

AQUATIC MACROPHYTES AS BIOINDICATORS OF METALS IN WATER RESERVOIRS IN VENEZUELA

Macrófitas acuáticas como bioindicadores de metales en reservorios de agua de Venezuela

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ABSTRACT

In Caracas, Venezuela, the water reservoirs that provide services to the city do not have a monitoring plan, and the concentrations of possible pollutants are unknown. This is of interest, considering that these areas have had uncontrolled growth and suffer from urban discharges, which are potential sources of contaminants such as heavy metals. Under this scenario, the study's objective was to assess the potential of the aquatic macrophytes *Eichhornia crassipes* and *Lemna minor* as bioindicators for metal contamination by accumulation. Plants, water, and sediment samples were collected in La Mariposa, Camatagua, and La Perezza reservoirs, at two sampling points per reservoir, three transects per point, and three samples per transect. The concentrations of Pb, Al, Zn, Ni, Cu, and Hg in the mentioned compartments and the bioavailable fraction of sediments were determined with inductively coupled plasma in optical emission spectroscopy and a direct mercury analyzer. The metal content of the analyzed samples was different between the reservoirs (pperm < 0.05). Metal concentrations in *E. crassipes* and *L. minor* were also different between water reservoirs (pperm < 0.05 and MC at 0.05, respectively). The plants' metal content had a similar pattern to the concentrations of metals in water, sediments, and bioavailable fraction (p < 0.05). The results suggest that both species are potential bioindicators for metal contamination by accumulation, especially *E. crassipes* since it is a perennial plant that could reflect environmental quality over a longer period than *L. minor*.

Palabras clave: bioacumulación, *Eichhornia crassipes*, metales pesados, *Lemna minor*

RESUMEN

En Caracas, Venezuela, los embalses de agua que brindan servicios a la ciudad no tienen un plan de monitoreo y se desconocen las concentraciones de posibles contaminantes. Esto es interesante, teniendo en cuenta el crecimiento incontrolado en estas áreas y las descargas urbanas que son entradas potenciales de contaminantes, como los metales pesados, a los embalses. Bajo este escenario, el objetivo del estudio fue

evaluar el potencial de las macrófitas acuáticas *Eichhornia crassipes* y *Lemna minor*, como bioindicadores de contaminación por acumulación de metales pesados. Se recolectaron muestras de las plantas, agua y sedimentos en los embalses La Mariposa, Camatagua y La Pereza, en dos puntos de muestreo por embalse, tres transectos por punto y 3 muestras por transecto. Las concentraciones de Pb, Al, Zn, Ni, Cu y Hg en los compartimentos mencionados y la fracción biodisponible de los sedimentos se determinaron con espectrometría de emisión óptica de plasma acoplado inductivamente y análisis directo de mercurio. El contenido de metales de todos los compartimentos fue diferente entre los embalses ($p < 0.05$). Las concentraciones de metales en *E. crassipes* y *L. minor* también fueron diferente entre embalses ($p < 0.05$ y MC a 0.05, respectivamente). Adicionalmente, el contenido metálico de las plantas tuvo un patrón similar a las concentraciones de metales en agua, sedimentos y fracción biodisponible ($p < 0.05$). Los resultados sugieren que ambas especies son potenciales bioindicadoras de contaminación de metales pesados por acumulación. Especialmente *E. crassipes*, que al ser una planta perenne podría reflejar la calidad ambiental durante un período de tiempo más largo que *L. minor*.

INTRODUCTION

The presence of pollutants in the environment is usually linked to wastes from anthropogenic activities, being oceans, rivers, and lakes the final destination of these products (Vareda et al. 2019). Heavy metals are among the most common contaminants since they are present in the Earth's crust, manufactured products, and industrial wastes (Vareda et al. 2019). These elements have a negative impact in the environment since once they are in water bodies like reservoirs, they tend to adsorb in sediments (Meena et al. 2017). However, a percentage of these contaminants are bioavailable to biota present in the system, leading to adverse consequences for certain organisms (Ali and Khan 2018). Due to the negative effects of heavy metals in biota, different organizations like the United States Environmental Protection Agency (EPA) have created guidelines that set the maximum concentration limit in water bodies that provide services to humans like reservoir. For that reason, they should be monitored regularly as an early alert system; a way to do it is with bioindicators of pollution (Manickavasagam et al. 2019).

Bioindicators can accumulate a specific contaminant and reflect environmental conditions, making them a reliable tool for monitoring systems (Bonanno et al. 2018). Plants have been used as indicators of heavy metals by accumulation in other studies due to their natural history and their ability to accumulate these elements in different tissues (Bonanno et al. 2018). The advantage of using a bioindicator and not water is that the second only shows contaminant levels at a specific time. Unlike bioindicators, which accumulate the pollutant of interest over time, mak-

ing analyses more robust (Manickavasagam et al. 2019). Additionally, the use of a bioindicator in most cases reduces sampling costs and effort, since some environmental data is not required to know the state of a certain study area.

Venezuela reservoirs like La Mariposa, Camatagua, and La Pereza are intervened with informal houses. Additionally, these areas usually lack of proper wastewater management (González and Ortaz 1998, González et al. 2003, Bentancourt and Mena 2012). On the other hand, the presence of plants like *Eichhornia crassipes* (water hyacinth) and *Lemna minor* (duckweed) has been reported in these reservoirs. These plants have properties that make them potential bioindicators of heavy metals. Both can accumulate and tolerate some metals such as Cu, Hg, Ni, Pb, and Zn (Bonanno et al. 2018). Additionally, their life cycle, size, and abundance make them potential bioindicators (Bonanno et al. 2018).

The study aims to determine the concentration of five metals in water and sediments of three reservoirs that provide services in Caracas, Venezuela, and evaluate the potential of water macrophytes *E. crassipes* and *L. minor* as bioindicators of heavy metals pollution by accumulation.

MATERIALS AND METHODS

Study area

La Mariposa

La Mariposa (LM; **Fig. 1**) is located in Miranda state and has a capacity of 8 million m³. Sportfishing and hiking activities are performed in the area. The tributaries of La Mariposa are El Valle river, Los

