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Heavy metals in soft tissue of blue crab (*Callinectes sapidus*) of Puerto Concha, Colon Municipality, Zulia State

(Metales pesados en tejidos blandos de cangrejo azul (*Callinectes sapidus*) de Puerto Concha, municipio Colón, estado Zulia)

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Abstract (english)

To aim was determine the concentrations of heavy metals in soft tissues of blue crab (*Callinectes sapidus*) in Puerto Concha, Colon municipality, Zulia state. The study was descriptive and experimental. For this, a sampling consisting of 37 catches were taken during almost three years, from April 2007 to February 2010. Determinations of heavy metals: lead (Pb), cadmium (Cd), nickel (Ni), chromium (Cr), cobalt (Co), arsenic (As), manganese (Mn), copper (Cu), vanadium (V) and thallium (Tl) by electrothermal atomic absorption spectrophotometry (ETAAS) were quantified. The results were expressed in mg/kg dry mass. The average concentrations of heavy metals did not exceed the permitted limits, however the presence of highly toxic metals such as Pb, Cd, As and Cr with unknown biological function is cause for concern and alert. The presence of toxic metals in soft tissues of blue crab, leads us to propose the study of these metals in living organisms, water and sediments near the affluent of the rivers, to detect the possible sources of pollution and take correctives to curb the entry of these elements into the environment (avoiding progressive deterioration of Maracaibo Lake). *Callinectes sapidus* is a key species in the studied ecosystem and could play an important role in transferring contaminants to higher trophic levels and the presence of these elements may raise concern for local biota.

Keywords (english)

Callinectes sapidus, heavy metals, lead, copper, atomic absorption spectrophotometry (source: MeSH)..

Resumen (español)

El objetivo fue determinar las concentraciones de metales pesados en los tejidos blandos del cangrejo azul (*Callinectes sapidus*) en Puerto Concha, municipio Colón, estado Zulia. El estudio fue descriptivo y experimental. Para ello, se tomaron muestras de 37 capturas durante casi tres años, desde abril de 2007 a febrero de 2010. Las determinaciones de metales pesados: plomo (Pb), cadmio (Cd), níquel (Ni), cromo (Cr), cobalto (Co), arsénico (As), manganeso (Mn), cobre (Cu), vanadio (V) y talio (Tl) mediante espectrofotometría de absorción atómica electrotermica (ETAAS) fueron cuantificadas. Los resultados se expresaron en mg/kg de masa seca. Las concentraciones promedio de metales pesados no excedieron los límites permitidos; sin embargo, la presencia de metales altamente tóxicos como Pb, Cd, As y Cr con función biológica desconocida es motivo de preocupación y alerta. La presencia de metales tóxicos en los tejidos blandos del cangrejo azul, nos lleva a proponer el estudio de estos metales en organismos vivos, agua y sedimentos cerca de los afluentes de los ríos, para detectar posibles fuentes de contaminación y tomar medidas correctivas para frenar la entrada de estos elementos en el medio ambiente (evitando el deterioro progresivo del Lago de Maracaibo). *Callinectes sapidus* es una especie clave en el ecosistema estudiado y podría jugar un papel importante en la transferencia de contaminantes a niveles tróficos más altos y la presencia de estos elementos puede generar preocupación por la biota local.

Palabras clave (español)

Callinectes sapidus, metales pesados, cobre, plomo, espectrofotometría de absorción atómica (fuente: DeCS).

Introduction

The environment and natural resources have always been of paramount importance for human development. The high degradation of natural resources, and consequently its progressive shortage, represent latent threats to the well-being of current and future generations, and this have demolished the old myth of the inexhaustibility of the natural resources (1-2). In Venezuela, environmental pollution has been poorly controlled, which depends on the amount and type of pollutants, the speed of introduction thereof, and the self-purification capacity of the medium.

