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Laboratory-Based Bioaccumulation Essay for Elements Associated to Particulate Matter in White Shrimps After *In Vivo* Exposure

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Abstract

Title: A laboratory-based effort to investigate the bioaccumulation of elements associated to the total airborne particulate matter in a globally representative crustacean, the White Shrimp (*Litopenaeus vannamei*).

Background: Adverse effects of air pollution are a health concern, especially those related to particulate matter (PM) exposure. However, potential ecotoxicological effects of this complex matrix are not well understood or even evaluated. We hypothesized that the bioaccumulation of elements (especially metals) could be observed in aquatic organisms as a primary ecotoxicological impact of air pollution.

Methods and Findings: Total suspended particles (TSP) were collected in two sites (Santa Cruz, SC, and Seropédica, SE, Brazil). SC was chosen due to its proximity to the Sepetiba Bay and the presence of an industrial complex, while SE was considered as a reference site. The industrial sources have a lower impact in SE, given a priori information about local pollution sources. Taking into account the global distribution of the white shrimp (*Litopenaeus vannamei*), it was chosen as a model organism. The evaluation of trace element levels associated with TSP exposure was carried out in gills, hepatopancreas, and muscles. Some elements frequently associated to toxic effects (e.g., Fe, V and Pb) presented low concentration (<0.003 mg kg^{-1}); however, we suggest that they may still cause damage through chronic exposure. Zn, Cu, Mn, and Ba presented high concentration (<1.67-32.8 mg kg^{-1}) in the saline extracts of PM composites which can be an indicator of greater bioavailability. Confirming the great bioavailability in saline conditions, the predominant metals in all organs were Zn and Ba. To the best of our knowledge, this is the first laboratory-based bioaccumulation study using PM as a complex source of contaminants to the white shrimp (*Litopenaeus vannamei*).

Conclusions: Our findings suggest that PM from urban areas, especially under influence of industrial activity, may represent a potential risk for aquatic organism due to the bioavailability of some toxic metals. Therefore, we reinforce the idea that guidelines for pollution assessment should be regionally validated.

Keywords: *Litopenaeus vannamei*; Particulate matter; Industry; Metals; Bioaccumulation

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Introduction

In recent years, air pollution has increased as a result of anthropogenic activities such as urbanization and industrialization. The deterioration of air quality has consequences for human and ecosystem health [1]. The atmosphere is the final destination for many pollutants, including particulate matter (PM). These particles have a diameter range that can vary from a few nanometers up to 100 µm. Frequently, PM is analyzed using fractions of the total suspended particles (TSP).

PM contains multiple chemicals, such as inorganic species (e.g., sulfates, nitrates, ammonia, and sea salt), organic compounds (e.g., carboxylic acids, amino acids, and Polycyclic Aromatic Hydrocarbons, PAH), and metals [2]. It is known that the metal fraction of PM induces several effects and damage to the aquatic environment [3]. Metals can be bioaccumulated, leading to many reactions and alterations in cells and tissues of the organisms [4]. Metals such as Fe, Cu, Pb, Ni, Cd participate in the generation of oxidative stress, since they catalyze Fenton reactions, producing the highly reactive hydroxyl radical [5]. In addition, studies relate air pollution exposure and cellular oxidative stress, which may be attributed to the presence of redox active metals in the PM composition [6].

Particulate matter samples were collected in two sites in the Metropolitan Region of Rio de Janeiro (MRRJ), Brazil. Santa Cruz (SC) is an industrial district with great influence of steelworks and an important marine estuary is also located in its surroundings, the Sepetiba Bay [7]. Trace metal inputs from rivers and atmospheric deposition are pollution sources for the Sepetiba Bay. Molisani et al. [8] reported that the bay is highly contaminated by Zn, Cd, Pb, and Hg. Seropédica (SE) is the reference site. During the period of this study, industrial and other urban sources were not as predominant in SE as in SC.

The aim of this study is to assess the bioaccumulation of trace elements associated with PM in white shrimps (*Litopenaeus vannamei*). Fishing is an important socio-economic activity in the region of Santa Cruz and the expansion of the industrial complex poses a potential risk to it. *Litopenaeus vannamei* was chosen as model organism due to its widespread use in aquaculture, both in coastal and estuarine/lagoon environments [9]. Besides that, their biological responses are sensitive to the presence of metals in aquatic environment, including bioaccumulation [10]. Thus, this laboratory-based study assessed the bioaccumulation of different chemicals associated with PM in gills, hepatopancreas, and muscles after *in vivo* PM exposure.

