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BIOMASS PRODUCTION AND HEAVY METAL ABSORPTION BY FOUR PLANTS GROWN AT THE MORAVIA DUMP, MEDELLÍN, COLOMBIA.

Producción de biomasa y absorción de metales pesados por cuatro plantas crecidas en el basurero Morovia, Medellín, Colombia.

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ABSTRACT

Dumps are sites where the presence of high heavy metal (HM) concentration is a common occurrence, creating the need for implementing restoration processes immediately after their closure. In the 7.6 ha and 45 m high Morro de Moravia dump, arose from the disposal of Medellín solid wastes from 1974 to 1984, previous studies have demonstrated high contents of contaminants, including HM, prompting the need to identify effective mechanisms to implement its restoration. The objective of this study was to evaluate the adaptation, growth and phytoremediation capacity of *Bidens pilosa*, *Lepidium virginicum*, *Brachiaria decumbens* and *Arachis pintoi*. Content of HM (mg/kg) in Moravia residue matrix went from 17 to 8193 for Pb, 44 to 564 for Cr, 0.2 to 339 for Cd and 77 to 1679 for Ni. Measurements of plant cover, plant height and dry matter production at all plant species studied suggested adequate growth and adaptation to the Moravia dump conditions. Plant absorption of HM showed the pattern Cr > Cd > Ni > Pb. Estimated bioconcentration factors were generally low, and maximum values were 0.36 in *A. pintoi* (Cr), 2.96 in *B. pilosa* (Cd) and 0.26 in *B. decumbens* (Ni). However, our estimations of the phytoremediation potential of the assayed species, suggested they possess low remediation efficiency. Further investigation should be carried out in order to identify more efficient HM accumulators, and to test the use of technologies such as modification of pH, rhizoremediation or the use of genetically enhanced accumulators to increase HM availability to plants

Key words: *Bidens pilosa*, *Brachiaria decumbens*, chromium, lead, phytoremediation.

RESUMEN

En los basureros se observan altas concentraciones de metales pesados (MP), creando la necesidad de conducir procesos de restauración en dichos lugares. En el Morro de basuras de Moravia, con 7,6 ha y 45 m de altura, conformado por la disposición de los residuos sólidos de la ciudad de Medellín entre 1974 y 1984, estudios anteriores demostraron alto contenido MP, evidenciando la necesidad de identificar mecanismos efectivos para su restauración. El objetivo de este estudio fue evaluar la adaptación, crecimiento y capacidad fitorremediadora de *Bidens pilosa*, *Lepidium virginicum*, *Brachiaria decumbens* y *Arachis pintoi*. El contenido de MP (mg/kg) en la matriz de residuos de Moravia varió entre 17 y 8.193 para Pb, 44 a 564 para Cr, 0,2 a 339 para Cd y 77 a 1.679 para Ni. Las mediciones de cobertura, altura y producción de materia seca mostraron que todas las especies evaluadas tuvieron un nivel adecuado de adaptación y crecimiento a las condiciones del basurero de Moravia. La absorción de MP presentó el orden Cr > Cd> Ni > Pb. Los factores de bioconcentración estimados fueron bajos, siendo los valores máximos: 0,36 (*A. pintoi*, Cr), 2,96 (*B. pilosa*, Cd) y 0,26 (*B. decumbens*, Ni). Sin embargo, nuestras estimaciones del potencial de fitorremediación de las plantas evaluadas sugieren que éstas poseen baja eficiencia fitorremediadora. Debe dirigirse investigación con el fin de identificar especies acumuladoras de MP más eficientes o introducir tecnologías que aumenten la disponibilidad de los MP, tales como la modificación del pH, rizorremediación o el uso de plantas acumuladoras genéticamente modificadas.

Palabras clave: *Bidens pilosa*, *Brachiaria decumbens*, cromo, fitorremediación, plomo.

INTRODUCTION

The term heavy metals (HM) is commonly used in literature to refer to a group of metals with a density greater than 6 g/cm^3, with biological functions not yet fully understood and recognized as human health and environmental threats. Among this group of contaminants, As, Cd, Hg, Pb, Tl y U represent a great threat to the environment, since accumulation of these elements is associated to soil, air and water pollution around the world. Although micronutrients such as Ni and Cr do not meet the criteria described above, they are also included in the HM group since high contents of these elements are associated with adverse effects on the environment (Alloway, 1995; Kota and Stasicka, 2000; Shanker *et al*., 2005).

Content of HM on non-polluted soils is low. However, in some regions of the world, considerable high contents have been reported as a result of several industrial or waste disposal activities. Concerns related to accumulation of HM relate to the fact that they might enter the food web through different mechanisms and could bioaccumulate in higher organisms. Such condition poses a threat not only to the environment but also to human health with several negative conditions such as neurological and respiratory system damages, lung and kidney cancer and dermatitis among others, being associated to the exposure, ingestion or inhalation of HM (World Health Organization, 2007).

