BIOREMEDIATION OF WASTEWATER ENRICHED WITH Cd, Cr AND Pb USING FLOATING MACROPHYTES.

Abstract

This study aimed to explore the effectiveness potential of *Eichhornia crassipes*, *Pistia stratiotes* and *Salvinia molesta* for the reduction of potentially toxic metalsfrom effluents enriched with Cd, Cr and Pb. Based on a21-day laboratory-scale experiment, a total of three concentrationsat 1,5 and 10 mg/L were selected to culture each of the macrophytes. In addition, kinetics and prediction models were used to analyze the metal reduction data to understand the behavior and removal rate of each species and with the Taguchi methodology to see what the right conditions for greater efficiency are. The results suggested that the three macrophytes was able to reduce metals *Eichhornia crassipes* manages to reduce 90.8-99.99% Cd, 90.8-99.9% Cr and 89.6-99.9% Pb, for *Pistia stratiotes* 74.6-99.9% Cd, 74.6-99.9% Cr and 91.2-99.9% Pb and *Salvinia molesta* 83.2-99.9% Cd, 62.2-99.9% Cr and 89.2-99% Pb. The first-order model it is adjusted appropriately to the removal of toxic metals for the three plants, having an adjustment of R^2 greater than 0.80. Taguchi analysis shows that optimal conditions for metal phytoremediation occur with 1 mg/L of toxic metals and after 21 days.

Keywords:macrophytes, wastewater, potentially toxic metals, first-order model

Introduction

One of the problems affecting developing countries is that more than 90% of domestic wastewater discharges directly into rivers and lakes (Zhou et al., 2020), causing serious ecological problems, such as deterioration of water quality and loss of biodiversity (Caspersen and Ganrot, 2018). The highest concentrations of toxic metals in river and lake water are found in Africa, Asia, and South America. Previous research shows that the toxic metals most prevalent in surface waters are Cd, Cr, and Pb (Newman, 2014;Yang et al., 2017; Gholipour et al., 2020;Wang et al., 2021; Chen et al., 2022; Mondal, 2023).

Methods commonly used to treat water contaminated with toxic metals areoften inefficient and expensive. There is little research focused on bioremediation for reducing the burden of organic and inorganic pollution (Gholipour et al. 2020). Therefore, it is necessary to develop alternative and sustainable methods for effluent treatment. Artificial Floating Islandsemerge as a method of bioremediation, it is a low-cost, sustainable, and efficient technologyfor the treatment of contaminated water (Lu et al., 2018; Singh, 2022). They have stood out for their easy operation, economic profitability, and improvement of regional landscapes (Ijaz et al., 2016a, Rehman et al., 2018, Wu et al., 2018).The use of floating macrophytes in IPA has been shown to be effective in absorbing toxic metals (Kumar et al., 2020; Wu et al., 2020; Zhang et al., 2020; Cheng et al., 2002; Kröpfelová et., 2009; Vymaza et al., 2016).Trends in the use ofArtificial Floating Islands have not adequately exploited the use of efficient species in the accumulation of toxic metals (Liu et al., 2017; Wang 2020a; Wang 2021).

Eichhornia crassipes, Pistia stratiotes L and *Salvinia molesta* are hyperaccumulating plant species that have a distinct ability to absorb various heavy metals from contaminated sites (Ibezim-Ezeani et al., 2020; Kumbar et al., 2018;Abhayawardhana et al., 2020;Kodituwakku, et al., 2020). They growin bodies of water such as lakes, rivers, dominated by a high content of nitrogen, phosphorus, minerals, and metals. Prediction and kinetic modeling studies are widely used to improve process performance (Kumar et al. 2019a, b, c). They are useful for understanding the steps of controlling the removal rate. In this way, the contaminant absorption behavior can be easily understood, and the wastewater treatment process can be designed to maximize performance (Eid et al. 2019a, b).

In addition to this, prediction models are also useful for the quantification of influencing, i.e.,intrinsic, and extrinsic factors, and for further improving the treatment process of polluted water (Eid et al. 2019a; Kumar et al. 2019a, b, c; Kumar et al., 2020). It is for all the above that the objective is to quantify the potential of *Eichhornia crassipes, Pistia stratiotes L* and *Salvinia molesta* for bioremediation of Cadmium, Chromium and Lead in different concentrations using waters enriched with these metals.

