Bioconcentration and translocation of Cd in water lily (*Eichhornia crassipes*) at different initial concentrations and pH values

Bioconcentración y traslocation de Cd en lirio acuático (*Eichhornia crassipes*) a diferentes valores iniciales de concentración y pH

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ABSTRACT
Eichhornia crassipes has a high tolerance to pollution, making it eligible for use in wastewater phytoremediation. This paper reports a laboratory study conducted under environmental conditions of the city of Toluca (Mexico), of the plant's suitability for cadmium accumulation, bioconcentration and translocation as a function of initial Cd concentration (1, 5, 10 and 130 mg Cd/L) and pH (pH_o= 3, 5, 7). Water lily showed high tolerance to high Cd concentrations. The plant neutralized the medium in less than 15 minutes (1 and 5 mg Cd/L), even when starting from pH_o 3. Water lily can be considered a Cd hyperaccumulator (from 1 to 10 mg Cd/L) in the root and the whole plant. Translocation factors (TF) were low in all experiments (< 1) and pH_o did not have significant effects on translocation.

Keywords: Water lily (*Eichhornia crassipes*), cadmium (Cd), bioconcentration, translocation, phytoremediation

RESUMEN
Eichhornia crassipes posee una alta tolerancia a la contaminación, convirtiéndola en una planta elegible para ser usada en fitorremediación de aguas residuales. Este artículo muestra los resultados de experimentos de laboratorio bajo condiciones ambientales de la ciudad de Toluca (México), en los que se muestra la capacidad de la planta para acumular Cadmio, bioconcentrarlo y traslocarlo, como función de la concentración inicial de metal (1, 5, 10, 130 mg Cd/L) and el valor de pH inicial (pH_o= 3, 5, 7). El lirio acuático mostró alta tolerancia a las altas concentraciones de metal. La planta neutralizó el medio en menos de 15 minutos (en los experimentos con concentraciones iniciales de 1 y 5 mg Cd/L), incluso comenzando desde un pH inicial de 3. El lirio acuático puede considerarse como hiperacumulador de Cd a concentraciones inferiores a 10 mg Cd/L, tanto en raíces como en planta completa. El Factor de traslocación (TF, por sus siglas en inglés) fue bajo, <1, en todos los experimentos. El pH inicial no tuvo un efecto significativo sobre la traslocación del metal.

Palabras clave: Lirio acuático (*Eichhornia crassipes*), cadmio (Cd), bioconcentración, translocación, fitorremediación

1 INTRODUCTION

Heavy metals are considered to be very dangerous to living beings in general, as they have high toxicity, partly due to their tendency to readily bioaccumulate. Bioaccumulation is the increase in the concentration of a chemical in a biological organism over a period of time, to the point where it becomes higher than the concentration of the chemical in the environment. Toxicity is often caused by the inability of the affected organism to maintain the necessary levels of excretion. The process is aggravated during the passage of the chemical through the food chain, because levels of incorporation increase sharply throughout successive levels. This process is called biomagnification. Contamination of water by heavy metals is mainly due to anthropogenic activity (Ali et al., 2013).

Jiménez-Moleón et al. (2012) reported the presence of various heavy metals in a water body in the Toluca Valley, State of Mexico, Mexico, including Cu, Cd, Pb, Ni, Mn, Zn and Fe. Cadmium (Cd) in particular is of environmental interest as it is a relatively common contaminant because its mobility is higher than that of most heavy metals (Moreno Grau, 2003; van Loon and Duffy, 2011) due to the relative solubility of its salts and hydroxides, especially in acidic media (this last characteristic is common to most metals). Cadmium has carcinogenic, mutagenic, and teratogenic effects, and causes renal failure and chronic anemia in humans (Ali et al., 2013). Hence, the interest in seeking solutions for removing Cd from water. Cadmium concentrations have been reported in Mexico at the Ignacio Ramírez dam (0.027 mg/L), State of Mexico (Contreras-Ponce, 2010), and in deep wells in the Comarca Lagunera region in the states of Durango and Coahuila (0.038–0.042 mg/L) according to Azpilcueta-Pérez et al. (2017). In both cases, Cd values were above the permissible Cd limit for water for human use and consumption (SS 2000).

