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Determination of Lead in Treated Crayfish *Procambarus clarkii:* Accumulation in Different Tissues

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The continual loading of trace metals into our environment represents a water pollution problem due to their toxic effects on aquatic biota. In addition, Metal ions can be incorporated into food chains and concentrated by aquatic organisms to a level that affects their physiological state (Bryan 1971).

Lead is a widespread non-essential element that is highly toxic to both humans (Haguenoer and Furon 1981) and animals (Baudouin and Scoppa 1974). It has became particulary important due to its relative toxicity and increased environmental contamination via automobile exhaust and highway runoff. In espite of lead salts having a low solubility in water, lead compounds may pose a hazard problem to the aquatic organisms.

There are several investigations on the toxic effects and bioaccumulation of lead in fishes (Reichert et al 1979), moluscs (Martincic et al 1984), and crustaceans (Anderson 1978; Gilles and Pequeux 1983). Recently, Tulasi et al (1987) studied the lead uptake in fresh water field crab, Barytelphusa guerini, after 30 days lead-exposure.

Lake Albufera (Valencia, Spain) and the surrounding rice-field waters are subjected to large loads of sewage and toxic industrial residues (including heavy metals) from many urban wastewaters in the area (Rosello 1983).

In 1978, the American red crayfish Procambarus clarkii (Girard) appeared in Lake Albufera. The crayfish have reached a high density producing ecological and agricultural economic problems in rice crops. The crayfish is being fished commercially for human consumption without adequate protection to human health

The purpose of the present study was to investigate the accumulation of lead in tissues of the crayfish P. clarkii following short terme lead exposure at several sublethal concentrations.

MATERIAL AND METHODS

Adult intermolt specimens of the crayfish P.clarkii were collected in April 1986 from Lake Albufera (Valencia, Spain) and carried immediately to the laboratory where they were transferred into 300-L aquaria and maintained for 15 days at 22°C with a daily diet of pork liver.

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Three groups of eigth crayfish were kept in 15-L experimental aquaria. Eigth more crayfish served as a control. Only crayfish weighing between 15 and 20 g were used. Desired lead concentrations were obtained by addition of appropriate amounts of stock solutions, which were prepared using distilled water and $Pb(NO_3)$ (E.Merck, West Germany). Conditions in the aquaria were as follows: hardness, 180-300 mg $CaCO_3$/L; alkalinity, 3.8-4.4 mmol/L, and chloride concentration < 0.1 mg/L.

Water quality in each aquarium was monitored daily for changes in pH and oxygen concentration, which ranged from 7.0-7.5 and 80% to saturation, respectively. All aquaria were kept a constant temperature (22°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 lead near the nominal level.

After 96 h of Pb-exposure (10, 50, and 100 mg of Pb/L) at 22°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 muscle of the control and treated crayfish were dissected with plastic materials that were washed with HNO_3 and rinsed with distilled and deionized water, in order to avoid metal contamination. Prior to analyses, the different tissues were lyophilised and homogenized. Digestion was performed as follows: 0.01-1 g of lyophilised tissue were introduced into a 100-mL erlenmeyer flask and 10 mL of concentrated HNO_3 were added. The samples were digested on a hot plate with temperature of 80-90°C until nitrous vapours disappeared. After cooling, solutions were quantitatively transferred to a 25 mL beaker and diluted with twice-distilled water to the mark. Absorbance measurements were made on a Perkin-Elmer model 5000 atomic absorption spectrophotometer equipped with a model 561 recorder, a deuterium background corrector for flame and an HGA 400 Heated Graphite Atomizer with Zeeman 5000 background corrector. Determination of lead was performed by flame method for all samples except the control tissues. These were analyzed with graphite furnace using the direct method with $(NH_4)_2HPO_4$ as matrix modifier (May et al. 1982; Medina et al. 1987). Measure conditions were: 283.3 nm with drying, charring and atomization temperatures of the 120, 500 and 1400°C, respectively (Medina et al. 1986) for graphite furnace using direct and standard additions methods. The measure conditions where 0.5% $(NH_4)_2HPO_4$ was used as matrix modifier were as follows: drying 120°C, charring 800°C and atomization 2400°C. In both cases argon was used as the purging gas. A final cleaning step at 2700°C was also used.

