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RESEARCH ARTICLE

Heavy metal bioconcentration factors in the burrowing crab *Neohelice granulata* of a temperate ecosystem in South America: Bahía Blanca estuary, Argentina

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Abstract

An extensive population of the burrowing crab, *Neohelice granulata*, inhabits the intertidal areas of the Bahía Blanca estuary, a moderately polluted temperate ecosystem located to the southwest of the province of Buenos Aires, Argentina. In order to determine the metal-accumulating ability of this species, concentrations of Cd, Cu, Pb, Ni, Zn, Mn, Cr, and Fe in soft tissues of adult specimens were measured. Subsequently, the bioconcentration factors (BCFs) of all heavy metals were determined using levels of concentrations previously obtained in intertidal sediments. The results showed concentrations above the detection limit in soft tissues of male and female crabs for all metals except Pb and Cr. BCF > 1 were obtained for Cd, Cu, and Zn, indicating that these metals are accumulated and biomagnified. However, BCF values < 1 were found for the rest of the metals (Mn, Ni, and Fe). The findings of metal accumulation in soft tissues of *N. granulata* is of great importance taking into account that this is a key species within this temperate ecosystem, playing a major role in the transference of pollutants to higher trophic levels.

Keywords *Neohelice granulata* · Heavy metals · Bioconcentration factor · Bahía Blanca estuary

Introduction

Aquatic invertebrates accumulate trace metals in their tissues both from the environment as well as via food sources. Although these organisms require certain levels of several metals as copper (Cu), zinc (Zn), manganese (Mn), and iron (Fe) playing essential roles in metabolism, excessive amounts of metals may have toxic effects (Rainbow 1997, 2002; Wang and Fisher 1999).

Marine and estuarine sediments act as a sink for a wide variety of metals sometimes reaching high concentrations. These pollutants are known to concentrate in fine grain sediments since fine particles, such as clay and colloidal materials, are generally surface-active and contain organic matter and Fe/Mn oxide surface coatings, playing an important role controlling the deposition of metals in sediments (Ip et al. 2007). To interpret the toxicological responses of organisms, it is necessary to understand the relationship between the concentration of chemicals in the environment (e.g., water, sediment, or air) and the concentration of chemicals in an organism, or in the target organ(s) of an organism, in contact with that environment (Gobas and Morrison 2000).

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Some benthic crustaceans can use the sediment not only to live in it but also as part of their diet; therefore, it is expected that the concentrations of contaminants in the sediment and in the tissues of these organisms have a certain degree of similarity (Marsden and Rainbow 2004). Studies of metal bioaccumulation from sediments have proved to be useful tools for assessing ecological risks from metal-contaminated sediment and researching the influences on bioavailability and mobility of metals in sediments (Ingersoll et al. 1994; Sundaray et al. 2011; Yang et al. 2012).

Neohelice granulata (Brachyura, Varunidae) is a semi-terrestrial estuarine crab widely distributed along the Atlantic coast of South America from southern Brazil (23°S) to the northern Argentinean Patagonia (41°S) (Spivak et al. 1994). This benthic organism is intimately associated with the sediment of both the supratidal and the intertidal zone, being found in the salt marshes vegetated by the *Spartina densiflora*, *S. alterniflora*, and *Sarcocornia perennis* as well as in tidal flats (Spivak et al. 1997; Escapa et al. 2008). The feeding of crabs differs according to where they are found. In the salt marshes, they are herbivorous–detritivorous while those found in the tidal flats are mainly deposit feeders (Iribarne et al. 1997). The incorporation of metals and other contaminants is intimately related to the close association of these organisms with benthos, which facilitates the income of metals and other contaminants present in sediments. This species is one of the most abundant macroinvertebrates in the estuary (Boschi 1964; Spivak 1997) and is a fundamental link in the trophic web since all stages of the life cycle of this species are important items in the diet of other species such as birds and fish among others. In this sense, *N. granulata* is a key species of this ecosystem, being a fundamental link in the transfer of contaminants through the food chain.

