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Comparison among native floating aquatic macrophytes for bioconcentration of heavy metals

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Abstract

This work was aimed to show the development of a comparative study of the capability of bioindicators to reveal the presence of heavy metals among species of floating aquatic macrophytes such as *Spirodela* sp (giant duckweed), *Pistia stratiotes* (water lettuce), *Salvinia* sp used as a parameter for bioconcentration factors plant / sediment. We performed a simple sampling at six sites in the region of Pelotas, southern Brazil, in which it was collected sediments and plants. The plants experienced the nitric perchloric acid digestion method, while the sediment suffered pseudo total acid digestion method. The determination of Cr, Cu, Pb and Zn in the extracts was conducted by atomic absorption spectrophotometry in flame. In general, the floating aquatic macrophytes studied showed better bioconcentration factor for metals from the sediment in the following order: Cu>Zn>Pb>Cr. For copper, the figures obtained with *Pistia stratiots* ranged from 5.7 to 82.8. The BCF for Zn ranged from 3.0 to 11.6 and *Salvinia* sp. For the Pb bioconcentration factor, it ranged from 5.4 to 0.6 in *Spirodela* sp. The in situ study showed that all species had high bioaccumulation potential, especially *Pistia stratiotes* that was employed to remove copper.

Keywords: Bioconcentration, heavy metals, macrophytes, bioconcentration factor.

INTRODUCTION

The city of Pelotas is located on the shores of the São Gonçalo Channel in the state of Rio Grande do Sul, in southern Brazil. This channel connects the Patos and Mirim lagoons, which are the largest ones in Brazil. This region has a humid subtropical climate, which is suitable for the development of some floating aquatic macrophytes species such as *Spirodela* sp, (giant duckweeds), *Pistia stratiotes* (water lettuce) and *Salvinia* sp.

The Macrophytes form an important group in the shallow freshwater ecosystems and these systems are common throughout the coastal plain of Rio Grande do Sul (Vieira & Rangel, 1988). This category of plant has a great potential for reducing or removing water pollutants, including phosphorus, nitrogen and heavy metals. On the other hand, these plants are regarded as weeds because they have high rate of biomass production setbacks, which may cause obstruction and changes in aquatic environments (Bini & Thomas, 2003). Contamination provided by toxic heavy metals is due mainly to the fact that they are quite stable in the environment and capable of being retained in the soil, plants, sediments and biological systems. Man cannot destroy heavy metals and in the environment, they usually appear in low concentrations. However, these concentrations when combined with human actions tend to increase as it damages the soil, water and human health consecutively (Garbisu & Itziar, 2001).

The regeneration of contaminated areas, carried out by human activities, can be conducted by several methods including phytoremediation, which features the reduction of levels of contaminants from soil and water and keeps consistent safe levels for protection of human health by preventing the dissemination of environmentally hazardous substances. Among the phytoremediation techniques, phytoextraction is one of them from which the plant assimilates the pollutant, and afterwards deposits it in the plant tissue, which favors the disposal of material that can be either ignited and used in co-processing or destined to a landfill (Andrade *et al*., 2007).

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The choice of the plant to be used for metal phytoextraction is important because it should have good absorbency and resistance to pollution, high growth rate in different environments, good biomass production, and be easy to be collected and removed from the chosen place (Coutinho & Barbosa, 2007).

In general, the pollutants when released into water systems bind to particulate matter, in which after eventually becoming decanted, are incorporated into the sediments. Thus, surface sediment is an important reservoir of heavy metals and other contaminants in the aquatic environment (Harguinteguy *et. al,* 2014)

The remobilization of metals found in the sediment can be driven to the water column through a process of physicochemical characteristics, being available to be incorporated by organisms that are present in this medium (Torres *et. al,* 2008; Lemes et al., 2003).

In general, the bioconcentration factor (BCF) is the ratio between a compound from one organism into the concentration and the environment in which it lies. Thus, the numerical value of the BCF indicates the partition degree of the pollutant among these divisions (Paraiba et al., 2006 , Lafabrie et al 2013).

Most studies evaluate the ability of bioconcentration by floating macrophytes from the waters (Pio *et. al,* 2013) Few data exist relating the concentration of floating macrophytes with sediment.

