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Freshwater crayfish *Astacus leptodactylus* (Eschscholtz, 1823) accumulates and depurates copper

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Abstract Cu accumulation and depuration in various tissues of the crayfish *Astacus leptodactylus* was investigated. Adult specimens were exposed to 0.5, 2.5 and 5.0 mg Cu/L under static conditions for three weeks. At the end of the 3rd week the specimens were divided into three groups and left in dechlorinated water for either 1, 2 or 3 weeks for depuration. After 7, 14 and 21 days, four crayfish from each group were instantaneously sacrificed. All crayfish were dissected into their hepatopancreas, gill, abdominal muscle and exoskeleton tissues for evaluation of Cu accumulation in each. The following accumulation pattern was obtained in decreasing order; hepatopancreas > gills > exoskeleton > abdominal muscles with values of 94.13, 84.86, 66.13 and 11.43 mg/L, respectively. The observed Cu depuration throughout the study was found to be time-dependent. Based on the present work we conclude that crayfish has a great potential for rapid accumulation and depuration of Cu in fresh water.

Keywords *Astacus leptodactylus* · Accumulation · Copper · Depuration

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Introduction

Cu is essential for several aquatic species such as *Cyprinus carpio* and *Oncorhynchus mykiss* (Eiseler 1998). Crustaceans accumulate some metals including Cu in their bodies directly proportional to the increase in bioavailability from water and food-chains (Rainbow 1997). Tissue levels of Cu can be regulated by decapod crustaceans at concentrations of dissolved metals below a threshold level (Bryan 1968; White and Rainbow 1982). However it may become toxic when present in high enough concentrations in the environment. It is among the most toxic heavy metals in freshwater biota and often accumulates and causes irreversible harm to some species, e.g. bay scallop (*Argopecten irradians*), lesser blue crab (*Callinectes similes*), zebra fish (*Brachydanio rerio*) at concentrations just above levels required for growth and reproduction (Eiseler 1998).

Convincing demonstrations have shown that in a polluted environment crayfish are able to accumulate a considerable amount of heavy metals in their organs and tissues (Anderson et al. 1997a, b; Allinson et al. 2000; Christopher et al. 2001; Mackeviciene 2002). The only reported investigation on the crayfish *A. leptodactylus* appears to be that of Bagatto and Alikhan (1987) in which an attempt was made to demonstrate only the accumulation of Cu and Cd in its body tissues. However, no attempt has yet been made to show depuration of Cu in *A. leptodactylus*.

Test subject, *A. leptodactylus* is naturally and widely distributed in lakes, ponds and rivers throughout Turkey. Today, there are 33 important *A. leptodactylus* harvesting areas throughout Turkey (Harlioğlu et al. 2004). The total harvest amount from these areas in 2002 was reported to be 1,850 tons (Anonymous 2002). Since they are widespread and consumed quite a lot, possible Cu accumulation by this species make it a potential health hazard for both humans and aquatic animals. Therefore, this study was undertaken to demonstrate the accumulation and depuration of Cu in *A. leptodactylus*.

Materials and methods

Adult *A. leptodactylus* specimens were obtained from DSI dam lake near Üsküp, Kırklareli city. They were transferred to glass aquaria (50×50×100 cm, 100 L) in the aquarium room and maintained at 20°C, 12:12 light:dark regime. This arrangement was left undisturbed for 7 days, during which animals became accustomed to room conditions. They were fed once on alternate days throughout the period of all experiments with a diet of trout pellets, carrot and potato. A total of 150 animals were used in the experiments. Injured animals were discarded and only active animals were included in the experiments. Crayfish were separated into four groups – 12 individuals for each group – and each group was exposed to a different concentration of Cu in replicate 100 L experimental glass aquaria. As a control, a group was kept in an aquarium filled with only filtered tap water. In order to avoid cannibalism, a number of short-lengthed polyvinylchloride pipes (5 cm diameter) were placed at the bottom of the aquaria. Prior to the experiments, aquaria were filled with the respective solutions of Cu for 48 h for initial adsorption of Cu onto inner tank surfaces. After 48 h, the solution was discarded and experiments begun with fresh Cu solutions.

Metal salt used for preparation of stock solution was $CuSO_4.7H_2O$ (Merck). Stock solutions of 1,000 mg/L. volumes were obtained by dissolving the Cu salt in tap water. The required volume of stock solution was added to the respective experimental aquaria to achieve the desired concentrations of 0.5, 2.5 and 5.0 mg/L. All aquaria were constantly aerated. Dead animals were removed immediately from the aquaria.

At end of the accumulation experiments' exposure period (after 7, 14 and 21 days of accumulation), four crayfish from each tank were sacrificed. All left animals at the end of the twenty-first day were left in filtered tap water for 1, 2 and 3 weeks to depurate. Water used was changed once in every 2 days during depuration period.

All crayfish were dissected into their hepatopancreas, gills, abdominal muscles and exoskeleton for evaluation of Cu accumulation. Before dissections, body weight and length of all animals were measured. All tissues were then weighed and digested by (1:1) concentrated nitric/perchloric acids (Merck) at 80–100°C until the return of yellow clear liquid.

