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Biomonitoring of Heavy Metals (Fe, Zn, Cu, Mn, Cd and Cr) in Oysters: Crassostrea rhizophorae of Mangrove Areas of Alagoas (Brazil)

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HIGHLIGHTS

- *Crassostrea rhizophorae* presented concentrations of the metals Fe, Mn, Cu, Zn, Cd and Cr.
- Zn and Cd presented results above Brazilian legislation regarding contaminants, noting that both environments are contaminated.

Abstract: Mangroves are tropical and subtropical flooded forests that generally develop in estuarine areas over unstable sediments protected from the action of waves in the intertidal zone with an environment characterized by a great diversity of fauna and flora. Thus, the present study aimed to evaluate the levels of heavy metals that are absorbed by mangrove oysters, in estuarine systems in the Alagoas coast by determining the concentrations of Fe, Cu, Mn, Zn, Cd and Cr. Two areas, which consisted of both a collection during the rainy season and the dry season, were selected for sampling of mangrove oysters. In each collection, seven samples were collected, at seven different points, where each sample contained four oysters. In the laboratory the oysters were dried at 105°C for 72 hours and then macerated. It was then digested with 10 ml of a solution comprising a combination of 4:1 nitric acid and hydrochloric acid, initially in 1h at 40°C, followed by 3 hours at 140°C. In General, the medians followed the order Zn > Fe > Mn > Cu > Cr > Cd in MMELC and Zn > Cu > Fe > Mn > Cr > Cd in Meirim River. It is concluded that the Crassostrea rhizophorae oysters from the studied environments presented concentrations of all the metals proposed in the research and demonstrates its accumulating and bioindicator character.

Keywords: Mangrove; Mangrove Oyster; Heavy Metals.

INTRODUCTION

Mangroves are tropical and subtropical flooded forests that generally develop in estuarine areas over unstable sediments protected from the action of waves in the intertidal zone [1].The forests of mangrove ecosystems are part of a highly complex transitional coastal ecosystem that includes 0.7% of tropical forests with a strong relationship with neighboring habitats. They hold a rich, peculiar structure that houses a wide variety of plants, animals and microorganisms [2].

Due to the presence of a predominantly tropical territory, Brazil has a great extension of mangroves, which justifies the great wealth of fish species on the Brazilian coast. In Alagoas, they are found practically across the entire coastal strip [3].

In the last four decades, the world's mangrove ecosystems have been severely impacted by a combination of natural and anthropogenic stressors. They have been especially targeted by an increasing anthropic pressure when near urban areas. As a result, this ends up altering its quality and compromising the health of the ecosystem as a whole, with a strong impact on the local biota. The main factors are deforestation for industrial, urban and touristic projects and contamination by sewage from aquaculture waste and chemical substances [4].

There are several ways to identify pollution of ecosystems which are highlighted with the biomonitoring of heavy metals. Besides having a cumulative effect [5] , metals naturally found in the environment, such as mercury, cadmium, lead and arsenic, are considered to be non-essential because of their high toxicity (they are able to interfere with enzymatic reactions by changing the conformation of the enzymes), and they do not to participate in metabolic processes.

Due to the ability to bioconcentrate trace elements and organic compounds, certain aquatic organisms have been used in recent years as biomonitoring of pollution in coastal environments. Some of these organisms are migratory, which makes them of little use in the study of the contamination of a certain area. Others, such as bivalve mollusks, live almost always fixed to a given substrate, and are therefore excellent environmental indicators [6].

Bivalve mollusks are widely used as indicators of pollution by trace elements because they are widely distributed in coastal ecosystems that are more susceptible to pollution, are abundant and easy to collect and are sessile organisms with a low level of enzymatic activity. So, they act less in the metabolism of these pollutants. In addition, these organisms arouse great economic interest because they are used by man as a source of food [5]. The objective of this study was to evaluate the levels of trace metals that are absorbed by mangrove oysters, (*Crassostrea rhizophorae*) in estuarine systems on the coast of Alagoas by determining the concentrations of iron, copper, manganese, zinc, cadmium and chromium. Another objective was to analyze and to correlate the possible variations of the concentrations of elements in the soft tissues of the oysters with the variations of the index of condition and with the seasonal variations.

