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Toxicity of cadmium on larvae of *Palaemon adspersus* Rathke, 1837 from the Black SeaLevent Bat^{1*}, Sabri Bilgin², Aysah Öztekin¹¹Fisheries Faculty, Department of Hydrobiology, Sinop University, TR57000 Sinop, Turkey²Fisheries Faculty, Department of Fishing Technology and Processing, Sinop University, TR57000 Sinop, Turkey

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ABSTRACT

Objective: To evaluate the suitability of *Palaemon adspersus* (*P. adspersus*) as a bio-indicator for 4 and 10 days of cadmium toxicity bioassays.**Methods:** Four and ten days experiments were designed. CdCl₂·2½H₂O was dissolved in distilled water and a stock solution was made. At 4-day bioassay, the larvae of Baltic prawns were exposed to nominal concentrations of 0, 0.02, 0.05, 0.1, 0.5, 1.0 and 5.0 mg/L of Cd for 4 days. The 4-day LC₅₀ was calculated by the probit analysis. At 10-day bioassay, the concentrations of 0.05, 0.1, 0.5, 1.0, 5.0, 10.0 and 20.0 mg/L were introduced into each of the jars in triplicate treatments and 0 mg/L as control. Each tank containing 20 larvae was exposed to test solutions.**Results:** Results from probit analysis showed that the 96-h LC₅₀ value was 0.14 mg/L for Cd. The 10 days bioassays were conducted with nominal concentrations of 0, 0.05, 0.1, 0.5, 1.0, 5.0, 10.0 and 20.0 mg/L Cd. Mortality increased in parallel with the increase in concentrations of Cd on Zoea – I stage of *P. adspersus* and time of exposure. The toxicity rate of the organism is concentration-dependent. All organisms except the control group died at the end of 10 days. Less than 25% of the animals survived at the 5 days of the exposure to concentrations of 0.5 mg/kg Cd or more. Only 20% of the organisms survived at the 7 days of the exposure to concentrations of 0.1 mg/kg Cd or less in seawater with clean sediment.**Conclusions:** The results showed that Cd was highly toxic to *P. adspersus*. When the larvae were exposed to concentrations of Cd, they become slightly excited and swam erratically, probably due to stress. However, behavioural and swimming patterns in control groups were normal, and there was not any mortality during the course of the experiment.

1. Introduction

Environmental contaminants especially heavy metals have detrimental effects on aquatic organisms[1]. Heavy metals as non-degradable contaminants are considered as a major problem for marine ecosystems that receive them through different anthropogenic and natural sources[2,3]. Non-essential metals such as Cd are very important in marine environments and many studies have focused on different aspects of their toxicity for marine organisms[4-11]. Crustaceans are widespread and important components of marine coastal ecosystems[12] and are thus interesting candidates for toxicological studies[13-

16]. A variety of toxicity test methods have been developed and 4 and 10 days bioassays have a considerable status helping to measure the susceptibility and survival potential of organisms to particularly toxic contaminants such as heavy metals[13]. Bat *et al.*[17] showed that *Palaemon adspersus* Rathke, 1837 (*P. adspersus*) is a suitable organism for Cu and Pb toxicity tests.

Baltic prawn, *P. adspersus* is a common inhabitant of the Black Sea and is known to have omnivorous feeding on small crustaceans, polychaetes, algae and detritus[18,19]. The growth and biology of the coastal population of *P. adspersus* has been studied in the Black Sea[19], and it has been shown that there is an association between this species and phanerogam beds which are considered as soft bottom habitats[17,20]. It tolerates a wide range of temperatures and salinities and it is distributed in shallow water, often in estuarine conditions[17].

The current experiments were carried out using the individuals of *P. adspersus* obtained from the Sinop Peninsula

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of the Black Sea. The objective of the present study is to evaluate the suitability of *P. adspersus* as a bio-indicator for 4 and 10 days of cadmium toxicity bioassays. The study was carried out at the Hydrobiology Laboratory of Sinop University. The experiment was conducted using standard static bioassay procedure. This involved controlled environmental conditions to define the response of the bioassay organism the Baltic prawn.

2. Materials and methods

2.1. Collection of animals

The crangonid shrimps appear during the winter and spring and the numbers of individuals increase to reach their highest abundance in mid-winter and mid-spring[21]. Specimens of the Baltic prawns were collected from Sinop Peninsula in 2013 (Figure 1).

