

## POTENTIAL OF TWO INVASIVE PLANT SPECIES, *LANTANA CAMARA* L. AND *WEDELIA TRILOBATA* L., FOR SELECTIVE HEAVY METAL UPTAKE

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### ABSTRACT

This study examined the potential of the alien invasive species *Lantana camara* L. and *Wedelia trilobata* L. to selectively remove ions of heavy metals, Cd, Cr, Cu, Co, Ni, Pb, Fe, Mn and Zn. The extent of removal of heavy metal ions by *L. camara* with reference to *Albizia odoratissima* (L.f.) Benth. was determined by using finely ground stems under static and dynamic conditions and by passing the metal ion solutions through fresh stems. The efficiency of heavy metal uptake by *W. trilobata* from polluted environments was investigated with reference to *Ipomoea aquatica* Forssk. Under static conditions, *L. camara* and *A. odoratissima* removed nearly 50% of Zn<sup>+2</sup>, Cd<sup>+2</sup>, Ni<sup>+2</sup> and Mn<sup>+2</sup>. The removal of Cr<sup>+6</sup>, Co<sup>+2</sup>, Cu<sup>+2</sup>, Fe<sup>+2</sup>/Fe<sup>+3</sup> and Pb<sup>+2</sup> was less than 50%. The removal ability for each metal ion can be enhanced under dynamic conditions and when fresh stem segments were used. *W. trilobata* absorbed more than 1 mgdm<sup>-3</sup> of Cu<sup>+2</sup>, Ni<sup>+2</sup>, Fe<sup>+2</sup>/Fe<sup>+3</sup> after 72 hours of treatment and Ni<sup>+2</sup>, Mn<sup>+2</sup>, Fe<sup>+2</sup>/Fe<sup>+3</sup>, Zn<sup>+2</sup>, Pb<sup>+2</sup>, Co<sup>+2</sup>, Cu<sup>+2</sup>, Cr<sup>+6</sup> after 120 hours of treatment. The high level of ion uptake ability by *W. trilobata*, *A. odoratissima* and *L. camara* can be attributed to the high amount of parenchyma in their tissues.

### Key words

Invasive plants, Phytoremediators, *Lantana camara* L., *Wedelia trilobata* L.

### INTRODUCTION

The level of pollution of wetlands of Sri Lanka is on the increase at an alarming rate (Anon. 1992). Among the many types of pollutants, heavy metals have made a significant contribution to water pollution, especially in industrial areas.

A significant amount of heavy metals has been introduced to the environment due to uncontrolled discharge of factory effluents. Further, the intensifying disposal of sewage-sludge on agricultural land has increased the accumulation of non-essential potentially toxic heavy metals in soils (Singh and Pandeya, 1998). When heavy metals enter food chains through domestic livestock, there are chances of reaching acute toxicity levels in animals as well as in humans. It has also been found that the heavy metal toxicity would affect the soil condition and crop yield (Raj, 1992).

The remediation of sites contaminated with heavy metals is a major concern, and suitable techniques are essential for the control of rising levels of heavy metal contamination in natural ecosystems (Senthilkumar *et al.*, 2000). In developed countries, most wastes are purified to varying degrees in treatment plants (Miller, 1992). Although recommended chemical and biological methods are available for the treatment of polluted systems, high operational cost is the

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main drawback in implementing them successfully in developing countries. Consequently, proper treatment and management of polluted water is still at an early stage in Sri Lanka. Due to these problems, there is a great demand for development of alternative low cost techniques for the treatment of polluted water.

During the past few decades, another serious but underestimated threat to natural and semi-natural habitats of Sri Lanka is invasion by alien plants. The spread of such plants is a potentially lasting and pervasive threat. Even when the introduction of alien plants is stopped, the existing aliens do not disappear from the environment they have already established; in contrast they continue to spread and consolidate (Cronk and Fuller, 1995). The Ministry of Forestry and Environment of Sri Lanka has ranked *Lantana camara* L. and *Wedelia trilobata* L. as two of the most threatening alien invasive species that have invaded and affected native biota in Sri Lanka (Bambaradeniya, 2001).

Certain plant species growing on metalliferous soils accumulate exceptionally high concentrations of metallic elements in their tissues. Recent interest in metal hyperaccumulation has been stimulated by the increased potential for phytoremediation of polluted environments by the use of metal accumulating plants to cleanse soils from contaminants (Pollard and Baker, 1997).

The objective of this study was to determine the potential of the invasive species *L. camara* L. and *W. trilobata* L. threatening the native flora of Sri Lanka for to selectively remove ions of the heavy metals Cd, Cr, Cu, Co, Ni, Pb, Fe, Mn and Zn from polluted environments. Evaluation of such a potential would help to utilize these two species in a profitable manner, by enticing the industrialists to extract these two species from their habitats to treat waste effluents and environments contaminated with heavy metal ions.