Plant absorption of HM depends on several factors inherent to soil and plant characteristics. High mobility and bioavailability of HM in soils is normally related to low pH values (<5) and low organic matter content in soils (Alloway, 1995). In turn, different groups of plants among ornamental, crops and wild species have been identified as HM accumulators. Such species include several families among which Asteraceae, Brassicaceae, Caryophyllaceae, Cyperaceae, Cunouniaceae, Fabaceae, Flacourtiaceae and Lamiaceae are the most common (Prasad y Freitas, 2003; Pilon-Smits, 2005; Wei *et al*., 2008).
Due to their purpose (final disposal of municipal wastes), landfills and dumps usually contain high amounts of HM, thereby becoming a threat to human health and to the environment. Associated risks are even greater in situations where dump operations do not include a residue separation program or a leachate treatment. Natural or man directed revegetation following the closure of landfills and dumps can be very important to control erosion, improve the aesthetic value and contribute to the decontamination of these places. The use of plants to clean polluted sites, known as phytoremediation, has received a great deal of attention during the lasts two decades. Among the advantages of phytoremediation its low cost in comparison to other cleaning methods and a public acceptance as a green technology, are reported. Using phytoremediation technologies the extent of pollution can be reduced and soil chemical properties and biological activity could be improved (Vangronsveld *et al*., 1995, Nagendran *et al*., 2006). A drawback of this body of scientific research is that a considerable fraction of these studies has been conducted under controlled conditions, adding HM to non-contaminated soils in greenhouse experiments, thus differing from actual field conditions.
The open dump known as Morro de Moravia (Moravia dump hereafter) was the designated final disposal site for all sources of municipal wastes in Medellín from 1970 to 1984. Starting as a 30 m mine pit, the residue matrix grew to a hill of 45 m high and became a serious threat to the environment and public health in the city. Since the beginning of the dump operation, Moravia dump has been inhabited, and a population of near 5000 people still resides there. After the dump's closure, municipal authorities conducted a series of preliminary studies in order to assess the pollution level as well as to decide about the adequate measurements to restore Moravia (Integral, 2000). In a more detailed study, Sánchez *et al*., 2009, aimed to determine the content of Hg, Pb, Cr, Cd and Ni in the local residue matrix (RM) and spontaneous vegetation samples. High levels of these five HM were detected and some samples (both, matrix and plant tissues) showed contents that greatly exceeded permissible limits in the Colombian and European normative (ICONTEC, 2004; ASCP Guidelines, 2001). The present work, aimed to evaluate *in situ* plant growth and HM absorption of the plant species *Bidens pilosa*, *Lepidium virginicum*, *Brachiaria decumbens* and *Arachis pintoi* grown at four sites in the Moravia dump, as a measure of their phytoremediation capacity.

MATERIALS AND METHODS

SITE DESCRIPTION

The hill-shaped Moravia dump, is located at 6° 13' N, 75° 34' W in Northern Medellín, Antioquia, Northwest Colombia, alongside the right margin of the Medellín River. The dump has an area of 7.6 ha and a height of 42.5 m from its base. The hillsides show mean slope values of 10° (North-South direction) and 25° (East-West direction). The phreatic level was determined in 19.8 m (highest quote). The residue hill started to grow as Medellín government designated a former mine pit as the place for the final disposition of solid residues of domestic, industrial, agricultural and health care origins. Superficial layers (0-40 cm) of the RM at Moravia show a dark sandy soil mixture with different proportions of plastics, broken glass, concrete, wood, metals, semi degraded fabrics and organic matter. These layers also contain various types of pipes for drinkable and residual water transport around the inhabited houses. As it is common in most open dumps, the Moravia dump does not have a drainage system or a system for the treatment of leachates. According to 2008 calculations, the dump is producing a total leachate effluent of 1.78m^3/day (Sánchez *et al*., 2009).

Due to the progressive reallocation of Moravia inhabitants conducted by Medellín municipal authorities, several plots in Moravia dump have been emptied and a revegetation process (both spontaneous and man influenced) is currently taking place. In such plots, it is evident the predominance of the herbaceous stratum. In our previous study (Sánchez *et al*., 2009), it was determined that *B. pilosa* and Urochloa maxima were the most abundant plant species.

A final feature of the Moravia dump is the presence of a clay - covered plots, originated when municipal authorities placed a 20 cm - thick clay layer on top of the RM, in an attempt to diminish soil erosion and thus, dispersion of pollution.

Selection of Planting Sites

Figure 1 shows the localization of the four sites selected for the evaluation. Criteria for site selection included presence/no presence of a clay superficial layer, slope gradient and exposure to leachates. Sites were designated by numbers 1 to 3 and had the following characteristics:

- Site 1: Local RM (this is residue matrix without a clay layer), 15° of slope and high exposure to leachates,
- Site 2: Local RM, 22° of slope and no leachate exposure
- Site 2 - clay: RM covered with a clay layer, 22° of slope and no leachate exposure
- Site 3: Local RM, slope of 34° and a medium leachate exposure.
- Selected sites were manually cleaned and weeded and large solid residues were removed.

At each site, four plots of 6 m^2 were arranged as to plant four different plant species, which were randomly allocated among the plots.

