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A toxicity scoring system for the 10-day whole sediment test with *Corophium insidiosum* (Crawford)

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Abstract This study developed a tool able to evaluate the potential contamination of marine sediments detecting the presence or absence of toxicity supporting environmental decision-making processes. When the sample is toxic, it is important to classify its level of toxicity to understand its subsequent effects and management practices. *Corophium insidiosum* is a widespread and frequently recorded species along the Mediterranean Sea, North Sea and western Baltic Sea with records also in the Atlantic Ocean and Pacific Ocean. This amphipod is found in high abundance in shallow brackish inshore areas and estuaries also with high turbidity. At Italian level, *C. insidiosum* is more frequently collectable than *Corophium orientale*, making routine toxicity tests easier to be performed. Moreover, according to the international scientific literature, *C. insidiosum* is more sensitive than *C. orientale*. Whole sediment toxicity data (10 days) with *C. insidiosum* were organised in a species-specific toxicity score on the basis of the minimum significance difference (MSD) approach. Thresholds to rank samples as non-toxic and toxic were based on sediment samples (n=84) from the Gulf of Taranto (Italy). A five-class toxicity score (absent, low, medium, high and very high toxicity) was developed, considering the distribution of the 90th percentile of the MSD normalised to the effects on the negative controls (samples from reference sites). This toxicity score could be useful for interpreting sediment potential impacts and providing quick responsive management information.

Keywords Whole sediment · 10-day toxicity test · *Corophium insidiosum* · Mortality · Minimum significance difference · Toxicity score

Highlights
- *Corophium* spp. is an interesting species to harvest for 10-day whole sediment test.
- *C. insidiosum* whole sediment toxicity data allowed to generate a toxicity score.
- Sediment samples can be ranked and managed according to the score.

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Introduction

Sediment is one the most important ecosystem compartment mainly because it is recognised both as a major sink and a source of contaminants and nutrients, depending on its physico-chemical properties and geochemical and hydrodynamic conditions (DelValls et al. 2004; Arizzi Novelli et al. 2006; Mamindy-Pajany et al. 2010). Sediment plays a key role in the ecological status of aquatic environments hosting diverse communities.

An important factor in the risk caused by sediment-bound chemicals is the degree of exposure encountered by sediment-dwelling organisms. However, the presence of contaminants does not necessarily mean that they are bioavailable that is just on the basis of their total concentration in sediment samples. In fact, sometimes, they show effects that are lower than expected (Hamelink et al. 1994; Kraaij 2001; Libralato et al. 2008).

For these reasons, ecological risk assessment and sediment quality assessment have been based not only on chemical measurements but also on biological endpoints. They integrate the effects of all contaminants including those not considered or detected by chemical analyses (Lors et al. 2010), reflecting the bioavailable fraction of contaminants, which can be very different from the total amount of chemicals (Hill et al. 1993). Since elutriate or pore water is not always good predictors of sediment toxicity (Guzzella 1998; Araújo et al. 2009), the use of at least one bioassay in which the organisms are exposed to whole sediment is strongly suggested in test batteries. Actually, the sediment direct exposure is more ecologically relevant for assessing its potential environmental effects (Lamberson and Swartz 1988; Araújo et al. 2009). Chapman and Wang (2001) stated that this approach should be the main tool in the verification of sediment toxicity, especially because it allows covering the largest variety of possible routes of exposure, involving minimal change in the physico-chemical conditions of the sediment.

Amphipods are the more widely exploited category of test organisms for whole sediment toxicity assessment. They are amongst the first species to disappear within contaminated areas, thus being sensitive indicators of sediment pollution (Long et al. 2001). They are abundant and ecologically important components of soft-bottom estuarine and marine benthic communities representing the preferred prey of fish, bird and larger invertebrate species. They live in direct contact with the sediment, being tolerant to a wide range of different environmental variables (mainly salinity and temperature). They showed a good discriminatory ability with sediments contaminated, preferentially, by organics and heavy metals (Bat and Raffaelli 1998). Many species are detritus feeders so the ingested sediment can directly expose them to bounded contaminants.

Two standard protocols for sediment toxicity assessment refer to estuarine and marine amphipods (USEPA 1994; ASTM 1993). Amongst the recommended species, *Rhepoxynius abronius* and *Ampelisca abdita* are frequently used in the USA, while *R. abronius*, *Eohaustorius washingtonianus*, *Eohaustorius estuarius* and *Amphiporeia virginiana* are recommended in Canada (Environment 1992).

