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Cadmium Accumulation in the Crayfish, *Procambarus clarkii*, Using Graphite Furnace Atomic Absorption Spectroscopy

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Cadmium is a ubiquitous, non-essential element which possesses high toxicity to both humans (Haguenoer and Furon 1981) and aquatic organisms (Lalande and Pinel-Alloul 1984; Lake et al. 1979).

In recent years, cadmium and cadmium compounds have been used extensively by various industries, and this has produced sharp increases in contamination of air, water and soil. Cadmium has been described as a perfect example of a trace metal which is very widespread in the biosphere, which is accumulated by plants and animals, and which induces acutely and chronically deleterious effects in organisms (Schroeder 1974).

Lake Albufera and the surrounding rice-field waters are being subject to very heavy loads of sewage and toxic industrial residues (including heavy metals and pesticides) from the many urban and wastewaters in this area (Dafauce 1975; Roselló 1983).

The American red crayfish Procambarus clarkii is native to the Louisiana marshes (USA). In 1978, the crayfish appeared in Lake Albufera near Valencia (Spain), and presently, without adequate sanitary controls, the crayfish is being fished commercially for human consumption.

In a recent paper, Laxen (1984) comments that the European Economic Community in 1975 included cadmium on its "black list" of substances requiring priority attention. The US Environmental Protection Agency has recently proposed water quality criteria as low as 0.5 ng/L (24 h average) and 600 ng/L (not to be exceeded) for soft waters (hardness < 400 μequiv/L), with concentrations expressed in terms of total metal (Laxen 1984).

In view of this interest, it is important to have accurate information on concentrations of cadmium in natural waters and cadmium levels of tissues of freshwaters animals used as human food, as well as the accumulation rates of this metal in this animal.

In the present study, we investigated the accumulation of cadmium in several tissues of the red crayfish, P. clarkii (Girard) from Lake Albufera following cadmium exposure. Determinations of cadmium were made by flameless atomic absorption spectroscopy and the standard additions method. Digestion of samples was made by wet ashing in open flasks with concentrated HNO_3, at 80-90 °C.

MATERIALS AND METHODS

Adult intermolt specimens (males and females) of the crayfish P. clarkii were collected in March 1985 from Lake Albufera (Valencia, Spain) and carried immediately to the laboratory where they were transferred into 300-L aquaria. They were maintained for 15 days at 20 °C and were fed a daily diet of pork liver.

Then, forty crayfish ranging in weight from 15.3 to 28.5 g were divided into four groups of 10 animals each. These were kept in 15-L experimental aquaria containing tap water. The cadmium stock for all experiments was reagent grade $CdCl_2 \cdot H_2O$ (E. Merck); a stock solution of 1 mg Cd^{++} per milliliter of water was prepared. Aliquots of this stock were added to each test aquaria to bring the Cd concentrations to the desired levels of 3.2, 10, 32, and 100 µg/L. All aquaria were kept at a constant temperature (20 °C) and on a 12 h light-dark photoperiod for the 4-day duration of the experiment. The water was changed every day to reduce the buildup of metabolic wastes and to keep the concentration of Cd near the nominal level. Ten more crayfish served as a control and were kept in 15-L of clean water.

After 96 h of Cd-exposure at 20 °C, the animals were transferred to clean water, free of any contamination, and kept there for an additional 5 h.

The gills, midgut gland, antennal glands and whole abdominal muscle of the control and treated crayfish were dissected using plastic materials, washed with HNO_3, and rinsed with distilled and deionized water, in order to avoid metal contamination and remove any surface contamination from the treated water.

Absorbance measurements were made on a Perkin-Elmer model 2380 atomic absorption spectrophotometer equipped with a model 561 recorder, a deuterium background corrector, and an HGA 400 Heated Graphite Atomizer.

A hot plate (Selecta model 157) with temperature control was used for digesting the samples by wet ashing.

All reagents used in the analyses were of analytical grade (Merck), and distilled and deionized water was used to prepare solutions.

A Cd(II) stock solution of 1000 µg/mL was prepared by dissolving 1000 g of cadmium metal in a minimum volume of HCl 1+1, and diluting to 1 L with 1% (v/v) HCl. The working standard solutions were prepared daily by appropriate dilution with 4+10 HNO_3.

