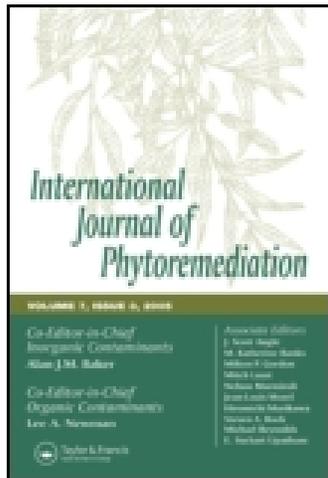


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Phytoremediation of Landfill Leachate with *Colocasia esculenta*, *Gynerium sagittatum* and *Heliconia psittacorum* in Constructed Wetlands

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This study assessed the accumulation of Cd (II), Hg (II), Cr (VI) and Pb (II) in *Gynerium sagittatum* (Gs), *Colocasia esculenta* (Ce) and *Heliconia psittacorum* (He) planted in constructed wetlands treating synthetic landfill leachate. Sixteen bioreactors were operated in two experimental blocks. Metal concentrations in the influent and effluent; root, stem, branch and leaves of plants were analysed, as well as COD, N-NH₄⁺, TKN, T, pH, ORP, DO, and EC. Average removal efficiencies of COD, TKN and NH₄⁺-N were 66, 67 and 72%, respectively and heavy metal removal ranged from 92 to 98% in all units. Cr (VI) was not detected in any effluent sample. The bioconcentration factors (BCF) were 10⁰–10². The BCF of Cr (VI) was the lowest: 0.59 and 2.5 (L kg⁻¹) for Gs and He respectively; whilst Cd (II) had the highest (130–135 L kg⁻¹) for Gs. Roots showed a higher metal content than shoots. Translocation factors (TF) were lower, He was the plant exhibiting TFs >1 for Pb (II), Cr (T) and Hg (II) and 0.4–0.9 for Cd (II) and Cr (VI). The evaluated plants demonstrate their suitability for phytoremediation of landfill leachate and all of them can be categorized as metals accumulators.

Keywords: bioconcentration factor, constructed wetlands, heavy metals, landfill leachate, translocation factor, Colombia

Introduction

Sanitary landfills are the most widely used method of solid waste disposal around the world. Landfill leachate (LL) is recognized as one of the most critical issues for landfill operators (Ziyang *et al.* 2009). Landfill leachate (LL) may contain large amounts of organic matter (biodegradable, and refractory to biodegradation), as well as ammonia-nitrogen, heavy metals (HM), chlorinated organic and inorganic salts (Renou *et al.* 2008). Lead, cadmium, mercury, and chromium can simultaneously prevail in the environment as a result of various human activities. LL is certainly one of the main anthropogenic sources for these heavy metals. Besides, HM are widely known to be non-essential elements for plants and they can cause adverse effects on the plant's photosynthetic system, chlorophyll synthesis, and antioxidant enzyme production, resulting in various forms of damage to the plants (Milone *et al.* 2003).

Phytoremediation applications have been recommended as cheaper and more effective alternatives for the removal and recovery of HMs from aqueous solutions (Mant *et al.* 2006). However, its use and application is often limited by the unavailability of appropriate plant species. Constructed wetland (CW), apart from applications to domestic sewage or biodegradable wastewaters, can also clean other wastewater types like agricultural run-off, acid mine drainage, storm water as well as industrial wastewater and landfill leachate (Vymazal 2009).

Several plants can take up HMs, translocate them into the shoots, and sequester them in non-metabolic-active tissues in less harmful forms (Kupper *et al.* 2007). It is worth noting that most known hyperaccumulators species are selective toward one metal and would not be effective at multi-metal mixes (Kamnev and van der Lelie 2000). The choice of plant species is an important issue in CWs because they should survive the potential toxic effects of the influent and its variability. Aquatic plant species, including free-floating species such as *Eichhornia sp.*, *Lemna sp.*, *Azolla sp.*, *Salvinia sp.*, submerged species such as *Potamogeton sp.*, *Myriophyllum sp.*, or emergent species like *Limnocharis flava*, *Typha sp.*, *Scirpus sp.*, *Spartina sp.*, *Phragmites sp.*, and *Cyperus sp.*, have shown potential for removing metals from different wastewaters (Dhir

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et al. 2009; Liu *et al.* 2010; Soda *et al.* 2012; Anning *et al.* 2013; Voijant Tangahu *et al.* 2013). Successful application of phytoremediation, however, depends of the identification of plant species (endemic and particularly indigenous species) with an appropriated suite of characteristics (Massa *et al.* 2010).