Conventional treatment systems that remove heavy metals from water, such as chemical precipitation, adsorption on activated carbon, ion exchange, and reverse osmosis, have two main limitations: their high cost and the sludge they produce, which requires treatment and final disposal (Priya and Selvan, 2014; Jiménez-Moleón et al., 2015). Green chemistry, including phytoremediation (removal of contaminants from natural or environmental matrices in water and soil by plants), is currently becoming increasingly important.

The mechanisms that plants use to remove metal contaminants in the aqueous phase are mainly phytoextraction, rhizofiltration and phytostabilization. The water lily (*Eichhornia crassipes*) is well suited for use in wastewater treatment ponds for its high rate of reproduction and high tolerance to pollution (Rezania et al., 2015; Priya and Selvan, 2014; Jiménez-Moleón et al., 2015). Water lily grows in a wide variety of environmental wetlands and prefers nutrient-enriched waters. However, it can tolerate considerable variation in nutrient levels, temperature and pH. Water lily can grow over a wide temperature range: 10–40 °C (optimal growth 25–27.5 °C) (Rezania et al., 2015; Malik, 2007; Wilson et al., 2005) although it is sensitive to cold (Rezania et al., 2015).

Bioconcentration and translocation factors are used to assess the absorption capacity of the water lily. The bioconcentration factor (BCF) measures a plant's efficiency in accumulating a metal in its tissues relative to the environment. The translocation factor (TF) measures the plant's efficiency in transporting the accumulated metal from its roots to its aerial parts (Liao and Chang, 2004; Wu and Sun, 1998; Ali et al., 2013).

The objective of this study was to determine the effect of initial concentrations of Cd and initial pH (pH_o) on the accumulation of the metal in water lily by calculating TF and BCF.

2 MATERIAL AND METHODS

2.1 WATER LILY COLLECTION

E. crassipes was sampled in three different water bodies invaded by water lily in the Toluca Valley (Fig. 1): Bordo de San Martín La Puerta (19º 23' 10.28'' N, l99º 43' 05'' W), Iztapantongo Dam (19º 10' 29'' N, 100º 17 35" W) and San Miguel Almaya Lagoon (19º 13' 00'' N, 99° 26' 11.94'' W). The sampling site selected for collecting the plants for the study was the site with the lowest concentration of Cd (Table 1), Bordo de San Martín La Puerta.

2.2 *E. crassipes* SELECTION AND PRETREATMENT

Healthy-looking plants were selected, with uniform color and waxy green leaves with no chlorosis or necrosis. Individuals of similar size (number of leaves and root volume) were collected. The plants were washed with water to remove the material attached to the roots. Subsequently, the plants were put in a neutral nutrient solution for three days: 900 mL of deionized water, 0.85 g/L $Ca(NO_3)_2{\cdot}4H_2O$, 2.00 g/L KH_2PO_4 and 3.69 g/L $MgSO_4{\cdot}7H_2O$ (reactive grade, Merck) as recommended by the Boyce Thompson Institute (2007). This solution was changed daily (Jiménez-Moleón et al., 2010).

2.3 CONTACT EXPERIMENTS

The water lily plants were placed in deionized water whose the initial concentration and pH conditions had been adjusted to the desired values. The initial concentrations of cadmium studied were 1, 5, 10 and 130 mg/L and each concentration was tested under three different initial pH values (3, 5 and 7), all of them in the acid-neutral range. Cd solubility in water increases in acidic media, while adsorption in soils and sediments is higher in basic media (VanLoon and Duffy, 2011). To adjust Cd concentrations, reactive grade $CdSO_4{\cdot}8H_2O$ (Merck) was used. The pH was adjusted with 0.1 N NaOH or 0.1 N HCl (Merck) as needed. The experiments were performed in duplicate and, in addition, in each of them, two types of controls were used: one under the same conditions as the test but without metal (the plant control, to observe possible acid damage to the plant due to pH levels), and another with metal, but without any plants (to measure any possible metal loss for reasons other than absorption).

The duration of the tests varied for the different initial concentrations, depending on whether the plant was damaged or whether the concentration of Cd in the water was detectable or not, or remained unchanged. pH was monitored throughout the contact time (Hanna pHmeter model HI991300). At the end of each test, the plant was sectioned into roots, petioles and leaves. The biomass was dried in porcelain capsules in an oven at 100 °C for 15 h (sufficient for obtaining dry weight). The dry biomass was then ground with a blender (Protect Silex) and digested in a microwave oven (MARS 5), using a 0.2500±0.0005 g sample and 10 mL of ultrapure concentrated nitric acid (Ultrex II, J. T. Baker), under the operating conditions and procedure shown in Table 2. Digestion was performed with temperature or pressure ramp depending on the available sensor. The digested samples were allowed to cool, filtered with number 41 Whatman paper and were increased in volume up to 50 mL with 0.5 N HNO_3 (J.T. Baker). Cadmium concentration was analyzed by the standard method (APHA, 2012) using an atomic absorption spectrophotometer (Spectra 600, Varian).