Methods

Samples of total suspended particulate matter (TSP) were collected in Rio de Janeiro State, Brazil. Two sampling sites were chosen. Santa Cruz (SC, 22°55′13″S and 43°41′6″W) is an industrial district, whose main activities are related to metallurgy. SC is nearby Sepetiba Bay area, an important ecosystem about 100 km west from Rio de Janeiro City. Seropédica (SE, 22° 44′ 38″ S and 43° 42′ 28″ W) was chosen as a reference site. SE presents a lower influence of industries, in comparison with SC, and the site is well ventilated. The sampling sites are showed in **Figure 1.**

Particulate matter samples were collected by the Environmental Institute of Rio de Janeiro State (INEA) over 24 h every 6 days using fiberglass filters (203 × 254 mm, 0.21 thickness, 0.3 µm diameter, Millipore, USA) in high-volume samplers (Energética, Brazil) with an average flow of 1.14 $m^3\ min^{-1}$ [11]. The sampling occurred in two campaigns between August 2010 and September 2011. The campaigns are related to low (1) and high (2) rainfall seasons.

Twelve sample filters (1/2 of each filter) were cut and weighed. All filters were extracted together (composite) in about 1,550 mL of saline water under mechanical stirring for about 1 h. Four blank filters (1/2 each filter) were also cut, weighed, and extracted with saline water as a control. The saline water used to extraction was prepared using a commercial sea-salt (Redcoral Aquatech, China) and based on the characteristics of Sepetiba Bay, such as: salinity (surface: 21-30 ‰, bottom: 29-30 ‰) and pH (surface: 6.8-8.2) [12,13] **(Table 1).**

In total, 6 groups were tested: 4 composites for TSP (2 for low rainfall season and 2 for high rainfall season), 1 for blank filters, and 1 for pure saline water. The composites were kept under refrigeration for circa 3 h after the preparation. An aliquot of 50 mL of each composite, acidified with 100 µL of bidistilled nitric acid, was kept under refrigeration for five days before analysis.

Table 1 Mean concentration levels ± standard deviation (mg kg^{-1}) for the saline extracts of PM samples and certified elements in the CRM DORM-3 (n=2), LOD, LOQ, and extraction efficiency for the measured concentration in the CRM (%).

Element	Blank filter	SE-2	SE-1	SC-2	SC-1	LOD (mg kg^{-1})		LOQ (mg kg^{-1})		DORM-3 (%)
						SW[b]	ST	SW	ST	
Al	12.2 ± 0.6	-	-	-	-	12.89	1.94	3.14	6.41	n.a.
Ba	11.4 ± 0.08	3.62 ± 0.16	3.03 ± 0.05	3.35 ± 0.11	4.73 ± 0.09	4.89	0.13	1.19	0.43	n.a.
Cd	-	-	-	-	0.23 ± 0.01	0.86	0.07	0.21	0.24	0.23 ± 0.007 (78)
Cr	-	-	-	-	-	3.96	0.17	0.97	0.56	1.61 ± 0.02 (85)
Cu	-	24.89 ± 0.26	19.87 ± 0.06	11.19 ± 0.09	10.48 ± 0.08	1.67	0.03	0.41	0.10	15.2 ± 1.32 (98)
Fe	0.23 ± 0.11	0.68 ± 0.04	0.62 ± 0.03	0.36 ± 0.02	1.18 ± 0.03	0.88	0.08	0.21	0.25	355 ± 5 (102)
Mn	-	4.29 ± 0.13	3.44 ± 0.01	4.11 ± 0.08	6.75 ± 0.09	2.67	0.05	0.65	0.10	n.a.
Ni	-	-	-	-	-	3.42	0.33	0.83	1.09	0.97 ± 0.07 (76)
Pb	-	-	-	-	-	15.28	0.05	3.72	0.18	0.520 ± 0.08 (132)
Sb		n.a.	n.a.	n.a.	n.a.	n.a.	3[a]	NA	12[a]	n.a.
Si	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	1.26	n.a.	3.34	
Ti	-	0.08 ± 0.01	-	0.09 ± 0.01	0.12 ± 0.01	0.21	1.99	0.05	0.14	n.a.
V	-	-	-	-	1.46 ± 0.04	3.34	3.73[a]	0.81	12.31[a]	n.a.
Zn	2.41 ± 0.23	23860 ± 210[a]	17690 ± 90[a]	18960 ± 130[a]	32850 ± 170[a]	3.51	0.30	0.85	0.99	54 ± 3 (106)

