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Heavy metals (Cd, Pb and Ni) in fish species commercially important from Magdalena river, Tolima tract, Colombia

Metales pesados (Cd, Pb y Ni) en especies de peces de importancia comercial del río Magdalena, tramo Tolima, Colombia

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Abstract. Water quality of the Magdalena River, main waterway of Colombia, has been severely damaged by the use of heavy metals from anthropogenic activity. Two commercially important fish *Prochilodus magdalenae* and *Pimelodus blochii*, were cap- tured in four of the most important fishing municipalities in Tolima-Colombia, to determine the levels of cadmium, lead (Atomic absorption spectroscopy) and nickel (colorimetric) in the muscle, gill and liver. *Pimelodus blochii* is the one who bioac- cumulates these chemical pollutants in major quantity, with concentrations of Cad- mium from 0.009 ± 0.001 to 0.340 ± 0.402; lead from 0 to 8.737 ± 1.299 and Nickel from 2.26 ± 1.59 to 31.69 ± 10.26 mg/kg; the port of Flandes stands out as one of the areas of major ecological impact. There was correlation between cadmium concen- trations accumulated and the animal organ which acts as a metal reservoir, showing elevated levels for the liver tissue.

Keywords: Heavy metal, Magdalena River, Pimelodus blochii, Prochilodus magdalenae.

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1. INTRODUCTION

The Magdalena River flows northward about 1.528 km from the Colombian massif to the Caribbean sea through the western half of the country which is 17% of its area (Agencia Internacional de la Oficina Francesa del Bosque et al., 2007); is the main river of the country, being navigable 886 km, thus becoming the setting of development of regions and the emergence and consolidation of its various cultures, communications, commerce, politics, arts, technological progress and modernity.

In spite of the fact that the Magdalena River is the main waterway of Colombia, it receives a diversity of harmful compounds from agricultural, mining, livestock, and other sources through direct dumping and runoff, posing risks to those whom base their diet on resources coming from the river (Mancera & Álvarez, 2006). Particularly, in the area of the study, about 83.4% of this land is devoted to crops rice, sorghum and cotton, farming and tourism are essential too (Durán, 2005).

In Colombia, particularly in the Tolima region, little is known about the problems caused by the disposal of heavy metals in water bodies and their effect on fishing resources, ecosystems and human health. From this perspective, there are concerns among the academic and scientific community about the environmental pollution effects and accumulation of these elements in biological ecosystems, which has come to be the case of the Magdalena River.

What makes the heavy metals toxic, is not their essential characteristics, but the concentrations that may occur, and most important, the type of shape given on an specific environment, its hazard is potentiated by not being chemically or biologically decomposed, once emitted metals can reside in the environment for hundreds of years, and for this reason, they are incorporated into aquatic organisms, like fish, as free metal cations being absorbed through the external respiratory organs (gills), and going straight to the blood, they can also be absorbed by the body and then spread passively through bloodstream. All this means that its concentration in living organisms increases when they are eaten by others and that therefore the intake of contaminated plants or animals can cause intoxication (Calcina, 2007).

Toxic metals such as lead and cadmium, among others, have taken great importance, because their anthropogenic contribution outweighs the one which is supplied through life cycles (Salamanca et al., 2004) and also because they show different toxic properties through different levels along the food chain. Although the interest in studying nickel accumulation in fish is not common in research related to environmental pollution, due to its low uptake and rapid elimination (Iniesta & Blanco, 2005), in this study we decided to determinate its concentration based on its ability to form a variety of coordination compounds that allows it to accumulate in aquatic organisms, however, the magnification along the food chain has not been proven (Marrugo & Lans, 2006).

This study aimed to evaluate the levels of Cd, Pb and Ni in liver, muscle and gills of *Prochilodus magdalenae* and *Pimelodus blochii*, species of commercial and cultural relevance in the most important fishing municipalities of Tolima region (Purificación, Flandes, Honda, and Ambalema). Moreover, this study sought to establish the potential risk to humans due to consumption of contaminated ichthyological products with the heavy metals mentioned above. Given the ability of fish to bioaccumulate and biomagnify high concentrations of these elements through the aquatic food chain, and the fact that these animals are constituted as representatives from various levels of the same chain they are consider excellent indicators of heavy metal pollution.

