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Acute Toxicity of Cadmium on *Ophelia bicornis* Savigny, 1822

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Research Article

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Abstract

Static bioassays to assess acute toxicity of cadmium on *Ophelia bicornis* were first time conducted. Organisms were exposed to the different concentrations of cadmium for 24, 48, 72 and 96-hr. The LC_{50} values for 24, 48, 72 and 96-h with 95% confidence limits were estimated by the probit method and were found as 26 (22.8-29.4), 18 (15-22), 12 (9.1-14.4) and 7.2 (6.8-7.5) mg/l for Group 1 (15 and 25 mm in length) and 28 (24.6-33.8), 24 (22.4-25.5), 18 (16.1-19) and 8.7 (7.8-9.6) mg/l for Group 2 (26 and 36 mm in length), respectively. Toxicity of Cd was dependent on concentration and exposure time. LC_{50} increased with increasing the length of organisms. Mortality of *O. bicornis* was increased by Cd concentration and exposure. Results showed that *O. bicornis* was sensitive to cadmium.

Key words: Lethal concentration, acute toxicity, cadmium, *Ophelia bicornis*

Kadmiyumun *Ophelia bicornis* Savigny, 1822 Üzerindeki Akut Toksisitesi

Özet

Ophelia bicornis türünde kadmiyumun akut toksisitesini değerlendirmek için statik biyolojik deneyler ilk kez yapılmıştır. Organizmalar 24, 48, 72 ve 96 saat süreyle farklı Cd konsantrasyonlarına maruz bırakılmıştır. % 95 güven aralığına sahip LC_{50} değerleri 24, 48, 72 ve 96 saatleri için probit yöntemiyle tahmin edilmiş olup, sırasıyla Grup 1 (15 ve 25 mm boy) için 26 (22,8-29,4), 18 (15-22), 12 (9,1-14,4) ve 7,2 (6,8-7,5) ve Grup 2 (26 ve 36 mm boy) için 28 (24,6-33,8), 24 (22,4-25,5), 18 (16,1-19) ve 8,7 (7,8-9,6) mg/l olarak hesaplanmıştır. Kadmiyum toksisitesi hem konsantrasyon hem de maruz kalma süresine bağlı olarak değişmiştir. LC_{50} değerleri organizmaların uzunluğu arttıkça arttı. *O. bicornis* bireylerinde mortalite Cd konsantrasyonu ve maruz kalma süresiyle artmıştır. Sonuçlar *O. bicornis* türünün kadmiyuma duyarlı olduğunu göstermiştir.

Anahtar kelimeler: Letal konsantrasyon, akut toksisite, kadmiyum, *Ophelia bicornis*

INTRODUCTION

Marine pollution which derived from toxic metals has become a threat to the coastal biota and is one of the matters of concern. These toxic metals are mainly entered the coastal waters from industrial effluent, domestic and agricultural areas and many of these compounds are highly resistant. The eco-toxicological tests with aquatic organisms help to protect the natural environment and people health from contaminants such as heavy metals in the water. The need to protect biota in the water ecosystems from contaminants released into the coastal environment have risen up during last few decades. As a results usage of the many standard methods for determining the acute and chronic toxicity of contaminants including heavy metals has increased. The common methods for assessing the toxicity of contaminants in wastewaters, effluents, water bodies and sediment are bioassays. There are many types of organisms that can be used as bio-indicators to determine the toxicity of chemicals including heavy metals but commonly used of them is with crustaceans and polychaetes (Bat, 2005).

The Marine Strategy Framework Directive (MSFD) necessitates that the European Commission should make criteria and methodological standards to permit consistency in approach in assessing the degree to which Good Environmental Status (GES) is being maintained (Official Journal of the European Union, 2008/56/EC). The concentration of contaminants including heavy metals in the marine environment and their effects need to be assessed taking into account the impacts and threats to the ecosystem.