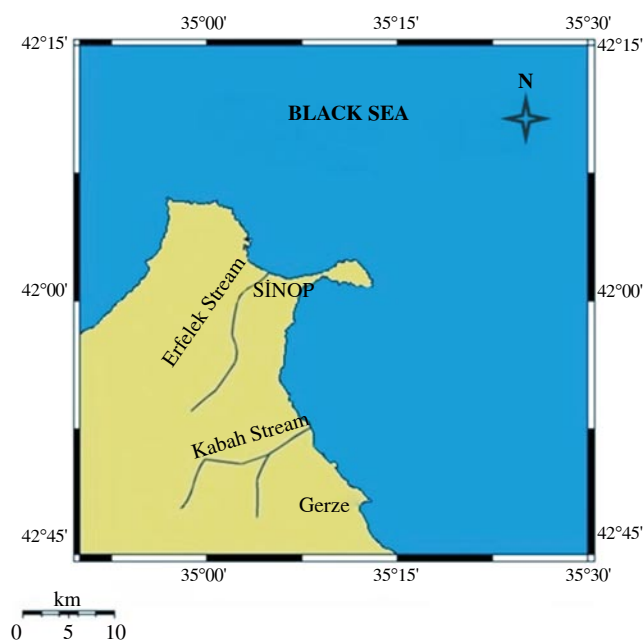


Figure 1. Collection area.

P. adspersus gravid females (20–35 individuals) were collected by beam trawl from Sinop Peninsula. They were stored in an aquarium tank (0.5 m × 1 m × 1 m) supplied by water recirculated through a gravel filter. The shrimps were fed pellet. Water temperature of the aquaria was kept at (23 ± 2) °C by a thermostatically controlled heater. The newly hatched larvae were stocked at a density of 10 larvae into 1-L round-bottom fiberglass tank. Larvae were fed with newly hatched *Artemia salina* nauplii at 10 mL⁻¹ during the experiment. Larvae of *P. adspersus* are carnivorous and they die in 2–3 days when they are starved in laboratory conditions (personal observations).

2.2. Experimental protocol

Four and ten days experiments were designed. CdCl₂·2½H₂O was dissolved in distilled water and a stock solution was made. The larvae of Baltic prawns were exposed to nominal concentrations of 0, 0.02, 0.05, 0.1, 0.5, 1.0 and 5.0 mg/L of Cd for 4 days. The 4-day LC₅₀ was calculated by the probit analysis[22]. At 10 days bioassays, the concentrations of 0.05, 0.1, 0.5, 1.0, 5.0, 10.0 and 20.0 mg/L were introduced into each of the jars in triplicate treatments and 0 mg/L as control[4,5]. Each tank containing 20 larvae was exposed to test solutions.

The pH of the solutions was daily measured with the pH meter, temperature with mercury-in-glass thermometer, dissolved oxygen with a digital DO₂. The exposure lasted for 96 h. Animals mortality was monitored and recorded hourly for the first 3 h and for the next 24 h and subsequently every 24 h for the next 4 and 10 days. If there was any dead individual within first 3 h, the experiment was terminated. The inability of the larvae to respond to external stimuli was used as an index of death. Dead organisms were removed immediately with a scoop net to avoid contamination due to rotting. Water temperature, pH and dissolved oxygen were determined every hour for the first 3 h and for the next 24 h and once every 24 h before the end of the experiment.

2.3. Statistical analysis

Statistical analyses were performed using SPSS software (ver. 21). Data were analysed by One-way of analysis of variance (ANOVA). Means were compared by Duncan multiple comparison test ($P < 0.05$).

3. Results

Control animal groups did not show significant differences during the present study. Figure 2 shows Zoea – I stage of *P. adspersus*, and Figure 3 shows mean size of *P. adspersus* during the course of the experiment.



Figure 2. Zoea – I stage of *P. adspersus*.