MATERIALS AND METHODS

Plant selection and adaptation

Eichhornia crassipes, Pistia stratiotes L, and Salvinia molesta were collected from a nearby freshwater body, located in Ixtaczoquitlán, Veracruz, Mexico (N18º 51' 2.524", W96º 59' 48.1"). Young and healthy plants were selected for the experiment and rinsed with tap water to remove particles, transported, and placed in culture cells to adapt in the new environment for seven days. The systems were arranged in a sheltered area, allowing adequate exposure to air and sunlight.

Experimental setup

The experiments were carried out under a natural day-night regime, that is, 12 h of light and 12 h of darkness. The experiments were performed in batch mode, using glass containers of 25 L capacity. Synthetic waters with different concentrations were formulated, having nine treatments varying the concentrations is 1, 5 and 10 mg/L for each metal (Table 1), the concentrations were established based on the most critical concentrations of toxic metals in wastewater (Ayaz et al., 2020; Singh et al., 22022; Bijuet al. ,2023).

The matrix of the experimental design (Table 1) corresponds to an orthogonal arrangement design of type $L_9(3)^4$, being four variables or factors at three levels, following the methodology of Taguchi (Loloide et al., 2016; Canales and Prieto, 2020).

The experiments were carried out in periods of 21 days, glass cells with a useful volume of 20 liters were used. The control parameters were pH, electrical conductivity, total dissolved solids (Hanna HI 98130, IN), which were monitored on days 7, 15 and 21 (Goswami and Majumder, 2015; Barajas et al., 2016; Queiroz et al., 2020). For the collection, preservation and analysis of the samples, the procedures established in the Standard Methods (APHA et al., 2012) and AOAC (2005) were followed.

Table 1. Formulation of treatments with toxic metals

	Control factors				Noise factors		
Levels	Cd^{2+} **mg/L**	Cr^{3+} **mg/L**	Pb^{2+} **mg/L**	HRT, **days**	***Pistia stratiotes L***	***Salvinia molesta***	***Eichornia crassipes***
1	1	1	1	7	1	2	3
2	5	5	5	15	4	5	6
3	10	10	10	21	7	8	9
4	1	5	5	21	10	11	12
5	5	10	10	7	13	14	15
6	10	1	1	15	16	17	18
7	1	10	10	15	19	20	21
8	5	1	1	21	22	23	24
9	10	5	5	7	25	26	27

Determination of toxic metals

Water samples were taken and digested with nitric acid, and measured by microwave-induced plasma atomic emission spectroscopy (MP-AES, 4200MP-AES, Agilent Technologies, New Castle, Delawere, USA) connected to a nitrogen generator (Peak Genius 3055, Agilent Technologies, New Castle, Delawere, USA)

The operating conditions (nebulizer flow and wavelength per element) of the MP-AES equipment were previously established by Herman-Lara et al. (2019). All multielement solutions were diluted in a concentration range of 0.1-5 mg/L. Calibration curves (correlation coefficient R^2 = 0.99 per element) using standard multielement solutions (Agilent Technologies, Delawere, USA) were performed for Cd, Cr and Pb. Determinations were performed three times to obtain reproducible and reliable results (Herman-Lara et al., 2019).

Removal kinetics

For the removal efficiency *of* each of the toxic metals contained *in* synthetic water, starting from the expression in equation 1 (Eq 1):

$$\text{Eq.1} \qquad \% E = \left(1 - \frac{C_t}{C_0}\right) \times 100$$

Where $C_0$is the initial concentration *of* Cd, Cr and Pb, $C_t$is the final concentration of the same metals in water at the respective times t.

The best-fit (first order) fit order model was used to express the kinetic absorption rate of the heavy metal transfer process (Kumar et al. 2019a). To do this, a graph of the logarithm of the concentration of metals in the effluent medium versus the retention time was drawn and the reaction rate constant (Eq. 2)was determined.

$$\text{Eq.2} \qquad Log[C] = Log[C]_0 - kt$$

where is the logarithm of the initial concentration, is the final $Log[C]_0$ logarithm *of the* $Log[C]$ concentration (mg/L) of toxic metals, k is the rate constant, and t is the sampling time (days)

Statistical analysis

The analysis was performed using statistical software, to know the significance of the results of each operational parameter, a one-way analysis of variance (ANOVA) with a significance level of 95% ($p<0.05$) was performed. To determine the contribution of the plants, a posteriori Dunnet test comparing treatments and control was carried out and complemented with the Tukey test to compare the means of all treatments.

