# Uptake and Accumulation of Cadmium by *Brassica juncea* and *Ageratum conisoides*

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## ABSTRACT

**Three *Brassica juncea* accessions i.e. 1440, 9726 and 1847 and *Ageratum conisoides* were tested for cadmium (Cd) uptake to determine their ability to accumulate Cd from a nutrient solution. Plants were exposed to 30 µM and 50 µM Cd in Hoagland nutrient solution for 14 days. Cadmium content in the plant shoots and roots were analyzed using an Atomic Absorption Spectrophotometer (AAS). *Ageratum conisoides* accumulated substantially high amount of Cd in their shoots and roots. All *B. juncea* accessions and *A. conisoides* were found to be hyperaccumulators when exposed to 50 µM Cd solution. Out of *B. Juncea* accessions, 1440 was the highest Cd accumulator. Root Cd content was four fold higher than shoot Cd accumulation for all genotypes.**

**KEY WORDS**: Ageratum *conisoides*, *Brassica juncea*, Cadmium uptake and accumulation, Heavy metal contamination, Phytoremediation

## INTRODUCTION

Indian mustard (*Brassica juncea* L., Family Brassicaceae), a high biomass crop plant, accumulates substantial amounts of cadmium (Cd), both in shoots and roots (Salt *et al.*, 1995). *Ageratum conisoides* (Family Compositae) is a hardy weed, growing on any soil in Sri Lanka.

Heavy metal contamination of the biosphere has increased sharply since 1900 (Nriagu, 1979) and poses major environmental and human health problems worldwide (Ensley, 2000). Among the heavy metals, Cd has a great potential to accumulate in the soil. Sources include the ashes from fossil fuel combustion, waste from cement manufacture and the disposal of municipal refuse and sewage sludge. As an agricultural application, phosphate fertilizer represents a direct input of Cd to arable soils (Freiberg *et al.*, 1992).

The necessity of degradation of a metal in the environment is dependent upon its level of toxicity. Particularly Cd has adverse effects on human health and the environment. Human uptake of Cd takes place mainly through food and tobacco transport of Cd into the lungs. Blood will transport it through the rest of the body. Breathing of Cd can cause severe damages to the lungs. Cadmium accumulation in kidneys damages the filtering mechanism. Diarrhea, stomach pains, severe vomiting, bone fracture, reproductive failure and even infertility, damage to the central nervous system and immune system, physiological disorders and cancer development are other possible effects of Cd.

Unlike many organic contaminants, most metals and radio nuclides cannot be eliminated from the environment by chemical or biological transformation (Cunningham and Ow, 1996, NRC, 1997). The various conventional remediation technologies that are used to clean heavy metal polluted environments are soil *in-situ* vitrification, soil incineration, excavation and landfill, soil washing, soil flushing, solidification and stabilization remediation (EPA, 1997, MADEP, 1993).

There is a relatively high potential for application of "Phytoremediation" as a soil and water clean-up method. Phytoremediation is a diverse collection of plant based technologies that use either naturally occurring or genetically engineered plants for cleaning environments (Cunningham *et al.*, 1997, Flathman and Lanza, 1998). It is an efficient clean up technology for a variety of organic and inorganic pollutants and an important subject for the restoration of the environment. Phytoremediation consists of four plant based technologies each having a different mechanism of action for the remediation of metal polluted soil, sediment or water. These are rhizofiltration, phytostabilization, phytovolatalization and phytoextraction.

The primary motivation behind the development of phytoremediative technologies is the potential for low cost remediation (Ensley, 2000). Using of standard or slightly modified agricultural equipment and practices makes the installation cheap. Because the primary energy input is solar, operating costs are low. Public acceptance of a phytoremediation project on a site can be very high, in part because of the park like aesthetics, including providing shade, dust control and bird and wildlife habitat. Once established these plants can be grown for many years, degrading compounds whilst providing a vegetative cap for the site.

Some plants have developed the ability to accumulate massive amounts of the indigenous metals in their tissues without exhibiting symptoms of toxicity i.e. hyperaccumulator plants. According to Brown *et al.*, (1995), the leaves of the hyperaccumulator plants may contain >100 mg $kg^{-1}$ cadmium, >1,000 mg $kg^{-1}$ Nickel (Ni) and Copper

(Cu) or >10,000 mg $kg^{-1}$ Zinc (Zn) and Manganese (Mn) a dry weight (DW) basis, when they are grown in soils with a high concentration of metals.