In Argentina, the Bahía Blanca estuary is considered a moderately polluted environment linked to an intense anthropogenic activity on the north shoreline that receives the contribution of diverse pollutants (Arias et al. 2010; Cifuentes et al. 2016), Biancalana et al. 2012, Oliva et al. 2015). There is a discharge of wastewater into the estuary without appropriate treatment, which comes from several urban settlements (Bahía Blanca, Punta Alta, General Cerri, 380,000 inhabitants) and industries (processing of chemicals, oil and plastic). In the inner area of the estuary, there are three sewage treatment plants in the city of Bahía Blanca, capable to treat approximately 215.000 m^3/day. One of the purification plants, called the "Third Basin," was recently installed in the most internal area of the estuary. The treatment plant of greater volume denominated "The big basin of Bahía Blanca" (181.440 m^3/day) owns only a preliminary treatment, and one of the localities of Ingeniero White is out of service. There is also a continuous atmospheric contribution of substances from the use of fossil fuels, fumes, particles in suspension, and products of industrial and urban activities (Cifuentes et al. 2016). Another source of contaminants to the estuary is agricultural activity through the continental runoff of fertilizers (Perillo et al. 2001; Botté et al. 2010).

Several studies have been conducted on the concentration of metals in this species inhabiting the Bahía Blanca estuary (Ferrer 2001; Ferrer et al. 2006; Simonetti et al. 2012, 2013). However, a relationship between the concentrations of metals in the sediment and in these organisms, namely bioconcentration factor (BCF), has not yet been established. Bioconcentration is the process of accumulation by which the concentration of a certain chemical in aquatic organisms exceeds the levels found in the environment (i.e. water or sediment) (Gobas and Morrison 2000; Mountouris et al. 2002). In this sense, the aim of this study was to assess the Cd, Cu, Pb, Zn, Mn, Ni, Cr, and Fe bioconcentration factors in the burrowing crab *N. granulata* from the Bahía Blanca estuary. Therefore, heavy metals were determined in the fine sediments and soft tissues of the crabs, to establish which metals and bioconcentrated in the tissues of these benthic invertebrates, trying to understand a potential trophic transfer through the corresponding food web.

Materials and methods

The Bahia Blanca Estuary is located in the southwestern Atlantic Ocean (38°45′–39°40'S and 61°45′–62°30'W). Is a mesotidal coastal plain estuary extended over 2300 km^2, formed by several tidal channels, islands, and extensive tidal flats with patches of low salt marshes dominated by crab caves of the burrowing crab *Neohelice granulata* (Piccolo et al. 2008).

Sampling of males and females of *N. granulata* was seasonally performed from April 2014 to September 2015 in two locations within the Bahía Blanca estuary (Fig. 1). Cuatreros Port (CP) located in the inner part of the estuary receives the contribution of the Sauce Chico River and the Saladillo de García stream, which drain an area of approximately 1600 km^2 that involve large extensions of agricultural fields (Perillo et al. 2001; Limbozzi and Leitao 2008). Rosales Port (RP), located in the middle part of the estuary, is influenced by Punta Alta city (~ 60 thousand inhabitants) and its sewage outfall, which discharges into the estuary with no treatment.

Samples of intertidal superficial sediment ($n = 3$ for each site) for determination of metal concentrations were taken with PVC cores (100 mm i.d.; 150 mm long). Afterwards, they were kept in polyethylene bags, carried to the laboratory in refrigerated boxes and stored up to maximum of 48 h at 4 °C. Sampling was done in the morning at low tide. In the laboratory, sediment samples were oven dried at 60 ± 5 °C for 4 days. Then, samples were ground in a porcelain mortar and they were sifted through stainless steel meshes until fine particles (< 63 μm) were obtained. Finally, they were stored in plastic desiccators until their analytical treatment.