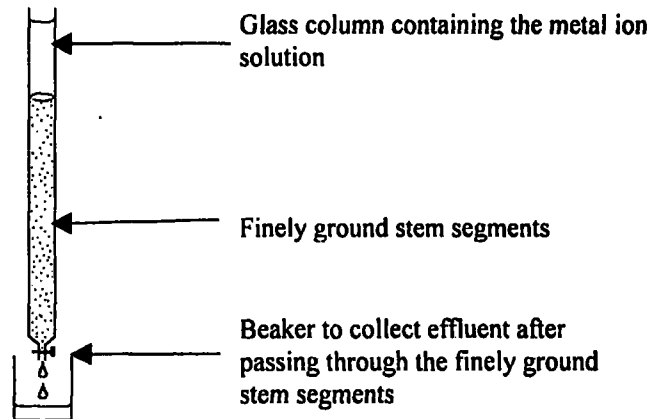
## MATERIALS AND METHODS

### Evaluation of *L. camara* as a potential candidate to treat waste effluents

*Albizia odoratissima* (L.f.) Benth., which had been extensively studied for its heavy metal uptake ability (Priyantha *et al.*, 1997) was used as the reference plant for this study. In this experiment three replicates of the materials of the test plant and the reference with the different heavy metal ions mentioned above, were studied.

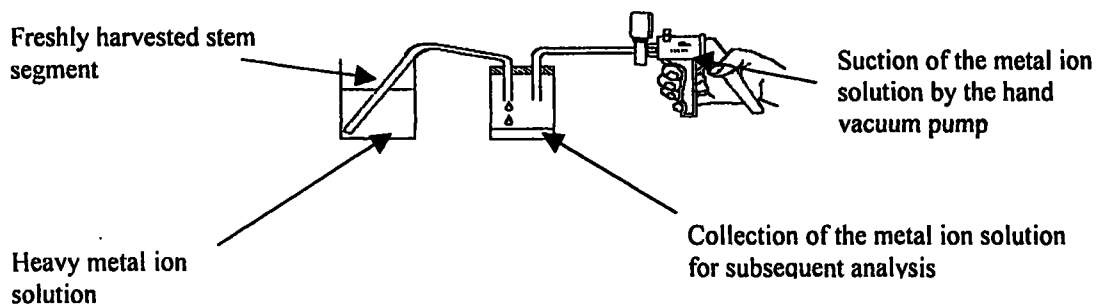
The amount of heavy metals absorbed by finely ground stem segments under static conditions was determined by mixing 5 g samples of finely ground stems with 50 cm<sup>3</sup> of each heavy metal ion (Cd<sup>+2</sup>, Cr<sup>+6</sup>, Cu<sup>+2</sup>, Co<sup>+2</sup>, Ni<sup>+2</sup>, Pb<sup>+2</sup>, Fe<sup>+2</sup>/Fe<sup>+3</sup>, Mn<sup>+2</sup> and Zn<sup>+2</sup>) solution of 2 mg dm<sup>-3</sup> concentration. The mixture was stirred for 5 minutes using a magnetic stirrer, and the amount of metal ions remaining in the solution was determined by analyzing the metal ions (Cd<sup>+2</sup>, Cr<sup>+6</sup>, Cu<sup>+2</sup>, Co<sup>+2</sup>, Ni<sup>+2</sup>, Pb<sup>+2</sup>, Fe<sup>+2</sup>/Fe<sup>+3</sup>, Mn<sup>+2</sup> and Zn<sup>+2</sup>) using the atomic absorption spectrophotometer (BUCK-Scientific-200A). This procedure was repeated after increasing the concentration of the metal ion solution in the beaker to 5 mgdm<sup>-3</sup> and using fresh, finely ground *L. camara* stems.

The amount of heavy metals adsorbed by stem segments under dynamic conditions was determined by passing  $2 \text{ mgdm}^{-3}$  metal ion solutions through a glass column packed with 10 g of finely ground stem segments (Figure 1). Then 10 ml samples of the filtrate were collected, and the amount of metal ions remaining in the effluent was determined using the standard atomic absorption spectrophotometric procedures (Christian, 1994).