The sources of this pollution comes from geochemical origins, or anthropogenic activities: mining, agriculture, industrial processes, urban development, consumption and exploitation of fossil resources, and others; that alter the different biological systems balance, generating environmental pollution (3). Currently there are programs for monitoring and control of environmental contamination by heavy metals, radionuclides and pesticides in several countries of the world (4).

In Latin America, there are investigations concerning the analysis of some biological organisms as potential pollution indicators (5). The quantification of trace elements in these organisms is an important aspect of environmental analytical chemistry. Investigations into the analysis of heavy metals in some biological organisms, potential indicators of pollution have begun (5). Recently, heavy metals have

attracted attention because of its harmful effects; hence it is imperative to have reliable analytical techniques. According to the Agency for Toxic Substances and Disease Registry into list of dangerous substances (6), the toxicity of heavy metals that threaten human health will have the following decreasing order: Pb> Cd> Ni > Zn> Cr> Cu> Mn.

There are many works that discuss the possibility of knowing the quality of aquatic systems from the analysis of contaminants in bivalves, sponges, clams, mussels and crabs, that can accumulate due to the respective eating habits of these organisms (4, 5, 7).

The blue crabs are opportunistic omnivores and detritivores, their diet consists of a wide variety of prey, including bivalves, fish, gastropods, and other crustaceans, as well as plant matter (7-11).

Maracaibo's Lake is located in the state of Zulia, in the western part of the Bolivarian Republic of Venezuela. It has 13,820 square kilometers, which makes it the largest lake in Latin America; due to its size it is considered an inland sea. The Maracaibo Basin is one of the areas with the greatest oil wealth in the world. The overfishing of the blue crab in Maracaibo's Lake is subject to artisanal fishing; although its capture, processing and commercialization, generates a significant amount of foreign exchange with a market in constant growth, given the current high demand, mainly in the population of the United States, which is established as the main market for the processed product.

Callinectes sapidus is an important crab in different localities of the area south of Maracaibo Lake, where it is consumed largely by humans. Because of this, these species play an important role in the transfer of contaminants other trophic levels (12). The aim is to determine the concentrations (mg/Kg on dry mass) of heavy metals, such: Pb, Cd, Ni, Cr, Co, As, Mn, Cu, V and Tl, in soft tissues of blue crab.

Materials and methods

The work was developed in an area between the south-western and eastern coasts south of Maracaibo Lake, which make up the vast floodplain in which empty the rivers: Santa Ana, Bravo, Catatumbo, Escalante, Chama, Capaz, Tucani, Caus and Motatán. The hydrographic systems that determine this plain, carrying the large volumes of sediment from the eastern section of the western branch of the Andes, which have given rise to tubular deltas like those seen at the mouth of the Catatumbo and Escalante rivers (9), and the coastal lagoons separated from the main body of the lake, by thin barriers of clay-sand sediments (13).

A total of 37 blue crab catches were taken, between April 2007 and February 2010. The fishing was carried out by members of the "Chamita Fishermen's Cooperative", and generally began at dawn, covering an average of six hours per day, which ended around noon. The long line technique was performed using chicken heads as bait. Two of the researchers accompanied the fishermen on their boats, during some of their working hours (to determine by geolocation by satellite, the geographical location of the capture sites). The data obtained were ordered following a lunar calendar. Table 1 shows the geographic coordinates of each of the stations. Three samplings per season of captures were made by each working group.

Treatment of samples

The samples were placed in plastic bags and transported on ice until further analysis in the laboratory. Once they arrived at the Molecular Spectroscopy Laboratory, at the University of Los Andes, Mérida, they were washed with distilled water to remove the sand and sediments that were found adhered to their body; subsequently, the weight, sex and respective morphometric of the blue crab were recorded (11). The samples were dried for 72 hours at 80 °C, and then subjected to lyophilization. 0.25 grams of dry pulverized homogenized soft material were weighed, and a 3:1 mixture of HNO₃/H₂O₂ was added,

to finally heat the preparations in a plate for one hour. A final volume of 25 ml was taken.