The values of lead content in the tissues studied were analyzed by one-way analysis of variance and multiple comparation test (Tukey-test) among treatments.

RESULTS AND DISCUSSION

Lead concentrations in the gills, midgut gland, muscle and antennal glands of the control and the crayfish exposed for 96 h to 10, 50, and 100 mg Pb/L are presented in Tables 1, 2, 3, and 4, respectively.

The digestion of samples with concentrated HNO_3 has been recommended by several authors (Hollak et al. 1972; Slavin et al. 1975; Bernhard 1976; May 1982; Capelli et al. 1982).

Table 1. Lead levels (ppm of Pb) in gills of crayfish after 96 h Pb-exposure at several concentrations.

	mg Pb(II)/L of water			
	0	10	50	100
	153	3940	12330	27039
	184	3070	56470	53113
	95	1730	36270	22338
	211	970	20520	30969
	125	1400	52200	13409
	233	6600	4570	59053
	410	4060	--	40486
	370	--	--	--
Mean	223	3110	30393	35199
SD	113	1966	21357	16547
	F=13.8; df=3,24; p<0.001			

The large number of samples makes 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 larger number of samples, so as to obtain comparable results for both digestion methods.

On the other hand, the recoveries of three standards of Pb(II) (subject to an analogous wet digestion in open flasks) being obtained: 1 µg/mL, 98.3%; 2 µg/mL, 103.2%; 4 µg/mL, 101.5%. In the same way, the addition of 4 µg of lead/L in a sample of muscle tissue (subject to the same process of digestion) shows a recovery of 101.8%. These results show that during the wet digestion no losses of lead occurred in open flask.

Table 2. Lead levels (ppm of Pb) in midgut gland of crayfish after 96 h Pb-exposure at several concentrations.

	mg Pb(II)/L of water			
	0	10	50	100
	6.1	380	70	150
	5.0	140	850	250
	4.2	160	430	300
	9.6	70	830	1500
	12.4	410	630	350
	6.5	220	60	600
	8.4	270	90	--
	3.1	--	80	--
Mean	6.9	236	380	525
SD	3.1	126	351	501
	F=3.9; df=3,25; p<0.05			

Table 3. Lead levels (ppm of Pb) in muscle of crayfish after 96 h Pb-exposure at several concentrations.

	mg Pb(II)/L of water			
	0	10	50	100
	16.2	40.0	30	710
	12.8	24.1	120	220
	9.0	9.9	250	70
	11.1	6.3	80	320
	14.2	19.0	220	200
	25.1	13.1	70	70
	8.6	15.3	40	100
	13.2	22.7	40	--
Mean	15.8	35.0	106	241
SD	6.1	16.0	85	226
	F=6.08; df=3,27; p<0.01			

The determination of lead by flame has been realized for all tissues exposed to lead, since its content (considering the necessary dilutions) has been the adequate to make use of this method. The direct and standard additions methods have been applied for all the tissues. Significant differences have not been observed between these methods, therefore the direct method has been used. In relation to control tissues, the gills possess a lead content which can be determinated by flame whereas the levels of lead in midgut gland and muscle are found near the detection limit, although significant measures have been realized. In the case of antennal glands (with a limited quantity of available sample) the determination of lead by flame cannot performed. For these control tissues the determination of lead has been realized basically by graphite furnace. For this technique, standard addition methods and direct method with and without 0.5% $(NH_4)_2HPO_4$ as matrix modifier have been applied. The results obtained in this comparative study, so as the results obtained by flame, are shown in the table 5. It must be emphasized that the values obtained by flame and graphite furnace with standard addition or in the presence of modifier can be considerated similar. As well significant differences have been found between the results obtained by graphite furnace using the direct and standard additions methods since a mean diminution of 45% has been obtained using the direct method. For this reason the determination of lead in the control samples has been performed by graphite furnace and direct method in the presence of 0.5% $(NH_4)_2HPO_4$.

