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ASSESSMENT OF HEAVY METAL ACCUMULATION IN *OPUSIA INDICA* (ALCOCK, 1900) (OCYPODOIDEA: CAMPTANDRIIDAE) WITH REFERENCE TO SEDIMENT CONTAMINATION FROM COASTAL AREAS OF PAKISTAN

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ABSTRACT

Opusia indica (Alcock, 1900) is the small and sluggish crab. They are common inhabitants of marine environments. Their burrowing behavior plays an important role in material cycling in the environment. It was hypothesized that these crabs are nourished by organic matter extracted from surface sediment; therefore, they may indicate the substrate contaminations and accumulate these contaminants in their body in higher amounts. Hence, to evaluate the heavy metal concentrations in crab as the reference to heavy metal contaminations in sediment, *O. indica* collected from two coastal areas of Pakistan, i.e. Port Qasim and Sonari. The significant differences were observed for Fe, Cu, Ni and Cr concentrations in sediment. All metal accumulations showed significant differences between the sites in crabs, except Co. The sediment biota accumulation factor (SBAF) of Cu, Zn, Co and Cd were evaluated greater than 1.0, as identified the active bioaccumulation of these metals in crabs. The linear relationship was observed between all metals in the sediments and crabs, except Co and Cd, which indicated that metals accumulation in crabs were highly associated with the substrate metal loads. The present results indicated the ecological significance of crab, *O. indica*, as potential accumulator and biomonitor species for metal contamination in coastal areas of Pakistan.

KEYWORDS: Bioaccumulation, Deposit feeder crab, Heavy metals, Sediment contamination, Ocypodid crab, Pakistan.

INTRODUCTION

The heavy metal pollution in the aquatic environment can be estimated through analyses of water, sediments and marine organisms (Morillo *et al*., 2005). Sediment acts as a primary sink and secondary source for the numerous contaminants in the marine environment, moreover they also reveal the chronological settlement of contaminants and their origination (Marchand *et al*., 2006; Sany *et al*., 2013; Saher and Siddiqui, 2016). These metals accumulate as contaminants in higher concentrations than those observed in the aqueous phase and produce negative effects on the benthic biota as well as on the organisms that feed on these sediments. Therefore, sediment is more appropriate in monitoring programs because of their persistence and ecological importance in marine environments (Morillo *et al*., 2005; Guerra-García *et al*., 2010). Crustaceans are well known to accumulate metals from surrounding waters and sediments by adsorption or direct ingestion from food, water and/or sediment (Bryan, 1971; 1979).

Opusia indica (Alcock, 1900) belonging to the super family Ocypodoidea and family Camptandriidae. These are small and sluggish crabs and a common inhabitant of mangrove and coastal areas (Tirmizi and Ghani, 1996; Ng *et al*., 2009). They are deposit feeder crabs, rely on organic matter for their food and are associated with either fine (clay or mud) or coarse sediment, therefore they are considered as a linkage between the primary consumers and consumers of higher trophic levels (Fielder and Jones, 1979; Skov and Hartnoll, 2001; Saher and Qureshi, 2011). They play an important role in the ecological functioning, nutrient cycling, and energy flow in the mangrove ecosystem through their feeding and burrowing activities (Fielder and Jones, 1979; Robertson, 1986; Saher and Qureshi, 2011). Several studies have reported on metal accumulation in crustaceans (Sanders, 1984; Rainbow, 1985; White and Rainbow, 1982; Rainbow, 1997; Bu-Olayan and Subrahmanyam, 1998; MacFarlane *et al*., 2000; Guerra-García *et al*., 2010; Reichmuth *et al*., 2010; Na and Park, 2012; Alvaro *et al*., 2016) and used to assess the contamination and bioavailability of heavy metals in coastal environment.

It was hypothesized that *O. indica* extract their nourishment (organics) from sediment, therefore they may be indicated the substrate contaminations and accumulate these contaminants in their body in higher amounts. Hence, the evaluation of heavy metal concentrations in crab with reference to heavy metal contaminations in sediment makes them a useful biomonitor species along the marine environment of Pakistan. The aim of the study was to evaluate the eight heavy metals concentrations in *O. indica* and sediment from two different sites of Pakistan coast and to compare the relationship between metals levels in crabs and sediments of their habitat.

