ACCUMULATION AND ELIMINATION OF Cr IN GILLS AND EGGS BY THE FRESHWATER CRAB *Zilchiopsis collastinensis* AFTER EXPERIMENTAL EXPOSURE

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ABSTRACT

Cr accumulation and elimination were studied in the freshwater crab *Zilchiopsis collastinensis* (Crustacea, Decapoda) exposed to 3 (T1) and 6 (T2) mg CrVI/L using 1000-L tanks with dechlorinated water and artificial sediment. The objectives of this study were to analyze the Cr-accumulating capacity of *Z. collastinensis* males and females in gills and eggs, as well as to relate this capacity with crab weight, and to determine the Bioconcentration Factor (BCF) in relation to water and sediment.

Significant differences in Cr concentration were found between the control and T1 and T2 ($p = 0.018$ and $p = 0.001$), and between T1 and T2 ($p = 0.025$). Significant differences in Cr concentration were found among all treatments in gills ($p < 0.0001$) and eggs ($p = 0.0247$), and when comparing Cr concentration between gills and eggs ($p= 0.0046$), but not between females and males ($p = 0.6035$). The tissues/water and tissues/sediment BCF values increased with Cr concentration, but was always higher in relation to water than to sediment. No relationship was found between crab weight and Cr concentration ($r = -0.025$).

The obtained results constitute a great contribution to the knowledge of Cr accumulation in freshwater crabs. We propose *Z. collastinensis* as biomonitor of aquatic systems contaminated with Cr.

KEYWORDS: bioaccumulation, chromium, freshwater crabs, *Zilchiopsis collastinensis*.

INTRODUCTION

The effects of Cr accumulation in different organs of marine and mangrove decapods have been studied by some authors [1-5], but studies on Cr bioaccumulation by freshwater crabs have not received the same attention, although they have been studied by some authors, e.g. Ip et al. [6] and the Environmental Protection Agency, that has monitored Cr concentration in the crab *Scylla serrata* [7]. Sediments enriched with heavy metals can potentially increase the concentration of metals in invertebrates and vertebrates associated to sediments [8, 9], some of which are consumed by men. Previous studies at different scales (bioassays, mesocosms and field) have demonstrated the loss of biodiversity, occurrence of malformations and alteration of biological parameters of planktonic and benthonic organisms linked to high Cr and sulfur levels in sediments and water of the Salado River basin in Argentina [10-13]. Although international regulations for biota protection indicates Cr values of 37.3 μg/g and 8.9 μ/L in sediments and water [14], in a previous work we found values up to 800 μg Cr/g and 13.6 μg Cr/L in the Salado river basin.

Most of the industrial production of the Salado river basin is composed by tanneries, together with leather production factories which use Cr as leather agent tanning. This entails a significant impairment of the water-courses as they mostly collect untreated effluents and discharges to the Salado River [15, 16].

The freshwater crustacean *Zilchiopsis collastinensis* (Pretzmann, 1968) lives in galleries excavated in gullies and clayey sediments of the Salado River, with high concentrations of Cr and other heavy metals, and is occasionally consumed by men. *Z. collastinensis* has a wide distribution from the south of the Amazon river basin in Brazil, Bolivia and Peru and the Paraguay, Paraná and Uruguay rivers' basins in Argentina [18, 19].

Oligochaetes and mollusks have traditionally been used as biomonitors of contaminated benthos [20-28]. However, since *Z. collastinensis* has a high residence time and territorial behavior, it could be used as indicator of Cr con-

tamination, being a better biomonitor than species with a higher mobility, such as fish.

