HEAVY METALS CONCENTRATION IN SEDIMENTS, MANGROVE LITTERFALL AND TISSUES OF *CARDISOMA CRASSUM*.

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ABSTRACT

Research in mangrove forests has recently become an important issue. Concentrations of Al, As, Ba, Cu, Cr, Fe, Ni, Pb, Sr, Si, V and Zn in sediments, mangrove litterfall, water and bioaccumulation in muscle tissue and shell of *Cardisoma crassum* were determined in the "Estero El Salado", Mexico, which is an important protected area located in the middle of Puerto Vallarta city. Samples were collected in two sites (A and B) and were analyzed by inductively coupled plasma optical emission spectrometry. Concentrations in sediments were: Al>Fe>Ba>Si>Sr>Zn>Pb>Cu>Cd in both sites. In water, samples concentration order was: Si>Sr>V>Fe>Al>Cu, with half of the elements analyzed below detection limits. Concentration order in mangrove litterfall was: Fe>Al>Si>Sr>Ba>Zn>V>Cu>As, for both sites; except that As was not detected in site B. In muscle samples of *Cardisoma crassum*, the metals detected were: Zn>Si>Cu>Sr>Fe>Al>V>Ba in site A with few differences in order for site B. In shell, concentrations were: Sr>Ba>Si>Al>Fe>V>Zn>Cu (both sites). Concentrations in muscle were higher than in other type of samples for Cu, Si and Zn, while concentrations of Sr and Ba were remarkably high in shell. There were significant differences between biological samples for Ba, Cu, Sr, V and Zn ($P<0.05$).

Keywords: Mangrove, Protected Area, Heavy Metals, Bioaccumulation Factor

INTRODUCTION

The mangrove forests in tropical coastal lagoons represent one of the most productive ecosystems in the biosphere, they provide important environmental services (storm surge protection, feeding grounds, coast erosion protection, nesting sites, fisheries, etc.), they have a high biodiversity, and recently their social and economic importance has been recognized. Mexico has around 5% of the world´s total mangrove forests area [1], [2]. Nowadays the principal threats to mangroves are anthropogenic activities, including pollution, aquaculture, urban and touristic developments and over-exploitation [3]. Designation of Natural Protected Areas (NPA) is one of the best tools for conservation of ecosystems in Mexico. Our study area is the "Estero El Salado" NPA, which is an "urban estuary", for being surrounded by the municipal area of Puerto Vallarta city, which is an important touristic destination in Mexico. Due to its location the Estero El Salado is threatened by solid wastes, municipal wastewater, heavy metals, hydrocarbons and feral fauna.

Heavy metals represent an important pollution threat to mangrove forests because their potential toxicity and its affinity to organic matter and fine particles that commonly compound sediments in these ecosystems [3]. Pollution research in mangroves has been done for several decades with the purpose of encourage their conservation, however the variability of metal pollution within the inherent complexity of trophic webs (mainly detritus food web) in these ecosystems has not been fully understood.

The "Estero El Salado" has an abundant population of the detritivores mouthless crab, *Cardisoma crassum*, which is relevant in this study for being traditionally extracted by the surrounded inhabitants for consumption purposes in the rainy season.

The aim of our study was the quantification of heavy metal concentrations in mangrove litterfall (detritus), sediments and water and also in muscle tissue and shell of the crab *Cardisoma crassum* to assess metals accumulation. We also evaluate if there is a potential risk exposure to metals derived from the traditional consumption of crabs by the inhabitants in this area.

MATERIALS AND METHODS

Study Area

The "Estero El Salado" NPA, is an urban estuary situated in the city of Puerto Vallarta, Jalisco, Mexico (Fig. 1). It is located on the Mexican Pacific coast, and is an important source of detritus and nutrients that are exported to the Banderas bay. This estuary was declared a Natural Protected Area in

July 2000, and in the last decades this area has been surrounded by the urban sprawl, being more vulnerable to urban activities.

