeding 10 days before testing (Bigongiari et al., 2001). Animals were not fed during holding.

2.2. Chemicals

Stock solutions of selected chemicals at 10,000 mg/L (for nickel and total ammonia) or 1000 mg/L (for cadmium and SDS) were obtained dissolving reagent grade salts ($CdCl_2 \cdot H_2O$, NH_4Cl, $Ni(NO_3)_2 \cdot 6H_2O$, and SDS) in water purified with Milli-Q® System (Millipore, Bedford, MA, USA). Artificial seawater used as dilution water was composed of NaCl, $MgSO_4 \cdot 7H_2O$, $MgCl_2 \cdot 6H_2O$, KCl, $NaHCO_3$, KBr, Na_2CO_3, H_3BO_3, $SrCl_2 \cdot 6H_2O$, KF, KI, and $CaCl_2 \cdot 2H_2O$ (Ocean Fish, Prodac International, Cittadella, Italy). This commercial salt mixture had been successfully used in several studies with sea urchin gametes and embryos (Arizzi Novelli et al., 2002, 2003; Losso et al., 2004a).

2.3. Toxicity testing: 96-h water-only exposure

Water-only exposure tests were performed by exposure of amphipods to 500-mL of test solution in a 1-L glass beaker using at least five toxicant nominal concentrations and a negative control (artificial seawater) (Onorati et al., 1999; Bigongiari et al., 2001). Reference toxicant tests were done using the following concentrations of Cd: 0.8, 1.6, 3.2, 6.4, and 12.8 mg/L (Onorati et al., 1999). Test concentrations for pure chemical tests were: 80, 160, 240, 320, 400 mg/L for Ni; 1, 2, 4, 8, and 16 mg/L for SDS; 25, 50, 100, 200, and 500 mg/L for total ammonia. All test concentrations were selected on the basis of preliminary range-finding tests. Nominal concentrations of ammonia were verified using the indophenol-blue method (Solarzano, 1969); measured concentrations were used for data analysis. Preliminary and definitive tests with ammonia were performed using artificial seawater buffered at pH 8.0 ± 0.1, in order to prevent biases due to the strong pH-dependency of ammonia toxicity.

All tests were done at least in triplicate, with 20 amphipods per test-chamber (Onorati et al., 1999). Temperature, salinity and pH were measured at the beginning and end of the test; during exposure, test chambers were continuously aerated and kept under constant illumination (>100 lux). All tests with pure substances (Ni, SDS, total ammonia) were performed with amphipods from the same batch in order to avoid biases due to possible seasonal changes in sensitivity.

2.4. Toxicity testing: 10-d sediment toxicity test

Sediments were sampled during summer–autumn 2003 in fifty-one stations distributed throughout the Lagoon of Venice (Fig. 1). Thirty-one surface sediment samples (0–15 cm depth) were collected from the shallows using a 5-cm diameter corer, following an integrated sampling design reported in detail in previous papers (Losso et al., 2004b; Volpi Ghirardini et al., 2005); twenty surface (0–15 cm) and ten deep (30–45 cm) sediment samples were collected from channels by scuba-divers using a 10-cm diameter plastic-liner. The 10-d sediment toxicity tests were performed exposing amphipods to 200-mL of 1-mm dry-sieved wet sediment and 750-mL of overlying water (artificial seawater) in 1-L wide mouth glass beakers, according to Onorati et al. (1999). The beakers were incubated under continuous aeration overnight at $T = 16 \pm 2\,^{\circ}C$ and $S = 35‰$ in a thermostatic chamber before amphipods were added. Four replicates per sediment were tested, using 25 amphipods for each replicate (Bigongiari et al., 2001). A negative control using native sediment was added to each batch of test sediments. Water temperature, salinity, pH, and NH_3 were measured at the beginning and end of the test. Exposure was under continuous aeration, constant illumination (>100 lux) and without water renewal (static test) (Bigongiari et al., 2001).

2.5. Chemical analyses

Dry-weight total-metals concentrations analyses were performed using inductively coupled plasma—atomic emission spectrometry (ICP-AES) for Cu, Cr, Ni, and Zn (EPA method 6010B), atomic absorption—furnace technique for As, Cd, and Pb (EPA methods 7060A, 7131B, and 7421, respectively) and atomic absorption spectrophotometry for Hg (EPA method 7473). Prior to analyses, samples were digested through microwave assisted acid digestion (EPA method 3052). SEM and AVS were determined on shallow sediments following the procedure by Allen et al. (1993).

2.6. Data analyses

For water-only exposure tests, data were analysed with Trimmed Spearman-Karber Method v1.5 for LC_{50} (lethal concentration 50) and 95% confidence limits calculation, whereas the EMSL Cincinnati-Dunnett v1.5 was used for NOEC (no observed effect concentration) and LOEC (lowest observed effect concentration) calculation, after arcsin square root transformation of raw data (USEPA, 1994, 2002). The 10-d sediment toxicity test results were reported as percentage of dead amphipods (PDA), normalised to control response using Abbott's formula (Finney, 1971). One-side *t*-test ($\alpha = 0.05$) on raw data was used to check for statistical differences between samples and control. Normality and variance heterogeneity were checked using Shapiro–Wilk's test ($\alpha = 0.01$) and *F*-test ($\alpha = 0.01$), respectively. When the normality test failed, Wilcoxon rank-sum test was performed for testing means equality (USEPA, 2002).

2.7. Toxicity-score development

The first step towards the definition of a toxicity-score for *C. orientale* 10-d sediment toxicity test was the designation of the most reliable threshold between toxicity and non-toxicity.

The toxicity threshold (TT) was defined using the minimum significance difference (MSD) criterion, proposed by Thursby et al. (1997) and Phillips et al. (2001). For every sample–control pair the MSD was calculated according to Eq. (1):

$$\mathrm{MSD} = t_{(\alpha,n+m-2)} \cdot [(s_1^2/n) + (s_2^2/m)]^{1/2}, \quad (1)$$

where t is the t-value derived from standard tables, α the significance value, n and m the number of replicates for control and sample, s_1^2 and s_2^2 the variances for control and sample.