Translocation factor (TF) is the ratio between the concentration of cadmium in the aerial parts and the roots (Eq. 1). TF must be greater than 1 for the plant to be considered an effective phytoremediator (Agunbiade et al., 2009; Carrión et al., 2012).

$$TF = \left[\frac{As}{Ar}\right] \quad (1)$$

where As is Cd accumulated in the aerial parts (mg Cd/kg DW) and Ar is Cd accumulated in the roots (mg Cd/kg DW).

The bioconcentration factor (BCF) measures the degree of enrichment of metal within the plant and was calculated by the ratio between metal concentration in the plant and the remaining concentration of the metal in the liquid phase at the end of the contact time (Eq. 2). The BCF must be greater than 1000 to consider a plant a hyperaccumulator (Zayed et al., 1998; Zhu et al., 1999; Liao and Chang, 2004; Ajayi and Ogunbayo, 2012). The BCF was determined in the different sections of the plant as well as in the whole plant.

$$BCF= \frac{A}{B} \quad (2)$$

where A is the cadmium concentration in the tissue (mg Cd/kg) and B is the cadmium concentration in the solution at the end of the test (mg Cd/L).

3 RESULTS AND DISCUSSION

The controls with metal and without water lily showed that Cd losses were less than 5%, indicating that the variations in concentration due to causes other than absorption were not statistically significant. In the plant control without metal, although Smolyakov (2012) reported slightly less growth of water lily at pH 6 than pH 8, no visible damage was observed for any of the tests involving contact times less than 75 hours.

The evolution of pH during the tests and in the respective control solutions is shown in Figure 2. The controls with the plants (Fig. 2e) were faster at neutralizing the medium than the corresponding Cd tests. They reached neutrality in less than one hour (varying between 15 and 45 min, for pH_o 5 and 3, respectively). As Cordes et al. (2000) reported, the initial concentration of metal influenced the ability of *E. crassipes* to neutralize the solution. While the solutions with 1 and 5 mg Cd/L were neutralized in 15 min (Fig. 2a and 2b), the 10 mg Cd/L tests required 90 and 30 min. starting from pH_o 3 and 5 respectively, while the 130 mg Cd/L tests with pH_o 3 and 5 never reached neutrality and stabilized at pH 6 after 120 min and 45 min respectively (Fig. 2d). At initial concentrations of 130 mg/L, even in the pH_o 7 test, pH decreased to 6.21 in 15 min. The inability of the water lily to neutralize the medium at Cd concentrations equal to or greater than 10 mg/L has been reported in literature for other metals, such as Mn and Ni (Jiménez-Moleón et al., 2010; Soltan and Rashed, 2003; Hussain et al., 2010). Soltan and Rashed (2003) suggested that the pH decrease in mediums with higher metal concentrations might be due to ion exchange and/or proton secretion from the water lily during metal accumulation.

Figure 3 shows the Cd concentrations within the sections and in the whole plants at the end of the contact time. It was observed that the highest concentration of Cd was found in the root (> 88 %). In addition, this study showed a clear relationship with pH; the most metal was accumulated in the root and in the entire plant at pH_o 5. This may be because although Cd is more soluble in a strong acid solution, its absorption was favored at a less extreme pH ($pH_o > 3$), where there are fewer hydrogen ions competing with the metal at root binding sites.

The concentration of Cd in the root and whole plant was higher at greater initial concentrations of the metal (Fig. 3). The same trend was observed by Aurangzeb et al. (2014), Carrión et al. (2012) and Hasan et al. (2007) (Table 3). At the highest concentration of Cd (130 mg/L), *E. crassipes* began to have chlorosis and dry leaves up to 4800 min. at pH_o 5, and at 8640 min. at pHo 7. In contrast, at pH_o 3 no visible damage was observed, although there could have been damage at the cellular level. Soltan and Rashed (2003), using a lower Cd concentration (100 mg Cd/L), reported damage to water lilies after 2880 min. of contact at pH 6. The bioconcentration at the root found in this study was 8 to 10 times greater (between 15558 and 21004 mg Cd/kg) than that reported by Soltan and Rashed (2003) at 100 mg Cd/L (2060 mg/kg). The differences between Cd accumulations in different studies may have been due to differential presence of metal-tolerant bacteria in the rhizosphere, since the presence of such bacteria promotes the accumulation of metals around the water lily root due to the secretion of organic acids (Abou-Shanab et al., 2007; Prabha et al., 2015).