MATERIALS AND METHODS

Study sites: Two study sites were selected for monitoring the heavy metal contamination load in sediments and their resident biota along the coastal areas of Pakistan (Fig. 1). The first study site located near the Port Bin Qasim (PQ), it is westernmost part of the Karachi coast. It is an intertidal muddy area with mangrove vegetation (*Avicenna marina*). The second site located at Sonari (So), which situated between Cape Monze and Gadani about 40 miles northwest of Karachi. It is extending two miles from the seacoast and is largely dominated by a tidal stream with the characteristic sand flat and no mangrove vegetation.

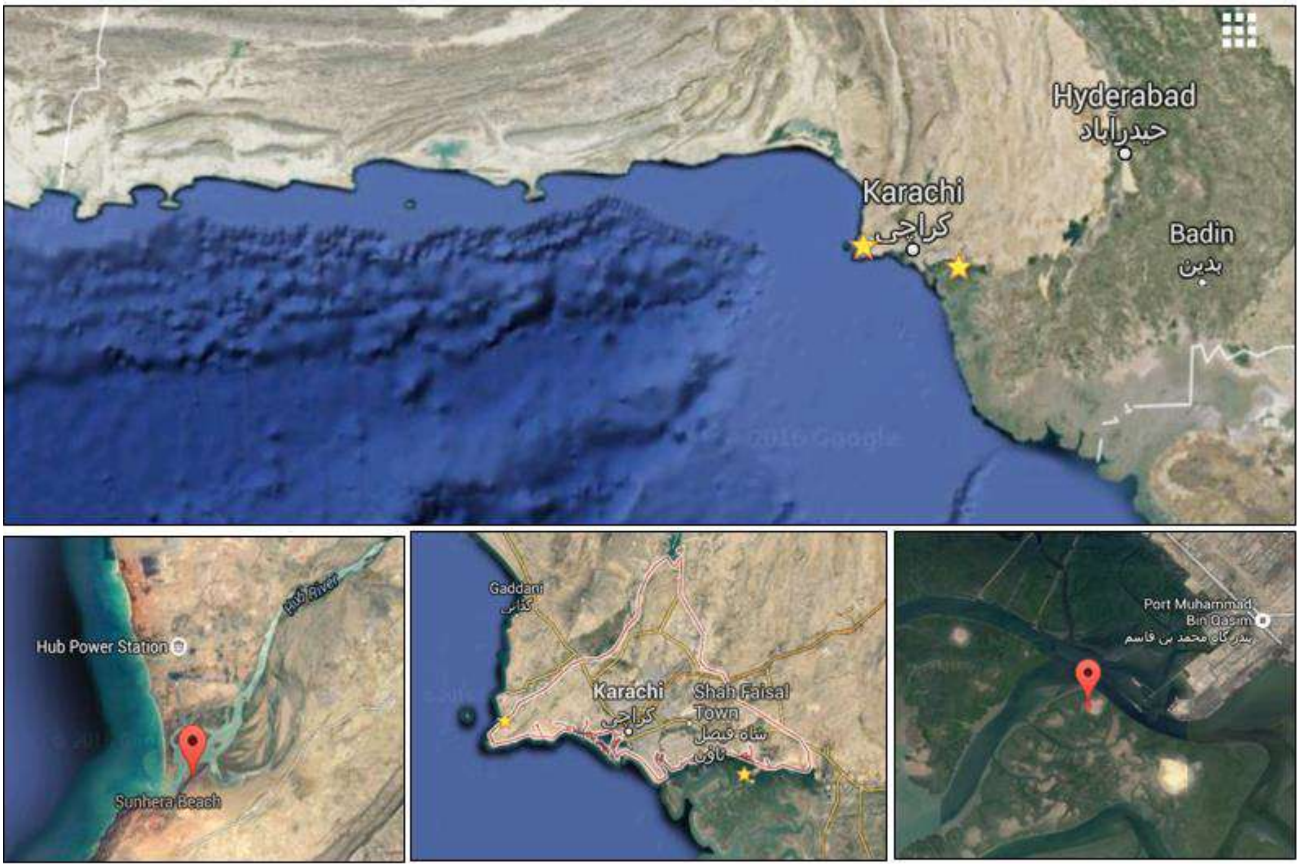

Fig. 1. Map of the coastline of Pakistan, magnified view of study sites along the coast of Pakistan (Google Map).

