Cey. J. Sci. (Bio. Sci.) 38 (2): 85-93, 2009

PHYTOEXTRACTIVE POTENTIAL AMONG MUSTARD (*BRASSICA JUNCEA*) GENOTYPES IN SRI LANKA

Shyama R. Weerakoon* and S. Somaratne

Department of Botany, Open University, P. O. Box 21, Nawala, Sri Lanka
Accepted 10 December 2009

ABSTRACT

Heavy metal pollution in soil and water is a global environmental concern. In Sri Lanka, accumulation of heavy metals in soil, water and plant/animal biomass has been widely reported. Phytoremediation, the use of plants and their associated microbes, is an emerging, low-cost, environmental-friendly approach in the cleanup and prevention of environmental pollution. Indian Mustard (*Brassica juncea*) has been widely used to extract toxic metals from contaminated soils worldwide. Sri Lanka has a genetically diverse mustard collection, but their phytoextarctive potentials are yet to be determined. Present study was conducted to evaluate variation in phytoextractive ability of soil-borne heavy metals by ten different mustard accessions. Heavy metal contents (Mn, Co, Pb and Zn) in 12-week-old mustard plants (whole plant) were estimated during Maha and Yala in 2007-2008 using Atomic Absorption Spectroscopy. Heavy metal content in soils where the plants were grown was also assessed during the two seasons. Soil Mn and Co concentrations were significantly high in Maha than in Yala. Difference in heavy metal concentrations in mustard accessions between the two seasons was not significant. However, significantly high concentrations of Mn were found in accessions 7788, 515 and 8831 (236, 225, 220x10^2 µg/g). Cobolt (Co) was significantly high in accession 5088 (238x10^2 µg/g). Lead (Pb) concentrations were significantly high in accessions 8831 (156x10^2 µg/g) and 5088 (148x10^2 µg/g) and Zn was significantly high in accession 501 (6413x10^2 µg/g). The study suggests that screening the entire mustard germplasm in Sri Lanka is worthwhile to identify potential heavy metal accumulators.

Key words: heavy metal hyperaccumulation, phytoremediation

INTRODUCTION

An extensive area of the world is contaminated with organic and inorganic pollutants including heavy metal pollutants (Ensley, 2000). Organic pollutants include solvents like trichloroethylene (TCE) (Newman *et al*., 1997), herbicides, atrazine (Burken and Schnoor, 1997), explosives like trinitrotoluene (TNT) (Hughes *et al*., 1997), hydrocarbons such as oil, gasoline, benzene, and toluene (Schnoor *et al*., 1995) and fuel additives, methyl tertiary butyl ether (Hong *et al*., 2001). Inorganic pollutants include plant macronutrients such as nitrates and phosphates, micronutrients, Cr, Cu, Fe, Mn, Mo, Ni and Zn and nonessential elements, As, Cd, Co, F, Hg, Se, Pb, V and radionuclides, ^{238}U, ^{137}Cs and ^{90}Sr (Dushenkov, 2003). Human activities such as mining, transport, agriculture, waste disposal and military actions frequently release these inorganic pollutants in high and toxic concentrations.

Due to their unassailable nature, metals are a group of pollutants of much concern. The danger of toxic metals is aggravated by their almost indefinite persistence in the environment. Heavy metals cannot be destroyed but can only be transformed from one oxidation state or organic complex to another (Marques *et al*., 2009).

In Sri Lanka and other regions in South Asia, the heavy metal and organic pollutant contamination already pose a severe threat to human and ecosystem health (World Health Organization, 2003). There are only a few published reports available on the nature of soil and water contamination in Sri Lanka (Bandara, 2003; Dissanayake *et al*., 2007). However, large areas of soil and water contain high levels of heavy metals such as Cd, Cu, Co, Ni and Zn and other pollutants due to various human actions (Dissanayake *et al*., 2002). Industrial effluents, agriculture residues, domestic waste water and solid waste have contributed largely to heavy metal contamination on land and in both surface and ground water resources (Bandara, 2003). Cadmium is available in considerable amounts

*Corresponding author's email: shy_ou_2003@yahoo.com.au

in soils, water, plant and animal biomass in the dry zone (Bandara, 2006).