The polychaetes are commonly used organisms for sediment testing around European seas (Bat, 2005) including the Black Sea (Bat et al., 2014). Literature has recommended polychaetes in eco-toxicological studies (Bat and Raffaelli, 1998; Bat et al., 2001). *Ophelia bicornis* is known to prefer clean habitats in Sinop coasts of the Black Sea (Dagli et al., 2015) and is also sensitive to a broad range of toxic chemicals (Bat et al., 2016). No literature is available concerning the toxicity of heavy metal on *O. bicornis* from the Black Sea coasts.

The polychaetes *O. bicornis* is important links in detritus food chains. The selection of *O. bicornis* has several advantages including its small size, its relatively short life cycle and reproduction, easy to collection and hold in the laboratory conditions and its wide distribution in the habitat. *O. bicornis* has been used as fish bait (Dagli et al., 2005) and is used as bio-indicator species in heavy metal pollution monitoring study (Bat et al., 2016). Cd is one of the most toxic metals for most of the aquatic organisms and human life even at very low concentrations. With this study, *O. bicornis* was first time examined eco-toxicologically.

MATERIALS and METHODS

Test solutions

A stock solution of cadmium was prepared by dissolving of weighted $CdCl_2.2½H_2O$ in bi-distilled water and used for the course of this study. Different concentrations of the stock solution were prepared from low values (0 mg Cd/l as control) to high values (64 mg Cd/l) on toxicity test. Preliminary tests were carried out to establish suitable concentration ranges. The stock solution was diluted to the desired concentration before beginning the toxicity tests. Nominal concentrations of $CdCl_2.2½H_2O$ were 0 (control), 0.1, 0.2, 0.4, 0.8, 1, 2, 4, 8, 16, 32 and 64 mg/l and were prepared freshly. Test concentrations were made up by serial dilutions of the stock solution with natural sea water which had been previously filtered through a filter (Bat et al., 2018a).

Collection and analysis of sediment

The sediment was taken from the surroundings of *O. bicornis* and was washed through a 0.5 mm mesh sieve into a tank to remove any associated macrofauna and particles. They were then washed again through a mesh sieve 0.5 mm to ensure a standard particle size for the sediment in all experiments. The area is known to be clean and where supported a healthy population of *O. Bicornis* (Bat et al., 2016).

Total organic content, pH, redox potential and water content of sediment samples were determined. Sediment samples for total organic content were dried at 105°C for 24 h. Five grams of dried sample were then treated with hydrochloric acid vapour overnight in a desiccating jar to convert any calcium carbonates to chlorides. Samples were then placed in a muffle furnace at 600°C for 4 hours. The loss ignition was taken as the organic carbon content of the sediment (Buchanan, 1984).

For the pH analysis, 1: 2.5 percent pure water were added to the sediment samples taken in a certain amount and the samples were thoroughly mixed in the water. After 30 min sediments were expected to settle in water, pH was measured by pH meter. Oxidation-reduction (Eh) values were measured with Portable redox meter. In order to determine the water content, dry weight was weighed by drying for 2 days. After cooling, water amount % calculated from weight differences (Bat et al., 2018b).

Test organisms

The used organisms for test are *O. bicornis* (Figure 1) which is deposit feeders. It is broadly distributed in the sediments at Sinop shores of the Black Sea and sampling area was shown in Figure 2. Samples were collected from the area up to 2 m depth by snorkelling.

Figure 1. *Ophelia bicornis*

Figure 2. Sampling area.

The sampled organisms were acclimated for 7 days in 10-l container containing clean seawater and sediment. The seawater used for the experiment was pumped from the natural sea in the surroundings of *O. bicornis* through a filter to avoid water containing suspended particles and organisms smaller than 10 µm. The photoperiod is adjusted to 16-h light and 8-h dark. Acute toxicity tests were carried out in 1 litre jars in the laboratory following static bioassay method. The jars were covered with black plastic bags to prevent the light from coming from the sides except for above. Clean sediment of 5 cm thickness was placed at the bottom of each jar. Required quantity of the chemical was weighed and was added directly to test medium for preparing different concentrations. Then the solution was stirred with a glass rod carefully for uniformity. All the bioassays carried out during the study were done following the methods outlined in American Society for Testing and Materials (ASTM, 1990 and 1991) and U.S. Environmental Protection Agency and U.S. Army Corps of Engineers (U.S. EPA/COE Manual, 1991). During acclimation and experimental period, the animals were not fed.