Sampling procedure: The sampling of crabs and sediment was carried out during Oct. and Nov. 2011 by transect and quadrat method from two coastal areas of Pakistan. The 0.25 m^2 quadrat placed (10 m apart) and excavated up to 30 cm depth and crabs were collected in labelled polyethylene bottles. Three sediment cores (20 cm long) collected from each site. The crab and sediment samples stored in the icebox and brought back to the laboratory.

Laboratory analysis: The individuals of *O. indica* were sorted and washed with plenty of distill water then were pooled to make a composite sample from each site for heavy metals analysis. All crab samples were oven dried at a constant weight, then grounded and homogenized. Dry crab samples (1.0 g) were mixed with 5 mL of concentrated hydrochloric acid (HCl) and 2 mL of nitric acid (HNO_3) and heated to near dryness then added 10 mL of hydrogen peroxide (H_2O_2). Samples were filtered and diluted to 50 mL using distilled water (Leung and Furness, 1999). Approximately, 1.0 g (<63 μm) dry sediment sample was mixed with 10 mL mixture of aqua regia and heated at 80ºC for an hour. After cooling at room temperature samples were filtered, then diluted to 50 mL using distilled water (Saher and Siddiqui, 2016). Crab and sediment samples were analyzed for the eight metals (Fe, Cu, Zn, Co, Ni, Cr, Pb, and Cd) by using Atomic Absorption Spectrometer (Perkin Elmer (USA), model A Analyst 700).

Data analysis: Spatial discrepancies of heavy metal concentration in sediment and crabs were executed by one-way ANOVA follow by Tukey's comparison test. Sediment biota accumulation factor ($SBAF = C_{Biota} / C_{Sediment}$) was used for investigating metal bioavailability in sediment to crab between the two habitats (Eca *et al*., 2013). Spatial differences in SBAF of heavy metals were also observed by one-way ANOVA follow by Tukey's comparison test. Regression analysis was performed to compare the heavy metal concentrations in crab and exposure concentration of metals in sediments.

RESULTS AND DISCUSSION

Metal contamination in coastal sediments: The mean heavy metal concentrations in coastal sediment of Pakistan are shown in Table 1. Fe, Cu, Ni and Cr showed significant spatial variability ($p < 0.05$) and observed higher in PQ as compared to So. Fe, Cu, Ni and Cr ranged from 986.8–1007.9, 14.97–28.50, 43.93–46.90 and 47.8–89.7 μg g^{-1}, respectively.

These metals showed comparable ranges in coastal sediment with literature reported from different coastal areas of the world (Siddique *et al*., 2009; Mashiatullah *et al*., 2013; Sany *et al*., 2013; Silva *et al*., 2014). These metals originate anthropogenically from various sources such as fertilizers, pesticide, agronomic and urban wastes (Cu), industrial discharges (Cu, Ni, Cr), varnishes, paints and pigments, electroplating, steel and alloys (Ni, Cr) (Cameron, 1992). However, Zn, Co, Pb and Cd concentrations showed no significant differences ($p > 0.05$) between the sites and indicates the similar circumstances of these metals sources in both areas. The main anthropogenic sources of Zn include paints, alloys, metal coatings, copying paper, glass, rubber, and cosmetics (Cameron, 1992). In the aquatic environments Co enter through mining and processing, alloys, cobalt containing chemicals, agricultural surplus, sewage and urban wastes (Hamilton, 1994). The significant anthropogenic sources of Pb are traffic exhaust, lead–zinc smelters, paints and batteries (Saher and Siddiqui, 2016). While, the leading sources of Cd contamination includes electroplating, alloys, coatings, batteries, fungicides, phosphate fertilizers, pigments, rubber, plastics, old motor oil, textile manufacturing and sewage sludge (Cameron, 1992). Most of these anthropogenic sources of above mentioned metals are present in the surrounding areas near monitoring sites.