Prior to analyses, the different tissues were lyophilized and homogenized. Digestion was performed by wet ashing to avoid possible losses of cadmium, which can occur during dry ashing (Sperling 1975). Concentrated HNO_3 was chosen because it has been successfully used for the digestion of biological samples and analyses of trace metals (Krinitz et al. 1974; Bernhard 1976; May 1982; Capelli et al. 1982).

The digestion procedure was as follows: 0.005-1.5 g of lyophilized tissue was

introduced into a 100-mL Erlenmeyer flask and 10 mL of conc. HNO_3 was added.

The samples were digested on a hotplate at a temperature of 80-90 °C during approximately 14 h. After cooling, solutions were quantitatively transferred to a 25-mL beaker and diluted with water to the mark. In all experiments several blanks were processed to ensure that contamination was not occurring.

Determination of cadmium was performed at 228.8 nm with drying, charring and atomization temperatures of 120, 250 and 1100 °C, respectively, using argon as the purging gas. A final cleaning step at 2700 °C was also used.

RESULTS AND DISCUSSION

Cadmium levels in the gills, midgut gland, antennal glands and muscle of the control and the crayfish exposed for 96 h to 3.2, 10, 32 and 100 µg of Cd(II) /L are presented in tables 1, 2, 3, and 4, respectively.

Table 1. Cadmium levels (µg/g dry weight) in gills of crayfish after 96 h Cd-exposure at several water concentrations.

µg Cd(II)/L of water				
0	3.2	10	32	100
1.93*	1.83	4.56	8.71	44.80
1.33*	1.26*	4.44	8.94*	31.18
1.41	1.14	3.43*	19.18	44.04
0.63	1.59*	2.81	6.20	27.85
1.16	1.10	2.57	20.16	41.25
1.22	2.00	4.75*	10.12	27.57
1.00*	2.13*	3.87	18.73	42.25
--	--	5.46	9.74	29.57
--	--	--	13.08	26.25
--	--	--	--	31.29
1.24 ± 0.40	1.58 ± 0.42	3.98 ± 1.00	12.76 ± 5.26	37.35 ± 10.56
F = 87.51; df = 4,36; $p < 0.01$		Linear regression: $r^2 = 0.90$; $y = 1.04 + 0.33\ x$		

Unless otherwise stated, each value corresponds to one sample.
(*) Pooled sample of gills from two animals.

The large number of samples would make the procedure of digestion in Teflon reactors under pressure very tedious. Therefore, we preferred to use open flasks, which allows us to work comfortably with a large number of samples. Using this procedure of digestion, we have proven that using the method of standard additions no losses of cadmium occurred; the recoveries of three standards of Cd(II) being obtained: 4 ng/mL, 105.2% ; 20 ng/mL, 95.5% ; 40 ng/mL, 95.8%.

Table 2. Cadmium levels (μg/g dry weight) in midgut gland of crayfish after 96 h Cd-exposure at several water concentrations.

μg Cd(II)/L of water				
0	3.2	10	32	100
0.65[x]	0.22[x]	0.15	0.45[x]	5.30
0.69	0.42[x]	0.40	0.33	0.91
0.44[x]	0.32	0.59[x]	0.39	4.86
0.37[x]	0.35[x]	0.33[x]	1.37[x]	0.79
0.44	0.63[x]	0.68	0.40	1.29[x]
0.42[x]	0.54	0.75	0.43	0.82
--	--	0.55[x]	1.38	3.25
--	--	--	1.05	2.63
--	--	--	--	1.83
0.50 ± 0.13	0.41 ± 0.15	0.49 ± 0.21	0.72 ± 0.46	2.41 ± 1.74
F = 7.08; df = 4,31; $p < 0.01$		Exponential fit: $r^2 = 0.56$; $y = 0.40\ e^{0.01\ x}$		

Unless otherwise stated, each value corresponds to one sample.
([x]) Pooled sample of midgut gland from two animals.

Table 3. Cadmium levels (μg/g dry weight) in antennal glands of crayfish after 96 h Cd-exposure at several water concentrations.