2. MATERIALS AND METHODS

2.1 Ecosystem Quality

The ecosystem quality was established following the protocol recommended by Barbour et al. (1999).

2.2 Sampling of Fish Species and Dissection

The accumulation of heavy metals was studied in different organs (liver, gills, and muscles) of two fish species (*Pimelodus blochii, Prochilodus magdalenae*) collected from upstream water of the Magdalena River, which receives direct discharge of untreated raw urban sewage from the cities boarding it.

All fish samples were collected in premon soon season (during January and February 2009) from four sites known as the most important fishing spots in the Tolima department: Purificación, Flandes, Ambalema and Honda, Figure 1 shows the location of the sampling areas.

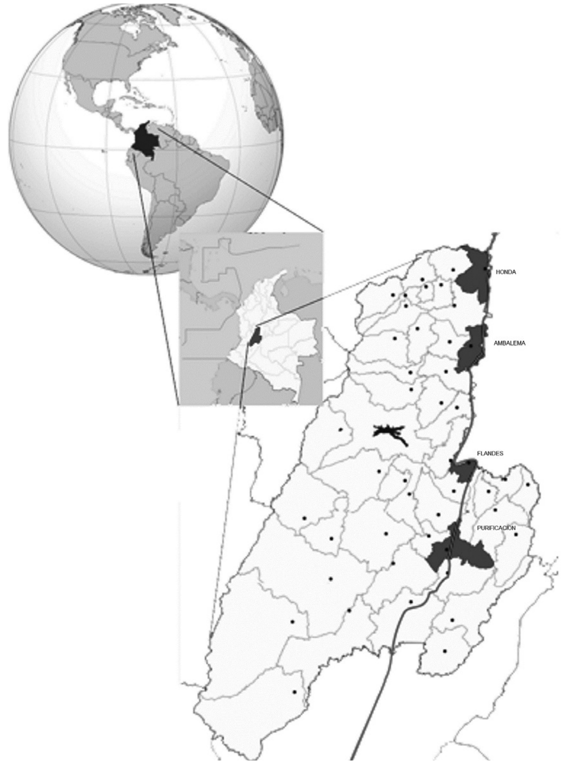

Fig 1. Location of sampling area. Main fishing towns on the Magdalena River in the Tolima department.
Source: Modified from Google Maps

Fish samples were collected from each site using fishing nets. After capturing, fish specimens were transferred immediately to the riverside where we determined morphometric parameters such as standard length (SL) and weight (W). A total of 139 fish specimens were collected (73 *Pimelodus blochii* and 66 *Prochilodus magdalenae*). To evaluate changes in energy reserves, fish health, and provide tools to facilitate the diagnosis in a population, the hepatosomatic index (HSI) was calculated by the next formula (Pardo, 2008).

$$\text{HSI}= \frac{\text{liver weight}}{\text{total fish weight}} \text{ X } 100$$

Each specimen was dissected with corrosion-resistant stainless steel knife, isolating gills, liver and muscle of each fish specimen, to ensure uniformity; all samples were taken from the dorsolateral muscle of the right side. Each dissected organ was placed in a polyethylene bag and provided with necessary information about identification code, date, locality, season, species name, and tissue type. Polyethylene bags containing different organs were placed in icebox, and transported to laboratory within 12 h for the purpose of further chemical analysis. In the laboratory, the organs were homogenized to form a composite sample for each specie and each organ, and then stored at –20ºC before digestion.

2.3 Heavy metal determination

Collected elements were grouped taking into account body weight and tissue type, in order to obtain samples that would yield reliable data. Sample preparation and analytical determination of heavy metals was made following the recommendations given by the AOAC (7.102 and 2.126, respectively) (Association of Official Analytical Chemist [AOAC], 1984). The concentration of nickel was evaluated by UV-VIS spectroscopy following the method proposed by Salesin & Gordon (1960).

2.4 Statistical analysis

Results were recorded in calculation matrices, allowed to relate the levels of metal concentrations obtained in each species, with the tissue type and collection site, examined to determinate descriptive parameters such as mean and standard deviation, so that they could be compare using variance analysis (ANOVA) and a comparison of means test, setting a significance level of 0.05. Comparisons were performed using the statistical software support InfoStat, free version 2008.