To report MSD values as proportion of control responses, the obtained MSD were divided by the respective negative control response, expressed in terms of "success" (survivors in sediment) (Phillips et al., 2001). These normalised MSD values were ranked in ascending order and the 90th percentile of the cumulative distribution was identified. The TT was then calculated by subtracting the 90th percentile MSD value (expressed in %) from 100:

$$\text{Toxicity threshold (TT)} = 100 - \text{90th percentile MSD (\%)}. \quad (2)$$

To verify if a sample should be considered toxic or not, it is necessary to take into account the sample normalised response (S):

$$S = 100 \cdot [(\%\ \text{success sample})/(\%\ \text{success control})]. \quad (3)$$

The S value is compared with the toxicity limit (TL), defined as follows:

$$\text{Toxicity limit (TL)} = \text{control response (\%)}\ \mathrm{TT}. \quad (4)$$

TL is the minimum sample response that could be regarded as significantly no different from the control. When $S > \mathrm{TL}$, the sample should be considered as non-toxic; when $S \leqslant \mathrm{TL}$, toxicity is statistically present. To develop a toxicity-score, detailed enough to distinguish between different pollution levels, 5 toxicity classes were chosen. Three more thresholds were established: (1) Medium toxicity threshold (MTT); (2) high toxicity threshold (HTT) and (3) extreme toxicity threshold (ETT). To take into account the relatively low sensitivity of the genus *Corophium* as compared with other infaunal amphipods (i.e. *R. abronius*) (Environment Canada, 1992; Bat and Raffaelli, 1998), MTT, HTT and ETT were calculated using

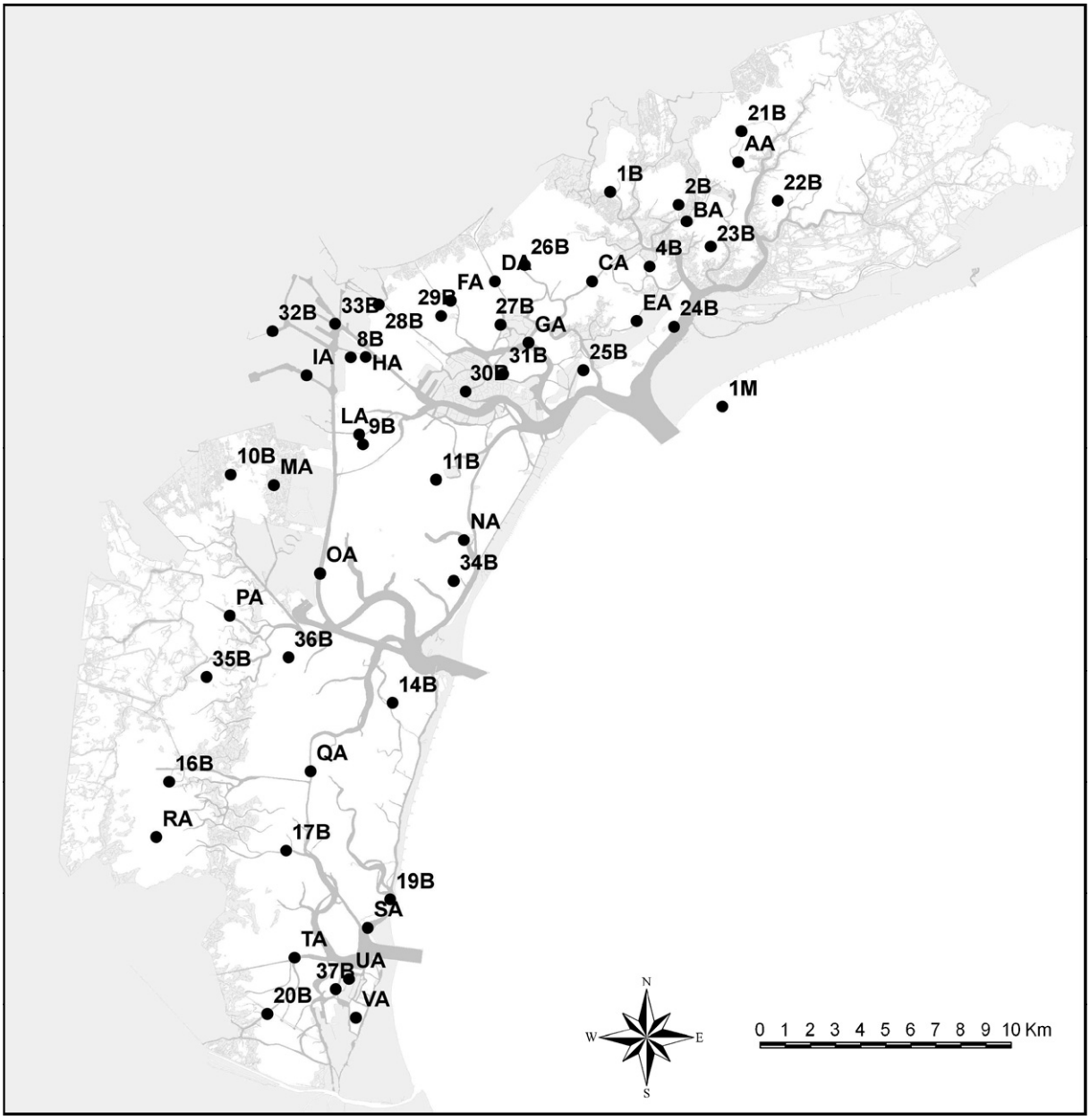

Fig. 1. Venice Lagoon map with sediment sampling sites.

three multiples of the 90th percentile selected in geometric progression with a common ratio of 1.5 (1.5, 3, and 6) in Eq. (2). The related toxicity limits were calculated by substituting TT with MTT, HTT, or ETT in Eq. (4):

Medium toxicity limit (MTL) = control response (%) MTT,

High toxicity limit (HTL) = control response (%) HTT,

Extreme toxicity limit (ETL) = control response (%) ETT.