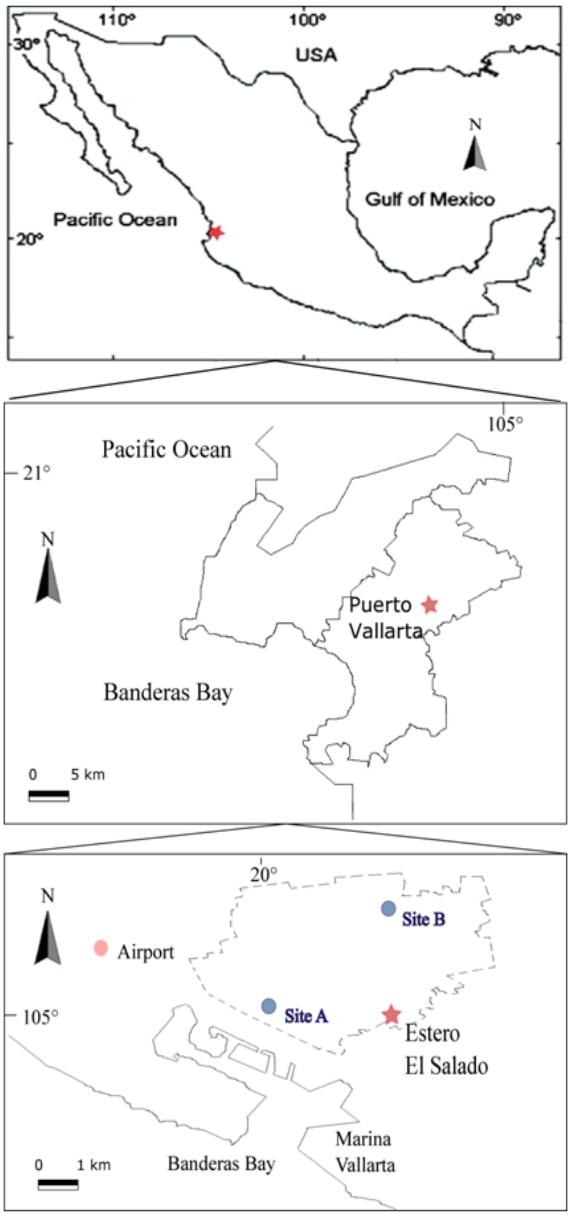

Fig. 1 Study area. Dashed line indicates the NPA; A and B are sampling sites in mangrove forest.

The NPA has 169 ha, of which 125.6 ha are mangrove forest, composed by three species: *Rhizophora mangle*, *Laguncularia racemosa* and *Avicennia germinans* (red, white and black mangroves, respectively). The mangrove forest is surrounded by salt marshes and urban areas.

The sediments are principally composed by fine sands, montmorillonite and kaolinite clays [4], which are typical in mangrove sediments.

Flora and fauna characterization

"El Salado" NPA has about 134 species of birds, 28 species of amphibians and reptiles, more than 25 species of mammals and 43 species of fishes. Several of these organisms are under protection status [5].

Sampling And Analytical Procedure

Samples of mangrove litterfall, water, sediment and organisms of the detritivorus mouthless crab, *Cardisoma crassum* were collected in two zones (A: 13Q 475531 2285975 and B: 13Q 475466 2285889), during the rainy and dry seasons (August and May, respectively). A sediment core sampler (30 x 6 cm) was used to collect sediments (n=12) and superficial mangrove litterfall (n=12), simple bottles were used for collecting the interstitial water (n=12) samples and plastic bags and fishing nets were used for collecting the organisms.

Mangrove litterfall samples were dried in a stove (45°C) until constant weigh was reached [6]. Sediment samples were also dried and then sieved (63µm mesh). Water samples were pre-treated with nitric acid (HNO_3) before transportation. Organisms were frozen and then transported to the laboratory where samples of muscle tissue (n=18) and shell (n=18) were taken, and then dried to constant weight (40° C). All samples were triturated in a mortar, homogenized and stored in plastic bags in a dry cool place [6].