Metal accumulation not only varies between species, but also within a species. For instance, there is considerable between-population differences in both Cd tolerance and Cd hyperaccumulation in *Thlaspi caerulescens* (Roosens *et al.*, 2003).

In this study, three accessions of *B. juncea* and plants of *A. conisoides* were tested for Cd uptake and accumulation to determine whether there is a difference between genotypes. Relationship between the root and shoot biomass production with the exposure to difference concentrations of Cd was investigated in *B. juncea* and *A. conisoides*. Finally the variation of Cd uptake and accumulation between two plant species was determined.

## MATERIALS AND METHODS

The experiments were carried out at the Plant Reproductive Biology laboratory of the Institute of Fundamental Studies (IFS), Hantana road, Kandy during the 20 week period.

Seeds of three accessions of *B. juncea* (1774, 1847, and 9726) were obtained from the Plant Genetic Resource Centre (PGRC), Gannoruwa. *Ageratum conisoides* seedlings were collected from the IFS premises.

### *1. Growing of Plants*

Seeds of the three accessions of *B. juncea* were sown on moistened filter papers in separate Petri dishes and supplied with a dark environment to accelerate germination. Three days after seed sowing, small seedlings were transferred to washed, pure sand medium in polythene bags and plastic cups, one seedling per vessel. They were kept in the green house for one month and supplied with different strengths of modified Hoagland nutrient solution (Hoagland and Arnon, 1950) at different growth stages. Then they were transferred to 0.5 M Hoagland solution prepared with tap water. Healthy, vigorous *A. conisoides* seedlings with five to six leaves were directly transferred to 0.2 M strength modified Hoagland nutrient solution. Plants were kept for two weeks in Hoagland solution replaced once in every three days.

### *2. Cadmium Treatments for Plants in Hydroponics Solution*

After 14 days in nutrient solution culture, Hoagland solution was supplemented with Cadmium sulphate ($CdSO_4$) solution to give final concentrations of 30 µM or 50 µM Cd. Each treatment was replicated in three vessels, each vessel containing four and five plants, of *B. juncea* and *A. conisoides*, respectively. Another two vessels were kept as controls with the same number of plants, but without Cd in the Hoagland solution. The nutrient solution in all the vessels was aerated continuously.

Any changes in the plants were noted after treatment such as wilting of plants, unusual falling down of leaves, burning of leaves, emergence of auxiliary shoots, discoloration of leaves, rooting, discoloration of roots and root drying until harvesting.

### *3. Harvesting, Drying and Grinding of Plant Samples*

Plants were harvested 14 days after Cd treatment. Roots were washed twice with 10 mM Ethylenediaminetetra acetic acid disodium salt ($Na_2EDTA$) solution (for five minutes each) and then twice with deionized water to remove surface bound cadmium as described by Roosens *et al.*, (2003).

The roots and shoots were separated and oven dried at 60 $^0$C to a constant mass and the samples were weighed and ground to a fine powder using mortar and pestle.

### *4. Ashing, Ash Dissolving and Sample Analysis Using Atomic Absorption Spectrophotometer*

From the sample, a known weight was ashed at 550 $^0$C for eight hours in a muffle furnance. Then the ash was dissolved in 5 ml of concentrated hydrochloric acid (HCl) in crucibles. It was volumerized up to 50 ml with deionized water. After filtering the solution through a filter paper, it was analyzed using flame atomic absorption spectrophotometer (Model GBC 933 AA) for cadmium.

### *5. Statistical Analysis*

Two-way analysis of variance (ANOVA) followed by Turkey test using Sigmastat statistical software (user's manual, 1995) was performed. The statistical significance was set at $P<0.05$ probability level.

## RESULTS AND DISCUSSION

Figure 1 shows the yield of the biomass for three accessions of *B. juncea* and *A. conisoides* exposed to 30 µM or 50 µM Cd. It is seen that the Cd has adversely affected the shoot and root growth at different Cd concentration levels. 50 µM Cd inhibited the growth of *B. juncea* and *A. conisoides* more than that of 30 µM Cd. Leaf chlorosis and auxiliary shoot emergence were observed in 50 µM Cd treated 1847 accession plants earlier while 30 µM Cd treated plant also showed that toxicity symptoms at a later stage of the treatment period. In the later stage of the growth, 50 µM Cd treated plants exhibited necrosis on young leaves and flowers appeared in an unusual light yellow colour. The accessions 1440 and 9726 showed necrosis on young leaves when exposed to both 30 µM and 50 µM Cd. Salt *et al.*, (1995) reported that the Cd toxicity in *B. juncea* L. produces chlorosis in the young leaves, where Cd accumulated. Chlorosis on young leaves and auxiliary shoots appeared seven and eleven days after exposure to 50 µM and 30 µM Cd respectively in *A. conisoides*. In these treatments distorted, chlorotic leaf production,

necrotic patches on lower leaves and falling down of leaves were also observed.