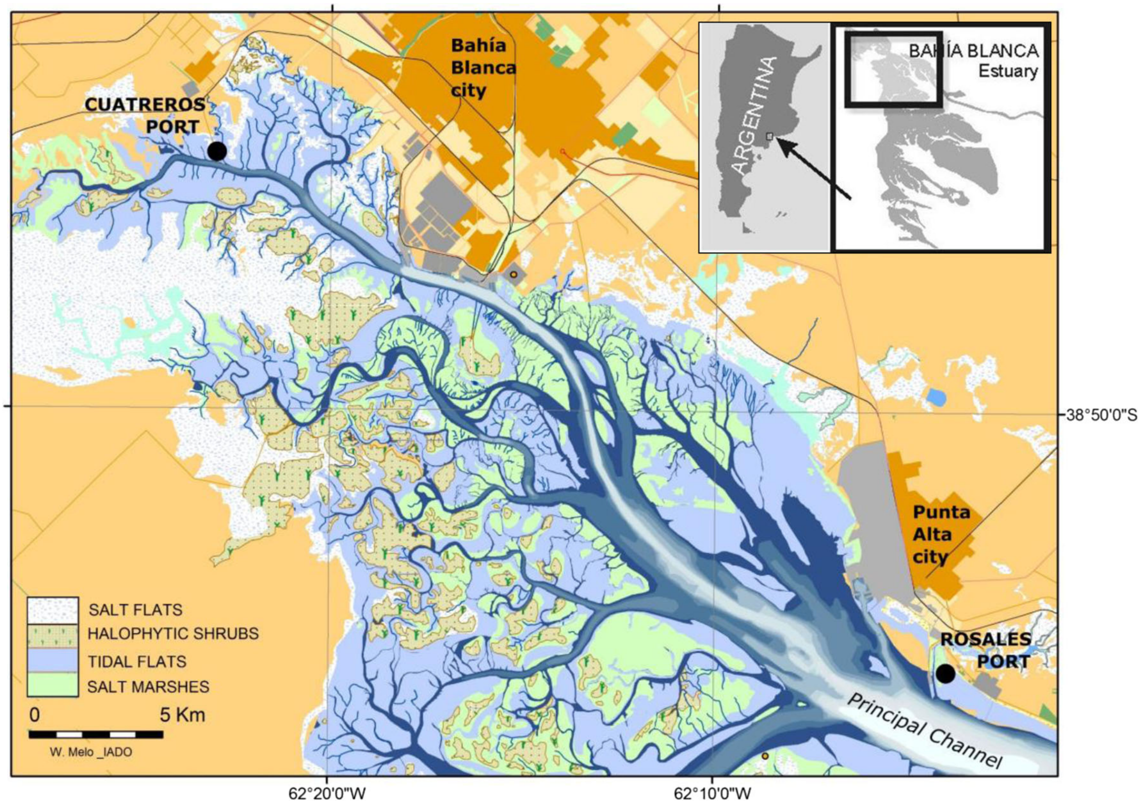

Fig. 1 General map of the study area. Black points are the sampling locations in the Bahía Blanca estuary

Crabs were caught using a crab trap with bait during high tide. Only adult males and females (i.e., maximum width carapace > 15 mm for males and > 17 mm for females, according to Luppi et al. (2004)) were selected. Individuals of each sex were placed in two plastic boxes with estuarine water and were transported to the laboratory where they were washed with distilled water and preserved at − 20 °C until analysis.

The soft tissues obtained by dissection of the crab samples were pooled (five or six specimens) according to sex and location and placed in Petri dishes in an oven at 50 °C until constant weight was reached. Then, dried samples were grinded in a porcelain mortar for homogenization. Acid digestion of crab tissues and fine sediment was performed according to the methodology described by Botté et al. (2010). Samples of 500 ± 50 mg were mineralized with 5 ml concentrated HNO_3 (65%, Merck) and 1 ml concentrated $HClO_4$ (70–72%, Merck) and placed in a heated glycerin bath at 120 ± 10 °C until complete mineralization. The residue was completed with diluted HNO_3 (0.7%) up to 10 ml into centrifuge tubes. All glassware and equipment used for dissection and drying of samples were cleaned with diluted nitric acid (5% *v*/*v*) to prevent contamination.