Sediment and water samples were digested using the 3051A and 3015A EPA methods respectively [7], [8]. The method for digestion of muscle tissue was validated comparing three digestion methods. Better recovery percentages (acceptable percentages between 80-120%) were obtained for the digestion with HNO_3 and HCl (INSTRA quality). DOLT-4 (NRC, Canada) Certified Reference Material (CRM) was used for correct standardization of methods. The 3050B EPA method was used for shell samples [6]. Digestion of mangrove litterfall was also validated comparing three analytical methods, the best recovery percentages were obtained for a mixture of HNO_3 and HCl, for this method the CRM used was 1573a tomato leaves (NIST, USA).

For validating the analytical methods, recovery percentages, linearity, limits of quantification (LOQ) and limits of detection (LOD) were calculated. All samples were analyzed by Thermo Scientific iCAP™ 6000 inductively coupled plasma optical emission spectrometry (ICP-OES) for Al, As, Ba, Cu, Cr, Fe, Pb, Ni, Sr, Si, V and Zn.

Operating conditions of the equipment are presented in Table 1. Standards were also analyzed during analysis, desionized water and high purity acids were used for better control of calibration.

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Table 1 Operational conditions

Parameter	Instrument operating conditions
Power	1150 W
Plasma gas flow	15 L min^{-1}
Auxiliary gas flow	2.0 L min^{-1}
Nebulizer pressure	270 kPa
Pump rate	50 rpm
Sample uptake rate	1.5 mL min^{-1}
Replicate read time	10 s
Replicate readings	3
Background correction	Fitted

One way analysis of variance (ANOVA) was performed to determine significant differences between sampling season in all the different matrix samples. Statistical analysis was performed using the software Statistica, version 13.

RESULTS AND DISCUSSION

Water

In water samples, the elements of As, Ba, Cd, Cr, Ni, Pb and Zn were below LOD and Al and Cu were slightly above the LOQ. The concentrations order was: Si>Sr>V>Fe>Al>Cu, for both sampling sites. Concentrations of elements found water samples are presented in Table 2.

Table 2 Mean and range of metals concentrations (mg kg^{-1}, dried weight) of elements detected in water samples in sites A and B, in both seasons. ND=not detected.

Element	Dry season		Rainy season	
	Site A	Site B	Site A	Site B
Al	0.26 (0.19-0.38)	0.029 (0.046.0.020)	0.19 (0.06-0.36)	0.41 (0.10-0.59)
As	ND	ND	ND	ND
Ba	ND	ND	ND	ND
Cd	ND	ND	ND	ND
Cr	ND	ND	ND	ND
Cu	0.043 (0.03-0.05)	0.037 (0.02-0.04)	0.011 (0.010-0.014)	0.014 (0.01-0.017)
Fe	0.78 (0.63-0.97)	0.68 (0.31-1.38)	0.51 (0.35-0.64)	0.57 (0.20-0.81)
Ni	ND	ND	ND	ND
Pb	ND	ND	ND	ND
Si	6.16 (6.85-5.24)	3.94 (3.37-4.58)	6.08 (1.91-9.35)	5.65 (5.04-6.36)
Sr	3.77 (3.13-4.32)	2.70 (2.39-3.22)	2.00 (1.38-2.52)	3.15 (2.30-3.61)
V	3.08 (2.69-3.64)	5.74 (5.20-6.75)	1.27 (1.33-1.82)	1.66 (1.65-1.99)
Zn	ND	ND	ND	ND

There were not significant differences by sampling season in any of the elements detected in water samples and concentrations range were narrow.