Amongst *Corophiidae*, *Corophiidae volutator* is a standard European species for acute sediment toxicity testing (Bat and Raffaelli 1998; Roddie and Thain 2001), but in southern Europe, *C. volutator* is not a common species. Therefore, SETAC (1993) guidelines, suggesting the use of locally available amphipods, opened great opportunities under this viewpoint.

Within the Mediterranean Sea, *Corophium orientale* is the only *Corophiidae* cited in the ISO protocol 16712 (ISO 2005), but in Italy, *Corophium insidiosum* is also a recommended species for the acute whole sediment toxicity test to assess dredged sediment from harbours or industrial channels (APAT/ICRAM 2007).

The suitability of *C. insidiosum* as a testing species has already been demonstrated by several laboratory experiments which showed a good acclimation to test conditions, high sensitivity to reference toxicants, tolerance to environmental variables such as temperature, salinity and a relatively high sensitivity to contaminants (Prato and Biandolino 2006; Prato et al. 2008). Moreover, sediment granulometry and total organic matter (TOM) did not affect its sensitivity (Prato and Biandolino 2006). *C. insidiosum* is a tube-building species, living in the brackish and estuarine water of the infralittoral zone, in which they are widely distributed. As components of marine macrobenthic fauna, it is exposed to sediment particles not only through direct contact but also through ingestion. This increases the ecological relevance of this species in sediment bioassays (Prato and Biandolino 2006).

C. insidiosum whole sediment test was successfully applied to sediments from the Southern Ionian Sea (Annicchiarico et al. 2007; Narracci et al. 2009; Prato et al. 2006, 2010, 2012) and Adriatic Sea (Guerra et al. 2007). This test is also routinely used for the assessment of coastal and marine sediments by different Italian Regional Environmental Protection Agencies, also in the perspective of the Marine Strategy Framework Directive (56/2008/EC).

The use of toxicity data for environmental management purposes still represents a great challenge because of their practical interpretation considering that just few examples of sediment toxicity ranking approaches are available (Bay et al. 2007; Picone et al. 2008; Libralato et al. 2010). The aim of this study was to improve the overall knowledge about *C. insidiosum*, including reference toxicants, as a 10-day whole sediment testing species, providing a tool such as an experience-based toxicity score to evaluate the relative hazard of sediments from coastal marine areas of Southern Italy.

Materials and methods

Sediment sampling and treatment

Fifty-seven sampling stations, distributed across the Taranto Gulf (Italy), were sampled between 2005 and 2013, for a total of 84 samples. Sediments were collected in triplicate using the Van Veen Grab Sampler. Unwanted materials (e.g. large particles, stones, trash and indigenous organisms) were removed from sediment samples using a tweezer. Specimens were stored in plastic bags, immediately transported to the laboratory facilities and kept at 4±1 °C in the darkness for no longer than 48 h before testing.

Granulometry: physico-chemical analysis

Sediment samples were analysed for total organic matter and grain size. TOM was estimated as the percentage of weight lost after the ignition of dry sediment at 550 °C for 4 h. Mechanical sieving allowed the separation of sediments into two grain size fractions (<0.063 and 0.063–2 mm).

At the beginning and the end of every toxicity test, the quality parameters of the overlying water included temperature, salinity, dissolved oxygen (DO) and pH in order to ensure the acceptability of toxicity tests.

Organism collection

The sampling sites of *C. insidiosum* were located in unpolluted intertidal areas along the Ionian Sea (Southern Italy) where a monospecific population lives. In the field, animal collection occurred by gently sieving small quantities of sediment through a 0.5-mm mesh in order to remove any associated macrofauna and larger sediment particles. Only the animals with 2–4 mm body length were kept for testing. Then, amphipods were placed into a clean plastic container with water from the sampling site and immediately transported to the laboratory, where they were isolated and transferred to 30-L plastic aquaria with their native water. They were acclimated at 16±2 °C and 36‰ for 3–4 days before the beginning of the test.