MATERIALS AND METHODS

Six 1000-L PVC tanks with dechlorinated water and artificial sediment were used [29]. $K_2Cr_2O_7$ was dosed to the water to attain final concentrations of 3 and 6 mgCrVI/L (T1 and T2, respectively). These concentrations were chosen according to preliminary tests of 14-days exposure to the same experimental conditions. After $K_2Cr_2O_7$ dosage, the artificial sediment was added. No Cr was added to the control, and one replicate per treatment, including the control, was done. After a 5-days stabilization period, 28 cm x 18 cm x 12 cm perforated plastic containers, covered on the inside with a 1-mm aperture plastic mesh, and separated by 30 cm from the sediment, were put. Two crabs were placed in each container, with two PVC tubes (10 cm length x 5 cm diameter) as refuges. Crabs were daily fed with animal protein. At day 28, and after taken the sample of the accumulation phase, the remaining crabs were transferred to similar tanks, but without Cr (elimination phase). Water and sediment samples as well as one crab were taken at 1, 7, 14 and 28 days during accumulation phase, and at 1 and 7 days in elimination phase. After washing the taken crabs twice with distilled water, they were frozen for 24 h to cryoanesthetize them. Crab weight and sex were determined, and then crabs were dissected with plastic instruments to analyze Cr content in gills and eggs.

Sediment and water samples were treated according to the EPA600/4-91/100 regulation, methods 200.2 and 200.9. Egg and gill digestions were carried out in nitric/ hydrochloric/peroxide acid [30]. Cr concentration was analyzed with a Perkin Elmer Analyst 800 atomic absorption spectrophotometer (detection limit: 5 µg/g).

The bioconcentration factors (BCF), considering the tissue/water and tissue/sediment relationships, were calculated according to Walker [31] as well as Spacie and Hamelink [32]: BCF = *C1/C2*, where *C1* = contaminant concentration in the organism (µg/g), and *C2* = contaminant concentration in water (mg/L).

The main physicochemical parameters of water, dissolved oxygen (mg/L), temperature (ºC), conductivity (µS/cm), pH, and salinity (%), were daily measured with a Horiba U 10 multiparametric monitoring model.

One-way ANOVA was used to determine the constancy of physicochemical variables during the experiment, and the possible significant differences between the control and both treatments in relation to Cr concentration in gills of males and females as well as eggs of *Z. collastinensis*. The possible correlation between Cr content and crab weight was tested using the Pearson correlation analysis [33].

RESULTS

Physicochemical variables of each tank during the accumulation and elimination phases remained constant during the experiment (Table 1). No significant differences were registered in dissolved oxygen, temperature, conductivity, pH, and salinity during the whole study period (ANOVA p = 0.8592; 0.6664; 0.132; 0.9494 and 0.2254, respectively).

Fig. 1 shows, in a logarithmic scale, Cr concentrations in water, sediment and *Z. collastinensis.* In spite of the low Cr level in water (highest values found in sediment), *Z. collastinensis* accumulated great quantities of Cr.

Cr values in water and sediments among control and T1 and T2 tanks showed significant differences (p = 0.001 and 0.0001, respectively). Fig. 2 shows Cr concentrations in *Z. collastinensis* during accumulation and elimination phases. In the control, Cr concentration was low during both phases. In T1, Cr concentration increased gradually during accumulation phase (except a slight decrease at day 14), and also during elimination phase, being always higher than in the control. In T2, Cr concentration was higher than in control or T1 during both phases. In T2, a slight decrease in Cr concentration, at the end of accumulation phase, was observed, but then also an increase, at the end of elimination phase. In the crabs, significant differences in Cr concentration were found between the control and T1 and T2 (p = 0.018 and p = 0.001), and between both treatments (p = 0.025).

TABLE 1 - Physicochemical variables in Control, T1 and T2 during the accumulation and elimination phases (means and standard deviations in parentheses are indicated).

