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Test for Acute Toxicity of Copper, Cadmium, and Mercury in Five Marine Species

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Abstract: : The acute toxicity of copper (Cu), cadmium (Cd), and mercury (Hg) to the marine invertebrates *Gammarus aequicauda*, *Corophium insidiosum*, *Idotea baltica*, *Sphaeroma serratum*, and *Mytilus galloprovincialis* were evaluated by static bioassays and calculation of the LC_{50} (lethality concentration for 50%). Hg was more toxic to *Gammarus aequicauda*, *Corophium insidiosum*, *Idotea baltica*, *Sphaeroma serratum*, and *Mytilus galloprovincialis* than Cu and Cd. Cu was the least toxic of the metals tested.

Key Words: Heavy metal, amphipods, isopods, *Mytilus galloprovincialis* larvae, acute toxicity test

Introduction

Concentrations of single compounds or complex mixtures of contaminants are continually released into the aquatic environment from commercial, industrial, and residential areas, representing a potential risk to the marine biota.

Particular attention should be given to heavy metals due to their persistence in nature (Leach et al., 1985), incorporation into the food chain (Broman et al., 1992; Suedel et al., 1994), and toxicity to wildlife and humans in certain concentrations (Travis and Frey, 1990). Heavy metals include both essential elements and metals with no known biological function, such as Cd, Hg, Ag, Sn, etc. In shallow, near shore waters where dispersion and dilution processes are less effective, the measured concentrations of heavy metals are generally noticeably higher than in open ocean waters. Chemical analyses determine the concentrations and nature of pollution, but they do not provide information on the deleterious effects upon living organisms (Chapman et al., 1987).

Toxicity tests allow the determination of these effects, providing direct evidence of the biological responses of marine organisms to contaminants.

Due to the fact that organisms from different species vary in their sensitivity towards chemical substances, it is difficult to set standards for protection of species with regard to pollutants in the environment. Extrapolation from one species to another is, therefore, difficult if their relative sensitivities are not known (Van Straalen et al., 1994).

Invertebrates, as organisms within a battery of tests to evaluate the quality of marine environments, have become increasingly important. The use of a single species for a correct evaluation of toxicity levels can be reductive; therefore, a battery of organisms belonging to different habitats, life stages, trophic groups, and evolutive levels should be used. The purpose of this investigation was to examine the sensitivity of different test-organisms in order to evaluate alternative or complementary test species for ecotoxicological studies in the Mediterranean ecosystem.

Materials and Methods

We tested the sensitivity of 5 species to 3 reference toxicants; 2 species of amphipods, *Gammarus aequicauda* and *Corophium insidiosum*, 2 species of isopods, *Sphaeroma serratum* and *Idotea baltica*, and *Mytilus galloprovincialis* larvae.

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Collection, holding, and acclimation of test organisms

Amphipods and isopods were collected from a single station to minimize the role of their origin as a potential source of distortion of response (Mersch and Pihan, 1993). This was a clean area located in Mar Piccolo in Taranto (Ionian Sea, southern Italy).

Gammarus aequicauda, *Sphaeroma serratum*, and *Idotea baltica* samples were collected from the macro-algae *Chaetomorpha linum*, *Ulva* sp., and *Enteromorpha* sp. If macro-algae are sparse or not present, the animals can still be found under stones.

Corophium insidiosum, being a tube building organism and found infaunally, mostly in fine muddy sediments, was collected by sieving the sediment in the field through a 0.5 mm mesh sieve.

The animals were immediately transferred to the laboratory, separated and placed in aerated glass aquaria with filtered natural seawater, and acclimated to the test temperature of 16 ± 2 °C for a minimum of 7 d prior to the start of the experiments.

In the aquaria, an excess of food was added in the form of macro-algae and organic detritus.

Toxicity tests

Water-only toxicity tests were carried out using 3 metal salts ($CuCl_2$, $CdCl_2$, and $HgCl_2$). The results of a preliminary test for each amphipod and isopod species were used to set the definitive concentrations of each toxicant.

