



Bioaccumulation of Copper and Zinc in Freshwater Crayfish (*Astacus leptodactylus*) Fed with Zebra Mussels (*Dreissena polymorpha*) Collected from Keban Dam Lake, Turkey

ONDER AKSU^{1*}, AZIME KUCUKGUL GULEC¹, VEYSEL DEMIR², DURALI DANABAS¹,
NUMAN YILDIRIM², MEHTAP OZCELIK³, ERKAN CAN¹ and MEHMET KOCABAS⁴

¹Fisheries Faculty, Tunceli University, 62000 Tunceli, Turkey

²Department of Environmental Engineering, Faculty of Engineering, Tunceli University, Tunceli, Turkey

³Vocational School of Health Sciences, Bingöl University, Bingöl, Turkey

⁴Department of Wildlife Ecology, Karadeniz Technical University, Trabzon, Turkey

*Corresponding author: Fax: +90 428 2131861; Tel: +90 428 2131794-1234; E-mail: onderaksu@tunceli.edu.tr

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This study is aimed to determine the bioaccumulation of copper and zinc in hemolymph of freshwater crayfish (*Astacus leptodactylus*) fed with zebra mussel (*Dreissena polymorpha*) collected from Keban Dam Lake. This is the first study on bioaccumulation of Cu and Zn in hemolymph of males and females of *A. leptodactylus*. The average concentrations of Cu and Zn in the hemolymph samples were found out as 0.54 and 35.07 ppm respectively for male and 0.42 and 15.59 ppm respectively for female control freshwater crayfish. The levels of Zn in the hemolymph of both male and female ones among control and diet groups were statistically significant ($P < 0.05$). The Cu levels among control and diet groups in female ones were statistically significant ($P < 0.05$) but not in the male ones ($P > 0.05$). The results of current study revealed that feed treatment with boiling was not effective at the levels of metal bioaccumulation in hemolymph. It was observed that this metal bioaccumulation is more related with sex differences.

Key Words: *Astacus leptodactylus*, Metals, *Dreissena polymorpha*, Bioaccumulation.

INTRODUCTION

The use of indicator species in aquatic ecosystems seems to be a suitable way of monitoring water quality due to the ability of some aquatic animals to accumulate metallic ions either directly from the surrounding water or indirectly through food sources¹. Since crayfish is solitary bottom dwellers which keep much of their bodies in contact with surrounding objects and tend to accumulate metals in their tissues, they can be used to monitor the aquatic environments for trace metal pollution^{2,3}.

Biologically essential trace metals such as zinc and copper are taken up by aquatic invertebrates both from food or/and water and they are also known as toxicants^{4,5}. Copper and zinc are essential trace elements for physiological functions of enzymes and beneficial to the health of all living⁶. Copper and zinc are also required for biological activities for some enzymes like SOD. In humans, copper deficiency may cause total activity loss and several sicknesses^{7,8}. In freshwater decapods, copper is a regulated metal due to biochemical role of this metal in the production of the respiratory haemocyanin^{9,10}. Decapod crustaceans typically maintain the tissue

and body concentrations of the essential metal zinc at approximately constant levels upon exposure to a wide range of ambient dissolved Zn concentrations^{10,11}.

Several studies have been performed to investigate Zn and Cu accumulation in body tissues and organs of different species of crayfish^{4,12}. Zinc and copper were bound by blood proteins metallothioneins and most of copper in decapods crustaceans was found in their blood and hepatopancreas⁹.

Crayfish utilize zebra mussels as a food source and may accumulate trace metals. Therefore, monitoring the possible heavy metal presence in crayfish edible tissues is important since crayfish can be part of the human diet¹³.

The objective of this study is to determine the bioaccumulation of copper and zinc in hemolymph of freshwater crayfish (*Astacus leptodactylus*) fed with zebra mussel (*Dreissena polymorpha*) collected from Keban Dam Lake.

EXPERIMENTAL

Freshwater crayfish (*A. leptodactylus*) from reference site and zebra mussels (*D. polymorpha*) from polluted site were collected at Keban Dam Lake in February 2011 (Fig. 1). Some properties and location of sampling sites were summarized in

Table-1. Carapace length and total weight of the crayfish were measured using a digital caliper in the laboratory. Crayfish were held in plastic tanks containing 40 L of water. The basic water parameters during the experiment were maintained at range of 10-15 mg/L dissolved oxygen and 14-16 °C temperature.

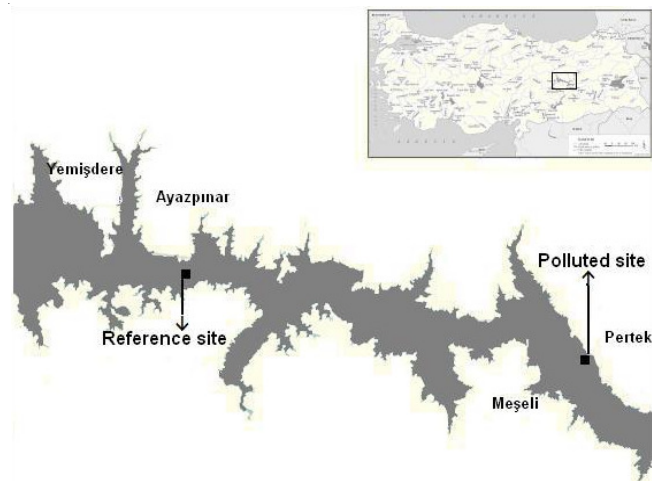


Fig. 1. Map of sampling sites on Keban Dam Lake

Total 30 adult crayfish with the average size of 46 mm carapace length and zebra mussels with the average size of 14 mm total length were used in the experiments. Crayfish were separated into three groups (10 individuals for each group). The content of trace metals in the hemolymph of the first group crayfish (control group) was measured after collected from reference site on Keban Dam Lake. The first experimental group of the crayfish (diet 1) was fed with boiled mussel while those in the second group (diet 2) were fed with not boiled mussel. Each diet was offered *ad libitum* to the crayfish for a period of 21 days.