Metal concentrations (Cd, Cu, Pb, Ni, Zn, Mn, Cr, and Fe) were determined by inductively coupled plasma-optical emission spectroscopy (ICP OES Optima 2100 DV Perkin Elmer). The method detection limit (MDL) was calculated as $MDL_m = t_{(n-1,\ 1-\alpha = 0.99)} \times S_m$, where m corresponds to each metal and S corresponds to the standard deviation of 15 method blanks (EPA 2016). The MDLs, expressed in $\mu g\ g^{-1}$, were as follows: Cd 0.017, Cu 0.136, Pb 0.209, Zn 0.276, Mn 1.854, Ni 0.214, Cr 0.185, and Fe 2.674.

Analytical quality was checked against certified reference material (pond sediment flour R.M.No. 82) provided by The National Institute for Environmental Studies (NIES) from Tsukuba University (Japan). The recovery percentages for all analyzed metals were as follows: Cu 71%, Pb 105.6%, Zn 107.5%, Mn 82.3%, Ni 105.1%, Cr 91.5%, and Fe 76%. All concentrations are expressed in parts per million (μg/g) on a dry weight (dw) basis. For samples that were lower than the applied analytical MDL, a value of one half the detection limit was assigned (Jones and Clarke 2005). These samples were considered in the dataset for statistical treatment only if they represented less than 40% of the total samples. Otherwise, only descriptive statistics was performed using the detectable values.

Two-way analysis of variance (ANOVA) was performed to analyze possible interactions between sites and sexes. Statistical analyses were carried out with appropriate software (IBM SPSS Statistics 22.0 and Infostat version 2016- Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina).

We used bioconcentration factors (BCFs) to analyze the relationship between heavy metals in crabs and sediment (Mountouris et al. 2002; Mendoza- Carranza et al. 2016). It was calculated as $BCF = C_{org}/C_{sed}$, where C_{org} is the concentration of metal in the organism and C_{sed} is the concentration of the same metal in the environment, intertidal superficial fine sediment in this case.

The results of heavy metal concentrations found in the intertidal sediments are analyzed in detail in Simonetti et al. (2017).

Result and discussion

Heavy metals in burrowing crabs (*Neohelice granulata*) and sediments as well as bioconcentration data are shown in Table 1. The concentration of all the metals (except Mn) in

Table 1 Metal concentration (μg/g dw, Fe mg/g dw) in intertidal fine sediment and burrowing crabs from the Bahía Blanca estuary (bioconcentration factor (BCF): tissue/sediment)

	Cd	Cu	Pb	Zn	Mn	Ni	Cr	Fe
Cuatreros Port								
Sediment ($n = 18$)[a]								
Mean	0.037	12.56	7.31	34.01	346.86	8.77	12.2	21.06
STD	0.003	1.61	2.17	9.36	82.11	2.09	3.14	21.37
Min	<MDL	8.86	2.98	13.61	224.45	5.18	5.18	18.02
Max	0.04	15.51	12.34	48.72	516.10	11.37	16.37	24.11
N°‹ MDL	9	0	0	0	0	0	0	0
Male soft tissue ($n = 12$)								
Mean	0.46	142.56	–	47.74	57.72	0.67	–	1.10
STD	0.13	32.1	–	7.42	15.03	1.01	–	0.41
Min	0.31	76	<MDL	35.37	40.50	<MDL	<MDL	0.39
Max	0.73	174.35	–	59	98.21	2.56	–	1.60
BCF	19.67	11.35	–	1.40	0.17	0.08	–	0.05
N°‹ MDL	0	0	12	0	0	9	12	0
Female soft tissue ($n = 11$)								
Mean	0.43	107.72	–	47.98	36.37	–	–	0.73
STD	0.13	26.99	–	6.93	10.15	–	–	0.44
Min	0.20	55.21	<MDL	34.10	20.87	<MDL	<MDL	0.12
Max	0.65	141.18	–	58.75	59.06	–	–	1.48
BCF	18.44	8.58	–	1.41	0.11	–	–	0.04
N°‹ MDL	0	0	11	0	0	11	11	0
Rosales Port								
Sediment ($n = 18$)[a]								
Mean	0.07	31.32	9.97	78.82	303.98	10.33	14.82	23.12
STD	0.01	6.40	2.49	20.72	46.02	1.32	3.25	27.73
Min	0.04	19.36	6.80	46.54	220	8.20	9.18	18.24
Max	0.11	43.69	11.85	111.35	383.65	12.22	19.48	26.85
N°‹ MDL	0	0	0	0	0	0	0	0
Male soft tissue ($n = 10$)								
Mean	–	166.64	–	49.49	68.22	2.80	–	1.09
STD	–	34.72	–	6.88	15.02	0.61	–	0.37
Min	<MDL	122.85	<MDL	34.30	49	2.10	<MDL	0.52
Max	–	232.65	–	58.10	97.69	3.82	–	1.59
BCF	–	5.32	–	0.63	0.22	0.27	–	0.05
N°‹ MDL	10	0	10	0	0	0	10	0
Female soft tissue ($n = 7$)								
Mean	–	110.32	–	50.96	58.45	2.68	–	1.03
STD	–	19.08	–	4.13	18.98	1.41	–	0.61
Min	<MDL	92.65	<MDL	44.02	40	<MDL	<MDL	0.56
Max	–	146.45	<MDL	56.49	85.03	4.40	–	2.13
BCF	–	3.52	–	0.65	0.19	0.23	–	0.04
N°‹ MDL	7	0	7	0	0	1	7	0

