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Cadmium and Lead Uptake by Red Swamp Crayfish (*Procambarus clarkii*) of Louisiana

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Environmental contamination by heavy metals such as cadmium and lead is generally reflected by an increase in the tissue residues of aquatic animals. This is also true for fresh-water crayfish as reported by Bagatto and Khan (1987) for Orconectes virilis collected in the vicinity of Canadian smelters. They found a positive correlation between the residues of Cd and Pb in crayfish tissues and the distance from smelters. Stinson and Eaton (1983) reported similar findings for another crayfish, Pacifasticus leniusculus, collected from a lake on the West Coast of the U.S. which was receiving urban runoff. Cadmium and lead were more concentrated in the viscera and exoskeleton, respectively. Madigosky et al. (1991) found that Procambarus clarkii collected from roadside drainage ditches of Louisiana contained greater amounts of Cd and Pb than commercially harvested control groups. Lead accumulation has been attributed to the use of farm machinery runoff in agricultural areas (Ward et al. 1978) and Cd to rubber tires (Madigosky et al. 1991).

Cadmium and lead are known to accumulate even in those crayfish where no known contamination can be established, e.g., Dickson et al. (1980) found metal residues in troglobitic crayfish (O. australis australis and Cambarus tenebrosus). Accumulation of Pb and Cd in the tissues of laboratory-exposed crayfish of different species has been documented by several investigators (Chassard-Bouchaud 1980; Dickson et al. 1980; Vereille-Morel and Chaisemartin 1982; Diaz-Mayans et al. 1986; Mirenda 1986; Roldan and Shivers 1987; Pastor et al. 1988).

These metals accumulate in exoskeleton, hepatopancreas, gills, antennal glands, mid-gut glands and abdominal muscles of crayfish. Generally, all studies mentioned

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above report metal uptake by crayfish but have not quantitated the amount of metal remaining after the crayfish are transferred to uncontaminated water (depuration). The purpose of this study was to: (1) assess Cd and Pb accumulation in laboratory-exposed male and female P. clarkii (total body wet weight basis), and (2) to determine how much metal is lost when crayfish are transferred to uncontaminated aged tap water.

MATERIALS AND METHODS

To insure that crayfish did not contain heavy metals in their tissues, adult crayfish (8-10 cm) were obtained from a relatively controlled environment (Ben Hur Experiment Station, Louisiana State University, Baton Rouge). A total of 240 crayfish were acclimatized in the laboratory for 96 hr (water temperature 22±2°C, dissolved oxygen 5.5-6.5 ppm, pH 7-7.8 and total hardness 32 ppm). Sixty male and 60 female crayfish were either exposed to 0.5 mg/L $CdCl_2$ or 100 mg/L $Pb(NO_3)_2$ for up to 12 wk and the same numbers were maintained in aged tap water as controls. We successfully used aged tap water to achieve very little or no mortalities in controls (Naqvi and Newton 1990). Test solutions were prepared by diluting 1% aqueous stock solution of either metal to desired concentrations. The actual amount of Cd or Pb in test solutions was determined by analyzing a 250-ml sample every 2 wk when test solutions were replaced in the all-glass aquaria (100 x 50 x 20 cm). After 4, 8 and 12 wk exposure, 10 males and 10 females were removed from the test and control groups and promptly frozen for future analyses. After 12 wk exposure, the remaining crayfish were transferred to metal-free water. A similar schedule was followed to remove crayfish for the next 12 (depuration period). Crayfish were fed Purina Top Choice^{R} dog food which was also analyzed for the presence of Cd or Pb residues.

Each crayfish was digested in a 3:1 mixture of concentrated nitric and perchloric acids for 2 hr at 103°C. Each sample was filtered through a 12.5 cm Whatman filter paper into a 100-ml volumetric flask and deonized water was used to bring the level to 100 ml. Cadmium and Pb analyses were performed with a Perkin-Elmer (Model 3030) atomic absorption spectrophotometer. Since the amount of Pb and Cd was high enough in test solutions, a graphite furnace was not used.

The same procedure was used for analyzing crayfish food. However, test solutions were not digested in acids. They were evaporated to 20 ml prior to quantitation by AAS. Cadmium and Pb standards were purchased from Mallinckrodt and Fisher Scientific Co., respectively. The

Table 1. Mean Cd concentration (mg/L wet wt.) in the tissues of red swamp crayfish (Procambarus clarkii) exposed to 0.5 mg/L $CdCl_2$ for up to 12 wk (uptake) and then transferred to aged tap water (depuration)

Treatment	Male Crayfish (N=10)			Female Crayfish (N=10)		
	4 wks	8 wks	12 wks	4 wks	8 wks	12 wks
12-Week Exposure Period						
Control	0.09	0.08	0.21	0.08	0.06	0.16
S.D.	(1.05)	(1.05)	(8.32)	(1.51)	(2.05)	(7.38)
0.5 mg/L $CdCl_2$	3.09	3.76	8.39	3.06	3.28	5.50
	(0.23)	(0.18)	(0.91)	(0.16)	(0.18)	(1.06)
12-Week Depuration Period						
Control	0.27	0.33	0.31	0.30	0.22	0.26
S.D.	(0.03)	(0.10)	(0.13)	(0.21)	(2.50)	(0.11)
0.5 mg/L $CdCl_2$	3.90	2.55	3.12	2.61	2.54	2.08
	(0.49)	(0.41)	(0.74)	(0.58)	(0.55)	(0.19)

below detection level for Cd and Pb were 0.025 and 0.5 ppm, respectively. The recovery was between 99.5 to 99.9%.