RESULTS AND DISCUSSION

Control parameters

Through phytoremediation, it is possible to bioremediate contaminated water, this through complex physical, chemical, and biological processes, which can be affected by the effects of nutrients or the types of metals contained in the water, and in the same way by surrounding factors. , such as pH, Electrical Conductivity (EC), Total Dissolved Solids (STD), and Chemical Oxygen Demand (COD) (Ali et al., 2020). For the present study, Table 2 shows the physicochemical characterization of synthetic wastewater before treatment (influent) and after treatment (effluent).

Table 2. Control parameters

Parameters	**Treatment**									
	N1	**N2**	**N3**	**N4**	**N5**	**N6**	**N7**	**N8**	**N9**	**Witness**
pH										
I($\bar{X} \pm \sigma$)	7.2 (0.4)	7.3 (0.5)	7.3 (0.6)	6.7 (0.1)	6.8 (0.3)	6.6 (0.4)	6.3 (0.2)	6.4 (0.3)	6.3 (0.2)	7.3 (0.1)
E ($\bar{X} \pm \sigma$)	8.2 (0.1)	8.2 (0.5)	8.2 (0.2)	8.2 (0.3)	8.4 (0.3)	8.2 (0.3)	8.3 (0.1)	8.2 (0.2)	8.3 (0.2)	7.5 (0.1)
EC (ms)										
I($\bar{X} \pm \sigma$)	1280 (2.8)	1281 (1.5)	1280 (2.5)	1790 (5.6)	1795 (2.8)	1798 (3.0)	2475 (2.8)	2475 (2.5)	2475 (2.7)	330 (1.7)
E ($\bar{X} \pm \sigma$)	2550 (7.0)	2560 (5.1)	2555 (4.6)	3649.5 (1.5)	3650 (2.0)	3652 (1.8)	4800 (2.0)	4805 (5.3)	4803 (3.2)	393.5 (2.1)
TDS (ppm)										
I ($\bar{X} \pm \sigma$)	641 (1.0)	641 (2.0)	640 (2.0)	895 (3.1)	893 (4.5)	892 (1.0)	1185 (3.5)	1180 (1.3)	1184 (4.0)	165 (1.8)
E ($\bar{X} \pm \sigma$)	1393 (3.0)	1390 (2.0)	1392 (1.8)	1950 (5.0)	1950 (2.5)	1952 (3.0)	2250 (5.5)	2253 (2.5)	2250 (2.3)	197 (2.0)
COD (mg/L)										
I($\bar{X} \pm \sigma$)	555 (2.2)	555 (3.0)	555 (2.4)	830 (1.6)	830 (1.3)	830 (2.4)	1250 (1)	1250 (1.1)	1250 (1.6)	2 (1.5)
E ($\bar{X} \pm \sigma$)	96 (3.11)	139 (4.1)	228 (12.9)	113 (9.6)	151 (4.5)	262 (6.0)	184 (6.7)	118 (5.5)	289 (13)	-
Phosphates (mg/L)										
I($\bar{X} \pm \sigma$)	150 (1.5)	150 (2.0)	150 (0.5)	200 (0.8)	200 (1.0)	200 (1.5)	300 (0.5)	300 (1.6)	300 (2.1)	8.6 (0.6)
E ($\bar{X} \pm \sigma$)	131.25 (3.6)	54.75 (5.8)	54.45 (4.8)	43.4 (1.8)	69.8 (4.9)	73.4 (2.4)	50.1 (1.2)	75 (3.5)	113.1 (1.5)	2.15 (0.8)
Nitrates (mg/L)										

I($\bar{X}$ ± σ)	100 (0.5)	100 (0.8)	100 (0.6)	300 (1.5)	300 (0.1)	300 (1.0)	400 (0.5)	400 (1.5)	400 (0.6)	5.2 (0.2)
E ($\bar{X}$ ± σ)	0.5 (0.6)	5.0 (1.5)	7.8 (6.4)	1.3 (0.5)	5.7 (6.8)	7.5 (5.0)	1.4 (1.5)	5.8 (2.5)	7.9 (3.6)	0.1 (0.1)

***I:** Influent , **E:** Effluent

pH monitoring

For the treatments with 1 mg/L the initial pH is 7.3, with 5 mg/L it is 6.75 and with 10 mg/L the pH is 6.39 for the control treatment the pH is 7.3 (Table 2). Emphasizing that the increase in the concentration of metals affects the pH of the synthetic waters. The pH variations are shown in Figure 1. In the first seven days of all treatments, there is a significant increase in pH. For treatments N7-N9 the pH increases by 37%, for treatments N4-N6 the increase is 31% and for treatments N1-N3 is 3%, for the control treatment the change is 0.7%.

