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Trace Metal Levels in Muscles and Exoskeleton of Pink Shrimp (*Penaeus brasiliensis and paulensis*) from a Subtropical Coastal Region, Laguna Estuarine System, Brazil

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Abstract

Contamination by heavy metals produced by either anthropogenic or natural activities represents a threat to man and marine life. The present paper aimed to carry out *in situ* monitoring of trace metals using shrimp species from an estuarine area as a bioindicator of environmental contamination. The shrimps were captured by fyke net during the rainy and dry periods, and the metal concentrations in the muscles and exoskeletons were determined. The results showed that the metal concentrations decrease in the following order Fe> Zn> Cu> Mn> Cr> As> Pb> Cd for both samples groups, muscles and exoskeletons, being the muscles presented lower concentrations. Metal concentrations were within the permissible limits allowed by the Brazilian legislation, except for Pb. Bioconcentration factor was below 1 for most of the metals, what it means that metals are not accumulating in the shrimp body.

Keywords: trace metals, contamination, biomonitoring, pink shrimp, estuarine system

1. Introduction

Industrial development and urbanization coupled with increasing population and global demand for food and consumer goods are causing a number of environmental changes, especially in water environments. The coastal zones are constantly under pressure from their ecosystems due to discharges from many polluting sources causing a negative impact on the life quality, diversity of aquatic organisms and the maintenance of species of commercial relevance in coastal marine ecosystems (Lu et al., 2018; Liu et al., 2019). Most of the environmental contamination in coastal regions come from different human activities, such as mining, tanning, industrial waste leakage, inadequate fertilization, domestic irrigation and sewage (Cai et al., 2016; Tang et al., 2019). Among them, heavy metals presence in the disposed materials are of great concern, due to their persistence in the environment and the tendency to concentrate in aquatic organisms (Lv and Liu, 2019).

Traditionally, the environmental management of coastal resources has always been focused on measurements of chemical pollutants (Capolupo et al., 2017). However, recent studies also emphasize the importance of ecologically relevant measures as a complementary and real tool, encouraging the use of bioindicators, since *in situ* biomonitoring is essential for maintaining environmental quality, followed by laboratory toxicity tests (Baltas et al., 2017; Zhou et al., 2008; Farias et al., 2018; Li et al., 2018). The determination of contaminants, such as heavy metals in aquatic organisms are currently widely used to identify critical areas and assessment of the degree of environmental pollution (Yi et al., 2018; Wang et al., 2019; Xiong et al., 2019; Tsaboula et al., 2019; Kaloyianni et al., 2019).

Shrimps are important seafood that is worldwide consumed and have been widely used as biological indicators of coastal pollution, as well as in the assessment of the influence of metals in the marine environment (Taylor et al., 2018; Frota et al., 2019). They have the potential to be used as sentinels since they can accumulate metals by absorption through the gills or by consumption of contaminated sediments, organisms, and debris. Therefore, information regarding to the metal concentrations in their tissues is potentially useful considering metal toxicity and concern that relies on the public health, in view of their widespread consumption by the population (Copat et al., 2013; Makedonski et al 2017; Fakhri et al., 2018; Shakouri and Gheytasi, 2018).

The pink shrimp (*Penaeus paulensis* and *Penaeus brasiliensis* is widely distributed along the east coast of the Atlantic Ocean and in the south of Brazil (Farias et al., 2019). In the Laguna Estuarine Complex (southern Brazil), such crustaceans breed in the lagoon, which presents a minimum degree of salinity brought by the ocean waters. Thereafter, when they get into the pound (larval phase), they are subsequently set apart to grow in protected areas, such as mangroves, due to the greater food supply. They remain in this environment until they become adults and thus return to the ocean as shrimp, giving rise to new larvae. Although pink shrimp have been the most economic relevance species in the region in the past, the excessive effort of fishing (by catching) and environmental problems affected the stability of this resource, generating fluctuations in production and reducing its economic importance in the region.

The main polluting sources in the study were the region enriched with the effluents from mining waste and coal processing, starch factories, wineries, potteries, ceramics, pig farming, food industries, thermoelectric plants, fluorite extraction and domestic sewage. Notably, the processing and tailings from coal extraction contribute to the depreciation of the environmental quality of the water of the Tubarão River in most of its extension (Lima et al., 2001). As a result of acid drainage, metals migrate to the lagoons where fish are caught for commercial purposes. In high concentrations, the metal ions dissolved on aqueous matrix (As, Cd, Cu, Ni, Pb, Zn, Al, Cr, Mn, Mg, among others) can cause severe contamination and stress in fishes and crustaceans, especially shrimp.

In this scenario, shrimp and sediment samples were captured and collected, respectively, in the Laguna Estuarine Complex (Imaruí Lagoon). Consequently, we aimed to study the concentrations of metals (Cu, Pb, Cd, As, Hg, Cr, Fe, Zn, and Mn) considering spatial distribution and temporal variability. Differences among concentrations of metals present in the hatchling, juvenile, and adult shrimps during the rainy or dry season were also evaluated. Subsequently, bioconcentration factor (BFC) was determined by the ratio between the concentration of metals in organisms (shrimp) and the concentration of sedimentary metals, in order to analyze the evolution of metal contamination in the sediments of the Imaruí lagoon.

2. Materials and Methods

2.1 Study Area

Laguna Estuarine System, a choked coastal lagoon in southern Brazil was selected for this study. The lagoon has an area of 184 km^2, a mean depth of 2 m, and it is isolated from the ocean by a sand barrier to the east, as shown in Figure 1 (Netto and Pereira, 2009). Small rivers to the west contribute freshwater input and sediment delivery. Water exchange between the lagoon and the ocean occurs through a single and narrow channel in the south. The particular geomorphology of the lagoon creates a west-east axis of sedimentary variation, with surface sediments varying from silty and poorly sorted grains with high organic matter. The system generated a series of cuspate divisions (septation) due to wind waves that build spits segmenting the lagoon into separate basins (Menegotto et al., 2019). The basins form strategic sites for intense fishing and tourism activity, the basin (lagoon) of Imaruí was the object of this study (Figure 1).

