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Determination of Chromium in Treated Crayfish, *Procambarus clarkii*, by Electrothermal AAS: Study of Chromium Accumulation in Different Tissues

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The American red crayfish Procambarus clarkii is native to the Lousiana marshes (USA). In the 70's, this crayfish was introduced into Spain through the Guadalquivir river swamps (Librero 1980). In 1978, the crayfish appeared in Lake Albufera near Valencia and in the surrounding rice fields. Presently, the crayfish have reached a high density due to their natural resistence, rapid adaptation, and growth; producing ecological and agricultural-economic problems in rice crops (Andreu et al. 1984). Without adequate sanitary control, the crayfish is presently being fished commercially for human consumption. 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).

Chromium, an essential trace element for humans and animals, is involved in normal carbohydrate metabolism (Mertz 1969; Anderson et al. 1983). It has been suggested that chromium may have an essential function in the regulation of glycogen metabolism of the crab (Sather 1966); although, it is toxic at higher concentrations and causes histopathological and ultrastructural changes in several tissues of shrimp (Doughtie and Rao 1984).

In the present study, we investigated the accumulation of chromium in muscle, hepatopancreas, antennal glands, and gills of Procambarus clarkii (Girard) from Lake Albufera following Cr(VI)-exposure. Determinations of chromium were made by using Electrothermal Atomic Absorption Spectroscopy and the standard additions method.

MATERIALS AND METHODS

Adult intermolt speciments of the crayfish Procambarus clarkii were collected in Lake Albufera (Valencia, Spain) and carried immediately to the laboratory where they were transferred into 300 l aquaria for 10 days and maintained, before treatment, at 19.5 ºC with a daily diet of pork liver.

50 crayfish ranging in weight 17.5 to 34.8 g were divided into five groups of 10 animals each. These were kept in 15 l experi-

mental aquaria containing 10, 37, 136, and 500 mg/l Cr(VI) as Na_2CrO_4 (Merck). 10 more crayfish served as a control and were kept in 15 l of clear water. After 96 hours of Cr-exposure at 19.5ºC, the animals were transferred to clean water, free of any contamination, and kept there for an additional 5 hours.

The gills, hepatopancreas, antennal glands, and tail muscle of the control and the treated crayfish were dissected using plastic materials in order to avoid metal contamination.

Prior to analyses, the different tissues were lyophilized and homogenized. Digestion was carried out as follows: 0.01 - 1 g of lyophilized tissue were introduced into the reaction flask and 10 ml of concentrated HNO_3 were added. The samples were digested on a hot plate at a temperature of about 80ºC until nitrous vapours disappeared (approximately 12 hours). After cooling, solutions were quantitatively transferred and diluted with twice-distilled water to a final volume of 25 ml.

The digestion of biological samples with concentrated HNO_3 for metal analysis has been recommended by several authors (Hollak et al. 1972; Krinitz et al. 1974; Slavin et al. 1975; Bernhard 1976; Hinderberger 1981; May 1982; Capelli et al. 1982).

The high 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 large number of samples. Precision (expressed as relative standard deviation) and accuracy of the latter method, were determined from six replicates of a homogenized sample of Mytilus galloprovincialis used for Intercalibration (Coordinator Center: Escuela Nacional de Sanidad, Madrid). Analyses of chromium were carried out by flameless AAS, obtaining a precision of 14.3% and an accuracy of 8.0% for a content of 2.12 ug/g dry weight. These values were similar to those obtained by carrying out the digestion with teflon reactors under pressure. For this reason, it may be considered that the digestion procedure applied in this work is adequate.

On the other hand, recoveries of three standards of Cr(VI) subjected to wet digestion were found to be as follows:

40 ng/ml -- 93.8% ; 200 ng/ml -- 105.5% ; 400 ng/ml -- 102.6%

These results show that during wet digestion no losses of chromium occurred.

Reagents used were of high purity appropiate for trace metal analyses and, to avoid contamination, the material used was made of Pyrex and high-density polyethylene.

A Perkin-Elmer Atomic Absorption Spectrophotometer 2380, equipped with a recorder 561, a deuterium background corrector, and a HGA 400 Heated Graphite Atomizer was used to measure atomic absorption.

Determination of chromium was carried out at 357.9 nm with drying, charring, and atomization temperatures of 120, 1100, and 2500ºC, respectively, using argon as purging gas.

Blanks subjected to digestion and blanks of the calibration curves gave similar absorbance values, and always lower than 0.020 units.

Absorbances of Cr(III) and Cr(VI) standards (between 20 and 200 ng/ml) were measured; finding that they were similar. The equations corresponding to the calibration curves were as follows:

Cr(III)	A = 0.006 + 0.882 c	r = 0.9995
Cr(VI)	A = 0.005 + 0.877 c	r = 0.9999

Potassium chromate standard for calibration curves was used for subsequent experiments.

