REMOVING ARSENIC, COPPER AND IRON FROM SEWAGE SLUDGE WITH REED (*Phragmites australis)*

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Abstract. Agriculture can use the sewage sludge resulting from wastewater treatment. In order to use sewage sludge, it is necessary to eliminate the content of toxic pollutants, including heavy metals through phytoremediation. The purpose of this research work was to assess the capacity of *Phragmites australis* to remove As, Cu and Fe in wastewater sludge of the municipal treatment plant in Cd. Victoria, Tamaulipas. We established four treatments: T1 = sewage sludge + soil + *Phragmites australis*; T2 = sewage sludge + soil; T3 = Sludge + *Phragmites australis*; T4 = Check test. Each treatment had six replicates. The phytoremediation process lasted eight months. We took monthly samples from the substrate and samples of *P. australis* every four months to determine the contents of As, Fe and Cu. The sludge + soil + P. australis treatment was able to remove higher percentages of Fe (96.63%) and Cu (47.67%); while sludge + P. australis was able to remove higher percentage of arsenic (81.18%), with a bio-concentration factor of 7.76. The amount of metals removed from the substrates from highest to lowest were: Fe > Cu > As. Furthermore, the final concentration of Fe, Cu and Arsenic in the sludge was below the allowable limit established by NOM-004- SEMARNAT-2002 Standard.
Keywords: *bio-concentration factor, heavy metals, Phytoremediation, translocation factor*

Introduction

Wastewater treatment is a worldwide strategy to recover water contaminated by residential and industrial use. The process does not only produce treated water, but also suspended solids known as sewage sludge or bio-sludge (Jing et al., 2017). Sewage sludge is treated and biologically stabilized to avoid harming living creatures and the environment. Once stabilized, sewage sludge turns into bio-solids used in fertilization, crop enhancement, forest remediation, composting and power generation (Castañeda et al., 2011; CRA, 2000).

Like other countries, Mexico has implemented wastewater treatment programs since 2008, including PROTAR (Wastewater Treatment Program), aiming to increase the volume of treated water and improve existing treatment processes. Thanks to these projects, the number of operating wastewater treatment plants in the country increased to 2447 by December 2015, representing an installed capacity of 177973.58 L s^{-1}. Nevertheless, those plants treat only 120902.20 L s^{-1} of wastewater, leaving behind 2911234 t $year^{-1}$ of bio-solids (CONAGUA 2015). CONAGUA (2011) reported that

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37% of the treated wastewater streams produce 640,000 to 10 million t year^{-1} of bio-solids. This difference is because sometimes the quantity is reported in terms of dry weight and in other cases the moisture content is not even mentioned; so in fact, there is no official data regarding sewage sludge and bio-solids production, and therefore, the percentage of treated bio-sludge is very low.

Sewage sludge obtained from wastewater treatment does not only have a high level of nutrients, but also several pollutants, such as heavy metals. The presence of trace elements transforms sewage sludge in a source of environmental pollution. The gases released when the sludge breaks down produce a fetid odor coming from a large number of fecal coliforms, including some pathogens like *Salmonella spp.,* and elements that are toxic in high concentrations. It is therefore important to treat sewage sludge and eliminate, reduce or form compounds that will not harm living organisms and the environment (Rojas and Mendoza 2012).

Stabilizing sewage sludge from wastewater plants implies very high operating costs. Phytoremediation is a solution to this problem, offering an effective, low-cost *in situ* solution. Wetlands retain trace elements in a natural way, in particular *Typha latifolia* L. and *P. australis* Cav. Trin. (Salema et al., 2014).

The cost of conventional stabilization and reuse of sewage sludge is unaffordable at Cd. Victoria, Tamaulipas' wastewater treatment plant. Therefore, untreated sludge is disposed inside the plant facilities, becoming a source of environmental pollution. This research work is aiming to determine *Phragmites australis'* capacity to remove arsenic, copper and iron from sewage sludge produced by Ciudad Victoria, Tamaulipas' wastewater treatment plant.

Materials and methods

The experiment was conducted from May 2015 to January 2016 at the hydroponic module of Ciudad Victoria's Technology Institute (ITCV).

500 kg of untreated sewage sludge were collected at Cd. Victoria, Tamaulipas' wastewater treatment plant and taken to ITVC facilities to fill 19 kg-pots which were used as individual experimental units, using a 5-cm gravel bed for planting and propagating *P. australis.* We established four treatments, each one with six replicates; 24 experimental units in total (*Table 1*).