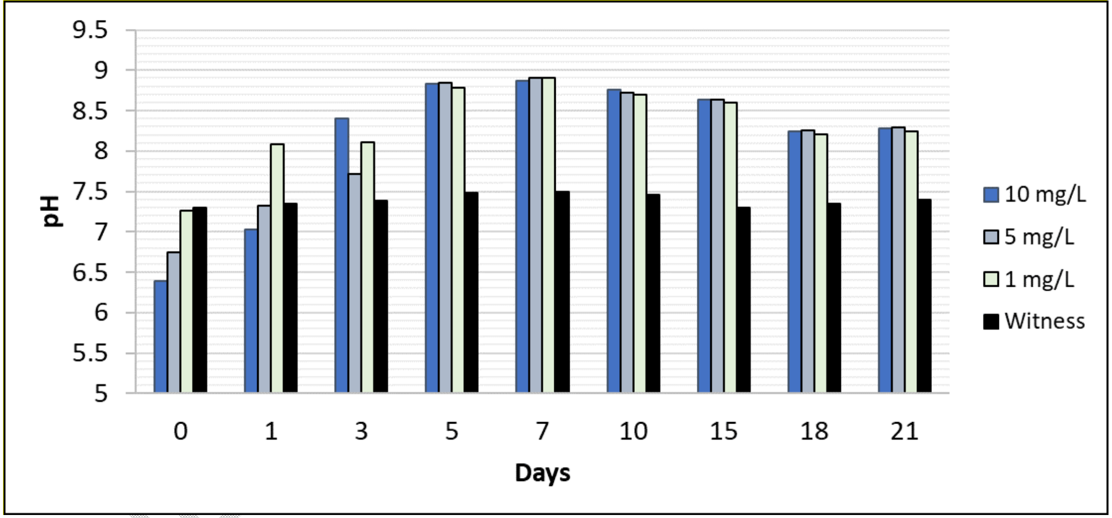


Figure 1. pH of the experiments

During the Phytoremediation process, a phenomenon occurs, where in the first seven days the pH increases, and after day eight the pH tends to decrease considerably until it stabilizes at values that fluctuate between 8.0 and 8.2. This phenomenon is mentioned in some other investigations mentioning that it is due to the consumption of CO_2 results of the photosynthesis carried out by each macrophyte (Mendoza et al., 2018; Li et al., 2015). Wang et al. (2021) studied the effect of the removal of aquatic plants on Cu, Zn, and Cd showing similar results where the pH values ranged between 7.9 and 9.4.

Removal of toxic metals

All treatments showed a marked decrease in toxic metals, shown in Figure 2. For this, the maximum removal efficiency is achieved with *Eichhornia crassipes* with a concentration of 1 mg/L manages to remove 99.9% of Cr, Pb: 99.9%; Cd: 99.9 %. The experiments where there is a concentration of 5 mg/L the following Cr removals are reached: 90.88 %; Pb: 97.30%; Cd: 95.38 % and for experiments with 10 mg/L the removal is Cr: 95.98 %; P.S.: 89.65%; Cd: 90.88 %. These results are consistent with Mojiri et al. (2018) study the removal of Cr and Cd using *Eichhornia crassipes*, with a water with a content of 50 mg/L of Cd and Cr finding that after 72 hrs. manage to remove 92%, for this study was included a support four layers of substrate of adsorbents which helped in the absorption of toxic metals.Singh et al. (2021) studied the phytoremediation of *Eichhornia crassipes* and *Pistiastratiotes*, in wastewater with a content of 0.92 mg/L of Cd removing 45 % and 1.16 mg/L of Pb removing 25%, ina hydraulic retention time of 41 days.