Cd concentrations were low in the aerial sections of the plant (Fig. 3) independently of pH or initial concentration. The highest concentration in the petiole (257 mg Cd/kg) occurred with 10 mg Cd/L and pH_o 3 and the highest concentration in the leaf (36 mg Cd/kg) was found at pH_o 5 and 130 mg Cd/L. Hasan et al. (2007) reported higher values than the present study, between 93 and 98% in aerial parts; while Aurangzeb et al. (2014) and Carrión et al. (2012) found values less than 99 and 95%, respectively, compared to the present paper.

Comparing the results of the present study to the literature, a high variability in Cd concentration in the plant can be seen. However, the references were in agreement that the section of the plant that accumulates the most metal is the root and that it can contain from two to five times (Carrión et al., 2012); one to ten times (Chunkao et al., 2012) or ten to twenty times (Zhu et al., 1999) more Cd than the aerial parts. In this study, the root accumulated 8 to 295 times more Cd in the roots than in the aerial parts (Fig. 3), so water lily is well suited for rizofiltration.

On the other hand, the TF did not show a clear trend by pH_o or the initial concentration of metal in the solution (Table 3); moreover, it can be observed that contact time did not influence TF values either. The highest TF value was obtained at 10 mg Cd/L, pH_o 3, followed by the test at 1 mg Cd/L Low translocation in plants has been explained by the immobilization of metals in vacuoles, by the formation of chelates (Carrión et al., 2012; Ali et al., 2013), or by the existence of transport mechanisms in the plant that prevent the passage of heavy metals (Williams et al., 2000).

BCF was determined for the different sections and for the whole plant (Fig. 4). When the initial concentration was < 10 mg Cd/L, independently of starting pH, the BCF was above 1000, the minimum accepted for a plant to be considered a hyperaccumulator (Zayed et al., 1998; Zhu et al., 1999; Liao and Chang, 2004; Ajayi and Ogunbayo, 2012), both in the root (where the metal was preferably absorbed) and in the whole plant, except at 5 mg Cd/L and pH_o 7 (whole plant). With an initial concentration of 130 mg Cd/L, this condition was met in the whole plant and root only at pH_o 7, while at pH_o 5, it was only met in the root. Thus *E. crassipes* proved to be a hyperaccumulator (mainly in the root) at extreme initial pH, in a range of 1–10 mg Cd/L in the initial solution, and at high concentrations (150 mg Cd/L) starting from neutrality.

The root BCF values obtained at 1 mg Cd/L were similar to those reported by Zhu et al. (1999) with the same initial concentration. The values of root BCF at this concentration increased with pH_o, a trend reported by Smolyakov (2012). The BCF values in aerial sections were less than those reported by these authors, which was expected, due to the low accumulation in leaf and petiole noted above.

A correlation matrix was calculated, which enabled the interdependence between variables to be measured (Table 4).

The criterion used to establish the relationship between parameters is shown in Table 5.

A very strong statistical correlation was found between initial concentration and accumulation. In addition, it is important to note that in both results and correlations, pH and plant size had no influence on accumulation or BCF. The rest of the correlations among the parameters under study were statistically weak or negligible.

4 CONCLUSIONS

Cd concentration inversely affected the neutralization capacity of water lily. At 130 mg/L, the plant was unable to neutralize. There was no visible damage after 3.5 days, even at pH 3. The roots were the main part of the plant accumulating Cd regardless of pH or concentration. Correlation between the initial metal concentration and full-plant Cd accumulation was virtually perfect. A weak pH-BCF correlation was observed. There was no influence of pH or plant size on Cd accumulation or BCF. Water lily was shown to be a Cd hyperaccumulator between 1 and 10 mg Cd/L, and accumulation was based on neutrality at 130 mg/L. The low TF values showed that *E. crassipes* qualifies as a rizofiltration plant.