We collected *P. australis* spikes from San Marcos River banks, Cd. Victoria, Tamaulipas. We selected the thicker spikes with the largest number of nodes for propagation. We made diagonal cuts every three nodes, in order to propagate the cuttings in every experimental unit. We organized the experimental units at random and we planted three cuttings in each experimental unit, watering them with drinking water. The experiment lasted eight months under uncontrolled conditions at an average temperature of 26 °C. We took monthly samples of the substrate (30 g) close to the roots and we selectted one plant per treatment every four months, to determine the concentration of As, Cu and Fe on the substrate and on *P. australis*. (The first sampling was in September).

Substrate characteristics

Table 1 shows the soil and sludge initial characteristics. The initial pH of soil and the sewage sludge was slightly acidic and had a small increase after mixing both substrates. As and Cu concentrations were lower than the maximum allowable limits of 41-

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75 mg kg^{-1} and 1500-4300 mg kg^{-1} respectively in dry weight, according to NOM-004-SEMARNAT-2002 standard.

Table 1. *Initial characteristics of the waste sludge, soil and the mixture of both substrates*

Parameter	Sludge	Soil	Sludge + soil
As (mg kg^{-1})	1.61	0.283	1.893*
Cu (mg kg^{-1})	12.411	1.29	13.701*
Fe (mg kg^{-1})	656.365	5.839	662.204*
pH	6.780	6.750	6.990
CE (dS m^{-1})	4.360	1.855	1.030
PR (mV)	-50.2	-45.9	-39.7
MO (%)	5.414	1.083	1.963
Nt (%)	1.072	0.142	0.389
P (mg kg^{-1})	33.161	1.005	15.576
K ($cmol_{(+)}$ kg^{-1})	0.189	0.492	0.082

CE = Electrical conductivity, Nt = total nitrogen, ORP = redox potential
*For the case of the mixture, the concentrations of As, Cu and Fe were not determined, so the value presented here is an approximation, based on the initial concentration of the metals in the waste sludge and the soil

Heavy metals' determination: We analyzed the substrate and *P. australis* samples to determine the content of metals using inductively coupled plasma atomic emission spectroscopy (ICP-OES Varian model 725-ES, Agilent, Mulgrave, Australia).

Data analysis

The bio-concentration factor (BFC) (Chandra, 2013), was calculated using *Equation 1:*

$$BFC = \frac{C_p}{C_i} \quad \text{(Eq.1)}$$

where: C_i = initial metal concentration in the substrate, and C_p= metal concentration in the plant.

The translocation factor (TF) (Chandra, 2013) was determined by *Equation 2:*

$$TF = \frac{C_a}{C_r} \quad \text{(Eq.2)}$$

where: C_a = metal concentration in aerial plant parts and C_r = metal concentration in plant roots.

pH and reduction-oxidation potential (PR) results were analyzed by linear regression, to determine their dependency. Metal concentrations in the substrates were submitted to repeated measures analysis of variance and Fisher's LSD test ($p < 0.05$). We analyzed metal concentrations, BCF and TF of the plants by discrimination functions, in order to determine the difference among these variables. Finally, we applied a canonical correlation to find the relation between metal concentrations in the plant and the

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substrates. We conducted the statistical analysis of the variables with PROC REG, MIXED, DISCRIM and CANCORR procedures, using SAS software (2002) and Statistica, version 7.

Results

The high temperatures in Cd. Victoria 18.5–31 °C dehydrated the substrates, reducing their initial volume. We had to add 12 kg more of sewage sludge to the experimental units in June and September in order to maintain the ratios that were originally proposed for each treatment (we determined the concentration of As, Cu and Fe in the sludge before the addition). The sewage sludge added during those months had lower concentrations of the three metals, than the initial concentrations of As, Cu and Fe found in the original substrates used for the experimental units (*Table 2*).

Table 2. *Concentration of As, Cu and Fe in waste sludge and irrigation water*

Sample	As (mg kg^{-1})	Cu (mg kg^{-1})	Fe (mg kg^{-1})
Sludge initial	1.61	12.411	656.365
Sludge july	0.000001	1.01	318.032
Sludge september	0.000001	1.143	270.181
Water sample irrigation	0.034	0.088	0.021

Only two, out of three plants that we planted in the experimental units survived. During the eight months that the experiment lasted, the plants showed phenotypic differences. Plants with sludge + *P. australis* had more foliage in the aerial parts; while plants growing on the sludge and soil + *P. australis* mix were taller.