[a] Values taken from Simonetti et al. (2017)

the fine sediment was significantly higher ($p < 0.005$) in RP than in CP (Simonetti et al. 2017). However, the concentrations found in burrowing crabs at both sites did not exhibit the same behavior.

Of the eight metals determined, Pb and Cr were found in concentrations below the detection limit in all crab tissue samples (Table 1) which coincide with previous studies of this species in the estuary (Ferrer 2001; Simonetti et al. 2012, 2013). These results place these organisms in a lower range than those registered for crabs from other parts of the world (Table 2). The availability of metals to be bioaccumulated is dependent upon the concentration of the element within the sediment as well as its geochemical behavior and physical-chemical conditions within that matrix. On the other hand, for crustaceans, several intrinsic factors, for instance age, size, stage in the molt cycle and reproductive cycle, may influence bioaccumulation process (Marsden and Rainbow 2004; Bjerregaard et al. 2005; Chen et al. 2005). Although recent information on the geochemical partitioning of metals in sediments of the Bahía Blanca estuary is not available, previous studies by Botté et al. (2010) in CP found, for potentially bioavailable fractions (i.e., PBF = F1 + F2, according to Marcovecchio and Ferrer (2005)), 55.6% for Pb and 16% for Cr. For RP, the study of Grecco et al. (2011) determined 62.4% for Pb and 18.7% for Cr. This suggests a moderate bioavailability for Pb whereas for Cr, a large part of this metal is found in non-bioavailable fractions, which could explain the results for Cr in crabs from both sites. Taking into account the greater potential bioavailability of Pb in the sediment, all the crab samples however showed concentrations below the limit of detection. The concentrations of Pb below MDL in soft tissues of *Neohelice granulata* could be explained considering both the facility of this metal to produce complex with organic matter in estuarine environments (Town and Filella 2002; Shank et al. 2004; Louis et al. 2009) as well as the high charge of organic matter as recorded within the Bahía Blanca estuary (Marcovecchio and Freije 2004; Marcovecchio et al. 2009). A second possible explanation could be that a detoxification of this metal is occurring. Previous studies indicate that molting as a mechanism for detoxification may vary depending upon the particular element and crustacean species (Bergey and Weis 2007). Moreover, Pb is known to be readily absorbed in calcium carbonate skeletons (Ahmed et al. 2011). Ferrer (2001), working with metals in soft tissues and shells of this same species in the Bahía Blanca estuary, found that for Pb, concentrations above the limit of detection were only obtained in shells of males and females, whereas in soft tissues of both sexes, Pb levels were not detectable. Based on this evidence, this species would be eliminating the Pb from its tissues through the molt cycles.

