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Determination of lead (Pb) in feces of *Lontra longicaudis* (Olfers, 1818) by flame atomic absorption spectrometry (F AAS)

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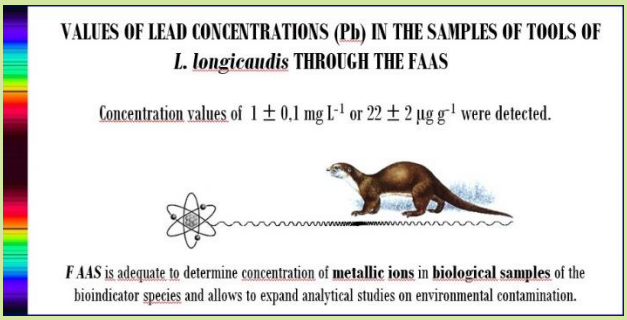
ABSTRACT: The determination of metallic ions by flame atomic absorption spectrometry reaffirms the success of the technique for ecological and toxicological studies of environmental contamination. In order to contribute to the scientific research on anthropogenic contamination, the study of lead in feces of *Lontra longicaudis* (Olfers, 1818) in the Guaraguaçu River Basin aims to understand the anthropogenic environmental contamination of the local ecosystem, located in one of the largest port areas in Brazil. Six samples were collected along the Guaraguaçu River. To determine the lead concentration by flame atomic absorption spectrometry method, samples were extracted with HNO_3 1 % v/v. Lead concentration values about 1 mg L^{-1} were detected in feces samples, indicating abnormalities. Thus, the analytical method allows the interpolation of fecal analysis of *L. longicaudis* in order to quantify the inorganic contaminants in water, secondary food web items and complementary data of elements that do not yet exist in normative tables. Thus, it converges to deepen the development of possible actions to control, monitor and inspect this element in the environment and in its main anthropogenic sources of contamination and for the presentation of possible conservation strategies of the ecosystemic biodiversity.



1. Introduction

The Guaraguaçu River Basin is an area characterized by divergent configurations of space where there are discordant relationships between social and natural determinants in relation to land occupation and the economic aspects of its use[1, 2]. The set of actions developed in its area of insertion destined to the production, distribution, consumption and financial market, mainly related to the agribusiness sector associated with the largest port complex in the region, represent a serious indication of environmental crisis[1-4]. Thus, the problem that drives the development of this work is based on the contamination of the physical and biological compartments of the Guaraguaçu River by metallic ions from the productive, logistic and operational processes of the Port of Paranaguá, located in the homonymous bay, on the coast of Parana. Also, it is considered that the coast of Parana does not currently present detailed knowledge about sanitary problems and ecological imbalance arising from metallic ions resulting from this type of contamination[5].

The analytical knowledge about the dynamism between the socioeconomic and socioenvironmental establishment for the occupation and use of the coastal space contributes to identify and to characterize territorial tendencies of risks to the ecosystemic health by productive processes potentially polluting. As a

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primary condition for the search for territorial development and quality of the natural space, such knowledge is strategic so that preventive, control, monitoring, inspection and remediation procedures are desired at the local level.

Thus, the use of biological material from the bioindicator species allowed not only to carry out the quantitative determinations of contamination by Pb from the productive activities of the local agricultural and port sector, but also to expose the understanding of the complexity and inseparability of the environmental impacts over the use and the disorderly and indiscriminate exploitation of common property.

With all the progress recently achieved, atomic absorption spectrometry is reaffirmed as an important and successful technique for the determination of metals and semi-metals in biological and environmental samples[6, 7]. As an available tool for assessing the environmental and toxicological effects of individual metallic ions available in water, sedimentological and/or biological compartments, its use has been widely inserted in environmental monitoring and control contexts.