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Figure 1. Water lily sampling sites in the State of Mexico

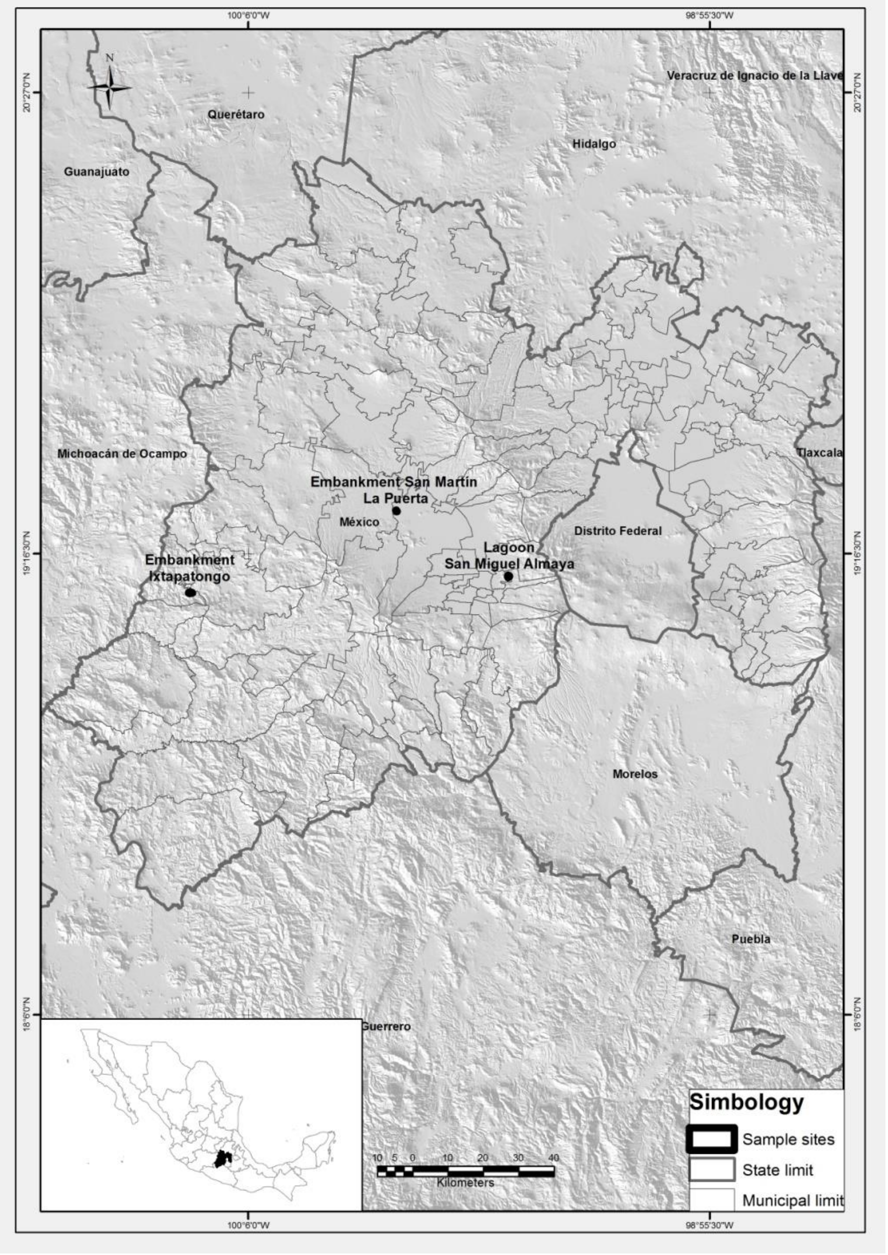

Figure 2. Evolution of pH