Cd concentrations in the fine sediment of CP and RP were mostly below the limit of detection (Table 1). Similarly, concentrations of this metal in crab tissues from RP were in all

Table 2 Comparison of metal concentrations (μg/g dw) measured in *N. granulata* with concentrations reported in marine crabs in other studies around the world

Species	Site	Cd	Cu	Pb	Zn	Mn	Ni	Cr	Fe	References
Neohelice granulata	EBB	<MDL–0.73	55.21–232.65	<MDL	34.10–59.0	20.87–98.21	<MDL–4.4	<MDL	124.0–2127.5	This study
Portunus pelagicus	Mersin Bay, Turkey	0.50–1.47	9.64–72.50	0.10–0.52	37.80–178.61	–	–	0.31–0.45	7.31–25.51	Ayas (2013)
Calinectes sapidus	Mersin Bay, Turkey	0.44–1.07	9.72–68.09	0.24–0.56	39.52–175.21	–	–	0.24–0.61	8.81–32.48	Ayas and Ozogul (2011)
Portunus pubescens	Daya Bay, China	0.38–0.68	16.60–26.70	0.40–1.80	49.70–69.90	–	–	–	–	Qiu (2015)
Chaceon quinquedens	Gulf of Mexico	0.48–2.24	–	4.56–33.84	161.0–326.0	–	0.96–325	1.02–630	–	Perry et al. (2015)
Callinectes danae	Santos Estuarine System, Brasil	0.012–0.02	3.50–20.10	0.003–1.73	20.10–33.80	1.2–3.49	<MDL–0.03	0.005–0.50	6.0–21.0	Bordon et al. (2012)
Sesarma mederi	Upper Gulf of Thailand	3.08–4.49	1.30–2.10	2.51–7.88	0.65–4.93	–	–	–	–	Chaiyara et al. (2013)
Macrophthalmus japonicas	Coastal areas of Korea	0.09–0.35	37.01–88.60	1.02–2.64	40.08–64.61	–	1.03–2.80	0.51–4.11	–	Na & Park (2012)

cases below the detection limit. In contrast, even though levels of this metal in crabs from CP were not high, all the samples were found above the limit of detection, in both females and males, and these concentrations are within the values found in the literature, except when is compared with *Sesarma mederi* crab (Chaiyara et al. 2013) (Table 2). It is evident that there is some other source of Cd from which the crabs would be incorporating this metal. A possible explanation could be the atmospheric deposition, which has been suggested by several authors as a possible source of heavy metals to the system (Galloway et al. 1982; Wong et al. 2003; Sabin et al. 2005; Kumar Sharma et al. 2008), but so far, there are no studies within this estuary proving the entry through atmosphere. A second possibility could be from agricultural sources. The agricultural and livestock activities carried out in the area make intensive use of fertilizers, herbicides, and other agrochemicals. In these farming activities, some herbicides such as glyphosate, that is a chelating agent for heavy metals and organic cations, could potentially affect the bioavailability, toxicity, and bioaccumulation of heavy metals when applied directly into the aquatic ecosystems (Tsui et al. 2005).

According to Vassiliki and Konstantina (1984), a BCF value of < 1 is expected for most of the metals; otherwise, bioconcentration of metals by organisms will occur. The results of this study showed a BCF < 1 for Pb, Cr, Ni, Mn, and Fe (Table 1). However, the BCF obtained for Cd (i.e., in crabs from CP), Cu, and Zn were greater than 1. In the case of Cd, the high BCF was due to greater concentrations found in the organisms than in the sediment, which allows to infer that there is some degree of active accumulation of this metal in the soft tissues of *N. granulata*. On the other hand, previous studies on this species in the Bahía Blanca estuary have shown