	Accumulation			Elimination		
	Control	T1	T2	Control	T1	T2
Dissolved Oxygen (mg/L)	9.21 (0.95)	8.95 (0.88)	8.96 (0.87)	8.85 (1.11)	9.14 (0.56)	9.01 (0.81
Temperature (ºC)	17.22 (2.51)	16.99 (2.43)	17.10 (2.42)	19.50 (2.74)	18.55 (2.17)	18.60 (2.19)
Conductivity (mS/cm)	1.28 (0.06)	1.19 (0.02)	1.21 (0.05	1.39 (0.17)	1.34 (0.17)	1.36 (0.16)
pH	8.33 (0.14)	8.29(0.13)	8.29 (0.12)	8.33 (0.23)	8.37 (0.17)	8.40 (0.18)
Salinity (%)	0.05 (0.0)	0.05 (0.0)	0.053 (0.005)	0.057 (0.007)	0.055 (0.011)	0.056 (0.011)

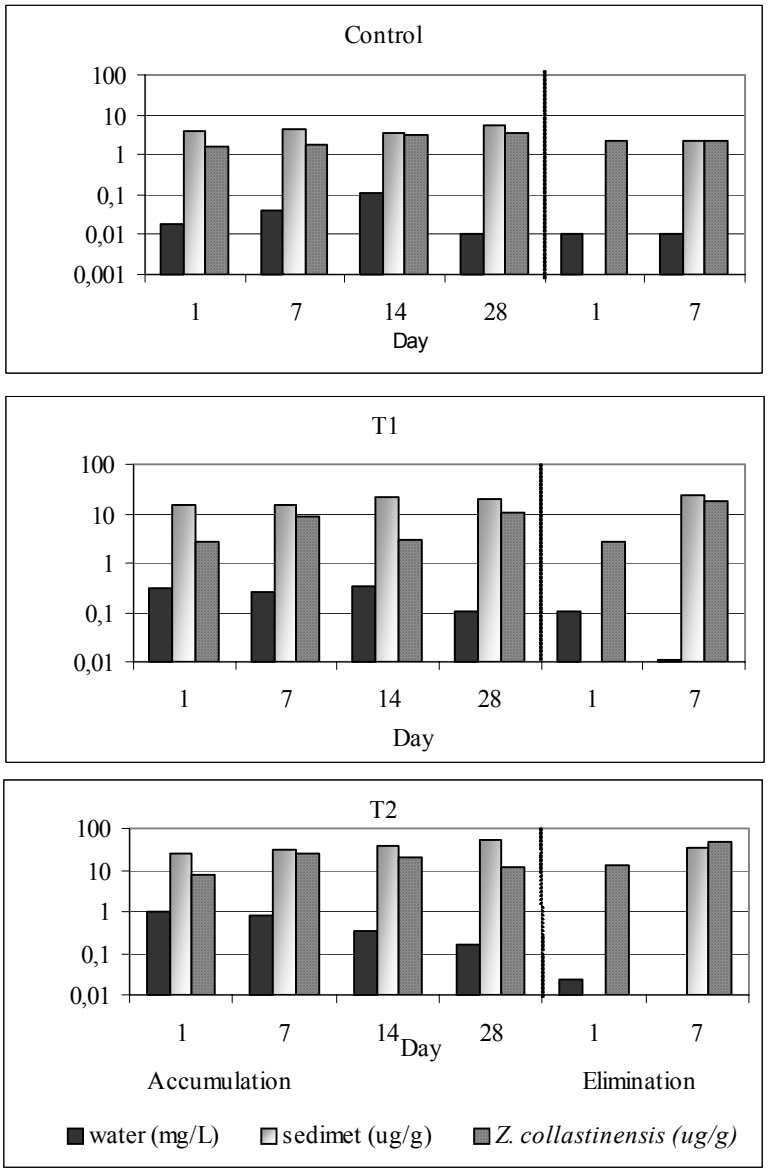

FIGURE 1 - Cr accumulation in water, sediments and *Z. collastinensis* (gill and egg data were grouped, 2 replicates per treatment).

