



# The response of plants growing in a landfill in the Philippines towards cadmium and chromium and its implications for future remediation of metal-contaminated soils

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## Abstract

During several visits to the Cebu City landfill in the Philippines, plants were observed growing within the area, including on top of the garbage piles. Studying the response of these plants is important in assessing which can be used in remediating metal contaminated soils. This study aimed to determine whether the plants in the Cebu City landfill excluded or accumulated cadmium (Cd) and chromium (Cr) in the plant tissues. The floristic composition of the landfill was analyzed prior to the sample collection. The samples were acid-digested before the desired elements were measured using atomic absorption spectrophotometry (AAS). The Cd and Cr concentrations in the plant root-zone soil were also measured using AAS. The results indicated that the landfill substrate was generally acidic based on the results of the pH measurement. Of the 32 plant species sampled, *Cyperus odoratus* showed potential for Cd uptake and internal transfer; *Cenchrus echinatus*, *Vernonia cinerea* and *Terminalia catappa* for Cr uptake, and *Cynodon dactylon* for Cr internal transfer. The plants in the landfill differed in their response towards the heavy metals. To confirm the behavior of *C. odoratus* towards Cd, and *C. echinatus*, *C. dactylon*, *V. cinerea*, and *T. catappa* towards Cr, controlled experiments are recommended, as the plant samples analyzed were collected from the field.

**Key words:** Cd/Cr uptake, metal contamination, metal transfer

## INTRODUCTION

For the past twenty-five years or so, studies have shown that plants growing in contaminated soils can tolerate otherwise toxic levels of certain pollutants. Plant communities respond differently to the presence of metals in the soil. Some plants have developed sensitivity to some metals even at very low concentrations while others have developed resistance to the toxic metal stress, which can be achieved either through avoidance or tolerance (Baker 1987). Tolerant plants can accumulate the contaminant in their tissue and this ability to take up pollutants including

a variety of metals differs among the plant species (Freitas et al. 2004, Szarek-Lukaszewska et al. 2004, Fischerova et al. 2006). On the other hand, excluder plants restrict contaminant uptake into their biomass (Chaudhry et al. 1998). Unlike organic compounds, the metals released from the decomposition of the waste are not degraded and can stay in the soil by sorption and precipitation for a long time (Jensen et al. 2000). During landfill stabilization, the metals present may be solubilized and become bioavailable (Cecen and Gursoy 2000). When oxygen and

<http://dx.doi.org/10.5141/ecoenv.2015.014>



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Received 17 November 2014, Accepted 26 January 2015

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oxygen-rich rain enters the landfill, metals will gradually be released from where they are bound (Ostman et al. 2006).

Plant species that have colonized an area in which other plants cannot survive have a potential role in remediating the area (Kim and Lee 2007). Studying the behavior of these plants growing on contaminated soils may be effective for selecting plants that can be used in remediating the soil. In the Philippines, studies on the type of vegetation found in landfills and the role they would play in future rehabilitation efforts seem to be limited or have not been conducted at all.

This study aimed to determine the level of cadmium (Cd) and chromium (Cr) in plant root-zone soil and in the roots, stems, and leaves of plants collected from a landfill; Cd and Cr are part of eight metals identified in polluted environments. Being highly urbanized, it is perceived that a big bulk of the waste thrown to the Cebu City landfill is composed of metal-containing household waste, and industrial and laboratory wastes. A study on the heavy metal content of combustible municipal solid waste in Denmark showed that Cd and Cr were present in the waste (Riber et al. 2005). Cd enters the environment through several activities including disposal of municipal solid waste; the metal is toxic and has a long biological half-life (Sankaran and Ebbs 2007). Cr is considered an essential element in trace amounts (Prasad and de Oliveira Freitas 2003), but the hexavalent form of Cr is relatively toxic (Shanker et al. 2005); in a study on the composition of a municipal solid waste deposition cell, it was found that 30% of the total amount of heavy metals including Cd and Cr in the wastes were in an available reactive form (Flyhammar et al. 1998).

The metal concentration values obtained were used to calculate the Bioconcentration Factor (BCF) and Translocation or Transfer Factor (TF), which were used to quantitatively express metal tolerance or avoidance.