3. Results

3.1. Toxicity testing: 96-h water-only exposure

Fifteen tests were performed with Cd as reference toxicant, with a mean $LC_{50} \pm SD$ (standard deviation) of 3.3 ± 2.46 mg/L Cd. The highest LC_{50} value (10.5 mg/L Cd) was found in March 2004, the lowest in September 2003 (1.44 mg/L Cd).The LC_{50}'s obtained in the definitive test for Ni, total ammonia and SDS were 352 mg/L, 126 mg/L, and 8.7 mg/L, respectively; the toxicity gradient for the chemicals tested was thus Cd > SDS > total ammonia > Ni. The NOEC, LOEC, and LC_{50} values with their 95% confidence limits for SDS, Ni and ammonia are reported in Table 1. The results regarding ammonia in the table are expressed both as total ammonia and unionised ammonia; unionised ammonia concentrations were calculated from test conditions (pH = 8.0; $S = 35$; $T = 16\,^{\circ}C$) using the Hampson model (Hampson, 1977), based on Whitfield's equations (Whitfield, 1974). The pH-values at the end of the tests with ammonia were within the range 8.0 ± 0.2.

Table 1
LC_{50} with 95% confidence-limits, NOEC and LOEC values obtained with 96-h water-only exposure on pure substances

Chemical	LC_{50}	95% C.L.	NOEC	LOEC
SDS	8.7	8.1–9.4	2.0	4.0
Total ammonia	126	114–138	51	95
(Unionised ammonia)	(2.78)	(2.56–3.06)	(1.13)	(2.10)
Nickel	352	325–382	80	160

All data are expressed in mg/L.

Negative controls with reconstituted seawater always had a mean survival percentage of $97.7 \pm 2.61\%$ well within the acceptability criterion (survival > 85%; Bigongiari et al., 2001; survival > 90%, ISO, 2005).

3.2. Toxicity testing: 10-d sediment toxicity tests

Temperature, salinity, and pH were always within the acceptability limits ($T = 16 \pm 2\,^{\circ}C$; $S = 35 \pm 4$; $pH = 8.0 \pm 0.5$) (PARCOM, 1995). Survival in controls with native sediment was always within the acceptability limits with a mean survival of $95.9 \pm 4.6\%$ ($n = 32$, c.v. = 4.8%). The results of the sediment toxicity tests are reported in Table 2 in terms of PDA, together with SD, *p*-values for one-side *t*-test, TOC content, grain-size analyses results, grain-size classification (Shepard, 1954), and toxicity classification following the score proposal. Survival success of the control (*S*) is also reported. Sediment samples showed very different grain-size (from sand to silty-clay sediments) and TOC content (from 0.4 up to 15%C). However, there was little difference in toxicity among samples, with the exception of four samples exhibiting a PDA of above 20% (QA2, SG, 28B, and DA2).

3.3. Toxicity-score development

Using the 61 MSD's data for every sample–control pair, a 90th percentile of MSD's normalised value of 10% was obtained. The TT for *C. orientale* 10-d sediment toxicity test is thus 90, and the toxicity limit is 90% of control survival, according to Eqs. (2) and (3). The values for the other toxicity limits are 85%, 70%, and 40% of control survival for MTL, HTL, and ETL, respectively. The sediment samples were then classified in the different classes as follows: if $S < TL$ (90% of control survival) toxicity is absent, if $85 < S \leqslant 90\%$ the toxicity is classified as low, if $85\% \leqslant S < 70\%$ toxicity is medium, if $70\% \leqslant S < 40\%$ we are in presence of high toxicity, whereas if $S \leqslant 40\%$ the toxicity is very high.

4. Discussion

4.1. Toxicity testing: 96-h water-only exposure

Test results showed that *C. orientale* sensitivity towards Cd varied during the testing period; the higher LC_{50} was found in early spring (March) and the lower during late summer (August–September). Up to 3× differences in LC_{50} could be considered as a normal variability observed during laboratory testing, but the differences measured during the whole testing period are generally higher (up to 7×) and the differences among the data obtained during summer–autumn and winter–spring are quite evident. The mean LC_{50} obtained with amphipods collected during late summer–autumn is 2.2 ± 0.82 mg/L and the values ranged from 1.44 up to 3.97 mg/L (about 3× difference), whereas the mean value obtained during winter and early spring is 5.9 ± 3.71 mg/L with a range of values from 1.9 up to 10.5 mg/L (more than 5× difference); the LC_{50} values were quite homogeneous and low during the period August–October, whereas a significant increase in the mean and in the variance was registered from November to March. The one-way ANOVA ($\alpha = 0.05$) on log-transformed data showed that the difference between the means obtained in the two periods is basically different ($p = 0.01$). The variance obtained with summer–autumn tests (approx. 3× difference among LC_{50}'s) can be due to normal laboratory variability, but for winter–spring tests results it seems that other factors should be taken into account. Although the available data are probably not sufficient (the data set covers a 8-months period only) and the statistical analysis is not conclusive to demonstrate an effective seasonal dependence of the sensitivity towards Cd, they are consistent with the literature data reported for *C. volutator* (Kater et al., 2000) and *C. orientale* (Lera et al., 2004). Kater et al. (2001) reported a low sensitivity peak in *C. volutator* for Cd in April, and Ciarelli et al. (1997) found an identical trend for lindane-spiked sediments. Moreover, data collected with a different *C. orientale* population showed that specimens collected in summer had higher sensitivity than those sampled during spring, with LC_{50} differing by a factor of between 2 and 2.5 (Picone, unpublished data). The reasons for these seasonal variations in *C. orientale* sensitivity to cadmium are still unknown. In the literature, little is known about changes in cadmium toxicity towards field-collected marine and estuarine amphipods. McGee et al. (1998) found that size, reproductive status and molting cycle affected the acute toxicity of cadmium in field-collected *Leptocheirus plumulosus*. On the other hand, Kater et al. (2000) performed test with field-collected and laboratory-reared *C. volutator* and suggested that field temperature, photoperiod, size and reproduction cycle cannot be the sole causative factors of seasonal changes in cadmium toxicity. Ciarelli et al. (1997) found a lack of correlation between *C. volutator* size and Cd toxicity. Among the factors that could be involved, it seems that attention should focus on lipids content (Meador, 1993) and molting cycle (McGee et al., 1998); the latter is particularly interesting because of the enhanced uptake of cadmium in postmolt crustaceans reported by different authors (Wright and Frain, 1981; McGee et al., 1998). Data reported in the literature using the same population of *C. orientale* and the same test protocol with