Therefore, this work was aimed to assess the suitability of three tropical plant species *Gynerium sagittatum* (Gs), *Colocasia esculenta* (Ce), and *Heliconia psittacorum* (He) for phytoremediation of metal contaminated landfill leachate Cd (II), Hg (II), Cr (VI), and Pb (II) using subsurface horizontal flow constructed wetlands at microcosm scale under tropical conditions. The specific objective was to determine the accumulation potential of the aforementioned heavy metals by these plants.

Materials and Methods

Location and Experimental Design

The experiment was carried out during 60 days in a greenhouse setting located at Universidad del Valle in Cali–Colombia- (3° 22' 23. 64'' N, 76° 31' 54. 15'' W) under natural light conditions (average radiation 542 $\mu\text{mol s}^{-1} \text{m}^{-2}$), photoperiod of 12:12 hr, air temperature of 27°C, and 66% relative humidity. The experiment followed a factorial design with two factors: i) plant species (three species) and ii) heavy metal concentration in water (two concentrations). The bioreactors were arranged in two experimental blocks to accommodate randomly the two treatments (T1 & T2), and they were run in parallel. Sixteen horizontal sub-surface flow CWs (0.60 × 0.30 × 0.50 m in length, width, and depth, respectively) were fitted in fibreglass tanks and operated at microcosm scale. Each block consisted of six planted (three native tropical species, each one in duplicate) and two unplanted (in duplicate) wetlands. Each unit was filled to a depth of 0.45 m with gravel ($\phi = 25$ mm and porosity, $\eta = 38.6\%$). All constructed wetlands were daily fed by gravity with synthetic landfill leachate under semi-continuous (8 hr d^{-1}) regime with a water flow of 0,01 $\text{m}^3 \text{d}^{-1}$, setting an average Hydraulic Retention Time (HRT) of 3 days. Additionally, six extra units with the same characteristics mentioned above were used as control for growth and physiological response of the plants (data no show), and were fed daily only with tap water and Hoagland solution.

Plant Species

Healthy-looking young plants of the three tropical species (Table 1) *Gynerium sagittatum* (Gs), *Colocasia esculenta* (Ce), and *Heliconia psittacorum* (He), were collected from a nearby local nursery. The selected plant species have not yet been tested extensively for wastewater treatment. However, some studies reported that these species showed good performance in a growth studies (Torres and Vasquez 2010) and Hg (II) and nutrient removal from wastewater (Skinner *et al.* 2007; Bindu *et al.* 2008). The above-mentioned species are readily found in the native flora near of the study area.

Transplanting of Test Plants

Forty plants (0.05–0.07 m in height) from each species were placed in plastic pots (one plant of each species per pot) and were irrigated three times a week (300 ml each) with water and modified Hoagland solution (Shiyab *et al.* 2009). After three weeks, three cuttings of each species (similar size, approximately 0.1–0.15 m in height) were randomly assigned to each wetland unit and were subsequently planted, setting an equivalent average plant density of 17 transplants or cuttings m^{-2} . The specific location of the constructed wetlands inside the greenhouse was also randomly allocated to minimise the error due to likely irradiance gradients.

Immediately after the transplantation, the plants were watered with modified Hoagland solution three times a week during three weeks. The composition of the nutrient solution was (mg L^{-1}): 1200 $\text{Ca}(\text{NO}_3)_2$, 505 KNO_3 , 135 KH_2PO_4 , 988 MgSO_4 , 2.86 H_3BO_3 , 1.81 $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 0.08 $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 0.22 $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.11 $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$, 3.00 $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$. Next, the wetlands units were operated for three weeks under batch conditions; a volume of effluent from a pilot constructed wetland treating domestic wastewater was added as inoculum (20 litres per unit). This was intended to allow for the establishment of a healthy microbial biofilm community on the gravel matrix. All these preliminary activities were the first steps of the acclimation period and then the wetlands units were gradually fed with synthetic LL first without heavy metals, and after five weeks of acclimation, the actual experimentation with metal amended in the landfill leachate started up.

Synthetic Landfill Leachate

Synthetic landfill leachate was used in this study in order to avoid potential interferences in the bioremediation process by toxic organic compounds present in LL. The key pollutants targeted in the leachate were COD, BOD, N (TKN, NH_4^+ -N), PO_4^{3-} , Cd (II), Hg (II), Pb (II), and Cr (VI). The COD consisted of short-chain fatty acids like acetic: propionic: butyric in proportions 73%: 23%: 4%, respectively (Moreno 2009). NH_4Cl was employed as ammonium source. With respect to HMs, HgCl_2 , PbSO_4 , $\text{K}_2\text{Cr}_2\text{O}_7$, and CdCl_2 salts were used. All the chemicals were purchased from Merck, Germany (purity of 99,99%). The experimental heavy metal concentrations were: T1: 60.37; 283.8; 86.33; 1183.64 and T2: 71.56; 593.87; 253.6; 2016.45, $\mu\text{g L}^{-1}$ for Hg (II), Pb (II), Cd (II), and Cr (VI), respectively. Each solution was prepared in 1-litre stock solution with distilled water. The landfill leachate was daily prepared for reactor feeding to avoid organic decomposition or precipitation of some salts. The main characteristics of the LL are shown in Table 2. Inflow and outflow was measured daily, using volumetric methods (bucket and chronometer). Evapotranspiration rates were calculated every day as a difference between inflow and outflow.