## MATERIALS AND METHODS

### The study area

The Cebu City Landfill is located in Barangay Inayawan, Cebu City (barangay is the smallest administrative division in the Philippines) in the Philippines (Fig. 1). The city is known to remain wet and humid throughout the year due to the rainfalls and the humidity at almost 80% all year; the rainy season is seen from June to December (Weather and climate: Cebu Philippines). The city-owned

landfill covered a reclaimed area equivalent to approximately 160,000 m<sup>2</sup>. It became operational in 1998 with a projected lifespan of seven years, but stopped operating only in late 2011. Although the landfill was designed as a sanitary landfill, it did not incorporate landfill and leachate treatment systems, and thus was considered a poorly controlled landfill. The soil cover was mainly limestone mixed with the soil from the garbage and drainage. Kim and Lee (2007) described a poorly controlled landfill as one with no landfill gas and leachate treatment system and having a final soil covering of minimal thickness and low quality.

### Analysis of Cd and Cr content in the root-zone soil and plant organs

A reconnaissance survey was conducted prior to the vegetation survey conducted in February 2010 to determine the sampling sites. Plant and soil samples were collected during the vegetation survey conducted late 2009 and early 2010, prior to the closure of the landfill. Patches of vegetation on top of the wastepile were identified based on the homogeneity of the existing plant communities, with each 1 m<sup>2</sup> patch considered as a sampling site (Banaticla and Buot 2005). Thirty-two plant species were collected from areas where dumping activities were no longer done (Fig. 2). The collection of plants was similar to the one conducted by Dogan et al. (2014). At each patch, 3 to 4 individuals were randomly collected (Freitas et al. 2004) and were mixed to form a composite of the plant species.

All the reagents used were of analytical grade, and deionized distilled water was used for all dilutions and for the washing of the plant samples. The glasswares used were soaked overnight in 5% nitric acid (HNO<sub>3</sub>) solution and rinsed thrice with deionized water prior to use.

Soils were collected from the same sites as the plant samples, from a 0-25 cm depth plant root zone (Baker et al. 1994, Gonzalez and Gonzalez-Chavez 2006); the roots of some of the plants were able to penetrate the garbage under the soil cover. The root-zone soil samples were mixed to form a composite (Sankaran and Ebbs 2007). Soil and debris attached to each individual plant were removed manually and added to the composite soil samples. The soil samples were air-dried for 5-7 days. Residues including tiny roots of plants were removed before the soil was ground and sieved (Wang et al. 2003). The soil samples were microwave-digested prior to the determination of the concentration of Cd and Cr using the Atomic Absorption Spectrophotometer (AAS) (AA-6300; Shimad-