Physico Chemical Properties And Heavy Metal Content Of The Residue Matrix

Four samples of the RM, corresponding to the four sites, were analyzed for physical and chemical properties following standard procedures: texture was determined by Bouyoucos methodology (IGAC, 1990), pH was determined in water (1:1), exchangeable Ca^2+, Mg^{2+}, K^+ contents were determined by the ammonium acetate method, the effective cation exchange capacity (ECEC) was estimated as the sum of Ca^{2++} Mg^{2++} K^{2++} in cmol/kg, and organic matter (OM) was determined according to the method reported by Walkley and Black, Pansu and Gautheyrou, 2006. These analyses were carried out as they may contribute to explain HM bioavailability.

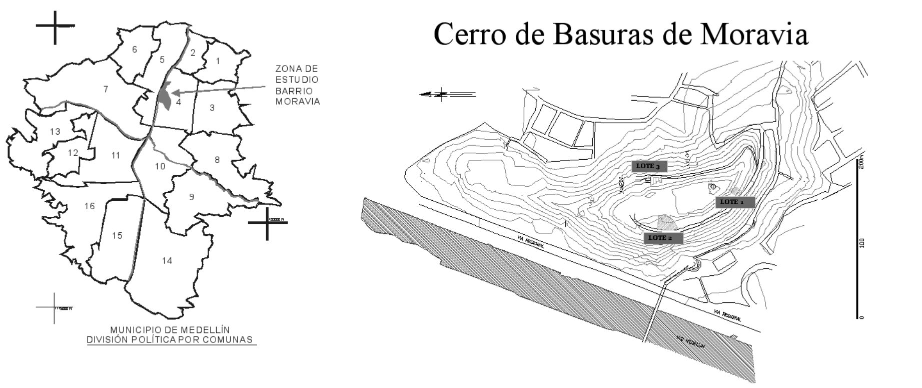

Figure 1. Moravia dump localization in Medellín, Antioquia, Colombia.

PLANT SPECIES SELECTION, ESTABLISHMENT AND EVALUATION

Plant species were planted in July 2008. Test species *B. pilosa* and *L. virginicum* were selected as our previous observations identified them as some of the most abundant plant species spontaneously growing at the Moravia dump area. The grass *B. decumbens* had been previously reported as HM accumulator (Santos *et al*., 2006). Even though to our knowledge *A. pintoi* has not been reported in any HM absorption study, the erosion control and esthetical advantages presented by this legume made *A. pintoi* an attractive alternative to revegetate dump sites.

In order to obtain the propagation material for planting, seeds of plant species *B. pilosa* and *L. virginicum* were harvested from plants growing at Moravia, *B. decumbens* seeds were purchased at a agricultural seed supplier and 45 days old *A. pintoi* cuttings were purchased from a local garden supplier. The species *B. pilosa*, *L. virginicum* and *A. pintoi* were planted to a final density of 6 plants/m^2 whereas *B. decumbens* was planted to a final density of 3 kg seed/ha. Plant species were grown under natural conditions, with no addition of amendments or fertilizers, although irrigation was provided for the first two weeks after establishment. This was done with the purpose of evaluating plant adaptation without adding extra cost to a future remediation process based on the use of these plants.

PLANT GROWTH

Plant height and plant cover were registered at five sampling times, corresponding to 45, 60, 75, 90 and 105 days after plant establishment (DAE). At each sampling time, five individuals per plot were randomly selected. Plant height was measured in cm by straightening individuals to their fullest length. Due to the prostrate growth habit of the species *A. pintoi*, this variable was not recorded in *A. pintoi* plots. Plant cover of *A. pintoi*, *B. pilosa* and *L. virginicum* was measured in cm^2, as the surface projection area of each individual plant, according to Steubing *et al*., 2001. Plant cover of *B. decumbens* was evaluated by means of visual scale on the four plots.

Visual observations for signs of phytotoxicity, disease and attack of plagues were also carried out at the same sampling times.

Production of dry matter (DM) in plant species was determined at 60 and 105 DAE. Briefly, the roots and aerial tissues of two randomly chosen plants per plot were collected, taken to the laboratory and gently washed with tap water. Tissues were dried (60 °C) up to constant weight and DM production estimated as g/m^2.

Heavy Metal Content in Residue Matrix and Plant Samples

Total content of Pb, Cr, and Ni on the RM was determined before planting and when planted species reached 60 and 105 days after establishment (DAE). In each plot, samples of the RM top layer (0-20 cm) were collected and transported to the laboratory, where large size fragments were removed. The remaining substrate was mixed to obtain a representative sample from each plot, thus collecting a total of 16 soil samples, corresponding to one sample per plot.

Content of Pb, Cr, Cd and Ni in plant tissues, was determined at 60 and 105 DAE. Two individual plants randomly selected per plot, were prepared in the same way as described for the DM determination.

Determination of HM both in residue matrix and plant tissues was carried out at the GDCON Laboratory, University of Antioquia, following standard procedure methodology SW-846 3050B (EPA, 1996). Briefly, 1 - g samples were subjected to calcination at 450 °C and the resulting ashes were subjected to acid digestion using a 1:1 mixture of HNO_3 and $HClO_4$. Concentration of HM was determined by means of atomic absorption, using a GBC 932spectrophotometer and HM content estimated as mg/kg of DM. Detectable limits (mg/kg) were 0.001 for Hg, 0.125 for Pb, 0.250 for Cr and Ni and 0.050 for Cd.