In this scenario, through the use of the flame atomic absorption spectrometry technique in the determination and analysis of lead in the stools samples of *Lontra longicaudis*, the objective of this study was to quantify the impacts of the environmental contamination originated from the Port of Paranaguá in the study area, as well as their direct and indirect interference relations on the organisms of the local ecosystem. It is possible to provide such a diagnosis considering that the bioindicator species, as a carnivorous predator of the chain, has its biological and ethological data coinciding space-temporally with other groups of species in the regional scope. Its traces reveal the integrality of the flows and dynamics of the metals in the environmental compartments in which it inhabits and shares with other groups of organisms.

Therefore, the research had a quantitative support and a qualitative understanding regarding the contamination of the Guaraguaçu River by inorganic contaminants from the use of an animal bioindicator and, according to the trophic web, in a regional way, of the animal species. That is, the results included the provision of data applicable to environmental surveillance and ecosystem health in the areas of direct and indirect influence of the Guaraguaçu River Basin, and the identification of irregular aspects for the future development of conservation strategies of specific and ecosystem diversity.

Therefore, it subsidizes the continuity of studies that aim at monitoring ecosystem and species conservation actions at a regional level, taking into account conservation plans for the species at the state and national levels.

2. Experimental

2.1 Study area and sampling points

Fieldwork and sampling campaigns consisted of the active fecal search along the 60 km of Guaraguaçu River, which were classified according to the influence of the tides. In order to understand quantitatively the impact of the environmental contamination originating from the Port of Paranaguá and, consequently, the possible scenarios under the different physicochemical conditions related to the local dynamics of tidal intake and tide, the points of collection were segmented according to a division in five areas: Area 1, control, mountain slope: without tidal influence; Area 2: low tidal influence; Area 3: intermediate tidal influence; Area 4, mouth in estuarine environment of the Bay of Paranaguá: high tidal influence; and, Area 5, Ditch or Watercourse DNOS: high tidal influence (Figure 1).

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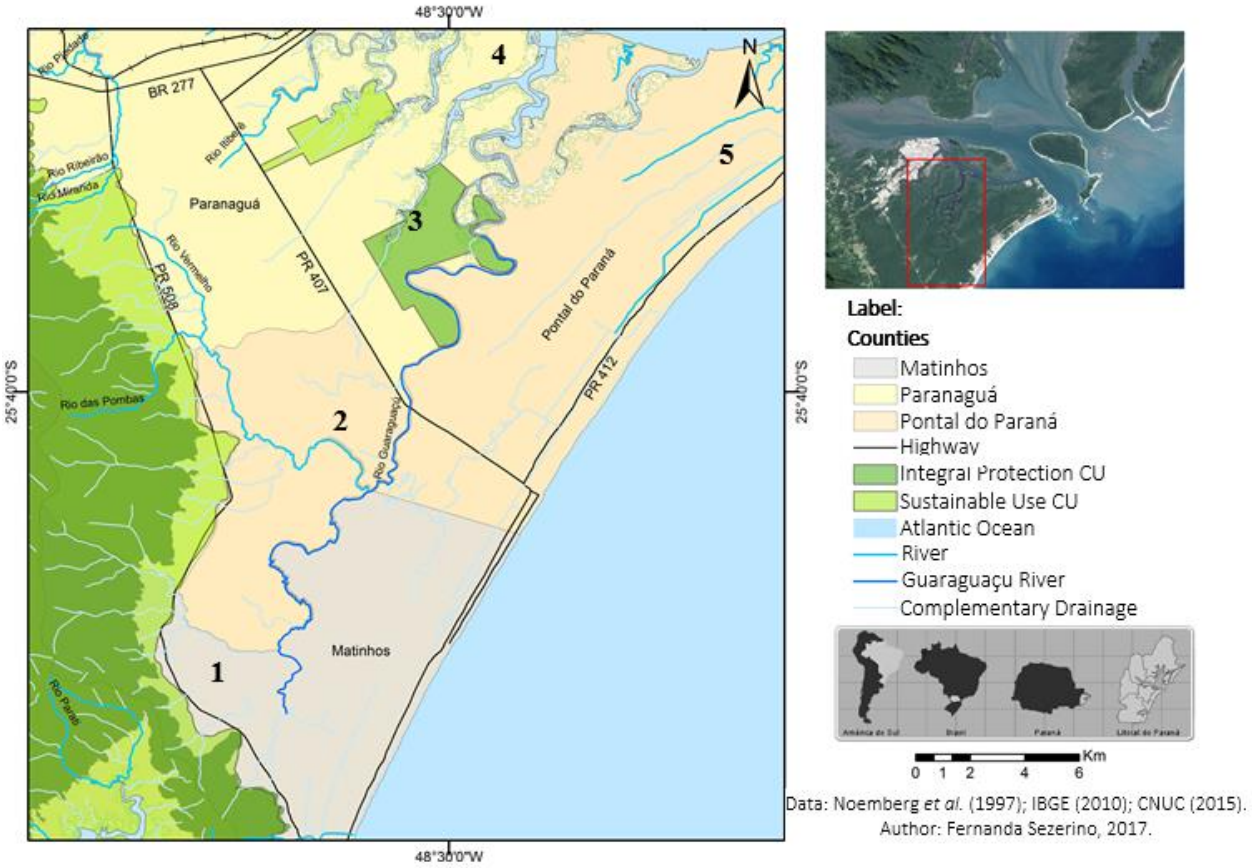