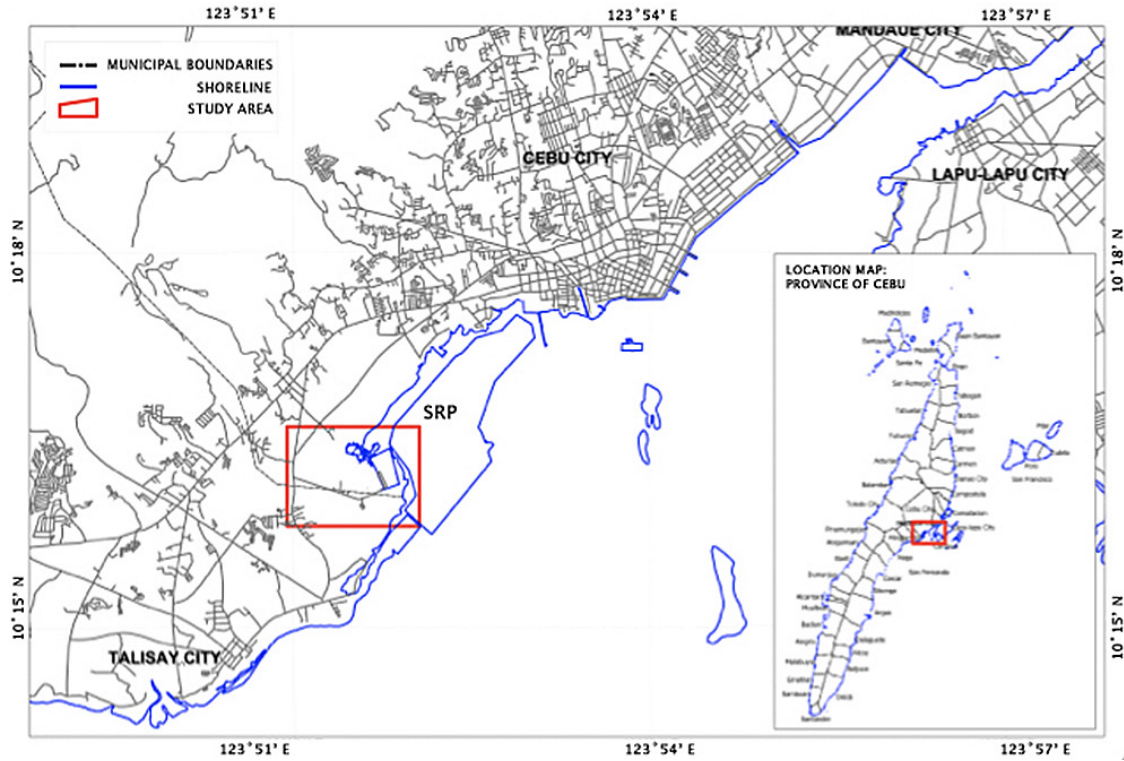


Fig. 1 Barangay Inayawan (boxed) in Cebu City, Philippines where the landfill is located.

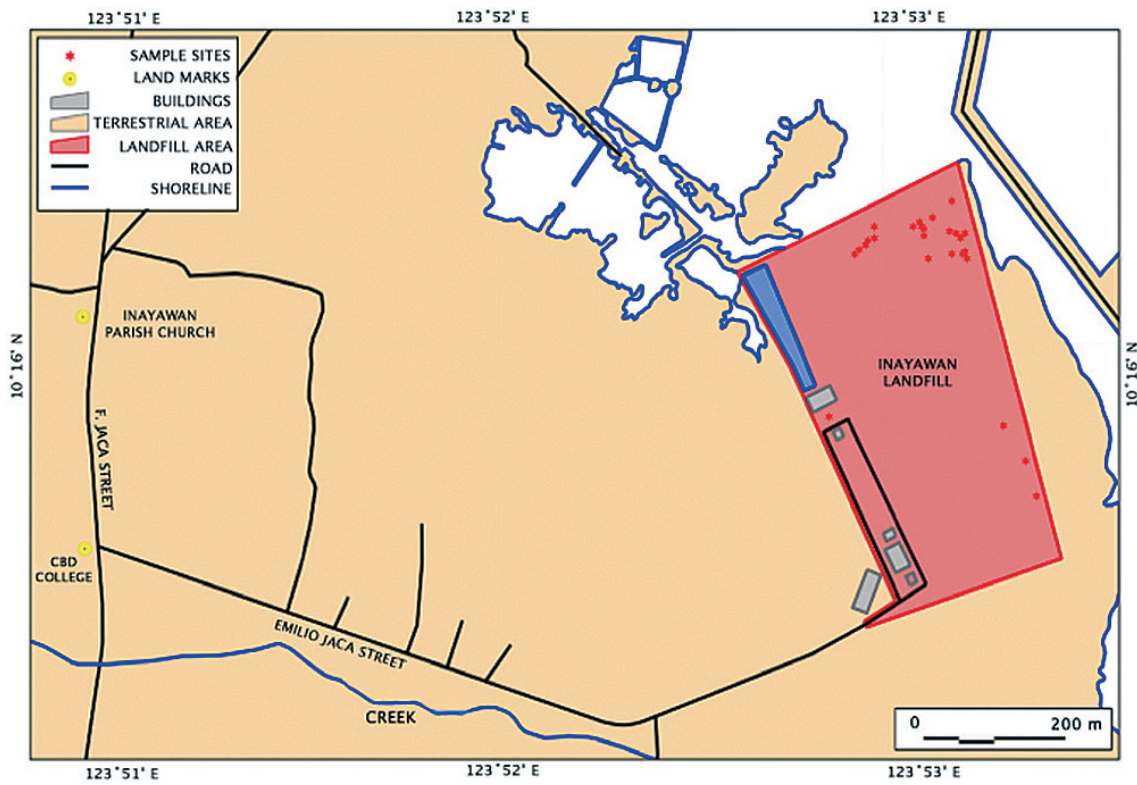


Fig. 2 Location of the sampling sites, Inayawan Landfill, Cebu City in the Philippines.