Ecotoxicity tests

Acute static toxicity tests with *C. insidiosum* considered two exposure scenarios: contact times of 96 h (water-based test for reference toxicants) ($Cd(NO_3)_2$ Baker, NL, 99.9 % purity) and 10 days (whole sediment). All the toxicity tests included negative controls and were performed at least in triplicate. For the 96-h test, 20 young-adult amphipods were randomly allocated in 1-L beakers containing 0.7 L of exposure medium. Because of their particular sensitivity, ovigerous females were excluded. Filtered natural seawater (GF/C Whatman, 0.45 μm) collected in unpolluted areas was used for negative controls and as dilution water. Toxicity tests occurred in 1-L beakers that were aerated and set at 16±2 °C with continuous light (>100 lx). Animals were not fed during the exposure period. The survivors were counted, considering apparently dead individuals as alive if any movement was exhibited after gentle stimulation. Missing animals were assumed to be dead. Tests were rejected when the control mortality exceeded 15 % according to Bigongiari et al. (2001), considering a maximum acceptable effect in the negative control of 15 %. This is less restrictive than ISO (2005) (maximum acceptable effect in the negative control of 10 %), but more limiting than other authors' suggestions (maximum effect of 20 % due to the number of organisms exposed (n=20) per test) (Ennas et al. 2002; Narracci et al. 2009). These limits were taken into account for both liquid- and solid-phase tests.

Acute static 10-day toxicity test on whole sediment with *C. insidiosum* was largely based on guidelines for conducting acute sediment toxicity tests with marine-estuarine amphipods (ASTM 1993; SETAC 1993; ISO 2005). The exposure occurred in 1-L beakers containing 200 mL of sediment and 750 mL of filtered seawater that was added from the top. Sediment and water were allowed to equilibrate before starting the test according to De Witt et al. (1989). Twenty young-adult (2–4 mm) amphipods were randomly selected from the aquaria and transferred to the exposure chambers. Only active and healthy organisms were used. Test chambers were continuously aerated through a glass tip placed at least 1 cm above the sediment surface to avoid animal disturbance. The testing temperature was set at 16±2 °C with continuous light (>100 lx). Three replicates were prepared per treatment. During the exposure, no food was provided. Water quality parameters (DO, salinity and pH of the overlying water) were measured at the start and at the end of the exposure period in all experiments to ensure that all replicates and treatments were exposed to the same conditions. After 10 days of exposure, the samples from each beaker were sieved through a 0.5-mm mesh screen to isolate the organisms. The survivors were counted, and apparently dead individuals were considered as alive if movement was exhibited after gentle stimulation. Missing animals were assumed to be dead. Tests were rejected when the control mortality exceeded 15 %. Negative control tests were carried on reference site sediment that was the native sediment inhabited by the testing organisms.

Data analyses and toxicity score development

The results of aqueous-phase tests with reference toxicants were analysed using the trimmed Spearman–Karber method (Hamilton et al. 1977) to determine the 96-h median lethal concentration values (LC_{50}) and 95 % confidence limits. Results from the 10-day whole sediment toxicity test were reported as a percentage of dead amphipods (PDA), normalised to control response using Abbott's formula (Finney 1971). The t test ($p<0.05$) was used to verify the statistical differences between the effects in the negative controls and treatments. Toxicity results were checked for normality (Kolmogorov–Smirnov test) and variance homogeneity (Bartlett's test). The statistical package software, SPSS (version 10.0) and XLSTAT (version 2008.4.01), was used for data analysis.

Toxicity data were used to define the toxicity score for *C. insidiosum* 10-day whole sediment bioassay. The first step was to discriminate between non-toxic and toxic samples. The procedure suggested by Thursby et al. (1997) and Phillips et al. (2001) was followed according to other specific case studies (Picone et al. 2008; Libralato et al. 2010). Samples were ranked as non-toxic or toxic on the basis of the 90th percentile of the minimum significance difference (MSD) normalised on the effect in the negative controls and further classified in five scores to distinguish between five potential adverse effect levels. In particular, three thresholds were established on the basis of Picone et al. (2008) and Libralato et al. (2010): (1) medium toxicity threshold (MTT), (2) high toxicity threshold (HTT) and (3) extreme toxicity threshold (ETT). The relative toxicity limits were calculated by substituting toxicity threshold (TT) with MTT, HTT and ETT. The MSD scaling took into account that the negative controls are acceptable if less than 15 % mortality is detected (Bigongiari et al. 2001), thus representing the lower limit of samples presenting low toxicity effect.