3. RESULTS AND DISCUSSION

3.1 Ecosystem Quality

As it is shown in the Table 1, surrounding Purificación predominates rice cultivation (*Oryza sativa*), which as a result of its development generates pollution sources (pesticide containers and farmers homes). As evidence of contamination in Flandes area we observed nearby houses and the mouth of the Bogotá River, which has been mentioned to discharge 39 m^3/s of highly polluted water into the Magdalena River (Alvear, 2005). In addition to that, Flandes introduces a vast array of polluting materials, both solid (79.7%) and liquid (91.3%) of urban, industrial and agrochemical waste to the water bodies (Alcaldía Municipal de Flandes, 2000). Regarding to Ambalema station, it exhibits sources of pollution such as urban housing, nearby roads and garbage dump. In Honda port there is a predominance of homes around body water, local transit and solid waste storage. Despite all of these, the water of the Magdalena River has normal smell, does not present oily surface, and typifies as cloudy or partially cloudy, in addition, the substrate or sediment is muddy or sandy, absent from oils and has normal smell, like water.

Table 1. General characteristics of the sampling locations

	Purificación	Flandes	Ambalema	Honda
Location	N: 3º46'3.7" W: 74º55'21.8" "El Puente"	N: 4º16'37.3" W: 74º49'49.5" "Mansogrande".	N: 4º46'37.4" W: 74º46'2.7" "La Carrilera".	N: 5º12'34.9" W: 74º44'3.4" "El Sámano".
Altitude (msnm)	309 m.s.n.m.	279 m.s.n.m.	242 m.s.n.m.	171 m.s.n.m.
Maineconomicactivity	Farming (rice), livestock and trade.	Farming (rice), mining, fishing, livestock and trade.	Farming (cotton, rice y sorghum).	Farming (rice), livestock, fishing, tourism and trade.

Source: Authors

From an environmental point of view, the sampling site showed moderately eroded soil with no aquatic vegetation in Purificación. In Flandes the ground cover was predominantly forested, with no local erosion or aquatic vegetation, the same as in Ambalema port. Magdalena River up to Honda is characterized by strong currents, abundant flow, does not have aquatic vegetation, or erosion, features and pastures predominate.

Based on that, there was a suboptimal condition in Flandes (58%), Ambalema (56%) and Honda (54%), while Purificación station presented optimal condition (80%).

3.2 Collected Samples

Fish caught in each port showed phenotypic characteristics of each species. Only *Pimelodus blochii* from Honda portshowed iridescent coloration higher and brighter than usual. The *Prochilodus magdalenae* animals collected at Purificación port and *Pimelodus blochii* from Honda showed the largest size. In any case, individuals who formed the study sample exceeded the minimum catch size established by environmental authority: (180 mm for *Pimelodus blochii* and 250 mm for *Prochilodus magdalenae*).

In a Colombian fishery and aquaculture report, Corporación Colombia Internacional (2006) says that the quantity and quality of fish species in the Magdalena River is affected by environmental factors such as deforestation, pollution by dumping of cities and towns, mining activity, presence of oil, mercury, pesticides, sediment increasing and abuse of fishing gear, which is reflected in declining catches.

The collected organisms are part of what in the region is known as "cabeza de subienda", which is the "subienda" prelude, being understood, that "subienda" is the reproductive migration process annually conducted by some fish species, including *P. magdalenae* and *P. blochii* (Torres, 2007). It should be noted that these fish migrate from marshes, meanders and lentic water bodies belonging to Magdalena River water system near to Purificación, Ambalema and Flandes ports, where they have remained four to six weeks, time during which they spawn. Conversely, samples captured in Honda port were from fish that migrated from the Middle Magdalena lagoon complex.

It is important to mention that the magnitude of HIS varies depending on deposits balance from intake or the transfer from other reserve organs and expenses of overfed, additionally, it can express the dynamic in endogenous energy use of organs such as gonads, liver and body mass (González & Oyarzún, 2002).

With this knowledge, data given in Table 2 allows us to affirm that individuals of *P. Blochi* and *P. magdalenae* species caught in Purificación have lipid and glycogen reserves accumulated in body, higher than the same specimens collected in other ports, which could be associated with habitat conditions in this port, regarded as "optimal" (80%).

Table 2. Hepatosomatic index of two commercially important fish species from Magdalena river-Tolima department.