Phytoremediation, the use of plants and their associated rhizospheric microorganisms to remove, degrade, or immobilize various contaminants from polluted soils, sediments, groundwater or surface water, is a novel strategy. Metal hyperaccumulator plant species are able to accumulate at least 0.1% of the leaf dry weight in a heavy metal (Baker and Whiting, 2002). This is a low cost and eco-friendly means of reclaiming heavy metal contaminated soils (Rajakaruna *et al*., 2006). The cost of growing crops is minimal compared to those of soil removal and replacement. As biological processes are ultimately solar-driven, phytoremediation is on average ten-fold cheaper than engineering-based remediation methods (Marques *et al*., 2009). A limitation of phytoremediation is that the plant roots have to be able to reach the pollutant and act on it. Therefore, the soil characteristics, toxicity level, and climate have to be amenable to plant growth. Furthermore, the pollutant must not only be within physical reach of the roots, it must be bio-available for absorption as well (Pilon-Smits and Freeman, 2006). Phytoextraction is the most commonly recognized of all phytoremediation technologies. This involves removal of heavy metals from soil by means of plant uptake. This technology is based on the capacity of the roots to absorb, translocate, and concentrate toxic metals from soil to the above ground harvestable plant tissues. This localization results in a reduction of the contaminated mass and also in the transfer of the metal from an aluminosilicate-based matrix (soil) to a carbon-based matrix (plants). The carbon in the plant material can be oxidized to carbon dioxide, further decreasing (and concentrating) the mass of material to be treated, disposed, or recycled (Blaylock and Huang, 2000). In practice, metal-accumulating plants are seeded or transplanted into metal-polluted soil and are cultivated using established agricultural practices. The roots of established plants absorb metal elements from the soil and translocate them to the above-ground shoots, in leaves and other parts of shoots. After sufficient plant growth and metal accumulation, the above-ground parts of the plant are harvested, a process referred to as phytomining. Phytomining is a "green" technology that uses metal-hyperaccumulating plant species to extract metals from soil, harvest the biomass and burn it to produce bio-ore (Brooks *et al*., 1998; Robinson *et al*., 2009). This results in the permanent removal of metals from the site. Phytoextraction is a long-term remediation effort which requires many cropping cycles to reduce metal concentrations (Kumar *et al*., 1995). As a plant-based technology, the success of phytoextraction is inherently dependent upon proper plant selection. The selected plants must be fast growing and have the ability to accumulate large quantities of environmentally important metal contaminants in their shoot tissues (Blaylock *et al*., 1997; McGrath, 1998).

Several plant families contain an unusually high number of hyperaccumulators. These plant families include Asteraceae, Brassicaceae, Euphorbiaceae, Fabaceae, Flacourtiaceae and Violaceae (Kumar *et al*., 1995; Dushenkov *et al*., 1995). Members of the Brasicaceae are promising candidates for phytoextraction of metals. Different genera of the Brassicaceae are known to accumulate heavy metals such as Pb, Cd, Zn and Ni (Prasad and Freitas, 2003; Robinson *et al*., 2009). Over the past decade, researchers have sought to perfect the phytoextraction remediation technology. The majority of phytoextraction research has focused on finding the ideal metal-accumulating plant and the means by which metals can be liberated from the soil for root uptake. At present, Indian mustard (*B. juncea*) is among the most viable candidates for the phytoextraction of a number of metals including Cd, Cr (IV), ^{137}Cs, Cu, Ni, Pb, U and Zn (Kumar *et al*., 1995; Blaylock *et al*., 1997; Zhu *et al*., 1999). An experiment was conducted by Kumar *et al*. (1995) to test many fast growing *Brassica* taxa for their ability to tolerate and accumulate metals, including mustard (*B. juncea* L.), black mustard (*B. nigra* Koch), turnip (*B. campestris* L.), rape (*B. napus* L.) and kale (*B. oleracea* L.). Although all *Brassica* taxa accumulated heavy metals, *B. juncea* showed the highest capacity to accumulate heavy metals in roots and translocate Cu, Cr (IV), Cd, Ni, Pb and Zn for shoot accumulation (Kumar *et al*., 1995; Jiang *et al*., 2000). Kumar *et al*. (1995) also investigated possible genetic variations in different *B. juncea* accessions in the hope of finding some that have greater phytoextraction potential than others.

The evolution of the hyperaccumulator trait is believed to have occurred as a trait beneficial to the plant (Baker *et al*., 2000). The biological mechanisms of metal accumulation are diverse. However, a requirement for hyperaccumulation is the ability to efficiently tolerate high concentrations of metals within plant tissues (Reeves and Baker, 2000). Further, the ecological role of metal hyperaccumulation is still uncertain. However, several studies have shown that metal-accumulating plants are better able to defend against fungal and insect attacks as well as herbivores (Boyd and Martens, 1998; Boyd, 2004). Another hypothesis for the benefit of metal hyperaccumulation is “elemental allelopathy”, which is the deterrence of colonization of other plants in the zone beneath hyperaccumulator plants (Zhang *et a*l., 2007).