A principal component analysis (PCA) based on Pearson's correlation matrix relationships between variables and the variation present in the dataset matrix were accounted via biplotting, identifying the major discriminating variables associated with a given principal component.

Results and discussion

Granulometric characterisation

Sediment grain size and organic matter content are considered confounding factors, and they can influence the capacity of adsorption of contaminants (Ligero et al. 2005). Generally, fine particles represent the main fraction in which the potential toxic substances can concentrate because of its large surface-to-volume ratio (Casado-Martínez et al. 2006). In this study, most part of sediment samples showed a high percentage of pelite (<0.063 mm) ($n=56$). About the TOM content, the samples significantly different from the control ($p<0.05$) showed values ranging from 0.34 to 22.81 %.

Ecotoxicity data

During liquid-phase test, temperature, salinity, pH and DO were always within the acceptability limits in all beakers ($T=16\pm2$ °C, 36±2‰, pH=8.0±0.5 and 80 % O_2 saturation). All negative controls ($n=84$) always had a mean survival percentage in line with the acceptability criterion (<15 % effect).

Toxicity tests performed with Cd as a reference toxicant showed a mean nominal LC_{50} value of 1.20± 0.71 mg/L ($n=30$, coefficient of variation (CV)= 59 %) with a maximum value of 2.00 mg/L and a minimum of 0.04 mg/L based on USEPA (2002) recommendation. Cadmium is a widely used reference toxicant in a liquid-phase test with amphipods (McGee et al. 1998; Onorati et al. 1999; Kater et al. 2000;

Bigongiari et al. 2001), and its stability during the 96-h bioassay is well documented (Kater et al. 2000). The mean sensitivity of *C. insidiosum* to Cd was comparable to that reported by Hong and Reish (1987), while Reish (1993) and Lamberson et al. (1992) reported LC_{50} values lower than the average one detected in this study as shown in Table 1. A wide range of LC_{50} values for Cd were reported by several authors for both *C. orientale* (Onorati et al. 1999; Bigongiari et al. 2001; Lera et al. 2008; Picone et al. 2008; Prato et al. 2010) and *C. volutator* (Ciarelli 1994) (Table 1). Rè et al. (2009) reported that *Corophium multisetosum* was the most sensitive *Corophiidae* against Cd with an LC_{50} range of 0.23–0.71 mg/L. The average sensitivity to Cd as mean LC_{50} values (Table 1) showed the following species ranking: *C. multisetosum* (0.47±0.34 mg/L of Cd, n=2, CV=72 %)<*C. insidiosum* (1.33±0.92 mg/L of Cd, n=10, CV=69 %)<*C. orientale* (3.40±1.91 mg/L of Cd, n=10, CV=56 %)<*C. volutator* (3.58±2.44 mg/L of Cd, n=2, CV=68 %).

During the whole sediment test, temperature, salinity, pH and DO were always within the acceptability limits in all beakers. The results of bioassays carried out on whole sediment with *C. insidiosum* showed a mean survival in negative control of 94±3 % (n=84, CV=3 %) meeting the acceptability criteria established for this type of sediment tests (Bigongiari et al. 2001; ISO 2005).

The results of the toxicity test as PDA±standard deviation are reported in Table 2, including the p values of t test, the percentage of survival in the sediment test (S), the percentage of survival success of the control (C) and the toxicity limit (TL). *C. insidiosum* evidenced that 53 samples responded in a way that was significantly different from the reference site controls ($p<0.05$).

In the pelitic sediment samples, *C. insidiosum* confirmed its high tolerance to grain size distribution and organic enrichment (Prato and Biandolino 2006; Prato et al. 2006) showing several no effect responses (n=37). This aspect makes *C. insidiosum* a key species in environments with a heterogeneous grain size and organic load becoming of great value for toxicity assessment of the whole sediment.

