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## Acute toxicity of zinc, copper and lead to three species of marine organisms from the Sinop Peninsula, Black Sea\*

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**Abstract:** Acute toxicity tests were performed on *Echinogammarus olivii* (Amphipoda), *Sphaeroma serratum* (Isopoda) and *Palaemon elegans* (Decapoda), from the Sinop Peninsula in the Black Sea. 96-h LC<sub>50</sub> values were estimated for copper, zinc and lead in these species using the static bioassay method. The LC<sub>50</sub> values of Cu for *E. olivii*, *S. serratum* and *P. elegans* were 0.25, 1.98 and 2.52 mg/l, respectively. The LC<sub>50</sub> values of Zn for *E. olivii*, *S. serratum* and *P. elegans* were 1.30, 6.12 and 12.3 mg/l, respectively. The LC<sub>50</sub> values of Pb for *E. olivii*, *S. serratum* and *P. elegans* were 0.62, 4.61 and 5.88 mg/l, respectively. The results indicated that Cu was more toxic to the species followed by Pb and Zn. *E. olivii* was more sensitive to the metals than *S. serratum* and *P. elegans*.

**Key Words:** Zinc, copper, lead, *Echinogammarus olivii*, *Sphaeroma serratum*, *Palaemon elegans*

### Çinko, Bakır ve Kurşunun Karadeniz'in Sinop Yarımadasında Yaşayan Üç Deniz Türüne Akut Toksisitesi

**Özet:** Karadeniz'in Sinop yarımadasında yaşayan AmFipod *Echinogammarus olivii*, İsoPod *Sphaeroma serratum* ve Dekapod *Palaemon elegans* akut toksisite deneylerinde kullanılmıştır. Bu türler kullanılarak 96 saatlik öldürücü konsantrasyon değerleri (LC<sub>50</sub>) statik biyolojik deneylerle çinko, bakır ve kurşun için tahmin edilmiştir. *E. olivii*, *S. serratum* and *P. elegans* türleri için bakır LC<sub>50</sub> değerleri sırasıyla 0.25, 1.98 ve 2.52 mg/l, çinko değerleri sırasıyla 1.30, 6.12 ve 12.3 mg/l, ve kurşun değerleri de sırasıyla 0.62, 4.61 ve 5.88 mg/l'dir. Bu türler için en toksik metal bakır olmuş ve bunu kurşun daha sonra da çinko izlemiştir. Bu sonuçlar *E. olivii*'nin bu metallere *S. serratum* ve *P. elegans*'lara göre daha duyarlı olduğunu göstermiştir.

**Anahtar Sözcükler:** Çinko, bakır, kurşun, *Echinogammarus olivii*, *Sphaeroma serratum*, *Palaemon elegans*

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## Introduction

Discharge of toxic pollutants into the marine environment presents a potential risk to the biota, unless the concentration of pollutants discharged are confined within certain limits. Toxicity tests carried out on representative components of the aquatic flora and fauna facilitate quantification of these limits.

Recent recommended water quality criteria for the protection of marine ecosystems have recognized that the proposed standards may be inadequate to protect some of the more sensitive invertebrates representing the major proportion of the diet of many species. The removal of components from the food web, due to pollution, may indirectly affect an ecosystem as a direct toxic effect.

Invertebrates are generally more sensitive to pollutants than either fish or algae. Among them, amphipods, isopods and decapods are important components of the marine intertidal and subtidal Fauna. Moreover, many marine invertebrates are cultured and used as food for young salmonids (1, 2). In spite of their abundance and importance, the use of these marine groups has been limited in marine toxicological research.

In conclusion, many schemes for the protection of the marine ecosystem give equal weighting to the results of toxicity tests with amphipods, isopods and decapods (3, 4). The aim of the present study was to investigate the effects of copper, zinc and lead on the survival of *Echinogammarus olivii*, *Sphaeroma serratum* and *Palaemon elegans*. These species were chosen for the study because they are abundant in the study area and are also a principle prey of many fish and larger invertebrates. They can also be transported easily and maintained in the laboratory. These characteristics make them ideal test species for toxicity bioassays.

## Material and Methods

Samples of amphipod, isopod and decapod crustaceans were selected primarily on the basis of the availability of large numbers of specimens which would survive under laboratory conditions.