Values with different capital letters represent significant differences ($p<0.05$), between HSI averages for each species between ports. (NC) No organisms were collected.

Specie	Port			
	Purificación	Flandes	Ambalema	Honda
P. blochii	1,26 ± 0,63 B	0,58 ± 0,28 A	0,67 ± 0,39 A	0,48 ± 0,26 A
P. magdalenae	1,12 ± 0,32 C	0,47 ± 0,10 A	0,62 ± 0,21 B	(NC)

Source: Authors

Likewise, Table 2 shows that *P. magdalenae* collected reveals significant differences between the sites of capture and that individuals of both species collected from Purificación are significantly different ($p<0.05$) to those captured in Flandes or Ambalema.

3.3 Levels of heavy metals

Table 3. Mean levels and standard deviation of Cd, Pb and Ni found in tissues of *Pimelodus blochii* individuals collected from four ports on the Magdalena River, department of Tolima.

Values with different capital letters represent significant differences ($p<0.05$) between the mean values of the levels of each heavy metal in each of the organs between the ports. Values with different lowercase letters represent significant differences ($p<0.05$) between average levels of each heavy metal between each of the organs in each of the ports.

METAL (mg/kg)	TISSUE	PORT			
		Purificación	Flandes	Ambalema	Honda
Cd	Gill	0,018 ± 0,009 A;a	0,034 ± 0,009 B;a	0,022 ± 0,013 AB;a	0,016 ± 0,004 A;b
	Liver	0,033 ± 0,00 A;a	0,340 ± 0,402 A;b	0,081 ± 0,065 A;b	0,072 ± 0,012 A;c
	Muscle	0,021 ± 0,007 B;a	0,010 ± 0,003 A;a	0,009 ± 0,001 A;a	0,011 ± 0,001 A;a
Pb	Gill	3,516 ± 0,867 A;a	8,737 ± 1,299 B;c	6,706 ± 1,806 B;b	4,282 ± 1,185 A;b
	Liver	1,418 ± 0,00 A;a	3,781 ± 1,968 A;b	0,420 ± 0,594 A;a	2,914 ± 0,00 A;b
	Muscle	1,136 ± 1,468 A;a	0,076 ± 0,169 A;a	0,069 ± 0,170 A;a	0,00 ± 0,00 A;a
Ni	Gill	10,92 A;b	31,69 ± 10,26 B;b	30,91 ± 9,84 B;b	16,14 ± 3,76 A;b
	Liver	10,12 A;b	27,50 ± 26,53 A;b	10,39 ± 5,46 A;a	9,29 ± 1,68 A;a
	Muscle	2,60 ± 1,06 AB;a	4,28 ± 1,40 AB;a	2,26 ± 1,59 A;a	4,73 ± 2,84 B;a

Source: Authors

Table 3 lists the values related to the levels of cadmium, lead and nickel detected in three organs of *P. blochii* specie, based on this information we could make some considerations such as:

Regarding cadmium, significant differences ($p<0.05$) are noticed in the accumulated levels in gills and muscle of samples collected, especially those coming from Purificación and Flandes; resulting liver as the major reservoir of this metal in fish caught in all the ports. The analysis revealed that *P. blochii* from Honda accumulated different significant amounts ($p<0.05$) of this chemical pollutant, according to the fish organ.

These observations suggest that the mechanism of entry in to the body is mainly through digestive tract, which seems justified by the ingestion of benthic invertebrates, crustaceans and similar organism associated with sediments that constitute their diet. Rodríguez et al. (2007) argued that liver is the main detoxification destiny, and one of the most important metal storage organs by the digestive tract. It should be kept in mind that the concentration of cadmium deposited in the kidney and liver depends on the intensity of exposure time and optimum state of renal excretory function (Ramírez, 2002). In cells, cadmium binds to metallothionein, a protein which contains 26 free sulfhydryl groups per molecule. As such, these metal-protein structures can be considered as specific molecular biomarkers (Benedicto et al., 2005). Experimental studies have shown that cadmium-metallothionein complex is more toxic to renal tubules than the cadmium itself (Ramírez, 2002).