Organisms (*Cardisoma crassum*)

Concentrations in muscle tissue

There were not found organisms during dry sampling season in the site A. Elements of As, Cd, Cr, Pb and Ni were below LOD and LOQ. Concentrations found in muscle by sampling season and sampling site are presented in Table 3.

The order of concentrations in muscle samples was: Zn>Si>Cu>Sr>Fe>Al>V>Ba for site A, with few differences in order for site B, but sill heaving remarkable high concentrations of Zn, Si and Cu.

The elements of Ba (df=1, 10; F=38.92; P<0.05), Cu (df=1, 10; F=28.29; P<0.05), Sr (df=1, 10; F=50.68; P<0.05) and V (df=1, 10; F=46.80; <0.05) presented significant differences by sampling season, while the other half of detected elements remained without significant differences.

Table 3. Mean and range of metals concentrations (mg kg^{-1}, dry weight) of elements detected in muscle tissue samples, in sites A and B, in both seasons. ND=not detected.

Element	Dry season		Rainy season	
	Site A	Site B	Site A	Site B
Al	-	92.90 (45.91-138.93)	34.58 (15.57-64.5)	8.03 (1.42-15.77)
As	-	ND	ND	ND
Ba	-	4.53 (3.23-6.47)	4.58 (1.66-6.60)	3.45 (1.40-5.63)
Cd	-	ND	ND	ND
Cr	-	ND	ND	ND
Cu	-	83.31 (70.57-102.4)	45.45 (21.78-77.93)	84.41 (812-164)
Fe	-	197.02 (73.79-347-05)	59.30 (40.44-77.45)	43.32 (25.51-56.21)
Ni	-	ND	ND	ND
Pb	-	ND	ND	ND
Si	-	105.12 (74.59-157.62)	126.79 (87.31-167.80)	65.79 (42.70-81.0)
Sr	-	51.32 (40.54-58.73)	42.56 (27.55-59.44)	26.58 (19.98-31.94)
V	-	15.36 (13.79.17.62)	10.20 (7.54-11.47)	8.31 (6.86-10.68)
Zn	-	262.11 (221.86-312.55)	310.79 (267.34-344.97)	293.91 (254.95-335.26)

Concentrations in shell

Concentrations found in shell are showed in Table 4. The elements below LOD were the same as in the muscle tissue samples.

The sequence of concentrations found in both sites was: Sr>Ba>Si>Al>Fe>V>Zn>Cu, with very high concentrations of Sr, even more than one magnitude order higher that concentrations found in muscle samples. In case of shell samples, in contrary to all the type samples, there were significant differences by season for most of elements: Al (*df*=1, 10; *F*=21.68; P<0.05), Ba (*df*=1, 10; *F*=5.33; P<0.05), Cu (*df*=1, 10; *F*=6.58; P<0.05), Fe (*df*=1, 10; *F*=35.04; P<0.05), Sr (*df*=1, 10; *F*=13.27; P<0.05), V (*df*=1, 10; *F*=114.2; P<0.05) y Zn (*df*=1, 10; *F*=9.03; P<0.05), Si was the only element that did not presented significant differences.

Table 4. Mean and range of metals concentrations (mg kg^{-1}, dry weight) of elements detected in shell samples, in sites A and B, in both seasons. ND=not detected.

Element	Dry season		Rainy season	
	Site A	Site B	Site A	Site B
Al	-	238.68 (115.55-354.64)	57.21 (46.85-77.92)	52.75 (35.51-59.29)
As	-	ND	ND	ND
Ba	-	102.36 (45.99-200.89)	191.67 (117.99-228.44)	175.18 (107.71-271.66)
Cd	-	ND	ND	ND
Cr	-	ND	ND	ND
Cu	-	23.91 (11.62-42.03)	10.38 (3.82-25.61)	13.11 (9.56-14.96)
Fe	-	184.26 (87.90-226.03)	26.33 (8.27-60.43)	28.21 (8.28-41.28)
Ni	-	ND	ND	ND
Pb	-	ND	ND	ND
Si	-	105.11 (61.12-157.62)	138.61 (99.70-172.41)	107.54 (77.36-152.37)
Sr	-	682.92 (506.03-785.47)	1047.12 (755.4-1157.2)	899.82 (722.44-1047.0)
V	-	61.72 (58.88-68.83)	39.51 (32.62-43.42)	38.83 (35.98-43.82)
Zn	-	6.70 (4.19-11.40)	11.44 (8.50-18.80)	13.29 (7.78-17.75)