Sri Lanka has a genetically diverse mustard (*B. juncea* (L.) Cazen) germplasm of over sixty accessions, stored in the gene bank of the Plant Genetic Resource Centre (PGRC) (PGRC Catalogue, 1999). The term accession refers to seeds that have been gathered from a particular area and are now a part of a collection at a seed bank or plant-introduction station (Kumar *et al*., 1995). Molecular studies with AFLP fluorescent markers on the genetic diversity of these mustard accessions showed the presence of highly diverse genetic variation among mustard germplasm in Sri Lanka (Weerakoon *et al*., 2008). However, their phytoextarctive potentials are yet to be determined.

A field study was conducted in a site at Nagollagama, Maho in the Kurunegala District, where wide agricultural practices are undertaken. It is anticipated that soil is contaminated with heavy metals due to heavy use of fertilizer, herbicides and insecticides. A water quality assessment carried out in 2007 reported that there were Cr and Cu contaminations in water bodies in the Kurunegala District (Dissanayake *et al*., 2007). In the present study, ten mustard accessions were selected from the mustard germplasm collection at PGRC and were used to assess the heavy metal phytoextractive ability of these accessions.

MATERIALS AND METHODS

Ten mustard accessions ((AC 501, 515, 580, 790, 1099, 1814, 2122, 5088, 7788 and 8831) were planted in a study site at Nagollagama, Maho in the Kurunegala District. There were ten replicates for each mustard accession and the planting was done using a random block design (RBD). The plants were grown during the two seasons Yala (October 2007 to March 2008) and Maha (April to September 2008). Plant materials (whole plant) were harvested 12 weeks after planting and washed five times with distilled water. Plants were air-dried and then oven dried at 60 $^{\circ}$C for 72 h and weighed. Dried plant tissue (0.5 g) was digested in 20 ml acid mixture of concentrated H_2SO_4 : $HClO_4$ (4:1) and the final volume was made to 25 ml. The heavy metal content of Mn, Co, Pb, and Zn in the plant biomass of each mustard accession was determined (in µg/g oven dry weight) for plants grown in Maha and Yala using Atomic Absorption Spectrum (AAS) (Lindsay and Norvell, 1978).

Ten soil samples were taken at 10 cm depth from the site at random and pooled into one sample. Three composite soil samples were taken for each season. Soil samples were crushed to pass through a 2 mm sieve and available heavy metals were extracted using Ammonium Bicarbonate-Diethyline Triamine Penta Acetic Acid as a multinutrient extractant (ABDTPA) (Soltanpour and Schwab, 1977). These soil extracts were used to estimate heavy metal content using Atomic Absorption Spectroscopy (AAS) (Linn and Doran, 1984). Soil heavy metal concentrations were expressed in µg/g of oven dried soil weight for Mn, Co, Pb and Zn.

For data analysis statistical software SAS 9.1 was used and comparisons were made at α = 0.05 level using Duncan’s Multiple Range Test (DMRT).

RESULTS

There was no apparent difference in the heavy metal content in different mustard accessions during Maha and Yala seasons (Table 1). However, different mustard accessions showed variation in their heavy metal accumulation capacity. Significantly high concentrations of Mn were found in accessions AC 7788, 515 and 8831. Cobolt (Co) was significantly high in accession AC 5088. Accessions AC 8831 and 5088 showed significantly high Pb concentrations and Zn was significantly high in accession AC 501 (Table 2).

Heavy metal concentrations in soil were higher during Maha season compared to Yala season (soil Mn and Co concentrations were relatively high). Available Zn concentration ($6515x10^2$ µg/g) in soil was higher than that of other heavy metals in both seasons (Table 3). Similarly, all mustard accessions had a higher Zn content in the biomass than other heavy metals during both seasons (Table 1).

Table 4 gives a comparison of accumulated Mn, Co, Pb and Zn in the plant biomass of ten mustard accessions (mean values of Maha and Yala seasons), available soil heavy metal content (mean values of Maha and Yala seasons) and the minimum standard concentrations of each heavy metal in µg/g dry weight expected to contain in plant biomass to consider a plant/species as a hyperaccumulator (Baker and Brooks, 1989). The results demonstrated that all ten mustard accessions have accumulated Mn, Co, Pb and Zn content exceeding the limits characterizing hyperaccumulation for those metals. Therefore, all mustard accessions are considered to be good hyperaccumulators with varied heavy metal accumulation potentials among them.