If attention is pay on the levels of lead and nickel shown in Table 3, we notice differences in the concentrations of these metals in gills, between ports ($p<0.05$). Purificación and Honda differ from Flandes and Ambalema, while Flandes has the highest levels. It is clear that the gill is the organ of accumulation for these metals; however, the amount of lead appears to be independent of capture site, which is not given for nickel case.

Bear in mind the comments presented in the preceding paragraph, we might think that the entry route of these two elements to the *P. blochii* is mainly the water way. It is recognized that the gills are a major organ of accumulation of heavy metals, since they are in direct contact with the water and therefore are the first barrier of defense, and also because gills are the first organs to be exposed to resuspended sediment particles, being significant sites of interaction with metal ions (Hosseinkhezri & Tashkhourian, 2011).

In the meantime, Table 4 reveals that *P. magdalenae* liver could be the major reserve organ of any of the three xenobiotics, so we may assume that the entry route of these elements to the animal is primarily digestive tract. It is important to highlight that this fish species diet is based on detritus and periphyton. Cadmium accumulates in this organ in a significant different way between the ports ($p<0.05$), and the fish collected in Flandes show the highest values. Since the observed levels of cadmium and lead in muscle were relatively lower than in the other potential organs, we conclude that the distribution of metals is uniform on them. While the lead in the gills differs between ports, the nickel content in liver and muscle does the same ($p<0.05$).

Table 4. Mean levels and standard deviation of Cd, Pb and Ni found in tissues of *Prochilodus magdalenae* individuals collected from four ports on the Magdalena River, department of Tolima.

Values with different capital letters represent significant differences ($p<0.05$) between the mean values of the levels of each heavy metal in each of the organs between the ports. Values with different lowercase letters represent significant differences ($p<0.05$) between average levels of each heavy metal between each of the organs in each of the ports. (-) no individuals were collected.

METAL (mg/kg)	TISSUE	PORT			
		Purificación	Flandes	Ambalema	Honda
Cd	Gill	0,013 ± 0,023 A;a	0,008 ± 0,002 A;a	0,009 ± 0,004 A;a	(-)
	Liver	0,012 ± 0,005 A;a	0,071 ± 0,022 B;b	0,037 ± 0,008 C;b	(-)
	Muscle	0,007 ± 0,001 A;a	0,008 ± 0,002 A;a	0,008 ± 0,004 A;a	(-)
Pb	Gill	0,102 ± 0,119 B;a	0,00 ± 0,00 A;a	0,00 ± 0,00 A;a	(-)
	Liver	1,437 ± 1,109 A;b	2,423 ± 1,248 A;b	1,229 ± 0,516 A;b	(-)
	Muscle	0,764 ± 0,144 A;ab	1,890 ± 1,972 A;b	0,989 ± 0,593 A;b	(-)
Ni	Gill	1,87 ± 0,65 A;a	2,70 ± 1,42 A;a	3,04 ± 2,40 A;a	(-)
	Liver	3,01 ± 1,38 A;b	10,36 ± 4,12 B;b	4,94 ± 1,71 A;a	(-)
	Muscle	1,10 ± 0,42 A;a	5,02 ± 0,86 B;a	4,40 ± 2,81B;a	(-)

Source: Authors

Corresponding to the best habitat conditions, samples coming from Purificación reveal middle and lower levels of the three metals, in relation to those found in the organisms collected on the other ports, which would be related to the higher values of Hepatosomatic Index found in organisms caught in that port, and define an inverse relationship between pollutants concentration levels and individuals sanitation.

In general, our results show a trend similar to what some authors have pointed out before around the world, about the accumulation of these metals in fish tissues (Canli & Atli, 2003; Bordajandi et al., 2003; Mazet et al., 2005; Eboh et al., 2006; Chi et al., 2007; Dural et al., 2007; Márquez et al., 2008; Shinn et al., 2009; Visnjic et al., 2010), who also determine significant differences in the concentrations of the metals analyzed in different tissues as well as between the gender of analyzed fish. Also, authors agree that gills and liver are where the highest concentrations of heavy metals analyzed are shown.

Ruiz et al. (1996) assessed *Pimelodus blochii* and *Prochilodus magdalenae* contamination risk by cadmium, copper, lead and zinc in the area near to Honda port on Magdalena River. They found cadmium concentrations between 104 and 256 mg/

kg, but no detectable levels of lead contamination, while *Pimelodus blochii* presented high mercury amounts.