Effective accumulation of Cu and Zn in muscle tissue of *C. crassum*, has been observed in other species by other authors [9]-[11]. These elements have enzymatic functions in crustaceans and their concentrations are well regulated by organisms, especially for zinc [12]-[14].

In the other hand, accumulation in shell of *C. crassum* is higher for the elements of Ba and Sr. These elements have been involved in several studies of marine organisms related with calcium; Ba/ Ca ratios, for paleo-chemestry reconstruction of estuaries and marine ecosystems [15], and the relation Ca-Sr has been observed in different species due to the Sr biochemical similarities with Ca [16], [17].

Mangrove Litterfall

In mangrove litterfall samples, elements of Cd, Cr, Pb and Ni were below LOD. The order of concentration in both sample sites were quite similar, but the element of As was only detected in site A. Concentration in both sites for each sampling season are showed in Table 5.

Table 5. Mean and range of metals concentrations (mg kg^{-1}, dry weight) of elements found in mangrove litterfall samples, in site A and B, in both seasons. ND=not detected.

Element	Dry season		Rainy season	
	Site A	Site B	Site A	Site B
Al	844.33 (541.3-1004)	2598.7 (1752.4-3445)	1002.42 (386-2042.6)	575.32 (479.3-722)
As	ND	ND	ND	ND
Ba	23.0 (5.83-33.4)	16.02 (13.23-18.81)	20.83 (2.42-27.7)	7.25 (2.4-15.68)
Cd	ND	ND	ND	ND
Cr	ND	ND	ND	ND
Cu	3.30 (2.52-4.13)	4.68 (2.69-6.98)	2.05 (1.08-3.83)	1.71 (1.43-2.19)
Fe	500.16 (434.5-592.2)	1723.21 (1775.2-2271.1)	1254.39 (896.6-2242)	806.52 (740.8-890.1)
Ni	ND	ND	ND	ND
Pb	ND	ND	ND	ND
Si	518.65 (256.4-794.4)	229.29 (218.43-240.1)	267.97 (138.2-376.9)	446.85 (84.8-928.9)
Sr	128.09 (125.9-132.3)	80.43 (74.98-85.8)	110.84 (85.79-124.9)	111.28 (108.3-114.6)
V	ND	25.77 (25.7-25.81)	2.86 (1.17-6.06)	2.12 (1.66-2.81)
Zn	19.35 (15.6-22.24)	11.21 (9.42-13.0)	14.31 (6.90-25.72)	11.80 (10.37-13.1)

Cu was the only element that presented significant differences by sampling seasons (*df*=1, 10; *F*=7.67; P< 0.05). Significant differences were also estimated by sampling site due to the inherent variability of the matrix of this type of sample and there were found significant differences for Ba (*df*=1, 10; *F*=8.69; P< 0.05), Sr (*df*= 1,1 0; *F*= 5.69; P<0.1) and V (*df*= 1,1 0; *F*= 5.39; P<0.1).

Sediments

The descendent order of concentrations of the elements found in sediments samples was: Al>Fe>Ba>Si>Sr>Zn>Pb>Cu>Cd in site A, while in site B order was: Al>Fe>Sr>Si>Zn>Ba>Cu>Pb>Cd. Concentrations found in sediment samples are presented in table 6.