Specimens were divided into two groups. Between 15 and 25 mm in length of individuals were formed as Group 1, whereas between 26 and 36 mm in length of them as Group 2. The organisms were exposed to the concentrations for 24, 48, 72 and 96-hr at 17℃±1. Ten worms were put each jar. The toxicity of the Cd was evaluated by estimation of the LC_{50} value. Mortality was recorded for daily observations. The dead worms were counted and removed immediately after death to avoid decomposition. Dissolved oxygen, pH and temperature were measured twice daily for water quality. Mortality data was used for calculating lethal concentration LC_{50}, 95% confidence interval using probit analysis (Finney, 1971) in SPSS software version 21.

The toxicity test was evaluated to be valid and acceptable if mean control mortality was lower than 10 percent and water quality parameters were maintained within acceptable limits (American Society for Testing and Materials (ASTM, 1990).

The bioassay was conducted with 3 replicates at each concentration and control. One-way analysis of variance (ANOVA) was carried out. If ANOVA results were significant, the control and treatment means or procedures were compared by Tukey test to determine which treatment(s) or procedure(s) differed from which (Zar, 1984).

RESULTS and DISCUSSIONS

The average total organic content, pH, redox potential, water content of the sediment at the start of the experiment were 0.81%±0.05, 7.92±0.09, 148 Eh and 24%±3, respectively. The average temperature over the experimental period in all tests was 17℃±1, dissolved oxygen was 7.4±0.5 mg/l, salinity was 17.2‰±0.3 and pH was 8.5±0.3.

There was no mortality in control organisms during the course of toxicity tests, indicating that the holding facilities, seawater, control sediment and handling techniques were acceptable for conducting such toxicity tests, as required in the standard U.S. EPA/COE protocol where mean mortality should be ≤10% (U.S. Environmental Protection Agency and U.S. Army Corps of Engineers, 1991).

LC_{50} values for 24, 48, 72 and 96 hours with 95% confidence limits were calculated by the probit methods and were found as 26 (22.8-29.4), 18 (15-22), 12 (9.1-14.4) and 7.2 (6.8-7.5) mg/l for Group 1 and 28 (24.6-33.8), 24 (22.4-25.5), 18 (16.1-19) and 8.7 (7.8-9.6) mg / l for Group 2, respectively (Figure 3). In all bioassays, the rate of *O. bicornis* mortality is increased corresponding to the high toxic concentrations and duration of exposure. No mortality was observed during cadmium exposure poisoning in the first twelve hours in both groups and the movement of the worms were normal. With time in higher Cd concentrations, the worms wrinkled, gradually became brittle and got decomposed. In lower Cd concentrations, mortality of the worms was late. Statistical analysis between mortality rate at different concentrations of Cd at 24, 48, 72 and 96 hours of exposures showed significant relationship ($p<0.05$). However, there was no significant differences ($p>0.05$) in mortality rates at 24-hr toxicity test.