zu, Kyoto, Japan) flame technique.

Soil pH in the water and in the calcium chloride (CaCl<sub>2</sub>) solution (Rajakaruna and Bohm 2002) was measured using a pH meter (Basic pH Meter PB-20; Sartorius, Göttingen, Germany). The latter was done in order to mask the variability in salt contents of soils and to maintain the soil in a flocculated condition (Jones 2001). About 2 g of each soil sample was placed in a 125 mL Erlenmeyer flask and was added with deionized water using a 1:5 proportion. After the soil pH in the water was determined, 1-2 drops of CaCl<sub>2</sub> were added to the soil-water suspension. The suspension was stirred several times within a period of thirty minutes and was then allowed to stand before taking the pH reading (Rajakaruna and Bohm 2002).

In the laboratory, the samples of the thirty-two plant species identified plants were rinsed with tap water to

remove loose materials on the surface and then rinsed twice with ethylene diaminetetraacetic acid (EDTA) for about a minute per rinsing to remove any possible adsorbed metals (Gothberg et al. 2002). Final rinsing with deionized distilled water was done thrice. The plants were then separated into roots, stems and leaves, and then air-dried (Robinson et al. 2000, Gothberg et al. 2002, Remon et al. 2005, Segura-Muñoz et al. 2006). An agate mortar and pestle was used to ground the air-dried samples and for hard plant parts an electronic food mill was used. The samples were then oven-dried at 80°C to constant weight (Carlosena et al. 1997, Robinson et al. 2000, Sharma et al. 2008).

The wet-digestion of the samples that followed was done under a fumehood using the procedure employed by Tüzen (2003). About 2 grams each of the samples were

**Table 1.** Soil pH and level of Cd and Cr in the plant root-zone soil, Cebu City landfill in the Philippines

Root-zone soil	pH <sub>H2O</sub>	pH <sub>CaCl2</sub>	[Cd] (mg kg <sup>-1</sup> )	[Cr] (mg kg <sup>-1</sup> )
<i>Andrographis</i> sp.	6.18	5.99	1.01	19.5
<i>Borreria articularis</i>	6.67	6.50	*bdl	21.4
<i>Cardiospermum halicacabum</i>	6.77	6.51	1.51	17.1
<i>Cenchrus echinatus</i>	6.85	6.76	*bdl	28.6
<i>Chloris barbata</i>	7.00	6.91	*bdl	7.93
<i>Chromolaena odorata</i>	6.59	6.53	*bdl	67.2
<i>Coccinia grandis</i> (L.) Voight	6.69	6.49	1.05	17.9
<i>Commelina benghalensis</i>	6.99	6.66	*bdl	9.89
<i>Corchorus olitorius</i> L.	7.05	6.93	*bdl	14.9
<i>Cucumis melo</i> L.	6.40	6.31	*bdl	82.8
<i>Cynodon dactylon</i>	6.72	6.70	*bdl	22
<i>Cyperus odoratus</i>	6.44	6.67	0.63	22.9
<i>Cyperus</i> sp.	6.31	6.60	*bdl	21.1
<i>Eleusine indica</i>	7.01	6.97	*bdl	16.2
<i>Eragrostis</i> sp.	6.50	6.41	*bdl	22.1
<i>Euphorbia hirta</i>	6.75	6.63	*bdl	26.8
<i>Ficus religiosa</i>	6.46	6.43	*bdl	29.1
<i>Ipomoea triloba</i>	6.75	6.73	*bdl	56.1
<i>Ipomoea</i> sp.1	6.78	6.53	*bdl	23.3
<i>Ipomoea</i> sp.2	6.34	6.18	0.99	124
<i>Leucaena leucocephala</i>	6.46	6.43	*bdl	29.1
<i>Portulaca oleracea</i>	6.48	6.46	*bdl	47.3
<i>Phyllanthus amarus</i> Schum.&Thom	7.11	6.93	1.58	27
<i>Ricinus cumunis</i>	7.24	7.02	*bdl	10.8
<i>Saccharum spontaneum</i>	6.65	6.50	*bdl	137
<i>Senna alata</i>	6.80	6.63	*bdl	32.1
<i>Sida cordifolia</i>	6.84	6.56	*bdl	8.36
<i>Stachytarpheta jamaicensis</i> (L.) Vahl.	7.03	6.82	*bdl	10.5
<i>Terminalia catappa</i>	6.46	6.43	*bdl	29.1
<i>Tridax procumbens</i>	6.52	6.49	*bdl	23.9
<i>Vernonia cinerea</i>	6.49	6.43	*bdl	26.5
<i>Zizyphus mauritiana</i> Lam.	6.84	6.67	*bdl	17.2