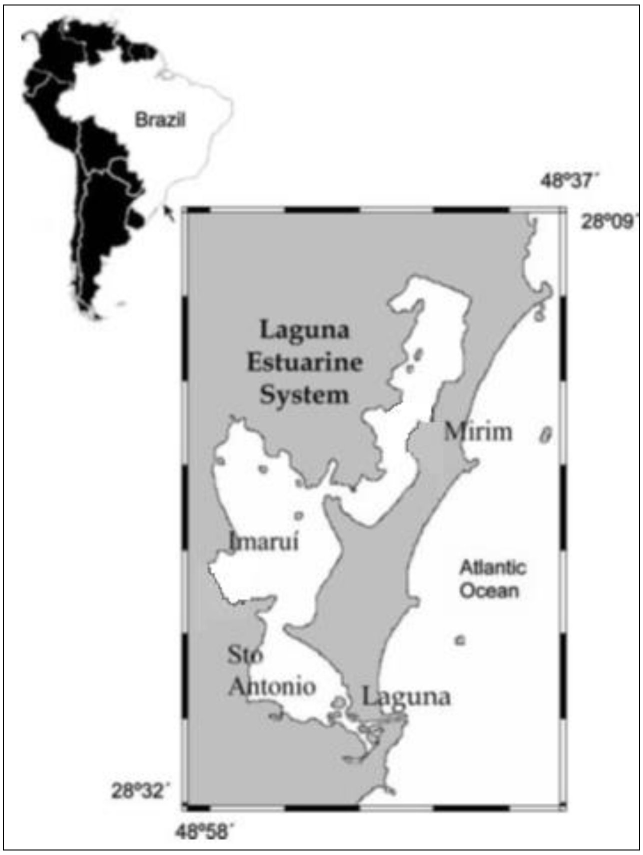

Figure 1. Location of the Laguna Estuarine System, South Brazil

2.2 Sampling Points

Three sampling points were strategically chosen, being 150 m apart as shown in Figure 2. The precise definition of sampling points at the Imaruí lagoon was: 28º22'18" S 48º48'40 W" determined by a pilot sampling, mapped by GPS.

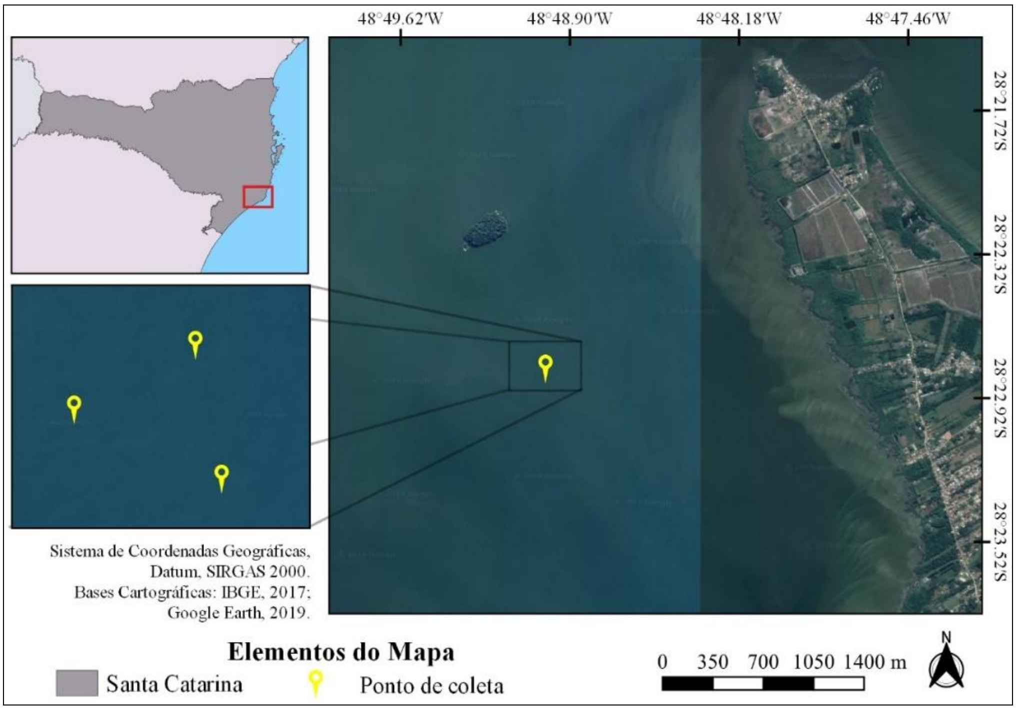

Figure 2. Sample collection points at the lagoon of Imaruí

2.3 Sampling

Artisanal fishing in the Laguna Estuarine System has been carried out using net traps (fyke nets) since the 1980s, which has led to a change in the fisherman's relationship with the fish resource and the environment since this type of net has a great impact on fauna.

According to Netto and Pereira, at Imaruí, the fyke nets are set in a group of 5–7 in contact to the bottom, fixed with stakes in shallow waters (1-2 m depth). Each group of nets forms a cage like structure. In the center of the enclosure, a fluorescent lamp produced by a car battery is placed on a stake, as shown in Figure 3 (Netto and Pereira, 2009). The positively phototropic shrimp are attracted to the light and enter the net. The body and sleeves of the nets have a 25 mm mesh size. Shrimps are harvested daily by changing the new nets. The area closed by each group of nets is around 30 m^2. The nets were always kept in place, except for occasional retrieval for cleaning. However, the stakes are kept in place as a way to mark the fishers' area.

For this study, samples were monthly collected during the legal period for shrimp fishing (November/2018 to May/2019), as established by the federal government in 2005. 0.5 kg of hatchling, juvenile and adult shrimps were collected, whilst about 1.0 kg for sediment sampling.