The amphipod *Echinogammarus olivii*, the isopod *Sphaeroma serratum* and the decapod crustaceans *Palaemon elegans* were collected from the littoral zone of the Sinop peninsula, Black Sea, Turkey (Fig. 1). Animals were collected from a variety of habitats. *Echinogammarus olivii* and *Sphaeroma serratum* were collected by hand from the algae *Ulva lactuca* and *Enteromorpha* sp. (mainly decaying leaves) present in the eulittoral and infralittoral zone at the Yuvam station. *Palaemon elegans* were collected by hand with a net from the rocky intertidal environment at the Akliman station. The animals were transported to the laboratory in sea water from the collection site.

In the laboratory, the animals were separated and placed in sea water at 15°C for a minimum of 7 days prior to the start of the experiments. All experiments were carried out at 15°C±2°C, at 17 ppt salinity, under a 12:12 hour light: dark regime with continuous aeration.

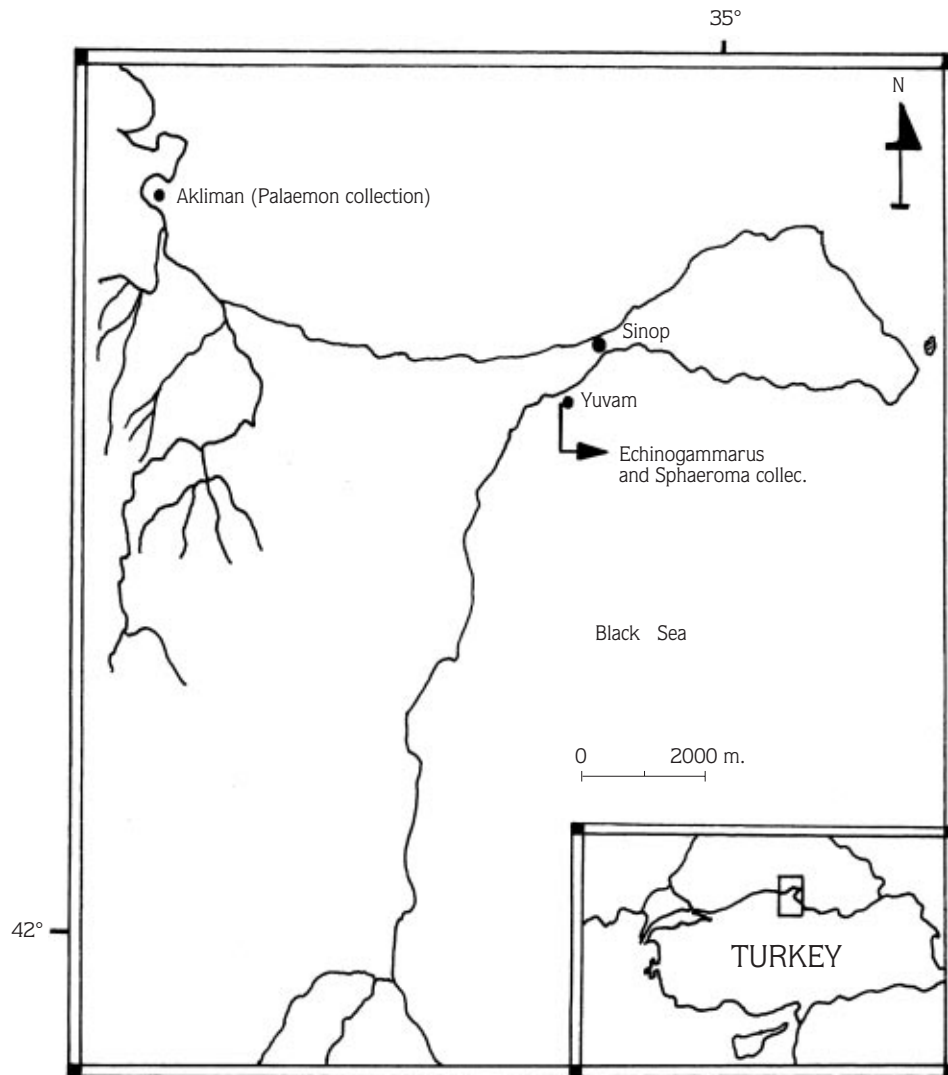


Figure 1. Map showing the location for collections of amphipod, isopod and decapod crustaceans.