TABLE-1
PROPERTIES AND LOCATIONS OF SAMPLING SITES

Sampling sites	Locations	Properties
References site	Ayazpınar province of Tunceli 38° 50' 24.27"N, 39° 17' 33. 69"E	Rural area, There is no municipal or industrial discharge point
Polluted site	Pertek province of Tunceli 38° 53' 20.33"N, 39° 02' 34. 10"E	Near the urban area and this site has a lot of municipal waste discharging points

Sampling of hemolymph: Samples of hemolymph were extracted with puncturing the arthroal membrane at the base of the most posterior walking leg with a disposable 1 mL syringe and a 24 × 0.5 mm sterile needle. The hemolymph was analyzed for total Zn and Cu concentrations according to AOAC 999.10 method, Atomic Absorption Spectrophotometry (Perkin Elmer AA- 800) after microwave digestion¹⁴.

Statistical analyses: Data were analyzed using the SPSS computer program (SPSS 15.0 for Windows, Chicago, Illinois, USA). Results are expressed as mean ± standard error (mean ± Se). Metal content of hemolymph was compared among the control and experimental groups using one-way ANOVA and Duncan's multiple range test. Independent t-test

was also used to analyze for association between metal content and sex differences.

RESULTS AND DISCUSSION

Bioaccumulations of Cu and Zn in hemolymph in fresh water crayfish, *A. leptodactylus* were given in Table-2. The average concentrations of Cu and Zn in the crayfish hemolymph samples were found as 0.54 and 35.07 ppm, respectively for male and 0.42 and 15.59 ppm, respectively for female control freshwater crayfish.

TABLE-2
MEANS ± SE, Cu AND Zn CONCENTRATIONS (ppm)
IN HEMOLYMPH OF FRESHWATER CRAYFISH,
FED WITH DIFFERENT DIETS

Parameters	Sex	Experimental groups		
		Control	D1	D2
Zn	Male	0.54 ± 0.04 ^b	0.31 ± 0.06 ^a	0.36 ± 0.04 ^{at}
	Female	0.42 ± 0.14 ^a	0.50 ± 0.09 ^{ab}	0.92 ± 0.13 ^b
	Mean levels	0.48 ± 0.07 ^a	0.40 ± 0.06 ^a	0.64 ± 0.09 ^a
	P values	0.424	0.110	0.002
Cu	Male	35.07 ± 2.64 ^{at}	25.30 ± 3.47 ^a	30.64 ± 3.67 ^a
	Female	15.59 ± 3.25 ^a	29.30 ± 1.11 ^b	23.60 ± 1.63 ^b
	Mean levels	25.33 ± 3.80 ^a	27.30 ± 1.84 ^a	27.12 ± 2.11 ^a
	P values	0.002	0.305	0.097

Different letters in the same line indicate significant differences among means of control and diet groups according to Duncan's multiple range test ($P \leq 0.05$). Independent T test indicates statistical differences between metal contents of male and female crayfish ($*P < 0.05$)

Levels of Cu and Zn among experimental groups were not statistically different ($P > 0.05$). But, considering the sexual status, there are statistically significant differences in metal levels in hemolymph among experimental groups ($P < 0.05$), except Cu levels in male of freshwater crayfish. The lowest Cu and Zn levels were measured in females of control group. On the other hands, in males the lowest Cu and Zn levels were measured in Diet 1. There were statistical differences in levels of Cu and Zn of both sexual status of freshwater crayfish among experimental groups ($P < 0.05$).

Heavy metal concentrations in aquatic ecosystems are usually monitored by measuring their concentrations in water, sediments and biota¹⁵, which generally exist in low levels in water and attain considerable concentration in sediments and biota¹⁶.

In a study on effects of dietary Cu on the Australian freshwater crayfish, *Cherax destructor*, Allinson *et al.*¹⁷ reported that high Cu concentrations were in gills, hemolymph and muscle. Similarly, certain levels of Cu were determined in hemolymph of *A. leptodactylus* in this study.

Conclusion

The results obtained in the present study, indicated that bioaccumulation of Cu in male freshwater crayfish (*A. leptodactylus*) was not significantly different among control and diet groups. In contrast, both Zn and Cu concentrations in hemolymph of female ones in diet groups were determined significantly higher than the control groups. Similarly Martín-Díaz *et al.*¹⁸ determined that metal accumulation differences

were found between male and female crayfish, *Procambarus clarkii*. Further studies are needed to investigate the molecular effects of sex and its supplements on bioaccumulation of metals.

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