**Figure 1.** The apparatus used to determine heavy metal absorption by finely ground *L. camara* under dynamic conditions

Instantaneous heavy metal absorption levels by *L. camara* was determined by passing  $2 \text{ mgdm}^{-3}$  heavy metal ion solutions through a freshly harvested, 15 cm long stem segment using a hand vacuum pump under a pressure of  $3 \times 10^6 \text{ Pa}$  (Figure 2) as described in Tennakoon *et al.*, (1997). An aliquot of  $5 \text{ cm}^3$  of the liquid that passed through the stem segment was subsequently collected and analyzed for metal ions using the atomic absorption spectrophotometric procedures (Christian, 1994).



**Figure 2.** Arrangement of the apparatus used to determine instantaneous heavy metal absorption by *L. camara* stem.

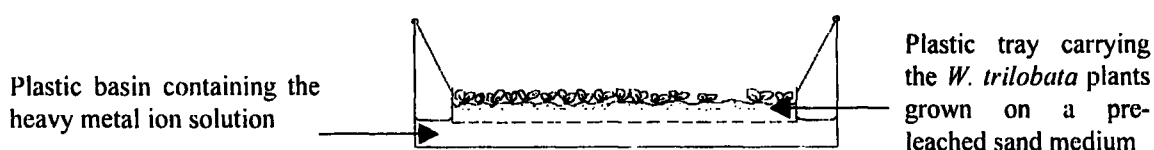
## Evaluation of *W. trilobata* as a potential candidate to treat waste effluents

Whole plants of *W. trilobata* were used to investigate the efficiency of heavy metal uptake from polluted environments/disposal areas. The reference plant for this study was *Ipomoea aquatica* Forssk., which has been extensively studied previously for its heavy metal absorption level (Perera, 2001). In this experiment three replicates of the live test plant and the reference with the different heavy metal ions mentioned above, were studied.

Live *W. trilobata* and *I. aquatica* plants were established on pre-leached sand devoid of metal ions spread on plastic trays with a perforated bottom. As depicted in Figure 3, the tray carrying *W. trilobata* plants was carefully placed on a heavy metal ion solution contained in a plastic basin to allow the plants to absorb the heavy metal ion solution from the bottom. The composition of the ion solution was as same as that used in the study of *L. camara*. Samples of 15 g leaf and stem matter were separately collected, at 72 hours and 120 hours after the initial treatment, for analysis. After 120 hours of treatment, another 15 g of root samples were also collected. For subsequent analysis, the samples were washed with tap water and then with distilled water. Then they were first air-dried (at room temperature) for 24 hours and then oven dried at 105 °C until a constant weight was obtained. The samples were then ground and were placed in an airtight, clean plastic bottle with proper identification.

The samples were then ashed by the following method: 1 g of the sample was transferred to a porcelain crucible and was ashed for 4 hours at 500 °C using a muffle furnace. Then it was cooled and wetted with a few drops of deionized water.

After that 5.00cm<sup>3</sup> of HNO<sub>3</sub>: HClO<sub>3</sub> (4:1) was added and heated gently and carefully on an electric hotplate. Then it was reashed at 430 °C for 2 hours and digested with 5.00 ml of 5M HCl. After that it was heated gently on a hot plate for a few minutes. The contents were filtered into a 50 ml volumetric flask, and left overnight. The amount of metal ions present was determined by atomic absorption spectrophotometric procedures (Christian, 1994).



**Figure 3.** The set-up used to examine the uptake level of heavy metal ions by *W. trilobata* plants

### Anatomy of the study species

Thin sections of stems and roots of the study species and reference plants were obtained using a rotary microtome (CUT 4055), stained with Safranin in 70% alcohol and mounted in glycerol. The parenchyma: vessel/tracheids ratio of stems and root segments was determined by examining the slides under a light microscope (Euromex-MIC 285).

## RESULTS

The extent of removal of metal ions from solutions containing of individual ions by different plant species, determined by taking the difference in atomic absorption measurements before and after treatment, is stated in Table 1.

*L. camara* and *A. odoratissima* showed the ability of removing more than 50% of heavy metal ions from the 2 mgdm<sup>-3</sup> solutions of Zn<sup>+2</sup>, Cd<sup>+2</sup>, Ni<sup>+2</sup> and Mn<sup>+2</sup> and showed similar absorption levels for Zn<sup>+2</sup>, Co<sup>+2</sup>, Cd<sup>+2</sup>, Ni<sup>+2</sup> and Mn<sup>+2</sup> from 5 mgdm<sup>-3</sup> heavy metal ion solutions (Table 1). The absorption levels of 2 mgdm<sup>-3</sup> Cr<sup>+6</sup>, Fe<sup>+2</sup>/Fe<sup>+3</sup>, Co<sup>+2</sup>, Pb<sup>+2</sup> and Cu<sup>+2</sup> and 5 mgdm<sup>-3</sup> of Cr<sup>+6</sup>, Fe<sup>+2</sup>/Fe<sup>+3</sup>, Pb<sup>+2</sup> and Cu<sup>+2</sup> were less than 50%. Percentage removal of metal ions by the reference plant (*A. odoratissima*) was higher than that by *L. camara* for all the metal ions (irrespective of the initial concentration of the metal ion solution) and Cu<sup>+2</sup> (when the initial concentration of the metal ion solution was 2 mgdm<sup>-3</sup>).