Comparatively, higher concentrations of Mn was found in mustard accessions AC 7788, 515 and 8831 and a higher Co concentrations in AC 5088. Lead (Pb) concentration was higher in AC 8831 and 5088. A higher Zn concentration was observed in AC 501 (Table 2).

Relatively, AC 501 is a good Zn accumulator while AC 5088 being a good Co and Pb accumulator. AC 8831 is comparatively a good Mn and Pb accumulator.

Table 1. Heavy metal concentrations (in µg/g) in plant biomass of ten mustard accessions in Maha and Yala seasons.

Mustard	Mn		Co		Pb		Zn	
Acc.	Maha	Yala	Maha	Yala	Maha	Yala	Maha	Yala
AC 501	$172x10^2$ ±0.03	$150 x10^2$ ±0.01	$116 x10^2$ ±0.01	$84 x10^2$ ±0.01	$108 x10^2$ ±0.10	$102 x10^2$ ±0.04	$6450 x10^2$ ±0.29	$6376 x10^2$ ±0.65
AC 515	$213 x10^2$ ±0.02	$236 x10^2$ ±0.01	$159 x10^2$ ±0.02	$49 x10^2$ ±0.01	$78 x10^2$ ±0.04	$30 x10^2$ ±0.01	$3286 x10^2$ ±0.84	$3609 x10^2$ ±0.58
AC 580	$144 x10^2$ ±0.02	$158 x10^2$ ±0.01	$106 x10^2$ ±0.01	$90 x10^2$ ±0.01	$35 x10^2$ ±0.02	$36 x10^2$ ±0.02	$4260 x10^2$ ±0.39	$3636 x10^2$ ±0.47
AC 790	$208 x10^2$ ±0.02	$184 x10^2$ ±0.01	$200 x10^2$ ±0.04	$128 x10^2$ ±0.01	$55 x10^2$ ±0.04	$21 x10^2$ ±0.01	$4222 x10^2$ ±0.42	$4286 x10^2$ ±0.53
AC 1099	$161 x10^2$ ±0.02	$232 x10^2$ ±0.01	$242 x10^2$ ±0.04	$124 x10^2$ ±0.01	$162 x10^2$ ±0.04	$61 x10^2$ ±0.04	$4096 x10^2$ ±0.57	$4112 x10^2$ ±0.34
AC 1814	$144 x10^2$ ±0.01	$179 x10^2$ ±0.03	$222 x10^2$ ±0.01	$148 x10^2$ ±0.04	$147 x10^2$ ±0.03	$83 x10^2$ ±0.06	$4728 x10^2$ ±0.69	$3890 x10^2$ ±0.20
AC 2122	$158 x10^2$ ±0.02	$164 x10^2$ ±0.02	$154 x10^2$ ±0.01	$128 x10^2$ ±0.02	$24 x10^2$ ±0.01	$61 x10^2$ ±0.05	$4522 x10^2$ ±0.69	$4658 x10^2$ ±0.66
AC 5088	$170 x10^2$ ±0.02	$139 x10^2$ ±0.01	$224 x10^2$ ±0.01	$252 x10^2$ ±0.16	$184 x10^2$ ±0.04	$111 x10^2$ ±0.05	$2892 x10^2$ ±0.40	$4718 x10^2$ ±0.35
AC 7788	$239 x10^2$ ±0.05	232 x10 ±0.01	$186 x10^2$ ±0.04	$112 x10^2$ ±0.01	$31 x10^2$ ±0.01	$58 x10^2$ ±0.04	$3994 x10^2$ ±0.35	$3508 x10^2$ ±0.39
AC 8831	$230 x10^2$ ±0.01	$210 x10^2$ ±0.03	$130 x10^2$ ±0.01	$196 x10^2$ ±0.05	$128 x10^2$ ±0.06	$183 x10^2$ ±0.03	$3938 x10^2$ ±0.47	$3298 x10^2$ ±0.84

Table 2. Mean heavy metal concentrations (in µg/g) in dry matter of plant biomass of ten mustard accessions (n = 10). (Means within a column followed by the same letter are not significantly different according to Duncan's Multiple Range Test at α = 0.05).