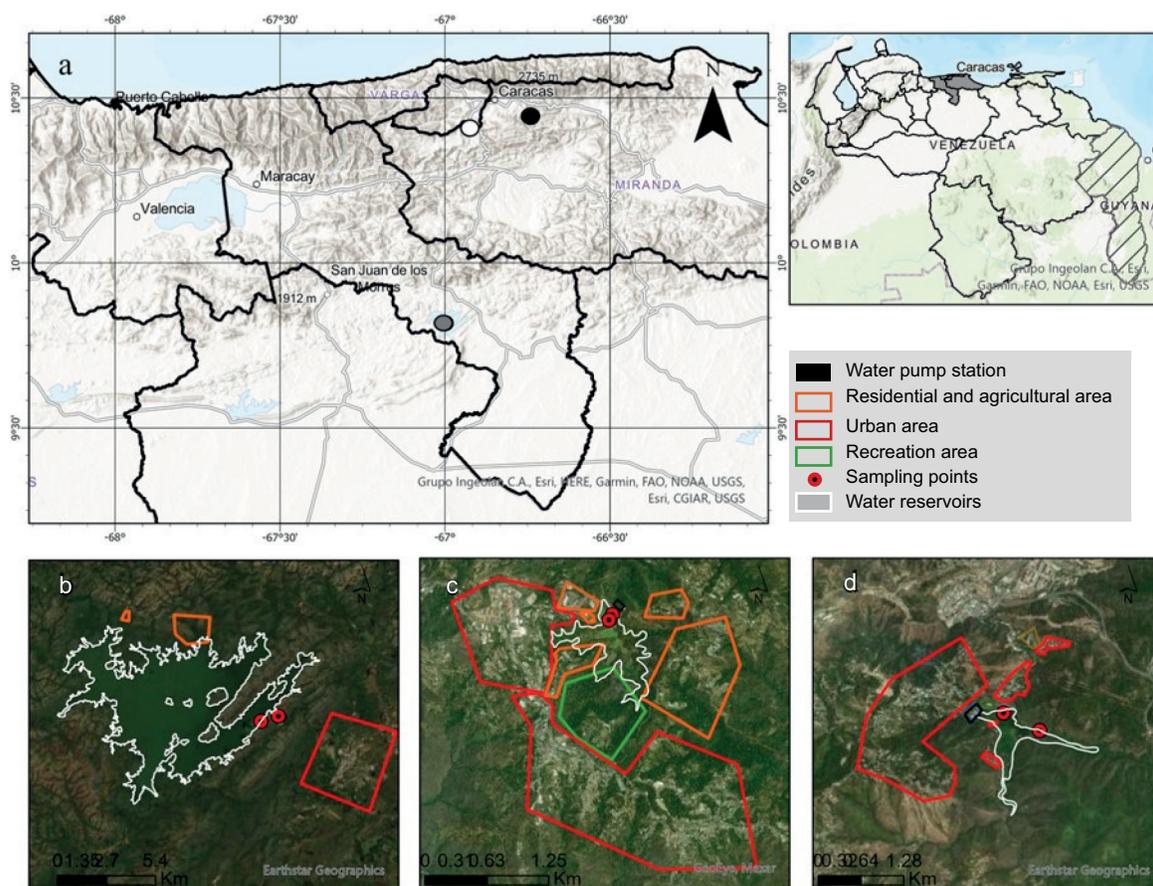


Fig. 1. (a) Water reservoirs sampled, the gray dot represents Camatagua, white circle La Perez, and black dot La Mariposa. Maps b-d represent the land use surrounding the reservoirs, Camatagua (b), La Perez (c), and La Mariposa (d).

Indios brook, and Camatagua and Lagartijo reservoirs. Slopes are affected by human activities and the lack of a proper sewer system (González et al. 2003, Bentancourt and Mena 2012). On the other hand, LM's tributaries have an input of organic matter and fertilizers coming from farmhouses, pig houses and nurseries (González and Ortaz 1998). The dominant species in LM reservoir was *E. crassipes* and in a lower proportion was found *L. minor*.

Camatagua

Camatagua (CM) is the biggest damn river in Venezuela, located in Aragua state (Fig. 1). It has a capacity of 1 573 million m³, being Guárico river its principal tributary. In the past, recreational activities were performed in the reservoir, nowadays these facilities are abandoned, and there is only the presence of sport fishermen. Slopes are also affected by human activities, informal houses, and some areas have been deforested (Bentancourt and Mena 2012).

Likewise, during the sampling, it was observed that some slopes were affected by a recent fire. In the water, the dominant species was *E. crassipes*.

La Perez

La Perez (LP) is a compensatory reservoir located in Miranda state (Fig. 1). It has a capacity of 8 million m³, being La Perez brook and other reservoirs from the Tuy system its principal tributaries. However, at the moment of the sampling, the rain was the only supply of LP. On the other hand, LP is highly intervened with livestock houses and farmhouses (González et al. 2003). The dominant species was *Pistia stratiotes* which cover the totality of the reservoir, except for the water pumps area, where *L. minor* was the dominant species.

Experimental design

There were three factors in the design, reservoir, sampling point, and transects. In each of the sampling

points three transects were set, and in each transect three samples were collected, as specified in **table I**. Samples were collected between December 2018 and June 2019. The sampling points were in the shoreline of reservoirs and classified as entrance and exit (**Fig. 1**). CM's entrance was a brook, and the exit was the Guárico river located outside the damn. LM's entrance was the river mouth of Los Indios brook, and the exit was near the water outlet. LP's entrance was the farthest point from the damn, and the exit was near the pumping station. On the other hand, sediments samples were collected using a steel collector at 6 m deep in LM's exit and 3 m deep in LM's entrance, and 0.5 m at LP and CM, and stored in polyethylene containers. Contrary to water samples which were from the surface and kept in polyethylene bottles. *E. crassipes* and *L. minor* were collected only in the sampling points where they were present as defined in **table I**. Additionally, they were washed with the reservoir's water before being transported to the laboratory in plastic bags, where they were

washed with distilled water and dissected in roots and leaves. Finally, they were stored at -20 °C until their digestion.

Samples treatment

Extraction of metals from sediments, water, tissue and bioavailable fraction

Water, sediment, and wet tissue were digested in a Milestone oven (Ethos Touch Control – Advanced Microwave Digestion Labstation, Milestone) before their analysis with inductively coupled plasma in optical emission spectroscopy (ICP-OES). The digestion of water was total, using the protocol proposed by Matusiewicz et al. (1989) with modifications. Each plant was digested without drying. Water samples were treated with nitric acid ($\text{HNO}_3 \geq 65\%$) and hydrogen peroxide ($\text{H}_2\text{O}_2 \geq 30\%$) 7:3. The same procedure was used for the digestion of roots and leaves of *L. minor* and roots of *E. crassipes*. The leaves of *E. crassipes* were digested with an aggressive protocol to achieve total digestion, with nitric

TABLE I. SAMPLES COLLECTION IN WATER RESERVOIRS.

Reservoir	Date	Sample	Sampling point	Number of samples
La Mariposa	December 2018	sediment	Entrance	18
			Exit	
		water	Entrance	18
	Exit			
	<i>E. crassipes</i>	Entrance	18	
		Exit		
April 2019	<i>L. minor</i>	Exit	9	
Camatagua	May 2019	sediment	Entrance	18
			Exit	
		water	Entrance	18
			Exit	
<i>E. crassipes</i>	Exit	9		
La Pereza	June 2019	sediment	Entrance	18
			Exit	
		water	Entrance	18
			Exit	
<i>L. minor</i>	Exit	9		

acid ($\text{HNO}_3 \geq 65\%$), hydrochloric acid ($\text{HCl} \geq 37\%$), ultra-pure water, and hydrogen peroxide ($\text{H}_2\text{O}_2 \geq 30\%$) 3:3:3:1. Sediments were digested with the protocol proposed by Hewitt and Reynolds (1990) with modifications, using nitric acid ($\text{HNO}_3 \geq 65\%$). The bioavailable fraction of metals were extracted from sediment samples using Ure et al. (1993) protocol. This consisted of shaking an aliquot of sediments with acetic acid for 16 hours until obtaining a solution containing the bioavailable fraction. All samples were stored at 4 °C until their analysis with ICP-OES.

Measurement of Pb, Al, Zn, Ni, Cu, and Hg

Pb, Al, Zn, Ni, and Cu concentrations were measured in an ICP-OES (Perkin Elmer Optima 2100 DV) with an AS 93 plus Autosampler. The calibration curve was made with the standard solution AccuStandard ICP multi-element IV, catalog number MES-04-1. Mercury was measured in a Milestone DMA-80. The concentration of all metals was standardized in ppm dry weight. This was made by drying the samples in a stove at 110 °C and calculating the dry weight fraction.

Total organic carbon (TOC) and the percentage of organic matter (OM)

Total organic carbon was estimated employing the methodology proposed by Walkley and Black (1934). Sediments were dried in a stove at 110 °C, after dehydration was added mercury sulfate, potassium dichromate (1N), sulfuric acid ($\text{H}_2\text{SO}_4 \geq 96\%$), and orthophenanthroline indicator. Later, samples were titrated with a solution of ferrous ammonium sulfate (0.1 N). Organic matter was determined by the ignition protocol (Ball 1964).

Conductivity and pH

Conductivity and pH were measured in a Contor 5 ph meter. Water samples were measured in 1 L of sample and sediments were diluted in distilled water in a proportion of 1:1.