The amount of Cd and Pb in control and treated crayfish was analyzed statistically using an IBM computer program for mean, standard deviation, sample variance and standard error. Analysis of variance was performed to see if there were statistically significant differences between control and treatment, male and female and exposure time (4, 8 and 12 wk).

RESULTS AND DISCUSSION

The actual concentration of Cd in 0.5 mg/L $CdCl_2$ solutions was 0.1 mg/L and in 100 mg/L $Pb(NO_3)_2$ solutions was 16.84 mg/L as determined by AAS technique. This indicates that only 1/5 of the Cd and 1/6 of the Pb were present in solution form to which crayfish were exposed. No Cd and Pb residues were detected in crayfish food. Table 1 shows the mean concentration of Cd in crayfish exposed to 0.1 mg/L Cd for up to 12-wk and also the depuration of this metal during the next 12 wk period. Cadmium residues are reported here on the whole-body wet weight of individual crayfish. The concentrations either of Cd or Pb were not determined in control crayfish prior to exposure.

The average concentration of Cd at the end of 12 wk in the control males was 0.21 and in females 0.16 mg/L while the treated crayfish accumulated 8.39 and 5.5 mg/L, respectively. The biomagnification factors (BF) for males and females were 84 and 55, respectively. The

Table 2. Mean Pb concentration (mg/L wet wt.) in the tissues of red swamp crayfish (Procambarus clarkii) exposed to 100 mg/L $Pb(NO_3)_2$ up to 12 wk (uptake) and then transferred to aged tap water (depuration)

Treatment	12-Week Exposure Period					
	Male crayfish (N=10)			Female crayfish (N=10)		
	4 wks	8 wks	12 wks	4 wks	8 wks	12 wks
Control	0.811	*	*	0.54	*	*
S.D.	(0.58)	-	-	(0.54)	-	-
100 mg/L $Pb(NO_3)_2$	2.04	25.34	47.30	1.80	23.22	46.33
	12-Week Depuration Period					
Control	*	*	1.44	*	*	0.61
	-	-	(0.53)	-	-	(0.48)
100 mg/L $Pb(NO_3)_2$	19.22 (9.63)	22.95 (14.23)	35.62 (8.90)	11.34 (7.33)	14.67 (8.97)	18.28 (12.58)

*Values below detection level

BF was calculated by dividing the average amount of Cd value below detection level in male or female crayfish with the actual amount present in the ambient water. This exhibits that P. clarkii accumulated a substantial amount of Cd in 12 wk. Similarly, Gillespie et al. (1977) exposed Orconectes propinquus propinquus to 1 mg/L Cd^{2+} for approximately 1 wk (190.5 hrs), resulting an accumulation of in 534.4 mg/L Cd.

The greater amount of Cd in male P. clarkii has also been reported in field-collected individuals from Guadelquivir marshes of Spain (Rincon-Leon et al. 1988). These investigators attributed this to larger chela in males. The accumulation of Cd in crayfish could also be due to the presence of cadmium-binding proteins in the mid-gut of P. clarkii and Austropotamobius pallipes (Lyon 1984; Del Ramo et al. 1989).

Cadmium was lost during the depuration period. However, at the end of the 12-wk period both males and females still had 36 and 38% of Cd remaining in their tissues, respectively (8.39 vs. 3.12 mg/L Cd in males and 5.50 vs. 2.08 mg/L Cd in females). This indicates that Cd does not rapidly depurate from crayfish tissues.

Table 2 shows the average concentrations of Pb in male and female P. clarkii during exposure and depuration periods. There was a time-dependent increase in Pb concentration in male and female crayfish. We did not find detectable levels of Pb in control crayfish after either 4 or 8 wk during the uptake period. However, a

Table 3. Analysis of variance of cadmium and lead uptake by red swamp crayfish (*Procambarus clarkii*) exposed to 0.5 mg/L $CDCl_2$ and 100 mg/L $Pb(NO_3)_2$)

SOURCE	Metal	DF	SS	F Value	PR/F	F level
Treatment	Cd	1	537.67	1117.58	0.0001	**
Sex	"	1	8.48	17.63	0.0001	**
Weeks	"	2	87.01	90.43	0.0001	**
Treatment	Pb	1	1963.89	158.29	0.0001	**
Sex	"	1	561.89	4.05	0.0470	*
Weeks	"	2	2921.42	58.35	0.0001	**

**P<0.01 *P<0.05

detectable level of Pb was present in both male and female crayfish at the end of the 12-wk depuration period (1.44 and 0.61 mg/L, respectively).