Figure 1. Map of location of the Guaraguaçu River in the coastal basin and sampling areas numbered from 1 to 5.

Each fecal sample found was referenced in a field book, identified and stored in plastic bags. All handling procedures ensured that samples were not contaminated by the use of utensils, packaging and tools. After the collection, identification and storage, the samples were sent to the Laboratories of Chemical and Biological Pre-Analysis and Physical-Chemical Analysis of the Federal University of Paraná - Littoral Sector for the appropriate specific laboratory procedures.

2.2 Sample preparation

Samples of feces of *Lontra longicaudis* contain variable water contents in their composition, as well as a great heterogeneity of components. As a result, the samples were dried in a conventional oven at 80 ºC until constant weight, fragmented and homogenized. The treatment procedures of the samples were established from the development complete factorial design 2^3 [8]. The multivariate statistical technique is based on the application of a fixed effects model for the optimization of factors and levels from a small number of experimental trials, as well as in the evaluation of interdependence effects and interrelations that they can cause in response of the final combinations[8, 9].

In the present work, the procedure of the purpose method was based on an experiment employing three factors (milling, HNO_3 volume and digestion time) and with each one of the variables tested in two levels. The response variable was evaluated from duplicate experiments leading to the 16 experiments. Replicas are of significant importance since they serve to determine experimental errors that can be evaluating the reproducibility of the method, as well as of the instrumental analysis[8].

The Table 1 indicates the factors and levels chosen to define the factorial design, and Table 2 shows the experimental conditions performed randomly as indicated by software Statistica® 7.0.

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Table 1. Factorial design 2^3 for optimization of the conditions of sample treatment.

	MILLING	HNO_3 mL	DIGESTION TIME/h
LEVELS	Hammer + Strainer	4	3
	Willey type knife mill	2	1.5

Table 2. Full factor design (2^3) for reactional conditions optimization in the sample treatment.

EXPERIMENTS	MILLING	HNO_3 mL	TIME DIGESTION/h
1	Hammer + Strainer	2	1.5
9	Hammer + Strainer	2	1.5
5	Willey type knife mill	2	1.5
13	Willey type knife mill	2	1.5
7	Hammer + Strainer	2	3
15	Hammer + Strainer	2	3
3	Willey type knife mill	2	3
11	Willey type knife mill	2	3
2	Willey type knife mill	4	1.5
10	Willey type knife mill	4	1.5
6	Hammer + Strainer	4	1.5
14	Hammer + Strainer	4	1.5
4	Hammer + Strainer	4	3
12	Hammer + Strainer	4	3
8	Willey type knife mill	4	3
16	Willey type knife mill	4	3

The results of the lead concentrations obtained from random variables showed no significant difference between the levels tested. The feces samples were fragmented and homogenized by automated method of the Willey-type knife mill, due to its greater agility, economy and safety.