Stock solutions of heavy metals were freshly prepared by dissolving the appropriate metal salts in distilled water in 1 litre glass volumetric flasks, which had previously been washed in 10% nitric acid and well rinsed with distilled water. They were then stored in darkness at 4 °C. None were prepared more than 2 d in advance of a test. Test concentrations were made of serial dilutions of the stock solution with natural seawater, which had been previously filtered.

Amphipod and Isopod tests

Preliminary tests were carried out to establish suitable concentration ranges. Five different concentrations (0.4, 0.8, 1.6, 3.2, and 6.4 mg/l) of cadmium and copper were used for *G. aequicauda*, *C. insidiosum*, and *I. baltica*, and (0.8, 1.6, 3.2, 6.4, 12.8 mg/l) of cadmium and copper for *S. serratum*. Concentrations of 0.1, 0.05, 0.025, 0.0125, and 0.00625 mg/l of mercury were tested on all species.

A control was used for each test with 5 replicates per treatment. The control water and dilution water used in the experiments consisted of natural seawater collected in an unpolluted area and filtered through a GFC Wathman (0.45 µm) filter.

Twenty young-adults (retained by a 0.5 mm mesh sieve) were randomly allocated in one-l beakers containing reference toxicant dissolved in 700 ml of filtered seawater; because of their particular sensitivity to ecotoxicological testing, ovigerous females were excluded from the experiments. All experiments were carried out at 16 ± 2 °C and 36‰ salinity under a 12:12 h light:dark regime with gentle continuous aeration. These conditions were selected according to the range of the parameter values annually registered in the Mar Piccolo. Temperature, pH, salinity, and dissolved oxygen were checked at the beginning and the end of every test to ensure the acceptability of the tests, following standard methods (Buchanan, 1984).

The exposure time of the organisms to each metal was 96 h, without adding any food.

At the end of the experiments, the survivors were counted. Apparently dead individuals were considered living if movement was exhibited after gentle stimulation. Missing animals were assumed to be dead. Tests were rejected when the control mortality exceeded 10%.

Mussel larvae toxicity tests

Adult stocks of *Mytilus galloprovincialis*, obtained from a commercial mussel culture of Mar Grande (Ionian Sea), were induced to spawn with hydrogen peroxide. This method was used based on Morse et al. (1977), with some modifications, who described the effects of this stimulation on *Haliotis rufescens* and on other molluscs.

The animals were stimulated in mass, not individually; the duration of the treatment was only 15 min. The hydrogen peroxide concentration (5 mM) and the pH value of the water (9.1) remained for the duration of stimulation. Every specimen that initiated the emission was individually transferred to a small bath with 500 ml

of 0.2 µm filtered seawater, where they continued to release gametes.

After filtration through a sterile 100 µm sieve to remove tissue debris, eggs from seven females were transferred to sterile 2-l graduated cylinders with filtered seawater moved lightly by means of air gurgled through an air stone at 20 °C. Males, during spawning, were transferred to cylinders and covered with filtered seawater. Freshly collected eggs and sperm were suspended in natural filtered seawater in a sterile glass beaker to achieve fertilization. After 30 min on 3 subsamples of 100 µl, the number of fertilized eggs was counted under a microscope.

All tests performed were static bioassays utilizing 0.2 µm filtered seawater in 50-ml test tubes. The volume of solution added (0.5-1 ml) for each experiment was calculated according to the number of organisms required, approximately 1000 fertilized eggs.

Mussel larvae were exposed to 5 concentrations of each metal in geometric progression plus a control as follows: Cu (0.025, 0.05, 0.010, 0.20, and 0.4 mg/l); Hg (0.00625, 0.0125, 0.025, 0.05, and 0.1 mg/l); Cd (0.003125, 0.00625, 0.0125, 0.025, and 0.05 mg/l); all with 5 replicates per treatment.

All static assays were run for 48 h, the period of embryonic development of *M. galloprovincialis* at 20 °C. The larvae were not fed during the duration of the experiment.

At the end of the assay, the larvae, fixed with 10% buffered formalin, were counted in lots of 100 from each treatment and were observed under an inverted microscope in order to determine the percentage of normal embryonic development in each tube test. Any larvae with atypical cell development or those exhibiting no shell growth (i.e., undeveloped larvae) were considered abnormal according to His et al. (1997). The test was rejected and repeated if an average of 20% of the control larvae were abnormal.

