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ORIGINAL PAPER

Total Mercury Distribution and Volatilization in Microcosms with and Without the Aquatic Macrophyte *Eichhornia Crassipes*

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Abstract Mercury (Hg) is one of the most toxic pollutants and spreads in the environment according to its affinity to several compartments. Aquatic macrophytes, such as *Eichhornia crassipes*, are known as sites for accumulation of Hg and methylmercury formation. The objective of this research was to observe Hg distribution among air, water and whole plants of the macrophyte *E. crassipes* for 17 days. The distribution of a single ^{203}Hg spike was evaluated by gamma spectrometry. Two experiments, with and without macrophytes, were made, and the compartments analyzed for the presence of Hg were air, 0.2-μm filtered water, suspended and settled particles, roots, leafs, petioles and adsorption on the desiccators walls. ^{203}Hg was detected in all analyzed compartments, and the highest total Hg concentrations were found in the roots and particles of the incubations with and without macrophytes that retained in average 68 and 34 % of added Hg, respectively. On the other hand, the lowest concentrations were found in air for both incubations, with higher volatilization (up to 2.5 % of added Hg) in the absence of macrophytes. The lower Hg values in leafs and petioles suggest this plant has mechanisms of Hg retention in the roots. Results suggest this macrophyte promotes changes in the Hg cycle since it attracts most Hg present in water and particulate to its roots and settled particles underneath and also reduces Hg volatilization.

Keywords Mass balance · Freshwater lake · Aquatic macrophyte · Gamma spectrometry

1 Introduction

Increasing concerns with anthropogenic sources of Hg to water systems has lead to extensive surveys of Hg levels and chemical speciation in various types of human and environmental samples. Despite the effort, the understanding of the overall dynamics of Hg in the aquatic environment is still far from satisfactory (Ullrich et al. 2001).

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The use of radiotracer techniques on model ecosystems offers a unique possibility to better understand the Hg cycling in various environmental conditions. This approach allows a great simplification of the experimental setup in studies of the distribution and transformation of a contaminant, such as Hg, in different compartments and its interaction with the biota. This is particularly true if a gamma emitter isotope is used, such as ^{203}Hg, since gamma spectrometry is non-invasive and non-destructive, allowing in vivo experiments in a micro/mesocosm scale.

Aquatic macrophytes, such as water hyacinths (*Eichhornia crassipes*), display high primary productivity, and they may be the main organic matter producers in their environment (Esteves 1988). Under favorable conditions, plants can double their dry mass in 3–7 days and can cover the entire surface of the lake (Chambers et al. 2008). The submersed parts of the macrophytes form a complex habitat called periphyton, which is composed of stems, roots, organic and inorganic detritus, all colonized by microorganisms, algae and invertebrates, offering shelter, feeding and breeding grounds to many fish species (Wetzel 1983; Sánches-Botero et al. 2003; Cazzanelli et al. 2008). Periphyton of Boreal lakes can accumulate high concentrations of Hg and methylmercury, and *E. crassipes* periphyton shows high rates of net methylmercury formation, in average an order of magnitude higher than found in the sediment below, thanks to the dense microbial community they host. (Desrosiers et al. 2006; Mauro et al. 1999, 2001, 2002; Guimarães et al. 2000).

E. crassipes can be used in the production of methane gas, in fertilizer industries, as food, animal feed, paper, among others (Wolverton and McDonald 1979; Singhal and Rai 2003; Verma et al. 2007). This plant is frequently used in studies involving domestic and industrial sewage depuration systems. Its use in depuration systems relies on its high rates of absorption of organic and inorganic substances through its roots, allowing the reduction of nitrogen, phosphorus, fecal coliforms and heavy metals including Hg in the outflow (Soltan and Rashed 2003; Maine et al. 2006; Zimmels et al. 2006; Ebel et al. 2007).