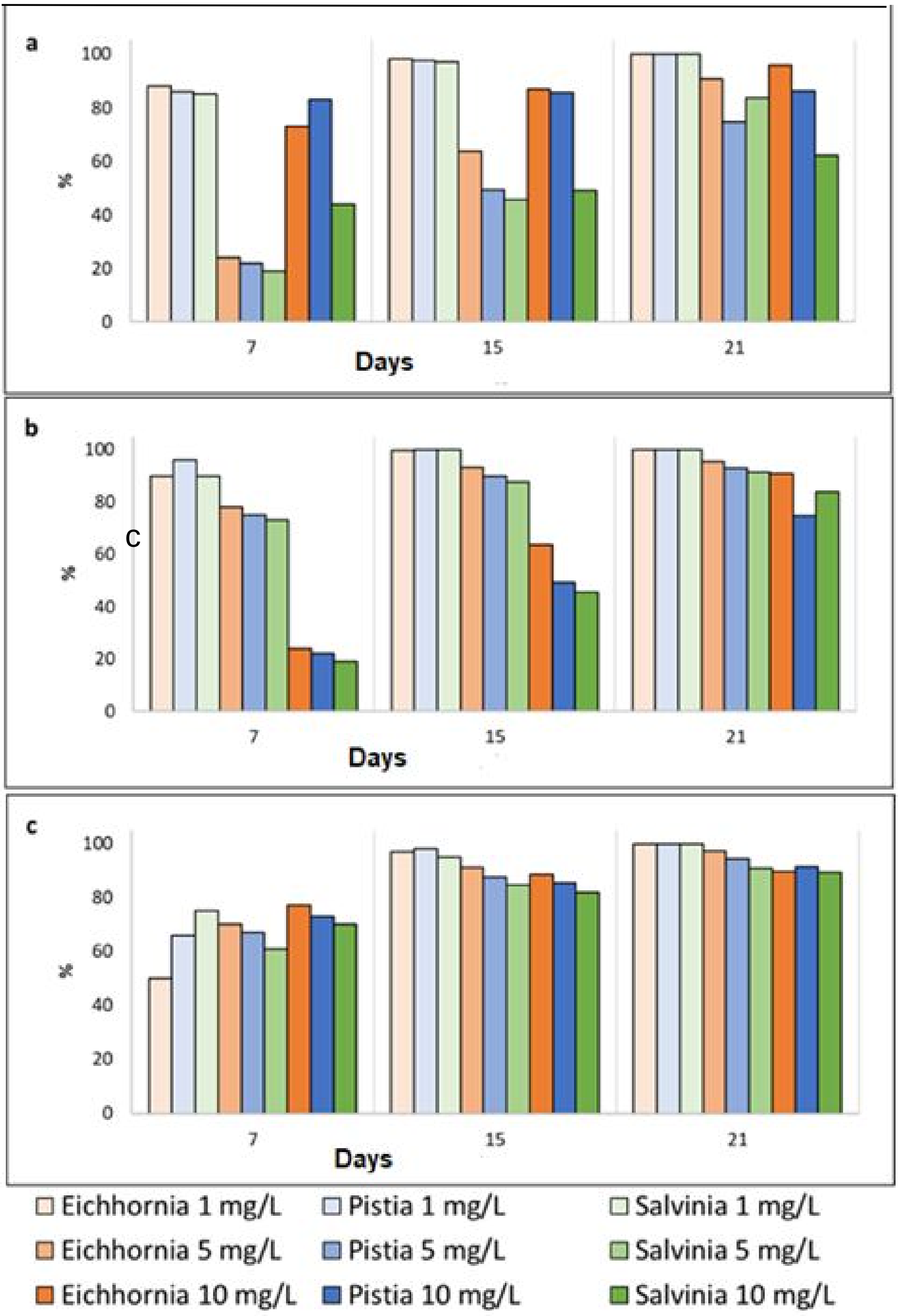


Figure 2. Removal of Cr(a), Cd(b) and Pb(c) in the different treatments

For experiments performed with *Pistia stratiotes* with a concentration of 1 mg/L the removal is Cr: 99.9, Pb: 99.9; Cd: 99.9, with 5 mg/L Cr: 86.46; P.S.: 94.36; Cd: 93.0 and 10 mg/L Cr: 74.65; P.S: 91.25; Cd: 74.65. D and similarly Ayaz et al. (2020) evaluated Cd and Pb removal in concentration ranges of 4. 5-8. 0 mg/L Cd, 6.81-8. 01 mg/L of Pb, using *Pistia stratiotes* and *Eichhornia crassipes* managed to remove 93.3% of Cd, 97.7 of Pb and in a time of 60 days. Xie et al. (2022) studied the phytoremediation of wastewater with a content of Cd, Cr and Pb using *Pistiastratiotes*, after45 days of culture the concentration was reduced to 0. 25–0. 90 mg/L and 0. 78–1.4 mg/L of 5 mg/L achieving removals of 72% to 84.4%.

Experiments with *Salvinia molesta* with a concentration of 1 mg/L achieve removals of Cr: 99. 0, Pb: 99. 0; Cd: 99. 0. for the concentration of 5 mg/L are removed from Cr: 83.72; PS: 90.64; Cd: 91.50 and with 10 mg/L removals are Cr: 62.29; P.S.: 89.20; Cd: 83.72.