Mustard Accessions	Mn	Co	Pb	Zn
AC 501	$161x10^2$ b	$100 x10^2$ b	$105 x10^2$ ab	$6413 x10^2$ a
AC 515	$225 x10^2$ a	$104 x10^2$ b	$54 x10^2$ b	$3448 x10^2$ b
AC 580	$151 x10^2$ b	$98 x10^2$ b	$36 x10^2$ b	$3948 x10^2$ b
AC 790	$196 x10^2$ ab	$164 x10^2$ ab	$38 x10^2$ b	$4254 x10^2$ b
AC 1099	$197 x10^2$ ab	$183 x10^2$ ab	$112 x10^2$ ab	$4104 x10^2$ b
AC 1814	$162 x10^2$ b	$185 x10^2$ ab	$115 x10^2$ ab	$4309 x10^2$ b
AC 2122	$161 x10^2$ b	$141 x10^2$ ab	$43 x10^2$ b	$4590 x10^2$ b
AC 5088	$155 x10^2$ b	$238 x10^2$ a	$148 x10^2$ a	$3805 x10^2$ b
AC 7788	$236 x10^2$ a	$149 x10^2$ ab	$45 x10^2$ b	$3751 x10^2$ b
AC 8831	$220 x10^2$ a	$163 x10^2$ ab	$156 x10^2$ a	$3618 x10^2$ b

Table 3. Mean heavy metal concentrations (in µg/g) in soil during Maha and Yala seasons.

Season	Mn	Co	Pb	Zn
Yala	$697x10^2$ ±0.07	$152 x10^2$ ±0.04	$111 x10^2$ ±0.02	$6025 x10^2$ ±0.35
Maha	$945 x10^2$ ±0.12	$181 x10^2$ ±0.05	$114 x10^2$ ±0.01	$7004 x10^2$ ±0.39
Mean Total	**$821 x10^2$**	**$167 x10^2$**	**$113 x10^2$**	**$6515 x10^2$**

Table 4. Mean heavy metal concentrations (in µg/g) in ten mustard accessions and mean (in Maha and Yala) soil heavy metal concentrations.
(STD - Minimum recommended standard heavy metals concentrations in hyperaccumulator plants.)

Mustard Accession	**Mn** (µg/g dry weight) STD value: >10x10³		**Co** (µg/g dry weight) STD value: >1x10³		**Pb** (µg/g dry weight) STD value: >1x10³		**Zn** (µg/g dry weight) STD value: >10x10³	
	In Plant Biomass	In soil	In Plant Biomass	In soil	In Plant Biomass	In soil	In Plant Biomass	In soil
AC 501	$161x10^2$	$821x10^2$	$100\ x10^2$	$167x10^5$	$105x10^2$	$113x10^2$	$6413x10^2$	$6515x10^2$
AC 515	$225\ x10^2$		$104\ x10^2$		$54\ x10^2$		$3448x10^2$	
AC 580	$151\ x10^2$		$98\ x10^2$		$36x10^2$		$3948x10^2$	
AC 790	$196\ x10^2$		$164\ x10^2$		$38x10^2$		$4254x10^2$	
AC1099	$197\ x10^2$		$183\ x10^2$		$112x10^2$		$4104x10^2$	
AC 1814	$162\ x10^2$		$185x\ 0^2$		$115x10^2$		$4309x10^2$	
AC 2122	$161\ x10^2$		$141x10^2$		$43x10^2$		$4509x10^2$	
AC 5088	$155\ x10^2$		$238\ x10^2$		$148x10^5$		$3805x10^2$	
AC 7788	$236\ x10^2$		$149\ x10^2$		$45x10^2$		$3751x10^2$	
AC 8831	$220\ x10^2$		$163\ x10^2$		$156x10^2$		$3618x10^2$	

DISCUSSION

Heavy metal pollution in soil and water is an environmental concern today. Metals and other inorganic contaminants are among the most prevalent forms of contaminants found at waste sites and their remediation in soil and sediments are among the most technically difficult. The projected cost for remediation of areas containing mixtures of heavy metals and organic pollutants by conventional means is estimated to be several billion US$ over the next decade (Prasad and Freitas, 2003). However, phytoremediation technologies are on average ten-fold cheaper than conventional engineering-based remediation methods (Marques *et al*., 2009).

Although all the mustard accessions present in Sri Lanka are *B. jun*cea (L.) Czern, they exhibit different phenotypes based on their geographic distribution in Sri Lanka (Weerakoon *et al*., 2008). Molecular analysis with AFLP markers and cluster analysis of the results have confirmed that these accessions fall into five major clusters showing diverse genetic variation (Weerakoon *et al*., 2008). Similarly, Kumar *et al*. (1995) found that Indian mustard accessions also exhibit considerable phenotypic variation. Indian mustard accessions 426308, 211000, 426314 and 182921 are among the best suited for phytoextraction compared to several other accessions. Several researchers have confirmed the phytoremediation potential of these accessions over other *B. juncea* accessions (Blaylock *et al*., 1997; Ebbs and Kochian, 1998).