Table 2
Summary of the results for sediment toxicity test, grain-size, and TOC analysis results

Sample	Typology	PDA ± SD	*p*-value	S	C	TL	TOC (%)	gravel (%)	sand (%)	silt (%)	clay (%)	Shepard sediment classification	Toxicity judgment
1B	SH (0–15 cm)	4 ± 4.2	0.095	96	91	82	0.45	–	28	48	24	Loam	Absent
1M	SH (0–15 cm)	3 ± 3.3	0.084	97	99	89	0.41	–	68	27	4	Silty-sand	Absent
2B	SH (0–15 cm)	3 ± 4.7	0.139	97	99	89	1.50	–	4	62	35	Clayey-silt	Absent
4B	SH (0–15 cm)	1 ± 2.3	0.268	99	99	89	0.74	–	15	70	15	Clayey-silt	Absent
8B	SH (0–15 cm)	4 ± 2.1	0.095	96	95	86	1.60	–	33	38	29	Loam	Absent
9B	SH (0–15 cm)	5 ± 2.4	0.060	95	95	86	1.20	–	14	69	17	Clayey-silt	Absent
10B[a]	SH (0–15 cm)	0 ± 2.4	> 0.05	100	98	88	4.50	–	21	39	40	Loam	Absent
11B	SH (0–15 cm)	6 ± 8.7	0.130	94	95	86	1.50	–	9	57	34	Clayey-silt	Absent
14B	SH (0–15 cm)	5 ± 5.1	0.060	95	98	88	0.86	–	47	42	10	Silty-sand	Absent
16B	SH (0–15 cm)	10 ± 8.8	0.033	90	98	88	5.00	–	20	39	40	Loam	Absent
17B	SH (0–15 cm)	4 ± 4.7	0.073	96	99	89	1.90	–	36	39	25	Loam	Absent
19B	SH (0–15 cm)	4 ± 5.3	0.104	96	98	88	0.43	–	88	12	–	Sand	Absent
20B	SH (0–15 cm)	6 ± 4.7	0.030	94	98	88	0.96	–	54	31	16	Silty-sand	Absent
21B	SH (0–15 cm)	16 ± 5.1	0.001	84	91	82	1.60	–	8	42	50	Silty-clay	Absent
22B	SH (0–15 cm)	1 ± 5.7	0.383	99	91	82	0.87	–	4	73	22	Clayey-silt	Absent
23B	SH (0–15 cm)	7 ± 4.2	0.034	93	91	82	0.75	–	6	64	30	Clayey-silt	Absent
24B	SH (0–15 cm)	2 ± 3.9	0.195	98	99	89	0.47	–	60	30	10	Silty-sand	Absent
25B[a]	SH (0–15 cm)	0 ± 2.0	> 0.05	100	99	89	0.51	–	56	31	13	Silty-sand	Absent
26B	SH (0–15 cm)	0 ± 2.5	0.404	100	94	85	0.98	–	14	51	35	Clayey-silt	Absent
27B	SH (0–15 cm)	2 ± 7.8	0.352	98	94	85	1.30	–	26	58	16	Sandy-silt	Absent
28B	SH (0–15 cm)	33 ± 19.1	0.008	67	91	82	1.60	–	2	55	43	Clayey-silt	Medium
29B	SH (0–15 cm)	0 ± 4.1	0.500	101	94	85	1.00	–	17	50	33	Clayey-silt	Absent
30B	SH (0–15 cm)	3 ± 5.7	0.178	97	99	89	1.90	–	22	44	34	Loam	Absent
31B	SH (0–15 cm)	5 ± 4.1	0.127	95	94	85	0.50	–	24	66	10	Sandy-silt	Absent
32B	SH (0–15 cm)	12 ± 6.1	0.005	88	99	89	2.40	–	46	25	29	Loam	Low
33B	SH (0–15 cm)	9 ± 5.2	0.009	91	99	89	1.60	–	32	34	34	Loam	Absent
34B	SH (0–15 cm)	2 ± 2.0	0.104	98	99	89	0.91	–	45	38	18	Silty-sand	Absent
35B	SH (0–15 cm)	6 ± 5.1	0.034	94	99	89	15.00	–	17	56	27	Clayey-silt	Absent
36B	SH (0–15 cm)	4 ± 5.1	0.095	96	99	89	1.70	–	47	33	20	Silty-sand	Absent
37B	SH (0–15 cm)	5 ± 6.1	0.085	95	98	88	2.40	–	34	38	29	Loam	Absent
SG	SH (0–15 cm)	29 ± 8.7	0.001	71	95	86	2.8	–	3	61	36	Clayey-silt	Medium
AA1	CH (0–15 cm)	14 ± 7.5	0.008	86	98	88	0.95	1	6	63	30	Clayey-silt	Low
BA1	CH (0–15 cm)	11 ± 3.9	0.004	89	98	88	0.56	1	60	28	11	Silty-sand	Absent
CA1	CH (0–15 cm)	10 ± 10.5	0.069	90	93	84	0.67	–	14	45	41	Clayey-silt	Absent
DA1	CH (0–15 cm)	9 ± 2.5	0.035	91	94	85	0.51	–	43	38	19	Silty-sand	Absent
EA1	CH (0–15 cm)	16 ± 5.3	0.001	84	98	88	0.77	–	4	74	22	Clayey-silt	Low
FA1	CH (0–15 cm)	5 ± 4.1	0.127	95	94	85	1.86	3	3	50	44	Clayey-silt	Absent
GA1	CH (0–15 cm)	3 ± 4.1	0.239	97	94	85	0.44	–	39	49	15	Sandy-silt	Absent
HA1	CH (0–15 cm)	0 ± 4.2	0.500	100	91	82	0.42	–	72	16	12	Silty-sand	Absent
IA1	CH (0–15 cm)	1 ± 8.4	0.412	99	91	82	1.21	–	3	62	35	Clayey-silt	Absent
LA1	CH (0–15 cm)	18 ± 6.6	0.002	82	91	82	1.68	28	20	27	25	Loam	Low
MA1	CH (0–15 cm)	12 ± 6.2	0.009	88	91	82	2.90	–	4	60	36	Clayey-silt	Absent
NA1	CH (0–15 cm)	1 ± 3.5	0.352	99	93	84	1.24	–	3	65	32	Clayey-silt	Absent
OA1	CH (0–15 cm)	1 ± 6.1	0.390	99	93	84	0.69	3	83	8	6	Sand	Absent
PA1	CH (0–15 cm)	0 ± 3.4	0.375	101	95	86	0.82	7	34	37	22	Loam	Absent
QA1	CH (0–15 cm)	2 ± 6.3	0.314	98	95	86	3.42	–	3	64	33	Clayey-silt	Absent
RA1	CH (0–15 cm)	9 ± 7.3	0.040	91	95	86	14.21	–	2	50	48	Clayey-silt	Absent
SA1	CH (0–15 cm)	2 ± 5.8	0.268	98	98	88	0.58	11	72	11	6	Sand	Absent
TA1[a]	CH (0–15 cm)	0 ± 2.4	> 0.05	100	98	88	1.61	20	38	27	15	Loam	Absent
UA1[a]	CH (0–15 cm)	8 ± 4.1	> 0.05	92	98	88	1.74	8	40	30	22	Loam	Absent
VA1	CH (0–15 cm)	7 ± 7.7	0.064	93	98	88	2.23	16	21	37	26	Loam	Absent
BA2	CH (30–45 cm)	12 ± 5.3	0.005	88	98	88	0.73	5	62	22	11	Silty-sand	Low
DA2	CH (30–45 cm)	62 ± 17.4	0.0003	38	94	85	1.11	–	15	50	35	Clayey-silt	Extreme
EA2	CH (30–45 cm)	10 ± 7.5	0.027	90	98	88	0.90	–	3	71	26	Clayey-silt	Absent
FA2	CH (30–45 cm)	11 ± 8.5	0.054	89	94	85	1.68	–	2	50	48	Clayey-silt	Absent
MA2	CH (30–45 cm)	13 ± 2.2	0.001	87	91	82	3.01	–	7	58	35	Clayey-silt	Absent
NA2	CH (30–45 cm)	6 ± 2.2	0.016	94	93	84	1.23	–	5	81	14	Clayey-silt	Absent
OA2	CH (30–45 cm)	0 ± 6.5	0.500	100	93	84	0.64	2	69	18	11	Silty-sand	Absent
QA2	CH (30–45 cm)	27 ± 8.0	0.001	73	95	86	4.02	–	4	61	35	Clayey-silt	Medium
RA2[b]	CH (30–45 cm)	7 ± 6.0	0.057	93	95	86	32.52	46	30	10	14	–	Absent
TA2	CH (30–45 cm)	4 ± 5.3	0.104	96	98	88	1.21	–	85	8	7	Sand	Absent