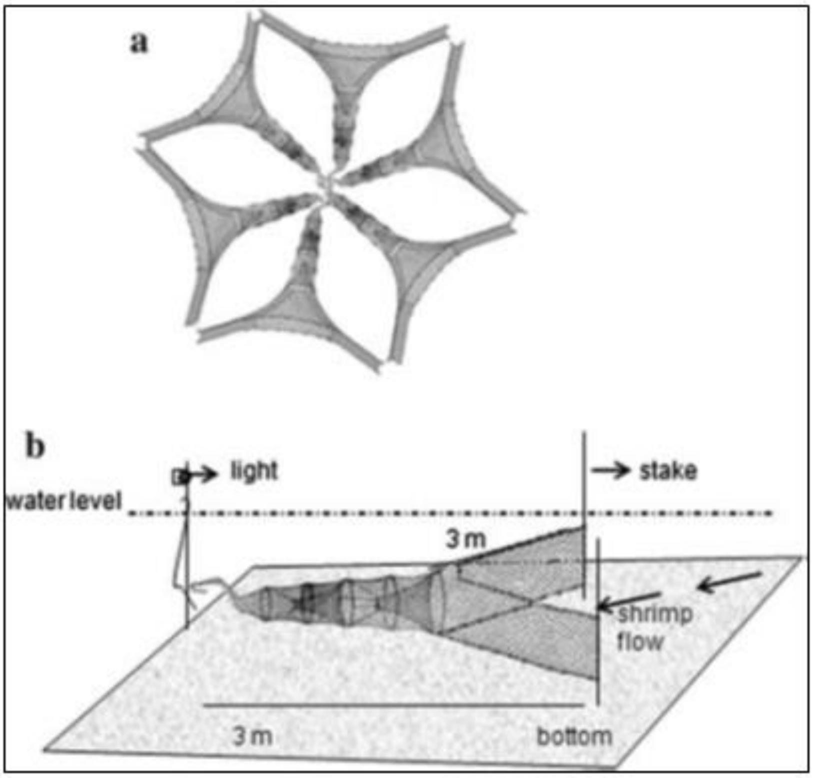

Figure 3. (a) Net enclosure ("aviãozinho") in an upper view (b) a detail of the net that composes the enclosure

2.4 Materials

All chemicals used were of analytical grade. All materials used were cleaned by using a 5% solution of alkaline detergent Extran (Merck), and later, washed with deionized water and then left to stand for 24 h, in a 10% v / v solution of nitric acid (analytical grade, Merck). Finally, materials were rinsed with deionized water. Hydrogen peroxide used was also from Merck (30% analytical grade).

2.5 Sample Preparation

For each sampling, with the aid of a small plane net, 0.5 kg of shrimp were captured. Shrimp samples were divided in groups considering their life cycle (30 hatchling, 20 juvenile or 10 adult), and thus were peeled and separated the exoskeletons of the muscles. Thereafter, samples were oven-dried (with air circulation) to determine the humidity. The water content was evaluated by the gravimetric method in an oven at 105 °C for 6, 12 and 24 hours. Finally, samples were digested and analyzed atomic absorption spectrometry, as described section 2.6.

2.6 Metal Analysis by Atomic Absorption Spectroscopy

Initially, 0.3 g of each sample were placed to a digestor flask (made of PTFE) and 4 mL of nitric acid 65% with 3 mL of hydrogen peroxide 30% was added. Sample digestion was done by using a digestor block in microwave oven DGT 100 Plus (Provecto Analítica, city and country name). After digestion in a microwave oven, samples were cooled to room temperature and 25 mL water was added to them. Samples were thus transferred to volumetric flasks (250 mL) and rest for a while, in order to guarantee that any remained solid particle to be at the bottom of the flask. Prior to the metal analysis (Cu, Fe, Al, Mn, Pb, Cd, As, Hg, Cr, Zn), samples were transferred to a polyethylene 250 mL flask.

The metals measurements were carried out in an atomic absorption spectrometer with electrothermal atomization with a graphite oven GTA 110 (Varian, model SpectrAA 220), equipped with a Zeeman background corrector and an automatic sampler. The data acquisition system was managed by a microcomputer in a Windows environment using Spectra AA data processing, as a radiation source by using hollow cathode lamps Pyrolytically coated Varian graphite tubes (Part N^{o}. 6310003700) and Varian L'vov platforms (No. VII / 113/78501) were used.

Analytical signals were monitored and evaluated through a linear calibration considering the measurements of the prompt area. For all metals analyzed, a 50% palladium/magnesium modifier solution was used and Argon (purity of 99.996%) was used as a shielding gas.

The determination of Cu, Fe, Mn, and Zn concentration were carried out by atomic absorption with flame (Agilent Technologies, 200 Series AA, SPS 3, city and country name of the instrument), by using a multi-element hollow cathode lamp. Background correction and deuterium lamp were similarly used for all metals.

2.7 Statistical Analysis

All analysis was carried out in triplicates and data were expressed in average values, which were taken for analysis of variance (ANOVA) at 5% of significance level and 95% of confidence level, by using Statistica ® (StatSoft) 7th edition.

Differences in univariate descriptors between treatments (inside and outside fyke nets), sites (mud and sand), and treatment and site interaction were tested by 2-way ANOVA. Cochran’s C tests were applied to test for homogeneity of variances, and data were log (x + 1) transformed wherever necessary. Tukey’s multiple comparison tests were used when significant differences were detected ($p< 0.05$), (Sokal and Rholf, 1997).

The bioconcentration factor (BCF) was measured by the ratio between the metal concentration found in the muscles and in the sediments (Nascimento et al., 2016).

3. Results and Discussion

Crustaceans are considered good bioindicators of metals in aquatic ecosystems (Farias et al., 2018). In this study, an integrated assessment was made on a temporal scale of the degree of contamination by traces of metals in shrimps as well as in sediments of Imaruí lagoon, South Brazil. The metals are normal constituents of the marine environment being some of them essential for crustaceans (Cu, Zn, Fe, Mn, Se, etc.), others can be classified as potentially toxic or non-essential metals such as As, Cd, Pb, Hg, and Ni.

This is important to notice that shrimp are the main source of income for fishermen it this region, although the drop in production of this species has increased the importance of fishing for crabs and fish, such as yellowtail and a juvenile mullet (family *Mugiliformes*).

The average concentrations of metals (Fe, Zn, Cu, Mn, Cr, As, Cd, Pb, and Hg) found in muscles and exoskeletons of the shrimp samples, are shown in Table 2.