The datasets generated during the current study are available in the OSF repository (<https://osf.io/5mbf4/>). Pb, Al, Zn, Ni, Cu, and Hg concentrations in water and sediments were compared with EPA (1996, 2002) guidelines, Canadians guidelines for sediments quality (1999), Dutch law guideline for environmental management (2008), Environmental quality standards for soils in Peru (ECA 2017) and national guidelines, Official Gazette 5021, Decree 883 for the water quality (1995). Enrichment factor for sediments was measured (Abraham and Parker 2008) using Al as normalizer element; baseline values

were extracted from Mogollón et al. (1990), Bifano and Mogollón (1995), and Mogollón et al. (1996). Those works were carried out in the Tuy river basin and Valencia lake, Venezuela. The toxicity index for the analyzed metals was measured (SQGQ; Fairey et al. 2001), and the referential values were extracted from the ARCS program (EPA 1996).

The bioindicator capacities of *E. crassipes* and *L. minor* were assessed with the bioconcentration factor (BCF) and the translocation factor (TF). The BCF is a relation between metal concentration in plant tissue with the metal concentration available in the environment (Rezania et al. 2016). The BCF was determined for leaves and roots, and using metal concentration in the bioavailable fraction as environmental data. On the other hand, the TF is a relation between metal concentration in leaves with the metal concentration in roots (Rezania et al. 2016). The BCF and TF indicate if metals are being concentrated in plants and to what extent. Additionally, they reflected which organ stores higher concentrations of those elements. Both factors were determined for each of the metals evaluated.

Data analysis

Environmental data, metals concentration, and bioconcentration factors were analyzed with PerMANOVas using Euclidean distance. Logarithmic transformations were carried out in case they were necessary. Also, principal components analysis was used as an ordination analysis. All tests were done in P 6 (version 6.1.16). Additionally, linear regression analysis was made with the concentration of metals in compartments (water, sediment, and bioavailable fraction) and environmental data (TOC, OM, pH, and conductivity). Likewise, linear regression analysis was carried out with the concentration of metals in organs and concentration of metals in compartments. All linear regression analysis was made in free software R.

RESULTS

Water

Pb concentration in water samples was below the detection limit (0.0005 ppm), and Al was only in LM's water samples. The concentration of Al was below the detection limit (0.001 ppm) in CM and LP water samples. In **figure 2** is appreciated an aggrupation according to the reservoir. Those differences were significant ($p_{\text{perm}}=0.001$). There were no differences between sample points of entrance

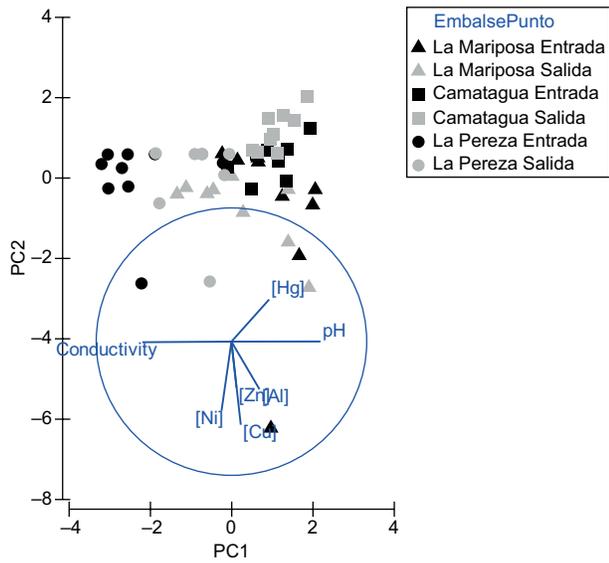


Fig. 2. Principal components analysis for water samples. PC1 represents pH and conductivity. PC2 represents Cu and Ni concentrations. Both principal components represent 55.5 % of variance.

and exit in the water reservoirs (**Fig. 2**). The highest concentrations of Ni, Cu, Zn, and Al were found in LM's water samples, followed by LP and last CM. However, Hg was more abundant in CM (**Fig. 2**). On

the other hand, the lowest pH and highest conductivity were found in LM's water samples, followed by LP and last by CM (**Fig. 2**).

Median concentration of metals in water are in **table II**, where it can be observed that the decreasing order in LM was $[Al] > [Cu] > [Zn] > [Ni] > [Hg]$, CM $[Cu] > [Zn] > [Ni] > [Hg]$ and LP $[Zn] = [Ni] > [Cu] > [Hg]$. Median concentrations of some metals in water were above EPA and Decree 883 guidelines (**Table II**).

Linear regression analysis with the metal concentration in water was not related to any of the environmental variables measured (pH, conductivity, metal concentration in sediments, and bioavailable fraction).

Sediments

Metals concentration in sediments were different between reservoirs ($p_{perm}=0.001$; **Fig. 3**). Additionally, using the Monte Carlo test, the sediments metal concentration was significantly different between sampling points (entrance and exit) of LM and LP ($MC = 0.001$; **Fig. 3**). Sampling points in Camatagua had no significant differences between the entrance and exit sample points; however, the exit point had the highest OM, TOC, and Hg concentrations. The relationship between metals concentration in sediments with pH and conductivity was significant for Zn, Ni, Hg, and Al with pH ($p < 0.03$) and Hg and

TABLE II. RANGE AND MEDIAN \pm STANDARD DEVIATION OF METALS CONCENTRATIONS IN WATER SAMPLES (PPM).

Reservoir	Sampling point	Al	Zn	Ni	Cu	Hg (ppb)
La Mariposa	Entrance	0.9 ± 2 0 - 4.3	0.2 ± 0.3 0 - 0.8	0.2 ± 0.6 0 - 1.8	0.3 ± 0.6 0 - 1.8	0.1 ± 0.1 0 - 0.4
	Exit	0.6 ± 2 0 - 5.8	0.4 ± 0.5 0 - 1.1	0.04 ± 0.08 0 - 0.3	0.4 ± 0.1 0.2 - 0.7	0.1 ± 0.1 0 - 0.3
Camatagua	Entrance	<LD ^(c)	<LD ^(c)	0.2 ± 0.3 0 - 0.9	0.03 ± 0.07 0 - 0.2	1 ± 2 0 - 6
	Exit	<LD ^(c)	1.2 ± 0.9 0 - 2.4	0.3 ± 0.5 0 - 1.5	0.1 ± 0.08 0 - 0.2	3 ± 3 0.1 - 8
La Pereza	Entrance	<LD ^(c)	0.04 ± 0.1 0 - 0.3	0.3 ± 0.7 0 - 2.1	0.1 ± 0.1 0 - 0.2	<LD ^(c)
	Exit	<LD ^(c)	0.5 ± 1 0 - 3	0.1 ± 0.2 0 - 0.5	0.06 ± 0.1 0 - 0.2	0.03 ± 0.06 0 - 0.1
EPA ^(a)		0.75	0.12	0.47	0.013	1.4
Decree No 883 ^(b)		0.2	5		1	10

^(a) EPA guidelines, 2002. EPA-822-R-02-047.

^(b) Decree N° 883, (1995) guidelines for waters subtype 1A and 1B.

^(c) Below detection limit.

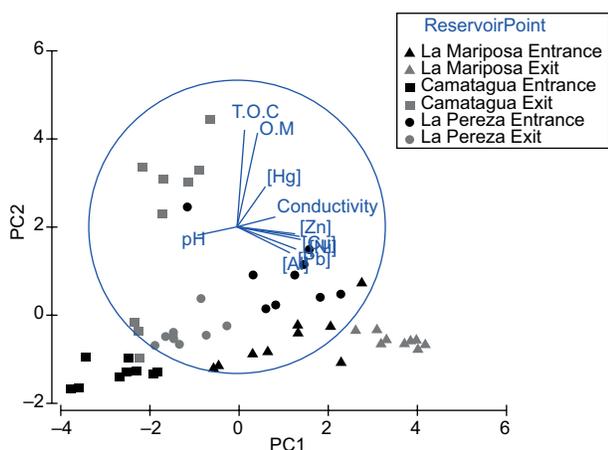


Fig. 3. Principal components analysis for sediment samples. PC1 represents the metal concentration of Pb, Al, Zn, Ni, and Cu. PC2 represents TOC and O. Both principal components represent 71 % of the variance.