In this paper, a comparative study of the ability of phytoextraction and bioindicators of metals was developed amongst floating aquatic macrophytes such as *Spirodela* sp, (giant duckweeds), *Pistia stratiotes* (water lettuce) and *Salvinia* sp., all used as a parameter for the bioconcentration factors plant/sediment in the region of Pelotas (RS).

MATERIALS AND METHODS

Sampling and digestion

The sites were chosen accordingly to their large facility for collection and great abundance of aquatic species. We selected six sampling sites in the region of Pelotas / RS, as shown in figure 1. Simple sampling was done in April 2013. Figure 2 shows images of aquatic plant species used in this study.

Point 1 - P1, (31 ° 46’25 .18 “ S and 52 ° 14’08 .69” w) the collected species was *Pistia stratiotes* (water lettuce); Point 2 - P2, (31°46’24.54” S and 52°14’11.28” W) the collected species were *Spirodela* sp, (giant duckweeds) and *Salvinía* sp; Point 3 - P3, (31º46’22.45” S and 52º14’24.00” W) the collected species were *Pistia stratiotes* (water lettuce), *Spirodela* sp (giant duckweeds) and *Salvinía* sp, and in Point 4 - P4, (31°45’31.65” S and 52°13’43.60” W), Point 5 - P5, (31°45’08.21” S and 52°17’25.77” W), and point 6 - P6 (31º41’53.46” S and 52º20’51.63” W) the collected species were *Spirodela* sp, (giant duckweeds).

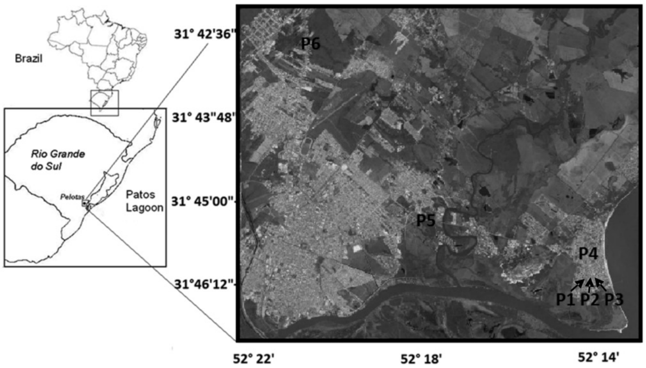

Figure 1- Location of the sampling area. fonte: google Maps.

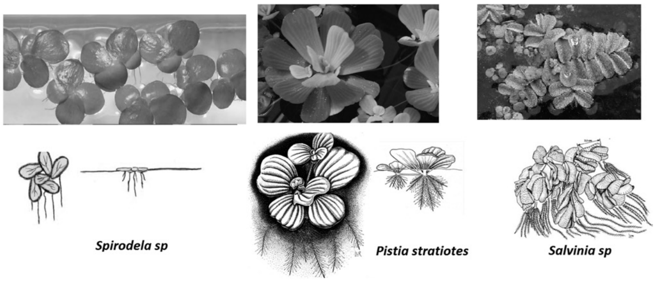

Figure 2- Shows images of aquatic plant species used in this study.

The P1, P2, P3 and P4 are points of channel drainage that cross the urbanized area resort, being located on the shores of the Patos Lagoon. In addition, points P5 and P6 are urban streams in the outskirts of the city.

The experiments were performed with plants and sediment. The collection of plants was carried out with the aid of a dip net made of satin and subsequently stored and previously transported in decontaminated polyethylene container.

Plant samples were separated by species and washed with water, being washed again in distilled water and, in kiln, dried at 60 ° C for 48 hours, weighed about 2 g of the sample in triplicate, which underwent through nitric perchloric digestion – Abreu’s based method (2004), 4 mL HNO_3, 1 mL of concentrated $HClO_4$ was added to the samples and carried out in glass tubes to the digester at 210 ° C block until there was no more shedding of white fumes. The filtered extracts were swelled to 25 mL with ultrapure water.

The preparation of blank was performed with a mixture of nitric and perchloric acids under the same conditions of the samples.