\*bdl - below detection limit

placed in a 250-mL Erlenmeyer flask. Ten mL of concentrated  $\text{H}_2\text{SO}_4$  was cautiously added and the mixture was shaken until no lumps remained. About 5 mL of concentrated  $\text{HNO}_3$  was added to the mixture and mixed. A glass funnel was placed on the Erlenmeyer flask and the reacting mixture was allowed to stand overnight. The reacting mixture was heated cautiously on a hot plate until the vigorous reaction subsided. Each sample was then allowed to cool. Five mL of concentrated  $\text{HNO}_3$  was added, replacing the cover and refluxed for about 30 minutes. If brown fumes were still produced, indicating the oxidation of the sample by the  $\text{HNO}_3$ , the addition of 5 mL concentrated  $\text{HNO}_3$  was repeated over until no brown nitrous fumes were given off by the sample, indicating the complete reaction with  $\text{HNO}_3$ . During this time, a transparent solution was obtained.

The mixture was then heated until white fumes were evolved and the solution was allowed to concentrate to about 5 mL by heating it at 80-85°C without boiling for two hours. A covering of solution was maintained over the bottom of the vessel at all times. The solution was then cooled and 2 mL of deionized water and 3 mL of 3% hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) were added. The glass funnel was put back to cover the vessel and the covered vessel was returned to the heater for warming and to start the peroxide reaction. This was done carefully to ensure no losses due to excessive vigorous effervescence. The mixture was heated until the effervescence subsided and the vessel was then cooled. The  $\text{H}_2\text{O}_2$  solution was continually added in 1-mL aliquots, with warming, until the effervescence was minimal. When the digest was clear, the funnel was removed and the resulting solution was heated at about 80°C nearly to dryness.

### Bioconcentration Factor (BCF) and Transfer Factor (TF)

Bioconcentration Factor is defined as the ratio of the total metal concentration in the roots to that in the soil (Elkhatib et al. 2001, Gonzalez and Gonzalez-Chavez 2006, Yoon et al. 2006):

$$\text{BCF} = [\text{M}]_{\text{roots}} / [\text{M}]_{\text{soil}}$$

where  $[\text{M}]_{\text{roots}}$  is the total metal concentration in the roots, and  $[\text{M}]_{\text{soil}}$  is the total metal concentration in the soil, and wherein for this particular study the metal refers to chromium and cadmium.

Translocation or Transfer Factor (Mocko and Waclawek 2004, Yoon et al. 2006, Sanghamitra et al. 2012) is defined as the ratio of the total Cr or Cd concentration in the

shoots to the roots:

$$\text{TF} = [\text{M}]_{\text{leaves}} / [\text{M}]_{\text{roots}}$$

where  $[\text{M}]_{\text{leaves}}$  is the total Cr or Cd concentration in the leaves, and  $[\text{M}]_{\text{roots}}$  is the total Cr or Cd concentration in the roots.