Cu values in each tissue were determined by flame atomic absorption spectrophotometer (Unicom 929 AA). The standards used to make calibration curve were 1, 3, and 5 mg/L. The SNK test was used to compare Cu concentrations among replicates, Cu treatments and tissues. As metal accumulation in replicate aquaria was not significantly different from each other, samples were pooled and a two-way Anova analysis was performed followed by a SNK test as a post hoc test. Groups were considered to be significantly different from each other if $p<0.05$. All analyses were performed using an SPSS program.

Result and discussion

Water parameters during the experiment remained constant as follows; dissolved oxygen 14.59± 2.75 mg/L, temperature 20±1°C, pH 8, 10±0.46 and conductivity 780.68±13.35 μhos. Total lengths for all female and male specimens used for all experiments were measured as to be 110.843±17.411 and 111.820±9.129 mm, respectively. Total weights for each sex were 43.483±17.061 and 48.026±16.967 g, respectively.

Highest survival was recorded in the control group while two animals survived even in the highest Cu treatment of 5.0 mg/L. Among 12 animals used for each group, only one from each died from the groups of 2,5 and 5,0 mg/L at the end of the third week. The mean Cu concentration in body tissues (hepatopancreas, gill, abdominal muscle and exoskeleton) of *A. leptodactylus* after 7, 14 and 21 days exposure is shown in Table 1.

Cu was accumulated by different body tissues of *A. leptodactylus* during the 3 weeks exposure period. The value of Cu accumulation in hepatopancreas tissues was greater than the values in the gill tissues and exoskeleton. During the 3 weeks of exposure the maximum accumulation occurred in hepatopancreas (94.13 mg/L) which was followed by gills (84.86 mg/L), exoskeleton (66.13 mg/L) and abdomen (11.43 mg/L). Mean Cu concentrations in tissues of all exposure groups were significantly different from that of the control. Pattern of accumulation was hepatopancreas > gills > exoskeleton > abdomen. The same pattern was reported by Alikhan and Zia (1989) for *Cambarus bartoni*. Gill and exoskeleton of *C. bartoni* showed increasing copper concentrations with increasing levels in the water, though the hepatopancreas and muscle showed decreasing levels of copper. Alikhan and Zia indicated that digestive gut and muscle were not considered to be specific physiological sites for the storage of copper. White and Rainbow (1982) indicated that body of concentrations of copper were regulated until 100 μg/L Cu in 3 weeks by *Palaemon elegans* but above this concentration copper was accumulated and tissues concentrations were increased.

SNK post hoc test showed a significant difference between gill and hepatopancreas ($P<0.05$) and between gill and exoskeleton ($P=0{,}0258$).

The hepatopancreas is considered to be storage organ for detoxification and storage of heavy metals in this organ has been reported by Christopher et al. (2001). Hepatopancreas is involved in a variety of physiological processes which include the secretion of digestive juices, absorption and storage of digested food and detoxification and storage of heavy metals (Icery and Noot 1992).

Although dissolved copper is generally considered toxic for many aquatic organisms, this metal is an essential constituent of the crayfish respiratory pigments (Allinson et al. 2000). The gills are in direct contact with the environment and are involved in exchange of gases and they regulate ion fluxes. S. M. Naqvi et al. (1998) reported the highest amount of Cu accumulation in gills of marine crustaceans as to be 87.45 mg/L and concluded that this metal was readily absorbed by gills and transported to other organs via hemolymph. Copper interferes with the respiration and osmoregulation functions of gills in crayfish (Anderson et al. 1997a, b). In the rusty crayfish (*Orconectes rusticus*), toxicity of copper at high concentrations is due to the coagulatory action on cellular proteins and to interference with respiratory processes. Many authors have also demonstrated that absorption of Cd and Cu metals by gills was in high concentrations (see Anderson et al. 1997a, b). The gills of aquatic animals are in direct contact with water and play an important role in gas exchange and are therefore the point of entry of dissolved metal into the body of crayfish. In addition, the crayfish has an impermeable exoskeleton. Gills have been shown to

Table 1 Cu (mg/L) accumulation in crayfish (*A. leptodactylus*) exposed to 0.0, 0.5, 2.5 and 5.0 mg/L copper sulfate for 3 weeks (μg Cu/g w.w.)