In the tanks an excess of food was available in the form of the algae *Ulva lactuca* and organic detritus for the amphipods and isopods. During this period, the shrimps were fed every second day with dried *Gammarus* sp. (crude protein min. 32%, crude fat min. 4%, crude fibre max. 5%, moisture max. 10%, crude ash max. 12%). The food was a floating type and never clouded the water. After feeding all remaining food was removed.

Stock solutions of heavy metals were freshly prepared by dissolving the appropriate metal salts ( $ZnCl_2$  for Zn,  $CuSO_4$  for Cu,  $Pb(NO_3)_2$  for Pb) in deionized water in 1 litre glass volumetric flasks which had previously been washed in 10% nitric acid and well rinsed with deionized water. The stocks were acidified by adding a few drops of concentrated Analar nitric acid in order to reduce precipitation/adsorption of the metal ions (3, 5, 6). They were then stored in darkness at 4°C. None were prepared more than two days in advance of a test. Test concentrations were made up by serial dilutions of the stock solution with natural sea water which had been previously filtered. The animals were exposed to a range of concentrations of zinc, copper and lead from 0.001 to 20  $mg\ l^{-1}$ .

Static acute bioassays were conducted using five test concentrations plus a control series. Each series consisted of three replicates with 20 animals for *Echinogammarus olivii* and *Sphaeroma serratum* and with 5 animals for *Palaemon elegans*.

Each toxicity test lasted 4 days and observations for mortality were made twice daily. The criterion for determining death was the absence of movement when the animals were gently prodded for 1 min. Dead animals were removed at each observation. Tests were rejected when the control mortality exceeded 10%.

Water quality with respect to such parameters as pH, temperature, salinity and dissolved oxygen has also been shown to greatly affect toxicity (7, 8, 9). Thus, all these parameters were measured.

The mean temperature during the experimental period in all bioassays was  $15^\circ C \pm 1$ , dissolved oxygen was  $85\% \pm 6$ , salinity was  $17\% \pm 1$  and pH was  $8.10 \pm 0.20$ .

#### Data Analysis

A computerized probit analysis was carried out according to the methods of Finney (10). Mortalities recorded in the three beakers for each concentration were pooled. The percentage mortality at each concentration was corrected for any control mortality using Abbott's formula. Weighted regression lines of probit (transformed percentage mortality) against log-dose were obtained for each metal independently and from these the lethal concentrations ( $LC_{50}$ ) and fiducial limits (FL) were calculated.

#### Results and Discussion

The 96-h  $LC_{50}$  values of copper, zinc, lead to three species of crustaceans are shown in Table 1.

The mortality of all animals tested increased with increasing copper, zinc and lead concentrations in sea water. None of the control animals except *Sphaeroma serratum* died. However, the survival of *Sphaeroma serratum* was 93% in the controls, demonstrating that the holding facilities, water and handling techniques were acceptable for the toxicity test, as required in the standard EPA/COE protocol, where mean survival should be  $\geq 90\%$  (11, 12). Even in the control, many *Sphaeroma serratum* tried to get out of the water and some of the dead *Sphaeroma serratum* was found outside the beakers. Their habitat is crevices and under stones

Table 1. The 96-hour LC<sub>50</sub> values with 95% fiducial limits (FL) for amphipod, isopod and decapod crustaceans exposed to copper, zinc and lead.

Species	Cu LC <sub>50</sub> (95% FL)	Zn LC <sub>50</sub> (95% FL)	Pb LC <sub>50</sub> (95% FL)
<i>Echinogammarus olivii</i>	0.25 (0.21-0.28)	1.30 (1.00-1.57)	0.62 (0.58-0.67)
<i>Sphaeroma serratum</i>	1.98 (1.72-2.27)	6.12 (5.51-8.11)	4.61 (3.81-5.22)
<i>Palaemon elegans</i>	2.52 (2.18-2.91)	12.3 (8.94-14.8)	5.88 (5.50-7.90)

usually on the middle shore. Therefore, it may be concluded that there was a sublethal stress caused by the absence of stones or sediment. It seemed likely that some explanation applied to the other species survival results such as those reported by Holmström and Morgan (13 and 14); Lawrie, (15); Bat, (16).

The results indicate that among the three metals studied copper is the most toxic followed by lead and zinc. At 10 mg<sup>-1</sup> concentration of copper, activity of all species tested was abnormal and 100% of the animals died within 24 h.