To collect sediment, a dredge used a stainless steel type “Van Veen” and there was a collection of surface sediment (0-5 cm depth). The material of the central part of the dredger was stored in a polyethylene pot previously decontaminated and removed afterwards. Later the collection samples were transported to the laboratory and refrigerated at ±4 ° C. For the determination of heavy metals from sediment samples, they were dried at 60 ° C for 48 hours, being avoided the dragging of metal caused by water vapor, and later on being ground and sieved. From less than 63 mm fraction, about 2 g of sample was weighed in triplicate that suffered pseudototal acid digestion method based on Hortellani (2005), 4 ml of ultra pure water, 4 ml of aqua regia (3:1 HCl: HNO3) and 1 mL of HClO4 placed in a water bath at 90 ° C for 30 min and filtered.

Later on it was swelled to 25 ml with water Ultra pure.

The pH of the slurry was measured by using 1:2 (sediment / water spot) as employed by Yoon et al (2006). Organic matter was determined after drying loss of volatiles at 450 ° C by Mannino & Orecchio, (2008) The moisture content was obtained by gravimetric method (APHA, 2005).

When preparing the blank, we used a mixture of perchloric acid with ultra pure water and aqua regia going under the same conditions.

Solutions and reagents

The materials used in handling and storage of the samples underwent through decontamination process in a solution of HNO_3 10% (v / v) for 24 hours and then dried at 105 ° C (Teódulo *et. al,* 2004).

All reagents used were of analytical grade. Concentrated nitric acid (65% w/w) and perchloric acid (70% w/w) were used in the digestion of the samples.

For the calibration curve (the range 0.2 to 4.0 mg L^{-1}) , standards were prepared from stock solutions of 1000 mg L^{-1} Cu, Cr, Pb and Zn all brand Titrisol® - Merck.

Determination of Metals

For the determination of Cr, Cu, Pb and Zn, the samples were subjected to analyses by atomic absorption spectrometry with a flame equipment, brand GBC, model 932 plus air/acetylene**.**

The blank reading was performed 10 times to calculate the limit of quantification (LOQ) and the limit of detection (LOD) of the equipment, which were calculated based on the average standard deviation (SD) for the LOQ blank signal plus 10 times the average signal blank, plus 3 times the SD for the LOD, according to IUPAC (1997). The ratio between concentrations in sediment and plants was evaluated by correlation coefficient Pearson using the software Microsoft Excel®.

To control the recoveries in the opening procedures and analyses, reference materials were used such as BCR-060 (*Lagarosiphon major*, Aquatic plant), BCR-596 (*Trapa natans*, Aquatic plant) and NMCR#4 (Natural Matrix Certified Reference) for soil and sediment obtained from Ultra Scientifc Analytical Solutions.

The bioconcentration factors was calculed was calculated using the following formula Cx/Cs, where Cx is the mean concentration in the organism (collected macrophytes) and Cs is the mean concentration in the sediment

RESULTS AND DISCUSSION

In general, copper, lead and zinc metals were present at high levels in the biomass of all the species collected in most of the sites. Along with, phytotoxic levels being considered normal for Pb, Zn and Cu were described by Levy et al. (1999), which was 0.5-10 and 30 - 300mg kg^{-1} to Pb - 20-30 and 100 mg kg^{-1} for Cu, 10-150 and > 100 mg kg^{-1} for Zn. Most of the collected macrophytes showed higher concentrations at normal levels, as it is shown in Table 1. These results may indicate that these species are quite tolerant to these metals. These results comply with those described by Verkleij and Schat (1990).

Table 1- Levels of metals (dry basis) in mg kg^{-1} ± SD sediments, plants and bioconcentration factor (BCF), coefficient of correlation (plant x sediment) r^2