MATERIAL AND METHODS

Study area

In order to carry out this study, two areas of mangrove forests were chosen in the metropolitan region of Maceió, the capital of the State of Alagoas. The first study area was the mangrove forest formed on the banks of the mouth of the Mundaú / Manguaba lagoon complex (MMELC), which is georeferenced by coordinates 9°69"66'S and 35°78"57.87'W (Figure 1).

Figure 1. Map displaying the study area and sampling site in the MMELC.

The second study area is located at the mouth of the Meirim River in the neighborhood of Pescaria, north coast of Maceió, Alagoas. The chosen point is georeferenced by the coordinates 9º30"26'S and 35º37"14'W. The Meirim River is born in the municipality of Messias, a forest area in the State of Alagoas. It cuts practically the entire capital, Maceió, until it empties into the Atlantic Ocean (Figure 2).

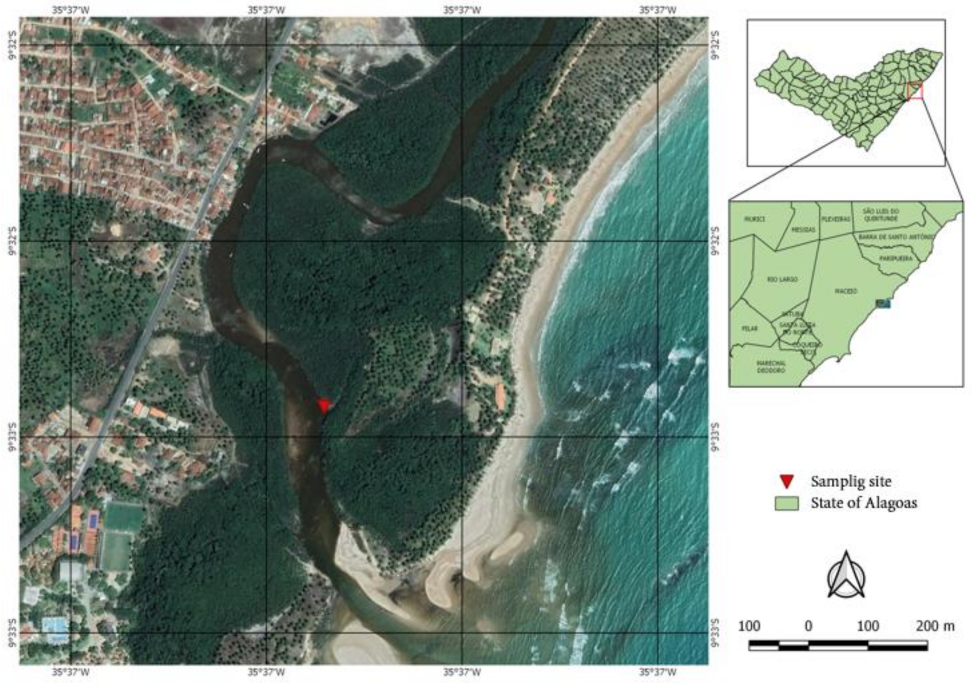

Figure 2. Map displaying the study and sampling site in the Meirim River.

Oyster collection

Oysters that are trapped in roots of the Rhizophora mangle were manually removed, with the aid of spatulas. The samples were collected at low tide at the medium height of the substrate. A collection was carried out during the rainy season (July) and another during the dry season (February). At each collection seven samples were taken at seven different points where each sample contained four oysters ranging in size from 3.0 to 5.0 cm, since sample size affected the concentrations of metals in bivalves [7].

Preparation of samples

The oysters were scraped to remove all the material fixed on the outside of the shells (for example, algae, barnacles etc.), were subsequently washed with distilled water, to then be measured in order to achieve the standardization of the samples. Then, they were removed from the shells and finally the soft part was heavily balanced for precision.