Table 2. Average values of metal concentrations (mg kg^{-1}) obtained for samples of muscles and exoskeleton of shrimp ($p < 0.05$)

Exoskeleton		Muscles	
Metals	**Average ± SD**	**Metals**	**Average ± SD**
As	$2.39^a \pm 0.82$	**As**	$2.27^a \pm 0.40$
Cd	$0.19^a \pm 0.02$	**Cd**	$0.19^a \pm 0.02$
Cr	$3.77^a \pm 2.53$	**Cr**	$2.44^a \pm 0.97$
Cu	$41.91^a \pm 12.17$	**Cu**	$49.69^a \pm 13.42$
Fe	$172.94^a \pm 48.24$	**Fe**	$114.91^a \pm 53.90$
Mn	$27.93^a \pm 6.88$	**Mn**	$6.99^a \pm 3.09$
Pb	$4.44^b \pm 2.58$	**Pb**	$1.62^a \pm 0.41$
Zn	$43.10^a \pm 10.99$	**Zn**	$54.64^a \pm 8.52$
Hg	ND	**Hg**	ND (Not detectable)

The results found on a temporal scale, was applied to the Tukey test. The data obtained represent the average of six collections ± SD ($n = 6$), where the values followed by the same letter in the column not show a significant difference ($p > 0.05$). However, values in the same column with different letters are significantly different by the Tukey test ($p < 0.05$).

The data show that the concentration of metals decreases in the following order Fe> Zn> Cu> Mn> Cr> As> Pb> Cd for both samples groups (muscles and exoskeleton). In addition, it is possible to identify that the highest concentrations were observed for essential metals Fe, Cu and Zn in both types of analyzed samples. The data also showed that the concentrations of the different metals are lower in the shrimp muscles than in the exoskeleton (shells), except for Cu and Zn. This shows that the shells are acting as metal adsorbent contributing to the reduction of contamination by traces of metals in the muscles. It is also important to notice that no Hg was detected in all analyzed samples.

The average concentrations obtained in the shrimp muscles for essential metals such as Fe were 114.91 mg kg^{-1}, Zn 54.64 mg kg^{-1}, Cu 49.69 mg kg^{-1}, and Mn 6.99 mg kg^{-1}. On the other hand, for non-essential metals, the average values obtained were less expressive, as expected, with average concentrations for Cr (2.44 mg kg^{-1}), As (2.27 mg kg^{-1}), Pb (1.62 mg kg^{-1}) and Cd of 0.19 (mg kg^{-1}).

The lower metal concentrations found in samples of shrimp muscle, when compared to the exoskeleton, is in agreement with the metals present in other fishes and crustaceans (Pourang et al., 2005; Darmono and Denton, 1990). It is probably associated to the hepatopancreas (digestive gland of many invertebrates) and to the filtering capacity of the shells (Yilmaz and Yilmaz, 2007). In decapod crustaceans, hepatopancreas has similar functions to the liver of vertebrates, being related to nutrient metabolism of essential elements, and in the removal of non-essential elements (Arulkumar et al., 2017). Therefore, due to its function, the exoskeleton may contain a more significant load of metals and, thus, the passively absorbed elements in this tissue will contribute to increased concentration of metals in the shrimps (Rainbow, 2007). It is important to mention that besides protein, calcium carbonate and pigments are the main chemical constituents of the shrimp exoskeleton. There is also a high chitin content, which is a biopolymer formed by *N*-acetylglucosamine units connected by covalent β-(1→4)-linkages. Therefore, chitin gives additional functioning to the metal filter and adsorbent to the shrimp shell for the protection of its muscles (Anastopoulos et al., 2017).

Table 3 shows the results of the statistical treatment regarding the concentration of metals obtained for the exoskeleton and muscles of the shrimp. As can be seen, the p values for As, Cd, and Cr did not show a significant difference ($p > 0.05$) when comparing the results obtained for both of the sample groups, the exoskeleton, and muscles of the shrimp. However, the metals Cu, Fe, Mn, Pb, and Zn showed a significant difference ($p < 0.05$) between the minimum and maximum values, with the highest values being observed for Fe, with values ranging from 38.63 to 223.33 mg kg^{-1} in the muscle and from 64.20 to 286.33 mg kg^{-1} in the exoskeleton. These values are in accordance with the high Fe concentration found in the sediments, where the average value obtained was 31401.26 mg kg^{-1} (Table 4). In addition, smaller values were observed for the elements Cu, Mn, Pb and Zn (Table 3).

Table 3. Minimum and maximum values found with statistic treatment of obtained values for metal concentrations found (mg kg^{-1}) in skeleton and muscle of shrimp samples ($p < 0.05$)

	skeleton		**muscles**		**skeleton and muscles comparison**	
Metals	**Min.**	**Max.**	**Min.**	**Max.**	**P value < 0.05**	
As	1.03	5.97	1.53	3.79	P > 0.72	NS
Cd	0.1	0.32	0.14	0.27	P > 0.60	NS
Cr	0	16.43	0.24	5.35	P > 0.24	NS
Cu	36.31	81.28	27.31	95.33	P < 0.016	P < 0.05

Fe	64.2	286.33	38.63	223.33	P < 0.0006	P < 0.05
Mn	14.63	43.91	2.33	14.32	P <0.00035	P < 0.05
Pb	0.41	8.49	0	6.26	P < 0.00051	P < 0.05
Zn	22.56	100.03	40	94.27	P < 0.00196	P < 0.05
Hg	ND	ND	ND	ND	-	-

In Table 4, we can see minimum, maximum, average, and respective standard deviation values of metal concentrations obtained for the sediments of Imaruí Lagoon. The results showed to the metal concentrations for the sediments decrease in the following order: Fe > Mn > Zn > Cd > Cr > Cu > Pb > As.

Table 4. Minimum, maximum, average, and respective standard deviation values of metal concentrations (mg kg^{-1}) obtained for the sediments of Imaruí Lagoon

Metals	**Min.**	**Max.**	**Average[*] ± SD**
As	3.50	14.07	8.82^{b} ± 3.42
Cd	0.05	157.95	52.67_{a} ± 52.63
Cr	0.70	60.80	28.6^{b} ± 18.92
Cu	12.35	29.65	23.27^{b} ± 5.60
Fe	19113.75	44202.94	31401.26^{b} ± 7858.07
Mn	291.61	692.46	477.88^{b} ± 126.60
Pb	10.43	33.09	17.30^{b} ± 7.50
Zn	41.52	114.27	79.07^{b} ± 22.00
Hg	ND	ND	ND

[*] Average of six collections ± SD (n = 6). Values followed by the same letter in the column not show a significant difference (P> 0.05) and with different letters are significantly different by the Tukey test (p <0.05).