in tests and metal-free control plant solutions

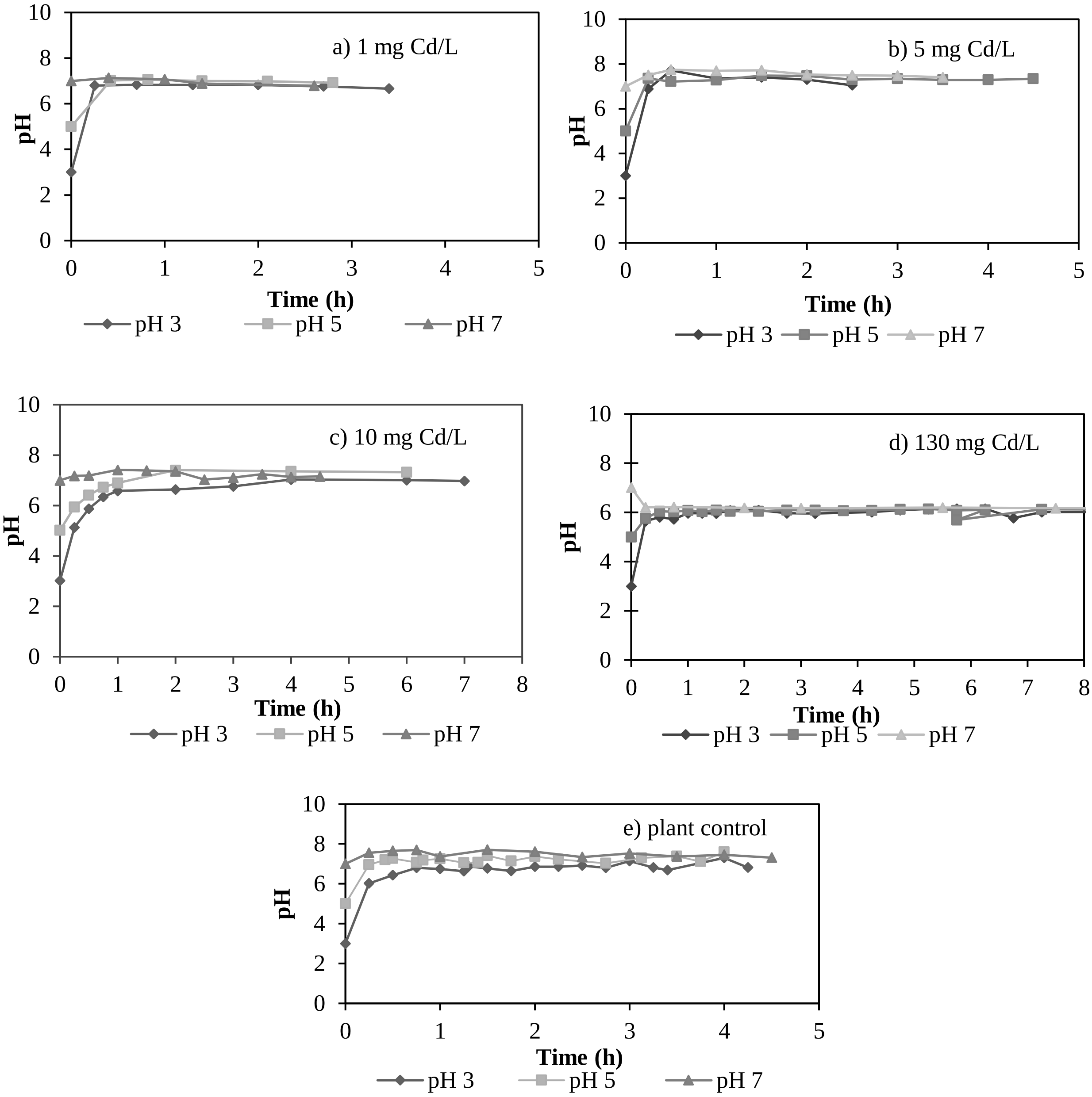

Figure 3. Cd Concentration in water lily (mg Cd/kg dw): a) pH_o= 3, b) pH_o= 5 y c) pH_o= 7