The oysters were dried at 105° C for 72 hours and then macerated using a mortar and pestle. Then it was digested with 10 mL of aqua regia, a solution comprising a combination of nitric acid and hydrochloric acid at a rate of 4:1, initially for 1 hour at a temperature of 40° C, followed by 3 hours at 140° C. The resulting solution obtained at the end was filtered using Whatman paper filter no. 17. Finally, the levels of Fe, Mn, Cu, Zn, Cd, and Cr were determined by using the Mehlich method and analyzed by means of atomic absorption spectrometry with the Analytik Jena, model novAA A300. After conversion, the data were presented in microgram per gram (μg.g-1) [7].

Human exposure assessment

The risk to human health was calculated according to equation 1 using the established data by FAO/WHO [8]:

$$EDI = C_{bivalve} \times \left(\frac{dc_{bivalve}}{bw}\right) \quad (1)$$

where EDI is the estimated diary intake, $C_{bivalve}$ = average trace metal concentrations

(mg kg−1 wet weight) in C*rassostrea rhizophorae*, $dc_{bivalve}$ is the daily bivalve consumption (g day−1) per capita for the Brazilian population as established by the FAO, and bw is the average body weight (kg) of the brazilian population.

The hazard quotient was obtained using equation 2:

$$HQ = \frac{EDI}{RfD} \quad (2)$$

where the EDI is divided by reference dose (RfD) as recorded by FAO. The HQ relates the heavy metal toxicity to the daily oyster consumption. HQ values less than 1.0 do not show a potential risk to human health [8].

Statistical analysis

The data were submitted to analysis of variance and the means were compared by Fisher's LSD test at 5% probability. The analyses were performed by using the software Genes [9,10].

RESULTS

Distribution of heavy metals

The mean concentrations of the Fe, Mn, Cu, Zn, Cd and Cr metals in the two studied regions, both in the dry season and in the rainy season, are shown in Tables 1 and 2. Concentrations of Fe, Cu, Zn, Cd, Cr, and Mn in MMELC in the dry period varied from 3.7 to 14.9, 0.18-0.42, 8.7-11.2, 0.06-0.19, 0.02-0.8 and 0.26-0.98, and in the rainy season 2.9-6.1, 0.19-0.43, 5.78-7.58, 0.01-0.14, 0.04-0.31 and 0.15-0.34, respectively. In the Meirim River in the dry season, the values ranged from 4.0-7.8, 0.17-0.42, 9.8-11, 0.08-0.14, 0.06-0.3 and 0.16-0.50, and in the rainy season 2.6-4.8, 0.16-0.32, 6.2- 7.7, 0.02-0.17, 0.03-0.14 and 0.15-0.29. In general, the averages followed the order Zn> Fe> Mn> Cu> Cr> Cd in MMELC and Zn> Fe> Cu> Mn> Cr> Cd in Meirim River.

Table 1. Concentration (mean ± standard deviation) of heavy metals in two mangrove regions - MMELC and Meirim River (dry mass concentration measured in $\mu g.g^{-1}$).

Metal	MMELC	Meirim River
Fe	278.06±120.41a	203.18±62.22b
Cu	14.33±7.02a	11.93±4.13a
Mn	17.20±5.54a	10.92±3.40b
Zn	413.58±122.00a	401.43±75.62a
Cd	4.65±4.48a	4.21±2.28a
Cr	13.14±10.66a	6.38±4.43b

Medium followed by the same letter in line does not differ statistically by the Fisher LSD test at 5% probability.

Table 2. Concentration (mean ± standard deviation) of heavy metals in dry and rainy period (dry mass concentration measured in μg.g^{-1}).

Metals	Dry	Rainy
Fe	219.08±108.44a	262.15 ±93.05a
Cu	10.48±4.22b	15.79±6.03a
Mn	13.61±5.81a	14.52±5.42a
Zn	357.61±91.44b	457.40±83.00a
Cd	4.37±1.41a	4.48±4.84a
Cr	10.49±10.65a	9.03±6.60a

Means followed by the same letter in the row did not differ statistically by Fisher's LSD test at 5% probability.

In comparing the dry and rainy period, a statistically significant increase in Cu and Zn concentrations from the dry to the rainy period occurred (Figure 3).