Sampling and Chemical Analyses

The influent and effluent were analyzed weekly for COD, NH_4^+ -N, NO_3^- -N, TKN and fortnightly for HMs, PO_4^{3-} -P,

Table 1. Main characteristics of the selected plant species used in this study

Family	Species	General description	Picture of the species at the beginning of the study.	Picture of the species at the end of the study.
Poaceae	<i>Gynerium sagittatum</i> (Aubl.) Beauv. (<i>Gs</i>)	Tall shrub with a grass-like habit. Stems are straight and erect, usually 5 or 6 m in height and 0.02 to 0.03 m in diameter.		
Araceae	<i>Colocasia esculenta</i> (L.) Schott. (<i>Ce</i>)	Perennial herb up to 1.5 m, with thick shoots from a large corm; slender stolon's are often produced, along with offshoot corms.		
Heliconiaceae	<i>Heliconia psittacorum</i> L. f. (<i>He</i>)	Perennial herb, flowers have 0.02 to 0.03 m long peduncles that rise above the pointed bracts The pinkish-red bracts arise from a central point on the stem. Plant indigenous to the Amazonian rainforest.		

Source: SIAC (2008).

and BOD₅. All of the parameters were determined according to APHA-AWWA (2005). Likewise, T, pH, ORP, EC, and DO were measured five times a week using a portable meter VWR symphony SP90M5 (OpticsPlanet, USA). All equipment was properly calibrated following the manufacturer instructions.

Table 2. Characteristics of the influent synthetic landfill leachate used in this study

Parameter	Influent Mean ± SD
COD (mg L ⁻¹)	735.5 ± 143.6
BOD ₅ (mg L ⁻¹) ^a	391.6 ± 25.4
TKN (mg L ⁻¹)	132.4 ± 46.4
NH ₄ ⁺ -N (mg L ⁻¹)	99.4 ± 28.2
NO ₃ ⁻ -N (mg L ⁻¹)	1.6 ± 0.6
PO ₄ ³⁻ -P (mg L ⁻¹) ^a	7.3 ± 3.4
Temperature ^b -T (°C)	23.2 ± 2.3
pH ^b (un)	5.3 ± 0.9
Dissolved Oxygen ^b – DO (mg L ⁻¹)	4.2 ± 0.4
Electric conductivity ^b -EC- (μS cm ⁻¹)	1482.5 ± 253.2
ORP ^b (mV)	222 ± 134.8

(N = 9; a. N = 5; b. N = 48)

Heavy Metal Determination in Plant Tissues

At the end of the experiment, all plants were harvested, washed thoroughly with tap water, and rinsed with deionized water. The whole plants were weighted using a digital scale (FENIX, LEXUS Electronics Scale, ±0.1 g). Then, the plants were divided into aboveground and belowground tissue and weighted again. Subsequently, the samples were dried at 80°C for 24 hours (MLW Warmeschrank WS oven) and were ground with a crushing machine (IKA 14 basic, A11-2 blades). Approximately, 0.5 g of sample material was digested in 10 ml of HNO₃ (65%) and underwent microwave digestion (CEM-Mars 5 X-press Duotemp, version 194A07). Cd (II), Pb (II), and Cr (total) concentrations present in the samples were determined by an ICP-MS (Inductively Coupled Plasma - Mass Spectrometer; Thermo scientific Type: X-series 2, ± 1 μg L⁻¹). Hg (II) was measured by using cold vapour atomic absorption spectrometry (Shimadzu AA 6300, ± 1 μg L⁻¹) with hydrides generation (Shimadzu HVG-1). Cr (VI) was determined colorimetrically using a spectrophotometer (Perkin Elmer, LAMBDA 20, ±2.8 μg L⁻¹) at 540 nm wavelengths. Certified standard sewage sludge (amended soil) samples from the Netherlands (No-143R. ID No 0811, May-04-2011) were used as quality control samples for the analyses.

Heavy Metal Determination in Water

50 ml of water sample was mixed with 0.5 ml of HNO₃ (65%) and then the HMs were determined by the same analytical method used for the plant samples.

Finally, in order to know the background heavy metal concentration of different matrixes used in these experiment, the background concentration of heavy metals was analysed in roots, stems and leaves of plants and gravel and found to be below the detection limit of the equipment.