Calibration curves up to 100 ng/ml of Cr were obtained by injecting 20 ul of standard solution and selecting Stop Flow in atomization step. For higher concentrations (100 - 500 ng/ml of Cr), 10 ul and Miniflow (50 ml/min) were used. Standard solutions of Cr(VI) and sample solutions were put in the same conditions of acidity.

In most analyses, it was necessary to use the whole sample for the digestion due to the little amount of sample available. Therefore, repeated analyses of a single sample could not be carried out. In control samples and in most low Cr-concentrations treated samples (especially in muscle), the Cr content was lower than the applicability range for flame AAS. Thus, to avoid the use of two different methods, depending on the chromium level to be determined, we have chosen the HGA technique because it allows one to analyze all the samples (when Cr concentration was higher than 500 ng/ml, an aliquot of the sample was diluted with 4:10 HNO_3).

Results obtained by the direct method were always lower than those of standard additions method. Mean differences of 35.2% (hepatopancreas), 34.8% (muscle), 19.6% (gland), and 18.9% (gills) indicated that an important matrix interference occurs. Consequently, the standard additions method is the most adequate to perform this study. Nevertheless, when Cr concentration was higher than 500 ng/ml, flame AAS was also applied to compare the results: using the direct method, concentration of Cr in all tissues analyzed by flameless AAS was always lower than those analyzed by flame AAS, with a difference of about 30%. However, the results obtained by flame AAS (using the direct method) and those obtained by flameless AAS (using the standard additions method) were more similar because a mean difference of 10% was obtained.

RESULTS AND DISCUSSION

Tissue chromium levels of the control and the treated crayfish exposed for 96 hours to 10, 37, 136, and 500 mg/l of Cr(VI) are presented in Table 1. The control crayfish showed chromium levels ranging from 0.4 ± 0.2 ug/g dry weight in muscle to 38.2 ± 5.0

ug/g d.w. in antennal gland.

The relative mean chromium level in control tissues were: gland > gills > hepatopancreas > muscle. It is important to indicate that the control animals showed amounts of Cr about 38 ug/g in glands and 13 ug/g in gills. This can be indicative of a chromium contamination in Albufera waters.

After 96 hours of Cr(VI)-exposure, the Cr levels in all examinated tissues increased with increasing Cr-concentration in the water. A one-way analysis of variance (ANOVA) indicated significant Cr-concentration effect on Cr-levels in all tissues examinated ($p < 0.001$). The highest accumulation occurred in antennal glands and gills, whereas, the lowest accumulation occurred in muscle.

Table 1. Chromium levels (ug/g dry weight) in some tissues of crayfish, after 96 h of Cr(VI)-exposure at several concentrations.

mg Cr VI/l.	GILLS	HEPATOP.	GLAND	MUSCLE	TOTAL
Control	13.1 ± 1.6	1.0 ± 0.4	38.2 ± 5.0	0.4 ± 0.2	52.7
10	67.2 ± 17.0	20.3 ± 3.5	37.5 ± 9.2	1.8 ± 0.4	126.8
37	89.4 ± 13.3	55.9 ± 25.0	147 ± 42	3.9 ± 1.2	296.2
136	230 ± 69	189 ± 99	286 ± 88	7.3 ± 1.5	712.3
500	541 ± 125	462 ± 102	1170 ± 202	32.0 ± 3.0	2205.0

Each value represents the mean ± SD of 10 crayfish, except for 500 mg/1 Cr(VI) (n = 4)

Figure 1 shows the % accumulation in tissues after 96 h of Cr-exposure, with respect to the total chromium amount detected in crayfish. 70% of Cr was present in glands of the control crayfish, whereas, Cr-content in muscle of animals treated with 500 mg/1 of Cr(VI) was only 2%. Relative % mean chromium levels in tissues of treated crayfish were as follows: glands > gills > hepatopancreas > muscle, as occurring in the control tissues.

Regression lines were fitted to the data presented in Table 1, for each of different tissues, using the general expression: $y = a + bx$ where y = chromium tissue levels (ug/g d.w.), and x = mg/1 of Cr(VI) in water. The following expressions were derived:

Gills	$y = 52.45 + 1.02\ x$,	$r = 0.86$
Gland	$y = 30.61 + 2.23\ x$,	$r = 0.93$
Muscle	$y = 0.72 + 0.06\ x$,	$r = 0.96$
Hepat	$y = 18.60 + 0.91\ x$,	$r = 0.87$
TOTAL	$y = 100.15 + 4.23\ x$,	$r = 0.99$