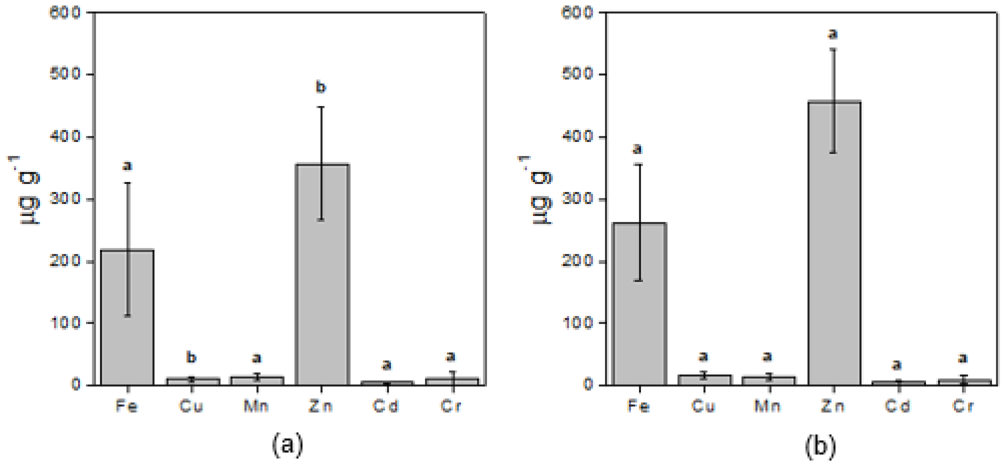

Figure 3. Concentration (mean ± standard deviation) of heavy metals in the dry (a) and rainy (b) period (dry mass concentration measured in μg.g-1).

Fe, Mn and Cr levels were significantly higher in MMELC than in Meirim River (Figure 4). An MMELC has a more diversified industrial complex, containing, in addition to agro-industry, a pole of chemical industries and a greater degree of urbanization, presenting several points of sewage disposal and irregular waste disposal, including inside the mangrove.

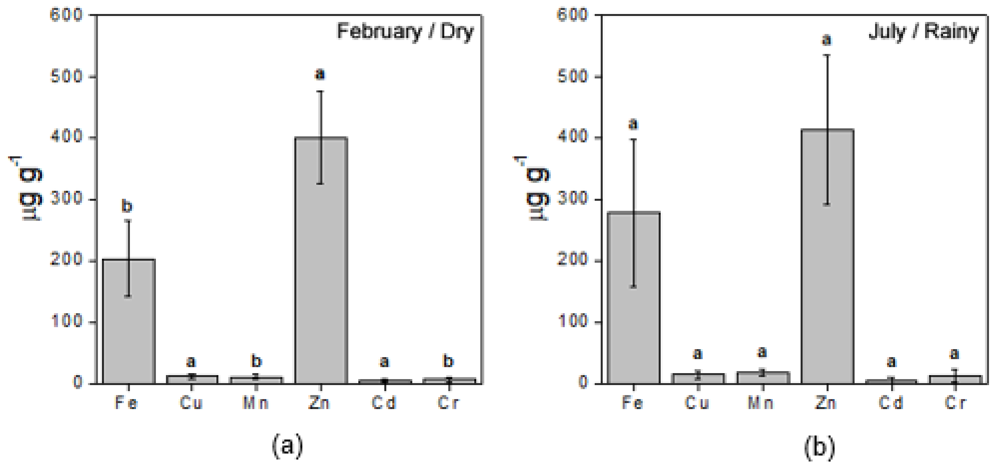

Figure 4. Concentration (mean ± standard deviation) of heavy metals in two mangrove regions – MMELC (a) and Meirim River (b) (dry mass concentration measured in μg.g-1).

Human exposure assessment

The mean trace element concentrations were used to estimate the daily intake of trace elements through bivalves consumption by the Brazilian population. Table 3 show the values of EDI and HQ for all studied heavy metals through oyster consumption.

Table 3. Estimated daily intake (EDI) (mg kg^{-1} bw day^{-1}), hazard quotients (HQs) and acceptable daily intake (ADI) (mg kg^{-1} bw day^{-1}) of heavy metals through *Crassostrea rhizophorae* consumption by people in Alagoas, Brazil.