μg Cd(II)/L of water				
0	3.2	10	32	100
2.31[xx]	5.68[xx]	1.96[xx]	3.72[x]	4.07
3.00[xx]	1.57[xx]	0.87	1.41[xx]	4.48[x]
4.23[xx]	0.67	0.75	1.23[x]	5.88[x]
2.78	3.10[xx]	1.43[xx]	1.95	7.45
--	--	1.63[x]	1.18	2.91
--	--	--	2.13	13.37
--	--	--	--	1.59
--	--	--	--	1.67
3.08 ± 0.82	2.75 ± 2.19	1.33 ± 0.51	1.94 ± 0.95	5.17 ± 3.87
F = 2.55; df = 4,22; $p > 0.05$		Not significant.		

Unless otherwise stated, each value corresponds to one sample.
([x]) Pooled sample of antennal gland from two animals.
([xx]) Pooled sample of antennal gland from three animals.

An accuracy of 6.5% was obtained for the method used by comparing the results obtained from six replicates of a standard sample of Mytilus galloprovincialis (Escuela Nacional de Sanidad, Madrid).

In most analyses, it was necessary to use the whole sample for the digestion, due to the small amount of sample available. Therefore, repeated analyses of a single sample could not be performed. However, in some cases, particularly in muscle and midgut gland, it was possible to make several digestions. A mean precision (expressed as relative standard deviation) of 12.3% was obtained in these cases.

Table 4. Cadmium levels (µg/g dry weight) in muscle of crayfish after 96 h Cd-exposure at several water concentrations.

µg Cd(II)/L of water				
0	3.2	10	32	100
0.01	0.03	0.08	0.51	1.46
0.03[xx]	0.04[x]	0.10[x]	1.00	0.40
0.03[x]	0.03[xx]	0.11[x]	0.26	0.75
0.02[xx]	0.03	0.07	0.71	0.54
0.02	0.03	0.05[x]	0.80[x]	1.50
--	0.04[x]	0.16	0.90	0.60
--	--	0.14	0.37	0.83
--	--	--	0.17	1.57
--	--	--	0.68	1.26
--	--	--	--	0.89
0.02 ± 0.01	0.03 ± 0.01	0.1 ± 0.04	0.60 ± 0.28	0.98 ± 0.43
F = 19.67; df = 4,32; p < 0.01		Exponetial fit: $r^2 = 0.66$; $y = 0.05\ e^{0.03\ x}$		

Unless otherwise stated, each value corresponds to one sample.
([x]) Pooled sample of muscle from two animals.
([xx]) Pooled sample of muscle from three animals.

Analyses of cadmium were made by direct comparison with aqueous standars, and also by the standard additions method, to demostrate the matrix interference in the four tissues examined. Three different calibration curves were prepared (0-5, 5-20, 20-50 µg of cadmium/L) depending on the range of concentrations of metal in each sample. Standard solutions of Cd(II) and sample solutions were put in the same conditions of acidity (HNO_3 4 + 10). Results obtained by the application of these two methods were very different, always being lower by applicating the direct method. Mean differences of 60.3% (antennal glands), 57.3% (gills), 55.4% (midgut gland), and 42.5% (muscle) indicated that an important matrix interference occurs, which would result in errors if the direct method were used as method of analysis. These high differences have also been observed when analyzing for cadmium in some marine organisms (Medina et al. 1985), and are much higher than those observed when analyzing for chromium in the crayfish P. clarkii (Hernandez et al. 1986). Consequently, the standard additions method is the most adequate to perform the present study.

In a very few cases, it was possible to measure absorbances by flame atomic absorption spectroscopy (only in gills and in some midgut gland of