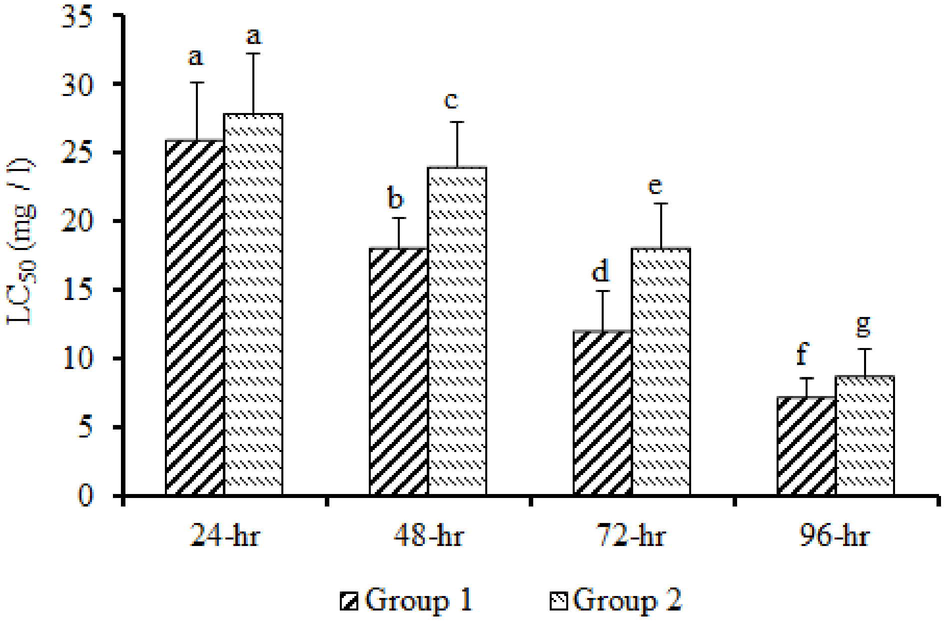

Figure 3. LC_{50} values with 95% confidence limits of $CdCl_2.2½H_2O$ in seawater with clean sediment to *O. bicornis* for two sizes at 24, 48, 72 and 96-hrs of exposure. a, b, c, d, e, f, g= The same letters beside the vertical bars in graph indicate the values are not significantly different ($p > 0.05$

The acute toxicity of cadmium to *O. bicornis* was dependent on both the concentrations and exposure times (Figure 4 and 5). Toxicity of Cd decreased with increase in the length of *O. bicornis*. On the other hand LC_{50} increased with increasing the length of organisms. It may be suggested that metabolic activity of *O. bicornis* played an important role in metal toxicity. Because metabolism of younger individuals is normally higher than that of older individuals thus, Cd toxicity is higher in younger individuals than the older ones. It means that, if Cd levels in water get higher with time it may be seen low metal toxicity owing to growth and lowered metabolic activity in older specimens. This trend indicates that smaller individuals of *O. bicornis* are more sensitive to Cd and hence have a greater toxic to them than larger species. The larger organisms may be developed a protective defence against the deleterious effects of Cd that produce degenerative changes like oxidative stress in the body. This tendency seems to specify that as smaller species in aquatic ecosystem, the sensitivity to heavy metals toxicity increases. In the study of Mendez and Green-Ruiz (2006) that supported this, the larvae of the polychaete *Capitella* sp. showed high sensitivity to Cd.

