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Assessment of EDTA in Chromium (III–VI) Toxicity on Marine Intertidal Crab (*Petrolisthes laevigatus*)

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Abstract Chromium poses a potential threat to coastal ecosystems. We used standard toxicity bioassays (semi-static, chronic) to evaluate EDTA as a chelating agent for reducing trivalent and hexavalent chromium toxicity on *Petrolisthes laevigatus*. Crab survival decreased linearly with increased chromium concentrations and dropped significantly beginning at 40 mg/L Cr (VI) and 80 mg/L Cr (III). No significant differences were observed with Cr (III) + EDTA as compared with untreated controls. Cr (VI) toxicity was greater than that of Cr (III), with low individual survival rates. The protective effect of EDTA in the medium increased crab survival by 41%–48%.

Keywords Chromium toxicity · EDTA · *Petrolisthes laevigatus*

Coastal areas receive large quantities of metals from natural continental leaching processes and industrial, mining, and urban activities. In Chile, mining affects the environment through massive material relocations, surface water usage, under-ground water pollution, coastal effluent of municipal and residual waste, and the transport of mineral concentrates (Rodríguez and Rivera 1995). Chromium is a highly toxic trace metal presenting various degrees of risk for coastal ecosystems (Scelzo 1997; Natale et al. 2000). Coastal Cr pollution is due mainly to dumping untreated or poorly treated industrial residues (Vutukuru 2003); this Cr is usually found in its trivalent and hexavalent forms (Bruhn et al. 1997). Cr (VI) is 30 times more toxic than Cr (III) and can be mutagenic and carcinogenic.

Cr occurs naturally off central Chile's coast (0.01–0.04 μg Cr L^{-1} in seawater; 35.3–51.5 μg Cr g^{-1} in sediments) (Ahumada Unpub. recent data). However, high concentrations of Cr are toxic. Natural or artificial chelating agents may modulate its harmful effects (Sperling et al. 1992), sequestering certain deleterious ions from solutions in the water (Lawrence et al. 1981). Ethylenediaminetetraacetic acid (EDTA), a well-studied chelating agent, can bond ions at two or more sites on the molecule. Although Spínola et al. (1999) demonstrated the high stability of the complex formed between Cr (III) and EDTA, few studies have considered the application and use of EDTA in marine environments.

San Vicente Bay in central Chile receives high levels of industrial input. Ahumada and Vargas (2005) estimated industrial effluents entering the bay to have total Cr levels of ~3.12 kg 20 h^{-1}. These authors used suspended sediment traps to determine the Cr sedimentation rate (1.97 mg m^{-2} h^{-1}) in one bay sector and the mean concentration of Cr (128.81 ± 1.10 μg g^{-1}) in sediments near a submarine effluent site. Here, bioaccumulation factors of 1,000× were determined for *Cancer coronatus* and *Geminus ovatus*, a bivalve filter feeder (Ahumada 1994). We selected a priori the porcelain crab *Petrolistes laevigatus*, a widely distributed coastal species (11° S-43° S) (Fig. 1), for Cr monitoring and ecotoxicological evaluations. This species is found throughout an extensive salt marsh near the mouth of San Vicente Bay, where average Cr levels in the sediments reach 35.5 ± 16 μg g^{-1} (n = 14) (Ahumada Unpub.). The present study evaluates the remediation effect of EDTA on the toxicity of trivalent and hexavalent chromium for *Petrolisthes laevigatus*.

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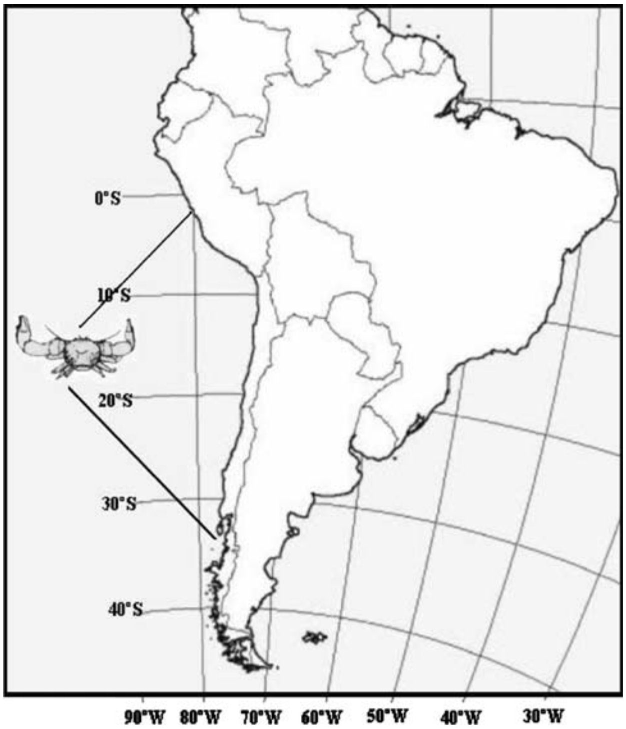

Fig. 1 Approximate distribution of *P. laevigatus* off the Chilean coast

Materials and Methods

Petrolisthes laevigatus (Porcelain crabs) were collected in winter 2005 and 2006 at low tide from an easily accessible, rock-strewn coastal site not subject to significant human impacts (36°45′S-70°10′W) (Moscoso et al. 2006; Fuentes et al. 2005). About 950 adult *P. laevigatus* specimens were collected manually (average carapace: 21.5 ± 1.5 mm × 20.45 ± 1.8 mm). These were acclimated to laboratory conditions for 7 days in aquariums (15 ± 1 °C, 33 ± 1 practical salinity unit, 8.1 ± 1.5 mg/L dissolved oxygen, pH 8.2 ± 0.2, 12:12 photoperiod). Tank water was constantly aerated during both acclimatization and experimentation in order to maintain saturated oxygen concentrations; the water was changed every 48 h following a 2-h feeding period (crabs were fed fish meal pellets ad libitum). Prior to the experiment, crab survival was 100%.