Location		Fe	Cu	Mn	Zn	Cd	Cr
MMELC	EDI	0.338762	0.017458	0.020955	0.503866	0.005665	0.016009
	HQ	0.483945	0.436457	0.149677	1.679553	5.665112	5.33617
Meirim	EDI	0.208909	0.012266	0,011228	0,412749	0,004329	0.00656
	HQ	0.298442	0.30666	0.080199	1.375831	4.32871	2.186633
	ADI	0.8*	0.5	0.14	0.21	0.001	–

*iron from all sources except iron oxide and hydrated iron oxides. Iron oxide and hydrated iron oxides ADI=0.5 [26].

DISCUSSION

Distribution of heavy metals

The concentrations found in the MMELC and in Meirim River for Cu (14.33 µg.g-1 - 11.93 µg.g-1) and Zn (413.58 µg.g-1- 401.43. µg.g-1), respectively, were below the studies in the Bay of Paranaguá (PR) and in the Bay of Guaratuba (PR) [6], which showed levels of Cu (331.50 µ.g g-1 - 121.98 µg.g-1) and Zn (2751.03 µg.g-1 - 1531.34 µg.g-1) as well as Botelho (BA) and Tapera (BA) [11], where the results for Cu were of (276.1 µg.g-1- 526.1 µg.g-1) and Zn (2099 µg.g-1 - 4733 µg.g-1). The same occurred in Buche (Venezuela) and Mochima (Venezuela) [12], for Cu (83 µg.g-1 - 31.6 µg.g-1) and for Zn (876.5 µg.g -1 - 719.3 µg.g-1).

However, exclusively for Zn (413.58 µg.g-1 - 401.43 µg.g-1), the data obtained here were higher than those obtained in the Gulf of Guacanayabo [13] (164 µg.g-1) and also in Paraty (RJ) [14] (256.5 µg.g-1). The presence of Cu and Zn in the environment, more specifically in the oyster-mangrove may be associated with natural and anthropogenic sources. The high values found for Zn can be explained by the strong human action to which the sites researched are exposed. The domestic sewage dump is an example of the type of action that these regions are subject to, as well as the effluent flow of the ethanol industry, since the state of Alagoas has several plants of this type.

According to the Brazilian Ministry of Health, the maximum limit for Zn is 50µg.g-1 [15]. Globally, this limit is quite variable, according to the legislation of each country. The Ministry of Agriculture, Forestry and Fisheries (MAFF) [16], in England, determines 50 µg.g-1, same value established by Brazilian law, but in Australia the maximum limit allowed for oysters is 1000 µg.g-1 [17]. Thus, the Zn levels found here were significantly elevated, which may be indicative of environmental contamination.

Regarding the limits for Cu established by the Ministry of Health in Brazil [15], the maximum limit is 30 µg.g-1, and the limits established by MAFF [16] is 20 µg.g-1. The result found for Cu, both in Brazil and in England, is satisfactory.

For Cd, the mean concentrations found were 4.65 µg.g-1 and 4.21 µg.g-1 for the MMELC and Meirim River, respectively. These values are similar to those found in Paraty (RJ) [13] (4,4 µg.g-1). However, they are lower in Altata-Ensenada del Pabellón

(Mexico) [18] (6.47 µg.g -1), and on the coast of Italy [19] (0.23 µg.g-1) and were superior on the South Korean coast [20] (0.59 µg.g-1) and in Baia de Aratu (BA) [21] (0.44 µg.g-1). With regard to the Cd, the MERCOSUR Technical Regulation [22] which determines the maximum levels of inorganic contaminants in food, establishes that for bivalve mollusks the maximum limit of 2 µg.g-1. The values found here are more than double in the two studied environments, suggesting that the environment is being contaminated, mainly by anthropic action.

The presence of Cd is associated with industrial pollution, the production of batteries, paints and plastics, and with urban pollution, with the burning of fossil fuels, urban waste and sewage sediments. Being a metal that does not participate in the physiological process and was detected in mangrove oysters, it can be seen that oysters are excellent bioindicators of environmental pollution [23]. Cd contamination can cause in humans, renal dysfunction, immune disorders, pulmonary emphysema and osteoporosis [24].