SH = Shallow, CH = Channel.
Toxicity test results are reported in terms of percentage of dead amphipods (PDA) together with standard deviation, *p*-values for *t*-test and survival adjusted to control (S), control survival (C), and Toxicity Limit (TL).
[a]Indicates samples analysed with Wilcoxon Ran–Sum test.
[b]For sample RA2 grain-size classification after Shepard (1954) was not performed due the high amount of gravel.

Cd are in good agreement with those obtained in this study (Onorati et al., 1999; Bigongiari et al., 2001; Lera et al., 2004). These data highlighted that *C. orientale* is a low-medium sensitivity amphipod when compared with other species commonly used for ecotoxicological surveys (Table 3), and tests performed with total ammonia confirmed this observation (Table 3). Indeed, when tested under comparable conditions, *C. orientale* seems to have similar sensitivity to *C. volutator* and *G. japonica* (both amphipods of the *Corophiidae* family), but it is usually less sensitive than *A. abdita* and *R. abronius* (USEPA, 1992; Kohn et al., 1994). Data reported by Environment Canada (1998) again showed the higher sensitivity of *R. abronius* (LC_{50} of 65 mg/L of NH_3–N) compared to *C. orientale*, and a relatively similar or rather lower sensitivity of *Eohaustorius washingtonianus* and *E. estuarius* (LC_{50} of 139 mg/L of NH_3–N and 156 mg/L of NH_3–N, respectively). Regarding ammonia, a direct comparison with *C. volutator*, an amphipod alike *C. orientale* morphologically and phylogenically is not possible, because the data obtained by Postma et al. (2002) (LC_{50} of 115 mg/L at pH 8.0) were with shorter exposure (72-h). The high LOEC and LC_{50} suggest that, although the data were obtained during a 96-h water-only exposure, *Corophium* is quite tolerant of ammonia but, when testing Venice Lagoon sediments, ammonia would probably not be a concern. Total ammonia concentrations in pore waters could reach values as high as 100 mg/L only in sites near the industrial area, whereas pore-water concentrations in the open Lagoon are generally much lower (Volpi Ghirardini, unpublished data, MAV-CVN, 2003). Moreover, when remaining buried in sediments *Corophium* sp. is not exposed directly to pore-water, but maintains a breathing current through its tube and so causes a dilution of the pore-water with the overlying water. However, Moore et al. (1997) reported a LC_{50} of 98.8 mg/L of NH_3–N for *L. plumulosus* (measured in the pore-water) in a 10-d toxicity test on sediment spiked with NH_4Cl; this value is not very different from the 96-h water only LC_{50} (88.9 mg/L of NH_3–N) found by the same authors. Equally, Environment Canada (1998) reported small differences between 10-d pore-water LC_{50} and 96-h water only LC_{50} for both *R. abronius* (65 mg/L of NH_3–N and 57.7 mg/L of NH_3–N, respectively) and *E. washingtonianus* (139 mg/L of NH_3–N and 112 mg/L of NH_3–N), whereas a marked difference was highlighted for *Amphiporeia virginiana* (151 mg/L of NH_3–N and 24.6 mg/L of NH_3–N). As regards nickel, *C. orientale* seems to be at least one order of magnitude less sensitive than *C. volutator*, although the data reported by Bryant et al. (1985) (LC_{50} of 34 mg/L) were obtained using different experimental conditions (thin layer of sediment in test chambers). Literature data on Ni toxicity towards other amphipods are in agreement with the data reported by Bryant et al. (1985) and Ewell et al. (1986) found an LC_{50} for *Gammarus fasciatus* of about 50 mg/L, whereas Rehwoldt et al. (1973) reported LC_{50} values ranging from 13 up to 15.2 mg/L for *Gammarus* sp. Similar results were obtained by Gajbhiye and Hirota (1990) with the shrimp *Artemia* sp. (LC_{50} of 11.2–15.6 mg/L). Data obtained in this work for *C. orientale* showed that nickel is about 100 times less toxic than cadmium, confirming that its contribution to toxicity could generally be considered negligible as compared with other metals. However, with increasing nickel concentrations (above 160 mg/L), an increasing number of the surviving amphipods showed a strongly limited motility (with only pereiopods and gnathopods exhibiting small movements after mechanical stimulation), highlighting a marked sub-lethal effect of nickel on *C. orientale*.