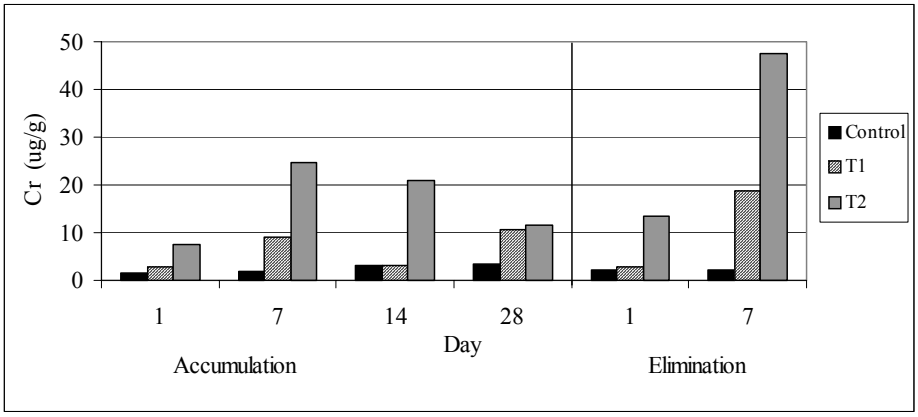

FIGURE 2 - Cr accumulation in *Z. collastinensis* during the accumulation and elimination phases (gill and egg data were grouped, 2 replicates per treatment).

TABLE 2 - Cr accumulation registered in field and experimental surveys at different scales for marine and estuarine crabs (d.w.=dry weight).

Reference	Species	Origin of Cr at field and experimental surveys	Critical organs
		Field surveys	
Sather (1967) in Eisler 1984 [34]	*Podophthalmus vigil*	USA	Cr in gills
Tennant and Forster (1969) in Eisler 1984 [34]	*Cancer magister*	USA	Cr in gills and hepathopancreas
Eisler. R. (1984) [34]	*Cancer irroratus*	USA	<0.3-0.6 µg Cr/g in muscle <0.5-1.2 µg Cr/g in digestive gland 0.8-2.5 µg Cr/g in gills
Mortimer and Cox (1999) [7]	*Scylla serrata*	Australia. Maroochy river. River with tributaries in industrial areas	0.200 - 0.540 µg Cr/g in muscle (d.w.) 0.190 - 0.633 µg Cr/g in hepathopancreas (d.w.) 0.820 - 3.620 µg Cr/g in gills (d.w.)
Ip et al (2005) [6]	*Portunus pelagicus*	China. Pearl River Delta. industrial area	0.14 - 0.76 µg Cr/g d.w.
Al-Mohanna and Subramanyam (2001) [4]	*Portunus pelagicus*	Kuwait. Alter the Gulf war	0.13 - 0.52 µg Cr/g in hepathopancreas (d.w.) 0.11 - 0.34 µg Cr/g in gills (d.w.) 0.10 - 0.21 µg Cr/g in gonads (d.w.) 0.15 - 0.66 µg Cr/g in muscle (d.w.) 0.12 - 0.58 µg Cr/g in stomach (d.w.)
		Experimental surveys	
Dias-Corrêa et al (2005) [5]	*Ucides cordatus*	53.3 g/L $CrCl_3$.6H20 10.4 g Cr/L	17.396.4 µg Cr/g in gills (d.w.) 316.5 µg Cr/g in hepathopancreas (d.w.) 47.3 µg Cr/g in muscle (d.w.)
This work	*Zilchiopsis collastinensis*	3 mg CrVI/L	0.24-1.26 µgCr/g in eggs (d.w.) 4.72-45.26 µgCr/g in gills (d.w.)
This work	*Zilchiopsis collastinensis*	6 mg CrVI/L	0.92-12.01 µgCr/g in eggs (d.w.) 11.03-47.44 µgCr/g in gills (d.w.)

Significant differences in Cr concentration were also found among all treatments in gills ($p < 0.0001$) and eggs ($p = 0.0247$). Cr concentration in gills and eggs also showed significant differences ($p = 0.0046$).

The BCF values (tissues/water) were 3.70; 27.92 and 67.72 in T1, and 5.59; 29.68 and 50.09 in T2, at days 1, 7 and 28, respectively. The BCF (tissues/sediment) reached lower values, 0.07; 0.47 and 0.35 in T1, and 0.24, 0.77 and 0.16 in T2, at days 1, 7 and 28, respectively.