We measured the pH and the reduction-oxidation potential (PR) in the samples of each substrate, four months after planting. The pH increased from 6.7 to 8.3, and significant differences were found in the correlation of pH with time (R^2 = 0.307 and p < 0.05) and PR (R^2 = 0.312 and p < 0.05). This parameter changed from -39.7 to 101.4 (*Fig. 1*).

Figure 1. *Plants of P. australis three months after sowing in the pots. T1 = sewage + soil + P. australis, T2 = sewage + soil, T3 = sewage + P. australis*

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We found significant differences in Arsenic concentration in the substrates throughout the eight months (F = 2.43 and $p < 0.05$). The higher concentration was in May, with 1.61 mg kg^{-1} and the lowest concentration was in November (0.08 ± 0.06 mg kg^{-1}). There were significant time differences among treatments (F = 2.74 and $p < 0.05$). In the second month (June) the sludge and soil + *P. australis* treatment had the highest concentration (0.86 ± 0.51 mg kg^{-1}), while the lowest concentration was found in October (0.10 ± 0.24 mg kg^{-1}). The sludge + soil treatment had the highest concentration in July (0.44 ± 0.38 mg kg^{-1}) and the lowest in November (0.11 ± 0.06 mg kg^{-1}). The sludge + *P. australis* treatment had the highest concentration in September (0.56 ± 0.46 mg kg^{-1}) and the lowest in November (0.11 ± 0.10 mg kg^{-1}). The check test (Sludge) had the highest concentration in August (1.04 ± 0.72 mg kg^{-1}) and the lowest in November (0.08 ± 0.05 mg kg^{-1}) (*Fig. 2a*). The sludge and soil + *P. australis* treatments, as well as the check test showed significant differences (t = 0.74 and $p = 0.0449$).

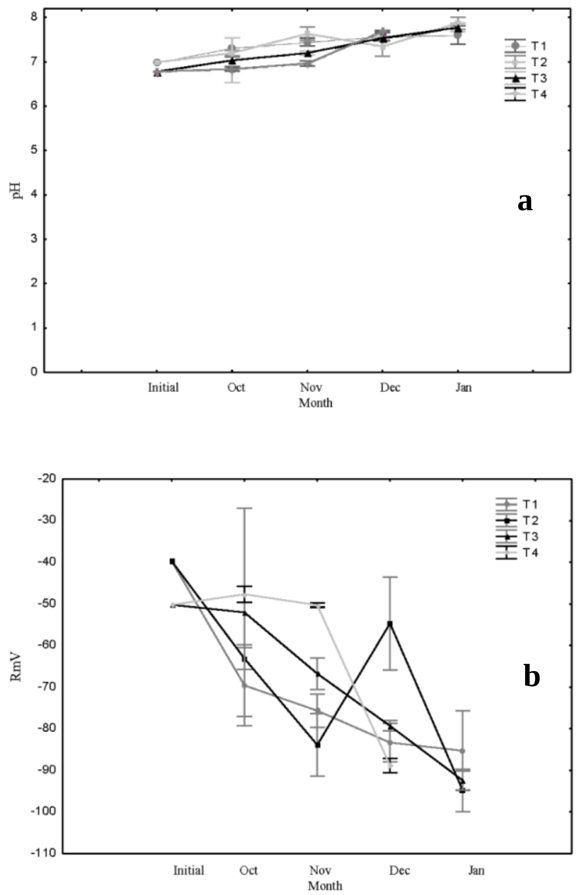

Figure 2. *Average values of a) pH and b) PR with SD bars, during the last four months of the phytoremediation process, for the treatments, T1 = sewage + soil + P. australis, T2 = sewage + soil, T3 = sewage + P. australis and T4 = sewage, (n = 6) with confidence interval of* ± *0.95*

Cu concentration in all treatments had significant differences throughout the months (F = 7.09 and $p < 0.05$). The highest concentration was in May 12.41 mg kg^{-1} and the lowest was in December (3.42 ± 2.34 mg kg^{-1}). The treatments had significant time differences (F = 3.06 and $p < 0.05$), (*Fig. 2b*). LSD analysis showed that there are

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significant differences in the sludge and soil + *P. australis* mix, and the check test (t = 2.92 and $p < 0.05$); as well as between the sludge + soil and the mix sludge and soil + *P. australis* (t = 4.06 and $p < 0.05$).

The mg kg^{-1} of Fe showed significant differences throughout the months (F = 44.98 and $p < 0.05$), with lower concentrations switching from one month to another, until reaching a final concentration (14.19 mg kg^{-1}) lower than the initial concentration (656.36 mg kg^{-1}). There were also differences among treatments and among the months (F = 13.25 and $p < 0.05$), (*Fig. 2c*). LSD analysis proved that there were significant differences among the four treatments with $p < 0.05$.