(n.a.): Not available; (SW): Saline water extracts; (ST): Shrimp tissue; (-): lower than the LOD (mg kg^{-1}); ([a]): µg kg^{-1}; ([b]): instrumental LOD (µg L^{-1})

Table 2 Mean concentration ± standard deviation of the measurement of elements (mg kg^{-1}, dry weight) in a pool (n=5) of white shrimp organs: gills (G), hepatopancreas (H), and muscle (M) exposed to saline extract of TSP.

Element	Organ	Saline water	Blank filter	SE-2[c]	SE-1[b]	SC-2[c]	SC-1[b]
Al	G	335 ± 19	92 ± 6	58 ± 2	855 ± 6	-	720 ± 19
	H	26 ± 16	26 ± 0.3	-	5.47 ± 0.31	11.9 ± 0.4	13.1 ± 1.4
	M	8.20 ± 0.71	7.27 ± 0.55	-	-	-	11.5 ± 0.1
Ba	G	66 ± 0.3	47 ± 0.8	232 ± 4	904 ± 2	74 ± 1	664 ± 7
	H	4.46 ± 0.22	4.21 ± 0.24	1.94 ± 0.02	23 ± 1	22 ± 2	19 ± 1
	M	486 ± 3[a]	1.37 ± 0.04	1.01 ± 0.05	1.13 ± 0.03	139.7 ± 0.4[a]	11.4 ± 0.3
Cd	G	0.44 ± 0.10	0.26 ± 0.04	-	-	-	32 ± 2
	H	50 ± 1[a]	0.30 ± 0.02	0.25 ± 0.05	0.19 ± 0.02	0.62 ± 0.24	0.31 ± 0.04
	M	-	-	-	-	-	-
Cr	G	4.96 ± 1.25	5.32 ± 3.90	-	0.82 ± 0.17	-	-
	H	-	0.83 ± 0.13	0.05 ± 0.02	-	0.14 ± 0.04	0.51 ± 0.13
	M	0.63 ± 0.03	0.45 ± 0.03	221 ± 4[a]	-	-	34 ± 3[a]
Cu	G	213 ± 1	295 ± 4	-	421 ± 1	65 ± 0.04	54 ± 1
	H	605 ± 1	723 ± 3	-	-	-	-
	M	17.8 ± 0.1	21 ± 0.1	10.5 ± 0.1	3.83 ± 0.07	-	9.71 ± 0.10
Fe	G	313.50 ± 5.07		-	583 ± 7	-	396 ± 5
	H	79 ± 4	106 ± 0.6	6.34 ± 0.05	-	9.83 ± 0.08	-
	M	9.46 ± 0.04	5.23 ± 0.07	-	-	-	3.52 ± 1.14
Mn	G	33 ± 20	9.03 ± 0.73	-	-	-	-
	H	-	4.48 ± 0.41	3.45 ± 0.12	3.66 ± 0.17	9.55 ± 2.87	4.47 ± 0.33
	M	-	-	-	-	-	-
Ni	G	-	-	-	-	-	-
	H	-	1.76 ± 0.12	0.98 ± 0.45	1.02 ± 0.65	1.76 ± 0.53	1.91 ± 0.66
	M	-	-	-	-	-	-
Pb	G	10.8 ± 2.8	4.47 ± 0.31	-	-	-	-
	H	-	2.02 ± 0.19	0.75 ± 0.04	0.47 ± 0.03	1.31 ± 0.24	1.28 ± 0.06
	M	5.67 ± 0.02	4.53 ± 0.01	-	-	-	-
Sb	G	1.48 ± 0.85	-	-	-	-	-
	H	-	0.12 ± 0.05	0.11 ± 0.05	-	0.75 ± 0.37	0.10 ± 0.04
	M	-	-	-	-	-	-
Si	G	777 ± 75	420 ± 21	1013 ± 68	3070 ±110	226 ± 13	3915 ± 136
	H	208 ± 63	95 ± 10	-	4.08 ± 0.25	-	36 ± 4
	M	25 ± 1	34 ± 7	11.6 ± 0.8	7.55 ±0.35	-	-
Ti	G	10.1 ± 0.9	2.28 ± 0.24	0.75 ± 0.05	23 ± 4	-	20 ± 1
	H	-	1.01 ± 0.09	0.68 ± 0.05	0.75 ± 0.05	1.27 ± 0.40	0.87 ± 0.07
	M	13.2 ± 2.7	13.6 ± 1.8	-	-	-	-
V	G	1.36 ± 0.98	0.26 ± 0.23	-	0.36 ± 0.03	-	1.27 ± 0.06
	H	0.61 ± 0.82	0.57 ± 0.11	-	-	-	0.13 ± 0.03
	M	0.03 ± 0.04	0.04 ± 0.01	-	-	-	-
Zn	G	114 ± 2	113 ± 0.2	155 ± 1	735 ± 57	91 ± 7	509 ± 22
	H	138 ± 1	144 ± 1	-	-	33 ± 0.3	2.61 ± 0.08
	M	50 ± 0.2	47 ± 1.1	3.43 ± 0.02	-	-	27 ± 0.5