Points	samples	Cr ± SD	Cu ± SD	Pb ± SD	Zn ± SD	r^2
P1	WL	0.2 <	56.2 ± 3.0	18.5 ± 2.6	109.8 ± 4.1	0.90
	BCF		5.7	1.7	1.9	
	Sediment	0.2 <	9.8 ± 0.8	11.2 ± 3.0	56.7 ± 4.2	
P2	DW	0.2 <	96.0 ± 3.3	20.0 ± 1.9	161.1 ± 7.4	0.84
	BCF	-	25.9	5.4	3.1	
	SL	0.2 <	92.8 ± 2.3	18 ± 0.7	154.8 ± 3.9	0.84
	BCF	-	25.1	4.9	3.0	
	Sedimento	0.2 <	3.7 ± 0.3	3.7 ± 0.1	51.7 ± 0.1	
P3	WL	0.2 <	99.3 ± 10.1	19.6 ± 2.9	163.4 ± 12.5	0.66
	BCF	-	82.8	5.6	15.1	
	SL	6.2 ± 1.8	68.3 ± 7.5	20.7 ± 1.0	125.1 ± 9.2	0.76
	BCF	62.0**	56.9	5.9	11.6	
	Sediment	0.2 <	1.2 ± 0.1	3.5 ± 0.5	10.8 ± 0.1	
P4	DW	0.2 <	90 ± 6.8	8.2 ± 1.5	151.7 ± 8.5	0.74
	Sediment	7.0 ± 0.3	7.8 ± 0.1	13.5 ± 2.1	50.2 ± 4.0	
	BCF	0.014**-	11.5	0.6	3.0	
P5	DW	0.2 <	< 0.2	2.3 ± 0.2	160.7 ± 7.0	
	Sediment	0.2 <	5.3 ± 0,8	< 0.4	20.9 ± 1.1	
	BCF	-	-	11.5**-	7.7	
P6	DW	< 0.2	< 0.2	19.6 ± 2,5	222.3 ± 12.7	
	Sediment	< 0.2	4.6 ± 0.3	< 0.4	7.8 ± 1.1	
	BCF	-	-	98.0**-	10.6	
LOD		0.2	0.2	0.4	0.06	
LOQ		0.6	0.8	1.2	0.2	
TEL*		52.3	18.7	35.0	124	
PEL*		160	108	91.3	271	

* Sediment quality criteria proposed by *Environment Canada* (1999).

TEL (Threshold Effect Level) = Threshold Effects of adverse biological community (possible effects); PEL (Probable Effect Level) Level of Probable Adverse biological community (probable effects); LOD: Limit of detection; LOQ: Limit of quantification; ND: Not detected; SD: Standard deviation; WL: water lettuce; DW: giant duckweed; SL: Salvinía sp

** used half of the DL for sediments and plant to calculate the BCF´s

Overall, the floating aquatic macrophytes showed better bioconcentration factor for metals from the sediment in the following order: Cu>Zn>Pb>Cr. This behavior is explained on the basis of Zn and Cu since they are essential nutrients, unlike the lead, which can be toxic for photosynthetic activity and chlorophyll synthesis antioxidadntes enzymes (Thomas and Eong, 1984, Kim et al. 2003). According to Mishra & Tripathi (2008), Cr is one of the most difficult metals to be removed from water due to the fact that these macrophytes are not used in their physiology. Since they are floating plants, the transport of these analytes is related to the distribution of coefficient water / sediment and water / biota (Paraiba et al., 2006). For copper, the results obtained for the BCF with water lettuce ranged from 82.8 to 5.7 at P3 and P1. The BCF for Zn ranged from 11.6 at P3 to 3.0 at P2. Pb obtained variations from 5.4 in duckweed P2 to 0.6 at P4. The Cr was not detected in most sediment, only in point 5 as shown in Table 1 It is important to highlight that the figures found do not depend only on the total concentration of metals in the sediment, but also in the chemical species present and their physicochemical conditions, including pH, organic matter and the cation exchange capacity of the soil, sediment and water as well as the plant species (Rosselli *et. al,* 2003, Yoon *et. al,* 2006).

In Table 1, we can see that the values for Cu, Cr, Pb and Zn found in the sediments of the sampling sites showed lower levels than the minimum limits set by the Guide to Quality sediment for protection of aquatic life in Canada (CCME EPC - 98E 1999), therefore, they are considered low sediment contamination. The pH values have not varied, as it is shown in Table 2. Points 1 and 4 had around 10.2 % of organic matter being the highest figure of this parameter when compared to the others. According to USEPA (2005), the higher of the content of organic matter, the greater is the possibility of environmental contamination.. The moisture content of the sediment indicates the presence of hygroscopic substances, which is related to the particle size. High values are generally associated with fine sediments (silt and clay), resulting in greater possibility of contaminants.