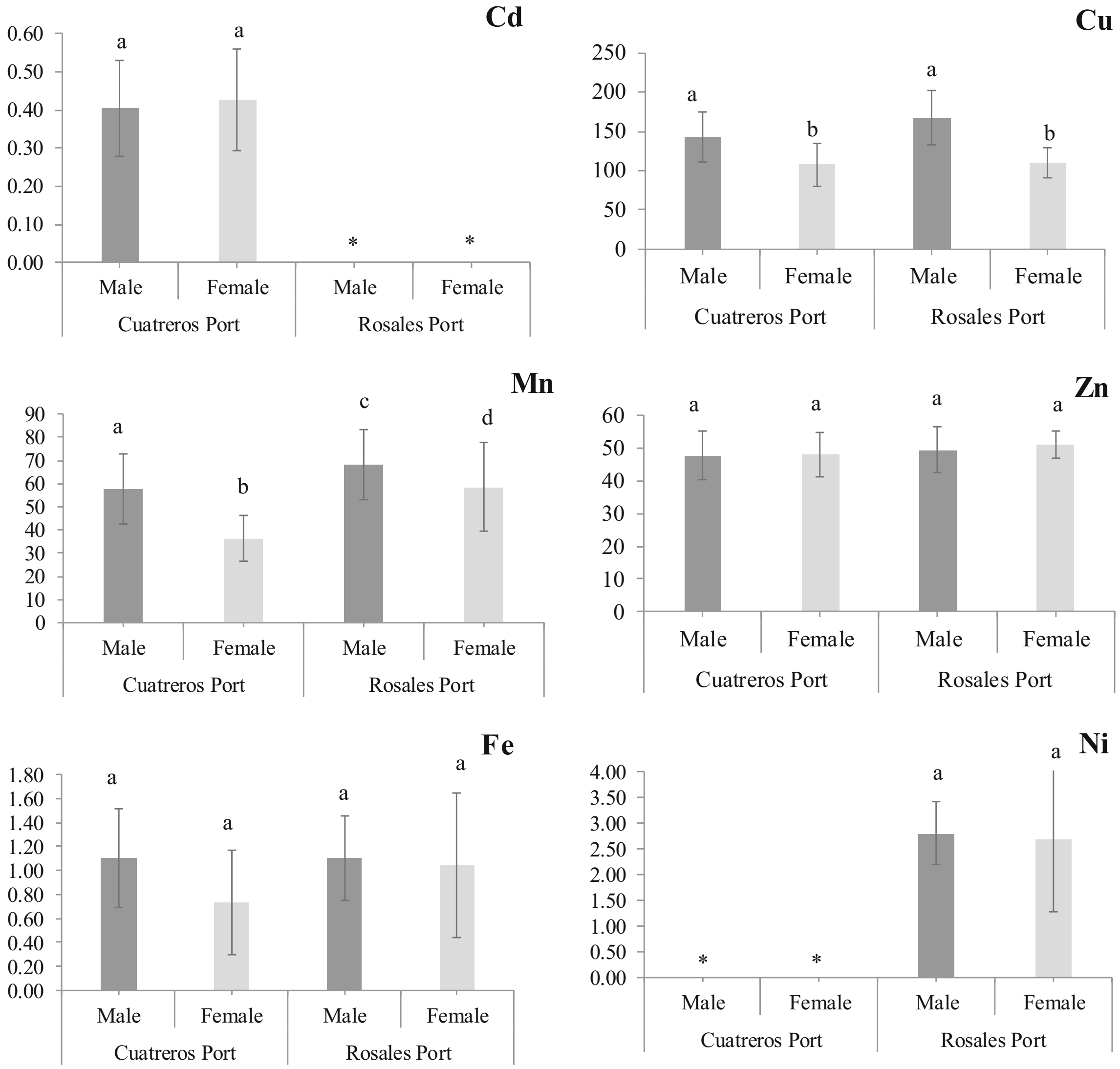

Fig. 2 Mean metal concentrations (μg/g dw, Fe mg/g dw) in soft tissues of males and females burrowing crabs *N. granulata* from both locations of the Bahía Blanca estuary. Different letters for each metal indicates significant difference ($p < 0.05$). *Concentrations below the limit of detection