The correlation of the results obtained for metals in the exoskeletons and muscles of the shrimp on a temporal scale, during all the collection months (November/2017 to April/2018), can be seen in Table 5 and 6. In general, the concentrations of essential metals (Cu, Zn, Fe and Mn) were significant in the shrimp muscles. The most expressive concentrations were observed for iron, ranging from 196.89 mg Kg^{-1} (January 2018) to 54.45 mg Kg-1 in April 2018. For the zinc element, the highest value was 75.84 mg Kg^{-1} (December 2017) and the lowest was 50.51 mg Kg^{-1} (April 2028), and for copper the highest obtained was 78.72 mg Kg^{-1} (March 2018) and the lowest 46.18 mg Kg^{-1} (April 2018). Cu shows a complementary behavior in regard to the temporal view, where Fe and Zn has a maximum value near January and decreases to April, the Cu has a maximum value near March. The main effect of January and February has a longer period indicate an effect at shrimp, probably associated with the metabolism for Cu for such type of crustacean.

However, the high concentrations of Mn observed in the shrimp muscles, can be explained by the chemical similarity of this element with Calcium. Mn competes with Ca during carapace carbonation and, once exposed to a deficient Ca medium, the organism tends to replace it with other metals that showed great availability in the medium, in this case, Mn (Phillips, 1977). The highest concentration of Mn in the shrimp muscle in Imaruí lagoon was found in January 2018, with a concentration of 11.54 mg Kg^{-1} and the lowest was in November 2017 with a concentration of 2.77 mg Kg^{-1}. The average value obtained over the six months was 6.98 mg Kg^{-1}.

For non-essential metals such as Cd, Cr, Pb and As, the concentrations found in the muscles of dehydrated shrimp (dry weight) were always less than 5 mg Kg^{-1}. The smallest were observed for cadmium, where the concentration varied from 0.144 mg Kg^{-1} (November 2017) to 0.20 mg Kg^{-1} (January 2018). The most significant concentrations were observed for chromium, where the average concentration observed over the six months was approximately 3 mg Kg^{-1}. For the arsenic element, the average value obtained was 2.26 mg Kg^{-1}, the highest value being observed in December 2017 with a concentration of 2.78 mg Kg^{-1} (Table 5). The behavior of As in estuarine waters is complex and is not yet fully understood. In the particulate fraction, arsenic may be associated with inorganic particles, mainly minerals, such as iron oxyhydroxides, which may come from sediment, soil erosion, deposition, naturally present in the environment, especially in rocks, and therefore the muscles of the shrimp analyzed will probably present this element. Intermediate values were observed throughout the collections, as can be seen in Table 5.

Table 5. Levels of metal concentrations found at muscles samples along time

Metals (mg Kg^{-1})	**Nov/17**	**Dec/17**	**Jan/18**	**Feb/18**	**Mar/18**	**Apr/18**
Cu	37.64 ± 12.26	41.56 ± 5.66	47.30 ± 4.72	46.73 ± 5.16	78.72 ± 19.13	46.18 ± 15.38
Fe	167.78 ±	126.59 ±	196.89 ±	57.39 ±	86.38 ±	54.45 ±

	43.7	9.84	135.04	13.85	5.73	17.67
Mn	2.77 ± 0.31	7.22 ± 0.57	10.325 ± 2.84	4.995 ± 0.72	11.545 ± 2.00	5.049 ± 1.11
Zn	57.43 ± 5.37	75.84 ± 17.79	58.00 ± 4.69	51.03 ± 1.65	53,26 ± 1.98	50.51 ± 10.24
As	1.61 ± 0,06	2.78 ± 0.88	2.56 ± 0.68	1.88 ± 0.13	2.25 ± 0.81	2.53 ± 0.40
Cd	0.144 ± 0.006	0.20 ± 0.03	0.20 ± 0.04	0.18 ± 0.02	0.18 ± 0.024	0.18 ± 0,02
Pb	3.62 ± 1.87	1.08 ± 0.36	1.45 ± 0.99	0.14 ± 0.12	1.72 ± 0.19	1.72 ± 0.19
Cr	2.79 ± 0.39	3.37 ± 0.79	2.651 ± 0.41	3.02 ± 1.67	3.48 ± 1.341	2.35 ± 0.79
Hg	ND	ND	ND	ND	ND	ND

For lead, the average value obtained was 1.78 mg Kg^{-1}, with the highest concentration observed in November 2017, 3.62 mg Kg^{-1}. These values may be related to the beginning of the opening of fishing. Probably these prawns had been in the lagoon longer and, therefore, the higher concentration of these metals present in the muscles of the prawns cleaned due to bioaccumulation. In addition, fishermen use lead batteries when fishing for small aircraft and often these batteries are lost due to winds or discarded in the lagoon, which increases the concentration of lead in the water. Lead has an affinity for molecules that have nitrogen and sulfur atoms, binding relatively easily to proteins and cellular macromolecules, thus being able to enter the metabolism of the shrimp organism.

Table 6 shows the results obtained for the concentration of metals in the shells (shells) over the six months of the study.

Table 6. Levels of metal concentrations found at exoskeleton samples along time

Metals ($mg\ Kg^{-1}$)	**Nov/17**	**Dec/17**	**Jan/18**	**Feb/18**	**Mar/18**	**Apr/18**
Cu	52.47 ± 5.30	62.69 ± 4.34	75.70 ± 4.75	71.02 ± 1.83	74.33 ± 1.12	42.40 ± 4.55
Fe	78.77 ± 17.18	183.40 ± 28.16	240.88 ± 43.34	181.39 ± 58.44	188.26 ± 46.77	164.98 ± 79.24
Mn	24.97 ± 23.90	16.98 ± 0.82	22.43 ± 6.86	32.50 ± 9.29	27.40 ± 8.09	29.89 ± 5.49
Zn	39.29 ± 2.48	48.26 ± 1.28	61.63 ± 27.44	37.07 ± 1.73	39.90 ± 3.65	25.52 ± 3.18
As	1.61 ± 0.06	2.78 ± 0.88	2.56 ± 0.68	1.88 ± 0.13	2.25 ± 0.81	2.53 ± 0.40
Cd	0.14 ± 0.006	0.22 ± 0.03	0.20 ± 0.04	0.18 ± 0.02	0.18 ± 0.024	0.18 ± 0.02
Pb	3.62 ± 1.87	1.08 ± 0.36	1.45 ± 0.99	0.14 ± 0.12	1.72 ± 0.19	1.72 ± 0.19
Cr	2.79 ± 0.39	3.37 ± 0.79	2.65 ± 0.41	3.02 ± 1.67	3.48 ± 1.341	2.35 ± 0.79
Hg	ND	ND	ND	ND	ND	ND