Al with conductivity ($p < 0.01$; **Fig. 3**). The highest metal concentration was LMs' sediment samples, followed by LP and last CM.

The median concentration of metals in sediments in LM was in decreasing order: $[Al] > [Zn] > [Cu] > [Ni] > [Pb] > [Hg]$; CM $[Al] > [Zn] > [Cu] > [Ni] > [Pb] > [Hg]$ and LP $[Al] > [Zn] > [Cu] > [Ni] > [Pb] > [Hg]$. Median concentrations of some metals in sediments were above EPA, Dutch and Canada guidelines (**Table III**).

LM's sediments had an EF between 5-20, and according to Sutherland's (2000) classification, it indicates significant contamination in this water reservoir. However, LP and CM had an EF < 2 , suggesting minimal or no contamination as specified by Sutherland (2000).

Bioavailable fraction

Pb was detected only in three samples of the bioavailable fraction, two from LP (2.14 and 1.72 ppm) and one from LM (4.9 ppm). There were significant differences between reservoirs ($p_{perm} = 0.001$; **Fig. 4**) and between sampling points (entrance and exit) of LM and LP (MC = 0.018 and 0.005, respectively; **Fig. 4**). The highest metal concentrations were LPs'

TABLE III. RANGE AND MEDIAN \pm STANDARD DEVIATION OF METALS CONCENTRATIONS IN SEDIMENT SAMPLES (PPM DRY WEIGHT).

Reservoir	Sampling point	Pb	Al	Zn	Ni	Cu	Hg
La Mariposa	Entrance	40 \pm 10 18 - 60	50000 \pm 20000 26147 - 69711	70 \pm 40 33 - 143	50 \pm 10 31 - 73	70 \pm 20 35 - 97	0.08 \pm 0.03 0.04 - 0.1
	Exit	70 \pm 8 54 - 81	40000 \pm 10000 24087 - 67013	200 \pm 30 177 - 268	70 \pm 10 61 - 93	100 \pm 10 97 - 131	0.10 \pm 0.01 0.09 - 0.1
Camatagua	Entrance	1 \pm 2 0 - 6	900 \pm 500 354 - 1692	30 \pm 10 10 - 53	3 \pm 2 0.8 - 6	6 \pm 4 1 - 11	0.012 \pm 0.005 0.004 - 0.02
	Exit	7 \pm 5 0 - 15	1300 \pm 500 658 - 2281	50 \pm 20 31 - 89	8 \pm 4 4 - 13	17 \pm 6 9 - 30	0.1 \pm 0.06 0.02 - 0.2
La Perezza	Entrance	20 \pm 7 0 - 24	3300 \pm 1000 11 - 4688	100 \pm 50 0.4 - 167	50 \pm 20 0 - 82	60 \pm 30 0 - 87	0.1 \pm 0.1 0.02 - 0.2
	Exit	8 \pm 6 0 - 19	2000 \pm 800 817 - 3220	70 \pm 30 39 - 118	10 \pm 3 8 - 18	30 \pm 9 16 - 46	0.05 \pm 0.04 0.02 - 0.2
EPA ^(a)		37		98	19.514	28.012	
Dutchland ^(b)		50		140	30	40	0.15
Canada ^(c)		35		123		35.7	0.17
Peru ^(d)			140				6.6

^(a) EPA, 1996. (ARCS) guidelines. EPA-905-R96-008.

^(b) Dutch law guidelines for environmental management, 2008. Baseline values.

^(c) Canadian guidelines for sediment quality for aquatic wildlife protection, 1999. ISQG.

^(d) Peru guidelines for environmental quality (ECA) for soils, 2017. Residential soils / parks.

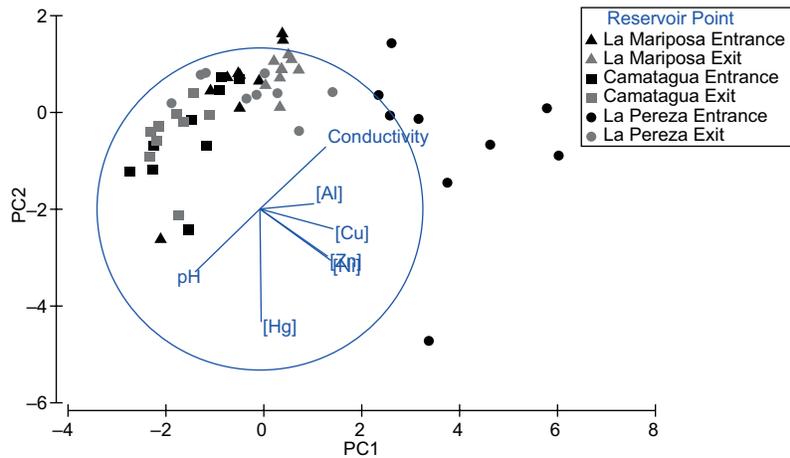


Fig. 4. Principal component analysis for bioavailable fraction of sediments. PC1 represents Pb, Al, Zn, Ni and Cu concentrations, pH, and conductivity. PC2 represents Hg concentrations. Both principal components represent 78.2 % of variance.

bioavailable fraction samples, follow by LM and CM. Except for Hg, which had higher concentrations in CMs' bioavailable fraction samples. In **figure 4** is a pattern where metal concentration in the bioavailable fraction increases with conductivity and lower pH. These relations were especially significant between Zn, Ni, and Cu ($p < 0.01$).

Mean concentrations of metals in bioavailable fraction are in **table IV**, the decreasing order in LM's entrance was $[Al] > [Cu] = [Ni] > [Zn] > [Hg]$; LM's exit $[Al] > [Cu] > [Zn] = [Ni] > [Hg]$; CM $[Al] > [Zn] > [Ni] > [Cu] > [Hg]$; LP's entrance $[Al] >$

$[Zn] > [Ni] > [Cu] > [Hg]$ and LP's exit $[Zn] > [Al] > [Cu] > [Ni] > [Hg]$. On the other hand, LP had an SQGQ > 1 , suggesting a toxicology risk for the mix of contaminants assessed, contrary to LM and CM's bioavailable fraction, which had an SQGQ < 1 (Fairey et al. 2001).

Eichhornia crassipes

The bioconcentration factor of *E. crassipes* had significant differences between organs ($p_{perm}=0.005$; **Fig. 5**) and reservoirs ($p_{perm}=0.049$; **Fig. 5**), being the highest sources of variation. Differences between

TABLE IV. RANGE AND MEDIAN \pm STANDARD DEVIATION OF METALS CONCENTRATIONS IN BIO-AVAILABLE FRACTION OF SEDIMENT SAMPLES (PPM DRY WEIGH).

Reservoir	Sampling point	Al	Zn	Ni	Cu	Hg (ppb)
La Mariposa	Entrance	90 \pm 30	1 \pm 1	5 \pm 1	5 \pm 2	4 \pm 9
		64 - 140	0 - 3	3 - 7	3 - 8	0 - 28
La Mariposa	Exit	200 \pm 30	4 \pm 2	4 \pm 1	8 \pm 1	4 \pm 3
		142 - 230	2 - 10	2 - 4	6 - 9	0.4 - 11
Camatagua	Entrance	40 \pm 20	10 \pm 3	2 \pm 0,8	2 \pm 0,9	10 \pm 10
		21 - 83	7 - 15	1 - 4	0.5 - 3	0 - 40
Camatagua	Exit	10 \pm 20	20 \pm 9	3 \pm 1	0.4 \pm 0.7	10 \pm 10
		0 - 47	8 - 40	2 - 4	0 - 2	0.2 - 28
La Pereza	Entrance	150 \pm 70	170 \pm 60	60 \pm 20	30 \pm 10	10 \pm 20
		63 - 276	83 - 241	31 - 89	15 - 42	0 - 63
La Pereza	Exit	20 \pm 30	40 \pm 40	8 \pm 6	8 \pm 7	2 \pm 2
		0 - 76	0 - 136	0 - 15	0 - 23	0 - 7