The percentage of Arsenic removal from the substrates did not show significant differences among the months and the treatments (F = 0.89 and F = 0.61 respectively; $p \geq 0.05$). We defined a range of 73-83% in the eight months and in the four treatments. We found significant differences among the months for Cu removal percentage; however, there were no differences among the treatments (F = 2.72, $p < 0.05$; F = 2.07, $p \geq 0.05$). The treatments did not show significant differences on Fe removal percentage (F = 3.38, $p < 0.05$; F = 2.98, $p \geq 0.05$). We determined the overall removal percentage during the eight months in the four treatments, showing a higher value for Arsenic in the sludge + *P. australis* treatment, as well as for Cu and Fe in the mix of sludge and soil + *P. australis* (*Table 3*).

Table 3. *Summary of the analysis of discriminant functions*

% Removal	Treatment							
	SSP		SS		SP		S	
As	80.18	±3.74	80.78	±3.09	83.67	±9.08	73.67	±6.75
Cu	47.67	±10.41	31.08	±6.47	42.30	±11.75	23.11	±11.09
Fe	96.63	±2.32	87.15	±3.05	83.88	±4.64	76.48	±2.48

Accumulation of As, Cu and Fe in P. australis

Metal concentration in *P. australis* organs was the following: As: 0.07- 1.96, Cu: 0.92-28.7 and Fe: 121.13-10224.01 mg kg^{-1} in dry weight. The discrimination analysis found significant differences among the plant parts, regarding the accumulation of heavy metals [F = 6.2994, Wilks' Lambda = 0.00019, $p < 0.05$)] (*Table 4*). We found differences in Arsenic concentration in the stem, and Cu concentration differences in the leaves (*Fig. 3*). There was a significant difference of $p = 0.1129$ at different times, in the sludge and soil + *P. australis* and sludge + *P. australis* mix treatments.

Estimated BCF values for Cu and Fe in the sludge and soil + *P. australis* and the sludge + *P. australis* mix treatments on September and January were greater than 1; while for As, the values were greater than 1 in September, and they were lower than 1 in January. TF for As was greater than 1 in the two samplings, on the sludge and soil + *P. australis* and sludge + *P. australis* mix treatments; while TF was below 1 for Cu and Fe (*Table 5*). BCF value was higher in Fe accumulation and lower in As with *P. australis.* The opposite happened with TF value, which was higher in Arsenic and lower in Fe (*Fig. 4*). Results of the discrimination function analysis showed that there were no significant differences among the months and the treatments regarding the BCF values for Cu and Fe (F = 1.4780, Wilks' Lambda = 0.15692, p = 0.1697). The only significant difference for BCF was Arsenic ($p < 0.001$) (*Fig. 5*).

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Table 4. *Summary of the analysis of discriminant functions*

	Wilks'	Partial	F-remove	p-level
		Root		
Cu	0.000726	0.261807	469.935	0.064384
		Stem		
As	0.002895	0.065653	2.371.949	0.002196
Cu	0.000377	0.504194	163.894	0.293155
Fe	0.000456	0.417124	232.895	0.191443
		Sheet		
As	0.000323	0.587784	116.884	0.408722
Cu	0.000598	0.317606	358.093	0.101736
Fe	0.000614	0.309561	371.730	0.095775