A plant is said to be a hyperaccumulator if it can concentrate the pollutants in a minimum percentage which varies according to the pollutant involved. For heavy metals Ni, Cu, Co, Cr and Pb, a hyperaccumulator plant needs to contain over 1000 µg/g of leaf dry weight. For Zn and Mn, hyperaccumulator plants should contain above 10,000 µg/g leaf dry weight (Baker and Brooks, 1989). The results of the present study have shown that all ten mustard accessions are effective accumulators of Mn, Co, Pb and Zn with varied heavy metal accumulation potentials among them.

Results of the present study indicate that different mustard accessions (genotypes) possess varying heavy metal accumulation capacity. All mustard accessions accumulated Zn than other heavy metals. This could be due to a higher content of Zn in soil than Mn, Co and Pb, due to a greater need for Zn in plants being an essential micronutrient or due to greater efficiency in absorbing Zn from soil than other heavy metals. Accession AC 501 showed a significantly high Zn accumulation than all other accessions. According to Ebbs and Kochian (1997), *B. juncea*, *B. napus* and *B. rapa* are more effective at removing Zn from soil than Cu. However, further laboratory and field studies are needed for complete understanding of the hyperaccumulation capacity of these mustard accessions. The findings of the present study suggest that it is worthwhile screening the entire Sri Lankan mustard germplasm for their variability in phytoextraction potential, in order to identify heavy metal accumulators of great phytoextraction potential.

Although difference in heavy metal concentrations in mustard accessions between two seasons was not significant, Mn and Co concentrations in soil were significantly high in Maha than in Yala. During Maha, there was no limitation in soil moisture due to abundant rainfall. However, soil moisture considerably varied during Yala season. Fluctuation of soil macro and micro nutrients during Maha and Yala may have contributed to depletion of soil organic carbon (SOC) and increase in the C:N ratio as well as variation of soil pH. Soil colloidal particles provide large interface and specific surface areas, which play an important role in regulating the concentrations of many trace elements and heavy metals in natural soils (Marques *et al*., 2009).

In Sri Lanka, mustard cultivations are common and they are well adapted to grow in the Dry, Intermediate and Wet zones. Identification of mustard genotypes within local mustard germplasm for higher phytoextractive capacity is very useful. Selection and cultivation of such accessions in heavy metal contaminated areas will enable reclaiming these lands for better use in an eco-friendly and cost-effective manner. The present study revealed that three mustard accessions out of the ten were superior Mn, Co, Pb and Zn hyperaccumulators. Studies by Rajakaruna and Bohm (2002) and Rajakaruna and Baker (2004) have shown high levels of heavy metal accumulation in whole-plant in several Sri Lankan taxa. These include *Crotalaria biflora* (Fabaceae), *Evolvulus alsinoides* (Convolvulaceae), *Hybanthus enneasermus* (Violaceae) for Ni; and *Clerodendron infortunatum* (Verbanaceae), *Croton bonpladianus* (Euphorbiaceae), *Geniosporum tenuiflorum* (Lamiaceae), *Tephrosia villosa* (Fabaceae) and *Waltheria indica* (Sterculiaceae) for Cu. However, prior to this study no work has been reported on phytoextractive potential of *Brassica juncea* (Brasicaceae) genotypes in Sri Lanka.

ACKNOWLEDGEMENTS

We acknowledge the Plant Genetic Resources Centre, Peradeniya for providing seed material for experimentation. Assistance given by Mr. L. A. D. D. Chaminda in conducting experiments is appreciated. We thank the reviewer Dr. Nishantha Rajakaruna of San Jose State University, U.S.A. for his comments, which helped to improve the manuscript.

REFERENCES

Baker, A. J. M. and Brooks, R. R. (1989). Terrestrial higher plants with hyperaccumulate metal elements – a review of their distribution, ecology and phytochemistry. *Biodiversity* **1**: 81-126.

Baker, A. J. M., McGrath, S. P., Reeves, R. D. and Smith, J. A. C. (2000). Metal hyperaccumulator plants: A review of the ecology and physiology of a biological resource for phytoremediation of metal polluted soils. In: N. Terry and G. Banuelos (Eds.), *Phytoremediation of Contaminated Soil and Water*, Lewis, Boca Raton, FL, USA, 85-108

Baker, A. J. M. and Whiting, S. M. (2002). In Search for the Holy Grail-another step in understanding metal hyperaccumulation? *New Phytologist* **155**: 1-7.