Bioconcentration and Translocation Factors

The Bioconcentration Factor (BCF, L kg⁻¹) was calculated as follows (Soda *et al.* 2012):

$$BCF = C_P / C_W \quad (1)$$

Where C_P is the metal concentration in the whole plant tissue (mg kg⁻¹-DW) and C_W is the metal concentration in water (mg L⁻¹). A large value of BCF implies a better phytoaccumulation capability.

The translocation factor (TF) was calculated by dividing the metal concentration in aboveground tissues by that accumulated in the underground tissue:

$$TF = C_A / C_u \quad (2)$$

Therein, C_A is the metal concentration in aboveground tissues (mg kg⁻¹-DW) and C_u is the metal concentration in underground tissue (mg kg⁻¹-DW). Larger values of TF >> 1, imply a higher translocation capability.

A two-way analysis of variance test (ANOVA) was performed on the heavy metal accumulation data sets in the plants. The least significant difference (LSD) was used for multiple comparisons at *p* < 0.05 between treatment means. The R programming language software (version 2.13-2012) was used for all statistical analysis. Shapiro wilk and Bartlett tests were conducted for normality and homogeneity of variance, respectively.

Results

Water Quality in the Experimental CWs

The experimental CWs showed a good pollutant removal capacity. Average removal efficiencies for COD, TKN and NH₄⁺-N were 66, 67, and 72%, respectively, for the studied conditions (T1 & T2). CWs removed a good proportion of the HMs, reaching average values from 92 to 98% for both experimental conditions (Table 3). A special condition emerged with Cr (VI), which was not detected in any of the effluent samples. It was also found that as the HMs concentrations increased in the influent, the unit's removal performance improved too.

Table 3. Effluent water quality (average concentrations) in the constructed wetlands along the study period

Parameter	Treatment	CW-Gs		CW-He		CW-Ce		Unplanted	
		Average	SD	Average	SD	Average	SD	Average	SD
COD ^a (mg L ⁻¹)	T1	246,6	172	277,4	167,6	230,2	128,9	311,9	178,2
	T2	349,4	190,1	333,3	213,8	292,4	167,6	330,5	232,9
TKN ^a (mg L ⁻¹)	T1	43,2	21,9	43,1	28	45,7	23,9	45,3	26,5
	T2	41,9	23,2	48,9	28,1	45,7	23,9	48,9	22,3
NH ₄ ⁺ a (mg L ⁻¹)	T1	27,5	16,1	31,1	16,1	28,3	15	32,3	17,7
	T2	32,5	17,1	32,7	17,9	31,5	16,7	34,6	16,2
PO ₄ ^{3-b} (mg L ⁻¹)	T1	0,7	0,9	1,7	1,5	1,7	1,2	1,2	1,1
	T2	0,9	0,9	1,3	1,3	1,9	1,7	0,8	0,9
Cd ^{2+b} (μg L ⁻¹)	T1	6,5	10,3	5,6	4,3	7,7	11,2	4,8	5,5
	T2	8,9	5,5	9,1	6,8	7,7	5,4	8,8	8,0
Pb ^{2+b} (μg L ⁻¹)	T1	45	125	24,4	39,3	23	47,5	21	42,6
	T2	13,9	26,4	14,6	32,9	10,3	20,3	13,8	26,4
Hg ^{2+b} (μg L ⁻¹)	T1	4,4	4,5	4,5	4,2	5,1	5,2	4,5	5,6
	T2	4,4	2,2	4,9	2,6	4,8	1,8	4,3	2,2
pH ^c	T1	8,0	0,2	8,1	0,3	8,1	0,2	8,1	0,2
	T2	8,0	0,3	8,0	0,3	8,1	0,2	8,6	0,2
T ^c (°C)	T1	25,1	2,6	25,2	2,4	25,1	2,5	25,1	2,4
	T2	25,4	2,1	25,510	2,4	25,4	2,2	25,4	2,2
DO ^c (mg L ⁻¹)	T1	2,1	0,7	1,7	0,7	1,9	0,7	1,8	0,8
	T2	0,8	0,7	0,6	0,6	1,2	0,8	0,8	0,7
EC ^c (dS m ⁻¹)	T1	1139,3	182,4	1179,3	189,5	1178,0	182,2	1167,6	182,9
	T2	1175,0	182,4	1203,2	175,6	1169,2	171,5	1180,5	176,2
ORP ^c (mV)	T1	-321,9	179,3	-356,6	139,3	-337,2	145,0	-354,8	133,1
	T2	-384,2	117,9	-404,4	74,0	-385,1	102,5	-391,8	108,0

(a: N = 9; b: N = 5; c: N = 48)