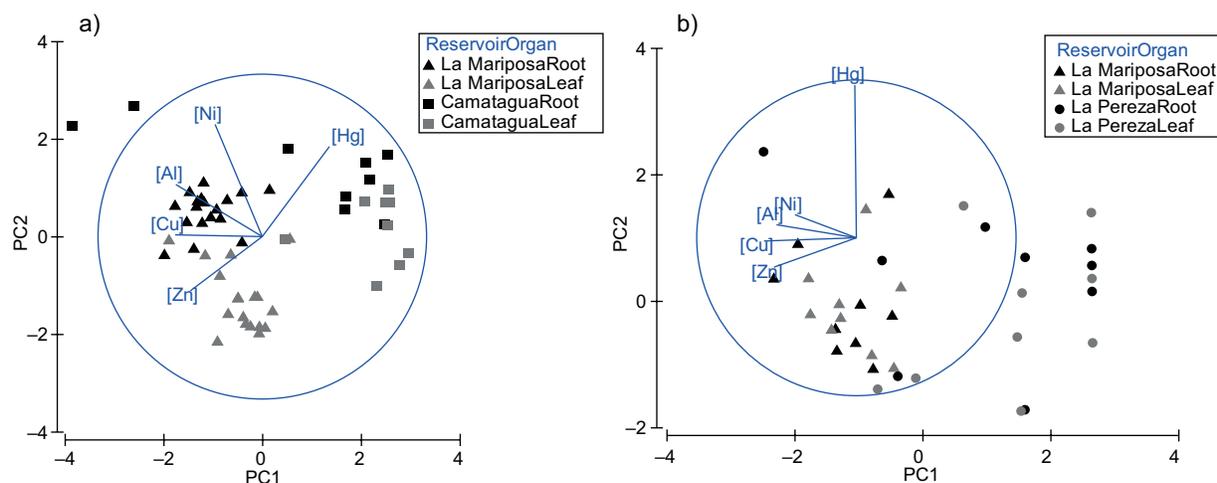


Fig. 5. (a) Principal components analysis for bioconcentration factor of *E. crassipes*. PC1 represents Cu and Al concentrations. PC2 represents Ni concentrations. Both principal components represent 78.5 % of variance. (b) Principal components analysis for bioconcentration factor of *L. minor*. PC1 represents Cu, Zn and Al concentrations. PC2 represents Hg concentrations. Both principal components represent 72.9 % of variance.

sampling points were not assessed due to an imbalance in sampling. In **figure 5** is appreciated that the highest concentrations of metals in *E. crassipes* were LMs' samples, except for Hg, which was more abundant in the CM (**Fig. 5**). On the other hand, roots had higher concentrations of Pb, Al, Ni, and Hg, while leaves had higher concentrations of Zn and Cu.

Differences between leaf and root of *E. crassipes* are in **figure 6**. Pb was found only in the roots. Ni, Hg, and Al were more abundant in the roots, and Al was present only in the leaves of LM. Zn and Cu were more abundant in LMs' leaves. Similar to CM, where Zn was more abundant in leaves, but Cu was more abundant in roots.

The decreasing order of metals in *E. crassipes* roots of LMs' samples were [Al] > [Zn] > [Ni] > [Cu] > [Pb] > [Hg] and in leaves [Al] > [Zn] > [Cu] > [Ni] > [Hg]. CM had a similar pattern, the decreasing order in roots was [Al] > [Zn] > [Ni] > [Cu] > [Pb] > [Hg] and in leaves [Zn] > [Cu] > [Ni] > [Hg]. There were significant relations between Al (root), Cu (root) and Zn (leaves) concentrations with metal concentration in sediments and bioavailable fraction ($p < 0.04$). These relations were only significant in *E. crassipes* samples from LM.

Lemna minor

There were no significant differences between organs in *L. minor* (**Fig. 7**) but there were between reservoirs (MC=0.035; **Fig. 7**), being this the highest source of variation. Ni, Al, Cu, and Zn were more abundant in LM. Mean concentration of metals in *L.*

minor is in **table V**, the decreasing order of metals in LM was [Al] > [Zn] > [Cu] > [Pb] > [Ni] > [Hg] and LP [Zn] > [Cu] = [Al] > [Cu] > [Ni] > [Pb] > [Hg].

There were significant relations between Al concentrations in *L. minor* with sediments and water concentrations ($p < 0.01$). *E. crassipes* and *L. minor* samples could not be compared due to unbalanced sampling. However, *L. minor* had the highest Pb concentrations in LM (**Fig. 8**). Al was more abundant in *E. crassipes*, contrary to Zn and Cu which had higher concentrations in *L. minor*. Ni concentrations were similar among *L. minor* and *E. crassipes* leaves, and Hg was more abundant in CMs' plants.

DISCUSSION

La Mariposa (LM), Camatagua (CM), and La Pereza (LP) reservoirs had significant differences in metal content in all compartments analyzed, being the highest source of variation. Despite the importance of these water bodies, this has not been studied before. LM and LPs' water samples had the highest metal concentrations; this could be related to the discharges rich in organic matter from farmhouses, pig houses, and informal houses located in the slopes of these reservoirs (González and Ortaz 1998, González et al. 2003, Bentancourt and Mena 2012). The water waste of these sources tends to be rich in metals like Cu, Ni, and Zn (Sutherland 2000). On the other hand, LM had high concentrations of Pb; a possible origin could be antiknocks of gasoline, before the abolition of its use

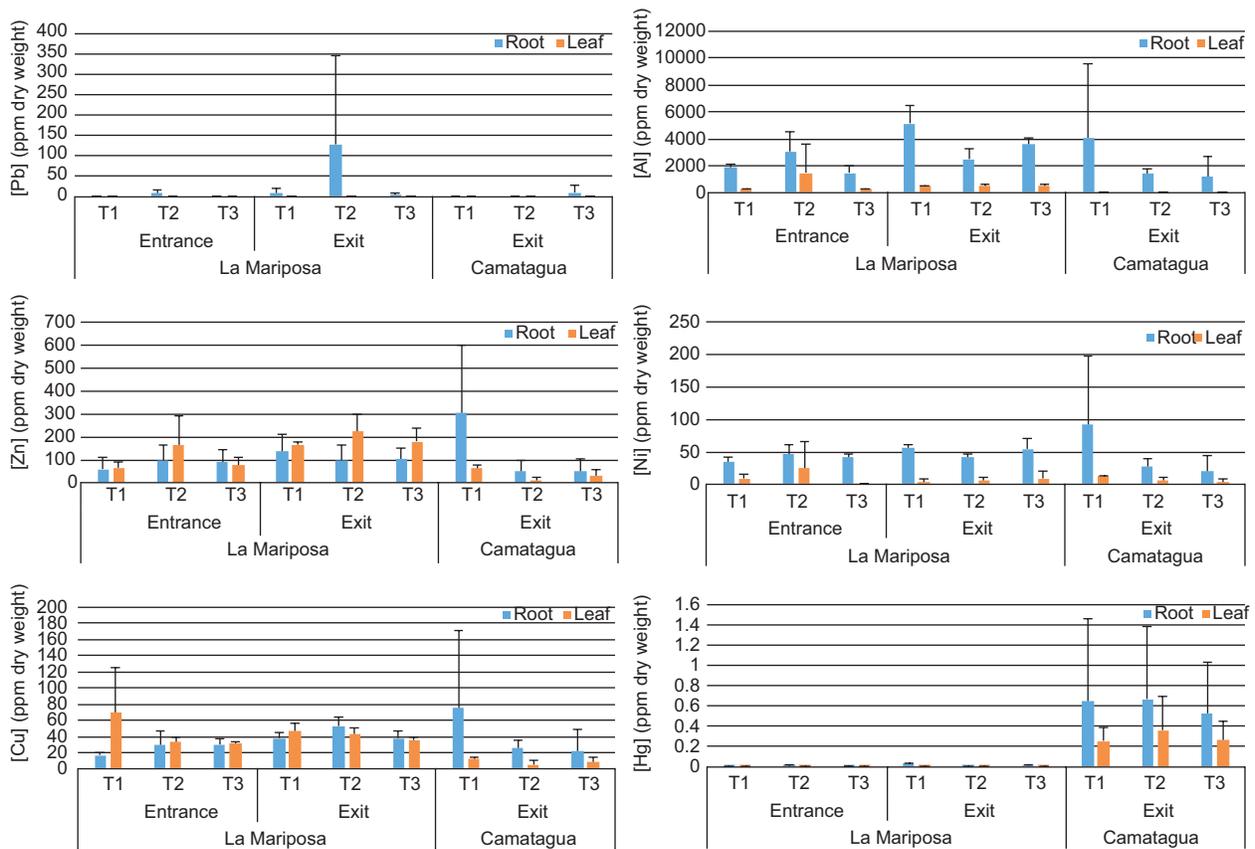


Fig. 6. Mean concentration and standard deviation of metals in roots and leaves of *E. crassipes*. (a) Pb, (b) Al, (c) Zn, (d) Ni, (e) Cu, and (f) Hg concentrations by transect and reservoir.

