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Sediment Toxicity Tests Using Two Species of Marine Amphipods: *Gammarus aequicauda* and *Corophium insidiosum*

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In the recent years, sediment toxicity tests are recommended procedures for evaluating the ecological hazards of toxicants in the aquatic environment.

Amphipods are small sediment–dwelling invertebrates, geographically widely distributed and constitute a major component of the aquatic food webs. Several species are used for sediment toxicity assessment, because of their ecological relevance, sensitivity to environmental disturbance and ease of handling (ASTM 1993; Conlan 1994). Although amphipod toxicity tests have been successfully used in the United States (EPA 1988; ASTM 1993), there are few tests based on European amphipods species. The SETAC – Europe guide (1993) particularly recommends the use of *Corophium volutator* or other locally available amphipods. This species is not available in the Ionian coastal area, but there are other endemic species, *Corophium insidiosum* and *Gammarus aequicauda* that could be used for tests.

Corophium insidiosum, is a tube-building species living in the brackish and estuarine water of the infralittoral zone, where it is widely distributed and available in large number and it feeds both on sediment and suspended particulate matter.

Gammarus aequicauda is an epibenthic, free-living widespread species, in the Mediterranean and Black Sea. It feeds on macroalgae, especially *Chaetomorpha linum*, *Ulva ssp*. and on sediment detritus. In muddy sediments *G. aequicauda* burrows into the sediment surface. These species are important in both transferring food to other organisms and the natural regeneration of organic matter in underlying sediment (Kevrekidis and Koukouras 1989).

The amphipods *G. aequicauda* and *C. insidiosum* were selected for this study because preliminary tests demonstrated their tolerance to non-contaminant variables (biotic and abiotic) and sensitivity to reference toxicants (Prato and Biandolino 2005 and unpublished data).

The objective of this research was to evaluate the quality of sediments from Mar Piccolo basin (Ionian sea, Southern Italy), to obtain information about correlations between metal contamination of sediment and toxicological effects in benthic organisms.

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MATERIALS AND METHODS.

Sediments were sampled in five stations (Fig. 1); four of them were located in the Mar Piccolo of Taranto (stations 1, 2, 3, 4), and one sediment sample (station 5) was collected in the north-west coast of the Ionian Sea in an area far from anthropogenic sources of contaminants. Sediments were collected with a "Van Veen" grab; after sampling they were wet-sieved through a 0.5 mm mesh sieve to remove amphipods and other small undesired macrobenthos. Sediments were stored under refrigeration for no longer than 48h before testing. Aliquots of samples were analyzed for total organic matter and trace metals (Hg, Cd, Cu, Pb, Ni). Total organic matter (TOM %) was estimated as the percentage of weight lost after ignition of dry sediment at 550°C for 4 hours.

Amphipods were collected from the control site using a 0.5 mm sieve; organisms were stored in polyethylene buckets containing seawater; they were immediately transported to the laboratory and placed in aerated glass containers with their native sediment. Experimental organisms were acclimated for 3-4 days before the beginning of different tests.

All reagents were of analytical-reagent grade and they contained only very low amounts of metals. All solutions were prepared with an ultra-pure water (conductivity of < 0.1 µS) obtained from a MILLI-Q® system (Millipore, Bedford, MA, USA). Nitric and fluoridric acid (Suprapur grade) were supplied by Merck (Darmstadt, Germany). Working standard solutions of metals were prepared by serial dilution of stock standard solutions (BDH, Poole, UK) containing 1000 mg/L of each metal. Normal precautions for trace analysis were observed throughout.

For metal determinations (Hg, Cd, Cu, Pb and Ni) 0.2 g of dry sediment was digested with 9 mL of concentrated nitric acid and 3 mL of concentrated fluoridric acid in a closed teflon vessel (EPA 1996) using a MARSX microwave oven (CEM Corporation, Matthews, NC). After mineralization, digests were cooled and the resulting solutions were diluted to a known volume (50 mL) with ultra-pure water and stored in polyethylene bottles until analysis.

Total mercury was determined by cold vapour atomic absorption spectrometry using a Perkin Elmer model 1100B equipped with a Perkin Elmer MHS-10 hydride generator. Other metals (Cd, Cu, Pb, Ni) were determined by atomic absorption spectrophotometry with a graphite furnace using a Perkin Elmer Zeeman 3030 spectrophotometer. Sediment samples were analyzed in triplicate. The detection limits were 0.06 $\mu g\ g^{-1}$ d.w. for Cd, 0.09 $\mu g\ g^{-1}$ d.w. for Hg, 0.11 $\mu g\ g^{-1}$ d.w. for Pb, 0.2 $\mu g\ g^{-1}$ d.w. for Cu and 0.18 $\mu g\ g^{-1}$ d.w. for Ni.