(-): Not detected; (a): µg kg^{-1}; (b): SC-1 and SE-1 are composites related to low rainfall; (c): SC-2 and SE-2 are composites related to high rainfall.

White shrimps (*Litopenaeus vannamei*) (~ 5.0 g) were conditioned into 30% salinity water over 24 h, before exposure. During the exposure period, they were individually maintained under laboratory condition in cylindrical glass flasks (each containing 300 mL of saline composite extracts of PM, pH 8.0 at room temperature), under aeration (7.20 mg O_2/L). For each treatment, five shrimps were used. Photo-period was fixed at 12L: 12D. Shrimps were individually exposed for 48 h to PM saline extracts. At the end of the experiment, shrimps were sacrificed and gills, hepatopancreas, and muscles removed to study the bioaccumulation of trace elements.

The organs were lyophilized, weighed, and prepared to an acid

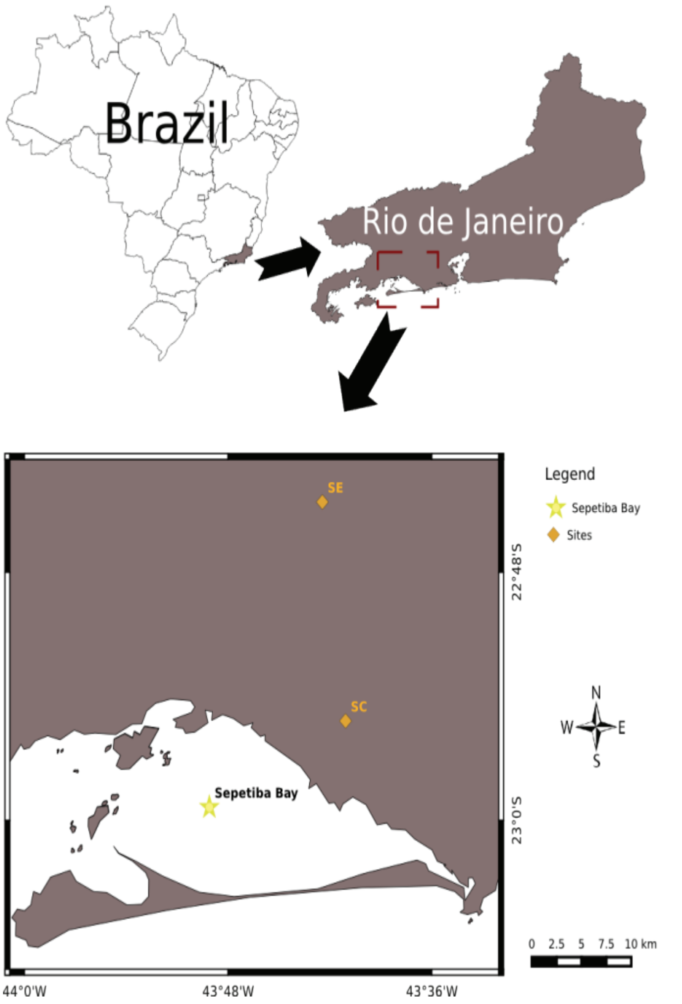

Figure 1 Sampling sites locations: Santa Cruz (SC), industrial area, and Seropédica (SE), reference area.