For Cr, the concentrations found were 13.14 µg.g-1 and 6.38 µg.g-1 for the MMELC and Meirim River, respectively. Values are higher in Botelho (BA), in Tapera (BA) [11], in Buche (Venezuela) and Mochima (Venezuela) [12], as well as to South Korea's coast [20]. With respect to Mn, concentrations of 17.20 µg.g-1 and 10.92 µg.g-1 for the MMELC and Meirim River were found respectively. Similar results to those found in Botelho (BA) and in Tapera (BA) [11] and also Mochima (Venezuela) [12] however, the values were lower in Buche (Venezuela) [12].

Regarding the presence of Fe (278.06 µg.g-1 - 203.18 µg.g-1) a similar result was found in Botelho (BA) [11], and in Mochima (Venezuela) [12] and was and inferior in Tapera (BA) [11] and higher in the Buche (Venezuela) [12].

The elements Cr, Mn and Fe are found naturally in the composition of the rocks, and artificially through anthropic action in the presence of sewage, garbage and industries, and in the use of fertilizers. The absence of guiding values in Brazilian legislation for Cr, Mn and Fe in bivalve mollusks makes it impossible to indicate contamination or not.

In the period from May to October, Alagoas presented a rainfall index of 252.95 mm [25]. The explanation for the increase in copper and zinc concentrations may be closely related to the high amount of suspended organic matter during this period, contributing significantly to the increase in the uptake and bioaccumulation of these metals [2]. Knowing that copper and zinc are easily found in rocks and sediments, their release through chemical weathering increases in periods of higher rainfall, which is proven with the difference in the concentrations of the metals mentioned.

These results are of the utmost importance, since it should be considered that particles present in the soil can be sources of potential contamination. As these metals were initially absorbed, small variations in environmental conditions (rainfall, salinity, organic matter, dissolved solids, among others) may cause them to be desorbed and consequently returned to the aqueous medium, increasing the bioavailability of these metals to organisms present in that ecosystem.

Human exposure assessment

The daily consumption of iron, copper and manganese is under the values established by the FAO/WHO, indicating that there are no significant risks in the intake of these metals through the consumption of Crassostrea rhizophorae at the MMELC and Meirim River. However, for the values of zinc and cadmium (Cr values were not found in the literature), it can be observed that they are above the levels established by the FAO/WHO, where zinc is 2.3 times higher while for cadmium a value almost 6 times higher was found.

An important fact to highlight is the effect of contamination by heavy metals of invertebrates, fish and humans. Significant amounts of these pollutants are discharged into rivers and ponds, where they can be heavily accumulated in water, sediments and the food chain, resulting in sub-lethal effects or even death in local fish and crustacean populations [27]. The toxicity of heavy metals has been a concern considering that they are not being removed from aquatic ecosystems and accumulate, thus potentially threatening human health and ecosystems through the food chain [28,29].

Zinc (2.3 times higher than Acceptable Daily Intake) is involved in numerous aspects of cellular metabolism. The zinc is required for the catalytic activity of more than 200 enzymes and it plays a role in immune function, wound healing, protein synthesis, DNA synthesis and cell division [30]. Already the copper is essential for maintaining the strength of the skin, blood vessels, epithelial and connective tissue throughout the body. Although zinc is an essential requirement for good health, excess zinc can be harmful excessive absorption of zinc suppresses copper and iron absorption. Acute adverse effects of high zinc intake include nausea, vomiting, loss of appetite, abdominal cramps, diarrhea, and headaches [31]. Impairment of zinc homeostasis is also associated with cardiac diseases such as ischemic cardiomyopathy [32], atherosclerosis [33], and ischemia/reperfusion injury [34].

CONCLUSION

It is concluded through the study carried out, that oysters Crassostrea rhizophorae from the MMELC, Marechal Deodoro / AL estuaries and the Meirim River estuary in Maceió / AL presented concentrations of all the metals studied, which demonstrates its accumulating and bioindicator character.

