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Toxicity of zinc and copper to the hermit crab *Diogenes pugilator* (Roux)*

Çinko ve bakırın keşiş yengeci *Diogenes pugilator* (Roux)'a toksisitesi

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Abstract

Several experiments were designed to investigate the separate and combined effects of zinc and copper in sea water on the survivorship of the hermit crabs *Diogenes pugilator* (Roux). Results showed that copper was more toxic than zinc to the test animals. Survival in sea water with dissolved zinc and copper was higher in the presence of sediment than without sediment. When copper was added together it zinc, this increased mortality of the test animals.

Key words: Hermit crab, toxic, zinc, copper, synergistic

Introduction

The protection of marine habitats from damage due to the release of toxic substances, can require toxicity test data on aquatic organisms, including macroinvertebrates. Many acute lethality tests have been conducted on the

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effects of metals on marine invertebrates (Bat, 1995a; Bat, 1996a). Such tests are used in two ways; firstly, to derive safe environmental concentrations using LC_{50} values and application factors in the absence of chronic toxicity information on the tested species, secondly to compare the sensitivities of different species and potencies of contaminants using LC_{50} values (Ahsanullah *et al.*,1981). Knowledge of the sensitivity of marine invertebrates to specific pollutants would allow a more meaningful interpretation of such analyses.

In the present study, the hermit crab *Diogenes pugilator* (Roux) was evaluated as a test organism for use in static toxicity tests by adapting the standard protocol developed by Bat (1996a) for conducting 96 hours. Hermit crabs live in the empty shells of a variety of prosobranch molluscs. When the crab becomes too big for the shell, it changes to another. Empty shells on the shore are clearly a valuable resource (Fish and Fish, 1996). *Diogenes pugilator* is found at extreme low water and below, in sandy sediments (Campbell and Nicholls, 1994; Fish and Fish, 1996). *Diogenes pugilator* was selected for the present study because of its ecological importance, wide geographical distribution, ease of handling in the laboratory, and its sensitivity to heavy metals. These characteristics make it an ideal test species for toxicity tests. Zinc and copper were chosen for the present study because their toxicity to aquatic organisms is well-documented (Hodson *et al*, 1979; Lewis and Cave, 1982; Bryan, 1984; Moore and Ramamoorthy, 1984; Mance, 1987). The two metals are essential in trace quantities, but are markedly toxic (Bat, 1995a,b; Bat,1996b).

Materials and Methods

Animals were collected by divers from the upper-infra littoral zone of Sinop coasts of the western Black Sea at a water depth of 1-2m. Total 1200 individuals of the *Diogenes pugilator* were collected. They are locally abundant during spring and summer, their frequent occurrence in the shells of *Hinia reticulata* (62.5 %) and *Cerithium vulgatum* (37.5 %) no doubt reflecting the availability of these shells. The animals were transported to the laboratory in tanks containing 10 L of clean sea water. At the time of collection of the samples in July 1997, the average water temperatures and salinities ranged between 22°C to 26°C and 17 to 18 ‰, respectively. In the laboratory the animals were kept at 24°C±2. Salinities during the experiments were kept similar to those at the time of collection. The sea water used for the experiment was poured through a filter (45 μm) into a tank to avoid water containing suspended particles and organisms larger than 45 μm. The water in the tank was continually aerated in order to maintain the dissolved oxygen levels above 60% of the air saturation value (ASTM, 1990 ; US EPA/COE Manual, 1991). The animals were stored in tanks with sediment for acclimatisation for a period of at least 5 days. Sediments were taken from the same area of collection of the animals. The sediment was washed through a 1 mm mesh sieve into a tank to remove any associated macrofauna and to ensure a standard particle size for the sediment in all experiments. Temperature, dissolved oxygen, salinity and pH were measured in all experiments and the design of the experiments ensured that all replicates and treatments were exposed to the same factors.

A stock solution of 1000 ppm of each metal was prepared by dissolving zinc (as $ZnCl_2$) or copper (as $CuSO_4.5H_2O$), in distilled water. Test solutions were made up by diluting the stock solution with sea water. Zinc concentrations of 0 (control), 5, 10, 20, 50 and 100 µg Zn L^{-1} and copper concentrations of 0 (control), 1, 5, 10, 20, 50 and 100 µg Cu L^{-1} were tested. To prevent precipitation of copper and zinc in sea water, copper and zinc solutions were acidified slightly, but the pH never dropped below 7 (Ahsanullah, 1976 ; Ahsanullah *et al.*, 1981). Preliminary tests were carried out to establish suitable concentration ranges. Hermit crabs were exposed to seven concentrations of zinc or copper in sea water as well as to uncontaminated sea water (control), in set-ups with and without clean sediment. Each set-up consisted of 6 replicate jars (8 cm in diameter, 14 cm deep) for each of the 7 concentrations plus 10 controls. 150 ml of clean sediment was added to half of these jars to create a 2 cm deep layer. All jars were aerated in order to maintain the dissolved oxygen levels above 60% of the air saturation value and covered by black material to exclude direct light except from directly above. Aeration, at a rate of approximately five or six bubbles per second, was provided by a Pasteur pipette so as not to disturb the sediment surface. Healthy adult animals (1.5-2.5 cm long) were randomly selected from the tank and five of them were placed in each jar containing 400 ml of solution. After 1 hour any hermit crab that was dead or showed abnormal behaviour were removed and replaced. *Diogenes pugilator* cauld survive in this manner for two months or more if there was no contaminant in the water. Experiments were conducted under static test conditions. No food was supplied during the course of the experiment. Jars were examined daily and any dead organisms removed and recorded. The LC_{50}s of zinc and copper in sea water with and without sediment were calculated by probit analysis (Finney, 1971). The per cent of animals responding was regressed against the logorithm of the zinc or copper concentration to determine the LC_{50}. Dead animals of each replicate were counted and placed together in separate vials which were previously numbered, dried and weighed so that each vial corresponded to a specific replicate. Vials containing hermit crabs without their shells were dried immediately at 70°C for 48 h. After drying, these were weighed on a balance and the dry weight of animals was calculated for each jar. The effects on dry weight of the treatments were estimated by calculating the statistical significant differences between the average weight of each treatment group and the controls using Analysis of Variance (ANOVA) followed by a multiple comparison test (Zar, 1984). Further experiment was designed to investigate the combined effects of zinc and copper in sea water on the survivorship of the hermit crabs. The sea water was treated with both metals with 3 replicate jars per concentration and 5 animals per jar. Mortality was recorded at intervals of 3, 6, 24, 48, 72 and 96 h.