Table 1. The heavy metal concentrations (μg/g) in sediments, crabs and sediment-biota accumulation factor from two coastal areas of Pakistan (unlike superscript indicates the significant differences between the sites at 0.05).

	Fe	Cu	Zn	Ni	Cr	Co	Pb	Cd
	Metal concentrations in sediment							
PQ	1007.9a	28.50a	58.97a	46.90a	89.75a	4.34a	31.63a	1.22a
So	986.8b	14.97b	38.52a	43.93b	47.83b	7.87a	26.11a	1.13a
Average	996.9	21.74	48.74	45.41	68.79	6.10	28.87	1.17
	Metal concentrations in Crab (*Opusia indica*)							
PQ	489.6a	97.11a	74.11b	22.77a	10.82a	23.30a	26.27a	1.87b
So	329.9b	41.10b	82.03a	17.04b	7.58b	20.89a	24.86b	2.02a
Average	409.8	69.10	78.07	19.91	9.20	22.10	25.56	1.95
	Sediment-Biota Accumulation Factor (SBAF)							
PQ	0.49a	3.57a	1.30b	0.49a	0.12a	6.23a	0.84a	1.54b
So	0.33b	2.76a	2.13a	0.39b	0.16a	2.92a	0.95a	1.80a
Average	0.41	3.17	1.72	0.44	0.14	4.57	0.90	1.67

Heavy metals concentrations in crabs (*Opusia indica*): The average metal concentrations in crabs follow the order Fe > Zn > Cu > Pb > Co > Ni > Cr > Cd for both sites, (Table 1), except Zn and Cu, they shift their positions as Cu > Zn at PQ and Zn > Cu at So. All metals showed significant differences ($p < 0.05$) between the two population of crabs, except Co. Moreover, concentrations of Fe, Cu, Ni, Cr and Pb were observed significant greater at PQ area as compared to So, however the levels of Zn and Cd were observed greater in crabs collected from So. Copper ranged from 41.10–97.11 μg g^{-1} in crab, and presented the 2nd and 3rd highest concentration in crabs among the eight metals collected from PQ and So, respectively. Cu is an essential nutrient in the synthesis of hemocyanin for decapods but above the requirement and/or at elevated concentration this regulatory process terminates and initiate metal accumulation (White and Rainbow, 1982). Zinc exhibited the 2nd and 3rd highest concentration in crabs among the eight metals collected from So (82.03 μg g^{-1}) and PQ (74.11 μg 6 g^{-1}), respectively. For marine crustaceans, the highest concentration recorded in muscle was 57 mg Zn/kg fresh weight in the king crab, *Paralithodes camtschatika* (NAS, 1974), and was associated with two metal binding proteins of molecular weight 11,500 and 27,000 (Eisler, 1981) and it's influenced by seasons and maturation. High zinc concentrations in crustaceans are usually associated with industrial contamination. Molting results in a 33–50% loss of Zn through excuviae and feces in marine crustaceans and play an important role in Zn circulation in the marine environment (Eisler, 1981).

The Ni, Cr and Pb are the non-essential metals and produce toxicity to the organisms, and these metals concentrations were detected greater than the values reported in decapod crustaceans (Bryan, 1976). These metals concentrations also exceeded from recommended levels of metals contamination in sea food by US Food and Drug Administration (USFDA). According to Eisler (1981), the muscle tissue of most marine biota seldom contains nickel concentrations in excess of 0.3 g/g wet weight, whereas Pb is potential toxic metal and tends to be detoxified by metallothioneins or phosphate granules and stored permanently in tissues (Rainbow, 1997; MacFarlane *et al*., 2000). The high levels of these metals in this deposit feeder crab species indicates the metals incorporation in food chain, which ultimately affect the higher trophic levels in the marine environment. The levels of Co and Cd varied from 20.89–23.30 μg g^{-1} and 1.87–2.02 μg g^{-1}, in two population of crabs, respectively. Cd concentrations in crabs was observed below than the US Food and Drug Administration (USFDA) recommended levels of sea food contamination. In the current study, most of the metals (such as Ni, Cr, Pb, Cd) in *O. indica* were observed greater than as compared to the metals level reported in other Ocypodid crab species, for instance *Macrophthalmus japonicas* (Na and Park, 2012), *Macrophthalmus depressus* (Bu-Olayan and Subrahmanyam, 1998) and *Uca pugilator* (Gbaruko and Friday, 2007). Although, the levels of Cu and Zn in *O. indica* were exhibited lower than the concentrations in *M. depressus* reported from Kuwait coastal areas.