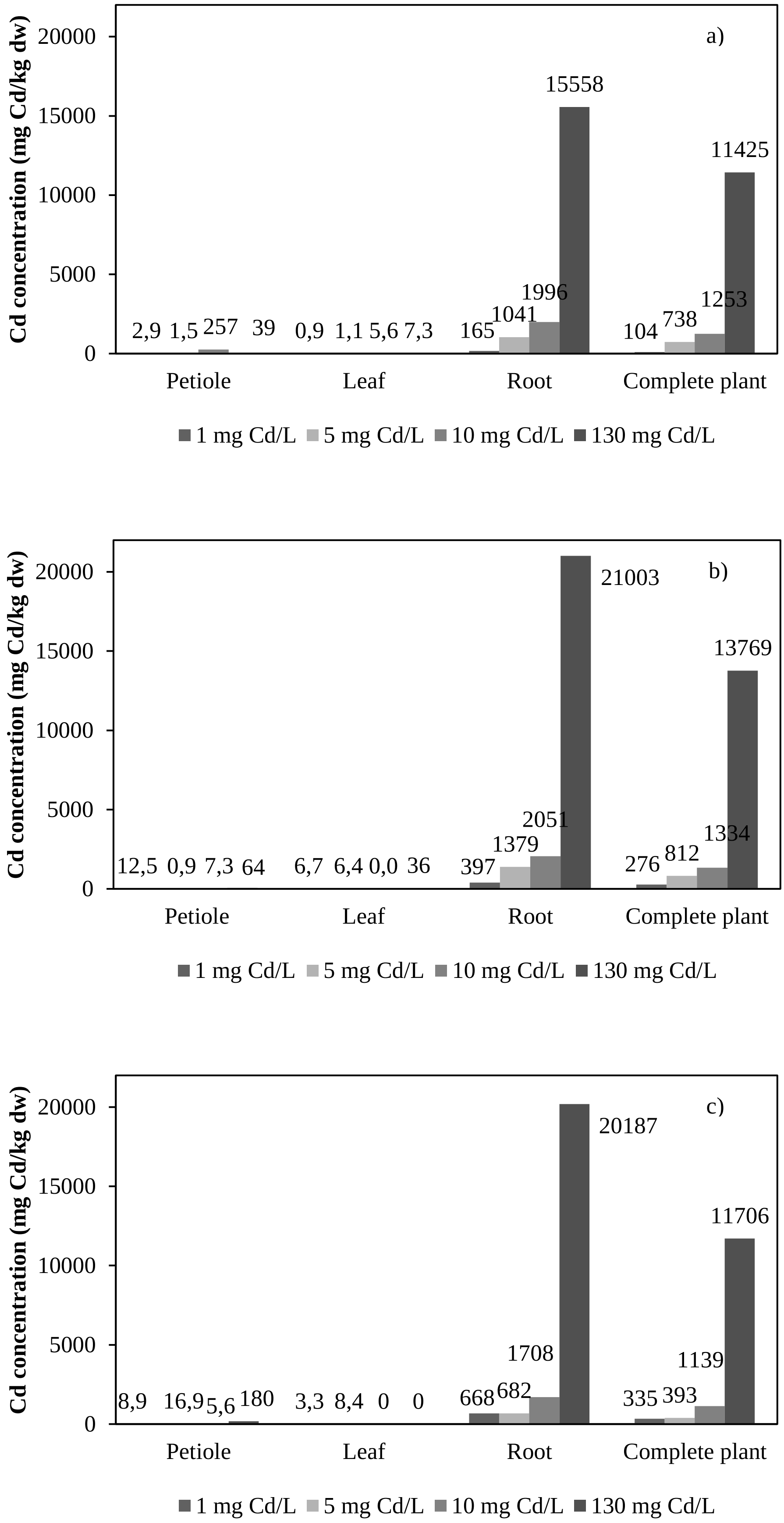

Figure 4. Bioconcentration factor: a) whole plant, b) root, c) leaf and d) petiole