Cadmium LC_{50} values, based on the lethal concentration at which 50% did not survive, was determined by Spearman–Karber method (Hamilton et al. 1977).

The general design of toxicity tests was based largely on the standard guides for conducting acute sediment toxicity tests with marine-estuarine amphipods, with some modification (ASTM 1993; SETAC-Europe 1993).

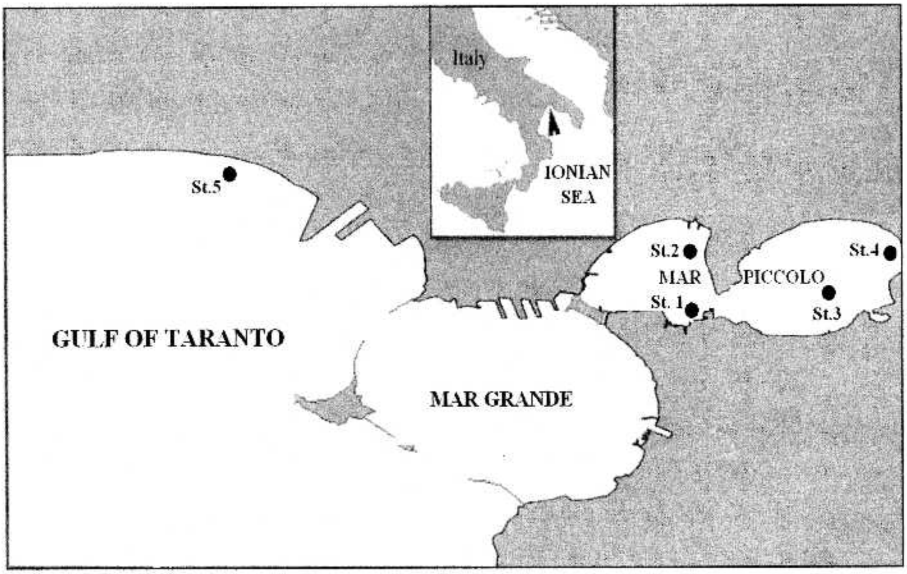

Figure 1. Location of sampling stations.

some modification (ASTM 1993; SETAC-Europe 1993).

Sensitivity to reference toxicant, cadmium chloride ($CdCl_2$), was verified monthly from April (2002) to March (2003). These controls, also designated as "positive control", consisted in the determination of 96h LC_{50} for toxicant in the seawater in absence of sediment. Twenty amphipods were added to the aerated beakers containing $CdCl_2$ dissolved in 500ml of 36‰ filtered seawater (0.45μm).Four different concentrations of 0.2, 0.4, 0.8 and 1.6 mg/L have used of $CdCl_2$ for *G. aequicauda* and 0.8, 1.6, 3.2, 6.4 mg/L for *C. insidiosum* and one seawater control with three replicates for each treatment. During the 96h exposures, temperature was maintained at 16 ± 2°C and the amphipods were not provided with additional food.

Tests were carried out inside a 1-litre glass beaker with collected sediment. The sediment layer was approximately 2 cm and filled with 750 ml of filtered seawater (0.45μm). Twenty young-adult amphipod were randomly selected and introduced into each beaker that was continuously aerated through a glass tip placed at least 1 cm above the sediment surface under constant temperature 16 ± 2°C. Three replicates were prepared per treatment. Only active and healthy organisms were used and gravid females were discarded. During 10-d exposure, no food was added to the test chambers. The dissolved oxygen concentration, salinity and pH of the overlying water in the test chambers were measured at the beginning and termination of the tests. At the end of exposure time the survivors were counted, missing organisms were considered dead and apparently dead individuals were considered living if movement was exhibited after gentle stimulation. The bioassay was considered valid if the mortality in the control sediment was < 20%, While the sediment was considered toxic if it significantly differed from control mortality (t-test; $p<0.05$).

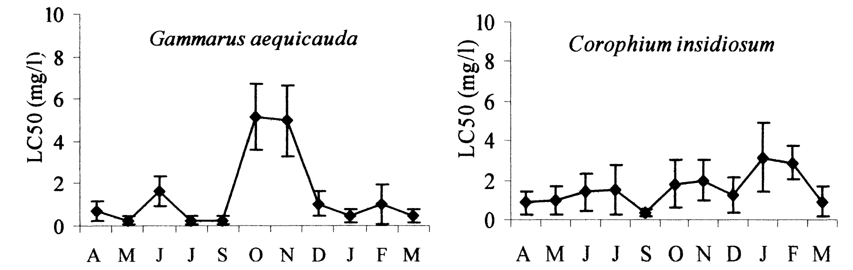

Figure 2. Monthly trend of Cd 96 h-LC_{50} for *G. aequicauda* and *C. insidiosum*.