In the semi-static toxicity assays, the incubation water was changed every 48 h (following feeding) for 168 h. Thirty crabs (three replicates, 10 crabs each) were used per test concentration in each system. Analytical grade stock solutions [Cr (III): $Cr(NO_3)_3$, Merck, lot 119779 and Cr (VI): $K_2Cr_2O_7$, Merck, lot 104862] were prepared in filtered seawater. The assayed test concentrations were 0 (control), 20, 40, 80, 100, and 160 mg/L seawater for both Cr (III) and Cr (VI). These concentrations were selected by preliminary testing and per bibliographic information (Boschi 1964); the same concentrations were used for Cr (III) + EDTA and Cr (VI) + EDTA assays (30 mg/L EDTA; Merck, lot 324503). In another control, the crabs were exposed to a solution of 30 mg/L EDTA in seawater.

The data's normality was analyzed with a Shapiro–Wilk test and its homogeneity with a Cochran test. Survival percentages in test and control systems were evaluated with two-way parametric ANOVA followed by a Tukey test to verify the differences between the treatment and control concentrations (STATISTICA version 6.0 2001).

Results and Discussion

No *P. laevigatus* mortality occurred in either control (seawater, EDTA-seawater). Crab survival decreased linearly with increased Cr concentrations in the different assays (Fig. 2) and significantly beginning at 40 mg/L Cr (VI) and at 80 mg/L Cr (III). Concentrations >80 mg/L Cr (VI) produced 100% *P. laevigatus* mortality. When EDTA was present in the treatments, however, a significant difference was observed. Survival values neared 40% in treatments with higher Cr (VI) concentrations. Boschi (1964) attained similar results in a study of *Chasmagnathus granulata*; survival decreased by ~45% with 80 mg/L Cr (VI).

EDTA was a more active chelating agent with Cr (III), which, at higher concentrations and without EDTA, caused mortality rates of up to 100%. With EDTA, however, crab survival resembled that of the controls, being comparable to systems with no Cr (III). Systems with EDTA did not differ significantly from the controls (2-way ANOVA; $F(15, 48) = 118.78$; $p = 0.00001$), with similar survival rates (99% ± 3%). This can be explained by the stability of the Cr (III) + EDTA complex (Spínola et al. 1999).

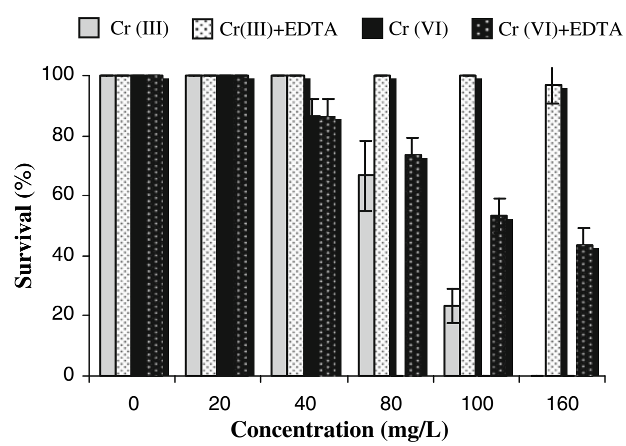

Fig. 2 Mean survival (% ±SD) after 168 h exposure of *P. laevigatus* to increasing concentrations of Cr (III), Cr (III) + EDTA, Cr (VI), and Cr (VI) + EDTA (n = 30)

P. laevigatus was less sensitive to Cr (VI) than *Ceriodaphnia dubia* (planktonic daphnid) (50% survival at 0.0053 mg/L) (Hickey 1989) but more sensitive than *Oncorhynchus tshawytscha* (50% survival at 189.6 mg/L) (Hamilton and Buhl 1990). The US EPA established range limits for Cr in different marine species, including fish (7–400 mg L^{-1}), daphnia (0.01–0.26 mg L^{-1}), and algae (0.032–6.4 mg L^{-1}). The values found for *P. laevigatus* herein exceeded the daphnia limits but were lower than the fish limits.

Various metal chelating agents have been tested experimentally (e.g. EDTA, Hydroxymethylaminoethane). These substances help achieve good water quality in culture experiments and for rearing marine organisms, confirming previous reports that found EDTA to facilitate molting in larval crustaceans (Sperling et al. 1992) by reducing the toxic action of metallic ions by chelation (Scelzo 1997; Anderson et al. 1997; Mahapatra and Mishra 2005). Cr (VI) was more toxic than Cr (III), producing low survival rates. EDTA additions increased crab survival from 41% to 48 %. Unnecessary operating costs can be avoided by determining working EDTA concentrations.

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