In the literature, other amphipods used as a test species were tolerant to different grain sizes such as *Leptocheirus plumulosus* (DeWitt et al. 1992), *Gammarus aequicauda* (Prato and Biandolino 2005) and *C. orientale* (Picone et al. 2008). Fine particles (silt and clay) are known to be able to clog amphipod gills, while coarser sediments may cause extra expenditure of energy to manipulate large particles when burrowing. In both cases, amphipod performance in test sediments may be affected in some way. The burrowing amphipod *R. abronius* and the tube dweller *Grandidierella*

Table 1 *Corophium* spp. 96-h LC_{50} for Cd with 95 % confidence limits reported in the literature

Test organisms	96-h LC_{50} Cd (mg/L)	References
Corophium insidiosum	1.27	Hong and Reish (1987)
	0.96	Lamberson et al. (1992)
	0.68 (0.3–1.8)	Reish (1993)
	1.68 (0.94–2.40)	Annicchiarico et al. (2007)
	1.38 (0.94–2.039)	Prato et al. (2008)
	From 0.35 (0.16–0.76) to 3.36 (1.72–5.74)	Prato et al. (2006)
	1±0.5; 0.9±20.6	Narracci et al. (2009)
	1.30±0.11	Prato et al. (2010)
Corophium orientale	From 2.91 (2.09–3.73) to 4.28 (2.96–5.63)	Onorati et al. (1999)
	From 1.56 (1.16–2.08) to 4.38 (2.69–7.12)	Bigongiari et al. (2001)
	3.3 (0.87–5.80)	Picone et al. (2008)
	1.21–1.36; 5.01–7.23	Lera et al. (2008)
	3.03±0.70	Prato et al. (2010)
Corophium volutator	From 1.85 (1.27–2.69) to 5.30 (3.72–7.54)	Ciarelli (1994)
Corophium multisetosum	From 0.23 to 0.71	Ré et al. (2009)

Table 2 Whole sediment toxicity test results with *C. insidiosum*; toxicity data are reported as a percentage of dead amphipods (PDA) together with standard deviation, *p* values for *t* test and survival adjusted to control (*S*), control survival (*C*), toxicity limit (TL) and sediment total organic matter (TOM) content as well as sediment sand or pelite composition (%); the sediment sample final toxicity classification is reported as well

Samples	PDA±SD (%)	*p* value	*S* (%)	*C* (%)	TL (%)	TOM (%)	Sand (%)	Pelite (%)	Toxicity rank
D1-8	−3±6	0.371	103	94	77	4.95	2	98	Absent
H1-11	−1±3	0.374	101	97	80	5.27	81	19	Absent
D1-9	0±3	0.371	100	94	77	6.10	0	100	Absent
D1-6	0±3	0.371	100	94	77	2.35	1	99	Absent
A1-4	1±3	0.333	99	98	80	0.94	33	67	Absent
H1-12	1±4	0.311	99	97	80	3.99	72	28	Absent
F1-4	2±3	0.333	98	90	74	0.86	13	88	Absent
A2-4	3±5	0.264	97	98	80	1.65	25	75	Absent
A4-4	3±5	0.092	97	98	80	3.86	38	62	Absent
E1-5	3±8	0.211	97	97	80	2.56	37	63	Absent
H1-8	4±5	0.054	96	97	80	6.75	91	9	Absent
H1-4	4±6	0.050	96	97	80	1.32	90	10	Absent
H1-7	4±8	0.228	96	97	80	3.64	69	31	Absent
H1-3	4±4	0.121	96	97	80	1.45	98	2	Absent
A1-2	4±3	0.113	96	98	80	1.77	20	80	Absent
C1-4	4±3	0.113	96	98	80	1.05	14	86	Absent
F1-3	6±5	0.239	94	90	74	1.67	8	92	Absent
A3-3	6±3	0.029	94	98	80	2.44	18	82	Absent
A2-1	6±3	0.029	94	98	80	2.79	19	81	Absent
A3-2	6±8	0.135	94	98	80	0.87	27	73	Absent
A4-1	6±6	0.135	94	98	80	1.87	17	83	Absent
H1-2	6±5	0.144	94	97	80	0.99	100	0	Absent
H1-5	7±6	0.092	93	97	80	1.60	85	15	Absent
H1-6	7±5	0.054	93	97	80	3.47	65	35	Absent
A2-2	8±9	0.100	92	98	80	2.96	28	72	Absent
A4-2	8±0	0.019	92	98	80	1.32	23	77	Absent
H1-9	8±2	0.008	92	97	80	0.51	69	31	Absent
D1-5	9±5	0.035	91	94	77	0.71	34	66	Absent
A3-1	9±3	0.007	91	98	80	1.12	29	72	Absent
C1-1	9±3	0.037	91	98	80	1.95	14	86	Absent
H1-1	10±9	0.006	90	97	80	0.43	92	8	Absent
B1-1	11±3	0.010	89	98	80	3.04	18	82	Absent
A3-4	11±6	0.059	89	98	80	3.11	15	85	Absent
C1-2	11±8	0.096	89	98	80	4.70	20	80	Absent
A1-3	11±6	0.059	89	98	80	0.97	32	68	Absent
A1-1	11±3	0.010	89	98	80	1.99	16	84	Absent
C1-5	13±5	0.001	87	98	80	2.79	18	82	Absent
D1-7	13±3	0.008	87	94	77	1.85	1	99	Absent
I1-24	13±3	0.010	87	90	74	1.10	23	78	Absent
E1-3	14±3	0.008	86	97	80	3.50	4	96	Absent
A4-3	15±3	0.018	85	98	80	1.03	28	72	Absent
A2-3	15±3	0.018	85	98	80	2.01	19	81	Absent