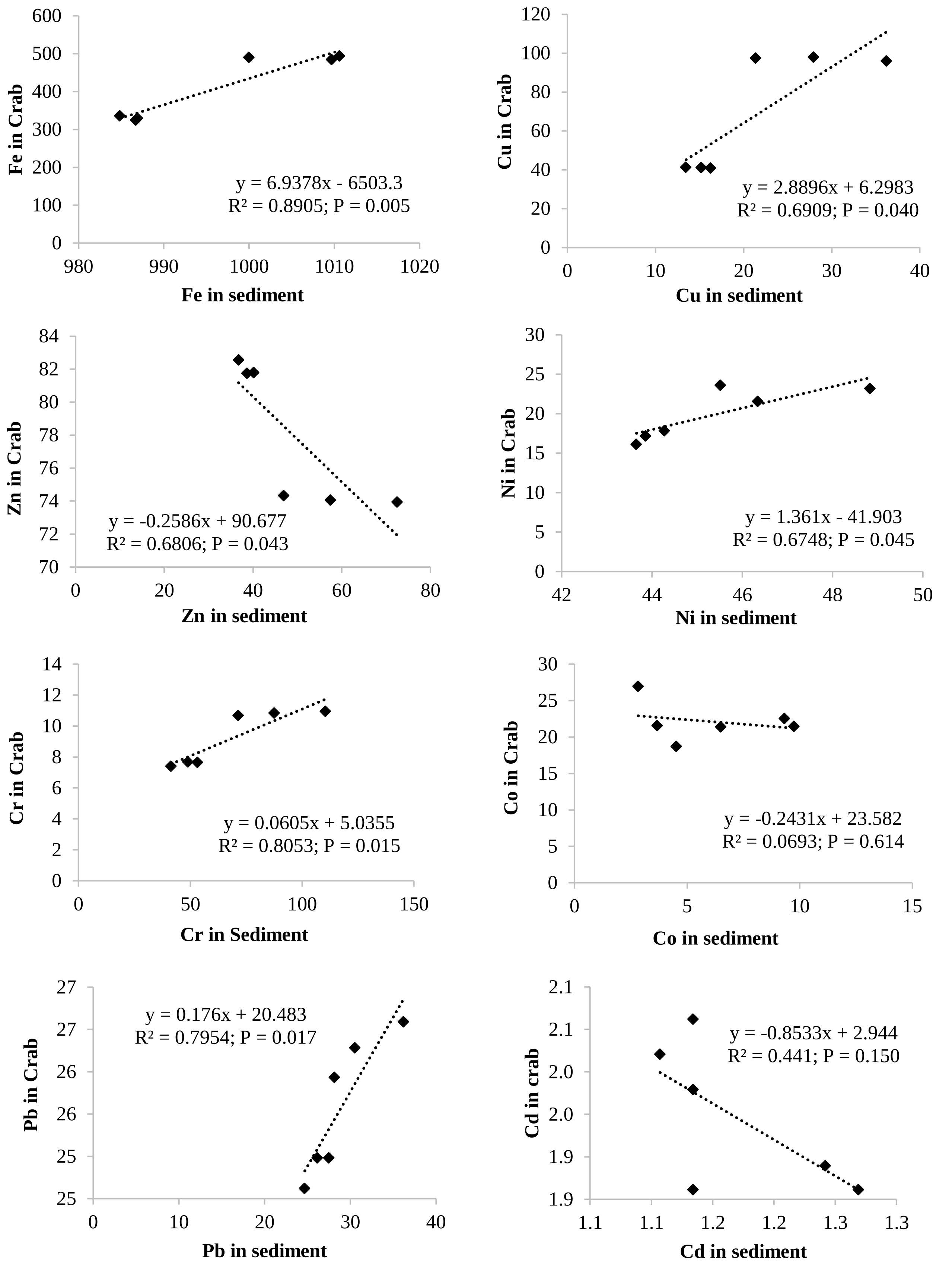

Fig. 2. Relationship between heavy metal concentrations in sediment and crab (significant relationship accepted at 0.05 probability level and shows in bold).