extraction. A pool (n=5) of each organ was kept in 2.5 mL of bidistilled nitric acid (Vetec, Brazil) for 12 h. Then, they were extracted in a hot plate for 4 h at 80 °C. According to the literature [14], fish gills and liver accumulate more metals than gonad and muscles and we also hypothesized these findings would hold for gills and hepatopancreas in shrimps, respectively. However, considering our model organism, the white shrimp (*Litopenaeus vannamei*), muscles were also seen as important organs to evaluate with regards to the metal bioaccumulation, since it is the edible organ.

Concentration levels of Al, Ba, Cd, Cr, Cu, Fe, Mn, Ni, Pb, Sb, Ti, V, and Zn were determined in all the organs by inductively coupled plasma mass spectrometry (ICP-MS, model NexION 300X, PerkinElmer-Sciex, USA). The analytical solutions were prepared by diluting individual (Titrisol®, Merck, Germany) and multielemental standards (PE-29, Perkin Elmer, USA) with concentrations of 1,000 µg L^{-1} and 10,000 µg L^{-1}, respectively. The analytical curves, prepared by the external method, ranged from 50 µg L^{-1} to 100 µg L^{-1}. The RF power was set to 1,100 W, argon plasma to 17.0 L min^{-1}, nebulizer and auxiliary gas to 1.0 L min^{-1}. The Limit of Detection (LOD) and Limit of Quantification (LOQ) were obtained using the standard deviation of the blank signal multiplied by 3 and 10, respectively; both divided by the slope of the analytical curve. A certified reference material (CRM) of fish protein for trace metals (DORM-3, NRC, Canada) was used to determine the extraction efficiency **(Table 1).**

Saline extracts were analyzed by inductively coupled plasma optical emission spectrometry (ICP OES, model Optima 4300 DV, Perkin Elmer, USA), using standard addition as calibration method. Due to high matrix background, only Al, Ba, Cd, Cr, Cu, Fe, Mn, Ni, Pb, Ti, and Zn were determined. The analytical solutions were prepared by diluting individual standards (Titrisol® - Merck, Germany) and multielemental standards (Certipur Multielement Standard IV, Merck, Germany) both with concentration of 1,000 mg L^{-1}. The analytical curves ranged from 0.05 to 0.5 mg L^{-1}. Besides determination of saline extracts composition, ICP OES was also used to determine Si levels in the organs of white shrimp. LOD and LOQ were calculated in the same manner as for ICP-MS **(Table 1).**

Results

PM levels, quality assurance and control

The TSP geometric mean concentration (± standard deviation) ranged from 39 (± 15) µg m^{-3} at SE to 63 (± 22) µg m^{-3} at SC. Both the ultrapure water and the bidistilled nitric acid exhibited low levels of the monitored elements, unlike the blank filters which presented high levels. The most representative elements in the blank filter were Al (12.2 ± 0.6 mg kg^{-1}), Ba (11.4 ± 0.08 mg kg^{-1}), Fe (0.23 ± 0.11 mg kg^{-1}), and Zn (2.41 ± 0.23 mg kg^{-1}) **(Table 1)**. In every 15 samples, the CRM DORM-3 was analyzed as a calibration check of both ICP OES and ICP-MS techniques. The certified elements (i.e., Cd, Cr, Cu, Fe, Ni, Pb, Zn) presented recoveries between 76 and 132%.

Saline extracts: Levels of PM-associated elements

With regard to the saline extracts from PM filters, the elements can be classified in two groups: 1) major elements (Zn, Cu, Mn, and Ba); and 2) trace elements (V, Fe, and Cd). Zn presented the highest concentration (32850 ± 170 µg kg^{-1}) while Cd presented the lowest one (0.23 ± 0.01 mg kg^{-1}). During the higher rainfall season, SE-2 and SC-2 composites, both Zn and Cu presented higher levels in SE than in SC. During the lower rainfall season, SC-1 and SE-1 composites, Fe, Zn, and Mn levels were higher in SC than in SE.

