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Cadmium Bioaccumulation and Depuration by Freshwater Crayfish, *Astacus leptodactylus*

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Abstract
The freshwater crayfish *Astacus leptodactylus* (Eschscholtz 1823) is naturally and widely distributed in the lakes, ponds and rivers throughout Turkey. They are omnivorous and thus are open to toxicant exposure from a variety of sources including the water column, adsorbed onto foodstuff, by bioaccumulation prey. Crayfish can serve as an excellent model species to increase the knowledge base for invertebrate ecotoxicology. Cadmium bioaccumulation and depuration in the various tissues of the crayfish *Astacus leptodactylus* were investigated. Adult specimens were exposed to 0.1, 0.5, and 1.0 mg Cd/L under static conditions for three weeks. At the end of the 3rd week, the specimens were divided into three groups and transferred into dechlorinated water for either 1, 2 or 3 weeks for depuration. After 7, 14, and 21 days, four crayfish from each group were sacrificed and were dissected into their hepatopancreas, gill, abdominal muscle and exoskeleton tissues for evaluation of Cd accumulation. The following accumulation pattern was obtained in decreasing order; hepatopancreas>gills>exoskeleton>abdominal muscles with values of 118.33, 661.63, 39.47, and 3.77 mg/L, respectively. Based on the present work we have concluded that crayfish have a great potential for rapid accumulation and depuration of Cd in fresh water.
Keywords: Accumulation, *Astacus leptodactylus*, cadmium, depuration.

Kerevitte (*Astacus leptodactylus)* Kadminyum Birikimi ve Eliminasyonu

Özet
Tatlı su kereviti *Astacus leptodactylus* (Eschscholtz 1823), Türkiye'nin birçok göl ve akarsuyunda doağl olarak yayılım gösterir. Omnivor olan kerevitler çeşitli besin kaynaklarında kullanabilirler ve buna bağlı olarak kirleticilerden etkilenirler. Kerevitler eko toksikolojide, omurgasızlar için mükemmel bir modeldir. Bu çalışmada kadmiyum farklı doku ve organlarında kadmiyum birikimi ve atılımı göstermiştir. Kerevitler statik deney koşullarında 3 hatfa boyunca 0,1, 0,5 ve 1,0 mg Cd/L maruz bırakılmış ve 7, 14, 21 günün sonunda hepatopankreas, solungaç, dış iskelet ve abdominal kaslarda kadmiyum birikimi belirlenmemiştir. Birikimin sırasıyla hepatopankreas>solungaç>dış iskelet>abdominal kaslarda 118,33, 661,63, 39,47 ve 3,77 mg/L olduğu bulunmuştur. Çalışmanın sonunda kerevitin kadmiyum hızla eliminine ettiği (atıldığı) sonucuna varılmıştır.
Anahtar Kelimeler: *Astacus leptodactylus,* atılım, birikim, kadmiyum.

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INTRODUCTION

Non-ferrous metal mines represent a major source of cadmium that is released into the aquatic environment (Rainbow 1993, 1997, 2007). Cd is a non-essential, extremely toxic trace element (Thorp et al. 1979, Thorp and Steven 1986, Dogan and Saygideger 2009) typically of low concentrations (i.e. parts per billion) in rivers, lakes and ponds. Cd is readily accumulated in the tissues of many aquatic organisms such as fish and crayfish (*Procambarus clarkii*) (Canli and Furness 1995, Anderson et al. 1997a). White and Rainbow (1982) investigated the accumulation of Cd in *Palaemon elegans* (Decapoda), *Echinogammarus pirloti* (Malacostraca) and *Eliminius modestus* (Ciripedia) in water with different concentrations of Cd (0.5 to 1000 g/L). The crustaceans accumulated non-essential Cd in all dissolved Cd concentrations without regulation (Wallace et al. 2003). Crayfish are not able to regulate Cd and therefore it is accumulated easily in their tissues and organs, and the amount of Cd increases with time (Thorp and Steven 1986).

Convincing laboratory experiments and field survey 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 about 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, although, no attempt has yet been made to show the

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depuration of Cd in *A. leptodactylus*.

A. leptodactylus is widely distributed naturally in the lakes, ponds and rivers throughout Turkey. A recent investigation (Harlioglu et al. 2004) reports 33 important *A. leptodactylus* harvesting areas throughout Turkey. The total harvest amount from these areas was reported to be 1850 tons in 2002 (Anonymous 2002). Large quantities of Turkish *A. leptodactylus* were harvested (approximately 7,000 tonnes annually) and exported to Europe before the crayfish plague (*Aphanomyces astaci*) was observed in these populations. The total harvest of *A. leptodactylus* in Turkey reduced dramatically to 320 tonnes in 1991 after the plague (Harlioglu and Harlioglu 2004).