in 2000 decade; other sources could be wastes from industries, mining, and smelters (Kabata-Pendias 2010). Although, the last ones have not been reported in previous works. Additionally, pointing a specific cause of metals pollution is hard, and it should be addressed in new projects. Unlike LM and LP, CM was the least intervened. This is one of the largest reservoirs of the country, and despite receiving water from contaminated sources such as the Guarico river it also receives from non-contaminated sources (Meléndez et al. 2006).

Zn, Ni, Cu, and Hg concentrations in water of the three reservoirs were higher than those reported for the Zuata reservoir, Aragua state, Venezuela (Álvarez et al. 2012). However, Pb concentrations in water samples from Zuata were higher than in LM, CM, and LP. There were no differences in water samples between sampling points in the three reservoirs, this could be due to water currents in the surface, a product of wind and convection movements by the gradient between day and night (Lewis 1983). On

the other hand, despite the regression analysis not being significant, it has been reported that pH and total suspended solids have a relation with the metal concentration in water (Eggleton and Thomas 2004).

LM and LPs' sediments had significant differences between sampling points, this could be due to differences in deep sampling. A deeper sample could have a higher content of metals due to sulfide production (Zhang et al. 2014). On the other hand, these differences could also be related to variables like organic matter, TOC, and pH (Kabata-Pendias 2010, Zhang et al. 2014), which were significantly related to the metal concentration in sediments.

Metal concentration in sediments of the three reservoirs was lower than the reported for the Zuata reservoir, except for Pb, which was higher in LM's sediments (Álvarez et al. 2012). On the other hand, metal concentration in sediments of the three reservoirs surpassed some international guidelines. Additionally, according to Sutherland (2000) classification, LM's sediments have significant

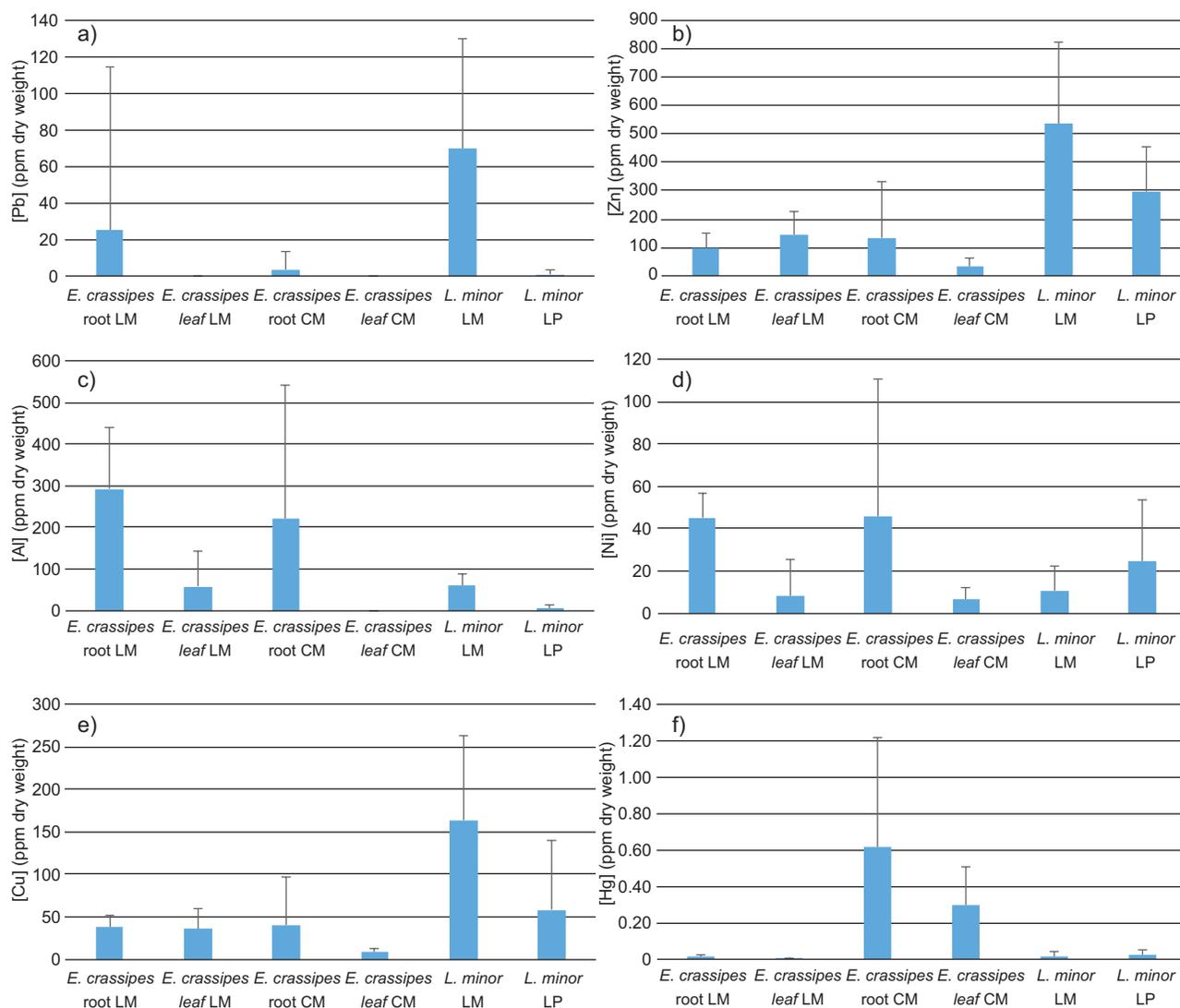


Fig. 7. Metals concentration in roots and leaves of *E. crassipes* and *L. minor* in three water reservoirs, La Mariposa (LM), Camatagua (CM), and La Perezza (LP). Bars represent the mean concentration with their corresponding standard deviation of Pb (a), Zn (b), Al (c), Ni (d), Cu (e), and Hg (f)

TABLE V. RANGE AND MEDIAN \pm STANDARD DEVIATION OF METALS CONCENTRATIONS IN *L. minor* (PPM DRY WEIGH).

Reservoir	Pb	Al	Zn	Ni	Cu	Hg
La Mariposa	70 \pm 60 0 - 191	600 \pm 300 246 - 1274	500 \pm 300 209 - 1116	10 \pm 10 0 - 30	200 \pm 100 68 - 477	0.02 \pm 0.03 0.006 - 0.1
La Perezza	1 \pm 2 0 - 6	60 \pm 100 0 - 374	300 \pm 200 0 - 697	20 \pm 30 0 - 100	60 \pm 80 0 - 303	0.02 \pm 0.03 0 - 0.1

contamination. Unlike CM and LP, which have minimum contamination also according to Sutherland (2000) classification.