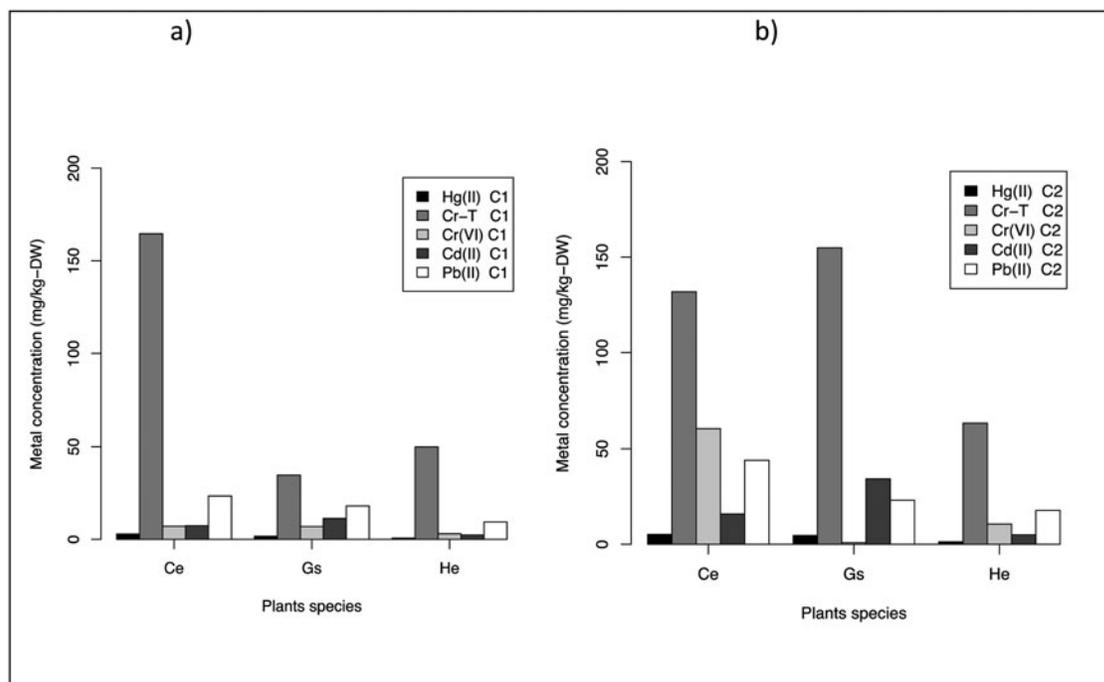


Fig. 1. Heavy metal contents in whole plant a) CWs T1, b) CWs T2.

Bioconcentration and Translocation Factors in the Native Plants

As heavy metal concentrations in the influent increased, the plant species accumulated greater amounts of metals in their tissues (Figure 1). This is in agreement with similar effects reported in other works with emergent plants (Skinner *et al.* 2007; Ye-Tao *et al.* 2009; Soda *et al.* 2012). Total Cr was the element with the highest accumulation for the three emergent species with values between 63.4 and 164.6 mg kg⁻¹ dry weight (DW). Cr (VI) was found in the emergent species, but not in all tissues, with *Ce* being the plant species that accumulated this metal most for both treatments with values of 7 and 60.5 mg kg⁻¹ DW, respectively. Hg (II) was the least accumulated element in the plant species investigated (0.7 mg kg⁻¹ DW).

Although the uptake of each metal by the plant species may be different, a small tendency was found: the metal with high concentrations in the landfill leachate showed high concentrations in the plant tissues, except for Cr (VI) in the *Gs* and *He* species.

The lowest BCF (Table 4) values were obtained for Cr (VI): 0.4 and 2.5 (L kg⁻¹) for *Gs* and *He*, respectively; whilst the highest BCF occurred with Cd (II) (130 to 135 L kg⁻¹) in *Gs*. The BCF tended to increase as the metal concentration in the water increased. Meanwhile, the BCF values for the remaining metals fluctuated between 10¹ and 10².

Metal translocation from the roots to other plant tissues showed different orders of magnitude (Table 4). *Ce* presented TF < 1 for all metals, showing that this plant species does not distribute much of the accumulated metals in its tissues, leaving the greatest amount in the roots; Cd (II) was even not detected in the shoots. Hg (II) was the element with the highest TF (1.7)

in the *Gs* species. *He* is an emergent plant that showed the best distribution of metals in its tissues, in treatment 1 (T1) two HM had TF values > 1 and for the rest of the heavy metal in treatments T1 & T2, the values fluctuated between 0.4 and 0.9. As for the BCF, TF values increased as the metal concentration in the leachate increased, but no relation was found ($p < 0.05$) to indicate that a high bioconcentration factor implied a high translocation factor. Hg (II) and Cd (II) BCFs in the studied species are affected by the concentration of the metal in water and the type of plant tissue ($p < 0.05$). None of the evaluated plant species revealed visible signs of chlorosis at the end of the experiment.