The actual amount of Pb in the 100 mg/L solution was 16.84 mg/L. By the end of the 12-wk exposure period, both male and female crayfish accumulated average concentrations of 47.30 and 46.33 mg/L Pb. Aftr 12- wk depuration the concentraitons decreased to 35.62 and 18.28 mg/L. Therefore, 75.3% of the Pb in males and 39.3% in females still remained after 12-wk of depuration.

The BF for Pb-exposed males and females were 2.8 and 2.7, respectively. In comparison to Cd these figures were 30 and 20 times lesser in males and females, respectively. This further confirms that Cd has a greater propensity for tissue uptake than Pb, perhaps due to a rapid binding with proteins. We noticed that the amount of Pb during depuration kept increasing, which is perplexing. This perhaps could be from the aged tap water itself, although, it did not seem to affect the control crayfish.

The analysis of variance for Cd and Pb uptake is given in Table 3. The difference between the amount of Cd and Pb accumulation by control and treated crayfish during the uptake period (12 wk) was highly significant ($P<0.01$). There was a highly significant difference between the Cd-treated males and females. Also, the difference in Cd and Pb uptake between sampling periods (4, 8 and 12 wk) was also highly significant, indicating that uptake was time-dependent.

We concur with Vermeer (1972), Stinson et al. (1983), and Bagatto and Khan (1987) that crayfish can be used for monitoring of heavy metal contamination in aquatic ecosystems, due to their ability to rapidly accumulate them and retain them in their tissues for a long period of time.

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REFERENCES

Bagatto G, Khan A (1987) Copper, cadmium and nickel accumulation in crayfish populations near copper-nickel smelters at Sudbury, Ontario, Canada. Bull Environ Contam Toxicol 38: 540-545

Chassard-Bouchaud C (1980) Effects of temperature on cadmium bioaccumulation by the crayfish Pontastacus leptodactylus: Microprobe and secondary ion micro-analysis. CR Seances Acad Sci Nat 290: 347-350

Del Ramo J, Pastor A, Torreblanca A, Medina J, Diaz-Mayans J (1989) Cadmium-binding proteins in mud-gut-gland of freshwater crayfish, Procambarus clarkii. Bull Environ Contam Toxicol 42: 241-246

Diaz-Mayans, J, Hernandez F, Medina J, Del Ramo J, Torreblance A (1986) Cadmium accumulation in the cray-fish, Procambarus clarkii using graphite-furnace atomic absorption spectroscopy. Bull Environ Contam Toxicol 37: 722-729

Dickson GW, Briese LA, Giesy JP Jr (1980) Tissue metal concentrations in two crayfish species cohabiting a Tennessee, USA, cave stream. Oecologia (Berl) 44: 8-12

Gillespie R, Reisine T, Massaro EJ (1977) Cadmium uptake by the crayfish Orconectes propinquus propinquus (Girard). Environ Res 13: 364-368

Lyon R (1984) Metal-binding patterns in the hepato-pancreas of the crayfish, Austropotamobius pallipes during short-term cadmium stress. Comp Biochem Biophys 78C: 415-418

Madigosky SR, Xavier AH, Glass J (1991) Lead, cadmium and aluminum in the red swamp crayfish Procambarus clarkii collected from roadside drainage ditches in Louisiana. Arch Environ Contam Toxicol 20: 253-258

Mirenda RJ (1986) Toxicity and accumulation of cadmium in the crayfish Orcontes virilis (Hagen). Arch Environ Contam Toxicol 15: 401-407

Naqvi SM, Newton DJ (1990) Bioaccumulation of endosulfan (Thiodan^{R}) insecticide in the tissues of Louisiana crayfish, Procambarus clarkii. J Env Sci Health (B) 25: 511-526

Pastor A, Medina J, Del Ramo J, Torreblanca A, Diaz-Mayans J, Hernandez F (1988) Determination of lead-treated crayfish, Procambarus clarkii: Accumulation in different tissues. Bull Environ Contam Toxicol 41: 412-418

Rincon-Leon F, Zurera G, Pozo-Lora R (1988) Lead and cadmium concentration in red swamp crayfish, Procambarus clarkii in the Guadalquivir marshes

(Spain). Arch Environ Contam Toxicol 17: 251-256
Roldan BM, Shivers RR (1987) The uptake and storage of iron and lead in cells of the crayfish, Orconectes propinquus hepatopancreas and antennal gland. Comp Biochem Physiol C 86: 201-214
Stinson MS, Eaton DL (1983) Concentration of lead, cadmium, mercury and copper in the crayfish, Pacifasticus leniusculus obtained from a lake receiving urban runoff. Arch Environ Contam Toxciol 12:693-699
Vereille-Morel C, Chaisemartin C (1982) Natural tolerance and acclimation of different populations of Austropotamobius pallipes to heavy metals (chromium and lead). Acta Oecol (Ser) Oecol Appl 3: 105-122
Vermeer K (1972) The crayfish, Orconectes virilis, as an indicator of mercury contamination. Can Field Nat 86: 123-125
Ward NI, Brooks RR, Roberts E (1978) Lead levels in sheep organs relating from pollution from automotive exhausts. Environ Pollut 17: 7-12

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