Table 1

The percentage removal of different metal ions by the finely ground stem segments of *L. camara* (test plant) and *A. odoratissima* (reference plant) under static conditions.

Metal ion	Percentage removal of heavy metal ions			
	* from 2 mgdm <sup>-3</sup> solution		* from 5 mgdm <sup>-3</sup> solution	
	<i>L. camara</i>	<i>A. odoratissima</i>	<i>L. camara</i>	<i>A. odoratissima</i>
Zn <sup>+2</sup>	71.0	77.5	73.0	75.4
Co <sup>+2</sup>	33.5	37.5	70.6	78.2
Pb <sup>+2</sup>	36.0	12.5	37.8	67.6
Cr <sup>+6</sup>	1.5	3.0	10.8	11.2
Cd <sup>+2</sup>	86.0	96.3	78.4	65.2
Cu <sup>+2</sup>	71.0	21.0	41.0	52.0
Ni <sup>+2</sup>	51.5	74.5	74.0	90.0
Mn <sup>+2</sup>	100.0	100.0	94.0	94.0
Fe <sup>+2</sup> /Fe <sup>+3</sup>	35.0	50.0	46.0	100.0

\* Concentration of the metal ion solution

The percent removal of metal ions from laboratory prepared 5 mgdm<sup>-3</sup> solutions under dynamic conditions was more than 80% for all the heavy metal ions except for Cr<sup>+6</sup> by both *L. camara* and *A. odoratissima* (Figure 4). The percent removal of metal ions by *A. odoratissima* was higher than that by *L. camara* for all the metal ions except for Cr<sup>+6</sup> and Fe<sup>+2</sup>/Fe<sup>+3</sup>. The percent removal of all the metal ions was higher under dynamic conditions than under static conditions.

When metal ion solutions of 2 mgdm<sup>-3</sup> were passed through stem segments, both species removed more than 80% of all the metal ions except Cr<sup>+6</sup> (Figure 5). The percent removal of

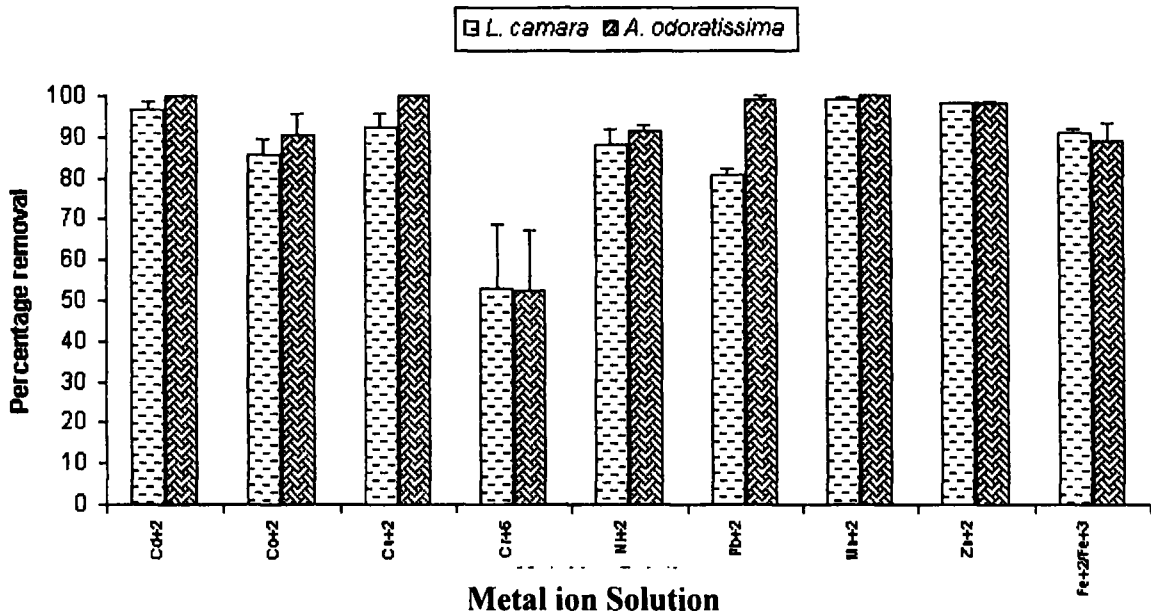


Figure 4. The percentage removal of different metal ions from a 2 mgdm<sup>-3</sup> solution by *L. camara* and *A. odoratissima* in a dynamic condition (Error bars indicate the standard error of the mean)