Equipment

The Thallium determination was performed using an atomic absorption spectrometer Analyst model 600 (Perkin-Elmer) equipped with a graphite atomizer with transverse heating and Zeeman background correction effect. THGA graphite tubes equipped with integrated platforms to pyrolytic graphite (Perkin-Elmer) were used. As radiation source, lamp hollow cathode lead, cadmium, nickel, chromium, cobalt, arsenic, manganese, copper, vanadium and thallium, of the Perkin-Elmer brand was used.

Reagents

A standard solution of 1000 mg/L of lead, cadmium, nickel, chromium, cobalt, arsenic, manganese, copper, vanadium and thallium was used. All working solutions were prepared by appropriate dilution of the above solution, with distilled and deionized water (18 MΩcm).

Results

The results obtained in our study are presented in Table 2, where the averages ± standard deviations can be observed for each of the eight heavy metals quantified in soft tissues of *C. sapidus* from Puerto Concha, Zulia state, during our sampling period. It should be noted that the levels of V and Tl were not detected in any of the samples corresponding to the 37 captures taken, in the soft tissues of the blue crab.

Discussion

The presence of heavy metals in the soft tissues of the blue crab can be related to the discharges of waste from the oil industry, served directly from the river basins and sub-basins, which flow into Maracaibo's Lake, representing the most important source of pollution for this ecosystem in Venezuela. The blue crab can be considered a bioaccumulative organism of different beneficial chemical substances and other toxic substances. Other species of crabs also show this potential of bioaccumulation, in this sense; the red crabs (*Cancer productus*) showed evidence of bioaccumulation of heavy metals, with a high variability in the levels of some polluting metals. It is important to note at this point in our discussion that the bioavailability of

Table 1. Sampling blue crab (*Callinectes sapidus*), Puerto Concha, Zulia state.

Station	Coordinates
Chamita	09°06'607"N and 071°43'036"W
La Boyera	09°07'685"N and 071°43' 539"W
Costa de Los Palos	09°09'936"N and 071°44'247W
La Hormiga	09°12'970"N and 071°44'512"W
Boca Zulia	09°14'092"N and 071°44'248"W
Frente de Birimbay	09°15'310"N and 071°43'890"W
La Lagunita	09°16'183"N and 071°43'605"W

metals in sediments did not always lead to the accumulation of red crabs in muscle tissue (14).

Another possible cause that could affect the levels of heavy metals found in our study is the use of antifouling paints, used for the cleaning and maintenance of the hulls of small boats and oil vessels, especially containing: Pb, Cd and Cr; which would fall directly into the water of Maracaibo's Lake. The presence of As (table 2) in the soft tissues of blue crabs, although at low levels, worries because it is highly toxic, for the biota of the ecosystem. Its origin can be related to the discharges of tributaries to Maracaibo's Lake containing high concentrations of agrochemicals, from different areas of agricultural production, used for the control of pests in agriculture.

By establishing possible comparisons between our data and those reported by other research groups, such as those presented by Santos et al. (2007) (8) in Maceio, Alagoas, Brazil, which is considered as "moderately contaminated area", all the elements analyzed presented low values for the elements analyzed. This was not the case in Puerto Concha, Zulia state (table 2); however, for Cd, Pb and As, the values were higher than other sites considered "uncontaminated" (7). Our values were (usually) higher than those reported by the group of Zotti et al., in 2016 (15) for metals in samples of *C. sapidus*, *Eriphia verrucosa* and *C. pagurus*.

This is also observed for Cd in soft tissues of blue crab (table 2), which were higher than some reports in some estuaries "unpolluted" in the United States (16). This may indicate the potential contamination by Cd, Cu and As in the area, from an unknown source. Cadmium is a toxic environmental pollutant that can disturb cell functions and even lead to cell death. At work of Ariano et al., in 2015 (17), the Cd concentrations in all samples of white crab meat, were found to be very low (below the limit of quantification), although brown crab meat showed

significantly higher Cd concentrations (up to 5.629 mg/kg wet weight; mean value, 1.465 mg/kg). Meanwhile in our results we obtained an average value 0.24 ± 0.05 mg/Kg dry mass, in blue crab.