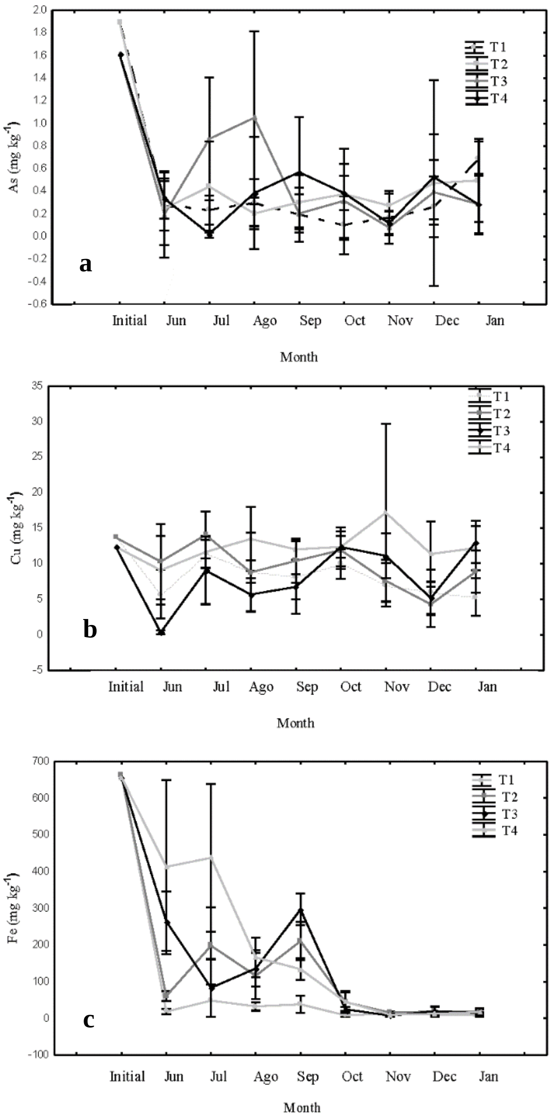

Figure 3. *Variation in the concentrations of a) As, b) Cu and c) Fe, during the eight months of treatment. T1 = waste sludge + soil + P. australis, T2 = waste sludge + soil, T3 = waste sludge + P. australis and T4 = waste sludge, (n = 6), with a confidence interval of* ± *0.95*

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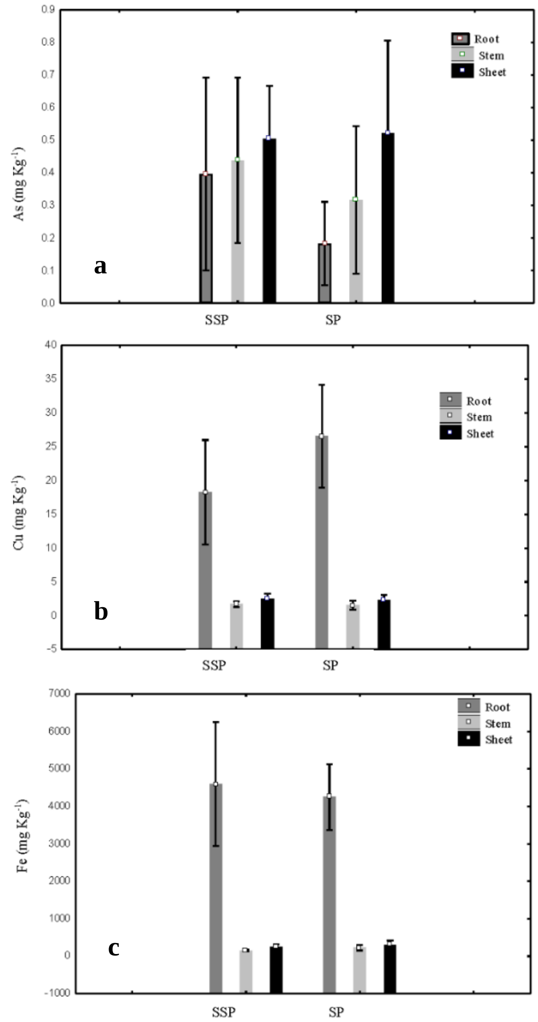

Figure 4*. Concentration of the metals studied (mg kg*$^{-1}$*) in root, stem and leaf of P. australis, a0 As, b) Cu and c) Fe. Bars represent the means ± SD (n = 6).*

Table 5. *Mean values of the bioconcentration factor and the translocation factor for the SSP and SP treatments between months*

Month	T	BCF						BF					
		As		Cu		Fe		As		Cu		Fe	
Sep	SSP	1.04	±0.33**	1.87	±1.23	7.77	±4.28	2.50	±1.80	0.32	±0.30	0.30	±0.53
Sep	SP	1.07	±0.46**	2.32	±0.42	6.93	±1.69	5.25	±2.46	0.22	±0.10	0.15	±0.06
Jan	SSP	0.34	±0.18**	1.41	±0.44	7.31	±3.95	9.15	±2.09	0.28	±0.15	0.11	±0.06
Jan	SP	0.35	±0.11**	2.55	±1.10	7.53	±2.09	6.17	±3.38	0.13	±0.09	0.12	±0.06

**p < 0.001

Canonical correlation among plants and substrates

Canonical correlation analysis was significant when we compared metal concentrations in plants versus metal concentration in the substrates (R = 0.9659,

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$Chi^2 = 23.806$, $p = 0.4727$). Results showed a low correlation between metal concentrations in the plants versus the substrates (*Table 6*).