The bioavailable fraction of LP had the highest metal concentration, despite LMs' sediments having the highest metal concentration. These differences could be related to the acid pH of LP, which could increase the solubility and mobility of metals like Zn and Ni (Zhang et al. 2014). Nonetheless, other variables can affect the observed pattern, like clay percentage and origin and nature of the metal (Eggleton and Thomas 2004). On the other hand, according to the toxicology index, LP's bioavailable fraction could be a toxicology risk to microorganisms (Fairey et al. 2001). Additionally, the fish consumed by locals in LM reservoir could have high concentrations of metal in tissue, being a potential risk for humans. Moreover, this is one of the first studies of this type. However, future studies in risk assessment are needed.

Pb concentrations in bioavailable fraction were below the detection limit, this could be due to its nature. Pb tends to be adsorbed in organic matter and clay, reducing its solubility and mobility (Eggleton and Thomas 2004). Ni and Zn were the most common elements in this compartment, following literature. Likewise, Cu in LM's bioavailable fraction was among the most common metals, this could be due to its origin. Cu from anthropic input is more mobile and soluble than compounds with lithological origin (Kabata-Pendias 2010).

The highest concentrations of Hg in sediments were in LM and LP samples. However, the water and the bioavailable fraction of CM had the highest concentrations of this metal. This pattern could be related to the aerobic conditions of the reservoir. The bioavailability of Hg in water systems is highly dependent on the methylation rates, which in turn is related to the oxygen levels (Boszke et al. 2008). In anoxic systems, anions of Hg are bound to sulfides and are less available, contrary to aerobic ecosystems, where the formation of cations that can participate in the methylation process is favored (Boszke et al. 2008, Kabata-Pendias 2010). According to González et al. (2003), and González and Roldán (2019) the three reservoirs studied are eutrophic due to the phosphorus and nitrogen ratio and phytoplankton biomass. However, the eutrophic conditions are less critical in CM (González and Roldán 2019). In other words, CM could have more oxygen availability, and in consequence favor the formation of more mobile forms of Hg than LP and LM (Boszke et al. 2008, Kabata-Pendias 2010).

There were differences in metal content between

E. crassipes and *L. minor* according to the reservoir. This could be a reflection of environmental variables like organic matter, TOC, pH, and metal concentration (Lu et al. 2004, Bonanno and Giudice 2010). However, other variables could affect the observed pattern, like antagonistic relations between metals, root area, grain size, and genetic factors (Kabata-Pendias 2010). On the other hand, the differences between organs in *E. crassipes* have been reported in other studies, aquatic macrophytes like *E. crassipes* tend to have 5 – 60 times higher concentrations of metals in roots than in leaves (Quian et al. 1999, Soltan and Rashed 2003). However, Zn and Cu were more abundant in leaves. This could be attributed to their nature, both metals are micronutrients of plants, involved in different metabolic pathways (Lu et al. 2004). For that reason, the translocation to leaves depends on the metal concentration in the environment (Kabata-Pendias 2010), being favorable in sites like LM where Zn and Cu were abundant in the bioavailable fraction. However, other variables like metal mobility, phosphorous content, and ligands presence could affect the observed pattern (Hadad et al. 2009).

Unlike *E. crassipes*, there were no differences between organs in *L. minor*, opposed to Kastratovic et al. (2015) study. The investigators found differences between roots and leaves for Cd, Cu, Co, Cr, Mn, Ni, Pb, Zn, V, and Sr. These differences between studies could be consequences of *L. minor* size. Metals entrance in plants occurs in roots and leaves, in a small plant-like *L. minor*, metal ingress in both organs could be similar, leading to little differentiation between the two. However, other studies should be carried out with a larger sampling size to evaluate this hypothesis.

There were some differences between *E. crassipes* organs, where roots accumulated more metals than leaves. In consequence, the roots are better suited for bioindicator studies by accumulation. In the case of *L. minor*, there were no differences between organs, and metal content can be assessed in the whole body. On the other hand, *E. crassipes* and *L. minor* had some differences, which could be a consequence of genetic and physiological factors. Nonetheless, metal content in *E. crassipes* roots and *L. minor* had a similar pattern to water, sediments, and bioavailable fraction. Moreover, many of those relations were statistically significant. Overall, these patterns suggest that both plants are potential bioindicators for the three water reservoirs studied, especially for water and the bioavailable fraction of sediments. Lastly, since *E. crassipes* is a perennial plant (Rezania et al. 2016) its roots could reflect environmental quality for a longer

period than *L. minor*. Which has a short period of life (15 days) and could reflect the momentary system conditions (Barks and Laird 2014).

CONCLUSIONS

This is the first study to evaluate the metal content in La Mariposa, Camatagua, and La Pereza reservoirs. There were significant differences between the three. Those differences are intimately related to environmental variables like organic matter, TOC, and pH. There were also differences between *E. crassipes* organs, where roots accumulated more metals than leaves. On the contrary, there were no differences between *L. minor* organs. The differences found between reservoirs are reflected in *E. crassipes* organs but are more clear in its roots. Likewise, these patterns were also clear in *L. minor*, suggesting that both plants have potential as bioindicators of metal contamination for some water reservoirs, where *E. crassipes* could reflect the environmental quality for a longer period than *L. minor*.

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REFERENCES

- Abraham G.M. and Parker R.J. (2008). Assessment of heavy metal enrichment factors and the degree of contamination in marine sediments from Tamaki Estuary, Auckland, New Zealand. *Environmental Monitoring and Assessment* 136, 227-238. <https://doi.org/10.1007/s10661-007-9678-2>
- Ali H. and Khan E. (2018). Bioaccumulation of non-essential hazardous heavy metals and metalloids in freshwater fish. Risk to human health. *Environmental Chemistry Letters* 16, 903-917. <https://doi.org/10.1007/s10311-018-0734-7>
- Álvarez M., Aru R., Barrero M., González E., González Y. and Sepúlveda G. (2012). Estudio biogeoquímico del embalse Suata, Venezuela, de acuerdo al contenido metálico de sus sedimentos de fondo, aguas y peces. *Avances en Ciencias e Ingeniería* 3, 103-114.
- Ball D.F. (1964). Loss-On-Ignition as an estimate of organic matter and organic carbon in non-calcareous soils. *J. Soil Sci.* 15 (1), 84-92. <https://doi.org/10.1111/j.1365-2389.1964.tb00247.x>
- Barks P.M. and Laird R.A. (2014). Senescence in duckweed: Age-related declines in survival, reproduction and offspring quality. *Functional Ecology* 29 (4), 540-548. <https://doi.org/10.1111/1365-2435.12359>
- Bentancourt W.Q. and Mena K.D. (2012). Assessment of waterborne protozoan passage through conventional drinking water treatment process in Venezuela. *Journal of Water and Health* 10 (2), 324-337. <https://doi.org/10.2166/wh.2012.118>
- Bifano C. and Mogollón J.L. (1995). Metallic contaminant profiles in sediment cores from Lake Valencia, Venezuela. *Environmental Geochemistry and Health* 17, 113-118. <https://doi.org/10.1007/BF00126079>
- Bonanno G. and Giudice R. (2010). Heavy metal bioaccumulation by the organs of *Phragmites australis* (common reed) and their potential use as contamination indicators. *Ecological Indicators* 10 (3), 639-645. <https://doi.org/10.1016/j.ecolind.2009.11.002>
- Bonanno G., Vymazal J. and Cirelli G.L. (2018). Translocation, accumulation and bioindication of trace elements in wetland plants. *science of the Total Environment* 631-632, 252-261. <https://doi.org/10.1016/j.scitotenv.2018.03.039>
- Boszke L., Kowalski A., Astel A., Baranski A., Gworek B. and Siepak J. (2008). Mercury mobility and bioavailability in soil from contaminated area. *Environmental Geology* 55, 1075-1087. <https://doi.org/10.1007/s00254-007-1056-4>
- CCME (1999). Canadian sediment quality guidelines for the protection of aquatic life: Summary tables. Canadian Council of Environment Ministers. Guideline. Winnipeg, Canada, 5 pp.
- Official Gazette. (1995). Gazette 5.021, Decree 833, 28 of December of 1995: Rules on environmental assessment of activities likely to degrade the environment. Extraordinary Official Gazette. Newspaper. Caracas, Venezuela, 14 pp.
- USEPA. (1996). EPA-905-R96-008. Program for the evaluation and remediation of contaminated sediments (ARCS). Great Lakes National Program Office, United States Environmental Protection Agency. Guideline, Chicago, USA, 474 pp.
- USEPA. (2002). EPA-822-R-02-047. Recommended national water quality criteria. Office of Water and