Sample treatment step was also optimized employing full factorial design 23 (Table 1). Again, due to significant non-differentiation between the volumes tested, the sample treatment with 2.00 mL of HNO_3 65 % (v/v) during 1.5 h in a digestion block was chosen due to the characteristics of economy and agility. The previously prepared samples were directed to the wet decomposition step with oxidant acid - HNO_3 65 % (v/v) - and hydrogen peroxide - H_2O_2 30 % (m/m). After optimized conditions the samples, about 450 mg, were treated with 2.00 ml of HNO_3 65 % (v/v) and allowed to stand for 30 minutes at room temperature. Then, 1.00 mL of 30 % H_2O_2 (w/w) and 2.00 mL H_2O were added on. The flasks were semi-sealed with parafilm protector and placed in a digester block at 80 °C for 1.5 h. After complete mineralization and cooling, the extracts were diluted to 10.00 mL with ultrapure water, filtered with 0.45 μm Teflon membrane filter and kept under refrigeration until the analysis of the samples by F AAS.

Automated Instrumental Analysis - F AAS after the acid digestion process, the extracts were sent to the Automated Instrumental Analysis Laboratory of the Federal University of Parana - Litoral Sector for analysis on AA240 Varian atomic absorption spectrometer and determination of the flame Pb contents in the following operating conditions: gas compressed air and acetylene in flow of 5 L min-1; lamp current equal to 12 mA; wavelength equal to 217 nm; slit equal to 1 nm and background correction with Deuterium lamp. The atomic absorption spectrophotometer analyzes followed the basic protocols of analytical chemistry and previous validation of the method, considering that the whole experimental process was reproducible, sensitive, precise, accurate and linear in the range of the analytical calibration curve. The range of the analytical calibration curve constructed using lead standard solutions in HNO_3 1 % v/v at eight calibration levels, were: 0.25; 0.50; 0.75; 1.5; 2.0; 2.5; 3.0; 4.0 mg L^{-1}. The linearity was evaluated by linear regression equation obtaining the determination coefficient ≥ 0.995 (Figure 2).

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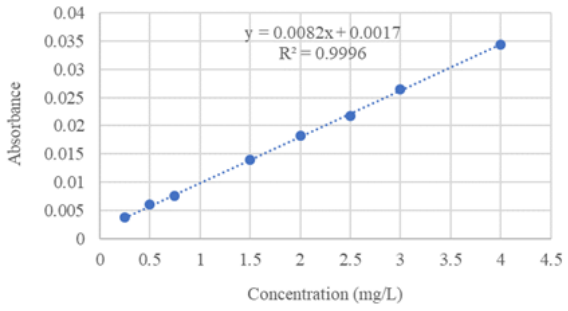

Figure 2. Analytical calibration curve for the lead (Pb) in HNO_3 1 % (v/v).

2.3 Sample treatment

From the data shown by the graph, the functional relationship between the variables was characterized and expressed by the present calibration analytical curve which, as a straight equation, is calculated from the measured signals at concentrations previously established through the linear regression model[7, 10, 11, 12]. Thus, it was possible to determine the concentrations of Pb in the sample, since its results were totally dependent and directly proportional to the concentration of the analyte in the sample[7, 10, 11, 12]. In all cases, the correlation coefficients showed a satisfactory linearity. That is, it showed direct proportionality to the concentration of the analyte in the sample, within the specific concentration range, and it can be proved from the values presented by the determination coefficients ($\mathbf{R^2}$ = 0.9996).

3. Results and Discussion

The results of the concentrations obtained through the F AAS technique are presented in Table 3.