Aside its capacity to absorb and concentrate high amounts of Hg, it is expected that the presence of this macrophyte alters Hg volatilization rates in the system since Hg would tend to form complexes with organic matter and particulates present in the plant roots and become trapped (Lawson et al. 2001). Therefore, in this study, an experimental approach based on the incubation of indoor microcosms was set up to investigate the possible effect of the presence of the aquatic macrophyte on Hg distribution in constructed freshwater ecosystems.

2 Materials and Methods

2.1 Sampling

Samples were collected on a managed garden pond, constantly fed with tap water, located within the campus of Fundação Oswaldo Cruz, Rio de Janeiro, southeast of Brazil (22°52′29.64″S 43°14′43.39″W). During the monitored period, this lake held an average pH, Eh and temperature of 7.0, 240 mV and 23 °C, respectively. Whole individuals of *E. crassipes* and water samples were manually collected, conditioned on plastic bags and bottles and readily transported to the laboratory.

2.2 Incubation

Two different unreplicated treatments were used to evaluate Hg distribution along a 17-day period. The first consisted of a microcosm filled with unfiltered lake water only. The second consisted of unfiltered water and macrophyte individuals (Fig. 1).

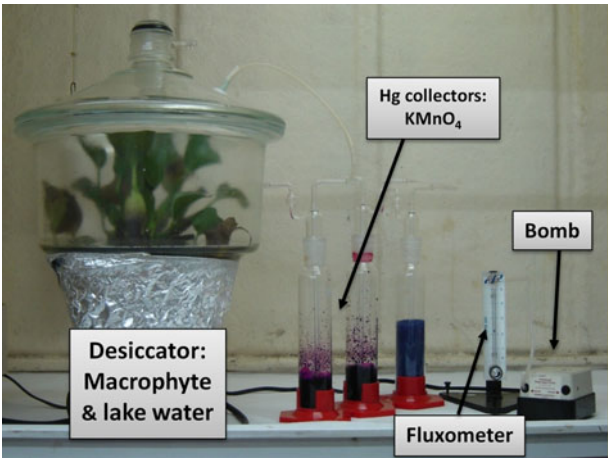

Fig. 1 Picture of the macrophyte microcosm setup

The incubations were performed in tightly sealed borosilicate desiccators of 30 cm of diameter with 4 L of lake water, with or without plants. The air of the desiccator head space was renovated through a negative pressure pump of constant flux (1.5 L/min) to prevent the loss of volatile Hg through the desiccators lid and bubbled through an acid potassium permanganate solution (0.125 mol/L in 0.5 % H_2SO_4 Spectrum Chemicals and QM respectively) that trapped all volatile forms of Hg. In similar experiments, Greger et al. (2005) and Moreno et al. (2008) used constant air fluxes of approximately 1L/min while Tessier et al. (2007) used 0.1 L/min. The microcosms were kept at room temperature (25 °C) and lighted by daylight type fluorescent tubes. To reproduce the natural photoperiod during the experiments, the lights were controlled by an outdoor photocell.

Inorganic Hg, as $^{203}HgCl_2$ (Eckert and Ziegler Isotope Products Laboratories), was added to the water column as a single initial spike of 2.02 and 0.53 μCi in incubations with and without macrophytes, respectively. Hg in the microcosms water was briefly homogenized with a glass rod before adding the macrophytes. In the microcosm with macrophytes, this spike would result in a total Hg concentration of 35 ngHg/g (d.w.) in the roots, if all added Hg was fixed by this compartment. In the microcosm with water only, the Hg spike would result in a concentration of 0.11 ngHg/mL. Hg spikes for each incubation were calculated to assure that Hg would be detectable in filtered water until the end of the experiments, which required higher Hg addition in microcosms with macrophytes, considering the much higher amount of Hg binding sites in macrophyte roots and their associated particulate material.