Table 2 (continued)

Samples	PDA±SD (%)	*p* value	*S* (%)	*C* (%)	TL (%)	TOM (%)	Sand (%)	Pelite (%)	Toxicity rank
E1-1	16±15	0.113	84	97	80	5.50	19	81	Absent
D1-1	16±24	0.189	84	97	80	1.94	9	91	Absent
C1-6	16±13	0.055	84	98	80	2.96	28	72	Absent
B1-2	16±8	0.032	84	98	80	3.55	24	76	Absent
C1-3	16±3	0.005	84	98	80	0.88	31	69	Absent
I1-16	19±8	0.055	81	90	74	1.78	37	63	Absent
I1-15	19±3	0.005	81	90	74	1.22	64	36	Absent
H1-10	21±6	0.311	79	97	80	4.78	85	16	Medium
E1-4	21±10	0.029	79	97	80	0.75	23	77	Medium
I1-5	22±5	0.010	78	90	74	0.45	63	37	Absent
E1-2	22±9	0.020	78	97	80	4.75	15	85	Medium
D1-2	24±3	0.010	76	94	77	1.92	9	91	Medium
D1-4	24±8	0.003	76	94	77	1.74	6	94	Medium
I1-27	26±8	0.036	74	90	74	0.55	41	59	Low
F1-1	30±3	0.008	70	90	74	1.95	7	93	Medium
D1-3	31±35	0.160	69	94	77	1.88	7	93	Medium
I1-17	31±8	0.007	69	90	74	0.56	78	22	Medium
I1-2	35±10	0.009	65	90	74	0.77	75	25	Medium
I1-11	39±9	0.003	61	90	74	0.34	47	53	High
I1-25	43±10	0.025	57	90	74	1.22	21	79	High
F1-2	44±0	0.003	56	90	74	1.99	9	91	High
I1-21	44±13	0.029	56	90	74	0.88	33	67	High
I1-18	46±6	0.006	54	90	74	0.66	72	28	High
I1-4	48±13	0.005	52	90	74	0.65	70	30	High
I1-22	48±16	0.025	52	90	74	1.02	33	67	High
I1-19	50±10	0.018	50	90	74	0.74	65	35	High
G1-1	52±10	0.007	48	100	82	17.92	15	85	High
I1-9	54±8	0.004	46	90	74	0.87	54	46	High
I1-12	54±6	0.004	46	90	74	0.72	63	37	High
I1-8	56±5	0.000	44	90	74	0.47	60	40	High
I1-20	56±9	0.007	44	90	74	0.95	67	33	High
I1-13	57±3	0.001	43	90	74	0.88	50	50	High
I1-14	59±8	0.002	41	90	74	1.04	58	42	High
G1-2	60±10	0.005	40	100	82	22.81	21	79	High
I1-1	61±5	0.005	39	90	74	0.85	64	36	High
I1-6	63±13	0.013	37	90	74	0.43	88	12	High
I1-10	63±6	0.000	37	90	74	0.60	36	65	High
I1-23	63±3	0.003	37	90	74	0.96	46	54	High
I1-26	65±13	0.014	35	90	74	0.45	16	84	High
I1-3	72±5	0.003	28	90	74	0.53	87	13	Very high
I1-7	76±8	0.000	24	90	74	0.96	56	45	Very high
E1-6	100±0	0.000	0	97	80	0.97	15	85	Very high

japonica exhibited reduced survival after 10 and 28 days of exposure to sediments with high content of silt and clay, respectively (DeWitt et al. 1988; Nipper et al. 1989).