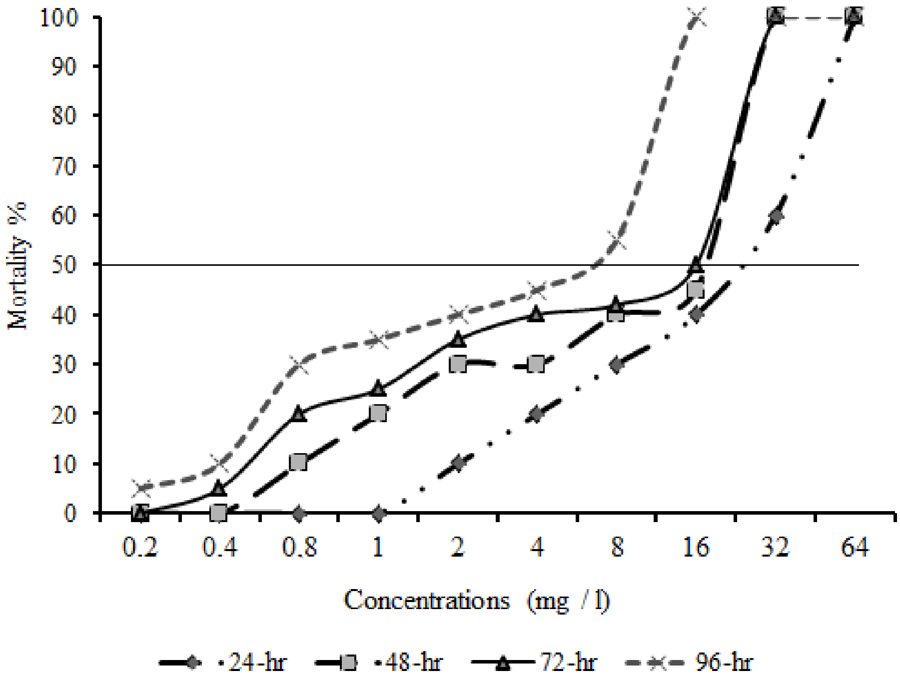

Figure 4. Relations between the percent mortality of *O. bicornis* for Group 1 (15-25 mm in length) at different concentrations and exposure times.

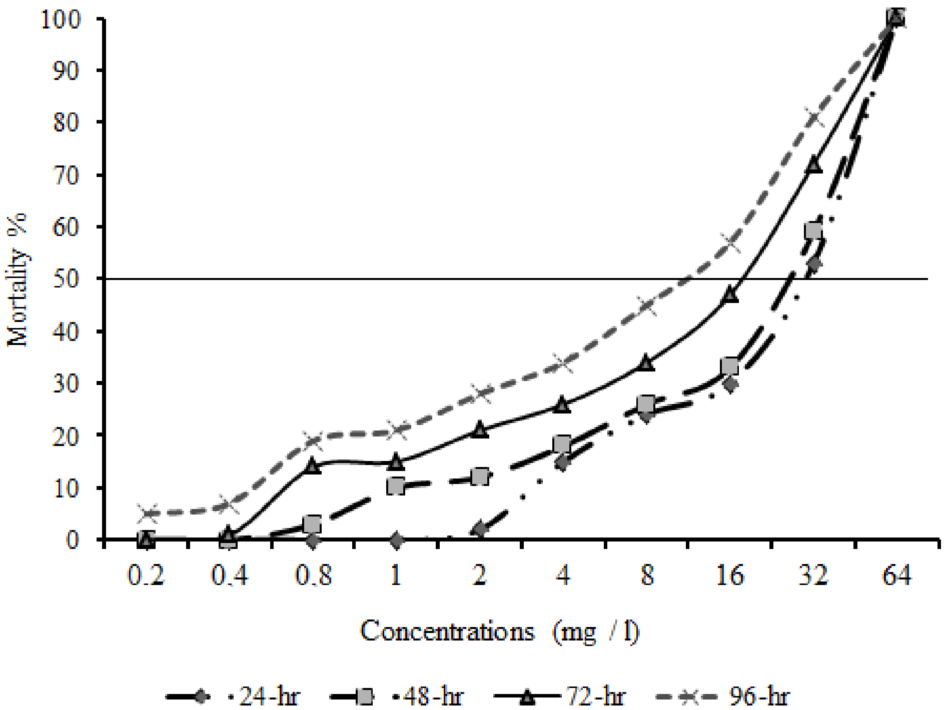

Figure 5. Relations between the percent mortality of *O. bicornis* for Group 2 (26- 36 mm in length) at different concentrations and exposure times.