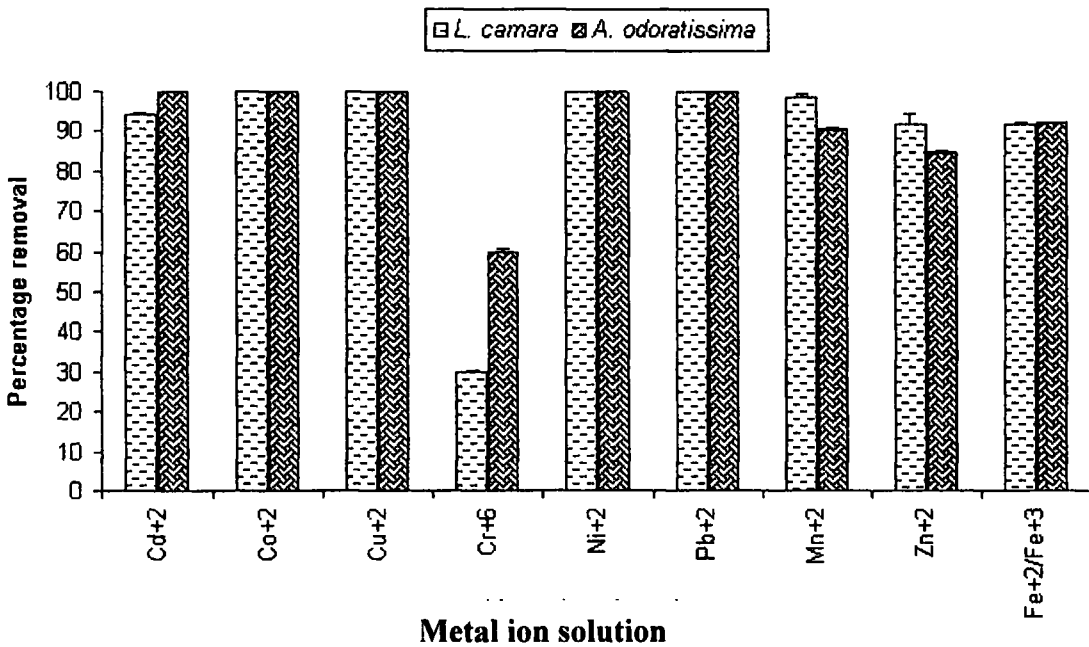


Figure 5. The percentage removal of different metal ions from a 2 mgdm<sup>-3</sup> solution by *L. camara* and *A. odoratissima* when laboratory prepared polluted water was passed through freshly harvested stem segments (Error bars indicate the standard error of the mean).

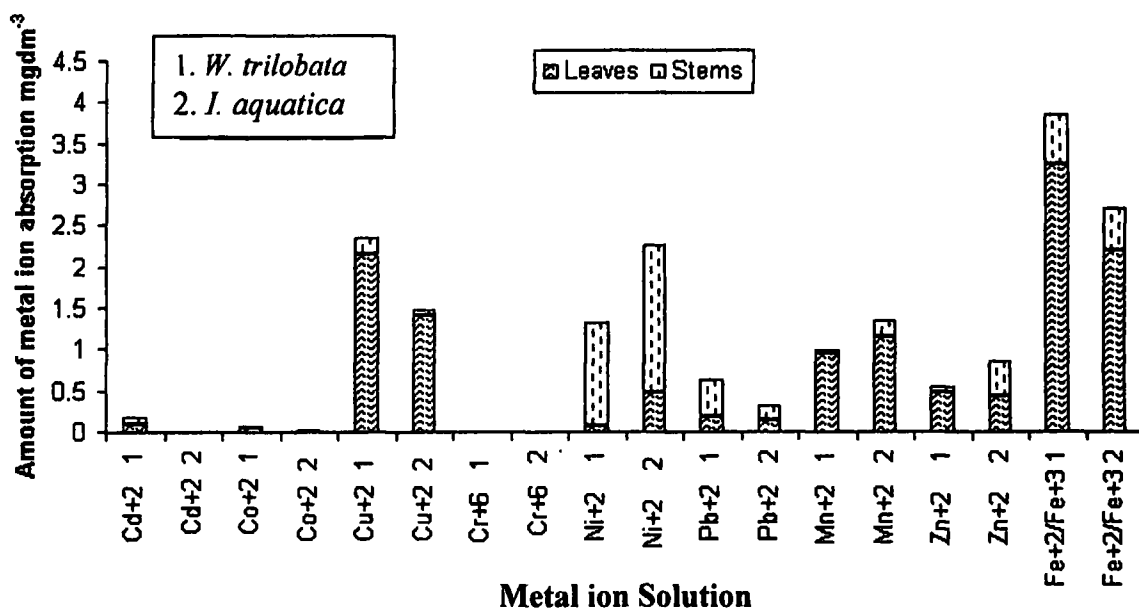


Figure 6. The amount of metal ions absorbed by *W. trilobata* and *I. aquatica* after 72 hours of treatment.