TF indicates internal metal transportation (Nouri et al. 2009). According to Yoon et al. (2006), both the BCF and TF can be used to estimate a plant's potential for phytoremediation; BCF is used to estimate a plant's ability to accumulate the metal in the roots while TF is used to estimate a plant's ability to translocate metals from the roots to above-ground parts. Plants exhibiting BCF and TF values less than one are unsuitable for phytoextraction (Yoon et al. 2006).

## RESULTS AND DISCUSSION

### Root-zone soil pH and concentration of Cd and Cr

The results of the determination of the soil pH in both deionized water and in  $\text{CaCl}_2$  solution, as well as concentration of metals in the plant root-zone soil, are presented in Table 1. Results show that the soil samples were under generally acidic conditions with  $\text{pH}_{\text{H}_2\text{O}}$  ranging from 6.18-7.24 and  $\text{pH}_{\text{CaCl}_2}$  ranging from 5.99-7.02. The mean  $\text{pH}_{\text{H}_2\text{O}}$  was 6.69 and mean  $\text{pH}_{\text{CaCl}_2}$  was 6.58, as presented in Table 2, which also shows the range, mean, and standard deviation for root-zone soil Cd and Cr concentrations. The soil chemical properties significantly affect the distribution of the metals among the different soil fractions (Mocko and Waclawek 2004). For instance, pH is an important factor influencing the availability of metals in the soil for plant uptake (Prasad and de Oliveira Freitas 2003, Bradl and Xenidis 2005). Jones (2001) stated that the  $\text{pH}_{\text{CaCl}_2}$  value is lower than  $\text{pH}_{\text{H}_2\text{O}}$  and this was evident in this study. Under acidic conditions (pH 4.0-8.5), metal cations are mobile (Saxena and Misra 2010).

Bert et al. (2002) considered soil to be contaminated if it contains greater than 2  $\text{mg kg}^{-1}$  Cd, based on the

**Table 2.** Summary of results for pH and soil concentrations of Cd and Cr, Cebu City landfill in the Philippines

Parameter	Range	Mean	Standard deviation
$\text{pH}_{\text{H}_2\text{O}}$	6.18-7.24	6.69	0.26
$\text{pH}_{\text{CaCl}_2}$	5.99-7.02	6.58	0.22
Cd	Not detected-1.58	1.08	0.33
Cr	7.93-137	24.08	29.06

French agricultural approved National Farmers' Union (NFU) 44,041 norm. The concentration of Cd in soil was relatively low, ranging from "below detection limit" to  $1.58 \text{ mg kg}^{-1}$  (for *Cardiospermum halicacabum* root-zone soil), and with a mean concentration of  $1.13 \text{ mg kg}^{-1}$ . Of the 32 plant root zone soils, only 6, or about 19%, had Cd. Cd is mainly used in alloys, in electroplating, in pigments, in batteries, and in protecting steel iron and steel against corrosion, and although it has been restricted worldwide due to its environmental impact, it can still be found in many consumer goods (Bradl et al. 2005).

The concentration of Cr in the soil ranged from  $7.93 \text{ mg kg}^{-1}$  (for *Chloris barbata* root-zone soil) to  $137 \text{ mg kg}^{-1}$  (for *Rottboellia cochinchinensis* root-zone soil), and with a mean of  $27.47 \text{ mg kg}^{-1}$ . For Cr, the commonly observed mean concentration in soils is 40 ppm (or  $\text{mg kg}^{-1}$ ) with a range of 10-150 ppm, and in sewage sludge, the average Cr concentration is 74 ppm with a range of 2-1100 ppm (Bradl et al. 2005). Cr is used in a wide variety of industrial applications including the paper industry, chemical industry, in fertilizers, metal works and foundries, leather tanning, and finishing, among others (Bradl et al. 2005). The relatively wide range of Cr concentration can be attributed to the heterogeneity of the waste. An evaluation made of the linear relationship between the concentrations of Cd and Cr in the root-zone soils using Pearson's correlation showed that there was no statistically significant linear relationship between Cd and Cr ( $r=0.2618$ ,  $P=0.141$ ).