2.3 Analytical Procedures

Water pH, redox potential and temperature were measured each sampling day with a Lutron PH-206 pH/mV/Temperature meter. Samples of different compartments were collected on days 1, 2, 7, 14 and 17 and were directly analyzed via gamma spectrometry on a Perkin Elmer Wizard 2470 with a 2″ NaI(Tl) detector. These compartments were as follows.

2.3.1 Water and Suspended Particles

One sample of 100 mL was extracted and filtered through 0.22-μm pore size membranes to separate all suspended particles from water samples. The Hg present in the filtered water was preserved with the addition of 0.5 mL of $KMnO_4$ (0.125 mol/L in 0.5 % H_2SO_4 Spectrum Chemicals and QM, respectively). Two filters and 10-mL samples of filtered water were then directly gamma-analyzed.

2.3.2 Volatilization

All volatilized Hg was captured in bubblers containing $KMnO_4$ (0.125 mol/L in 0.5 % H_2SO_4 Spectrum Chemicals and QM, respectively). The Hg recovery rate of the potassium permanganate solution was 100 % in all microcosms. To avoid permanganate precipitation, the solution was replaced every 2 days. Two 4-mL samples were then separated and analyzed at each replacement day and discarded after measurement. Total volatilized Hg was calculated as the sum of the activities of each permanganate solution and of the hydroxylamine chloride solution used at the end of the experiment to dissolve the $KMnO_4$ precipitates that formed on the bubbler walls.

2.3.3 Leafs and Petioles

Samples of leaf and petiole (average wet weight of 2.5 and 6 g respectively) were excised, rinsed in distilled water to remove detritus and analyzed at each sampling day.

2.3.4 Roots

Three samples of roots (4 g wet weight) were excised, placed in a vial and counted directly by gamma spectrometry. Periphyton attached to roots was not removed before analysis, resulting in periphyton–root composite sample that is called roots from now on for simplification.

2.3.5 Settled Particles, Adsorption on Microcosm Walls and on Silicone Seals

All three compartments were sampled only in the last day of incubation. The particulate matter deposited in the bottom of the desiccators was separated by repeated centrifugations. Samples of 0.3 and 0.5 g were taken (water and macrophyte treatments respectively) and posteriorly analyzed. The Hg adsorbed on the walls of the desiccators was recovered by means of successive swabs of the walls with $KMnO_4$ soaked cotton until a blank value was obtained. The silicon grease used for desiccator sealing was removed with a spatula, placed in vials and analyzed for the presence of Hg.

The results in net CPM of the gamma spectrometry were later corrected to DPM as a function of the counting efficiency (variable according to the sample geometry) and ^{203}Hg decay. DPM data were then used to calculate the proportion of Hg found in each compartment taking into account each site total mass or volume. The minimum detectable activity was 5.4 dpm, and the samples with lower activity (volatilized Hg) were 25 times higher than this threshold.

3 Results

3.1 Physicochemical Parameters

Values of the physicochemical parameters monitored in the water column are presented in Fig. 2. The data collected revealed minor variations between the treatments over the exposure period for the three parameters analyzed. The more apparent variation is the pH increase of 6.02–6.98 in the macrophyte treatment.

3.2 Total Hg Distribution

Hg was found in all compartments analyzed on both treatments. In the incubation without macrophytes, the higher percentages of total added Hg were found in the settled and suspended particles. Conversely, in the incubation with macrophytes, the roots were the compartment with higher values of Hg, and all other sites showed considerably lower percentages (Fig. 3).