RESULTS AND DISCUSSION.

Figure 2 shows seasonal variation of amphipod sensitivity to cadmium in the aqueous phase. Mean survival (%) in the controls of both species in all experiments (n=10) was high (95%; SD= 1). The 96 h LC50 monthly value (95% confidence interval) for cadmium in the aqueous phase ranged from 0.35 mg/L to 3.36 mg/L with Cv (Coefficient of variation) from 10% to 59% for *C. insidiosum*. *G. aequicauda* shows a range from 0.26 mg/L to 5.16 mg/L and Cv from 19% to 75%. There wasn't a significant difference in sensitivity between the populations ($t = - 0.17$; $P > 0.05$).

Table 1 shows that the sensitivity of *G. aequicauda* and *C. insidiosum* to aqueous cadmium was similar to that of other amphipod species used in sediment toxicity assessment.

Table 1. Comparison of amphipod sensitivities to cadmium in 96-h (95% confidence limits) water only exposure.

Test - organism	96 h- LC_{50} mg/l	Source
Gammarus aequicauda	from 0.26 (0.24-0.18) to 5.16 (7.35-3.57)	Present work
Gammarus locusta	0.59 (0.43-0.82)	Costa et al 1996
Corophium insidiosum	from 0.35(0.16-0.76) to 3.36 (1.72-5.74)	Present work
Corophium orientale	from 2.91 (2.09-3.73) to 4.28 (2.96-5.63)	Onorati et al 1999
Corophium volutator	from 1.85 (1.27-2.69) to 5.30 (3.72-7.54)	Ciarelli 1994
Ampelisca abdita	1.32 (1.22-1.43)	Kohn et al 1994
Rhepoxynius abronius	1.92 (1.47-2.51)	Kohn et al 1994
Ehoaustorius estuarius	9.33 (7.20-12.09)	ASTM 1993
Grandidierella japonica	1.17 (0.94-1.46)	ASTM 1993
Leptocheirus plumulosus	1.06 (0.85-1.33)	ASTM 1993

The mortality and t values between each sample and control sediment are reported in Table 2. Mortality of either species to field sediments in stations 3 and 4 was low, with no significant differences ($p>0.05$). Populations examined did not tolerate sediments from stations 1 and 2 for which mortality was very high compared with the control sediment, but there were significant differences in the mortality between the species for sediment collected at station 1 (68.3% for *G. aequicauda* and 34.6% for *C. insidiosum*) ($p<0.05$).

Table 2. Mortality (%) and t values between each sediment sample and control sediment.

	G. aequicauda			*C. insidiosum*		
Stations	Mortality (% ± SD)	t	p-value	Mortality (% ± SD)	t	p-value
1	68.3 ± 1.2	t= 16.54	p<0.001	34.6 ± 0.6	t=7.07	p<0.01
2	51.6 ± 1.5	t= 9.54	p<0.002	51.0 ± 0.6	t= 13.4	p<0.001
3	11.6 ± 0.6	t=2.12	p>0.05	15.0 ± 1.0	t= 1.41	p>0.05
4	6.6 ± 0.6	t= 1	p>0.05	11.6 ± 0.6	t=2.12	p>0.05
5 Control	10 ± 1.0	t= 0		11.6 ± 0.6	t= 0	

Table 3. Metal and TOM (total organic matter) percentage in analysed sediments.

		STATIONS				
		1	2	3	4	5
TOM (%)		5.50	3.50	1.23	0.85	0.70
Metal	**Cd**	0.29±0.02	0.26±0.01	0.12±0.01	0.43±0.19	ND
($\mu g\ g^{-1}$d.wt)	**Hg**	11.55±0.05	2.04±0.04	0.16±0.01	0.36±0.27	ND
	Pb	137.90±0.36	129.30±0.97	16.38±0.18	28.98±0.59	10.64±0.12
	Cu	86.88±0.05	149.86±0.08	23.22±0.72	35.34±0.10	5.57±0.10
	Ni	57.88±0.12	53.67±0.17	48.21±0.15	0.97±0.32	2.01±0.15

ND = not detectable

Table 3 shows metal concentrations in the analysed sediments. The highest levels of Hg, Pb, Cu, Ni were found in sediments from stations 1 and 2 while the lowest concentrations occurred at stations 4 and 5.

Table 4. Metals concentration ($\mu g \cdot g^{-1}$ dry weight) and recovery in certified marine sediment (CRM 277).