- Office of Science and Technology, United States Environmental Protection Agency. Guideline. Washington, D.C., USA, 35 pp.
- Eggleton J. and Thomas K.V. (2004). A review of factors affecting the release and bioavailability of contaminants during sediment disturbance events. *Environ. Int.* 30 (7), 973-980. <https://doi.org/10.1016/j.envint.2004.03.001>
- Fairey R., Long E.R., Roberts C.A., Anderson B.S., Phillips B.M., Hunt J.W., Puckett H.R. and Wilson C.J. (2001). An evaluation of methods for calculating mean sediment quality guideline quotients as indicators of contamination and acute toxicity to amphipods by chemical mixtures. *Environmental Toxicology and Chemistry* 20 (10), 2276-2286. <https://doi.org/10.1002/etc.5620201021>
- González E.J. and Ortaz M. (1998). Efectos del enriquecimiento con N y P sobre la comunidad del fitoplancton en microcosmos de un embalse tropical (La Mariposa, Venezuela). *Revista de Biología Tropical* 46 (1), 27-34.
- González E.J., Ortaz M., Peñaherrera C., Montes E., Matos M.L. and Mendoza J. (2003). Fitoplancton de cinco embalses de Venezuela con diferentes estados tróficos. *Limnetica* 22 (1-2), 15-35. <https://doi.org/10.23818/limn.22.02>
- González E.J. and Roldán G. (2020). Eutrophication and Phytoplankton: Some generalities from lakes and reservoirs of the Americas. In: *Microalgae - From physiology to Application* (M. Vítová, Ed.). IntechOpen, United Kingdom. <https://doi.org/10.5772/intechopen.89010>
- Environmental Management Act. (2008). Guidelines for Part 17.2: Measures in case of environmental damage or its imminent threat. Dutch Environmental Law. Guideline. Netherlands, 83 pp.
- Hadad H.R., Maine M.A., Pinciroli M. and Mufarrege M.M. (2009). Nickel and phosphorous sorption efficiencies, tissue accumulation kinetics and morphological effects on *Eichhornia crassipes*. *Ecotoxicology* 18, 504-513. <https://doi.org/10.1007/s10646-009-0308-3>
- Hewitt A.D. and Reynolds C.M. (1990). Dissolution of metals from soils and sediments with a microwave-nitric acid digestion technique. *Atomic Spectroscopy* 11 (5), 187-192.
- Kabata-Pendias A. (2010). Trace elements in soils and plants. 4th edition. CBC Press, Florida, USA, 505 pp. <https://doi.org/10.1201/b10158>
- Kastratovic V., Jacimovic Z., Durovic D., Bigovic M. and Krivokapic S. (2015). *Lemna minor* L.: As bioindicator of heavy metal pollution in Skadar Lake (Montenegro). *Kragujevac Journal of Science* 37, 123-134. <https://doi.org/10.5937/KgJSci1537123K>
- Lewis W.M. (1983). A revised classification of lakes based on mixing. *Canadian Journal of Fisheries and Aquatic Science* 40 (10), 1779-1787. <https://doi.org/10.1139/f83-207>
- Lu X., Kruatrachue M., Pokethitiyook P. and Homyok K. (2004). Removal of Cadmium and Zinc by Water Hyacinth, *Eichhornia crassipes*. *Science Asia* 30, 93-100.
- Manickavasagam S., Sudhan C. and Bharathi A.S. (2019). Bioindicators in aquatic environment and their significance. *Journal of Aquaculture in the Tropics* 34, 73-79
- Matusiewicz H., Sturgeon R.E. and Berman S. (1989). Trace element analysis of biological material following pressure digestion with nitric acid – hydrogen peroxide and microwave heating. *Journal of Analytical Atomic Spectrometry* 4, 323-327. <https://doi.org/10.1039/JA9890400323>
- Meena R.A., Sathishkumar P., Ameen F., Yusoff A.R. and Gu. F.L. (2017). Heavy metal pollution in immobile and mobile components of lentic ecosystems – a review. *Environmental Science and Pollution Research* 25, 4134-4148. <https://doi.org/10.1007/s11356-017-0966-2>
- Meléndez R., Romero-Cazeaudumec Y. and Blavia F. (2006). Livestock facilities and pollution of water resources in Venezuela: Current status. *Veterinaria Tropical* 29-30 (1-2), 99-111.
- Mogollón J.L., Bifano C. and Davies, B.E. (1996). Geochemistry and anthropogenic inputs of metals in a tropical lake in Venezuela. *Journal of Applied Geochemistry* 11 (4), 605-616. [https://doi.org/10.1016/0883-2927\(96\)00033-9](https://doi.org/10.1016/0883-2927(96)00033-9)
- Mogollón J.L., Ramirez A.J., Guillén R.B. and Bifano C. (1990). Heavy metals and organic carbon in sediments from the Tuy River basin, Venezuela. *Environmental Geochemistry and Health* 12, 277-287. <https://doi.org/10.1007/BF01783452>
- Quian J.H., Zayed A., Zhu Y.L., Yu M. and Terry N. (1999). Phytoaccumulation of trace elements by wetland plants: III. Uptake and accumulation of ten trace elements by twelve plant species. *Journal of Environmental Quality* 28 (5), 1448-1455. <https://doi.org/10.2134/jeq1999.00472425002800050009x>
- Rezania S., Taib S.M., Din M.F., Dahalan F.A. and Kamayab H. (2016). Comprehensive review on phytotechnology: Heavy metals removal by diverse aquatic plants species from wastewater. *Journal of Hazardous Materials* 318, 587-599. <https://doi.org/10.1016/j.jhazmat.2016.07.053>
- Soltan M.E. and Rashed M.N. (2003). Laboratory study on the survival of water hyacinth under several conditions of heavy metal concentrations. *Advances in Environmental Research* 7 (2), 321-334. [https://doi.org/10.1016/S1093-0191\(02\)00002-3](https://doi.org/10.1016/S1093-0191(02)00002-3)

- MINAM. (2017). Supreme decree N 011-2017-MINAM, Environmental Quality Standards for Soil (ECA). Environment Ministry. Guideline. Lima, Peru 4 pp.
- Sutherland R.A. (2000). Bed sediment-associated trace metals in an urban stream, Oahu, Hawaii. *Environmental Geology* 39, 611-627. <https://doi.org/10.1007/s002540050473>
- Ure A.M., Quevauviller P., Muntau H. and Griepink B. (1993). Speciation of heavy metals in soils and sediments. An account of the improvement and harmonization of extraction techniques undertaken under the auspices of the BCR of the Commission of the European Communities. *International Journal of Environmental Analytical Chemistry* 51 (1-4), 135-151. <https://doi.org/10.1080/03067319308027619>
- Vareda J.P., Valente A.J. and Durães L. (2019). Assessment of heavy metal pollution from anthropogenic activities and remediation strategies: A review. *Journal of Environmental Management*. 246, 101-118. <https://doi.org/10.1016/j.jenvman.2019.05.126>
- Walkley A. and Black A. (1934). An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. *Soil Science* 37, 29-38.
- Zhang C., Yu Z.G., Zeng G.M., Jiang M., Yang Z.Z., Cui F., Zhu M.Y., Shen L.Q. and Hu L. (2014). Effects of sediment geochemical properties on heavy metal bioavailability. *Environment International* 73, 270-281. <https://doi.org/10.1016/j.envint.2014.08.010>