It was observed that Zn and Cd presented results above Brazilian legislation regarding contaminants, noting that both environments are contaminated. In addition, there was a variation in the concentrations Cu and Zn in the comparison between the rainy and dry periods.

Finally, it is suggested that biomonitoring studies be periodically carried out aiming at preservation, conservation and recovery actions, as well as subsidizing studies related to the risk to human health. Although Brazil has more than 26,000 km2 of mangrove areas, studies in these ecosystems are still scarce.

REFERENCES

1. Elabie JAHCD, et al. As formigas como indicadores biológicos do impacto humano em manguezais da Costa Sudeste da Bahia. *Neotrop. Entomol*. 2006;35(Oct):602–15.
2. Aguirre-Rubi JR, *et a*l. Chemical contamination assessment in mangrove-lined Caribbean coastal systems using the oyster *Crassostrea rhizophorae* as biomonitor species. *Environ Sci Pollut R*. 2017;1–20.
3. Correia MD, Sovierzoski HH. Ecossistemas marinhos: recifes, praias e manguezais. Maceió (Brasil): Edufal; 2005. 55 p.
4. Belarmino PHP, *et al*. Resíduos sólidos em manguezal no rio Potengi (Natal, RN, Brasil): relação com a localização e usos. RGCI. 2014;14(3):447–57.
5. Otchere FA, Joiris CR, Holsbeek L. Mercury in the Bivalves Crassostrea tulipa and Perna perna from Ghana. *Environment*. 2003;304:369–75.
6. Castello BDFL. Avaliação das teorias de As, Cu, Cd, Ni e Cd em ostras, Crassostrea Rhizophorae (Guilding, 1828), nas baías de Paranaguá e Guaratuba [dissertação]. Paraná: Universidade Federal do Paraná, Setor de Ciências da Terra; 2010. 69 p.
7. Yap CK, Azmizan AR, Hanif MS. Biomonitoring of trace metals (Fe, Cu, and Ni) in the mangrove area of Peninsular Malaysia using different soft tissues of flat tree oyster Isognomon alatus. *Water Air Soil Pollut*. 2011;218(1–4):19–36.
8. Yang F, *et al*. Bioaccumulation of Trace Elements in *Ruditapes philippinarum* from China: Public Health Risk Assessment Implications. *Int J Environ Res Publ Health*. 2013,1392–405.