high concentrations of Cu and Zn, two essential metals for these organisms (Ferrer 2001; Simonetti et al. 2012, 2013). Cu is an integral part of the respiratory pigment hemocyanin of decapod crustaceans and Zn is associated with the activity of several enzymes involved in various metabolic pathways and is also an activator of other enzyme systems (White and Rainbow 1985, Rainbow 1997, MacFarlane et al. 2000; Harris et al. 2018). The concentrations of Cu found in *N. granulata* tended to be higher than the crabs of other coastal areas around the world; meanwhile, Zn concentrations were within the range of concentrations described in the literature (Table 2). Although the metabolic requirements for this species are still unknown, taking into account the concentrations of Cu and Zn found in sediment and organisms, it could be inferred that, as with Cd, there is a certain level of accumulation of these metals in the soft tissues of *N. granulata*.

Considering that the BCF does not have the same meaning for organisms if the metals that are being bioconcentrated are essential or not essential, it is worth noting that those metals that presented BFC > 1 in this study were Cu, Zn (i.e., essentials), and Cd (i.e., non-essential). Although non-essential metals are toxic to organisms even in traces, it is important to note that the essential metals can also produce toxic effects if the levels to which they are accumulated are excessively high. In this sense, it is necessary to determine the metabolic requirements of these metals in *N. granulata* to be able to establish if bioconcentration levels found are potentially toxic for this species.

Differences between sites and/or sexes were analyzed (i.e., two-way ANOVA for Cu, Zn, Mn, and Fe; student *t* test for Cd and Ni). Figure 2 shows the differences between sites and sexes for each metal. Although it was not possible to perform a statistical analysis for sites, the figure shows clear differences for Cd and Ni. Meanwhile, significant differences between sites were only found for Mn, with concentrations significantly greater in crabs from RP (Mn: 64.20 ± 16.93 μg/g dw vs 47.51 ± 16.69 μg/g dw, $p = 0.002$).

According to the literature, there is no consensus regarding sex as a biological intrinsic factor that may influence the bioaccumulation of metals in crustaceans. Several studies with various species of crabs found a lack of relationship between sex and the accumulation of metals in the tissues. Examples include the researches of Kannan et al. (1995) working with the marine crab *Tachypleus tridentatus*, Olusegun et al. (2009) with the crab *Callinectes amnicola*, or of Ayas (2013) studying adult blue swimmer crabs (*Portunus pelagicus*), among others. Meanwhile, in the case of the shrimp *Pleoticus muelleri*, Jeckel et al. (1996) found sex differences for Cu and Zn. Chen et al. (2005) also found differences between sexes for the accumulation of certain metals in the rock crab *Thalamita crenata*.

In this study, significant differences between sexes were found for Cd (RP only), Cu, Mn, and Ni in both sites ($p < 0.003$ in all four metals) being superior in males than females in all cases (Fig. 2). Previous studies in crabs of CP showed no differences between sexes for any of these metals (Simonetti et al. 2012, 2013). However, in those studies, the sample size was quite small, so that the lack of differences could be related to that factor. For RP, to date, there are no data published in journals on the metal concentrations in crabs; thus, there are no previous data to compare the results obtained in this study.

Conclusions

The results of this study showed concentrations above the detection limit in soft tissues of male and female crabs for six of the eight analyzed metals. This suggests that metals would be bioconcentrated in the tissues of these benthic invertebrates. On the other hand, when the concentrations of metals in the sediment were taking into account, BCF > 1 was obtained only for Cd and the essential metals Cu and Zn so it can be inferred that there is a certain level of bioconcentration of these metals in *N. granulata* from the Bahía Blanca estuary. In this study, differences between sexes for the concentration of Cd, Cu, Mn, and Ni have been recorded. It would therefore be advisable to keep them separate in future studies.

The knowledge of metal accumulation in soft tissues of *N. granulata* is of great importance taking into account that this is a key species within the Bahía Blanca estuary, playing a major role in the transference of pollutants to higher trophic levels. In this sense, it is worth noting that these are the first results obtained for this species in Rosales Port; therefore, they will be of great utility for subsequent studies of environmental monitoring.

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