These findings were in agreement with Bat et al. (2001) who study on the toxicity of zinc and lead in water with sediment was made to identify to tolerance limit of the hazardous substances to the polychaete worm *Hediste diversicolor*. It was found that small worms are more sensitive to Zn and Pb than bigger worms. Bat et al. (2017) showed that Cd was highly toxic to larvae of Baltic prawn *Palaemon adspersus*. Similarly, in another study of Reish et al. (1976) 96-hr LC_{50} of Cd was 7.5 ppm for adult polychaetes *Capitella capitata* and 0.22 ppm for trochophore larvae. Ray and McLeese (1983) also found Cd levels in polychaetes *Nereis virens* increased with increasing Cd levels in sediment. Smaller *N. virens* accumulated higher Cd levels (per unit wt.) and at a greater rate than larger worms. Bryan and Hummerstone (1973) found that Zn was regulated by the worm *Nereis diversicolor*, whereas Cd was not, in laboratory, rising concentrations in solution the rate of absorption of Cd rises more quickly than that of Zn. In the field, Zn levels in the worm *N. diversicolor* vary less than Cd and populations from high-Zn sediments are better at regulating Zn than normal populations and *N. diversicolor* more resistant to Zn than normal ones.

On contrary Bat et al. (2013) studied the acute toxic effects of copper, lead and zinc in seawater on the survival of the Mediterranean mussel *Mytilus galloprovincialis*. They found that the 96-h LC_{50} values for the medium and larger mussels were lower than that of the smaller individuals. It is concluded that smaller individuals were more tolerant to metals than bigger sizes. Further bioassays are needed to evaluate such trends for much smaller sizes or other small benthic organisms.

Comparison of polychaete LC_{50} results involving Cd exposures in water are given in Table 1.

Table 1. Comparison of polychaete LC_{50} results involving Cd exposures in water.

Species	Time (hr)	Cd-LC_{50} (ppm)	References
Capitella capitata	96	7.5 for adults; 0.22 for trochophore larvae	Reish et al., 1976
Capitella capitata	96	0.6-1	Reish et al. 1983
Neanthes arenaceodentata	96	12 for adults; 12.5 for juveniles	Reish et al., 1976
Neanthes vaali	168	6.4	Ahsanullah, 1976
Nereis virens	24,48,96	25, 25, 11	Eisler, 1971
Nereis virens	24,96,168	56, 9.3, 0.7	Eisler and Hennekey 1977
Laeonereis acuta	96	8.2349	Carricavur et al., 2018
Perinereis aibuhitensis	96	0.7934–13.5673	Wang et al., 2008
Ophrgotrocha labronica	48	4	Røed, 1980

The results of the present study in agreement with Reish et al. (1976), Eisler and Hennekey (1977), Wang et al. (2008) and Carricavur et al. (2018) who found similar LC_{50} values for 96-hr for the polychaetes *C. capitata, Nereis virens, Perinereis aibuhitensis* and *Laeonereis acuta*. In the present study 24 and 48-hrs LC_{50} values were also similar to *Nereis virens* (Eisler, 1971). On the other hand *Ophrgotrocha labronica* (Roed, 1980) was the most sensitive organisms when compared with *O. bicornis* and *N. virens* (see Table 1).

In general, the use of bioassays to straight measurement of toxicity of contaminants is a correct and reliable method to detect its effect. Here, the toxicity of Cd on *O. bicornis* by static method has been studied. The results provide baseline information in formulating strategy for controlled release of treated industrial effluents into the receiving water bodies. The estimated LC_{50} values for Cd with nominal concentrations for the *O. bicornis* show that Cd is toxic to this species. For application of toxicity data in control of wastewater discharges and estimate of environmental affects both acute and chronic toxic levels have to be determined to protect ecosystem.

CONCLUSION

The polychaete worm (*O. bicornis*) was used as the test organism for the static bioassay method. The worms were exposed up to 96-hr to Cd concentrations ranging from 6.6 to 8.1 mg/l in seawater with clean sediment. As heavy metals including cadmium binds to sediments and gradually becomes available to the bottom dwelling organisms, *O. bicornis* was selected as the test organism in the present study. *O. bicornis* is a primary consumer of the detritus-based food chain of marine coastal areas. It feeds on organic matter, particles of detritus, algae and other microorganisms, indicating that it plays a major role in the biomagnification of toxic heavy metals from bottom sediments to the higher trophic levels. In conclusion, *O. bicornis* is sensitive to cadmium than other many polychaetes (Bat, 2005).

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