The results show that *Salvinia molesta* was efficient in reducing the concentration of metals in wastewater, so the data obtained are purchased with George et al. (2017) study the potential of *Salvinia molesta* finding that wastewater with Cd and Pb, after ten days of treatment removes a large amount of these metals so its detection is very low, is below the limit of (0.01 and 0.05 ppm). On the other hand, Biju et al. (2023) studied wastewater from the textile industry which contained 20 mg/L of Cr and after eight days of treatment with *Salvinia molesta* reached a removal of 82 %.

Toxic metal removal kinetics

The kinetic behavior of the experiments shown in Figure 3 was considered that the model for the removal of toxic metals, is of the first order. In table 3 we can see the kinetic parameters, where in most experiments has an adjustment of R^2 higher than 0.80, only in the removal of lead with 10 mg/L made by *Salvinia molesta* the value is lower reaching 0.77. For the treatments carried out with Cd we can highlight that the highest values of the constant k are obtained in the experiments carried out with *Eichhornia crassipes* where the values are 0.047, 0.064, 0.228 (mg/L.d) with 1, 5 and 10 mg/L respectively.   In case of experiments with Pb happens a similar behavior *Eichhornia crassipes* reaches the highest values ranging from 0.046, 0.073, 0.223 (mg/L.d) with 1, 5 and 10 mg/L respectively. Finally, the experiments carried out with Cr, the highest values of k are 0.145, 0.047, 0.218 (mg/L.d) with 1, 5 and 10 mg/L respectively, which also belong to *Eichhornia crassipes*.It is evident that *Eichhornia crassipes,* has a higher assimilation rate of Cd, Cr and Pb compared to other macrophytes.

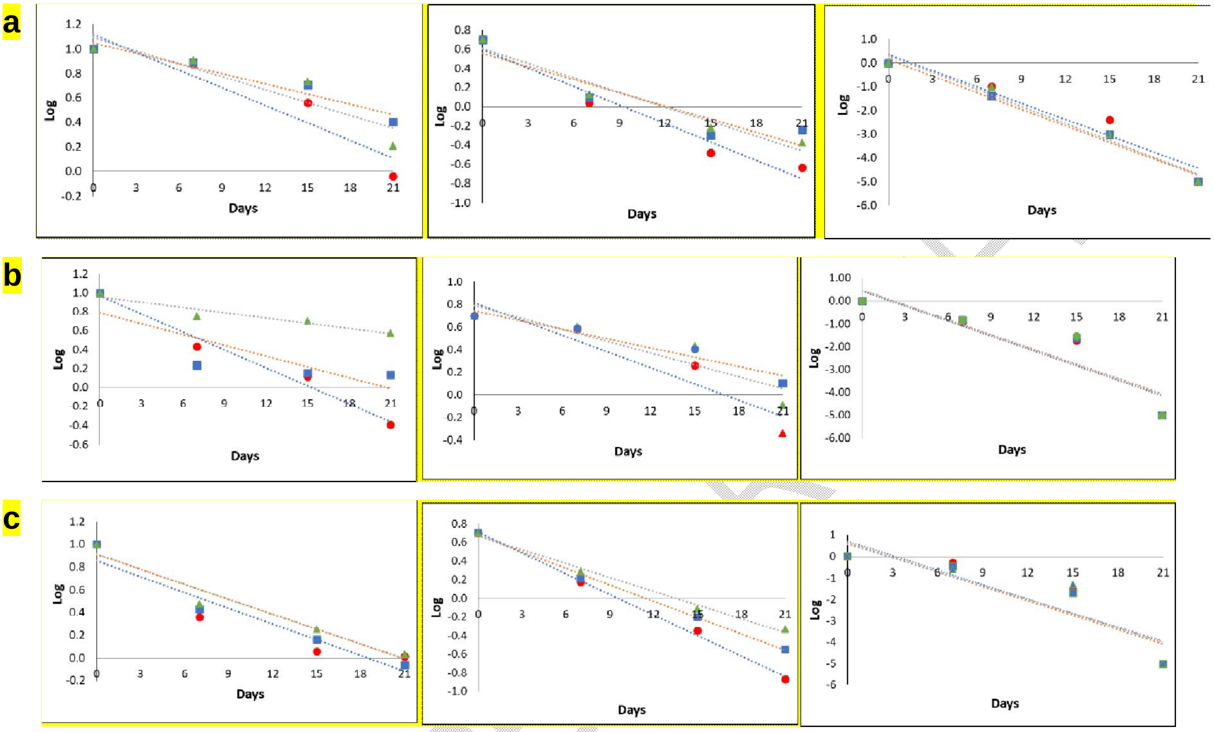


Figure 3. First-order model correlation with removal kinetics for Cd (a: 1 mg/L, 5 mg/L,10 mg/L), Cr (b: 1 mg/L, 5 mg/L,10 mg/L) and Pb (c: 1 mg/L, 5 mg/L10 mg/L) in the different treatments