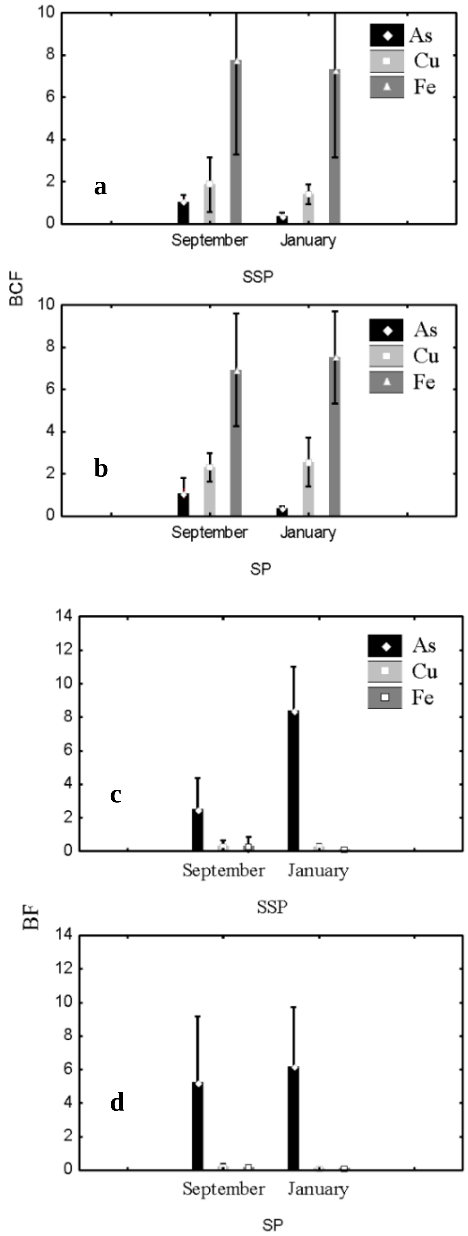

Figure 5. *Bioconcentration factor of the metals studied for September and January a) SSP treatment; b) SP treatment Translocation factor of metals in aerial part/roots of the plant per month, c) SSP treatment and d) SP treatment Average (n = 6)*

Table 6. *Correlations between metals for plant and substrate*

Plants	Substrates		
	As	**Cu**	**Fe**
As	0.378599	-0.218674	-0.358279
Cu	-0.632306	0.482277	0.547177
Fe	-0.240246	-0.224118	-0.408420

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Discussion

General characteristics of plants during the phytoremediation process using P. australis

The optimal temperature range for *P. australis* plants is between 12–33 °C, according to Cooper (1996). However, in this essay, the plants were exposed to ambient temperature of 03–42 °C; exceeding by 8 °C the maximum value of said temperature. The temperature had an impact on the oldest leaves, which turned yellowish before wilting, a potential sign of heat stress (Yepes and Buckeridge, 2011). Although plants received consistent watering, the high temperatures affected the structure of substrates, leading to dehydration, compaction and low water availability (Paradelo, 2013).

Substrate characterization

pH was slightly acidic in every treatment, allowing the mobility and retention of As, Cu and Fe, since most metals have better availability at acidic pH (As, Ni, Cu, Fe, Pb) (Wilson et al., 2010; Bolan et al., 2014). Ansari et al. (2014) indicate that Cu solubility increases at pH = 5.5. In this case, the initial and final pH of substrates led to low solubility of Cu and Fe. Plants therefore showed a lower uptake of these metals (Pha et al., 2014); while As mobility increased at alkaline pH, as shown by the pH increase in the substrates, from 6.75 to 8.36 (Bolan et al., 2011). pH increases in this essay did not show significant differences among treatments. This pH increase was due to organic matter (MO) breakdown, the content of N in the sewage sludge, and the rainfall events where water leached some cations, afterwards replaced by acidic elements, such as iron (Valentín-Vargas et al., 2014).

PR is another property affecting metal bioavailability by changing the state of ions. PR value in this essay was low, allowing As, Fe and Cu to be available in the soil, at more soluble forms (Wilson et al., 2010; Bolan et al., 2014).

Heavy metal concentrations in initial substrates (As = 1.61, Cu = 12.411 and Fe = 656.365 mg kg^{-1}) did not exceed the values established by NOM-004-SEMARNAT-2002 (As = 41 and Cu = 1500 mg kg^{-1}). Nevertheless, it is important to minimize the metal content in sewage sludge, since metals have high capacity of bioaccumulation and biomagnification that increase their toxicity in trophic chains (Sabir et al., 2015; David et al., 2012).