The LC_{50} for SDS is consistent with the data reported by Lera et al. (2004) using two populations of *C. orientale* (LC_{50} ranging from approx. 4 up to 12 mg/L) and in agreement with the LC_{50} range (5.16–9.56 mg/L) found by Carr et al. (1996) for *A. abdita*.

Table 3
Reference toxicant (Cd) and ammonia 96-h LC_{50} with 95% confidence limits reported in literature for amphipods used in bioassays

Specie	Cd		Ammonia	
	LC_{50}	95% C.L.	LC_{50}	95% C.L.
C. orientale	3.3[a]	0.87–5.80	125.7 (2.78)[a]	114.3–138.3 (2.56–3.06)
	2.91[b]	2.09–3.73		
	4.2[b]	2.96–5.63		
A. abdita	0.33[c]	0.29–0.38	49.8 (0.83)[d]	45.6–54.4 (0.76–0.92)
	0.94[d]	0.83–1.05		
	1.32[d]	1.22–1.43		
	1.09[e]	–		
E. estuarius	9.33[c]	7.20–12.09	126.7 (2.52)[d]	114.2–168.4 (2.26–3.38)
	6.42[e]	4.90–8.30		
	11.41[e]	8.90–14.70		
G. japonica	1.17[c]	0.94–1.46	154.7 (3.48)	133.4–203.8 (3.05–4.46)
	3.14[d]	2.36–4.17		
R. abronius	0.92[c]	0.68–1.25	78.7 (1.59)[d]	73.3–84.5 (1.46–1.72)
	1.92[d]	1.47–2.51		
	0.79[e]	0.5–1.1		

Data are expressed in mg/L of Cd.
All data are reported in mg/L. For ammonia, number within brackets represents the values for the unionised form.
[a]Present work.
[b]Onorati et al. (1999).
[c]ASTM (2003).
[d]Kohn et al. (1994).
[e]USEPA (1992). 95% C.L. for data with Cd obtained in this work represent mean ±SD obtained during the experimental period.

4.2. Toxicity testing: 10-d sediment toxicity test

Of the 61 samples tested, 21 were statistically different from the control when using one-side *t*-test or Wilcoxon rank-sum test. The sediments giving a not statistically different response from the control ranged from silty-clay to sands, with all the main grain-size classes of the Lagoon (silty-sands, loams, sandy-silts, clayey-silts) being well represented in this "no-effect" class.

Furthermore, samples not statistically different from the control had a TOC content ranging from 0.4% to 4.5%. These results lead to the conclusion that grain-size and TOC are not an issue when testing Venice Lagoon sediments with *C. orientale*. This lack of evidence of non-contaminant factors contribution to the toxicity is a key element to establish the applicability of the method in an extremely heterogeneous environment such as the Venice Lagoon. The tolerance of *C. orientale* to a very wide range of grain-size thus makes it a powerful tool for toxicity assessment over the whole Lagoon.

Samples from the shallows generally resulted as not toxic for *C. orientale* following the toxicity-score proposed in this paper: 90% of samples showed an absence of toxicity, whereas only 1 sample exhibited low toxicity (32B) and 2 were classified as medium toxicity sediments (SG and 28B). These results are not surprising due to the fact that these samples are located in or close to the industrial area of Porto Marghera, the most contaminated area of the Lagoon, where metals and organic pollutants (PAH, PCDD/F, and PCBs) are up to 2 order of magnitude higher than in the less contaminated areas (MAV-CVN, 1999). However, not all the samples from this area evidenced toxicity (i.e. site 33B) and although the measured concentration of metals were higher than in the whole lagoon (Table 4), the difference SEM-AVS was less than 0 for all the sample toxic towards the amphipods, excluding effects due to Cd, Cu, Ni, Pb and Zn (Table 5). The toxicity is probably due to other pollutants (i.e. Hg and organic micropollutants).

In general, channel sediments showed a more heterogeneous state than shallows: 17 surface sediments (85%) were not toxic, whereas 3 samples located in the northern lagoon (AA1 and EA1) and near the industrial area (LA1) showed low toxicity. As regards deep sediments, BA2 exhibited low toxicity, QA2 showed medium toxicity, whereas DA2 was extremely toxic towards *C. orientale*.

There was a generally good agreement between surface and deep sediments toxicity responses, with the exceptions of site EA, where surface sediments were slightly more toxic than deeper ones, and sites BA, QA, and DA, where deeper sediments were more toxic than surface ones. In QA and DA sites the difference in toxicity response was quite marked, deeper sediments being much more toxic. The available chemical data showed no marked difference in sediment contamination between surface and deep samples nor among shallows and channels (Table 4).