Organs: Levels of PM-associated elements

Silicon, Ba, Zn, Al, Cu, and Fe were the major elements observed in gills while Al, Ba, Si, and Zn were the highest measured elements in hepatopancreas, their average concentration ranged from 5 to 36 mg kg^{-1} **(Table 2).**

With regard to the levels in hepatopancreas, Fe, Mn, Ni, Ti, and Zn were higher at SC-2 and Fe and Zn were only determined in the SC treatment. SE-1 and SE-2 composites presented similar levels to Ni and Mn. Mn level quantified in SC-2 is higher than reported in the literature to liver tissues of intensive aquaculture [15]. During the lower rainfall season, Ti did not show difference between SE and SC. Barium presents similar levels to all treatment, except for SE-2. Antimony presented similar concentration levels in three treatments. Copper was not detected at any treatment.

As observed in other studies, the metal content in muscles was lower than in the other organs. The concentration of elements presented the following order Zn>Ba>Cu>Fe>Cr **(Table 2)**. The other elements were either detected in only one composite (i.e., Al and Fe) or not detected in any composite (i.e., Cd, Mn, Ni, Pb, Sb, and V).

Discussion

The geometric mean for TSP at SC exceeded the secondary annual Brazilian standard (60 µg m^{-3}), which is designed to protect the public welfare, including vegetation, animals, and material. The difference in concentrations is expected since SC is under influence of more pollution sources than SE.

With regard to the saline extracts from PM filters, Zn presented the highest concentration (32850 ± 170 µg kg^{-1}) among the samples and it is very often listed as one of the traffic related elements (TREs). However, in this study its concentration may also be influenced by metallurgical process [11]. It is also noteworthy some degree of seasonality for the concentration levels of Cu, Fe, Mn, and Zn. During the higher rainfall, SE-2 and SC-2 composites, both Zn and Cu presented higher levels in SE than in SC. During the lower rainfall, SC-1 and SE-1 composites, Fe, Zn, and Mn levels were higher in SC than in SE. The lower rainfall relates to the opening of a large steel plant in SC. Given that Fe, Zn, and Mn are commonly associated with steelworks, the undesirable emission of the company may explain these results. In spite of the good extraction efficiency of some elements, provided by the analysis of the CRM DORM-3, a potential source of problems for our methodology is the assessment of Al, Ba, Fe, and Zn in the saline extracts, since these are major elements in fiberglass filters **(Table 1)**. The use of filters from other materials may yield to better results.

Due to the constant contact with external environment, the concentrations of pollutants in gills, as expected, were higher than in other organs **(Table 2)**. Several authors described lesions in the gills of different organisms as a result of metal exposure: 1) fishes [16], 2) mollusks [17], and 3) shrimps [18]. In fishes, there is a concerning about the absorption and metabolism of metals, via gills, before reaching the liver [19]. Authors reported histological damages to gills, but these findings are related to higher concentration of single metals (i.e., Cd, Cu, Pb, and Zn) in different species of shrimps [18,20,21]. In our study, the major elements observed in gills were Si, Ba, Zn, Al, Cu, and Fe.

As expected, high concentration levels of known toxic metals (i.e., Mn, Ni and Pb) were found in hepatopancreas. These levels were higher than in other organs as observed in other studies [22]. In crustaceans, hepatopancreas is important for the metabolism and storage of xenobiotics [23]. Although is not common to detect exposure to high Zn concentrations in aquatic organisms, it does occurs, mainly in coastal areas, and it has been previously reported [24].

This study suggests that the industrialization of coastal areas may represent a potential risk for aquaculture, given the exposure to TSP. In terms of public health, TSP is not associated to direct damages, since they have high aerodynamic diameter and cannot penetrate in the respiratory system. However, the same does not apply to environmental health. Due to the similarity to sediments in both composition and particle size, shrimps ingest TSP and that explains the higher bioaccumulation in gills, despite of the short-term exposure of this study.

As a consequence of the pilot design of this study, a limited number of shrimps yielded in a small mass for each pool and the standard deviation provided are related to the instrumental measurement of the same pool. Therefore, we could not perform formal statistical comparisons. We strongly believe that future studies applying our method can be even more successful through the use of a bigger sample size. Even though some trace elements were in low concentration levels, they may still be toxic through chronic exposure. Besides, shrimps present small size but are relatively abundant, which may imply a trophic level transfer of contaminants.

Acknowledgements

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