### Behavior of the landfill plants towards Cd and Cr

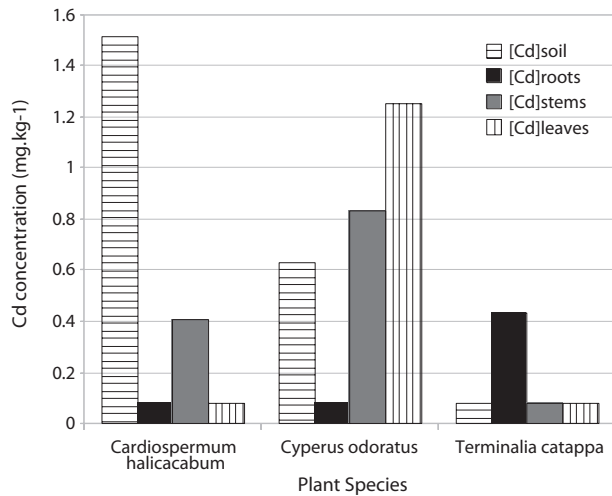
The plant species in the landfill differed in their Cd and Cr content, which indicated that they differed in their capacities for metal uptake, as also observed in other studies on metal contaminated sites (Freitas et al. 2004, Nouri et al. 2009).

**Cadmium.** Of the thirty-plant species analyzed, only 3 showed to have Cd either in the roots, leaves or stems. Cd was detected in the roots of *Terminalia catappa* ( $0.425 \text{ mg kg}^{-1}$ ), but not detected in the stems, leaves or root-zone soil. The root-zone soil of *T. catappa* was slightly acidic. Studies show that under acidic conditions, Cd solubility increases, and very little adsorption of Cd by soil colloids take place (McLean and Bledsoe 1992). Cd was also detected in the stems of *C. halicacabum* ( $0.408 \text{ mg kg}^{-1}$ ), but not in the leaves and roots, and in both the stems ( $0.828 \text{ mg kg}^{-1}$ ) and leaves ( $1.25 \text{ mg kg}^{-1}$ ) of *Cyperus odoratus*, but not in the roots. As shown in Fig. 3, the highest Cd concentration was in the leaves of *C. odoratus*. *C. odoratus*

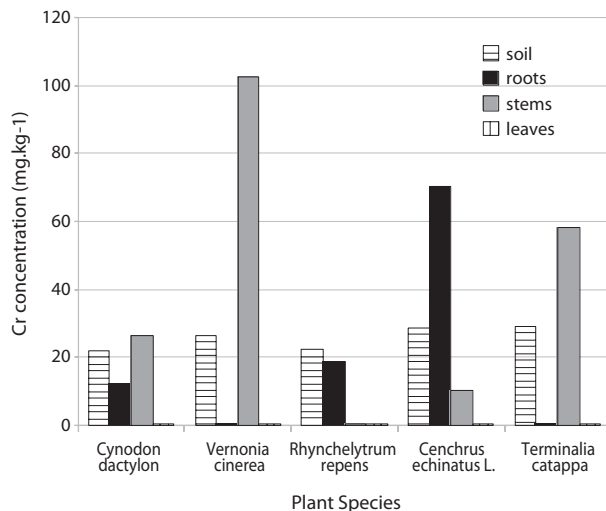
showed the potential for Cd uptake from the soil to the stems, as expressed in its  $\text{BCF}_{\text{Cd}} > 1$ . And only *C. odoratus* had a  $\text{TF}_{\text{Cd}}$  value equivalent to 1.51. The translocation of Cd from the stems to the leaves of *C. odoratus* was relatively efficient with  $\text{TF}_{\text{Cd}} > 1$ . Studies though have shown that species from the Brassicaceae family are found to be the most tolerant to Cd (Prasad 1995). Based on the survey, there were no plant species in the landfill that belonged to the Brassicaceae family. For Cd, foliar concentration above  $100 \mu\text{g g}^{-1}$  (equivalent to  $100 \text{ mg kg}^{-1}$ ) dry weight is considered exceptional, and is used as a threshold value for Cd hyperaccumulation (Bert et al. 2002, Assunção et al. 2003). Based on the analysis conducted, no plant species qualified as a hyperaccumulator of Cd.