The toxicity-score proved to be a valuable tool for sediment toxicity classification as it could distinguish industrial area samples from open lagoon sites. However, although it was possible to identify statistically toxic sediments using the score, and even if the *C. orientale* sediment test was reported as able to discriminate between harbour sediments from the Tyrrhenian coast (Onorati et al., 1999), there is still concern over the real discriminatory ability of *C. orientale* when testing Venice Lagoon sediments. Indeed, the ''absolute'' differences in mortality

Table 4
Total metal concentrations (mg/kg dry weight) in shallows and channels

Site	As	Cd	Cr	Cu	Hg	Ni	Pb	Zn
1M	17.2	0.24	18	8	1.04	9	8	35
1B	7.4	0.12	33	16	0.35	15	27	63
2B	7.9	0.45	50	23	0.94	25	31	79
4B	9.0	0.35	29	24	0.51	20	25	84
8B	16.6	2.81	45	54	1.69	19	37	429
9B	12.3	1.69	47	32	1.12	21	42	208
10B	24.5	2.43	65	43	0.60	35	37	265
11B	7.7	0.82	51	24	0.98	18	35	82
14B	7.1	0.24	48	13	0.29	17	15	66
16B	15.9	0.80	64	24	0.27	38	34	120
17B	10.2	0.52	53	19	0.40	26	22	90
19B	4.5	0.23	66	4	0.14	15	11	50
20B	7.3	0.27	52	15	0.13	22	23	88
21B	6.1	0.27	45	22	0.20	24	29	63
22B	6.9	0.21	38	15	0.93	19	25	70
23B	6.0	0.25	50	20	0.66	26	25	73
24B	5.4	0.20	28	13	0.20	14	12	38
25B	5.5	0.21	34	10	0.45	12	18	59
26B	9.3	0.42	40	19	0.07	24	25	120
27B	7.0	0.77	27	25	0.77	14	31	110
28B	14.5	5.66	47	55	1.39	22	50	1072
29B	8.9	1.45	42	22	0.67	27	37	129
30B	10.1	2.58	45	106	2.00	21	49	310
31B	4.4	0.66	28	18	0.57	11	22	91
32B	13.9	1.87	144	97	3.28	115	65	375
33B	23.8	7.06	54	86	3.66	19	78	830
34B	6.6	0.33	37	13	0.91	15	16	70
35B	15.2	0.57	36	17	0.31	19	26	89
36B	10.6	0.34	62	16	0.15	28	22	92
37B	8.8	0.52	88	24	0.37	44	28	114
SG	9.0	4.00	65	93	1.97	20	60	736
AA1	7.1	0.69	44	22	1.10	20	19	55
BA1	6.7	0.52	27	25	0.25	13	12	35
CA1	5.8	0.26	35	14	0.52	15	15	63
DA1	10.3	1.06	36	23	0.34	16	27	132
EA1	8.4	0.54	35	30	0.83	19	32	108
FA1	14.8	1.56	54	50	0.98	28	53	289
GA1	6.0	0.72	35	21	0.43	23	42	81
NA1	7.3	0.43	44	22	1.09	18	22	87
OA1	4.9	0.27	16	13	0.09	22	11	51
PA1	9.7	0.95	36	26	0.75	18	52	213
QA1	12.4	0.81	52	30	0.65	30	31	153
RA1	12.3	0.57	23	14	0.12	14	15	111
GA1	6.0	0.72	35	21	0.43	23	42	81
HA1	6.5	1.54	21	20	0.62	10	22	163
IA1	12.7	1.58	50	42	1.34	22	43	238
LA1	84.1	14.41	292	80	1.43	131	289	1055
MA1	13.4	1.57	52	32	0.68	24	33	197
SA1	5.7	0.30	52	15	0.10	24	11	29
TA1	10.2	0.75	55	18	0.22	31	24	71
UA1	9.2	0.56	80	53	0.28	40	36	140
VA1	10.3	1.03	77	57	0.35	36	37	135
BA2	7.3	0.32	23	14	0.42	13	12	31
DA2	13.5	1.36	48	40	0.81	25	41	171
EA2	8.7	0.57	36	34	1.17	19	42	89
FA2	14.9	2.35	56	55	1.38	30	58	375
NA2	8.0	0.54	47	23	0.99	20	25	111
OA2	6.0	0.45	21	12	0.09	17	11	46
QA2	12.6	0.86	55	28	0.81	31	33	154
RA2	3.2	0.13	7	11	0.03	6	5	28
MA2	13.6	1.70	53	32	1.19	24	34	221
TA2	6.5	0.25	43	4	0.06	27	15	33

Table 5
Simultaneously extracted metals, AVS concentrations in shallows (mg/kg dry weight)