Table 3. Values of lead concentrations (Pb) obtained in the feces samples of *Lontra longicaudis* by F AAS

Samples	Area	Pb mg L^{-1}	Mean ± Std Dev	Pb mg kg^{-1}	Mean ± Std Dev
1	Intermediate tide influence	0.89	0.98 ± 0.09	19.78	22 ± 2
2	Intermediate tide influence	1.06		23.58	
3	Without tide influence	1		22.22	
4	Without tide influence	0.90		20.05	
5	Without tide influence	0.94		20.87	
6	Without tide influence	1.11		24.66	

The analysis of the results of the concentration values was based on comparative checks and knowledge of acknowledgments with the legislation of reference organisms, such as ANVISA[10], MAPA[11], CONAMA[12] and MERCOSUL[13] (Table 4 and 5). These legislations with their respective standards and values regarding inorganic contaminants in water and food samples are used as guiding parameters since specimens of this nature make reference to the biology, habitat and way of life of the species. In addition, there is still no data to ensure the maximum limits allowed in *Lontra longicaudis* feces. That is, the comparative proposal is based on confronting the concentration values found in feces with the maximum values allowed by current legislation in samples that incorporate part of the ecosystem, biology and ethology of the bioindicator species.

Therefore, the determination and analysis of the Pb ions metal in the feces samples of *Lontra longicaudis* involved a certain complexity about the concentrations as a function of the different spatialities between the areas determined as points of sampling and collection, as well as in relation to the nature of the samples and the comparative values of the reference legislations.

Despite the gap in the results, significant non-differentiation points to the fact that environmental contamination in the study area may not be only timely for the activities of the Port of Paranaguá. There are also some other activities such as agriculture, livestock, fish farming, tourism, leisure and navigation, which are already described in the literature as potentially contaminating activities due to their uncontrolled and irrational use[1-4]. This context of insertion of the collection and sampling points will be investigated through the study between detailed examinations of the region of the study area and their respective surroundings, concomitantly with the data obtained by the analytical technique of flame atomic absorption spectrometry.

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Table 4. Guidelines and guidelines on maximum lead limits (Pb) in foods.

Part	Regulatory Agency	Normative	Samples	Maximum
MERCOSUL[13]	Ministério da Saúde (MS) Agência Nacional de Vigilância Sanitária (ANVISA)[10]	Resolution Nº 12/11	Fishes	0.3 mg kg^{-1}
			Cephalopod mollusks	1 mg kg^{-1}
			Bivalve mollusks	1.5 mg kg^{-1}
			Crustaceans	0.5 mg kg^{-1}
SDA/MAPA[11]	Defesa Agropecuária (SDA) Ministério da Agricultura, Pecuária e Abastecimento (MAPA)[11]	Normative Instruction Nº 09/17	Catch fish	0.3 mg kg^{-1}
			Growing fish	0.3 mg kg^{-1}
			Shrimp	0.5 mg kg^{-1}
Present study	-	-	Feces extract	21.9 mg kg^{-1}

Table 5. Guidance standards and values on lead limits (Pb) in fresh, salt and brackish water.

Part	Regulatory Agency	Normative	Samples	Maximum limit
CONAMA[12]	Conselho Nacional do Meio Ambiente (CONAMA)	Resolution Nº 357/05	Class Sweet Waters 1 and 2	0.01 mg L^{-1}
			Class Sweet Waters 3	0.033 mg L^{-1}
			Salt water class 1	0.01 mg L^{-1}
			Salt water class 2	0.21 mg L^{-1}
			Brackish water class 1	0.01 mg L^{-1}
			Brackish water class 2	0.21 mg L^{-1}
Present study	-	-	Feces extract	21.86 mg kg^{-1}