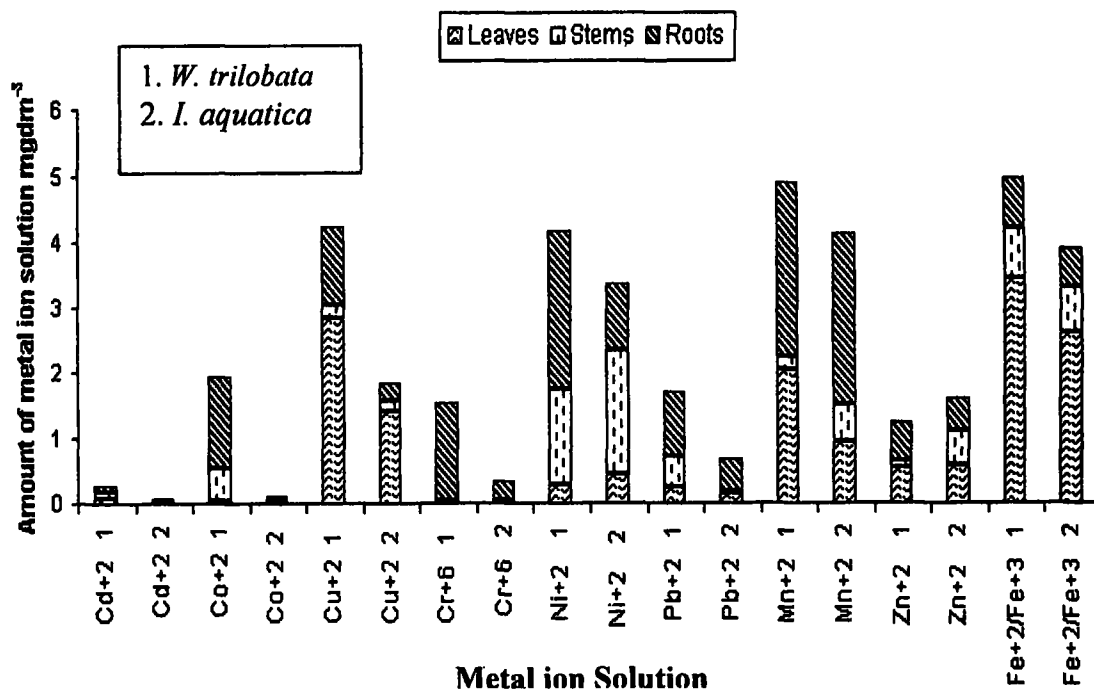


Figure 7. The amount of metal ions absorbed by *W. trilobata* and *I. aquatica* after 120 hours of treatment.

metal ions by *A. odoratissima* was higher than that by *L. camara* for all metal ions except for  $\text{Cr}^{+6}$  and  $\text{Fe}^{+2}/\text{Fe}^{+3}$  as in dynamic conditions. The percent removal of  $\text{Cr}^{+6}$  by *A. odoratissima* was however, more than 50%, which is much higher than that under dynamic conditions. Furthermore, the percent removal of metal ions was much higher for all the metal ions when freshly harvested stem segments were used as compared to finely ground dried stem segments used either under static or dynamic conditions.