Estimation of the Bioconcentration Factor and Heavy Metal Extraction

The bioconcentration factor (BCF) is defined as the ratio between the HM concentration in the plant tissue and the soil or substrate in which the plant is growing. BCF is an estimate of species ability to take up HM from soils. Plant species with BCF greater than 1 are considered to have phytoremediation potential, Sun *et al*., 2009; Zhang *et al*., 2007. In this study, the estimation of BCF was conducted at 60 and 105 DAE.

The extraction of HM in terms of mg/plant and mg/m2, were calculated on a DM basis, and were also carried out at 60 and 105 DAE. For the first estimate (mg/plant), the DM value was multiplied for the mg/kg of HM in dry plant tissues, whereas for the second estimate (mg metal/m^2) the mg metal/plant was multiplied by the density of plants per m^2.

Statistical Analysis

Data obtained for the different variables and estimates were subjected to an analysis of variance (ANOVA) using a randomized block design with four treatments corresponding to the four plant species evaluated. For each variable (plant cover, plant height and plant HM content), an independent analysis for each sampling time was conducted. Where significant differences were observed, mean values were separated by means of the Duncan test. When the ANOVA analysis showed a significant ($P<0.05$) interaction between sites and plant species, linear contrasts were calculated in order to compare mean values for both, sites and plant species. Linear contrasts (L) for sites were conducted by the criteria described below:

L1: Site 2c (clay covered) *vs.* local RM sites 1, 2 and 3

L2: Sites 2 and 3 (slope >20°) *vs.*
L3: Sites 2, 2c and 3 (with slope < 20°) *vs.* Site 1 (with slope < 20°)
L4: Sites within the same localization within the dump (2 and 2 - clay) *vs.* distant plots (3+1)
L5: Site 1 *vs.* Site 3
L6: Site 2 *vs.* Site 2 - clay
For plant species there were two linear contrasts made:
Ls1: *B. pilosa* and *L. virginicum vs. B. decumbens* or *A. pintoi*, this is the comparison between plant species previously found among the spontaneous vegetation *vs.* plant species introduced to the Moravia dump
Ls2: *B. pilosa vs. L. virginicum*.

RESULTS AND DISCUSSION

Physical and Chemical Properties

There were great differences in physical and chemical properties (Table 1) between samples obtained from the RM sites and the sample collected from the site covered with clay. Samples of the RM were classified as sandy loam, whereas the clay-covered site was classified as clay loam. In what refers to cation content, Ca contents were high in all RM samples, being almost three times higher than the clay-covered site. In turn, contents of Mg were low in RM (average 1.8 cmolc/kg) and about 6 times higher in samples obtained from the clay - covered site. Potassium content was high for RM sites and low in the clay - covered site. ECEC values ranged from 21.9 cmolc/ kg (site 2 - clay) to 33.3 cmolc/kg (site 2). Organic matter content varied greatly going from 7.8 to 12.9% in RM samples with the corresponding value for site 2 - clay being only 1%. According to Cuesta, 2006, OM content values greater that 5% in semitropical climate are considered high. Soil pH showed a narrow range, going from 7.0 (site 3) to 7.6 (sites 1, 2 and 2c). Since properties such as texture, pH and OM content are good estimators of fertility; it appears that Moravia, being closed for more than 20 years, still provides more than adequate plant growth conditions for plants (Cuesta, 2006). The site covered with clay showed very low OM content, which in turn is associated to low availability of N, and thus plant growth could be reduced.

Property	Site 1	Site 2	Site 2c	Site 3
Sand (%)	66	68	32	60
Lime (%)	24	20	36	24
Clay (%)	10	12	32	16
Classification	Sandy loam	Sandy loam	Clay loam	Sandy loam
Ca (cmolc/kg)	29.6	28.5	11.0	27.8
Mg (cmolc/kg)	1.6	2.5	10.7	1.4
K (cmolc/kg)	1.8	2.28	0.16	1.87
ECEC (cmolc/kg)	32.6	33.3	21.9	31.3
% Organic matter	12.9	9.1	1.0	7.8
pH	7.6	7.6	7.3	7.0

Table 1. Physicochemical properties of the Moravia residue matrix.

Total HM Content in Residue Matrix Samples

The total HM content in samples collected at the four sites of the Moravia dump is shown in Table 2. There were not significant differences in content of any of the assayed HM among sites, as SEM values of HM concentration both, between sites and sampling times were high.

Sampling Time	Plot	Pb	Cr	Cd	Ni
First (0 DAE)	1	3.391.0	154.5	7.30	87.3
	2 clay	16.6	476.7	0.20	1.679.0
	2	4.850.0	98.2	7.96	124.5
	3	3.794.0	323.6	4.65	153.2
Second (60 DAE)	1	722.3	147.2	33.91	8755.8
	2 clay	34.8	219.2	1.47	254.0
	2	462.4	82.1	4.48	76.6
	3	505.4	145.9	7.69	88.8
Third (105 DAE)	1	8.193.5	564.0	3.98	151.8
	2 clay	94.2	221.0	1.15	130.3
	2	426,8	273.4	5.64	124.6
	3	603.8	367.4	7.96	173.6
	Mean Value	1.924.6	256.1	7.20	983.3
	SEM	747.0	43.8	2.55	718.2
Normal range in soils (World Health Organization, 2007)		10-30	14-70	0.07-1.1	3-1000
Reported mean values in a landfill in Eritrea (Tesfai and Dresher, 2009)		597.5	186.4	3.3	87.7
Colombian Regulation for Maximum HM limits in compost (ICONTEC, 2004)		300	1200	39	420
Swiss Regulation for Maximum HM limits in compost (ASCP Guidelines 2001)		120	100	1	30

Table 2. Total HM content (mg/kg DM) in samples collected at four plots of the Moravia dump at three different times after plant establishment (0, 60 and 105 DAE).