Toxicity score generation

The choice of percentiles for sediment toxicity classes' characterisation was suggested on the basis of similar experiences (Picone et al. 2008; Libralato et al. 2010) in order to reduce the expert judgement to a minimum. In Fig. 1, the cumulative distribution of MSD values normalised on negative control data was reported after its normalisation to the average relative negative controls for all sediment toxicity tests. The 90th percentile of normalised MSD values for every sample–control pair ($n=84$) was 18 %. The toxicity limit should be set at 82 % of control survival. This is in accordance to the main authors and protocols (Bigongiari et al. 2001; Ennas et al. 2002; ISO 2005; Narracci et al. 2009) that set the acceptability for negative controls between 10 and 20 %. Swartz et al. (1995) indicated that a sediment sample is toxic if >24 % of amphipod mortality can be detected. Thursby et al. (1997) and Phillips et al. (2001) reported a threshold value of 20 % for *A. abdita*.

In our case, the maximum effect detected in sediment samples from reference sites never exceeded 10 %. The values for MTT, HTT and ETT were 27, 36 and 54 % of effect, respectively. These values were obtained considering the 90th percentile value (18 %) increased using an amount of the same value that is proportionally cumulative in geometrical order (0.5, 1 and 2). A concise judgement, a score from 0 to 4 and a colour accompanied all classes. Thus, the classification of sediment samples resulted as follows: if $S<18$ %, the effect (82 % of control survival) toxicity is absent (0, blue); if 18 %$\leq S<$27 %, the toxicity is ranked as low (1, green); if 27 %$\leq S<$36 %, the toxicity is medium (2, yellow); if 36 %$\leq S<$54 %, the toxicity is high (3, orange); and if $S\geq$54 %, the toxicity is very high (4, red).

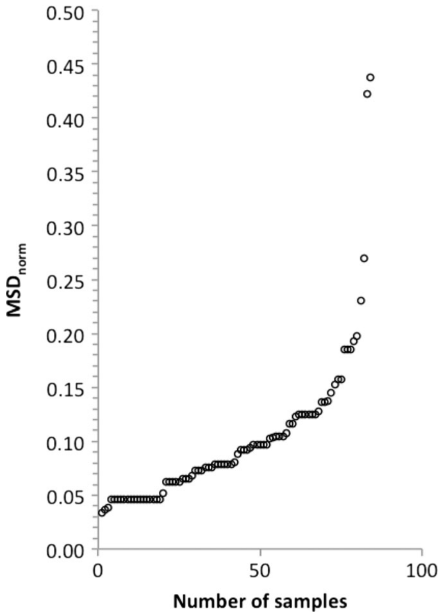

Fig. 1 Cumulative distribution of the minimum significance difference values normalised to the average negative controls ($n=84$)

The thresholds obtained in this study represent valuable approaches to classify sediment toxicity into multiple clearly delineated categories. Indeed, the bioassays with *C. insidiosum* identified 60 % of sediments as non-toxic, 1 % as low toxic, 11 % as medium toxic and 25 and 4 % as high and very high toxic, respectively.

To establish a threshold representing a test response associated with moderate to strong toxicity, other authors have used thresholds based on the MSD values. The 90th percentile of the MSDs reported by Picone et al. (2008) for *C. orientale* was 10 %, whereas *C. insidiosum* showed the same values reported for *E. estuarius* and *R. abronius* for sediment from the California Coast (USA) with a 90th percentile of the MSDs of 18 and 17 %, respectively (Bay et al. 2007). The values found for *A. abdita*, *E. estuarius* and *R. abronius* by Phillips et al. (2001) were slightly above 20, 25 and 23 %, in that order.

In Fig. 2, a biplot summarised the PCA results on sample TOM, granulometry and toxicity both as PDA and toxicity rank. The first two principal components accounted for 46.85 and 33.25 % of the variation, respectively. Therefore, the two-axis ordination diagram described 80.10 % of the variation. Samples were grouped into two main clusters (A and B) on a granulometric basis evidencing that the whole sediment toxicity was not influenced by its granulometric distribution, meaning that both pelitic and sandy sediment samples presented very low or very high toxicity levels in a way that was independent of their grain size composition. Thus, sediment granulometry is not sufficient to allow speculation about its potential toxicity. Pelitic sediments presented the highest levels of TOM, as expected, but it showed to be still independent from the toxicity level of samples.