The partition coefficients for total Hg particles and roots to whole water (Log K_D and K_R in L Kg^{-1}) in the macrophyte treatment were 4.2 and 4.7 respectively while the particle-water coefficient in the treatment without macrophytes was 3.9. The bioconcentration factor (BCF) for total Hg calculated as [Hg]whole plant/[Hg]water, the source of

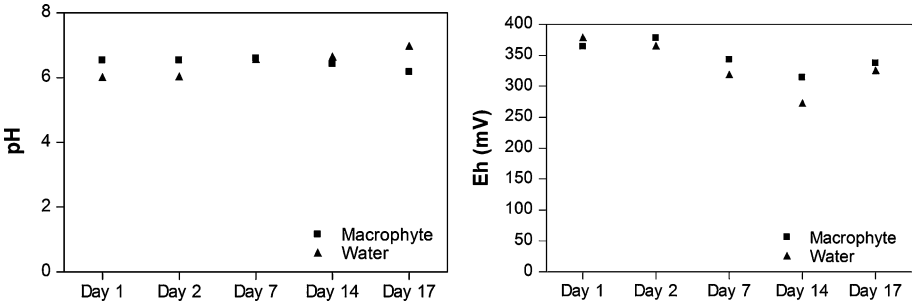

Fig. 2 Values of pH and Eh measured in the water column at each sampling day for the treatments with and without macrophytes

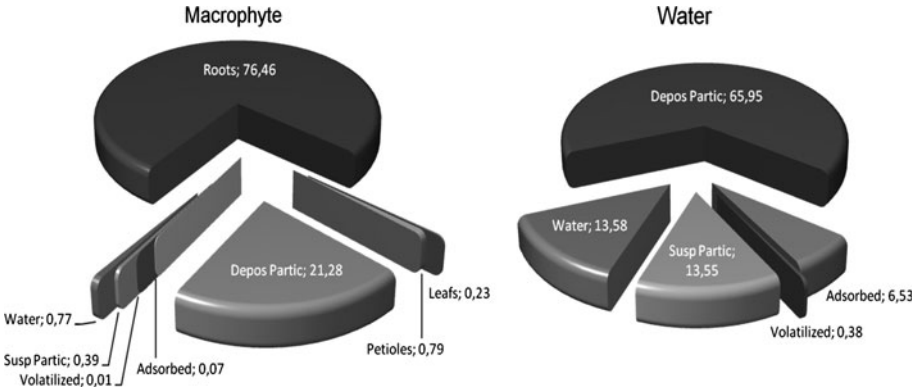

Fig. 3 THg distribution for all compartments in the last sampling day

contamination, was 194, 704 and 1,447 L kg^{-1} in the 1, 7 and 17°days, respectively. And the root: shoot transfer factor (TF) calculated as [Hg]sum of leafs and petioles/[Hg]roots, was 0.02, 0.11 and 0.02 kg kg^{-1} in the same days described above.

3.2.1 Hg Volatilization

Volatilized Hg showed low percentages throughout the experiment for both treatments. Total Hg volatilization represented 0.48 and 5.26 % of the total Hg spike in the treatments with and without macrophytes, respectively (Table 1 and Fig. 4).

Table 1 THg distribution throughout the treatments with and without the macrophyte

THg distribution	Day 1		Day 2		Day 7		Day 14		Day 17	
	Plant	Water	Plant	Water	Plant	Water	Plant	Water	Plant	Water
Volatilized	0.25	0.56	0.04	0.67	0.12	1.29	0.05	2.45	0.01	0.30
Water	4.56	15.14	2.05	10.15	1.02	10.22	1.12	12.25	0.88	10.88
Suspended particles	3.75	63.74	2.23	53.67	0.67	23.79	0.32	16.96	0.45	10.86
Settled particles	–	–	–	–	–	–	–	–	24.41	52.84
Roots	69.29	–	75.14	–	50.25	–	59	–	87.72	–
Leafs	0.47	–	0.23	–	0.92	–	6.1	–	0.26	–
Petioles	0.41	–	2.17	–	2.55	–	1.86	–	0.91	–
Total recovered	78.73	79.44	81.86	64.49	55.53	32.3	68.45	31.66	114.64	74.88

Values represent the percentage of the total added Hg found in each compartment at each sampling day