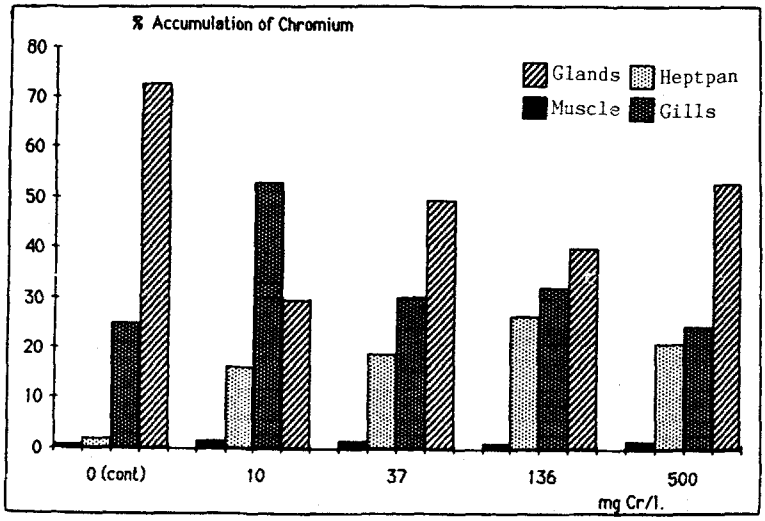

Figure 1. % accumulation of chromium (with respect to the total chromium amount) in muscle, hepatopancreas, gills, and antennal glands of the control and the treated crayfish.

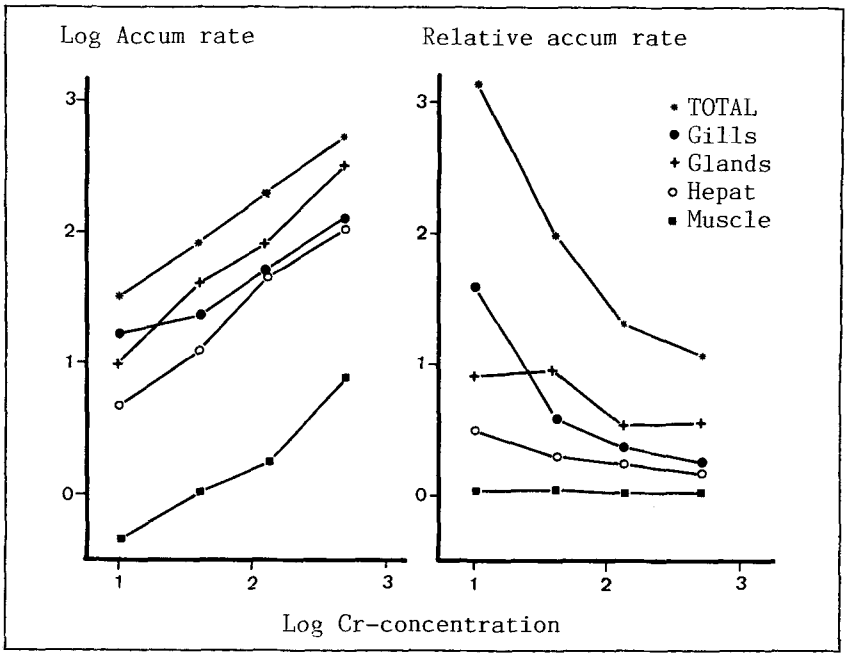

Figure 2. Cr-accumulation rate (ug Cr/g d.w./day) and relative Cr-accumulation rate (accum rate/mg Cr/l) of muscle, gills, hepatopancreas, and antennal glands of the crayfish treated with several Cr-concentrations.

Chromium concentration in tissues, expressed on a dry weight basis, increases linearly when increasing the chromium concentration of the test solution. Animals at the higher Cr-concentrations continue to accumulate chromium.

The Cr accumulation rates (ug Cr/g d.w./day) and relative accumulation rates (accum rate/mg Cr/l) of different tissues, after Cr-exposure, are presented in Figure 2. The accumulation rates increase when increasing Cr-concentration in the water for all tissues examinated. Whereas, the relative accumulation rate decreases with increasing contamination in the water, occurring especially in the gills and the hepatopancreas.

It was not expected because the accumulation rate curve is almost a straight line, at least in the range of the Cr concentrations used in the current study (see Figure 2). However, it is probable that the curve runs into saturation at higher Cr concentrations. Unfortunately, these Cr levels are not accesible experimentally for as the lethal dose for this crayfish is attained at concentrations near 500 mg/l of Cr(VI).

On the other hand, the relative accumulation rates of gills and hepatopancreas show a tendency to become equal when the chromium concentrations in the water increases (see Figure 2). This suggested that metabolic activity increased at higher Cr concentrations in order to metabolize chemicals when they are at toxic levels. In support of this interpretation is the observation that high Cr concentrations resulted in a higher rate of translocation of chromium from the gills to hepatopancreas. This was proved by the decrease in the gill/hepatopancreas chromium ratios when increasing the Cr concentration in the water: 13.22, 3.31, 1.60, 1.22, and 1.17 after exposure at 0, 10, 37, 136, and 500 mg/l of Cr(VI), respectively.

As it has been demonstrated,the crayfish Procambarus clarkii presented a high capacity for chromium accumulation, which is not dependent upon the size and sex of animals ($p > 0.05$).

Amounts of chromium as high as 38 ug/g were found in antennal gland of the control animals. This is probably indicative of Cr contamination in Lake Albufera waters. We highly recommend the use of sanitary conditions for raising these crustaceans since they are being utilized for human consumption.

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