Data analysis

The copper, cadmium, and mercury LC_{50} values, based on the lethal concentration at which 50% of amphipods and isopods did not survive, and EC50 values of the concentration of test substances, which caused death or abnormally-developed larvae in 50% of tested organisms, were determined according to Spearman-Karber method (Hamilton et al. 1977).

A one-way ANOVA analysis was used to evaluate significant reaction differences between each species towards the 3 reference toxicants. The Tukey test, used for multiple range comparisons, was performed to evaluate differences of sensitivity between the species.

Results

All controls resulted in low mortalities, fewer than 5%, which indicated the acceptability of the experiments. Figure 1 shows the 96 h LC_{50} values of copper, cadmium, and mercury to all species tested.

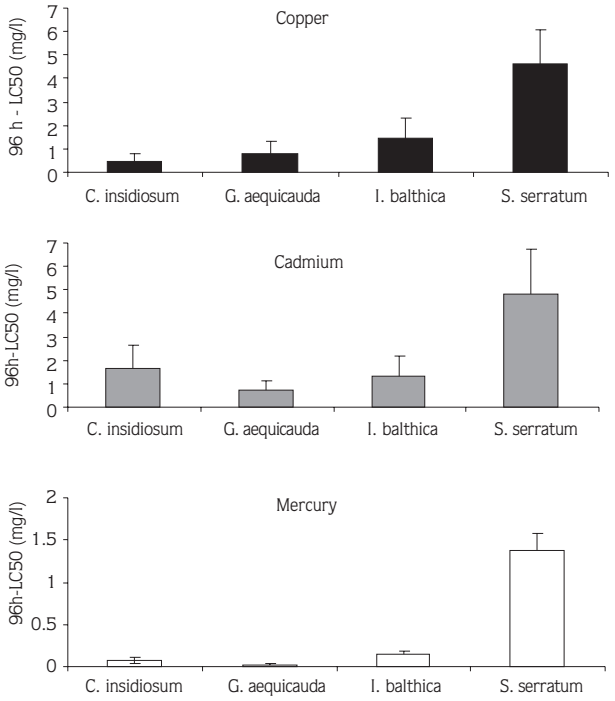

Figure 1. Crustacean sensitivity test results (LC_{50} ± SD) for the reference toxicants copper chloride ($CuCl_2$), cadmium chloride ($CdCl_2$) and mercury chloride ($HgCl_2$).

For the 96-h copper experiment, *C. insidiosum* and *G. aequicauda* were particularly sensitive species with LC_{50} values of 0.47mg/l (0.33-0.66) and 0.82 mg/l (0.53-1.28), followed by *I. baltica* with an LC_{50} value of 1.45 mg/l (0.90-2.35), and finally, *S. serratum* was the most tolerant with an LC_{50} value of 4.60 mg/l (10.93-1.93). The lowest LC_{50} values were obtained for mercury experiments in all crustacean species, with the exception

of *S. serratum* with a LC_{50} value of 1.38 mg/l (11.71-0.06).

For the experiment with cadmium, once more, *G. aequicauda* appeared much more sensitive with an LC_{50} of 0.71mg/l (0.44-1.14), followed by *I. baltica* (LC_{50} value of 1.29 mg/l (1.92-0.86)), and *C. insidiosum*, (LC_{50} value of 1.68mg/l (0.94-2.40),) while *S. serratum* was much more tolerant with an LC_{50} value of 4.79 ml/l (5.75-3.99).

There were significant differences in the LC_{50} values for *M. galloprovincialis*, *G. aequicauda*, *C. insidiosum*, and *I. baltica* to all reference toxicants (ANOVA $P < 0.001$), while *S. serratum* showed no significant differences ($P > 0.05$).

The Tukey test, used for multiple comparison between means, showed that the sensitivity of *I. baltica* to mercury was significantly different than other species ($P < 0.05$) and, again, the sensitivity of *S. serratum* to copper was significantly different than the other species ($P < 0.05$).