Initial concentration of Arsenic in sewage sludge decreased from 1.61 to 0.345 mg kg^{-1}, which is equal to 79.57% removal. Sludge + *P. australis* treatment had the highest removal percentage (83.67%). Among the treatments of sludge and soil + *P. australis;* sludge and soil and sludge + *P. australis* there were no significant differences, indicating that *P. australis*, is not the only factor leading to Arsenic reduction in these treatments. These results were confirmed by the correlation analysis among plants and substrates, where we found a low correlation between the concentration of Arsenic in the substrate and Arsenic concentration in the plant (R = 0.37). However, we found significant differences between the treatment of sludge and soil + *P. australis* mix, and the Sludge treatment. Therefore, the type of substrate and the presence/absence of *P. australis* allowed us to determine that Arsenic removal was impacted by the reduction-oxidation potential; leaching during rainfall events and the mobility of Arsenic at the bottom of pots. The release of Arsenic particles depends on the formation of strong bonds between As particles and soil particles due to PR. In our case, soil aeration produced at low PR, led to a depletion of electron acceptors and the development of

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anoxic conditions forming iron oxides and oxygen hydroxides requiring reduction and dissolution, minimizing the uptake of arsenic from the sludge solution where it can be leached (Elekes, 2014; Punshon et al., 2017).

Initial Cu concentration in sewage sludge decreased from 12.411 to 9.437 mg kg^{-1}, corresponding to an average removal percentage of 79.57%. The treatment of sludge and soil + *P. australis* mix showed a higher removal percentage of 47.67%. We observed significant differences among treatments, due to the type of substrate and the presence of *P. australis.* This is because alkaline pH affects trace elements like Cu, in terms of solubility and bioavailability of the element, besides transportation at soil level (Avci and Deveci, 2013). These results coincide with the reports of Torres et al. (2010), who used similar treatments to remove Cr with vetiver, finding that the most effective treatment was the plant + sewage sludge + organic manure. Canonical correlation was R = 0.482 when comparing Cu present in the substrates versus Cu present in the plants. This result explains the low removal percentage of Cu.

Likewise, in case of Fe, initial concentration of the sewage sludge decreased from 656.36 to 91.90 mg kg^{-1}, corresponding to an average removal percentage of 86.35. Sludge and soil + *P. australis* mix treatment achieved the largest percentage of Fe removal (96.63%). We found significant differences among the four treatments, showing that in the case of Fe, the variables of plant and type of substrate (only sewage sludge or sludge and soil mix) had an impact on Fe removal. Canonical correlation indicated a low negative relation, when Fe decreased in the substrate, Fe increased in the plant (R = -0.408). However, the percentage of removal in substrates was high, maybe due to the leaching of Arsenic and the affinity to form iron sulfide under reduction conditions, such as the conditions found at test substrates. Other reasons might be a pH close to 7 and MO content, leading to low Fe solubility in the soil (Willscher et al., 2017).

Accumulation of As, Cu, Fe in P. australis

The test results showed significant differences among treatments (sludge and soil + *P. australis* mix and Sludge + *P. australis*) regarding Arsenic concentration in the stems and Cu concentration in the leaves. These results may imply that the type of substrate, pH, MO and PR can have an impact on the affinity of these elements towards specific plant organs (Willscher et al. 2017). We found significant differences in metal concentrations along the time, for the sludge and soil + *P. australis* mix (As = 1.96 to 0. 65 mg kg^{-1}, Cu = 25.56 to 14.47 mg kg^{-1} and Fe = 5142.22 to 722.23). The decrease in metal accumulation by the plants from September to January was due to the metals availability, depending on the different physical/chemical properties of substrates (Willscher et al., 2017; Avci and Deveci, 2013), and the variability of such properties, (pH, PR, MO) as a consequence of the sludge addition before the first sampling in September. This addition modified As, Cu and Fe concentrations, as well as the substrate characteristics (like it happened when mixing sewage sludge and soil), enabling better uptake of the three metals. The treatment sludge + *P. australis*, did not have any significant differences along the sampling months. We attributed this to the reports by Torres et al. (2010), indicating that the addition of the same substrate, in this case sewage sludge, does not imply a higher rate of metal uptake by the plants.