In general, the concentration of metals in the shrimp shells is approximately 30% higher when compared to the results obtained for the clean shrimp muscle, regardless of the metal and the sample analyzed. The highest concentrations were observed in the month of January, where the concentration of iron found in the carapace was 240.88 mg Kg^{-1}, zinc 75.70 mg Kg^{-1} and copper 61.43 mg Kg^{-1}. However, the lowest values were observed in the month of April, where the iron concentration was 188.26 mg Kg^{-1}, zinc 39.90 mg Kg^{-1} and copper 74.33 mg Kg^{-1}. Intermediate values were obtained in the other months. For cadmium, the concentration (dry weight) varied from 0.17 mg Kg-1 in November 2017 to 0.24 mg Kg^{-1} in

January 2018. The most significant concentrations were obtained for the chromium and lead metals. For lead the highest concentration was 7.31 mg Kg^{-1} and chromium 8.15 mg Kg^{-1} observed in January 2018.

For the arsenic element, the average value obtained for shrimp exoskeleton was 2.39 mg Kg^{-1}, with the highest concentration value observed in December 2017, with a concentration of 3.68 mg Kg^{-1} (Table 6).

When evaluating the shrimp samples, it was observed that there is no significant difference (exoskeleton and muscles) regardless of samples age (hatchlings, juveniles, and adults) and season. Therefore, to ensure such behavior of the absorption of essential and non-essential metals between the exoskeletons and the muscles, a factorial ANOVA was run using the results of the averages of all months comparing with the three ages analyzed (hatchlings, juveniles, and adults), both for muscle and for carapaces, as shown in Figures 5 and 6.

As shown in Figure 5, samples of different ages of shrimp can have common characteristics. Variations between hatchlings, juvenile, and adult shrimp were evident for all metals, although for Fe, Zn, Mn and Cu have higher concentrations in juvenile shrimp.

In general, for the iron, hatchlings shrimp samples showed have higher concentrations of Fe in the muscles and exoskeletons (Figure 5A, blue), while juvenile (Figure 5A, red) and adult (Figure 5A, green) shrimp samples showed relatively equal concentrations in muscles and exoskeletons. However, concentration of Zn found in the muscles of the hatchlings, juvenile, and adult shrimp was higher when compared to the values found for concentrations in the exoskeletons. In the muscle, juvenile prawns showed a higher concentration of Zn, while hatchlings (red) and adult (green) shrimp samples showed relatively equal concentrations in muscles (Figure 5B). Mn concentration found in the exoskeleton were greater than in the concentration obtained muscles, regardless of the age of the shrimp (Figure 5C). However, for Cu, the exoskeleton showed a higher concentration compared to the muscles. In addition, the muscles of adult prawns showed a higher concentration of Cu when compared to the shells of young shrimps. In the exoskeleton of adult shrimp samples, there was a higher concentration of Cu in relation to the exoskeletons of offspring (Figure 5D, blue) and juvenile shrimp (Figure 5D, red).