As stated, Cu was absorbed by a metal that was studied in most species, which is observed in point 1, the water lettuce had almost three times higher levels than the other metals BCF. This behavior shows that Cu agrees with the results of Mishra & Tripathi (2008) by assessing the concentration of this element from fortified water samples. In point 2, the bioconcentration factor for copper using duckweed and *Salvinía* sp showed values of 25.9 and 25.1 respectively. The duckweed and *Salvinía* sp in this environment showed the same behavior for bioconcentration in all metals studied. The ability of phytoextraction of duckweed has already been proved by other authors such as Zayed, *et al*., (1998), and Zhu *et. al*. (1999). In Point 3, we observe that the *Salvinía* sp presented the same four metals studied but Cr was not detected in the sediment When comparing with with the P4, Duck Weed showed no detectable levels although sediments did. So, for Cr, SL resulted a better bioindicator than DW. However, more data is needed to purpose the higher bioavailability of Cr than the other metals studied.

Table 2 - Characterization of the sediment

Physicochemical Properties			
Points	pH	organic matter (% ± SD)	Moisture (%± SD)
P1	6.5	10.2±0.4	63.8%±5.1
P2	6.4	2.6±0.4	38.5%±3.1
P3	6.4	4.4±0.8	42,1%±3.2
P4	6.7	10.2±4.1	65.1%±3,5
P5	6.4	4.2±0.2	40.2%±2.1
P6	6.5	2.3±0.4	35.8%±2.1

SD: standard deviation

Again, we can confirm high bioconcentration factors for Cu when compared to water lettuce and *Salvinía* sp with values of 82.8 and 56.9 and Zn 15.1 and 11.6 respectively. In point 4, the duckweed showed Cu, Pb and Zn in the sediment and all metals studied were quantified. The Pb at this point displayed a higher concentration when compared to the other points, although its bioconcentration factor was low. This behavior can be explained by the low bioavailability of this element. The results suggest that the high organic matter content in the sediment reduces the displacement of the metal in the water, thus, hindering plant uptake and resulting in low values for BCF.

In points 5 and 6, only Zn and Cu were found in the sediment. When duckweed was studied, Pb and Zn were found. These results suggest that even at these points, Pb undetectable concentrations are bioavailable unlike Cu.

The recovery values obtained from the BCR-060 (Lagarosiphon major, Aquatic plant), BCR-596 (Trapa natans, Aquatic plant) and NMCR#4 (Natural Matrix Certified Reference) for soil and sediment ranged from 75.1% for Chrome to 103.6% for copper in sediment, and 85.1% for chrome to 96,4% for Zn in aquatic plant. These results are shown in Table 3.

The values of Pearson correlation coefficients between metals and plants for different points were above 0.70, indicating a direct relation between sediment contamination and the potential for bioaccumulation of macrophytes.

CONCLUSION

The in situ study was able to prove the ability to bioaccumulate and its consequent phytoextraction of floating macrophytes. Species showed similar behavior against different metals. The Cu was the easiest element absorbed. The bioconcentration factors from the sediment are not dependent only on total metal concentration, as they are also influenced by the physico-chemical characteristics of the environment. Thus, the use of floating aquatic macrophytes

Table 3 - Recovery figures in mg kg^{-1} ± RSD (in %) obtained from the BCR-060 (Lagarosiphon major, Aquatic plant), BCR-596 (Trapa natans, Aquatic plant) and NMCR#4 (Natural Matrix Certified Reference- soil and sediment)

	Lagarosiphon major			Trapa natans			NMCR#4		
	VC	FV	rec	VC	FV	rec	VC	FV	rec
Cr				36.3±1.7	30.9±0.8	85.1	48.1±6.6	36.1±3.7	75.1
Cu	51.2±1.9	45.5±1.1	88.9				36.4±2.2	37.7±1.7	103.6
Pb	94.0±4.0	90.6±2.1	96.4				95.3±7.7	90.3±1.2	94.8
Zn	313.0±8.0	301.9±3.8	96.5				133.5±10.6	112.2±0.12	84.0

CV: Certified value; FV : Found value; rec: % recovery

allowed an assessment of the chemical forms with higher mobility. The study of native species is a tool for selecting appropriate plants already in an ecosystem and its consequent use in phytoremediation.

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