Discussion

Water Quality

The removal efficiencies of heavy metal of all constructed wetlands were between 84 and 95% (Table 3). This removal capacity in all reactors remained almost constant during the whole study period. The data suggest that due to the high metal concentrations entering the reactor; a large amount of metals is removed, indicating that a higher concentration of pollutants in the LL improves the response of the reactor. Furthermore, the quality of the CW effluent for the case of Cd, Pb, and Cr were below the Colombian standards for wastewater treatment plant effluents discharging into a fresh watercourse intended for human consumption downstream (Cd (II) 10, Pb (II) 50, and Cr (VI) 50 $\mu\text{g L}^{-1}$). For Hg (II), however, the concentration in the effluent was still twice higher than the Colombian standard (2 $\mu\text{g L}^{-1}$) (Ministerio de Salud de Colombia, 1984).

Table 4. Metal concentration in the root and shoot (mg kg^{-1}), bioconcentration factor-BCF (L kg^{-1}) and translocation factor-TF in the native plants investigated

Plant	Treatment		Hg (II)	Cd (II)	Pb (II)	Cr (VI)	Total-Cr
<i>Colocasia Esculenta</i>	T1	Root	1,9 ^a	7,3 ^a	12,5 ^b	ND	112,6 ^a
		Shoot	0,8 ^a	ND	9,8 ^a	4,6 ^a	47,3 ^a
		BCF	48 ^a	85 ^c	82 ^d	6 ^b	100 ^b
		TF	0,4 ^c	—	0,8 ^e	—	0,1 ^e
	T2	Root	2,6 ^a	15,9 ^a	20,6 ^b	28,8 ^c	95,4 ^a
		Shoot	2,3 ^a	ND	23,2 ^a	31,8 ^a	25,7 ^a
		BCF	73 ^a	63 ^c	74 ^d	30 ^b	58 ^b
		TF	0,9 ^e	—	1,1^e	1,1^e	—
<i>Gynerum Sagittatum</i>	T1	Root	1,5 ^a	7,5 ^a	9,3 ^b	ND	24,6 ^a
		Shoot	0,2 ^a	3,9 ^a	8,5 ^a	3,5 ^a	11,9 ^a
		BCF	27 ^a	130 ^c	64 ^d	5,9 ^b	21 ^b
		TF	0,1 ^e	0,6 ^c	0,9 ^e	—	0,4 ^e
	T2	Root	1,7 ^a	28,2 ^a	18,1 ^b	0,4 ^c	139,0 ^a
		Shoot	2,9 ^a	6,0 ^a	5,7 ^a	ND	15,8 ^a
		BCF	64 ^a	140 ^c	39 ^d	0,4 ^b	68 ^b
		TF	1,7 ^e	0,2 ^c	0,3 ^e	—	0,1 ^e
<i>Heliconia psittacorum</i>	T1	Root	0,4 ^a	2,3 ^a	4,6 ^b	1,7 ^c	22,6 ^a
		Shoot	0,3 ^a	ND	6,1 ^a	1,3 ^a	27,3 ^a
		BCF	$1,2 \times 10^{1a}$	$2,6 \times 10^{1c}$	$3,0 \times 10^{1d}$	$2,5 \times 10^{0b}$	$3,0 \times 10^{1b}$
		TF	0,9 ^e	—	1,3^e	0,7 ^e	1,5^e
	T2	Root	0,6 ^a	5,0 ^a	10,6 ^b	7,6 ^c	47,0 ^a
		Shoot	0,7 ^a	ND	7,1 ^a	3,0 ^a	16,5 ^a
		BCF	19 ^a	20 ^c	30 ^d	5,3 ^b	28 ^b
		TF	1,2^e	—	0,7 ^e	0,4 ^c	0,4 ^c

ND: not detected. TF > 1.0 is in bold. Different letters as superscripts in the same type of column indicate significantly different values at $P < 0.05$.

Plant Accumulation

The emergent plants used in the CWs revealed considerable variations in the uptake and HM translocation abilities through phyto-extraction. This fact shows that the appropriate selection of the plant species can be crucial to improve the efficiency of HM removal in CWs (Brisson and Chazarenc 2009). In this study, Table 4 and Figure 1 demonstrated that native emergent species could tolerate and accumulate heavy metals like Cd (II), Hg (II), Cr (VI), and Pb (II), and still showed healthy growth (data not shown) in waters containing multiple HM. This feature is similar to previous reports with emergent plants employed in constructed wetlands such as *Phragmites australis*, *Arabis paniculata*, *Brasica juncea*, *Limnorcharis flava*, *Thalium geniculata*, *Typha latifolia*, and *Colocasia esculenta* (Lesage *et al.* 2007; Liu *et al.* 2007; Skinner *et al.* 2007; Moreno *et al.* 2008; Soda *et al.* 2012; Anning *et al.* 2013).