Time	Tissues	0.0 $\overline{X} \pm S_{\overline{X}}$	0.5 $\overline{X} \pm S_{\overline{X}}$*	2.5 $\overline{X} \pm S_{\overline{X}}$*	5.0 $\overline{X} \pm S_{\overline{X}}$*
7. day	Muscle	N.D. **xa**	3.07±0.25 **xb**	6.22±0.31 **xc**	6.26±0.62 **xd**
	Exoskeleton	N.D. **xa**	11.39±0.28 **yb**	22.21±1.04 **yc**	25.69±0.63 **yc**
	Gill	N.D. **xa**	30.57±2.01 **zb**	57.06±1.37 **zc**	73.77±2.30 **zd**
	Hepatopancreas	1.44±0.07 **ya**	44.77±2.11 **tb**	71.77±3.08 **tc**	83.35±0.84 **td**
14. day	Muscle	N.D. **xa**	5.55±0.48 **xb**	5.78±0.32 **xc**	7.14±0.41 **xd**
	Exoskeleton	N.D. **xa**	12.90±1.23 **yb**	25.77±1.03 **yc**	52.85±3.07 **yd**
	Gill	N.D. **xa**	40.42±1.77 **zb**	61.58±0.78 **zc**	74.48±2.29 **zd**
	Hepatopancreas	1.86±0.34 **ya**	65.37±1.68 **tb**	76.97±1.13 **tc**	85.80±1.73 **td**
21. day	Muscle	N.D. **xa**	6.94±0.42 **xb**	9.21±0.29 **xc**	11.43±0.47 **xd**
	Exoskeleton	N.D. **xa**	15.55±1.13 **yb**	27.98±0.65 **yc**	66.13±0.59 **yd**
	Gill	N.D. **xa**	50.53±0.54 **zb**	67.17±1.53 **zc**	84.86±0.16 **zd**
	Hepatopancreas	1.23±0.01 **ya**	73.84±1.39 **tb**	84.45±1.20 **tc**	94.13±1.16 **td**

* a, b, c and d letters are used for concentrations; x, y, z and t letters are used for tissues ($P<0.01$), $\overline{X} \pm S_{\overline{X}}$: Mean ± Standard error, N.D.: Not Detectable, w.w.: wet weight.

Table 2 Depuration values at different times in *A. leptodactylus* exposed to 0.5 mg/L Cu concentration (μg Cu/g w.w.)

Tissues (μgCu/g)	Accumulation $\overline{X} \pm S_{\overline{X}}$*	7. day (D) $\overline{X} \pm S_{\overline{X}}$*	14. day (D) $\overline{X} \pm S_{\overline{X}}$*	21. day (D) $\overline{X} \pm S_{\overline{X}}$*
Muscle	15.55±1.13 **a**	13.36±0.98 **b** ↓	9.01±0.46 **b** ↓	7.04±0.69 **c** ↓
Exoskeleton	6.94±0.42 **a**	2.08±0.55 **b** ↓	4.44±0.64 **c** ↓	5.22±0.39 **d** ↓
Gill	50.53±0.54 **a**	54.82±0.88 **b** ↑	21.47±0.52 **c** ↓	10.11±0.30 **d** ↓
Hepatopancreas	73.84±1.39 **a**	54.13±4.47 **b** ↓	59.05±1.96 **c** ↓	49.54±0.55 **d** ↓

* a, b, c, d letters for different between depuration and accumulation ($P<0.01$), $\overline{X} \pm S_{\overline{X}}$: Mean ± Standard error, w.w.: wet weight. ↓,↑, increase or decrease according to accumulation.

be the first organ, which exhibit the symptoms of sublethal exposure of metals (Naqvi et al. 1998; Torreblanca et al. 1989).

The absence of Cu accumulation in white muscle is not surprising as many studies have shown that abdominal muscle accumulates low concentrations of most metals (Alikhan and Zia 1989; Anderson et al. 1997a, b; Naqvi et al. 1998). Whether this is a result of the absence of binding molecules for storage in abdominal muscle tissue remains to be elucidated.

Depuration of Cu was time-dependent until the end of the experimental period. Cu in exoskeleton depurated from 6.94 in the third week of accumulation to 5.22 mg/L at the end of 3rd week of depuration period (24.87% decrease), gills from 50.53 to 10.11 (80.0% decrease) muscle from 15.55 to 7.04 (54.7%) and hepatopancreas form 73.84 to 49.54 (32.9%). Although gill tissues showed a significant decrease in Cu accumulation, other tissues (hepatopancreas, exoskeleton, muscle) showed a significant increase during to first week for depuration time. SNK post hoc test showed a non-significant difference between the first and second week of depuration at muscle tissues ($P>0.05$) (see Table 2).

Based on earlier studies we conclude that crayfish have a great potential for rapid accumulation and depuration of Cu in fresh waters. If these animals from a contaminated area are consumed in large quantities they could cause adverse health consequences. We agree with Rainbow and White (1989) in that certain decapods (amphipods, barnacles and crayfish) are not suitable for long-term monitoring of heavy metal contamination due to their rapid depuration capabilities. Cu presence in exoskeleton of crayfish could have a survival value as a possible elimination mechanism through molting.

A. leptodactylus has the ability to take Cu from surrounding medium in its body tissues. Gill and hepatopancreas tissues could be used to detect the presence of Cu level in contaminated waters. But because of the rapid depuration of Cu, they should not be used as an organism for biomonitoring of Cu pollution in contaminated waters. Furthermore, the rapid depuration and lack of Cu accumulation in muscle tissues demonstrate that *A. leptodactylus* could be safe for human consumption provided that only the muscle is utilized.

Based on our present work we conclude that crayfish has a great potential for rapid accumulation and depuration of Cu in fresh waters.

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