Table 3. Kinetic parameters

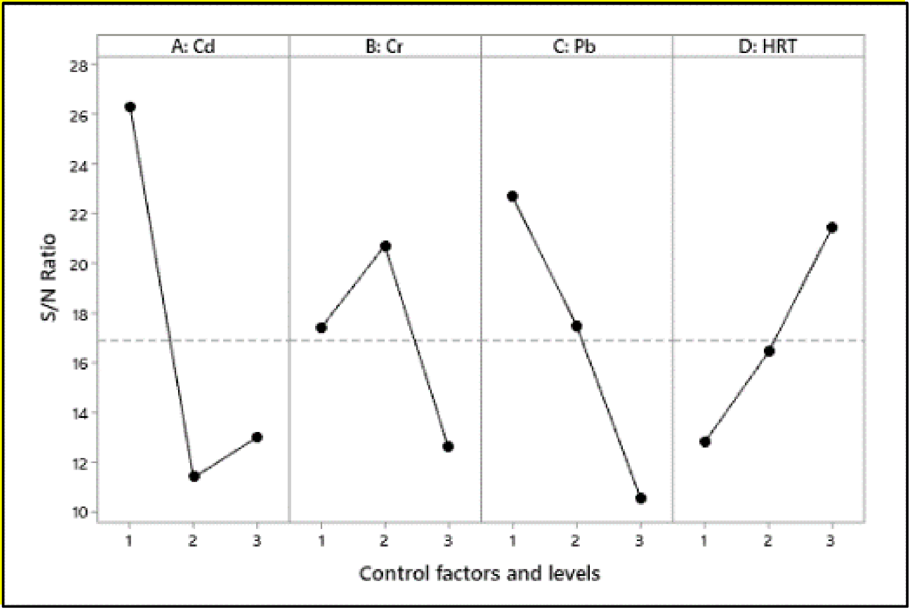
	K (mg/L.d)			**R^2**		
Cd	**1 mg/L**	**5 mg/L**	**10 mg/L**	**1 mg/L**	**5 mg/L**	**10 mg/L**
Eichhornia crassipes	0.047	0.064	0.228	0.88	0.95	0.93
Pistia stratiotes L	0.027	0.046	0.232	0.93	0.84	0.98
Salvinia molesta	0.035	0.05	0.238	0.83	0.94	0.97
Pb						
Eichhornia crassipes	0.046	0.073	0.223	0.87	0.99	0.8
Pistia stratiotes L	0.044	0.058	0.224	0.94	0.99	0.83
Salvinia molesta	0.044	0.049	0.215	0.94	0.99	0.77
Cr						

Eichhornia crassipes	0.145	0.047	0.218	0.98	0.88	0.84
Pistia stratiotes L	0.038	0.027	0.217	0.7	0.93	0.82
Salvinia molesta	0.018	0.035	0.216	0.9	0.83	0.81

Taguchi Design Analysis

The conditions for achieving the highest removal efficiency for each factor are shown in Figure 4. The conditions for cadmium (Figure 4.A) are reached when you have 1 mg/L of Cd, 5 mg/L of Cr, 1 mg/L of Pb in a time of 21 days. To achieve optimal levels of chromium removal (Figure 4.B) it is necessary to have the concentration of 1 mg/L of each metal Cd, Cr, and Pb, for this case HTR does not play such a relevant role, but it is more significant the TRH of 21 days. Finally, to achieve the highest levels of lead removal (Figure 4.C) you can have a concentration of 5 mg/L of Cd, while chromium can contain between 10 to 5 mg/L and Cd 1 mg/L of chromium is needed, for this case if it is very marked the removal in a HRT of 21 days is more significant.