When comparing total HM contents of the matrix samples against the maximum limits permitted by the Colombian normative for compost (ICONTEC, 2004), the highest Pb content was 27 times that limit and the highest Ni concentration was 20 times that limit. It should be considered that this normative is not as rigorous as similar normative (ASCP guidelines, 2001), under this normative, our results are more disquieting not only for Pb and Ni, but also for Cr and Cd.

The highest concentrations of Pb Cr, Cd and Ni values (mg/kg) were found at site 1, which could obey to high exposure to leachates, as samples collected at this site came from a region localized under a very steep slope, thus receiving a great deal of water infiltrated through the upper ground. Besides this, there was no other association between HM content and site characteristics.

The large variation in HM content could be explained in terms of the high heterogeneity of the Moravia dump, itself a reflection of the miscellaneous nature and the random disposal of municipal wastes. According to Nagendran, 2006, landfills constitute a heterogeneous environment as a result of the diversity of the residues they receive. Likewise, Kasassi *et al*., 2008, explained the high variation in Ni values observed in landfills as the result of the random disposition of batteries and electric equipment waste. Such condition could also explain the extremely high Pb values observed in the present study.

A practical consideration for this high variation in HM content across Moravia is that it adds difficulty to the selection of a single plant species for any phytoremediation approach, being more likely for a plant consortium to be needed for the efficient extraction of the HM present there.

Characteristics of the Moravia residue matrix such as OM content, pH and ECEC values can be used to estimate HM bioavailability. In this regard, several studies have reported that soils with high clay content have superficial negative charges that efficiently adsorb soil cations, including HM. By contrast, low (<5) soil pH values are associated to high concentrations of H^+ ions that can replace HM cations, leaving these in solution and ready to be absorbed by plants. Normal ECEC values ranged from 0 to 60 cmolc/kg and exhibit an inverse relationship with cation availability. High contents of OM are inversely correlated to HM bioavailability since OM increases the soil absorption capacity towards metals (Alloway, 1995; Yin *et al*., 2002; WSTB, 2003; Pilon-Smits, 2005). According to our determinations for these parameters, it should be expected for HM bioavailability from Moravia RM to be low.

However, our expectation of low HM availability should not be interpreted as if the environmental threat that Moravia represents is low. In this regard, it is important to note that toxic metals can enter the food web not only by plant tissue absorption, but also through direct ingestion of particulate material (World Health Organization, 2007). Hence, adequate control measures must be taken as soon as possible to reduce the risk that the Moravia dump currently represents for both human beings and the environment.

Plant Growth and HM Absorption

Plant cover. Mean plant cover values (average of 15 observations) are shown in Table 3. As the number of DAE increased plant cover increased going from 3.5 to 4.5 $cm^2/day/m^2$ between 45 and 105 DAE ($R^2 \geq 0.78$). Our observations showed that at the end of the evaluation period, most (ca. 90-95%) of the plots were covered with the different plant species studied.

Days after establishment	Moravia Plots				Linear contrasts
	1	2 clay	2	3	
45	248.2b	265.13b	455.6a	275.53b	
60	448.6	538.0	803.8	507.9	High slope (2 *vs.* 3)* (1+3) *vs.* (2 native + 2 clay)* 2 *vs.* 2c*
75	667.1	821.6	1149.5	955.0	High slope *vs.* Flat (2+3 *vs.*1)*
90	1206.1	1711.2	2189.3	1370.1	
105	1792.9	1308.4	1723.6	1904.2	

Table 3. Average plant cover (cm2) at the four Moravia Plots. *Means with different letters are significantly different from each other (P < 0.05) according to the Duncan test.

At 45 DAE, the highest plant cover (455.6 cm^2) was found at site 2 (P<0.05). At 60 DAE, linear contrasts 3, 4 and 6 showed significant differences (P<0.05), with plant cover on plot 2 being higher (803.3 cm^2) than the mean value of the other three sites (498.2 cm^2). At 75 DAE, sites 2 and 3 showed the highest values (1150 and 955 cm^2), compared to 667 cm2 for site 1 (P<0.05). At 90 and 105 DAE there were not significant differences in plant cover among sites.

Plant cover values for plant species are shown in Table 4 (average of 20 observations). At 45 DAE, *A. pintoi* had a higher plant cover than *L. virginicum* (P<0.05), which could be associated to the *A. pintoi* initial biomass being greater than that of the other studied species. At 60 and 75 DAE, linear contrasts showed no significant differences between local and introduced species, but at 60 DAE *B. pilosa* had higher plant cover (685 cm^2) than *L. virginicum* (P<0.05). At 90 and 105 DAE, *B. pilosa* continued to show higher plant cover than *L. virginicum* (P<0.05).