The amphipod *Echinogammarus olivii* was the most sensitive species tested and the decapod *Palaemon elegans* was the most tolerant. Nugegoda and Rainbow (17 and 18) showed that *Palaemon elegans* regulates the body concentrations of zinc to a constant level when exposed to a wide range of dissolved zinc concentrations. Nugegoda and Rainbow (19) concluded that zinc uptake and regulation in decapods are affected by intrinsic adaptations of the species concerned and physico-chemical factors. The shrimp, *Palaemon elegans* is not consumed by humans but is a major prey organism of, and is used as bait for, commercially important edible fish. Concentrations of heavy metals in this shrimp could be amplified up through the food chain to man.

Although *Echinogammarus olivii* and *Sphaeroma serratum* were collected from the same habitat (see Fig. 1), their 96-h LC<sub>50</sub> values were different (Table 1). This indicates similar or different results can be measured with marine invertebrates living in the same niche.

LC<sub>50</sub> values for heavy metals for several invertebrates are also shown in Table 2. Some of the LC<sub>50</sub> values for zinc, copper and lead recorded for different invertebrates in other studies are similar to those recorded in the present study, whereas some LC<sub>50</sub> values for some metals are not. These differences may be attributed to different collection sites and periods, different species, different experimental salinities, different size beakers and different laboratory conditions.

The LC<sub>50</sub> values obtained from these toxicity studies provide data on the comparative effect of pollutants especially heavy metals and are useful in screening potentially toxic substances. Moreover, the present study with amphipod, isopod and decapod indicate the usefulness of these animals in toxicity testing. Their small size makes them convenient test organisms. Their widespread distribution, ease of collecting from the field, ease of handling in the laboratory makes it possible to use large numbers of specimens per replicate giving greater statistical validity to the results. They are also recommended as worthy of further research due to their ecological importance.

Table 2. Literature LC<sub>50</sub> values of heavy metals for some invertebrates.

Species	Metal	Time (h)	Tem. (°C)	Sal. (‰)	LC50 (mg/l)	Ref
<b>Decapoda</b>						
<i>Paratya tasmaniensis</i>	Cd	96	15	--	LC <sub>50</sub> was 0.06.	20
<i>Crangon semtemspinosa</i>	Cd	96	20	20	LC <sub>50</sub> was 0.32.	21
<i>Palaemoneter vulgaris</i>	Cd	96	20	20	LC <sub>50</sub> was 0.42.	21
<i>Pagurus longicarpus</i>	Cd	96	20	20	LC <sub>50</sub> was 0.32.	21
<i>Carcinus maenas</i>	Cd	96	20	20	LC <sub>50</sub> was 4.1.	21
<b>Isopoda</b>						
<i>Asellus aquaticus</i>	Cu, Pb, Zn	96			LC <sub>50</sub> s were 9.2, 64, and 18.2 in order listed.	3
<b>Amphipoda</b>						
<i>Crangonyx pseudogracilis</i>	Cd, Cu, Cr, Pb, Hg, Mo, Ni, Sn, Zn	48 72 96	13	--	48h LC <sub>50</sub> values were 34.6, 2.4, 2.2, 43.8, 0.47, 3618, 252, 72 and 121 in order listed; 96h LC <sub>50</sub> s were 1.7, 1.3, 0.42, 27.6, 0.001, 2623, 66 (72h), 50 and 19.8 in order listed.	3
<i>Allorchestes compressa</i>	Cd, Zn	96-120	16.8-20.5	34.5	120h Cd LC <sub>50</sub> =0.2-4; 96h Zn LC <sub>50</sub> =0.58, this amphipod was more sensitive than the crab, shrimp, mollusc and worm.	5
<i>Austrochiltonia subtenuis</i>	Cd	96	15±1		LC <sub>50</sub> was 0.04 ppm.	20
<i>Corophium insidiosum</i>	As, Cd, Cr, Cu, Pb, Hg, Zn	96	19±1		LC <sub>50</sub> s were 1.1, 0.68, 11, 0.6,>5, 0.02 and 1.9 in order listed.	22
<i>Elasmopus bampo</i>	As, Cd, Cr, Cu, Pb, Hg, Zn	96	19±1		LC <sub>50</sub> s were 2.75, 0.9, 3.4, 0.25,>10, 0.02, and 12.5 in order listed.	22
<i>Rhepoxynius abronius</i>	Cd	96	19.5	35	LC <sub>50</sub> was 0.24.	23
<i>Elasmopus bampo</i>	Cd	96	19.5	35	LC <sub>50</sub> was 0.57.	23



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