Sample	As	Cd	Cr	Cu	Hg	Ni	Pb	Zn	AVS	SEM-AVS
1B	1.77	0.11	1.05	2.51	<0.02	1.08	9.75	16.17	81.8	−0.27
1M	1.48	0.13	0.81	1.18	<0.02	1.25	2.16	10.15	15.2	−2.20
2B	1.93	0.18	1.37	2.58	<0.02	1.30	14.57	23.98	839.7	−25.68
4B	2.35	0.26	1.37	6.13	<0.02	1.02	9.65	34.47	338.9	−9.88
8B	4.69	1.84	1.41	8.11	<0.02	1.79	22.55	222.47	400.5	−8.80
9B	3.02	0.97	1.15	9.22	<0.02	1.41	15.21	94.17	95.7	−1.29
10B	5.05	1.40	1.70	3.33	<0.02	4.19	26.21	146.94	461.8	−11.89
11B	1.92	0.22	0.85	5.41	0.03	4.74	9.40	24.23	28.6	−0.31
14B	2.41	0.14	0.90	4.14	<0.02	2.15	6.04	19.56	16.1	−0.07
16B	5.80	0.52	0.62	9.74	0.03	4.10	22.28	45.23	73.0	−1.25
17B	3.28	0.28	1.18	5.66	0.03	2.93	11.64	30.63	15.5	0.18
19B	0.66	0.10	0.64	1.86	<0.02	0.91	3.78	11.94	30.4	−0.70
20B	1.37	0.16	1.24	5.33	0.02	2.85	8.00	21.28	11.5	0.14
21B	1.11	0.16	1.27	<0.1	<0.02	1.16	7.21	16.07	180.9	−5.34
22B	2.98	0.21	1.41	5.26	0.02	1.63	8.92	61.16	286.0	−7.83
23B	2.03	0.20	1.36	5.45	<0.02	1.30	10.74	18.90	99.8	−2.66
24B	1.19	0.19	1.01	3.37	<0.02	0.95	4.50	12.68	40.9	−0.99
25B	0.81	0.20	1.35	2.21	<0.02	0.77	4.68	17.48	114.8	−3.24
26B	2.91	0.36	1.00	5.74	<0.02	1.39	8.73	40.18	42.7	−0.56
27B	3.15	0.46	1.38	11.01	<0.02	1.24	10.63	46.07	6.9	0.74
28B	3.89	2.67	2.20	29.80	0.08	1.70	32.90	738.00	407.0	−0.72
29B	2.99	0.56	1.04	6.28	<0.02	1.12	11.46	61.14	156.0	−3.75
30B	4.73	1.43	2.64	45.21	<0.02	3.17	34.98	192.97	493.3	−11.48
31B	2.28	0.49	1.24	7.48	<0.02	0.71	9.87	47.02	82.8	−1.68
32B	6.37	0.68	9.76	38.51	<0.02	8.29	47.88	153.32	243.4	−4.26
33B	6.60	6.08	3.54	0.16	<0.02	2.25	59.80	544.10	635.0	−11.10
34B	3.32	0.27	1.23	6.72	<0.02	2.44	7.47	40.00	4.6	0.66
35B	3.17	0.49	<0.1	7.16	0.05	3.66	20.50	43.20	17.6	0.39
36B	2.41	0.20	1.20	7.10	0.02	3.91	7.46	21.73	3.0	0.45
37B	2.99	0.28	2.78	10.54	<0.02	5.46	15.88	49.11	313.6	−8.69
SG	6.03	2.32	2.56	15.62	<0.02	2.06	42.57	470.03	9.0	−18.54

SEM-AVS is reported in μM.

among samples were relatively small when results are expressed as PDA: only 2 samples out of 61 showed an effect higher than 30%, even if chemical analyses displayed relatively strong differences in contamination levels (Tables 4 and 5) (MAV-CVN, 1999). Toxicity tests performed with embryos of the sea-urchin *Paracentrotus lividus* on elutriates extracted from the same sediments showed a strongly marked toxicity difference among the samples: 14 (45%) samples from shallows and 18 (60%) samples from channels showed a highly reduced embryo–larval development (<50% normal plutei on undiluted elutriate) and were classified as being from medium up to extremely highly toxic samples (Picone, unpublished data). These data highlighted a toxicity distribution closer to that expected from chemical analyses (not shown). Sperm-cell tests on the same samples were not as sensitive as the embryos development test, but could highlight a hot-spot of toxicity in 3 samples. The data obtained with *P. lividus* are in agreement with the results of earlier studies on some of the sites investigated (Losso et al., 2004b; Volpi Ghirardini et al., 2005). Moreover, the 90th percentile of the MSD's found (10) is rather lower than the values reported in the literature for the marine and estuarine amphipods *A. abdita*, *E. estuarius* and *R. abronius* (20, 25, and 23, respectively) (Phillips et al., 2001). This lower value was probably due to low among-replicate variation obtained with Venice Lagoon sediments. On the one hand this reveals the good replicability of the test, but on the other it could be an indicator of lower sensitivity. The lower sensitivity of the *Corophium* genus compared to *R. abronius* and *A. abdita* reported in the literature (Environment Canada, 1992; Bat and Raffaelli, 1998) could be in part explained by different ecological characteristics of *R. abronius* (which is a free-burrowing amphipod directly exposed to pore-water and sediment and not a tube-dweller like *Corophium*) and the different test-temperature (20 °C) often used when testing sediments with *A. abdita* (USEPA, 1994; ASTM, 2003). For all the above-mentioned features, when developing the toxicity-score proposed in this paper, we focussed our efforts on providing a tool able to take this lower sensitivity of *C. orientale* into account (i.e. choice of 90th percentile multiples). Following this design, even a relatively small deviation from "non-toxicity" conditions leads to the assignation of a higher toxicity judgment. The discriminatory power of this acute test protocol might be enhanced by extending the exposure period (up to 28 days) or shifting the test temperature up to 20 °C.

5. Conclusion

C. orientale exhibits a good discriminatory ability when testing pure substances, although the data confirmed that *Corophium* are less sensitive than other species. Sediment toxicity test results highlighted the applicability of the method to Venice Lagoon sediments; the tolerance to a wide range of sediment typologies and grain-sizes is a valuable advantage when heterogeneous environments are studied. Moreover, *C. orientale* was very resistant to ammonia, which is one of the most critical confounding factors when testing sediments from highly productive or organic matter-rich ecosystems. The site specific toxicity-score, developed taking into account (1) the variance obtained for every sediment-control pair and (2) the relative sensitivity of *C. orientale*, allowed the samples to be discriminated on the basis of their toxicity, even if the discriminatory power of the test is still of concern. The sediments tested showed very different chemical contamination, which increased from sea-inlets towards the industrial area. Higher mortality was expected in the more polluted sites, especially those in or near the industrial area, whereas there was very little difference in mortality between the supposed control sites (2B, 19B, 22B, 24B) and the others. A comparison with the tests performed on sea-urchin early life stages confirmed the relatively lower discriminatory ability of the *C. orientale* 10-d mortality test. However, these tests were carried out on a different matrix (elutriate) and the endpoints are well known as being more sensitive than mortality (especially embryo development). Nevertheless, the data obtained displayed acute toxicity towards the autochthonous amphipods only in a few sites located close to the industrial area and in deep sediments from channels, indicating that most of the Lagoon is characterised by sediment which is not toxic toward *C. orientale* in the short-term.

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