An accuracy of 8.5% was obtained with graphite furnace and matrix modifier by comparing the results obtained from six replicates of the standard sample of Mytilus galloprovincialis (International Atomic Energy Agency, Monaco. Standard sample MA-M-2/TM). The precision, as relative standard deviation, was 12.2%.

The control crayfish showed lead levels ranging from 6.9 ± 2.8 ppm dry weight in midgut gland to 261 ± 114 ppm dry weigth in gills. This may be indicate of high lead contamination of Lake Albufera.

As can be seen in Tables 1, 2, 3, and 4, the four analysis of variance show a significant effect of lead concentration, but they are different according to tissue.

Table 4. Lead levels (ppm of Pb) in antennal glands of crayfish after 96 h Pb-exposure at several concentration.

	mg Pb(II)/L of water			
	0	10	50	100
	162	2640	1870	990
	78	1170	8350	2180
	115	3390	1860	1240
	55	1370	1510	8180
	104	3780	1600	990
	154	5050	2660	7670
	82	4060	--	--
	135	4520	--	--
Mean	110	3249	2975	3542
SD	38	1418	2664	3427
	F=3.62; df=3,23; p<0.05			

Table 5. Values of lead concentration (ppm) in control tissues by flame and graphite furnace.

	FLAME	Direct method	Standard additons	0.5% $(NH_4)_2HPO_4$
	93	63	100	95
Gills	190	99	188	184
	365	226	382	370
	4.4	2.3	4.1	4.2
Midgut gland	8.6	5.1	8.8	8.4
	11.7	7.1	11.9	12.4
	--	41	84	78
Antennal glands	--	71	110	115
	--	85	138	154
	10.3	4.9	9.1	9.9
Muscle	16.7	8.3	15.5	15.3
	22.4	13.9	23.3	24.1

Two groups can be considered: effect on gills and the rest of tissues. In gills two groups of lead exposure can be considered: a Tuckey test shows that mean values of lead levels are different among control-10 mg Pb/L and 50-100 mg Pb/L. The other tissues, however, shows only significant differences among control-10-50 mg/L and 100 mg Pb/L. Of course there are not significant differences among the three different treatments.(control-10-50 mg/L).

In the present study we have used sublethal lead concentrations, but these were near the LC50 value (96-h LC50=127 ppm at 22°C). This fact allowed us to find the lead

saturation levels in antennal gland of this crayfish (see Table 4). This is in accordance with the excretory function of antennal gland.

In control and treated crayfish the highest % accumulation (with respect to the total amount lead detected) was present in gills. Near to 90% of lead was present in gills of crayfish treated with 100 mg of Pb/L, whereas the lead content in other tissues, as midgut gland and muscle was less than 1%.

Similar results have been obtained in other crustaceans by several authors. Anderson (1978) found that gills were the most important site of lead accumulation when Orconectes virilis were exposed at several concentrations of lead; lead accumulations in muscle and midgut gland were lower than in gills. Crayfish tend to accumulate lead primarily in exoskeleton and gills (Anderson 1978). Dickson et al. (1979) examined the concentration of lead in tissues of two species of crayfish obtained from natural water that contained only 2.3 ppm of lead. They found the highest lead concentration in gills and antennal glands and the lowest in midgut gland and muscle. Tulasi et al. (1987) show that the lead content in gills was very higher that lead content in muscle and midgut gland of crabs after 4 days lead exposure.

The crayfish Procambarus clarkii has a high capacity for lead accumulation. The gills are the most important tissue of lead accumulation, as evidenced by increasing lead concentrations in the gills with increasing water concentrations. Anderson (1978) postulated that there was some type of physiological compensation and crayfish are able to acclimate to the metal concentration by compensating for decreased gill efficiency. In previous study we have found that high concentrations of lead caused some decrease in the oxygen consumption, so as histopathological alterations in gill tissue (Torreblanca et al. 1986).

Since the crayfish used as controls in this study appear to be able to accumulate large quantities of lead without apparent lethal consequences, these animals may be potentially toxic and harmful in human and natural food chains.

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