When *W. trilobata* and *I. aquatica* were treated with  $5 \text{ mgdm}^{-3}$  heavy metal ion solutions for 72 hours, both species showed the presence of more than  $1 \text{ mgdm}^{-3}$  of  $\text{Cu}^{+2}$ ,  $\text{Mn}^{+2}$ ,  $\text{Ni}^{+2}$  and  $\text{Fe}^{+2}/\text{Fe}^{+3}$  in their leaves and stems. When total absorption levels by leaves and stems were considered, the amount of  $\text{Cd}^{+2}$ ,  $\text{Co}^{+2}$ ,  $\text{Cu}^{+2}$ ,  $\text{Pb}^{+2}$  and  $\text{Fe}^{+2}/\text{Fe}^{+3}$  absorption by *W. trilobata* was higher than that by the reference plant, *I. aquatica*. Metal ions such as  $\text{Cu}^{+2}$ ,  $\text{Mn}^{+2}$ ,  $\text{Fe}^{+2}/\text{Fe}^{+3}$  showed tendency to accumulate in the leaves, while  $\text{Ni}^{+2}$  showed tendency to accumulate in the stems.  $\text{Cr}^{+2}$  (by both species) and  $\text{Cd}^{+2}$  (especially in *I. aquatica*) showed no accumulation in either stems or in leaves (Figure 6). As the plants had to be maintained in the trays for 120 hours to investigate the ion absorption levels 120 hours after the initial treatment, entire plants were not harvested in this instance. Hence the roots could not be analysed to examine the heavy metal uptake level 72 hours after the metal ion treatment. Neither species showed the development of toxicity symptoms due to the presence of heavy metal ions in the substrate during the study period.

When the substratum of trays of *W. trilobata* and *I. aquatica* was treated with  $5 \text{ mgdm}^{-3}$  heavy metal ion solutions for 120 hours, unlike *I. aquatica*, *W. trilobata* showed removal of more than  $4 \text{ mgdm}^{-3}$  of  $\text{Cu}^{+2}$ ,  $\text{Ni}^{+2}$ ,  $\text{Mn}^{+2}$  and  $\text{Fe}^{+2}/\text{Fe}^{+3}$  ions (Figure 7). However, *W. trilobata* absorbed less than  $1 \text{ mgdm}^{-3}$  of  $\text{Cd}^{+2}$  from the solution. When the total amount of metal ions absorbed by leaves, stems and roots is considered, the overall metal ion absorption by *W. trilobata* was higher than that by *I. aquatica* for all except for  $\text{Zn}^{+2}$ . Higher amounts of  $\text{Mn}^{+2}$  accumulated in the roots when compared to stems and leaves of both species. During this experiment too, toxicity symptoms were not developed during the period of study.

The parenchyma (non lignified tissue): vessel/tracheid (lignified tissue) ratio in the vascular tissues of roots in *W. trilobata* was much higher than that in *I. aquatica*. As expected the results showed a higher level of metal ion absorption by tissues with a higher proportion of parenchyma than vessels/tracheids.

## DISCUSSION

When *L. camara* was investigated for its ability to remove heavy metal ions, the removal can be attributed to several reasons. Ion absorption into the apoplast of tissues would have taken place passively by diffusion. Usually when the concentration gradient between the metal ion solution and the apoplast of the plant tissues increases the amount of metal ions absorbed through the cell wall increases proportionately (Taiz and Zeiger, 1991). However, in some cases (i.e.,  $\text{Zn}^{+2}$ ,  $\text{Pb}^{+2}$  and  $\text{Fe}^{+2}/\text{Fe}^{+3}$  by *L. camara*), the percent removal has increased only by a small proportion when the strength of the solution was increased from  $2 \text{ mgdm}^{-3}$  to  $5 \text{ mgdm}^{-3}$ . This may be due to the saturation of metal binding sites in the cell walls. However, for metal ions such as  $\text{Co}^{+2}$  and  $\text{Ni}^{+2}$  the proportionate increase of absorption may be due to the relatively large quantity of metal binding sites remaining unattached specifically for these metals that results in the increase of ion absorption with increase of external heavy metal ion concentration. Ion absorption

across the plasma membrane of living cells may also take place by an active process and usually it is a selective process. Thus, *L. camara* cell membranes may allow the transport of only certain metal ions across them, and this phenomenon can be attributed to the varying levels of selective ion absorption by both *L. camara* (test plant) and *A. odoratissima* (reference plant) with the increase of the concentration of the heavy metal ion solution from 2 mgdm<sup>-3</sup> to 5 mgdm<sup>-3</sup>.

The decrease in Pb<sup>+2</sup> and Cu<sup>+2</sup> absorption levels by *L. camara* and that of Cd<sup>+2</sup> and Ni<sup>+2</sup> by *A. odoratissima* when the concentration of the metal ion solution was increased from 2 mgdm<sup>-3</sup> to 5 mgdm<sup>-3</sup> may be due to the release of selected metal ions earlier stored in the cell cytosol back to the external medium. Some metal ions absorbed actively by the plant cells are stored at a high concentration in the cytosol. High concentrations of metal ions in the cytosol readily intoxicate the cells, which lead to progressive death coupled with the inactivation of the plasma membrane. When cells are dead due to increasing strength of external solution, some metal ions earlier stored in the cell using metabolic energy, are released back to the external solution. When the stems are ground to obtain small particles, the cellular structure of the tissues may be damaged. Since active uptake of heavy metals by plant cells takes place only when the tissues are alive, the amount of heavy metals absorbed by ground stem segments which comprise dead cells should always be lower than that by the plant parts comprised of live tissues where both active and passive absorption take place. This phenomenon can be attributed to the high level of ion absorption exhibited by the fresh stem segments as opposed to static and dynamic conditions where stem segments are finely ground before treatment.