Days after establishment	*L. virginicum*	*B. pilosa*	*A. pintoi*	Linear contrasts
45	222.4b	321.9ab	389.1a	
60	431.5	685.9	606.5	*L. virginicum vs. B. pilosa**
75	843.2	1055.9	795.9	
90	1132.4b	2062.6a	1662.6ab	
105	1271.6b	2068.8a	1706.5ab	

Table 4. Plant cover (cm^2) as average among four plant species. *Means with different letters are significantly different from each other (P < 0.05) according to the Duncan test.

In general, plants growing on sites not covered with clay had greater plant cover values and this could be related to the higher OM, Ca and K contents observed in this site as compared to the clay covered site. It is accepted that OM could enhance plant growth. On the other hand, it is noteworthy to observe that the site with the highest leachate and HM content was the one that had the lowest plant cover for most of the evaluation period.

Plant height. As observed with cover, plant height increased as the number of DAE increased, with this increase being in average 0.93 cm/day/plant for all plant species from 45 to 105 DAE (R2 ≥ 0.98).

Mean plant height values for sites (average of 15 observations) are shown in Table 5 and those for plant species (average of 20 observations) are show in Table 6. There were significant interactions (P<0.01) between plots and plant species at all sampling times. At 45 and 60 DAE, height of plants growing on the clay site was greater than those growing on sites not covered with clay (P<0.05). However, at 105 DAE this situation was reversed, with plants growing on local sites being in average 13% higher (P<0.05). At 75 DAE, plants growing at site 1 had the highest height values (26.3 and 51.3 cm^2; P<0.05). When comparing plant height across species, linear contrasts showed significant differences between *B. pilosa* and *L. virginicum* at 60, 90 and 105 DAE (P<0.01) with *B. pilosa* showing the greater plant height at all these sampling times. There were no significant differences between local (*B. pilosa* and *L. virginicum*) and introduced plant species (*B. decumbens* and *A. pintoi*) during the period evaluated.

Judging by plant cover and height, all plant species studied showed adequate adaptation

Days after establishment	Moravia plots				Linear contrasts
	1	2 clay	2	3	
45	16.2	14.9	9.9	7.7	Natives *vs.* Clay* High slope *vs.* Flat (2+3 *vs.*1)** 1 *vs.* 3** 2 *vs.* 2c**
60	26.3	28.1	22.17	21.4	Natives *vs.* Clay* High slope *vs.* Flat (2+3 *vs.*1)* 2 *vs.* 2c*
75	51.3	42.9	39.1	37.9	High slope *vs.* Flat (2+3 *vs.*1)* 1 *vs.* 3*
90	61.7	53.1	62.7	49.2	High slope (2 *vs.* 3)** 1 *vs.* 3** 2 *vs.* 2c*
105	72.3	59.7	65.5	65.6	Natives *vs.* Clay* (1+3) *vs.* (2 native + 2 clay)*

Table 5. Plant height (cm) as average of four plots in Moravia dump. *P < 0.05. **P < 0.01.

Days after establishment	*L. virginicum*	*B. pilosa*	*B. decumbens*	Linear contrasts
45	11.43	14.30	10.90	
60	21.90	28.20	23.35	*L. virginicum vs. B. pilosa**
75	40.55	50.55	37.40	
90	41.60	71.15	57.30	*L. virginicum vs. B. pilosa***
105	42.70	85.05	69.60	*L. virginicum vs. B. pilosa***

Table 6. Plant height (cm) observed as average among four plant species. *P < 0.05. **P < 0.01.

to the Moravia conditions, suggesting all these four species could be successfully used in the revegetation process needed at Moravia. However, based on plant height and cover, *B. pilosa* appears to be the most promising plant species.

Dry matter production. Values of DM production (g/m^2) are presented in Table 7. Plant DM production increased throughout the evaluation period in all plant species, with mean values among plant species going from 22.77 (*B. decumbens*) to 68.47 (*B. pilosa*) g/m^2 at 60 DAE and from 112.8 (*L. virginicum*) a 357.9 (*B. decumbens*) at 105 DD. Although no significant differences were observed (sites or species), *B. decumbens* DM production was higher indicating that this Poaceae is a promising alternative for rehabilitation processes due to its vigorous DM production. Biomass accumulation by *B. pilosa* and especially *L. virginicum* was not favored by plant structure, given their smaller foliar area as compared to *B. decumbens*, which grew erect with numerous and vigorous stems. Additionally, a severe attack of Lepidoptera larvae reduced the biomass production in *L. virginicum* growing in site 3 and although plants recovered from this attack, their biomass production was clearly reduced. It is clear that DM production strongly affects both HM extraction and the efficiency of the phytoremediation process.