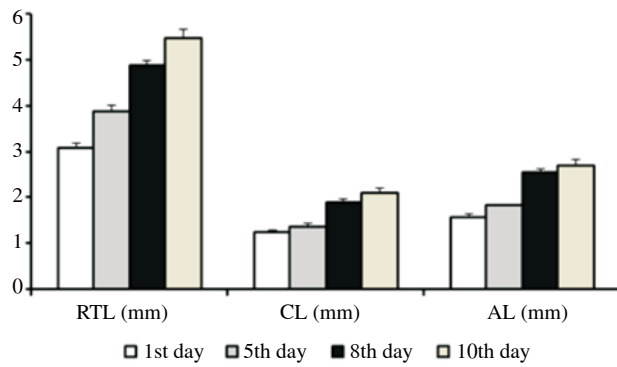


Figure 3. Size of *P. adspersus* during the course of the experiment. RTL: Total size with rostrum; CL: Carapace length; AL: Length of abdomen.

The 4 days static bioassay was used to determine the median lethal concentration (LC_{50}) and the value was 0.14 mg/L. Mean mortality was 0%, 30%, 47%, 55%, 65% and 95% in the concentrations of 0.02, 0.05, 0.1, 0.5, 1.0 and 5.0 mg/L, respectively.

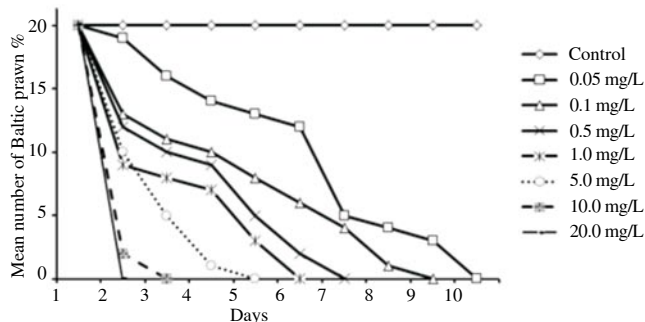


Figure 4. Mean numbers of *P. adspersus* surviving in seawater containing cadmium concentrations. Each concentration included three replicates.

There was no mortality in the control treatment after 10 days of exposure, demonstrating that the holding facilities, water, control sediment and handling techniques were acceptable. There were significant differences ($P < 0.05$) on the effect of concentration. Mortality increased in parallel with the increase in concentrations of Cd on Zoea – I stage of *P. adspersus* and time of exposure. The toxicity rate of the organism is concentrate-dependent. All organisms except the control group died at the end of 10 days (Figure 4). Less than 25% of the animals survived at the 5 days of the exposure to concentrations of 0.5 mg/kg Cd or more. Only 20% of the organisms survived at the 7 days of the exposure to concentrations of 0.1 mg/kg Cd in seawater with clean sediment or less.

4. Discussion

The primary criterion of a toxicity test is the survival after exposure to water with contaminated Cd concentrations and control[23,24]. Environmental conditions which affect the toxicity

of heavy metals include salinity, temperature, and dissolved oxygen. These factors may either influence the physiology of the organism especially larvae or may alter the form of the Cd in seawater. None of the control larvae died, indicating that the holding facilities and handling techniques were acceptable for conducting such bioassays, as required in the standard EPA/COE protocol where mean survival should be 90%[23]. Mortality of *P. adspersus* increased in parallel with the increasing Cd concentrations in the seawater.

Results obtained from the 4 and 10 days bioassays obviously demonstrated that Cd is highly toxic to larvae of *P. adspersus*. The most important effects of sub-lethal exposure of organisms to the metals include changes in growth, development, morphology and behaviour[4,5]. It was found in the present study that, when the larvae were exposed to concentrations of Cd, they become slightly excited and swam erratically, probably due to stress. However behavioural and swimming patterns in control groups were normal, and there was no mortality during the course of the experiment. These results agree with the toxicity studies conducted on other invertebrates[25-27]. Moreover, Bat *et al.*[17] showed that Cu was 4.25 times more toxic than Pb to *P. adspersus*. In this study, the toxicity of Cd for larvae of *P. adspersus* increased with increasing concentration and exposure time. The 4 day LC_{50} values of Cd for aquatic organisms differ from species to species and according to the type of heavy metals[13]. These results also agree with the finding by other authors[4,5,25-27]. Chandurvelan *et al.*[7] found that acute 96 h lethal concentration of 0.405 mg/L was derived for *Paratya curvirostris*, placing it among the most tolerant of fresh water shrimp species.

It is known that fish feed on these contaminated benthic invertebrates, thereby accumulating heavy metals. These toxic contaminants are then further accumulated by fish consuming people. Therefore, these toxicants should not be allowed to enter the water bodies of the coastal marine ecosystems.

Conflict of interest statement

We declare that we have no conflict of interest.

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