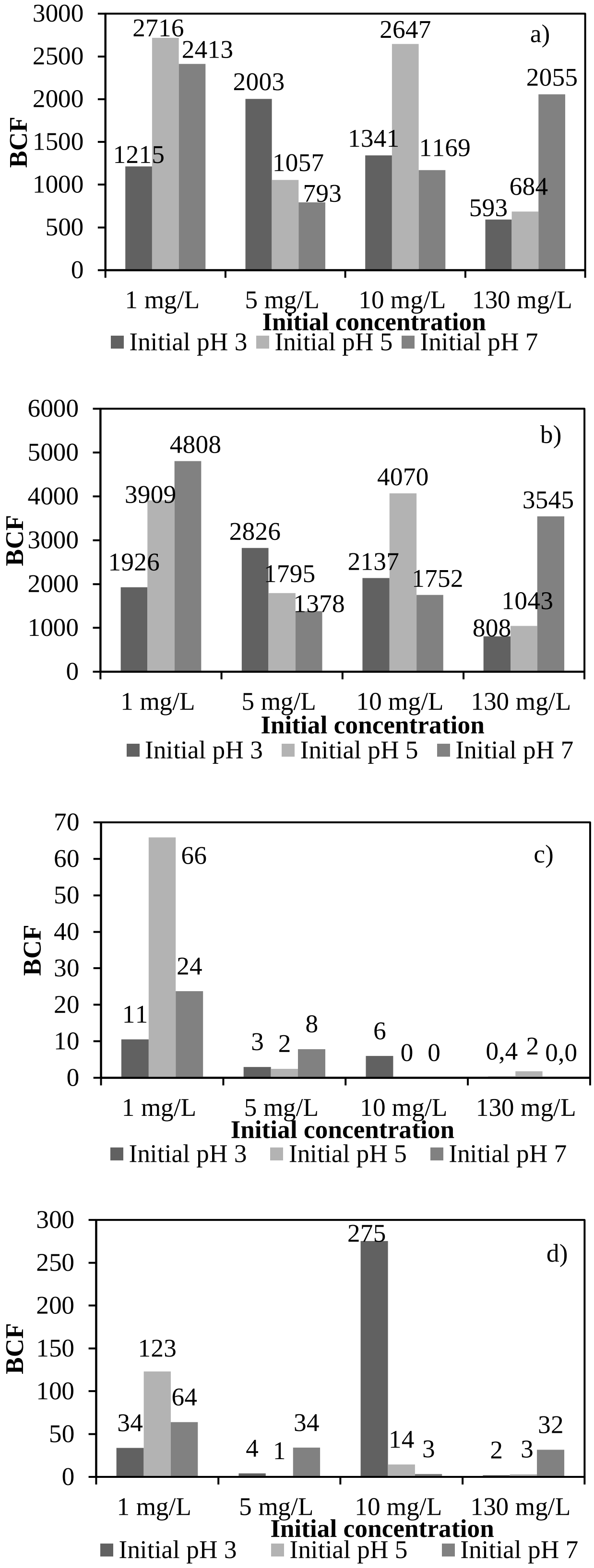

Table 1. Cd concentration (mg/kg) in water lily in the sampling areas

Plant o section	Cd concentration (mg Cd/kg dw)		
	Bordo San Martín La Puerta	**Iztapantongo Dam**	**San Miguel Almaya Lagoon**
Whole plant	1.21 ± 0.17	5.87 ± 1.10	32.48 ± 0.67
Root	0.40 ± 0.00	4.84 ± 1.6	53.77 ± 1.33
Petiole	2.30 ± 0.71	9.31 ± 0.30	3.04 ± 4.29
Leaf	4.49 ± 2.1	5.41 ± 1.46	4.45 ± 4.55

dw: dry weight.

Table 2. Mars 5 microwave operating conditions

a) Temperature (Jiménez-Moleón et al., 2010)

Stage	Power (%)	Ramp (min)	Temperature (ºC)	Retention time (min)
1	100	10:00	200	05:00
Time until temperature drops to 50 ºC. Approximate time: 30 min.				
2	100	10:00	200	05:00

b) Pressure

Stage	Power (%)	Ramp (min)	Pressure (psi)	Retention time (min)
1	100	10:00	150	05:00
Time until pressure drops to < 60 psi. Approximate time: 60 min.				
2	100	10:00	150	05:00

Table 3. Translocation factors in plants

Concentration	Translocation (TA)					
	pH 3		**pH 5**		**pH 7**	
	TF	**Time (min)**	**TF**	**Time (min)**	**TF**	**Time (min)**
1 mg Cd/L	0.023	206	0.048	166	0.018	135
5 mg Cd/L	0.002	150	0.002	270	0.030	210
10 mg Cd/L	0.132	420	0.004	360	0.002	270
130 mg Cd/L	0.003	1515	0.005	1515	0.009	8670

Table 4. Matrix of correlations between accumulation, BCF, TF, and full plant (dry weight)

	Initial concentration	**Accumulation (mg Cd/kg)**	**BCF**	**TF**	**Dry weight of plant**	**pH**
Initial concentration	1					
Accumulation (mg Cd/kg)	0.99	1				
BCF	−0.36	−0.36	1			
TF	−0.28	−0.27	0.04	1		
Dry weight of plant	0.19	0.15	0.03	0.28	1	
pH	0.00	0.00	0.18	−0.29	−0.06	1

Table 5. Correlation matrix interpretation scale

Values	r
Less than (+ or −) 0.2	Positive or negative insignificant
Between (+ or −) 0.2 and (+ or −) 0.4	Positive or negative weak
Between (+ or −) 0.4 and (+ or −) 0.6	Positive or negative mean
Between (+ or −) 0.6 and (+ or −) 0.8	Positive or negative strong
Between (+ or −) 0.8 and (+ or −) 1.0	Positive or negative very strong