Plant heavy metal content and extraction. Values of HM content (mg/kg), HM plant extraction (mg/plant) and HM extraction (mg/m^2), all calculated on a dry matter basis

Days after establishment	Plant Species	Dry matter	HM content (mg/kg)			HM Plant Extraction (mg/plant)			HM Extraction (mg/m^2)		
		(g/m^2)	Cr	Cd	Ni	Cr	Cd	Ni	Cr	Cd	Ni
60	*A. pintoi*	50.07	23.7	1.3	NA	0.21ab	0.01	NA	1.17	0.08	NA
	B. pilosa	68.47	32.8	3.6	NA	0.40a	0.07	NA	1.91	0.32	NA
	B. decumbens	22.7	29.8	8.2	NA	0.03b	0.01	NA	2.97	0.26	NA
	L. virginicum	54.50	21.1	3.9	NA	0.24a	0.01	NA	1.11	0.23	NA
105	*A. pintoi*	147.42	17.2	NA	8.9	0.29ab	NA	0.24	1.47b	NA	1.37
	B. pilosa	194.50	8.9	0.7	4.4	0.49a	NA	0.16	2.33b	NA	0.79
	B. decumbens	357.85	24.4	NA	11.0	0.31ab	NA	0.13	30.78a	NA	12.97
	L. virginicum	112.83	7.4	0.09	4.4	0.14b	NA	0.18	0.64b	NA	0.81

Table 7. Dry matter production (g/m^2), HM content (mg/kg) and HM extraction (mg/m^2) in plant species grown in four plots at Moravia dump.

are presented in Table 7. No acute toxicity due to HM absorption was evident in *B. pilosa*, *B. decumbens* and *L. virginicum*. However, there was an initial chlorosis in *A. pintoi* plants which occurred at 45 and 60 DAE, after which plants fully recovered. With regard to the tolerance of plant species to HM contamination, Sun *et al*., 2009, reported high tolerance of *B. pilosa* to soils artificially contaminated with 100 mg/kg of Cd. In their study, plants only had a 22% reduction in chlorophyll content, and other physiological parameters did not change significantly from the values observed in the control plants.
Absorption of HM exhibited the following patterns:
Lead: In all samples collected at 60 DAE, Pb content was under the detection limit (0.05 mg/kg). However, at 105 DAE in five of the 16 plant tissue samples detectable Pb concentrations were found, which ranged from 0.77 to 32.38 mg/kg (data not shown).
Nickel: Ni absorption in plant tissues followed a similar pattern as that of Pb with Ni not being detected in plant tissues collected at 60 DAE. However, at 105 DAE most of the analyzed samples showed different contents of this metal with values ranging from 4.4 to 11.0 mg/kg, although no significant differences were observed across plant species or sites (P>0.05).
Chromium: Most collected samples contained detectable amounts of Cr at 60 and 105 DAE, ranging from 21.1 to 32.8 (60 DDE) and from 7.4 a 24.4 (105 DDE) mg/kg on a DM basis. There were no significant differences among sites or plant species. However, there were differences between the two sampling times (P = 0.007).
Cadmium: Detectable Cd contents were observed at 60 DAE, with values ranging from 1.3 mg/kg (*A. pintoi*) to 8.2 mg/kg (*B. decumbens*). However, Cd content decreased considerably at the end of the evaluation (105 DAE) and was only found in detectable concentrations in *L. virginicum* and *B. pilosa* 0.09 y 0.71 mg/kg, respectively. Statistical analysis for 60 DAE samples did not show significant differences (P>0.05) between plant species or sites, but showed significant differences between the first and second sampling times (P = 0.004).
Plants can be classified as HM hyperaccumulators if they accumulate more than 100 (Cd), 1000 (Ni, Pb y Cu) or 10000 (Zn o Mn) mg/kg of DM (Dahmani-Muller *et al*., 2000). Sun *et al*., 2009, reported *B. pilosa* as a hyperaccumulator, as more than 100 mg/kg of Cd were detected in aerial organs of this plant species. In our study, *B. pilosa*

had high absorption of Cd and Cr, but not to the same extent as described by Sun *et al*., 2009. These differences could be associated to differences in soil properties and microenvironment, since the reported study was conducted on non-contaminated soils that had recently received and addition of Cd and our study was conducted under field conditions on soils contaminated with other HM in addition to Cd, in an ongoing process of more than 20 years.

The pattern of Cd content in plant tissue suggests that the plants tested absorb Cd very actively during their first stages of growth, thus resulting in early accumulation of this metal in their tissues. However, as plant development continues, Cd absorption stops and via an apparent dilution effect, Cd concentration diminished in plant tissues.

Bioconcentration factors: BCF were calculated as the ratio between HM content in plant tissue and that in the residue matrix (data not shown). Although there were no statistical differences among BCF values estimated for either plant species or sites, great variability was observed for these values. For example, the maximum BCF values observed were 0.36 in *A. pintoi* in the case of Cr, 2.96 in *B. pilosa* in the case of Cd and 0.26 in *B. decumbens* in the case of Ni. Overall, HM absorption by plants under the conditions of the Moravia dump had the following order: Cr > Cd > Ni > Pb.

It is commonly understood that high HM content in the soil does not necessarily relates to high content of HM in plant tissues ever since the process of plant absorption depends upon several interacting physicochemical soil properties (Pilon-Smits, 2005). In our study, HM absorption in plant tissues was low when compared to the HM contents in the residue matrix. This observation was corroborated with the calculations of BCF, which were very low, and only in exceptional cases greater than 0.2. This low mobility of HM from the matrix to plant species corroborated our previous suggestion of low HM mobility within Moravia dump matrix.