Since they are widespread and consumed extensively, possible Cd accumulation by this species makes it a potential health hazard for both humans and other aquatic animals. Therefore, this study was undertaken to demonstrate the accumulation and depuration of Cd in *A. leptodactylus*.

MATERIALS AND METHODS

About 150 adult *A. leptodactylus* specimens at the intermolt stage were collected from the DSI (The General Directorate of State Hydraulic Works) Dam Lake (N 41° '44 E 27° '22) near Üsküp, Kırklareli Turkey. They were transferred to four glass aquaria (50x50x100 cm, 100 litres) and maintained at 20°C, with a light: dark regime of 12:12. This arrangement was left undisturbed for seven days, during which the animals became accustomed to the room conditions. They were fed once on alternate days throughout the period of all experiments with a diet of trout pellets, carrot and potato. Injured animals were discarded and only active animals were used in the experiments.

In the beginning, we conducted an experiment to determine the 96-h LC_{50} values for crayfish subjected to experimental conditions (Hamilton et al. 1977). Crayfish were separated into four groups (12 individuals for each group) and each group was exposed to a different concentration of Cd 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, the aquaria were filled with the respective solutions of Cd for 48 h for the initial absorption of Cd onto the inner tank surfaces. After 48 h, the solution was discarded and all experiments begun with fresh Cd solutions.

The water parameters during the experiment remained constant as dissolved oxygen 14.59±2.75 mg/L, temperature 20±1°C, pH 8.10±0.46, and conductivity 780.68±13.35 μhos. The total lengths for female and male specimens used for all experiments were measured at 110.843±17.411 mm and 111.820±9.129 mm respectively. The total weights for each sex were measured at 43.483±17.061 g and 48.026±16.967 g respectively.

The metal salt used for the preparation of the stock solution was $CdSO_4.7H_2O$ (Merck). The stock solutions of 1000 mg/L. were obtained by dissolving the Cd salt in tap water. The required volume of stock solution was added to the respective experimental aquaria to achieve the desired concentrations of 0.1, 0.5, and 1.0 mg/L. All aquaria were constantly aerated and dead animals were removed immediately from the aquaria during the experiment.

Four crayfish from each tank were sacrificed after 7, 14, and 21 days to assess the Cd accumulation. The animals remaining in the exposure tanks after 21 days were transferred to filtered tap water to assess the Cd depuration from the animals. The water was changed once on alternate days during this period, and animals were sampled after 7, 14, and 21 days.

All crayfish were dissected to obtain their hepatopancreas, gills, abdominal muscles and exoskeleton for evaluation of Cd accumulation. Before dissections, the body weight and length of all animals was measured. All tissues were then weighed and digested in concentrated nitric /perchloric acids (2 mL: 2 mL) (Merck) in a closed falcon tube at 80-100°C in an autoclave (approximately 3 h) until they turned to a yellow clear liquid.

The Cd values in each tissue were determined by flame AAS (Unicom 929 AA atomic absorption spectrophotometer). The standards used to make the calibration curve were 1, 3, and 5 mg\L. The SNK (Student-Newman-Keuls) test was used to compare Cd concentrations among replicates, Cd treatments and tissues. Since the metal accumulation in the replicate aquaria were not significantly different from each other, samples were pooled and a two-way ANOVA was performed followed by a SNK test. Groups were considered to be significantly different from each other if $p<0.05$. Statistical analyses was performed using an SPSS

(version 10.0) program.

RESULT and DISCUSSION

Raindow and White (1993) investigated the accumulation strategies of the decopod for the essential metal, copper and for the nonessential metal cadmium. The concentration of copper appeared to be the body of the decopod, but verification of regulation awaits the separate identification of incoming and already accumulated metal. The decopod are net accumulators of the nonessential metal cadmium (Bryan 1968). Indeed, regulation as a metal accumulation strategy does seem to be restricted to essential metals.

Cd is toxic to all crustaceans, although the LC_{50} values are usually much lower for freshwater versus marine benthic species (Dobson 1992, Canli and Furness 1995). Depending on the species, the LC_{50} values for marine benthic crustaceans varies from 0.32 mg/L to 16.7 mg/L but, are usually above 1 mg/L (Thorp and Steven 1986, Canli and Furness 1995). The toxic effect of Cd is dependent on the variety of environmental variables. Factors that reduce the free ionic concentration are water hardness and salinity (Dobson 1992). Crayfish seem relatively more tolerant to Cd in comparison with smaller crustaceans. This is evident in the LC_{50} values for *Daphia pulex* at 0.1 mg/L and *Paratya tasmanies* at 0.04 mg/L (Dobson 1992). Moreover, *A. leptodactylus* possessed much higher LC_{50} values (14.42 mg/L) than *Orconectes virilis* LC_{50} values (6.1 mg/L) (Miranda 1986).