It is commonly known that plants naturally take up metals from the substrates and store them in their tissues. Furthermore, some plants are capable of absorbing and accumulating a wide spectrum of non-essential elements at concentrations several times higher than that in the soil solution. According to Lombi *et al.* (2000), accumulation of exceptionally high levels of metal ions into roots can take place by three mechanisms, viz. (i) enhanced absorption of metal into the roots, (ii) secretion of protons, organic acids and / or chelators by the roots, which can solubilize the metals, (iii) foraging for metals by the roots, involving preferential allocation of root biomass into regions of metal enrichment. But certain metal ions (such as Cu<sup>+2</sup> and Fe<sup>+2</sup> / Fe<sup>+3</sup> as exhibited by *W. trilobata* and *I. aquatica*) are translocated from roots to shoot at high rates (Lombi *et al.*, 2000) and in such instances the metal ions get accumulated preferentially in the shoots.

The amount of Cr<sup>+6</sup> absorbed by all the species investigated was generally very low. This can be either due to the restriction of the transport of Cr<sup>+6</sup> across the plasma membranes of cells or due to the absence of chromium specific carrier proteins in the membranes of all four species investigated.

High concentration of all metals above the normal levels, including those essential for growth and metabolism, exert toxic effects on the metabolic machinery of plants (Metha and Gaur, 1999). Surprisingly none of the plant species exhibited toxic symptoms during the investigation period. This may be due to the ability of these plants to detoxify the heavy metal ions by degradation or sequestration before they cause damage (Gregory and Bradshaw, 1995), or alternatively the plants may not have had sufficient time to exhibit toxic symptoms as the harvesting was carried out 72 hours and 120 hours after the start of the experiment.

When the parenchyma: vessel/trachied ratio of a plant is high, the rate of transport of water through the xylem is usually low, because the transport of water occurs mainly via the vessels and/or trachieds. Typically, the walls of vessels and trachieds are heavily impregnated with lignin and the cells are dead at maturity. The wood parenchyma are living cells with cell walls mainly of cellulose (Pandey, 1996). The hyperaccumulation mechanisms of metals involve living cells comprising cellulose walls. Consequently, the rate of water transport and uptake is low in *W. trilobata* roots due to the high parenchyma: vessel/ trachied ratio, while the amount of heavy metal ion binding sites may be very high due to the presence of a high proportion of parenchyma tissues. These phenomena are thus related to the ability of metal ion uptake by *W. trilobata*.

Even though *L. camara* does not show an overall percentage of heavy metal ion removal as high as the reference plant, *A. odoratissima*, it can satisfactorily be used for selective removal of  $Zn^{+2}$ ,  $Co^{+2}$ ,  $Cd^{+2}$ ,  $Ni^{+2}$  and  $Mn^{+2}$ . Liquid industrial effluent carrying such heavy metal ions can be transported through columns filled with finely ground stem segments of *L. camara* to remove these specific metal ions before the effluents are disposed of to the environment. The heavy metal ions absorbed by *L. camara* could then be disposed of in safe and well-managed areas designed for dumping such contaminants to minimize environmental hazards.

On the other hand, *W. trilobata*, which is a herb, has shown the capability of absorbing a high amounts of  $Cu^{+2}$ ,  $Ni^{+2}$ ,  $Mn^{+2}$ , and  $Fe^{+2}/Fe^{+3}$  than by the reference plant, *I. aquatica*. Hence *W. trilobata* could be specifically used to remove such metal ions from contaminated soils by planting them in such areas. After it is ensured that the heavy metal ions have been efficiently absorbed by *W. trilobata* from the environment where they have been grown, the entire plant can be easily uprooted and disposed of, or else if required, metals that have been absorbed by the plant can even be recovered for recycling.

The results of this study clearly exhibit the overall ability of both *L. camara* and *W. trilobata* to remove selected heavy metal ions. Hence, these two invasive species could be recommended as phytoremediators that could be used in a profitable manner to treat heavy metal ion contaminated waste effluents and environments. On the other hand, the use of *L. camara* and *W. trilobata* for such ventures will help to minimize the spread of these alien invasive species into the natural habitats of Sri Lanka.

## ACKNOWLEDGEMENTS

The financial support provided by the Postgraduate Institute of Science (Grant No: PGIS/RG/02/99) is gratefully acknowledged.

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