A



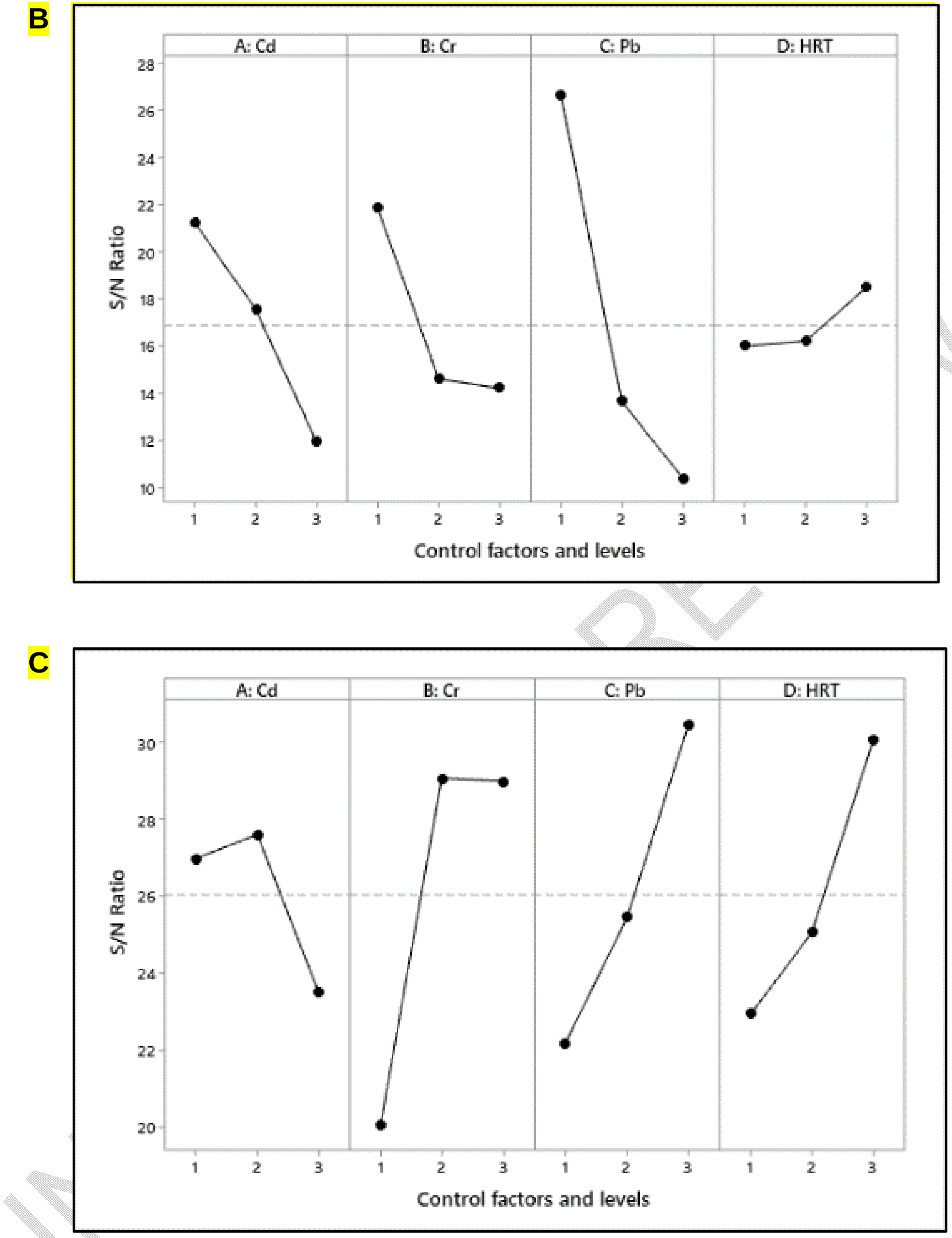


Figure 4. Optimal levels for higher % removal efficiency of a) Cd b) Cr c) Pb

The Taguchi analysis showed the best conditions to perform phytoremediation Table 4, the optimal conditions are at low concentrations when you have 1 mg/L of each of the toxic metals and the most suitable HRT is after 21 days.

Table 4. Optimal values in Taguchi analysis

Cd^{+2}	**Cr^{+3}**	**Pb^{+2}**	**TRH**
mg/L	**mg/L**	**mg/L**	**días**
1	1	1	21

CONCLUSIONS

The findings of this work show that *Eichhornia crassi, Pistia stratiotes* and *Salvinia molesta* are efficient in the removal of toxic metals (Cd, Cr and Pb). Although different removal efficiencies are shown for each toxic metal, the removals of *Eichhornia crassipes* stand out, achieving a higher removal rate*. Eichhornia crassipes* is the optimal macrophyte to efficiently remove compounds such as Cd, Cr and Pb in a time of 21 days. In general, the days from 15 to 21 were where the greatest elimination of d e Cd, Cr and Pb was achieved. Being the day 21 the day considered the most appropriate.

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