9. Cruz CD. Genes: a software package for analysis in experimental statistics and quantitative genetics. *Acta Sci. Biol. Sci*. 2013;35(3):271-6.
10. Cruz CD. Genes Software-extended and integrated with the R, Matlab and Selegen. *Acta Sci. Biol. Sci*. 2016;38(4):547-52.
11. Amado-Filho GM, *et al*. Heavy metals in benthic organisms from Todos os Santos Bay, Brazil. *Braz J Biol*. 2008;68(1):95-100.
12. Alfonso JA, *et al*. Temporal distribution of heavy metal concentrations in oysters *Crassostrea rhizophorae* from the central Venezuelan coast. *Mar. Pollut. Bull.* 2013;73(1):394–8.
13. Días-Rizo O, *et al*. Copper, zinc and lead enrichments in sediments from guacanayabo gulf, cuba, and its bioaccumulation in oysters, *Crassostrea rhizophorae*. *Bull Environ Contam Toxicol*. 2010;84(1):136–40.
14. Wanick RC. *et al*. Use of the digestive gland of the oyster *Crassostrea rhizophorae* (Guilding, 1828) as a bioindicator of Zn, Cd and Cu contamination in estuarine sediments (south-east Brazil*). Chem EcoL.* 2012;28(2):103–11.
15. Brasil. Ministério da Saúde. Decreto n° 55.871 de 26 de março de 1965. Modifica o Decreto nº 50.040, de 24 de janeiro de 1961, referente a normas reguladoras do emprego de aditivos para alimentos, alterado pelo Decreto nº 691, de 13 de março de 1962. Diário Oficial da União. 1965 abr. 09.
16. Steering Group on Food Surveillance. Working Party on the Monitoring of Foodstuffs for Heavy Metals. Survey of Copper and Zinc in Food: The Fifth Report of the Steering Group On Food Surveillance, the Working Party On the Monitoring of Foodstuffs for Heavy Metals. London: H. M. S. O, 1981. 50 p.
17. Hungspreugs M, Yaunghthong C. The present levels of heavy metals in some mollusks of the upper Gulf of Thailand. Water, *Air and Soil Poll.* 1984;22:395-402.
18. Frías Espericueta MG, *et al*. The metal content of bivalve molluscs of a coastal lagoon of NW Mexico. *Bull Environ Contam Toxicol*. 2008;80(1):90–2.
19. Burioli EAV, *et al*. Trace element occurrence in the Pacific oyster Crassostrea gigas from coastal marine ecosystems in Italy. Chemosphere. 2017;187:248–60.
20. Mok JS, *et al*. Bioaccumulation of heavy metals in oysters from the southern coast of Korea: Assessment of potential risk to human health. *Bull Environ Contam Toxicol.* 2015;94(6):749–55.
21. Da Araújo CFS, *et al*. Cadmium and lead in seafood from the Aratu Bay, Brazil and the human health risk assessment. . Environ Monit Assess. 2016;188(4).
22. Brasil. Ministério da Saúde. Resolução nº 42 de 29 de agosto de 2013. Regulamento técnico mercosul sobre limites máximos de contaminantes inorgânicos em alimentos. Diário Oficial da União. 2013 ago. 30.
23. Rocha AFDA. Cádmio, chumbo, mercúrio: a problemática destes metais pesados na Saúde Pública [dissertação]: Porto: Faculdade de Ciências da Nutrição e Alimentação, Curso de Ciências da Nutrição; 2009. 63 p.
24. Lima DPDE, *et al*. Contaminação por metais pesados em peixes e água da bacia do rio Cassiporé, Estado do Amapá, Brasil. *Acta Amaz*. 2015;45(4):405–14.
25. Alagoas. Secretaria de Estado do Meio Ambiente e dos Recursos Hídricos - Semarh/AL. Dados de Precipitação Mensal. [cited 2018 Mar 18]. Available from: http://dados.al.gov.br/it/dataset/dados-de-precipitacao-mensal.
26. Seiler H, Sigel A, Sigel H, editors. Handbook on metals in clinical and analytical chemistry. Basel, Switzerland: CRC Press; 1994.
27. Arantes FP, Savassi LA, Santos HB, Gomes MVT, Bazzoli N. Bioaccumulation of mercury, cadmium, zinc, chromium, and lead in muscle, liver, and spleen tissues of a large commercially valuable catfish species from Brazil. *An. Acad. Bras. Ciênc*. 2016;137-47.
28. Hariku PS, Nasir UP. Ecotoxicological impact assessment of trace elements in core sediments of a tropical estuary. *Ecotoxicol Environ Safe*. 2010;73:1742-7.

29. Nadmitov HS, In KS, Chu JM, Gomboev B, Janchivdorj L, Lee CH, Khim JS. Largescale monitoring and assessment of metal contamination in surface water of the Selenga River Basin (2007-2009). *Environ Sci Pollut Res Int*. 2015;22(4):2856-67.
30. Osredkar J, Sustar N. Copper and Zinc, Biological Role and Significance of Copper/Zinc Imbalance. *J Clin Toxicol*. 2011;1–18.
31. Sandstead HH. Understanding zinc: recent observations and interpretations. *J Lab Clin Med*. 1994;124;322-7.
32. Shokrzadeh M, Ghaemian A, Salehifar E, Aliakbari S, Saravi SS, Ebrahimi P. Serum zinc and copper levels in ischemic cardiomyopathy*. Biol Trace Elem Res*. 2009;127:116–23.
33. Beattie JH, Kwun IS. Is zinc deficiency a risk factor for atherosclerosis? *Br J Nutr.* 2004;91(02):177.
34. Karagulova G, Yue Y, Moreyra A, Boutjdir M, Korichneva I. Protective Role of Intracellular Zinc in Myocardial Ischemia/Reperfusion Is Associated with Preservation of Protein Kinase C Isoforms. *J Pharmacol Exp Ther*. 2007;321(2):517–25. doi:10.1124/jpet.107.119644.