The apoptosis induced by Cd in gill cells of crab was observed, which was evidenced by apoptotic DNA fragmentation, activations of caspases-3, -8 and -9 and the presence of apoptotic morphological features. The Cd elevated the intracellular concentration of Ca^{2+} , the protein concentration of calmodulin (CaM) and the activity of Ca^{2+} -ATPase in the gill cells of the crabs. The set of above results therefore indicate, that Cd evokes gill cell apoptosis through activating Ca^{2+} -CaM signaling transduction pathway (18).

The accumulation of lead is toxic to humans and crabs. The acute Pb exposure led to a reduction of survival rate of sperm and harmful effects at the cellular level of crab testes and accessory glands, which are most likely linked to Pb-induced oxidative stress (19). In the work of Cruz, Ramos and Ablan-Lagman (2015) (20) were determined heavy metal levels (Pb and Cu) in mud crabs (*Scylla* spp.) from East Bataan Coast. The average concentrations in the samples were 3.37×10^{-3} and 1.01 mg/L, both within WHO acceptable limits.

Meanwhile, the group Lavradas et al. (2014) (21) reported the presence of metals (Cu, Pb, Zn and Cd) in muscles, gills, soft tissues and eggs in specimens of male crabs; as well as ovigerous and non-ovigerous female specimens of *Callinectes* sp. in a reference site in the southeast of Brazil. The levels of metallothionein (MT) and reduced glutathione (GSH) were also determined. Results demonstrate that sex has a significant influence on metal, MT and GSH concentrations. Regarding human consumption, metal concentrations were lower than the maximum permissible levels established by international and Brazilian regulatory agencies, indicating that this species is safe for human consumption concerning this parameter. The presence of metals in *Callinectes* sp., however, is still of importance considering that this is a key species within the studied ecosystem and, therefore, plays a major role in the transference of pollutants to higher trophic levels (21).

Our results (table 2) have shown that the average values obtained for heavy metals in no case exceeded the permissible limits for human and animal consumption (established in the COVENIN standards of Venezuela). However, the presence of highly toxic metals such as Pb, Cd, As and Cr, which do not fulfill any biological function, are a cause for concern and it is recommended to carry out other complementary

Table 2. Levels of heavy metals in soft tissues of blue crabs (*Callinectes sapidus*) expressed in mg/Kg dry mass, Puerto Concha, Zulia state.

Elements ^a	Sampling 1	Sampling 2	Sampling 3	Averages
Cu	89.14±4.46	98.72±4.93	85.90±4.29	91.25±4.56
Co	0.26±0.05	0.37±0.01	0.43±0.05	0.35±0.03
Mn	2.39±0.11	3.60±0.18	3.97±0.19	3.32±0.16
Ar	0.31±0.05	0.42±0.02	0.53±0.02	0.42±0.03
Cr	0.29±0.01	0.39±0.02	0.45±0.03	0.38±0.02
Ni	0.37±0.01	0.41±0.02	0.57±0.02	0.45±0.02
Cd	0.26±0.13	0.25±0.01	0.20±0.01	0.24±0.05
Pb	1.05±0.05	0.89±0.04	0.71±0.03	0.88±0.04

Values are expressed as mean ± standard deviation. ^aEach determination was performed in triplicate.

studies for the determination of these metals in biocontrol organisms (such as *C. sapidus*), in water samples and in the sediments of the study area, to detect which are the sources of contamination? and take the corresponding measures to stop the entry of these toxic elements into the environment and prevent further deterioration of Maracaibo Lake. As a key species in the ecosystem, the blue crab could play an important role in the transfer of environmental contaminants to higher trophic levels and the

presence of these elements can generate concern for local biota and environmental balance.

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