No mortality was observed in the control group while only one died from each of the 0.5 and 1.0 mg/L groups at the end of the 3rd week (mortality 8.3%). The mean Cd concentration in the body tissues (hepatopancreas, gill, abdominal muscle and exoskeleton) of *A. leptodactylus* after 7, 14 ,and 21 days of exposure is shown in table 1.

Cd was accumulated by different body tissues of *A. leptodactylus* during the 3 weeks exposure period. Cd accumulation in the hepatopancreas tissues was greater than in the abdominal muscle, gill and exoskeleton tissues. During the 3 weeks of exposure, the maximum accumulation occurred in the hepatopancreas (118.33±0.88 mg/g), which was followed by the gills (61.63±1.08 mg/g), the exoskeleton (39.47±0.62 mg/g), and the abdomen (3.77±0.38 mg/g). The Cd concentrations in the control group were below the detection limit. The mean Cd concentrations in the tissues of all exposure groups were significantly different from that of the control group. The pattern of accumulation was hepatopancreas>gills>exoskeleton>abdomen muscle.

Cd in fish (*Orechromis aureus* and *Tilipia zilli*) was retained 5 weeks after the Cd exposure was stopped (Cogun at all. 2003). The highest concentration of the Cd was found in the kidney and liver, and the lowest concentration was in the muscle. Naqvi et al. (1998) found similar rapid depuration of As, Cd and Pb during the first 2 weeks of deputation in *Procambarus clarkii*.

The 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, in general, is involved in a variety of physiological processes which includes the secretion of digestive juices, absorption and storage of digested food and detoxification (Icely and Noot 1992). Studies carried out with various aquatic species have shown that the liver (hepatopancreas) is the prime organ for metal accumulation and also plays an important role for storage, redistribution, detoxification or transformation of metals (Dobson 1992, Christopher et al. 2001, Cogun et al. 2003).

Naqvi et al. (1998) reported the highest amount of Cd accumulation in the 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 the hemolymph. Many authors have also demonstrated that gills absorb Cd to high levels (Anderson et al. 1997). The gills of aquatic animals are in direct contact with water and therefore they acts as a gate for the entrance of dissolved metals into the body (Torreblanca et al. 1989, Naqvi et al. 1998).

The value of Cd accumulation in the exoskeleton was lower than the values found in the hepatopancreas and gill. We assume that this lower level of Cd might be a result of contamination from the other tissues during shell the dissection process. This is because the crayfish has an impermeable exoskeleton and ecdysiast is not frequent.

The absence of Cd accumulation in the white muscle is not surprising since many studies have shown that abdominal muscle accumulates low concentrations of most metals (Alikhan and Zia 1989, Anderson et al. 1997, Naqvi et al. 1998). Muscle tissue generally accumulates the lowest concentrations of Cd during many exposure times. For many fresh water fish species, muscle is not

considered to be a metal accumulator (Cogun et al. 2003). Whether or not this is a result of the absence of binding molecules for storage in abdominal muscle tissue remains to be elucidated.

In invertebrates, two major mechanisms of detoxification involving intracellular ligands have been well documented: metal-binding to cytosolic compounds including metallothioneins (or metallothionein-like proteins) and biomineralisation (Mason and Jenkins 1995, Marigomez et al. 2002). The accumulated cadmium is necessarily detoxified, typically as metallothionein. The metallothionein will be broken down in lysosomes (Langston et al. 1998), but cadmium is rarely visualised in lysosome residual bodies (type B granules) unlike copper or zinc. Recent studies indicate that cadmium may compete with zinc, an important cofactor in many enzymatic reactions, replacing it and effectively denaturing the enzyme (Torra et al. 1995). Acute exposure to cadmium results in several effects at the cellular level: the disruption of protein synthesis and the breakdown of mitochondrial metabolism (Behra 1993).

Depuration of Cd was time-dependent until the end of the experimental period. Cd in exoskeleton depurated from 6.94 mg/L at the start of the depuration period 5.22 mg/L at the end of the 3rd week of the 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 the hepatopancreas from 73.84 to 49.54 mg/L (32.9%). Alikhan and Zia (1989) reported the same pattern for *Cambarus bartoni*. Moreover, Naqvi et al. (1998) found similar rapid depuration of As, Cd, and Pb during the first 2 weeks of depuration in *Procambarus clarkii*.