Bandara, N. I. G. J. (2003). Water and waste water issues in Sri Lanka. *Water Science and Technology* **47**: 305-312.

Bandara, S. (2006). "Rajarata Goveen Wakugadu Roogeen Karana Pohora". *Lakbima*, December 10, Pp. 21.

Blaylock, M. J. and Huang, J. W. (2000). Phytoextraction of metals. In: I. Raskin and B. D. Ensley (Eds.), *Phytoremediation of toxic metals. Using plants to clean up the environment*. J. Wiley & Sons, New York, USA, 53-70.

Blaylock, M. J., Salt, D. E., Dushenkov, S., Zakharova, O., Gussman, C., Kapulink, Y., Ensley, B. D. and Raskin, I. (1997). Enhanced accumulation of Pb in Indian mustard by soil-applied chelating agents. *Environmental Science and Technology* **31**: 856-860.

Boyd, R. S. and Martens, S. N. (1998). The significans of metal hyperaccumulation for biotic interactions. *Chemoecology* **8**: 1-7.

Boyd, R. S. (2004). Ecology of metal hyperaccumulation. *New Phytologist* **162**: 563-567.

Brooks, R. R., Chambers, M. F., Nicks, L. J. and Robinson, B. H. (1998). Phytomining. *Trends in Plant Science* **3**: 359-362.

Burken, J. G. and Schnoor, J. L. (1997). Uptake and metabolism of atrazine by popler trees. *Environmental Science and Technology* **31**: 1399-1406.

Dissanayake, P., Clemett, A., Jayakody, P. and Amarasinghe, P. (2007). *Report on Water Quality Survey and Pollution in Kurunegala, Sri Lanka.*

Dissanayake, U. S., Tennakoon, K. U. and Priyantha, N. (2002). Potential of two invasive plant species, *Lantana camara* L. and *Wedelia trilobata* L. for selective heavy metal uptake. *Ceylaon Journal of Science (Bio. Sci*.) **29**: 1-11.

Dushenkov, V., Kumar, P. B. A. N, Motto, H. and Raskin, I. (1995). Rhizofilteration: the use of plants to remove heavy metal from aqueous streams. *Environmental Science and Technology* **29**: 1239-1245.

Dushenkov, S. (2003). Trends in phytoremediationof radionuclides. *Plant and Soil* **249**: 167-175.

Ebbs, S. D. and Kochian, L. V. (1997). Toxicity of zinc and copper to *Brassica* species: Implications for phytoremediation. *Journal of Environmental Quality* **26**: 776-781.

Ebbs, S. D. and Kochian, L. V. (1998). Phytoextraction of zinc by oat (*Avena sativa*), barley (*Hordeum vulgare*), and Indian mustard (*Brassica juncea*). *Environmental Science and Technology* **32**: 802-806.

Ensley, B. D. (2000). Phytoremediation for toxic metals – using plants to clean-up the environment. In: I. Raskin and B. D. Ensley (Eds.), *Rational for use of phytoremediation*, John Wiley & Sons, Inc., 3-13.

Hong, M. S., Farmayan, W. F., Dortch, I. J., Chiang, C. Y., McMillan, S. K. and Schnoor, J. L. (2001). Phytoremediation of uranium-contaminated soils: role of organic acids in triggering uranium hyperaccumulation in plants. *Environmental Science and Technology* **35**: 1231-1239.

Hughes, J. B., Shanks, J., Vanderford, M., Lauritzen, J. and Bhadra, R. (1997). Transformatiom of TNT by aquatic plants and plant tissue cultures. *Environmental Science and Technology* **31**: 266-271.

Jiang, W., Liu, D. and Hou, W. (2000). Hyperaccumulation of Lead by roots, hypocotyls, and shoots of *Brassica juncea*. *Biologia Plantarum* **43(4)**: 603-606.

Kumar, P. B. A. N., Dushenkov, V., Motto, H. and Raskin, I., (1995). Phytoextraction: the use of plants to remove heavy metals from soils. *Environmental Science and Technology*, **29**: 1232-1238.

Lindsay, W. L. and Norvell, W. A. (1978). Development of a DTPA soil test for Zn, Fe, Mn and Cu. *Soil Science Society of America Journal* **42**: 421-428.