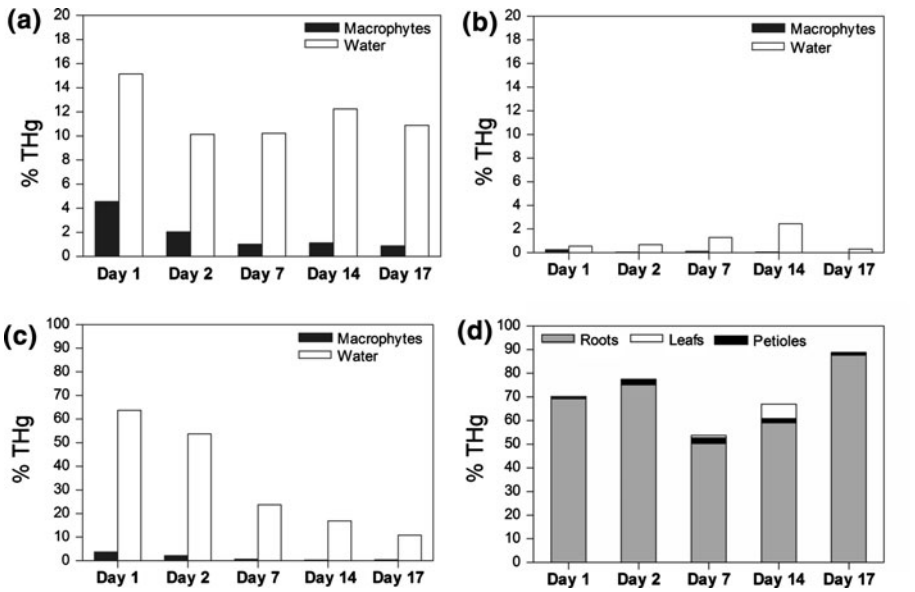

Fig. 4 THg distribution throughout the experiment. **a** Percentage of the THg found in the water. **b** Percentage of THg volatilized. **c** Percentage of THg found in the suspended particles. **d** Percentage of THg found in the roots, leafs and petioles of the macrophyte

3.2.2 Filtered Water

In both treatments, the percentage of added Hg found in filtered water was relatively stable and always below 20 %.

3.2.3 Suspended Particulate

In the treatment with water only, suspended particles retained up to 60 % of added Hg while in the treatment with macrophytes, this proportion was lower than 4 %, and in both treatments it decreased during the course of the incubation.

3.2.4 Leafs, Petioles and Roots

THg found in leafs presented values under 2 % at all days except for day 14 when it reached 6 %. The highest values of Hg found in petioles ($\sim$2.5 % of total added Hg) occurred in the middle of the incubation period. The roots of the macrophyte retained considerably higher amounts of Hg than the other analyzed compartments, reaching 80 % of added Hg, in the last sampling day.

3.2.5 Other Compartments

Small amounts of Hg were found adsorbed on the glass walls totaling 0.08 and 5 % in the treatment with and without macrophytes, respectively. No Hg was found on the silicone grease used to seal the desiccators.

3.2.6 Mass Balance

Mass balance calculations indicate that the total mercury budget was not recovered in all microcosms. After the first day of exposure, 79 % of the spike was recovered on both treatments, and after 17 days of incubation, 115 and 75 % were recovered for the treatments with and without macrophytes, respectively (Table 1).

4 Discussion

4.1 Volatilized Hg

As expected, Hg volatilization was apparently more intense in the water treatment (average of 1 %) when compared to the macrophyte treatment (average of 0.09 %). The same pattern was found in all preliminary experiments (data not shown). The most probable cause for this difference is that Hg complexed with organic and particulate matter becomes less available to volatilization (Lawson et al. 2001). During the incubation with macrophytes, Hg accumulation in the roots diminished its accessibility to Hg^{2+} reduction reactions and subsequent volatilization.