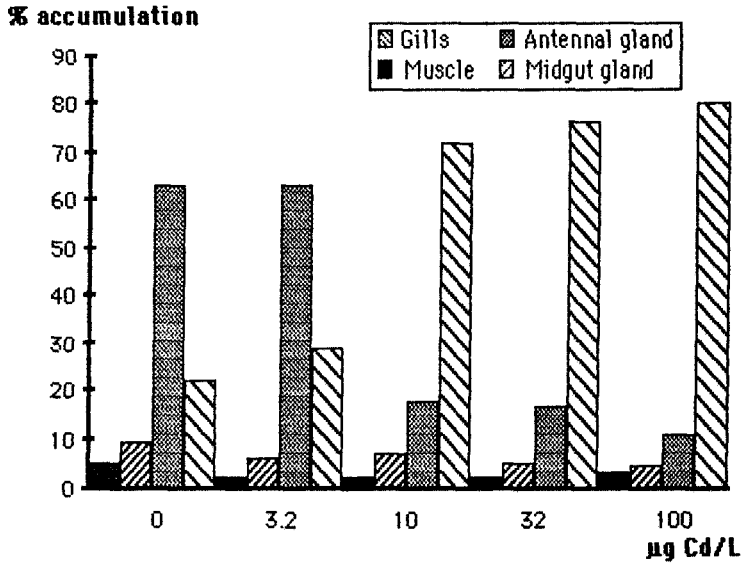

Figure 1. % Accumulation of Cadmium (with respect to the total cadmium amount in muscle, midgut gland, antennal glands and gills of the control and treated crayfish.

the crayfish treated with 100 µg of cadmium/L). In these instances, using the direct method, results obtained by flame AAS were higher than those of flameless AAS, but much lower than those obtained by the standard additions method and flameless AAS (mean differences of 50.4% for gills, and 43.8% for midgut gland).

The control crayfish showed cadmium levels ranging from 0.02 ± 0.01 µg/g dry weight in muscle to 3.08 ± 0.82 µg/g dry weight in antennal gland. It is important to indicate that the control animals showed total amounts of Cd about 2 ppm localizated in the four tissues examined. This can be indicative of a cadmium contamination in Albufera waters.

After 96 h. Cd(II)-exposure, the cadmium levels in all examined tissues increased with increasing cadmium concentration in the water.

A one-way analysis of variance (ANOVA) indicated significant cadmium concentration effects on cadmium levels in gills, midgut gland and muscle ($p < 0.01$). There were not significant treatment effects on the antennal gland concentration of cadmium ($p > 0.05$).

Regression curves were fitted, by the least squares method, to the data presented in tables 1, 2 and 4 corresponding to gills, midgut gland and muscle, respectively. The regression to the gills data was linear, whereas it was exponential for the midgut gland and muscle data.

Figure 1 shows the % accumulation in tissues after 96 h of cadmium exposure, with respect to the total amount of cadmium detected in crayfish. In controls, 62 % of cadmium was present in antennal glands, whereas cadmium content in muscle was only 5 %. In crayfish treated with 10, 32

and 100 µg Cd/L, near 80 % of cadmium was present in gills.

Relative % mean Cd levels in tissues of control and treated crayfish with low cadmium concentration (3.2 µg/L) were as follows: Antennal gland > Gills > Midgut gland > Muscle; whereas in tissues of crayfish treated with high cadmium concentration, they were as follows: Gills > Antennal glands > Midgut gland > Muscle.

The experiments with cadmium suggested that the concentration of this metal in the tissues was a function of metal concentration in the water. Similar results have been obtained for a number of crustaceans and other invertebrates (Ahsanullah et al. 1981; Nimmo et al. 1977).

O'Hara (1973) found that in Uca pugilator, the midgut gland and gills were important sites of cadmium accumulation when animals were exposed to 15 mg of cadmium/L in water, both these tissues reached a maximum cadmium concentration of approximately 110 ppm after 48 h; although the bioaccumulation was highest in the green gland tissue, with maximum concentrations of 380 ppm in tissue from crabs exposed to 25 ppm of cadmium. Gillespie et al.(1977), observed a great accumulation rate in Orconectes propinquus, where a mean of 18 ppm of cadmium was accumulated over a period of 190 h from water containing 10 ppb Cd. Our results show variations in the accumulation of Cd in different tissues. We found 4 and 37 ppm in gills from waters containing 10 and 100 ppb, respectively, whereas in muscle the accumulation was of 0.1 and 1 ppm, respectively, from the same waters.

As it has been demostrated, the crayfish Procambarus clarkii presents a high capacity for cadmium accumulation. Since these animals are ingested directly by man, a potential human health hazard exists.

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