Linn, D. M. and Doran, J. W. (1984). Effect of water-filled pore space on carbon dioxide and nitrous oxide production in tilled and non tilled soils. *Soil Science Society of America Journal* **48**: 1267-1272.

Marques, A., Rangel, A. O. S. S. and Castro, P. M. L. (2009). Remediation of Heavy Metal Contaminated Soils: Phytoremediation as a Potentially Promising Clean-up Technology. *Critical Reviews in Environmental Science and Technology* **39**: 622-654.

McGrath, S. P. (1998). Phytoextraction for soil remediation. In: R. R. Brooks (Eds.), *Plants that hyperaccumulate heavy metals: their role in phytoremediation, microbiology, archeology, mineral exploration and phytomining*, CAN International, New York, 261-288.

Newman, L. A., Strand, S. E., Choe, N., Duffy, J., Ekuan, G., Ruszaj, M., Shurtleff, B. B., Wilmoth, J., Heliman, P. and Gordon, M. P. (1997). Uptake and biotransformation of trichloethylene by hybrid poplars. *Environmental Science and Technology* **31**: 1062-1067.

PGRC Catalogue. (1999). Plant Genetic Resources Catalogue Passport Information. Plant Genetic Resource Centre (PGRC), Gannoruwa, Sri Lanka. Pp. 10-12.

Pilon-Smits, E. A. H. and Freeman, J. (2006). Environmental cleanup using plants: Biotechnological advances and ecological considerations. *Frontiers in Ecology and Environment* **4(4)**: 203-210.

Prasad, M. N. V. and Freitas, H. M. O. (2003). Metal hyperaccumilation in plants- Biodiversity prospecting for phytoremediation technology. *Electronic Journal of Biotechnology* **6**: 285-321.

Rajakaruna, N. and Bohm, B. A. (2002). Serpantine and its vegetation; a preliminary study from Sri Lanka. *Journal of Applied Botany* **76**: 20-28.

Rajakaruna, N. and Baker, A. J. M. (2004). Serpentine: A model habitat for botanical research in Sri Lanka. *Ceylaon Journal of Science (Bio. Sci*.) **32**: 1-19.

Rajakaruna, N., Tompkins, K. M. and Pavicecevic, P. G. (2006). Phytoremediation: An affordable green technology for the clean-up of metal-contaminated sites in Sri Lanka. *Ceylaon Journal of Science (Bio. Sci*.) **35**: 25-39.

Reeves, R. D. and Baker, A. J. M. (2000). Metal Accumulating Plants. In: I. Raskin and B. D. Ensley (Eds.), *Phytoremediation of toxic metals. Using plants to clean up environment*, J. Wiley & Sons, New York, USA, 193-229.

Robinson, B. H., Banuelos, G., Conesa, H. M., Evangelon, W. H. and Schulin, R. (2009). The Phytomanagement of Trace Elements in Soil. *Critical Reviews in Plant Sciences* **28(4)**: 240-266.

Schnoor, J. L., Light, L. A. Mccutcheon, S. C., Wolfe, N. L. and Carreira, L. H. (1995). Phytoremediation of organic and nutrient contaminants. *Environmental Science and Technology* **29**: 318-323.

Soltanpour, P. N. and Schwab, A. P. (1977). A new soil test for simultaneous extraction of macro and micro nutrients in alkaline soils. *Communications in Soil Science and Plant Analysis* **27**(7 & 8): 945-657.

Weerakoon, S. R., Weerasena, O. V. D. S. J., Peiris, P. K. D. and Somaratne, S., (2008). Differentiation of Sri Lankan mustard **(***Brassica juncea***)** accessions using FAFLP markers and Agro-Morphological data. Proceedings of *International Society of Bio Technology Conference (ISBT-2008), Gangtok, Skkim, India*, Pp. 239-243.

World Health Organization (2003). Health as a cross-cutting issue in dialogues on water for food and the environment. Report for an international workshop. http://whqlibdoc.who.int/2004/WHO_SDE_WSH_04.02.pdf.

Zhang, L., Angle, J. S. and Chaney, R. L. (2007). Do high-nickel leaves shed by the Ni-hyperaccumulator Alyssum murale inhibit seed germination of competing plants? *New Phytologist* **173**: 509-516.

Zhu, Y. L., Pilon-Smits, E. A. H., Jouanin, L. and Terry, N. (1999). Overexpression of gluthathione synthetase in *Brassica juncea* enhances cadmium tolerance and accumulation. *Plant Physiology* **119**: 73-79.