It is also known that in matrices of different natures there are variations to the maximum limits allowed by virtue of the unique biogeochemical and physicochemical characteristics. In sediment samples and organic materials, the tendency is to have higher concentrations due mainly to the adsorption process, because the adsorption variability influences the mobility, availability, retention and, therefore, the concentration of metallic ions and their toxicity. In the case of lead, toxicology consists of the interaction of its ionic form with organic groups present in the soil, sediments and biological tissues, and the introduction into the organism through atmospheric air, contact with soil, water and through skin[14]. The toxicity of lead results mainly from its interference in the functioning of cell membranes and enzymes, since it is capable of forming stable complexes with binders containing sulfur, phosphorus, nitrogen or oxygen, for example with the groups $-SH_2$, $-H_2PO_3$, $-NH_2$ and -OH, which function as electron donors[14, 15]. In this way, most of the lead, initially present in the blood, goes up to reach a plateau. The excess penetrates the soft tissues and organs, among which the brain stands out, and finally it deposits in the bones, teeth and hair[14, 15], where it replaces the calcium due to the similarity of size between the Pb^{2+} and Ca^{2+} [15]. From this perspective, therefore, the high concentrations of lead in the samples of this work are consistent when considering that the constitution of the feces samples is mostly composed of bones and scales originating from the food items of a diet such as otter, predominantly piscivorous. In this way, it is possible to provide a diagnosis considering the absorption by the alternative routes to the digestive route and, finally, the incorporation of metallic ions in the food web since the bioindicator species is a chain-top carnivore. When comparing the results and comparing the Pb analyzed in related studies, a significant equivalence of the data can be observed when analyzed on the normalized form of minimum and maximum values of the determined concentrations: the routing to a worsening of the ecosystem health due to natural processes and/or potentially polluting. As with the guideline values of the maximum limits allowed and established by SDA/MAPA[11], CONAMA[12] and MERCOSUL[13],

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the presence of irregularities is mainly correlated with the determinations obtained in all mentioned studies (Table 6)[14].

Table 6. Pb Concentrations in *Lontra longicaudis* in related studies

Species	Matrix	El	Conc. (min. - max.)	References
Lutra lutra	Feces	Pb	12.4 - 20.9 mg kg^{-1}	Mason and MacDonald (1986)[16]
Crysocyon brachyurus	Pelage		0 - 4.3 mg kg^{-1}	Brait, Antoniosi Filho and Furtado (2009)[17]
Cerdocyon thous	Pelage		0.9 - 3.6 mg kg^{-1}	Brait, Antoniosi Filho and Furtado (2009)[17]
Leopardus pardalis	Pelage		0.3 - 2.5 mg kg^{-1}	Brait, Antoniosi Filho and Furtado (2009)[17]
Cathorops spixii	Dorsal muscle		0.004 - 0.48 mg kg^{-1}	Trevizani (2011)[18]
Lontra longicaudis	Feces		1.35 - 27.327 mg kg^{-1}	Present study (2018)

In order to emphasize the study developed by Trevizani (2011)[18] in comparison with the present study (2018), both carried out in the Bay of Paranaguá, it is possible to investigate and recognize the minimization of the threats and risks involved, as well as the concealment for future trends and projections regarding territorial development and the quality of natural territorial spaces. In addition, the analytical knowledge about the dynamism between the socioeconomic and socioenvironmental establishment for the occupation and use of the coastal space contributed to identify and characterize the externalities to the ecosystemic health by potentially polluting productive processes, as is the case of the Port Terminal of Paranaguá. Thus, the use of biological samples from the bioindicator species allowed not only to carry out the quantitative determinations of Pb contamination from the productive activities of the local port sector, but also to explain the understanding of the complexity and inseparability of environmental impacts for long periods in the disorganized and indiscriminate use and exploitation of natural property in common use. Therefore, the importance of detailed knowledge about sanitary problems and ecological imbalance from metallic ions that rely on the direct and indirect relations of interference on all the environmental segments of the territory, among them the hydric, sedimentological and/or biological compartments.

4. Conclusions

Quantitatively, there are no significant differences in the Pb concentrations between collection points. This requires more in-depth studies based on a larger sample size as a function of the main anthropogenic sources of contamination for each of the sampling and collection areas. It also needs to investigate potential contaminants in water, sediment, food items such as fish and crustaceans, and biological tissues such as liver, brain, kidneys and lungs of dead animals. As well, to identify individuals or populations through DNA analysis aiming the clarification of the mobility by a certain area and, consequently, of the provenance of the feces.
However, the determination of lead in feces of *Lontra longicaudis* by flame atomic absorption spectrometry (F AAS) was adequate to determine concentrations of metal ions in biological samples of the bioindicator species. This fact makes it possible to use these procedures to extend the analytical studies on contamination in animal samples present in the territory.

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