The lowest EC50 values were obtained for $HgCl_2$ and $CdCl_2$ (Figure 2). As illustrated in the concentration-response curves for all metals, there was an evident decrease in the percentage of normal embryonic development with increasing metal concentrations. Mercury induced the development of more abnormal larvae at levels greater than 6.25 µg l^{-1} (Figure 3).

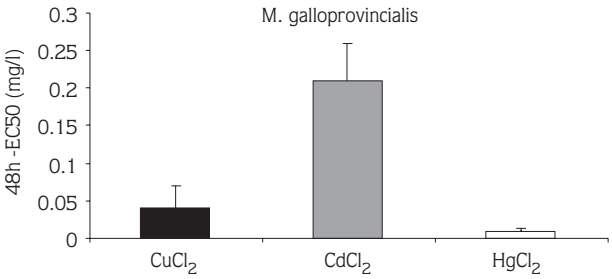

Figure 2. *Mytilus galloprovincialis* larvae sensitivity test results (EC50 ± SD) for copper chloride ($CuCl_2$), cadmium chloride ($CdCl_2$), and mercury chloride ($HgCl_2$).

Discussion

The primary intent of this study was to have some information on different sensitivities of some marine organisms to 3 toxicants in order to use them in the sediment toxicity test.

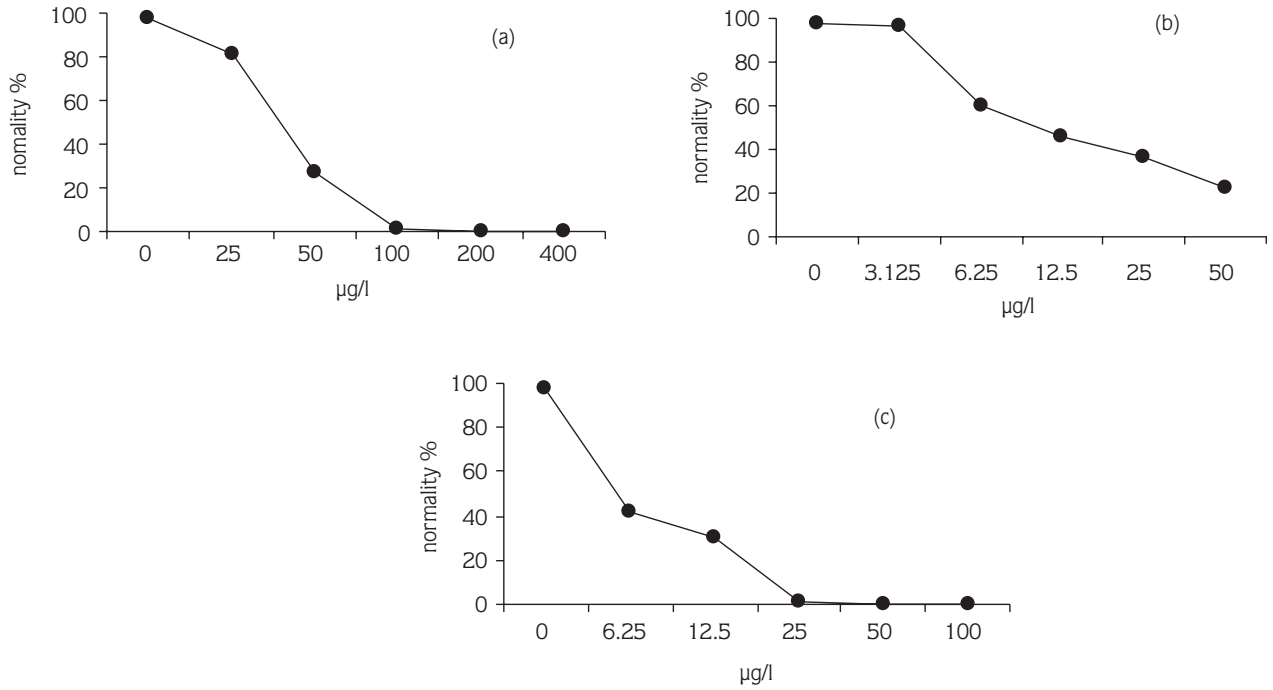

Figure 3. Concentrations-response curves tests on *Mytilus galloprovincialis* larvae, incubated for 48 h, for copper chloride ($CuCl_2$) (a), cadmium chloridE ($CdCl_2$) (b), and mercury chloride ($HgCl_2$) (c)

Although several standard methods have been developed for assessing the toxicity of contaminants using several species from Atlantic and Pacific coasts, no such tests have been reported for use in Europe, more specifically, for species from the Mediterranean Sea.