**Chromium.** Only *C. dactylon* and *Cenchrus echinatus* showed levels of Cr in both the stems and roots (with  $26.38 \text{ mg kg}^{-1}$  in the stems and  $12.17 \text{ mg kg}^{-1}$  in the roots of *C. dactylon*; and  $10.02 \text{ mg kg}^{-1}$  in the stems and  $70.07 \text{ mg kg}^{-1}$  in the roots of *C. echinatus*, but none in the leaves). *Vernonia cinerea* and *T. catappa* showed levels of Cr in their stems at  $102.4 \text{ mg kg}^{-1}$  for *V. cinerea* and  $58.2 \text{ mg kg}^{-1}$  for *T. catappa*, but was not detected in the roots; while only *Rhynchosytrum repens* showed the presence of Cr in the roots at  $18.17 \text{ mg kg}^{-1}$ . A number of the plant species that grew in the landfill substrate with a relatively elevated Cr concentration did not have Cr in the roots, stems or leaves. None of the plant species exhibited the potential to hyperaccumulate Cr given that the highest Cr level in plants was only  $102.4 \text{ mg kg}^{-1}$ . This is unlike reports of relatively high concentrations of chromium in some plant species. For example, chromium has been found to be relatively high in concentration in the leaves of *Dicoma nicotifera* ( $1,500 \mu\text{g g}^{-1}$  dry weight) and *Sutera fodina* ( $2,400 \mu\text{g g}^{-1}$ ) found in Zimbabwe (Hossner et al. 1998). Fig. 4 shows the level of Cr in five of the plant species, showing that the highest Cr concentration was in the stems of *V. cinerea*. None of the plant species exhibited the potential to hyperaccumulate Cr since the highest Cr level in plants was only  $102.4 \text{ mg kg}^{-1}$ .

*C. echinatus* is more effective in accumulating Cr in the roots than *C. dactylon*, as shown by the former's BCF, which was greater than 1. Thus, *C. echinatus* is a potential candidate for phytostabilization of Cr in the landfill substrate, since it exhibited the potential to immobilize and contain Cr in the roots. The poor translocation or transfer of Cr from roots to shoots is one of the difficulties in using plants and trees for the mitigation of chromium contamination. A study of temperate trees confirmed that Cr was poorly taken up in the aerial tissues, but was held predominantly in the roots (Shanker et al. 2005, Segura-



**Fig. 3** Level of cadmium in plant and root-zone soil, Cebu City landfill, Philippines.



**Fig. 4** Level of chromium in plant and root-zone soil, Cebu City landfill, Philippines.

Muñoz et al. 2006).

In a phytosociological analysis of roadside communities in Kerala, India, *C. dactylon*, *V. cinerea*, and *C. halicacabum* were also found in the area being studied; this indicates that these resilient species growing in contaminated environments like roadsides or a landfill, would serve as reliable indicators of pollution (Ray and George 2009). *C. dactylon* is part of the biodiversity list of Prasad and de Oliveira Freitas (2003) exhibiting resistance to metals with potential to clean up toxic metals.

## CONCLUSION

The soil in the landfill was generally acidic and under this condition metals are more mobile and become more available vertically upward for plant uptake. Results show that the plant species in the landfill differed in the metal content, which indicated that the plant species differed in their behavior towards the presence of Cd and Cr. Of the 32 plant species sampled, *C. odoratus* showed the potential for Cd uptake and internal transfer; *C. echinatus*, *V. cinerea*, and *T. catappa* for Cr uptake; and *C. dactylon* for Cr internal transfer.

To confirm the Cd accumulation and internal transfer of *C. odoratus*, the Cr accumulation of *C. echinatus*, the Cr internal transfer of *C. dactylon*, and the Cr accumulation in the stems of *V. cinerea* and *T. catappa*, controlled experiments are recommended, as the plant samples analyzed were collected from the field.

## ACKNOWLEDGMENTS

This study is part of a research funded through a research grant from the International Foundation for Science (IFS, Sweden) with the Organization for the Prohibition of Chemical Weapons (OPCW), The Hague, Netherlands, as a co-sponsor.

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