Crab weight was not related with Cr concentration ($r = -0.025$). On the other hand, there were no significant differences in Cr concentration between female and male tissues ($p = 0.6035$).

DISCUSSION AND CONCLUSIONS

The results of this study allowed to determine the capacity of freshwater crabs to accumulate Cr, confirming what was registered previously by other authors for marine and estuarine crabs in different scale studies (Table 2) [4-7, 34]. These authors studied Cr accumulation in different organs, and agreed in that organs are more sensitive if they are metabolically active. They also found, in experimental and field studies, that gills and hepatopancreas accumulated more Cr than other organs in marine crabs. In marine organisms, elimination of accumulated Cr differs among the different taxa, and the process generally has a complex pattern of elimination showing great differences between metals and invertebrate groups [5], but these patterns have only been studied very superficially in freshwater organisms. This study also found that Cr accumulation is higher in gills than in eggs, but it does not differ between females and males, nor it is related to weight.

A recent study developed by Rainbow [35] stated that toxicity does not depend on total concentration of the accumulated metal, but would be more related with the threshold concentration of the metabolically available metal. In Argentina, the first studies analyzing Cr concentration in different invertebrates are very recent [17, 36, 37]. The mentioned authors also obtained higher Cr values in organisms subjected to lower concentrations. This behaviour would be indicating that there is a threshold value for different invertebrates and fish, above which Cr incorporation is not proportional to the exposed concentration.

Dias Côrrea et al. [5] obtained microphotographs of transversal sections of branchial filaments of *Ucides cordatus* using TEM, and, through x-ray microanalysis spectra, he found a precise pattern of Cr distribution in the external side of gills. These results indicate that Cr is deposited and immobilized in the external surface of gills. An identical pattern was found by Saha et al. [28] in mantle and gills of mollusks. These organs are responsible for transferring Cr towards the organism, as well as for de-

toxification, since they are in contact with the external medium. In *Z. collastinensis,* Cr increased in gills in the detoxification phase, showing that they take part in the elimination. According to Viarengo [38], the organs that have an important role in metabolism are the same ones that take part in metal detoxification. The mechanism involved was proposed by Dias Correa [5], who states that Cr dissolved in natural waters is generally loaded as tetrahydroxy chromate(III) ion $[(CCr(OH)_4]^-$. This anionic form has high reactivity and can be transported to the organism through the permeable surface of gills that contain sulfate channels. The flux of Cr that mainly occurs in the gills turns this organ into the first site, in which the first Cr aggregates are formed with organic molecules of glutathione, ascorbic acid and saccharides, forming thus organometallic complexes.

Z. collastinensis accumulated little Cr in eggs in relation to gills, since their internal localization gives them a very limited contact with contaminants, being the carapace, the most important barrier. However, the fact that the toxic metal reaches the reproductive cells could have important negative effects in the genetic and population constancy of this species. Cr detected in eggs could have reached them through hemolymphs. This was also suggested by Sather [39], when he studied the ways of absorption and the metabolism of Cr(VI) in the crab *Podophtalmus vigil.*

When comparing the accumulation capacity of *Z. collastinensis* with oligochaetes and fish under identical conditions [40], a direct relationship between the medium concentration (water and sediment) and that of organisms was shown. In oligochaetes and fish, there were no significant differences between both assayed concentrations, but between the control and each one of them. In crabs, there were significant differences between both assayed concentrations and the control, as well as between the two concentrations, which shows the high degree of sensitivity of freshwater crabs to Cr contamination. The obtained results indicate that *Z. collastinensis*, since it is a territorial species that accumulates Cr in its gills and, to a lower extent, in the eggs, is a good biomonitor of aquatic systems contaminated with Cr. As this species is occasionally consumed by men, it could also act in Cr trophic transfer.

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