The SETAC-Europe guide (1993) recommends the use of indigenous species because, although standardized test species are extremely important as screening tools to provide information on contaminant effects, they do not always answer the question of local relevance (Chapman et al., 1983).

The different sensitivities of organisms may be related to their habitats, or may be due to the utilization of standardized sensitive organisms not indigenous to the study area. Thus, in these kinds of studies, comparison must be treated with caution.

Furthermore, the species should be selected on the basis of the availability of large numbers of specimens, since a laboratory toxicity test requires a large number of organisms and often the field population shows significant variations in abundance; thus an experiment may be interrupted because test organisms from the field are not available.

The results of this preliminary study show that every species tested in these experiments reacted differently to the metals tested; *G. aequicauda* appeared one of the most sensitive, while *S. serratum* seemed the most tolerant; therefore *G. aequicauda*, *I. baltica*, and *C. insidiosum* can be suitable for toxicity determination in the Mediterranean Sea.

Jones (1975) and Bryant et al. (1985; 1985a) have pointed out that metal toxicity to marine crustaceans, including isopods, varies with environmental conditions such as temperature, salinity, and metal type, and it would be useful to monitor test species around of the year in order to assess if the sensitivity changes with these variables.

Comparing our data with those available in the literature, we can see that the sensitivity of *C. insidiosum* to cadmium seems to be lower than that from Reish (1993). Indeed, Onorati et al. (1999) reported an LC_{50} range of 2.91 to 4.28 mg/l of Cd for *C. orientale* and Ciarelli (1994), and for *C. volutator*, found an LC_{50} range of 1.85 to 5.30 mg/l of Cd. The sensitivity of *C. insidiosum* towards copper was higher than that reported by Reish (1993). Comparison of the LC_{50} values for mercury with values determined for other *Corophidae* are not possible considering the lack of studies.

The sensitivity of *G. aequicauda* to aqueous cadmium was within the range of values reported for other amphipod species proposed for use in estuarine or marine sediment toxicity tests (DeWitt et al., 1992; Schlekat et al., 1992). Insufficient information was found about the toxicity of copper in seawater; Costa et al. (1998) reported a 96-h LC_{50} value of 0.3 mg/l for *G. locusta*.

I. baltica is very sensitive to long-term exposure to sub lethal levels of copper, but no background information was found in the literature about acute toxicity tests (De Nicola et al., 1989; Bat et al., 1999).

The early life stages of marine bivalves, in particular *Mytilus* spp. and *Crassostrea gigas*, are most commonly used in acute tests to assess the toxicity of micro-pollutants such as heavy metals (Robert and His, 1985; Beiras and His, 1995).

The toxicity of the reference toxicants used towards mussel larvae was higher than peracarida; on the other hand, they responded faster, took up less space, and may be useful for toxicity tests. *M. galloprovincialis* larvae EC50 was similar to that observed by other authors (Beiras and His, 1995; Volpi Ghirardini et al., 2004).

The selection of organisms may be an important tool for evaluating the effects of contaminants; most of the 5 species used in our experiment demonstrated their potential for use in bioassays.

In conclusion, comparing the sensitivity of these species to common reference toxicants, we suggest using *G. aequicauda*, *I. baltica*, *C. insidiosum*, and *M. galloprovincialis* larvae in a bioassay batterie for toxicity determinations in the Mediterranean Sea. On the other hand, *S. serratum*, which didn't show an elevated sensitivity toward the metals tested, doesn't seem to fit to this purpose.

Clearly, there is a need to conduct further experiments with specific contaminants on these species to assess their suitability for detecting toxicity, as well as experiments involving a complex mixture of contaminants, in order to determine the real discriminatory power of these species.

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