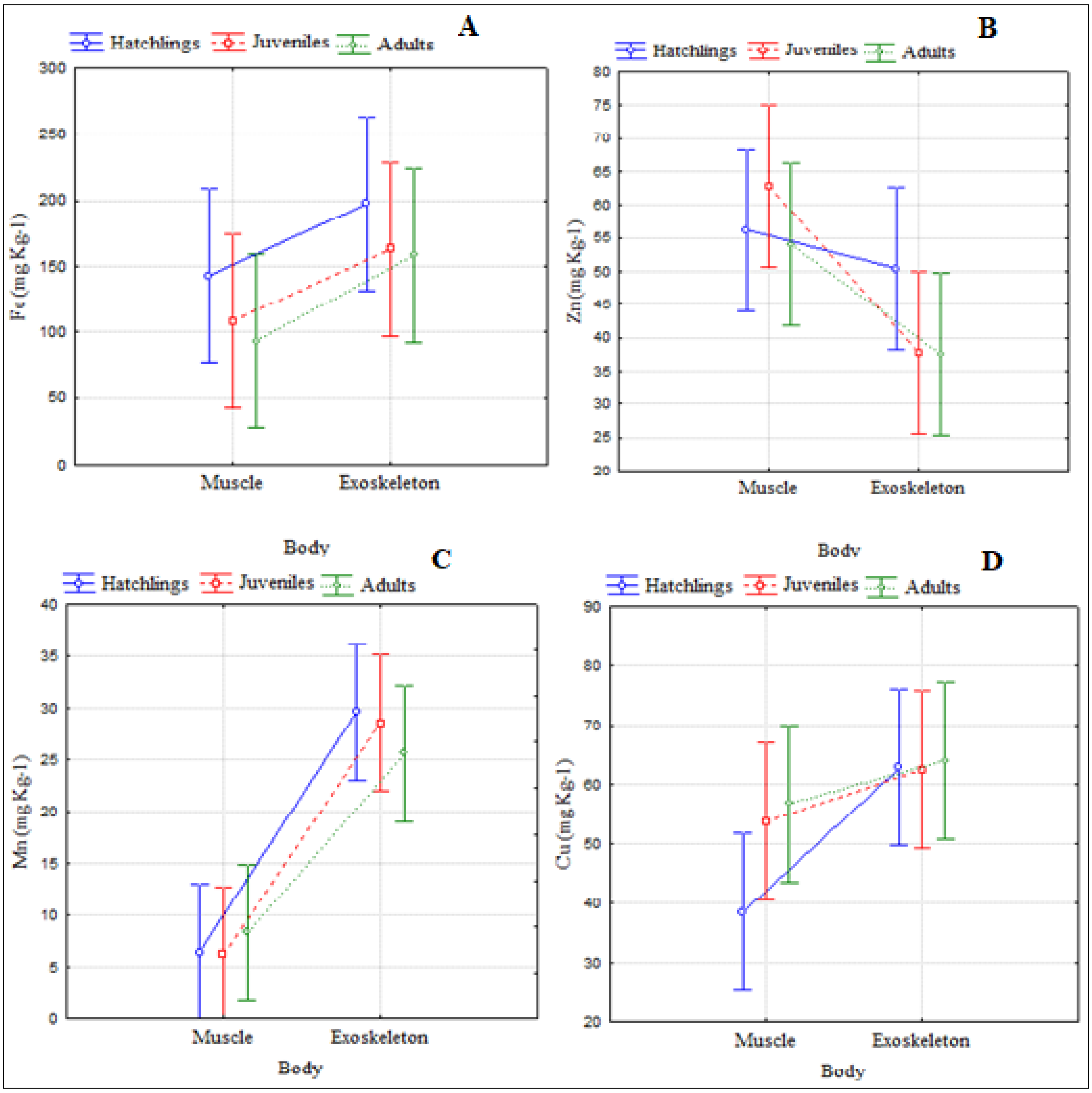

Figure 5. Correlation the average values obtained for of essential metals between the exoskeletons and the muscles of hatchlings, juveniles and adults shrimps, analyzed during the months of November/2017 to April/2018

Figure 6 shows the main characteristics observed with respect to metals As, Cd, Pb and Cr, in the different sizes of the shrimps. It was observed that the muscles of juvenile (Figure 6A, red) shrimp samples showed a higher concentration of As in relation to the muscles of hatchling (Figure 6A, blue) and adult (Figure 6A, green) shrimp. However, in exoskeletons the concentrations of As were higher in hatchling samples, followed by juvenile and adults. For Cd, concentrations were very different, among samples of different ages. The Cd concentrations found in the exoskeletons and muscles of the hatchling (Figure 6B, blue) samples higher than those found in juvenile (Figure 6B, red) and adult (Figure 6B, green) shrimp samples.

The concentration of Pb in the muscles showed no difference regarding the sample age. However, it can be seen that concentrations found in exoskeleton were higher when compared to those found in muscles, mainly in adult shrimps (Figure 6C). However, for chromium, muscles of adult shrimps showed to have higher concentrations followed by juveniles and hatchling, respectively. In this case, it is important to mention that higher concentrations in the muscles of adults shrimps can be a result of their need for such metals as a coenzyme functioning in cellular components. Exoskeletons of juveniles showed a contrary behavior, presenting the highest concentrations (Figure 6D, red), followed by hatchling (Figure 6D, blue) and adult (Figure 6D, green) shrimp.

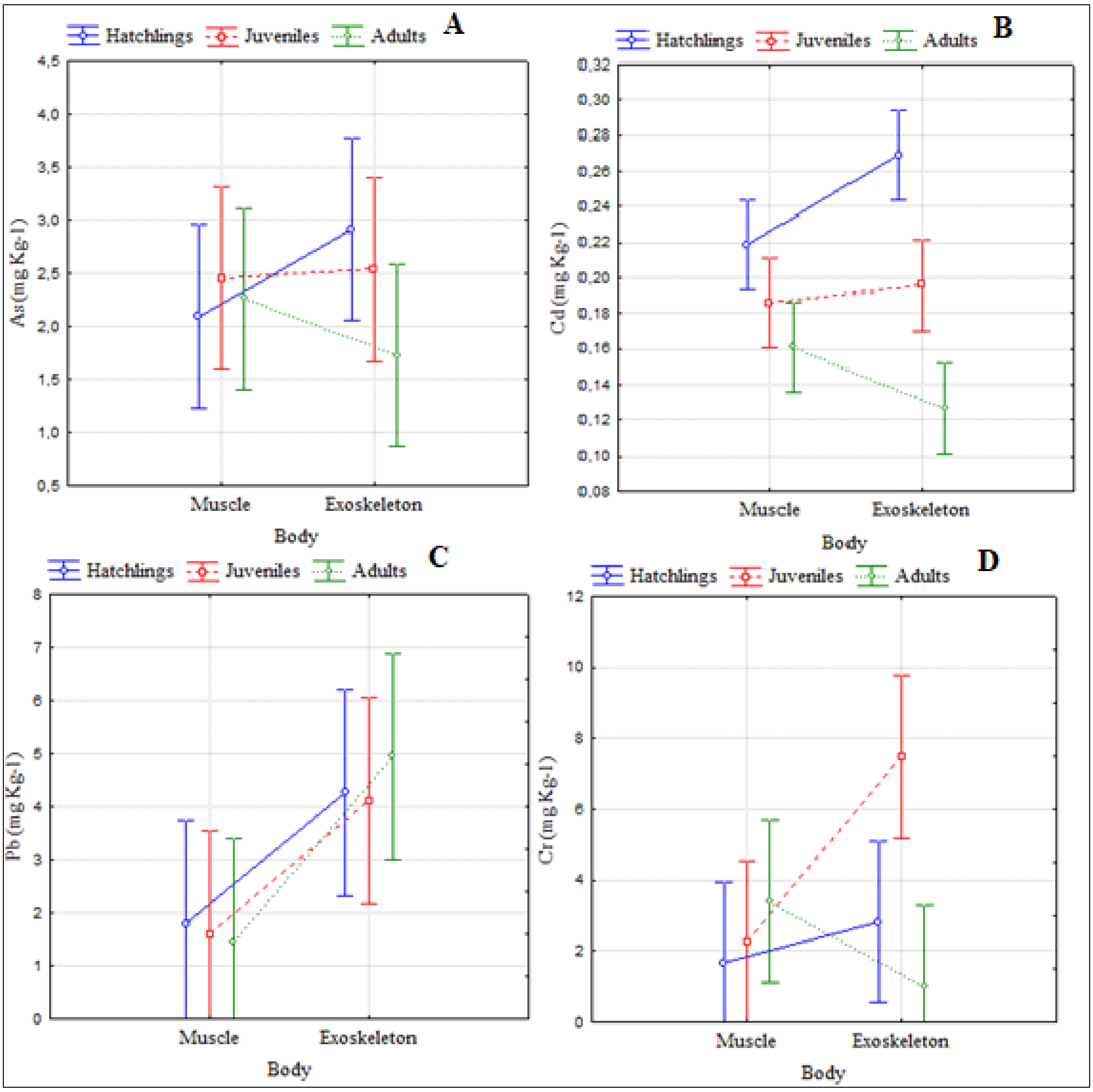

Figure 6. The correlation the average values obtained for of non-essential metals between the exoskeletons and the muscles of hatchlings, juveniles and adult shrimps, analyzed during the months of November/2017 to April/2018

3.1 Non-Essential Metals: Aspect of Human Consumption

Table 5 shows the established limits allowed by Brazilian law according to the ANVISA (National Health Surveillance Agency, Brazil), resolution of the collegiate board - RDC Nº 42, of August 29, 2013, which provides for the MERCOSUL (SOUTHERN COMMON MARKET) countries technical regulation for verification and cross-checking of obtained data. This resolution establishes the maximum content of inorganic contaminants, especially for metals such as As, Pb, Cd, and Hg in crustaceans, where the head and thorax are excluded. Therefore, for comparison with the maximum permitted metal concentrations, the results will be expressed in wet weight, calculated based on an average humidity of 71.74%.