Plant tolerance to heavy metals like As, Cu and Fe is due to the fact that these metals are transition metals with oxidizing capacity, as well as with an the capacity to reduce different biomolecules (Choppala et al., 2014). Therefore, these effects in the reduction-

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oxidation state of the cells enhanced due to the bonding reaction of these metals with biomolecules. This capacity can explain that the average concentration of As, Cu and Fe in the organs of *P. australis* in dry weight. These elements, (As: 0.61, Cu: 11.56 and Fe: 1420.99 mg kg^{-1}) were found within the typical ranges in plants reported by Larcher (2003) = Cu: 4 – 20 but not typical for Fe: 2 – 700 mg kg^{-1}. Although Cu is within the typical value range, it exceeds the value required by plants, just like Fe (1 – 5 mg kg^{-1} Cu and 100 mg kg^{-1} Fe).

As, Cu and Fe accumulation was found in certain plant organs. We found accumulated arsenic in the leaves (0.497 mg kg^{-1}). These results do not coincide with literature reports. *P. australis* belongs to the Poaceae family, characterized for having a great deal of roots promoting the accumulation of trace elements like As (Desjardins et al., 2018). According to Zhang et al. (2009) reports, there was an increase of As the roots of *Phragmites communis* (Trin., 1763).

Metals accumulated in the following order: root > stem > leaf. Higher concentration in the roots is due to the uptake of elements from the substrate, whereas the low concentration found in the stems may be due to their function as carriers of nutrients and minerals from the soil to aerial parts (Avci and Deveci, 2013).

BCF was determined in order to assess the accumulation efficiency of As, Cu and Fe by *P. australis.* For Fe and Cu the values were greater than one, in sludge and soil + *P. australis* and Sludge + *P. australis* treatments, during the two sampling months. These data indicates that *P. australis* is a hyper-accumulating species of these two metals, following the criterion established by Baker and Brooks (1989). The discrimination analysis showed that there were no significant differences in the BCF of Cu among the sampling months. However, the Sludge + *P. australis* treatment had a slightly higher value, indicating that Cu uptake efficiency is better in soilless substrates. The reason is the changes in the substrate characteristics, such as the pH increase and the bonding affinity of metal particles to the substrate (Avci and Deveci, 2013). In the case of arsenic, BFC value changed along the time and the treatments. We observed significant differences in this metal among different months and treatments, which can be attributed to leaching, and may be also to the chemical similarity between P and As contents in the substrate, as mentioned by Yan et al. (2017); Escutia-Lara and Lindig-Cisneros (2012). The roots were not able to uptake arsenic and the presence of other metals reduced the accumulation capacity of arsenic, coinciding with the reports of Desjardins et al. (2016).

BCF value for Cu (2.03) in *P. australis* was higher, compared to the reports from other species, including *Malva parviflora* (0.85), *Datura stramonium* L. (0.79), *Citrullus colocynthis* L. (0.84), *Lycium shawii* (Roem and Schult., 1990) (0.94) (Ibrahim et al., 2013). BCF value in Fe (7.38) was higher in *Elodea canadensis* (Michx.) (3.93), but it was lower, compared to *Polytrychum commune* (Hedw.) (0.9) and *Spirogyra* sp. (Link.) (10.25) (Busuioc et al., 2012). In both cases, Cu vales depended on the species, type of substrate, pH, PR and metal bioavailability (Desjardins et al., 2018; Ezeudo, 2014).

The translocation factor determining the capacity of metals to move from the roots to aerial parts was higher for arsenic (5.76 > 1), showing that Arsenic has great capacity to move into the leaves. This capacity can be the result of physiological factors of the species. *P. australis* has an extensive root system that can favor Arsenic permeability. Furthermore, this plant has the capacity to accumulate and transfer Arsenic from the roots to aerial parts (Yan et al., 2017). With regards to Cu (0.23) and Fe (0.17) TF

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values were smaller than one, indicating that these metals are not easily taken to *P. australis* aerial parts. According to Padmavathiamma and Li (2009), immobilization may be due to metal scavenging by the vacuoles or by the cell wall in the roots, inhibiting the interaction with high molecular weight compounds of the cytoplasm.

Conclusions and recommendations

-The order of heavy metals removal by *P. australis* was the following: Fe > Cu > As
-pH, PR and MO affected the mobility and availability of As, Cu and Fe.
-*P. australis* is a tolerant species capable of accumulating large concentrations of Fe and Cu in its organs, according to the following order: root > stem > leaf.
-The values of high BCF and low TF indicate that *P. australis* is a plant species that can be used to phytostabilize substrates polluted with Fe and Cu.

In the process of phytoremediation metal removal was observed not attributed to the plants so it may be appropriate to carry out leaching tests such as the Tessier scheme, in the waste sludge, soil and mixture in order to identify if it is because of these processes that decrease the concentration of metals in the waste sludge.

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