Metals	**Cd**	**Hg**	**Pb**	**Cu**	**Ni**
Certified value	11.9 ± 0.4	1.77 ± .06	146 ± 3	101.7 ± 1.6	43.4 ±1.6
Measured value	10. 9 ± 2.5	1.67 ±0.08	152 ± 1.2	99.5 ± 0.45	41.8 ± .8
Recovery %	92	94	104	98	96

Method accuracy was evaluated by analysing sediment reference material (CRM 277) provided by the Community Bureau of Reference (Commission of the European Community) (Table 4).
The retrospective evaluations of the biological significance of chemical residues in sediments is fraught with difficulties in absence of contemporary biological measurements. However, a variety of attempts have been made to set numerical sediment quality guidelines (SQGs).

They either involve "weight-of-evidence" approaches, based on field and laboratory observations of biological effects (MacDonald et al. 1996), or methods based on variations of the equilibrium partitioning (EqP) approach, which calculates the interstitial water concentration at equilibrium and compares it with "safe" or "effect" concentrations for the relevant chemicals in water (Swartz et al.

1995). In particular, the Threshold Effect Level (TEL)/Probable Effect Level (PEL) weight-of-evidence approach, adopted by MacDonald et al. (1996), seems to have produced guideline values which are protective against the majority of negative effects on the organisms.

The TEL/PEL method is based on large datasets of effects and no-effects arranged in ascending order of chemical concentration. The TEL is defined as the geometric mean of the 15th percentile concentration of the set of effects and the 50th percentile concentration of the no-effects dataset. Similarly, the PEL is the geometric mean of the 50th percentile of the effects data and the 85th percentile of the no-effects data. Compared with these guidelines, mercury, lead, nickel and copper showed PEL exceedances for the most contaminated stations (stations 1 and 2) in the Mar Piccolo (Table 5).

In short, the TEL/PEL analysis suggested that, especially for stations 1 and 2, sediments in Mar Piccolo should contain acutely toxic concentrations of metals. Sediment bioassay data obtained in tests (Table 2) with the amphipod *Corophium insidiosum* and *Gammarus aequicauda* support this prediction although metals are not thought to be the only cause of this toxicity.

Table 5. Comparisons of effect-based sediment quality guidelines (TEL/PEL) with metal concentrations (μg g^{-1} dry wt).

METALS	STATIONS 1	2	3	4	5	TEL Threshold Effect Level (μg g^{-1} d.wt.)	PEL Probable Effect Level (μg g^{-1} d.wt.)
Cd	0.29	0.26	0.12	0.43	ND	0.68	4.21
Hg	11.55	2.04	0.16	0.36	ND	0.13	0.7
Pb	137.90	129.30	16.38	28.98	10.64	30.24	112.18
Cu	86.88	149.86	23.22	35.34	5.57	18.7	108.2
Ni	57.88	53.67	48.21	0.97	2.01	15.9	42.8

ND = not detectable

Many substances accumulate in sediments, leading to elevated concentrations which may (or may not) cause adverse environmental effects. Sediment toxicity tests provide information about biological consequences of contamination that can only be inferred from chemical or benthic community analyses.
The literature indicates mortality thresholds for standardized amphipods bioassays is scarce. Mearns et al. (1986), EMAP (1994), Lourens et al. (1995) suggested that sediment samples which caused ≤ 24-25% mortality in tests with *Rhepoxynius abronius*, *Ampelisca abdita* and *C. volutator*, were considered not toxic.

Values obtained in this study for the sediment samples from stations 1 and 2 show the mortality rate > 24% and therefore were considered toxic.

The pollutants in the sediments are available to amphipods either through resuspension at the sediment water interface or through feeding and uptake across the digestive tract (Burton 1992). Other factors, e.g., physiology and ecology, may be responsible for contributing to differences in sensitivities. In our study, *C.*

insidiosum was slightly less sensitive than *G. aequicauda,* probably due to different ecologies as *C. insidiosum* is tube-building and although it remains in its tubes during sediment exposure, it is exposed to overlying water which is pumped through the burrow so that contact between *Corophium* and the sediment is limited, thereby reducing direct exposure to particle-bound contaminants in the sediment (Nair and Anger 1979). *G. aequicauda,* being a free-living organism is exposed to environment contaminant concentrations and their route of accumulation of contaminants may be from interstitial water via the gills and through feeding. It feeds upon sediment detritus, as picking up pieces of organic material or scraping the surface of mineral particles (Kevrekidis and Koukouras 1989).

The results of this study confirmed that these species may be suitable for use in sediment bioassays in order to attain valuable conclusions to be applied in global scale biomonitoring programs. Findings from the current study will have implications for future developments in ecotoxicological testing with these species. Further study in the Ionian Sea will be conducted, aiming to identify the critical areas of concern for remedial actions and to establish gradients related to the contamination sources.

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