For shrimp caught in the Imaruí lagoon, average concentrations of metals were found below the maximum limits allowed by Brazilian legislation for As, Cd, and Hg, except for Pb in November 2017.

Table 5. Average values of the non-essential metal’s concentration present in the shrimp samples from the Imaruí lagoon and the maximum limits allowed by the legislation of Brazil

Metal	**Average concentration (Imaruí Lagoon)**	**Maximum limit allowed [1]**
Arsenic (mg kg^{-1})	0.640	1.000
Cadmium (mg kg^{-1})	0.051	0.500
Lead (mg kg^{-1})	1.020	0.500
Mercury (mg kg^{-1})	ND	0.500

[1] *ANVISA - resolution of the collegiate board - RDC nº 42, of August 29, 2013.*

For As, the allowed limit is 1.0 mg kg^{-1}, and the average value was 0.64 mg kg^{-1}, with the highest value of 0.79 mg kg^{-1}. For Cd and Pb, the allowed limits are of 0.5 mg kg^{-1}, with the average values of 0.05 mg kg^{-1} and 0.5 mg kg^{-1} for Cd and Pb, respectively. In this case, the highest value for Cd was 0.064 mg kg^{-1}, whilst for Pb, it was 1.020 mg kg^{-1} (Table 5). Based on that, it is worth informing that Cd, As and Hg concentrations are in agreement with the established law in terms of environmental concerns, however, Pb is a metal that requires a more frequent and longer monitoring in such lagoon complex. It is important to mention that the muscle, as clean shrimp, is the most widely consumed form by the population, and may, therefore, present a risk warning sign to consumers, due to the effects of Pb in modifying human metabolism.

Another point to be considered is that fishermen use Pb batteries when fishing with net traps and often these batteries are discarded or thrown by them in the middle of the lagoon, which increases the concentration of Pb in the sediments and the probability of existing fauna to be contaminated by this metal.

3.2 Bioconversion Factor (BCF)

In addition to the physiology of the aquatic organism, the bioavailability of sedimentary metals also interferes with their incorporation into the biota. In the environment, metals can be released in response to changes in redox conditions, pH and saturation of the medium. Therefore, such conditions can interfere by inducing the chemical form of metals and their availability, resulting in metal bioaccumulation in shrimps.

BCF is the ratio between the concentration of metals in the muscle and in sediment samples. Table 6 shows the results of metal concentration and respective BCF for the Imaruí lagoon. It is important to note that if the denominator is much larger than the numerator, the ratio will be very low, which was observed for most metals in this study. However, if the BFC value is greater than 1, it means that metal is accumulating in the body.

Table 6. Metal concentrations for muscles and sediment samples and the bioconcentration factor (BCF) values

Metal (mg L^{-1})	**[*]Average value of metal concentration**		**BCF**
	Muscle	**Sediment**	
As	2.27^{a}	8.82^{b}	0.26
Cd	0.19^{a}	52.67^{a}	0.004
Cr	2.44^{a}	28.60^{b}	0.08
Cu	49.69^{a}	23.27^{b}	2.13
Fe	114.91^{a}	31401.26^{b}	0.004
Mn	6.99^{a}	477.88^{b}	0.01
Pb	1.62^{a}	17.30^{b}	0.09
Zn	54.64^{a}	79.07^{b}	0.69
Hg	ND	ND	ND

[*] Average of six collections ± SD (n = 6). Values followed by the same letter in the column not show a significant difference (P> 0.05) and with different letters are significantly different by the Tukey test ($p < 0.05$).

According to the obtained results, the lowest BCF values were close to 10^{-3} for the elements Cd and Fe, while for Mn, Pb and Cr showed a more expressive range of concentration from 10^{-1} to 10^{-2}, while for Zn and As BCF were even 10 times higher, ranging from 0.26 to 0.29, respectively. In addition, the obtained value for Cu was 2.13, showing that there is an accumulation of this metal in the muscles of the shrimps from Imaruí Lagoon in the Lagunar Complex, Santa Catarina, Brazil.

4. Conclusion

According to the results, it can be concluded that the concentrations of metals decrease in the following order Fe> Zn> Cu> Mn> Cr> As> Pb> Cd for the two groups of samples analyzed, for muscles and exoskeleton of shrimp. Data also showed that the concentrations in the muscles of different metals were lower than the concentrations found in the exoskeleton (shells), except for Cu and Zn, which had higher average concentrations in the muscle samples compared to the exoskeletons. This fact indicates that shells are acting as metal adsorbent contributing to the reduction of contamination by metal traces metals in the muscles. Considering the legislation for metals in the environment, the average concentrations of metals found in the muscles of shrimps were below the maximum limits allowed by Brazilian legislation for As, Cd, and Hg, but not for Pb.

In regard to the bioaccumulation of metals, BCF values were lower than 1 for metals Cd, Fe, Mn, Pb, Cr, As and Zn, indicating that there is not bioaccumulation at shrimp muscles. On the other hand, a BCF of 2.13 was found for Cu, showing the bioaccumulation of such metal in shrimps at Imaruí Lagoon in the Lagunar Complex, Santa Catarina, Brazil.

Finally, in spite of all the diversities that may cause heavy metal contamination (natural causes, anthropogenic activities, etc.) in the region, it can be concluded that the Imaruí lagoon presented a good quality of shrimp (shrimp muscles), but, it is worth raising a warning about the contamination of heavy metals in shrimp in general, especially arsenic and lead.

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