It is important to mention that water systems can be extensively contaminated with heavy metals released from domestic and industrial activities as well as other anthropogenic actions, which reach high toxicity rates and are efficiently absorbed through biological membranes due to its high chemical affinity by the sulfhydryl group of proteins. Toxicity results when the body is subjected to an excessive concentration of the chemical element during a prolonged period, when the metal appears in a biochemical way or when the body absorbs it by an unusual route.

However, the deciding factor in the total persistence of a contaminant is its own nature and its characteristics; such as water solubility and polarity, which influence the lipophilic nature of the compound and its volatility, chemical reactivity and stability. It seems that if the primary entry route of the xenobiotic is through the water, the highest concentration will be on gills, whereas if the intake is predominantly for food, the accumulation will occur in the digestive system tract tissues or organs (Sánchez, 2008).

It should not be forgotten that among all the animal species, fish are natural water inhabitants, therefore they cannot escape the harmful effects of heavy metals (Olaifa et al., 2004; Vinodhini & Narayanan, 2008), causing its retention in certain structures at different rates and for different causes such as metabolic, mode of entry, fitness, etc. (Sánchez, 2008). Other factors such as weather station, animal length and weight, physical and chemical characteristics of water, also influence metals accumulation in fish tissues (Dural et al., 2007), in quantities that obey ecologic, metabolic and eating patterns needs (Chi et al., 2007).

The chemical nature of metal and its concentration cause local or systemic effects on the animal body, both mechanisms are involved in the uptake and transport of metals. If it is dissolved the possible areas of absorption are gills (respiratory arrest), bowel (intestinal inclusion), and skin (transcutaneous capture) (Amundsen, 1997). Metal ions such as cadmium, are usually absorbed, through passive diffusion or transport mediated by carriers across the membrane, whereas metals associated with organic compounds are ingested and absorbed through gut endocytosis (Sabath & Robles, 2012). However, the ionic form of these metals is generally not metabolized, but can be stored in the body causing chronic effects.

In what concerns to the human body, it is known that certain heavy metals such as cadmium, lead and chromium, accumulate in tissues like kidney and lung, altering basic functions and causing toxic effects such as pneumonia, kidney failure and emphysema. However, the evidence of pathogenicity in humans is still not enough (García, 2000). Now, we understand that fish are used as a biological model to assess the health of aquatic ecosystems since pollutants accumulate in food.

It is worth pointing out that the organisms collected in this study do not exceed the permissible limits of cadmium and lead established by Food and Agriculture Organization (0.5 mg/kg), for both. It is possible that short-term overexposure to these contaminants do not cause immediate health threats to humans. Eventually, however, it could cause weight loss, anemia, heart and liver damage, respiratory and digestive problems, and possibly adverse effects on blood and kidneys (Marrugo & Lans, 2006).

It should be mentioned that the variability of the results seen in Tables 3 and 4 is explain taking into account that the regulation of heavy metals in teleosts and inductive response varies significantly, depending on factors associated with variability of the metal, residence time in the medium, time of exposure, concentration, fish physiology, metabolism, morphology and age of the fish, among others (Filipovic and Raspor, 2007). Deviller et al (2005), expose that this response depends as well of the variability of metal, time of exposure, concentration, concentration of particulate material and the physical and chemical characteristics of the water. Some researchers argue that there also exist interindividual responses of organisms, even warn that responses may be due to the adaptive capacity of individuals to polluted environments (Al Sabti and Metcalfe, 1995; Lemos et al., 2001; and Hurtado, 2007).

4. CONCLUSIONS

The results of the present study showed that *Pimelodus blochii* is the specie that bioaccumulates the most heavy metals quantity, regardless of the depositing organ. Cadmium preferable concentrates in liver tissue. In addition, we can say that in the two species of interest, health status variable appears to influence the accumulation levels of metals under study. On the other hand, Flandes port stood out as the most environmentally affected. Also, it is important to note that influence of the Bogotá river and its contaminants on the Magdalena river, contribute to this event. Related to this, *Prochilodus magdalenae* and *Pimelodus blochii* captured there had the largest heavy metals reserves. Our research team will continue to develop research related to the cumulative process of the metals studied, trying to increase knowledge about biochemical and toxicity of these environmental pollutants.

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