Around the world, over 400 species have been identified as heavy metal hyper-accumulators, many of which hyper-accumulate Ni (Baker *et al.* 2000). Fourteen and two species are hyper-accumulators of Pb and Cd, respectively (Ye-Tao *et al.* 2009). This feature has led to research on species that are not hyper-accumulators, but which possess rapid growth (data not shown) like the three native tropical species evaluated in this study. In fact, these plants grew healthy (data not shown) in spite of the environmental conditions in which they were kept for 60 days.

Under the studied conditions, no significant differences were noted among the concentrations of the HMs evaluated in the water and the accumulation in roots and shoots of the plants (Table 4). Likewise, translocation factors did not present any significant difference (Table 4), suggesting that the concentration of each heavy metal taken up does not interfere with the transfer of the three other metals in the plant tissues.

The total metal concentration is considered a poor indicator of the metal availability for the plants (Yoon *et al.* 2006). This was also true for our study, where no correlation between the concentration of Hg, Cd, Pb, and Cr in plants and their concentration in the water was found (data not shown). A tendency was noted for increased contents of the metals in the plant (root and shoots) when the metal concentrations in the landfill leachate increased, though the metal uptake was not linear with the increased concentrations (Table 4).

Bioconcentration Factor

The bioconcentration factors values were between 100 and 200 times higher than those reported by Ye-Tao *et al.* (2009) for Pb and Cd, who worked in a hydroponic setting with *Arabis paniculata*. According to data by Soda *et al.* (2012), the BCF for the *Acorus gramineus* and *Cyperus alternifolius L* species found for Cr and Pb were respectively 44 and 200 times higher than those found in this study. Meanwhile, Skinner *et al.* (2007)

worked with Hg (II) (concentrations in water of 0.5 and 2 mg L⁻¹) in four plant species, including *Colocasia esculenta*. They reported bioconcentrations factor values (the Hg (II) content was only determined in the root) between 20 and 48 orders of magnitude below those values found for this species in the current study. This condition surely confirms that different plant species develop diverse mechanisms to tolerate or accumulate heavy metals, most surely depending upon specific environmental conditions (Liu *et al.* 2010).

A concentration ratio of belowground (BG) and aboveground (ABG) ((BG/ABG)_{HMs}) between 0.9 and 15.9 was found for the heavy metal, with the highest value for Cd(II) in *Gs* and lowest for Hg(II) in *He*. (BG/ABG)_{HMs} is nearly always greater than 1 for the different HM, which confirms that roots are the primary site of metal uptake and therefore are usually found to be much higher than in leaves or shoots (Vyazal *et al.* 2009; Galleti *et al.* 2010). *Gs* was the species whose average Hg and Cd concentration in the shoot was the highest at 2.87 and 5.98 mg kg⁻¹, respectively. *Ce* accumulated the highest amounts of Pb (II), Cr (VI), and total Cr with values at 23.2, 31.79, and 47.32 mg kg⁻¹, respectively (Table 4). This accumulation capacity in the case of Pb and Cd is well below (43 and 22 times lower, respectively) the threshold defined for hyper-accumulators of these metals (Soda *et al.* 2012). This condition was confirmed by the finding that many of the average translocation factor values were <1 (Table 4), indicating that the capacity of the plant species to translocate the metals from the root to the shoots was rather limited.

Translocation Factor of Mercury

The key characteristic of the metal hyper-accumulator plants is the efficient transport of metals from the root to the shoots, characterized by TFs >1 (Zhao *et al.* 2006). In our study, the translocation factor for Hg (II) in *He* was >1. In *Gs* for the highest Hg concentration (T2) in water, translocation factor was >1 and it is 17 times higher than the TF for the lower concentration of Hg in the water. For *Ce*, this factor was below one. These results indicate that the Hg (II) concentrations in the root compared to those in the shoots were higher by at least one order of magnitude for *Ce* in both experimental conditions. In the two other species evaluated, this condition appeared for the lowest concentration of mercury in water. Hg (II) has a greater tendency to be translocating within the plant when the metal concentration in the water increases (Table 4); this is particularly interesting, considering the high toxicity and persistence of the metal in the environment. Giri and Patel (2011) established that for *E. crassipes* at Hg (II) concentrations in the water above 5 mg L⁻¹, translocation and bioconcentration factors diminish. Moreno *et al.* (2008) and Skinner *et al.* (2007) found a similar situation, but with Hg (II) concentrations in water at 2.5 and 2.0 mg l⁻¹ for *B. juncea* and *C. esculenta*, respectively.