Table 6 Mean and range of metals concentrations (mg kg^{-1}, dry weight) found in sediments in sites A and B, in both seasons. ND=not detected.

Element	Dry season		Rainy season	
	Site A	Site B	Site A	Site B
Al	14057.26 (13459.7-14097.64)	16402.83 (13459.69-18191.2)	10207.25 (8266.8-12359.2)	19821.98 (17736.222627.7)
As	ND	ND	ND	ND
Ba	61.14 (42.94-71.66)	44.65 (43.34-55.26)	59.76 (52.31-67.75)	33.49 (32.05-35.78)
Cd	0.86 (0.82-0.95)	1.87 (1.51-1.81)	0.71 (0.69-0.75)	1.51 (1.37-1.64)
Cr	ND	ND	ND	ND
Cu	10.99 (9.18-13.26)	21.46 (19-72-21.19)	9.80 (8.90-10.53)	17.21 (16.48-18.03)
Fe	15616.5 (13884.31-19022.4)	8837.7 (7082.76-9100.7)	8925.2 (8832.9-9029.4)	14531.5 (13322.5-16968.3)
Ni	ND	ND	ND	ND
Pb	12.62 (10.76-14.85)	11.74 (10.32.11.30)	11.95 (10.63-13.96)	15.28 (13.12-16.57)
Si	39.94 (31.62-40.13)	139.10 (41.39-238.33)	58.13 (44.17-74.27)	129.77 (109.37-154.87)
Sr	92.21 (53.94-153.29)	112.15 (96.25-127.68)	48.91 (47.62-50.94)	148.41 (144.58-163.23)
V	ND	36.68 (35.01-38.67)	ND	ND
Zn	37.24 (32.0-43.52)	29.88 (29.15-30.66)	29.03 (27.43-31.38)	57.16 (49.79-63.19)

As, Cr and Ni concentrations were below LOD, nevertheless, the elements of Pb and Cr were only detected in sedimentary phase. The concentrations in the site B were slightly superior to those in site A, for all elements found. V was the only element that presented significant differences by sampling season (df=1, 10; F=4.98; $P<0.05$).

CONCLUSIONS

An estuary has maritime and continental influence in the incorporation of elements and heavy metals cycle, which is clearly reflected in most of the elements found during the analysis (Al, Cu, Fe, Si, Sr, Ba and Zn), with exception of Pb and Cr, however, the concentrations found of these elements are relatively low and they were only detected in the sedimentary phase. This fact points the importance of this stratum as a "trap" of pollutants and the role of the whole ecosystem as a filter, but is necessary to considerate they can be resuspended "up-estuary" in a turbulent period or any other climatic or anthropogenic perturbation.

There were not significant differences by sampling season for any of elements found in water samples, while there were significant differences for V in sediment samples; Cu in mangrove litterfall samples; for the elements of Ba, Cu, Sr, and V in muscle tissue samples and for all the elements found in shell samples, with exception of Si.

The accumulation of Cu, Zn and Si seems to be an effective in *C. crassum* muscle tissue, while its accumulative capacity is higher for Ba and Sr in shell. The elements found in muscle tissue (Al, Ba, Cu, Fe, Si, Sr, V and Zn) are mostly essential for organisms and have clear enzymatic functions, with exception of V. If there might be an exposure to the elements found derived from the traditional consumption of *C. crassum*, it is require a more extensive evaluation and collection of data to define if there is a potential risk from this consumption.

ACKNOWLEDGEMENTS

We gratefully acknowledge the Consejo Nacional de Ciencia y Tecnología (CONACYT) for the fellowship of the first author, and also the Programa de Apoyo a Proyectos de Investigación e Innovación Tecnológica (Project PAPIIT # IN211213), UNAM, the Centro de Investigación en Materiales Avanzados (CIMAV), and especially to Silvia Miranda Navarro, Jaime Torres Guerrero and all the colleagues of the NPA "Estero El Salado" for supporting this research.

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