Liang *et al*., 2009, suggested that high BCF values could be good indicators of the potential for heavy metal accumulation. From this point of view, plant species tested in this study appear not to be good HM accumulators. Likewise, the parameters established to classify a plant species as hyperaccumulators, do not demonstrate the presence of hyperaccumulators among the plant species evaluated in this study.

Nonetheless, apart from BCF and accumulation capacity, additional criteria must be considered when selecting plants for a remediation process. Among others, a trait that can significantly modify the plant ability to act as an effective HM accumulator is biomass production. According to Liang *et al*., 2009, high-biomass plants can become effective HM accumulators on a per area basis.

Plant extraction (mg/kg and mg/m^2). Extraction of Cr (mg/plant) showed significant differences among plant species both at 60 and 105 DAE, with *B. pilosa* extracting the most Cr (0.4 and 0.49 mg/plant at 60 and 105 DAE, respectively). When expressing extraction of Cr in terms of mg/m^2, statistical differences were only found among plant species at 105 DAE, with *B. decumbens* extracting around 31 mg Cr/m^2 and absorption significantly greater than that of the other species evaluated ($P<0.05$).

Extraction of Cd (mg/plant) was low when compared to that of Cr and there were not significant differences among plant species at 60 DAE. Plants extracted from 0.001 to 0.07 mg Cd/plant. In turn, extraction of Ni (mg/plant and mg/m^2) did not show significant differences among plant species at any sampling time. Plant Ni extraction varied from 0.13 to 0.24 mg/plant and from 0.79 to 12.97 mg/m^2, with the highest value corresponding to *B. decumbens* collected at 105 DAE. Finally, absorption of Pb was very low regardless the plant species or the site evaluated. This was expected, as Moravia residue matrix properties are not associated with high HM bioavailability. However, a very high level of Pb was detected in *A. pintoi* sample collected at site 1, suggesting that microenvironment conditions such as leachate exposure could favor the absorption of lead into plant tissues.

The extraction/plant and extraction/m^2 could give a better idea of the phytoextraction potential of a given plant species. In our study, *B. pilosa* demonstrated the highest capacity to extract considerable amounts of Cr. However, *B. pilosa* Cr extraction/plant did not relate to its Cr extraction/m^2. This was due to differences in biomass production and plant density, as high biomass production noticeably increases HM extraction per unit of area. In this regard, Chaney *et al*., 1997, concluded that the success of phytoextraction depends mostly upon identification of suitable plants that not only concentrate metals to high levels, but also produce abundant biomass. In this study, the highest Cr extraction (ca of 31 mg/m^2) was observed with the plant species of the greatest dry matter production (*B. decumbens*). In previous reports, the grass *B. decumbens* has been shown to tolerate high Al contents in soils while displaying high biomass production, fast growing characteristics and absence of phytotoxicity symptoms, suggesting its potential as a phytoremediator species (Santos *et al*., 2006).

CONCLUSIONS

Few of the HM phytoremediation studies found in the literature have been conducted under field conditions, where the effect of soil buffering capacity influences nutrient availability to plants (Chaney *et al*., 1997). This could obey to the fact that under real conditions, results are difficult to understand given the high number of potential interactions. As the current study was conducted *in situ*, and in spite of the fact that under such experimental conditions predictions of plant HM absorption are often difficult, our observations are quite valuable in providing a realistic idea about the adaptation, growth and phytoremediation potential of the evaluated plant species.

Current HM concentrations in Moravia residue matrix represent a great pollution threat to soils, water bodies, plants and animals, including human beings and indicate an excessive pollution level that should be controlled as soon as possible. Fertility-wise, the matrix at Moravia present little limitations to plant growth, as mineral and OM contents, pH values and physicochemical properties approximate those of good agricultural soils, and that was corroborated by the acceptable growth performance of *B. pilosa*, *B. decumbens*, *A. pintoi* and *L. virginicum*. In addition, these local and introduced plant species demonstrated their tolerance to high levels of HM as no significant phytotoxicity symptoms were observed. Hence, it is possible to state that the evaluated species are good alternatives to revegetate Moravia dump area and to improve its esthetical value.

Our different approaches to estimate the phytoremediation potential of the assayed species, suggested that they possess low remediation efficiency. For example, results for rough calculations for Cr extraction by *B. decumbens* assuming 11 harvests a year suggest that a total of 4 kg of Cr/ha/year could be extracted under Moravia conditions. Since Cr

concentration in the first 20 cm of soil is around 1100 k/ha, it would take 286 years for complete removal of this HM. However, phytoremediation ability should be analyzed considering several criteria. On one hand, high phtytoextraction capacity is highly desired, even if the addition of an enhancer is required. On the other hand, economical factors as the management of harvested plant material containing high levels of HM should also be evaluated and additional factors such as the value of revegetation for erosion control and improvement of esthetical characteristics must be factors to consider when deciding the goals of a plant based landfill/dump restoration program.
Finally, it should be kept in mind, that low HM plant absorption observed within this study does not eliminate other risks of entrance of toxic metals to the food web, such as ingestion of particulate material (World Health Organization, 2007). Further investigation should be carried out in order to identify more efficient HM accumulators, and to test the use of technologies designed to increase HM availability to plants such as modification of soil pH, rhizoremediation or the use of genetically enhanced accumulators.

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