Translocation Factor of Lead and Cadmium

Regarding Pb (II) and Cd (II), the concentrations of these metals in the root were higher than in the shoots by more than

one order of magnitude, except for *Ce* and *He* for Pb, where the root-shoot relation was <1 in the two cases, indicating mobility of this metal toward the plant's aerial tissues. *Gs* was the only species where Cd was detected in all of the organs with a root-shoot relation >1, confirmed with TF values <1, meaning low mobility of this metal toward other plant tissues.

Cd or Pb bears numerous harmful effects in the biochemical machinery required for cell survival. Both Cd and Pb have many action sites within the plant (Niu *et al.* 2007). It is more probable that accumulation of these metals is associated to a sequestration mechanism in a less toxic form. The accumulation mechanism of these heavy metals and the plant response to those toxic metals is quite complex and cannot be explained without more profound research. In general terms, Cd is more toxic than Pb and the damage this metal causes is greater than the damage generated by Pb (Niu *et al.* 2007), affecting plant growth and their tolerance to other metals. In our study, the three native species evaluated accumulated more Pb than Cd (Figure 1), thus suggesting a plausible defence mechanism against Cd toxicity.

Cell sequestering Cd or Pb may have major effects on free Pb or Cd levels in the symplast and this may potentially influence movement of these metals throughout the plant (Salt *et al.* 1995). However, average levels of Cd and Pb found in tissues (root) of the species evaluated (Table 4) was below the phytotoxic range: Cd 5–700 mg kg⁻¹ (Chaney 1989, cited by Bonanno and Lo Guidice 2010) and Pb 30–300 mg kg⁻¹ (Bonanno and Lo Guidice 2010). In this sense, the native species never reached the thresholds defined for the species hyper-accumulating Cd and Pb, but these ones can be classified as accumulators because they showed good bioconcentration and translocation factor values between 0.4 and 1.3, respectively.

Translocation Factor of Chromium

This metal was not found in none of the tissues of the species studied, except for *He*. Concentrations of Cr (VI) in roots were above data reported in other emergent species used in CWs (Bonanno and Lo Guidice 2010; Yadav *et al.* 2010). Translocation of this metal to the plants aerial parts was not good; only in *Ce*, for the highest Cr concentration in the landfill leachate, this factor was >1, indicating on the one hand this metal has a good mobility capacity in plant tissues, but on the other, that uptake was not linear with the increased metal concentration in the leachate. For the other two plant species, the translocation factor was <1, but with a slight decrease as the metal concentration in the water increased.

The oxidation state of Cr strongly influences its uptake rate by the plants. Cr (VI), can easily cross the cell membrane and the sulfate and phosphate fluxes can help transporting the chromate anions (Giri and Patel 2011). This poor translocation of Cr (VI) to the shoots could be due to sequestration of Cr in the vacuoles of the root cells to render it non-toxic, which may be a natural toxicity response of the plants. It must be noted that Cr is a toxic and nonessential element to plants, and hence, the plants may not possess any specific Cr transport mechanisms (Shanker *et al.* 2005) Chromium concentrations above 0.5 mg kg⁻¹ in plant tissue generate toxic effects (Yadav

et al. 2010). In this study, the roots and shoots of the *Ce* and *He* species had concentrations above this value (Table 5), but did not show toxicity symptoms like chlorosis or wilting. For *Gs*, the values accumulated were below the threshold in both tissues (roots and shoots).

Conclusions

This study demonstrated the capacity of the three tropical native species to treat LL with significant concentrations of Cd (II), Pb (II), Cr (VI), and Hg (II). The concentration of these metals was different in each species and depends significantly on the type of plant tissue. The underground tissues (root) showed a greater accumulation capacity compared to the shoots.

The important difference in levels of Cd, Hg, Cr, and Hg found in these tissues may imply low mobility of these metals from the root to the plant's aerial tissues, a condition that was equally validated against the low TF values that in general terms remained <1. In spite of the good bioaccumulation of the metals, these revealed a decreasing tendency in the order of Pb > Cd > Cr (VI) > Hg; and in general terms accumulation decreased in organs in the following order: root > stem > leaf.

The species evaluated in this study did not reach the thresholds to be classified as hyper-accumulators, but nevertheless showed good performance so as to label them as accumulators of the heavy metals investigated. *G. sagittatum* was the species with the best performance for the four metals, followed by *H. psittacorum* and *C. esculenta*, respectively.

This work indicates that biological systems like the constructed wetlands investigated, planted with the tropical species *Ce*, *Gs*, and *He*, may accomplish water quality requirements according to the current Colombian standards (heavy metal concentration) and attain reduction levels similar to those obtained with highly mechanized systems, but and most importantly with potentially lower costs. Hence, development of this types of constructed wetlands at full-scale is an attractive technology for landfill leachate treatment in countries with low resources and high necessities to protect the environment and public health.

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