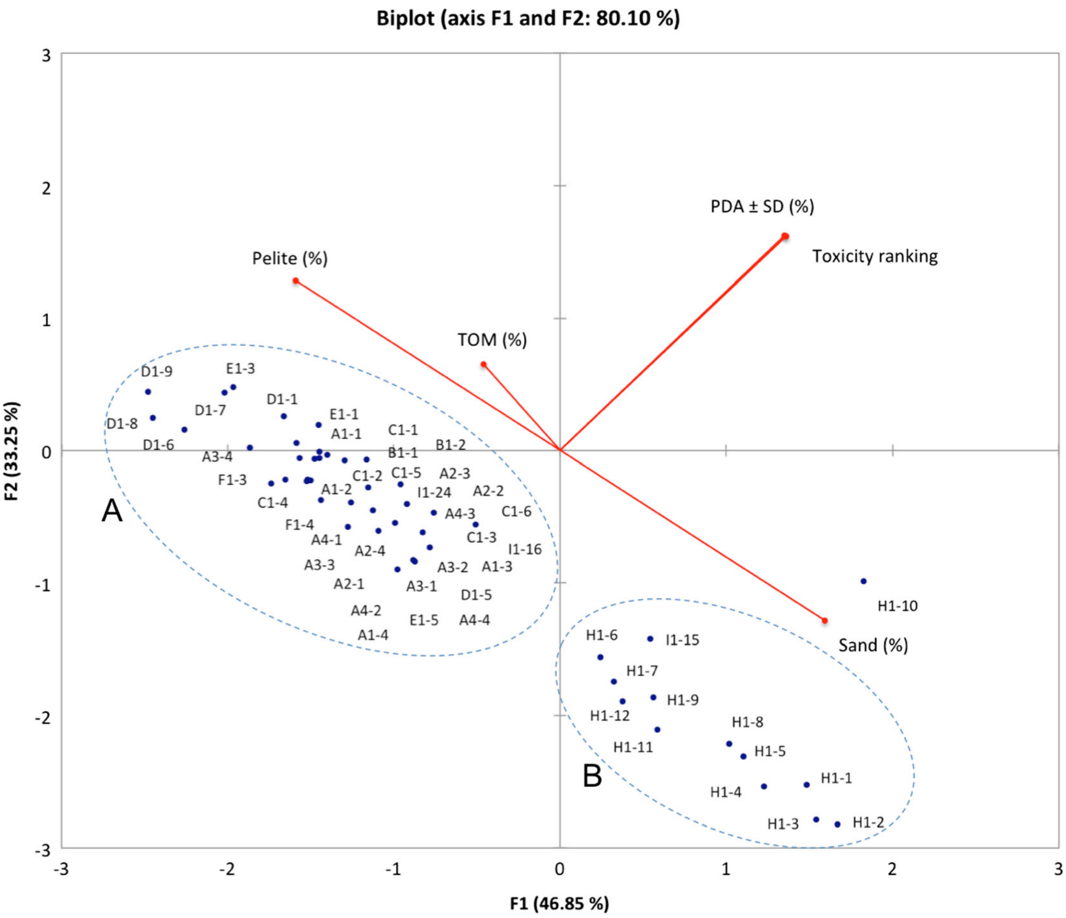

Fig. 2 Principal component analysis biplot of granulometric data with loadings and scores in the coordinates of the first two principal components (F1 and F2); clusters A and B grouped samples with a prevailing pelitic or sandy composition, in that order

Conclusions

An overview of the sensitivity of *C. insidiosum*, as the amphipod for southern Europe testing purposes, was provided about Cd (96-h test), evidencing a substantial similarity in the response with existing information. A large number of whole sediment toxicity data (10 days) allowed the determination on a statistical basis of a specimen classification tool mainly based on the 90th percentile of the MSD. This species-specific assessment score is now ready for verification and application in sediment ranking activities for its management practices as long as with other similar tools already present in the scientific literature. The proposed threshold limits allow an easy and immediate understanding and comparison of results also by non-experts. Additional investigations are needed to increase the validation level of this scoring system in order to verify the stabilisation of the statistics with further data and increase the feasibility with other species.

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