Although the hepatopancreas and exoskeleton tissues showed a non-significant increase in Cd accumulation, other tissues (gill and muscle) showed a significant increase during the first week of the depuration time. The SNK post hoc test denotes significant difference between the first and second week of depuration in all tissues ($P>0.05$) (see table 2).

Table 1. Cd accumulation in crayfish (*A. leptodactylus*) exposed to 0.0, 0.1, 0.5 and 1.0 mg/L cadmium sulfate for 3 weeks (μg Cd/g w.w.).

Time		0.0	0.1	0.5	1.0
	Tissues	$\overline{X} \pm S\overline{x}$ ⋆	$\overline{X} \pm S\overline{x}$ ⋆	$\overline{X} \pm S\overline{x}$ ⋆	$\overline{X} \pm S\overline{x}$ ⋆
7. day	Abdominal muscle	n.d. xa	0.65 ± 0.12 xb	1.27 ± 0.16 xc	2.45 ± 0.12 xd
	Exoskeleton	n.d. xa	3.68 ± 0.17 yb	6.50 ± 0.92 yc	24.25 ± 0.69 yd
	Gill	n.d. xa	21.45 ± 0.58 zb	36.40 ± 0.69 zc	44.97 ± 1.43 zd
	Hepatopancreas	0.56 ± 0.25 ya	22.61 ± 0.52 zb	44.55 ± 1.17 tc	57.80 ± 0.91 td
14. day	Abdominal muscle	n.d. xa	1.06 ± 0.04 xb	2.41 ± 0.15 xc	3.44 ± 0.12 xd
	Exoskeleton	n.d. xa	5.68 ± 0.43 yb	8.83 ± 0.34 yc	34.80 ± 1.10 yd
	Gill	n.d. xa	25.22 ± 0.82 zb	45.34 ± 1.48 zc	56.93 ± 0.58 zd
	Hepatopancreas	0.44 ± 0.26 xa	27.75 ± 0.80 zb	55.29 ± 1.81 tc	62.03 ± 0.63 td
21. day	Abdominal muscle	n.d. xa	1.63 ± 0.17 xb	2.75 ± 0.15 xc	3.77 ± 0.38 xd
	Exoskeleton	n.d. xa	5.34 ± 0.13 yb	8.50 ± 0.41 yc	39.47 ± 0.62 yd
	Gill	n.d. xa	27.92 ± 0.94 zb	48.74 ± 0.82 zc	61.63 ± 1.08 zd
	Hepatopancreas	0.24 ± 0.04 ya	30.74 ± 0.68 tb	105.40 ± 1.56 tc	118.33± 0.88 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 0.1 mg/L Cd concentration (μg Cd/g w.w.).

Tissues (μg Cd/g w.w.)	Accumulation	7. day (D)	14. day (D)	21. day (D)
	$\overline{X} \pm S\overline{x}$ ⋆	$\overline{X} \pm S\overline{x}$ ⋆	$\overline{X} \pm S\overline{x}$ ⋆	$\overline{X} \pm S\overline{x}$ ⋆
Abdominal muscle	15.63 ± 0.60 a	5.82 ± 1.60 b	5.75 ± 0.19 c	1.30 ± 0.72 d
Exoskeleton	1.81 ± 0.16 a	1.17 ± 0.18 a	0.82 ± 0.04 b	n.d b
Gill	52.90 ± 0.89 a	26.81 ± 0.92 b	22.24 ± 0.50 c	19.74 ± 0.27 d
Hepatopancreas	56.56 ± 0.49 a	53.98 ± 3.79 a	41.64 ± 11.17 b	35.00 ± 0.40 c

⋆ a, b, c, d letters for different between depuration and accumulation ($P<0.01$), $\overline{X} \pm S\overline{X}$: Mean ± Standard error, n.d.: Not Detectable, w.w. : wet weight.

Table 3. Inland water Cd standards (mg/L).(USPHS: U.S. Public Healty Service Com. , SABS: EPA, Science Avdisory Board, NAS: National Academy of Science, FRG: Germany Socity of Human Ecology).

	USPHS 1962	Japan 1968	USSR 1970	WHO 1970	WHO 1971	SABS 1971	NAS 1972	Australia 1973	EPA 1975	FRG 1975
Cd	10	-	10	10	10	50	10	10	10	6

Consequently, our results are in agreement with the earlier studies (Alikhan and Zia 1989, Anderson et al. 1997, Naqvi et al. 1998) that crayfish have a great potential for rapid accumulation and depuration of Cd in fresh waters. We agree with Rainbow and White (1989) that certain decapods (amphipods, barnacles and crayfish) are not suitable for long-term monitoring of heavy metal contamination due to their rapid depuration capabilities. If these animals from a contaminated area are consumed in large quantities, they could cause adverse health consequences.

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