Hg^{2+} reduction can occur in water, particulate and roots through the activity of microorganisms such as algae and associated bacteria (Mason et al. 1995; Deng et al. 2009) and through abiotic reactions promoted by light (Devars et al. 2000; Zhang and Lindberg 2001) and humic substances (Allard and Arsenie 1991). Since only the total volatilization of the system was analyzed, it is not possible to evaluate the relative importance of each of

these reactions. There is also the possibility that Hg^0 was released by the leafs; however, studies have shown that the participation of leafs in the Hg volatilization process is small or absent in many cases (Weis and Weis 2004; Greger et al. 2005) and that the major part of the volatilization is derived from root-associated microorganisms in conditions where the abiotic reactions are minimized (Moreno et al. 2008). Therefore, it is likely that in the water treatment, photo-reduction, humic substances, planktonic and adhered microorganisms were the main causes of Hg volatilization. In the macrophyte incubation however, considering that the presence of roots and associated periphyton increases significantly the amount of microorganisms and diminishes the incidence of light, the results suggest that, in this case, Hg^{2+} reduction is mediated mainly by microorganisms.

4.2 Water

Hg percentages found in this compartment correspond to dissolved (<0.22 μm) Hg. The dissolved fraction in water includes dissolved organic matter (DOM), comprised of a heterogeneous mixture of organic compounds such as humic substances (Ravichandran 2004). Humic substances and DOM have been reported as important complexing agents for Hg and methylmercury and affect their mobility and bioavailability in aquatic systems (Mierle and Ingram 1991; Driscoll et al. 1995; Cai 1999; Benoit 2001; Tipping 2007; Brigham et al. 2009). DOM has great affinity for Hg^{2+} in the pH range observed during our experiments (Lu and Jaffe 2001), and Hg found in the filtered water was very likely associated with DOM.

4.3 Suspended Particles

Hg has a high tendency to be adsorbed on surfaces. For this reason, in natural systems, Hg is mostly found adhered to the sediment, periphyton and suspended particles (Ullrich et al. 2001). The latter is characterized by mineral particulates, detrital organic matter and organisms in suspension, which are retained in a 0.22-μm pore size filter. Hg adsorption in this fraction became clear in the water treatment, where 60 % of added Hg was found adhered to the suspended particles.

Both treatments showed a gradual decline of the Hg percentages in the suspended particles and the formation of a settled particulate layer at the bottom of the desiccators. This settled particles held approximately 52 and 24 % of all Hg added to water and macrophyte treatments respectively in the last sampling day. The particles settling in both treatments once again points to its importance as a vertical Hg transport mechanism in aquatic systems. The sedimentation of the particulate matter is considered as the larger Hg input mechanism to the water–sediment interface, which in turn is one of the most important Hg methylation sites (Hurley et al. 1991, 1998).

4.4 Roots

As previously reported, the macrophyte roots accumulated high concentrations of THg throughout the experiment. The capacity of *E. crassipes* roots to accumulate Hg, as shown by the partition coefficient, and other contaminants is well known in the literature (Wolverton and McDonald 1979; Lenka et al. 1990; Riddle et al. 2002; Skinner et al. 2007; Rai 2009). Since periphyton was not detached from the roots prior to the analysis, we cannot estimate how much of the detected Hg was associated specifically with the roots

and their associated periphyton. Regarding the roots, some studies have shown that, in many plants, the larger amount of contaminants including Hg was bonded to cell structures such as cellular walls and membranes, working as a protection barrier and inhibiting high metal concentrations in the cytoplasm (MacFarlane and Burchett 2000; Castro et al. 2009). However, a study with *E. crassipes* showed that some metals such as copper, zinc and lead held higher concentrations inside the cells as compared to their external structures (Vesk et al. 1999). The behavior of Hg in roots of *E. crassipes* has not been studied yet, and its exact location in the root cells is not known.