Sediment-Biota Accumulation Factor (SBAF): The sediment biota accumulation factor (SBAF) for each metal in the crabs is summarized in Table 1. Average SBAF in crabs follow the order Co > Cu > Zn > Cd > Pb > Ni > Fe > Cr. No significant differences were observed for SBAF values of Cu, Cr, Co and Pb between the sites, indicates the similar bioaccumulation pattern of these metals in crabs for both sites. The SBAF values showed significant differences between the sites for Fe, Zn, Ni, and Cd. Moreover, the values of Fe and Ni were observed significantly higher at PQ and having the SBAF values less than 1.0, but both metals reflect the sediment concentrations. Whereas, Zn and Cd were observed significantly higher at So as compared to PQ, having the SBAF values greater than 1.0. Interestingly, both metals had no significant differences in sediment but this species showed the variability in accumulating these metals in similar substrate concentrations.

SBAFs higher than 1.0 were found for Cu, Zn, Co and Cd, indicating potential biomagnification. Cu and Zn are essential elements for decapods in different enzymatic activities and hemocyanin synthesis, thus these metals are regulated at a particular level by decapod crustaceans (Bryan, 1971; Rainbow, 1985), but once these requirements are accomplishing, the regulatory process becomes saturated and bioaccumulation starts (Engel and Brouwer, 1991; Rainbow, 1985). This could explain the low concentrations of Cu and Zn in the sediment but high concentrations of Cu and Zn in the crabs as well as their SBAFs values. Co and Cd are non-essential metals, but also were higher than 1.0 for the reason that non-essential metals are not regulated and accumulation can happen at all exposure levels (Brouwer and Lee, 2007; Rainbow, 1985). The molting of the crab *Carcinus maenas* did not decrease Cd concentrations (Bondgaard and Bjerregaard, 2005), which may also explain the high SBAF for Cd observed in this study.

Relationship between metal concentrations in sediment and crab: It would be expected that the higher concentration of a certain metal in sediments, greater the quantity of metal will be in the organisms; due to the influence of that particular sediment. The metal concentrations in crabs were compared with metal levels in sediments, which showed that this species presented significantly higher values for Cu, Zn, Co and Cd than those observed in sediment (Table 1). This is an indicative of the occurrence of bioaccumulation of these elements since *O. indica* live associated with the sediment. However, Cu and Zn are the essential elements, while, Co and Cd present no biological function for them. The crabs were significantly lower ($p < 0.05$) concentrations of Fe, Ni, and Cr than sediments, whereas no significant differences were noticed between matrices in the case of Pb concentrations.

Corresponding correlation coefficients derived to describe the relationships between metals concentration in sediment and crabs are provided in Fig. 2. The significant linear relationship was evaluated for Fe ($R^2 = 0.8905$), Cu ($R^2 = 0.6909$), Zn ($R^2 = 0.6806$), Ni ($R^2 = 0.6748$), Cr ($R^2 = 0.8053$) and Pb ($R^2 = 0.7954$) concentrations in the crabs with those in the sediments. The linear correlations between metal concentrations in the surface sediments and that found in the crabs indicate that some of the metals held in the sediments may become available to the crabs (Na and Park, 2012). The inverse correlations were assessed between Zn concentrations in the sediments and crabs, may indicate the Zn regulation in crabs (White and Rainbow, 1982) or inhibit accumulation at elevated concentrations (Simkiss and Taylor, 1989). However, significant relationships were not observed for Co ($R^2 = 0.0693$) and Cd ($R^2 = 0.441$). This difference may be attributed to different relationships between sediment and crab for different heavy metals.

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