Aside its capacity to accumulate metals through adsorption to its organic and inorganic material and through absorption by organisms (Lakatos et al. 1999; Desrosiers et al. 2006; Guimarães et al. 2006), the periphyton can also stimulate the accumulation of metals by roots. de Souza et al. (1999) report that in the absence of adhered bacteria (inhibition with ampicillin), the roots of *Scirpus robustus* and *Polypogon monspeliensis* accumulated less Hg than in treatments without any inhibition.

4.5 Leafs and Petioles

The detection of Hg in leafs may result from Hg translocation from roots (Riddle et al. 2002), Hg capture from the surrounding air via stomata and surface deposition followed by incorporation (Stamenkovic and Gustin 2009).

The experimental arrangement used does not allow us to separate Hg levels found in leafs into these three incorporation pathways. However, in the present study Hg levels found in leafs were generally low (<2 %) when compared to root Hg concentrations, suggesting that the analyzed macrophyte has mechanisms that block the ascension of Hg to leafs and petioles (Weis and Weis 2004). Riddle et al. (2002) found similar results for the same macrophyte where the levels of Hg on roots were two orders of magnitude higher than in leafs.

The observed 6 % peak in the 14° sampling day can be simply a statistical error or can possibly be due to a tolerance mechanism where the excess of Hg is translocated to senescent leafs (Weis and Weis 2004) since from this sampling day on, we encountered and analyzed senescent leafs along with younger ones.

Due to the reduced size of the desiccators and to the need to keep plants alive throughout the incubation time, it was not possible to extract more than one leaf and petiole at each sampling day. Weis et al. (2003) observed a variance of metal levels in leafs of plants from the same species exposed to the same metal concentrations, suggesting that individual leafs may not be representative of the plant as a whole.

No literature was found considering Hg concentrations on macrophyte petioles; however, it is possible that all the considerations pertaining macrophyte leafs are valid for petioles considering they are linked and constitute the aerial part of the macrophyte, with the exception of Hg uptake via stomata.

4.6 Distribution Factors

K_D values represent the equilibrium distribution of a given element, in this case Hg, between the particulate and dissolved phase. K_D values calculated for both treatments are high indicating a high affinity for the particulate phase. BCFs are ratios of concentrations of a contaminant in the media and dissolved in water. Plant BCF can be calculated to report the concentration of elements, such as Hg, in the whole plant relative to the surrounding water. When BCF values are greater than 1, there is an indication of Hg bioconcentration.

In this report, all BCF values were greater than 1 and also showed an increase in bioconcentration levels through time. TF values are calculated to compare the concentrations of elements in roots versus the aerial parts of the plant. When TF values are greater than 1, there is an indication of significant cross-membrane transport of elements from roots to petioles and leafs. In our case, TF values were always smaller than 1, indicating there was no translocation of Hg in this macrophyte species. Our results are in agreement with the results of other macrophyte species (Sundberg-Jones and Hassan 2007).

5 Conclusion

The size of the microcosms allowed a restricted number of samples to be taken at each day. A shorter incubation period would allow a more intensive sampling but with less representative results. However, the use of a Hg radiotracer allowed to study the distribution of Hg among all the compartments of the system, in conditions that are close to the natural ones, and without the analytical uncertainties involved in experiments with stable Hg, where it would be difficult to distinguish added from preexisting Hg, especially in the dissolved fraction.

The results of these experiments suggest that the studied macrophyte promotes changes in the Hg cycle since it attracts most of the Hg to its roots/periphyton and settled particles underneath reducing by the same token the availability of Hg for volatilization.

Considering the efficient retention of Hg in the roots of this macrophyte, some of the anthropic uses of *E. crassipes* can be detrimental to human and environmental health, such as its use as food for cattle and humans and as a fertilizer in plantations in which cases Hg would be directly ingested or run off to water bodies nearby. Hg degassing from *Eichhornia* biomass when the latter is used for biogas production also deserves further investigation.

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