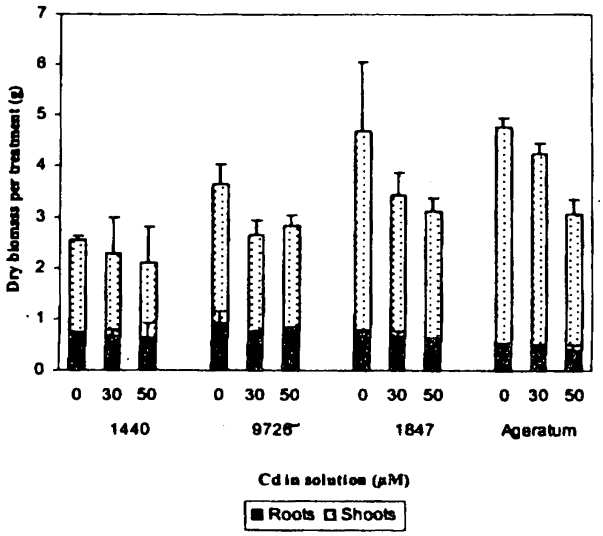


**Figure 1 - Shoot and Root dry biomass for three accessions of *B. juncea* and *A. conisoides* exposed to 30 μM or 50 μM Cd. Values are means ± SE (n=2 for 0 Cd and n=3 for 30 μM and 50 μM Cd):**

Two way ANOVA showed that there was a significant difference in shoot Cd accumulation when exposed to 30 μM and 50 μM Cd solutions ($P<0.05$). *Brassica juncea* 1440 and 9726 accessions and *A. conisoides* accumulated more than 100 μg $g^{-1}$ (DW) of Cd in their shoots when exposed to both 30 μM (0.03 μg $ml^{-1}$) and 50 μM (0.05 μg $ml^{-1}$) Cd that exceed the defined level for a Cd hyperaccumulator. The hyperaccumulators that have been examined for use in phytoextraction of Cd are able to take up more than 5,000 μg $g^{-1}$ Cd into their shoots from a 5 μg $ml^{-1}$ Cd solution (Baker *et al.*, 1994).However, shoots of accession 1847 did not accumulate 100 μg $g^{-1}$ Cd in 30 μM Cd solution, but in 50 μM Cd solution (Table 1).

Shoot Cd accumulation in accessions 1847 and 9726 was not significantly different. Accession 1440 had accumulated two fold Cd in shoots as 1847 and 9726 accessions. *A. conisoides* had taken up significantly high Cd amount into shoots compared to 1847 and 9726 accessions ($P<0.05$). Shoot Cd concentration in *A. conisoides* and 1440 *B. juncea* did not vary significantly.

The roots of *B. juncea* are effective in the removal of Cd from hydroponics solutions (Dushenkov *et al.*, 1995). This investigation proved the roots of *A. conisoides* is also effective in removing Cd from hydroponics solutions. Root Cd uptake was significantly varied among genotypes and solution Cd level ($P<0.05$). Root Cd uptake by *A. conisoides* was higher than that of investigated *B. juncea* accessions. Comparison within *B. juncea* accessions showed accession 1847 did not vary significantly from either 9726 or 1440 accessions. But, Cd uptake by 1440 and 9726 accessions was significantly different (Table 2).

Root Cd uptake did not varied significantly among of the four genotypes in 30 μM Cd solution. Uptake of Cd by *A. conisoides* was higher compared to *B. juncea* when exposed to 50 μM Cd. Out of *B. juncea* accessions the highest root Cd concentration was found in 1440 accession.

Though Cd uptake was higher at 50 μM Cd treatment, plants showed more toxicity symptoms. Therefore, sites which are heavily contaminated cannot be cleaned through phytoremediation means because harsh conditions will not support plant growth.

**Table 1 - Shoot Cd uptake in *B. Juncea* and *A. conisoides* when exposed to 30 μM and 50 μM Cd. Values are means ± SE (n=3)**

| Genotype | Cadmium in solution | |
|---|---|---|
| | 30 μM | 50 μM |
| 1440 | 205.8 ± 37.58[a] | 338.1 ± 152.19[a] |
| 9726 | 126.6 ± 72.76[b] | 166.0 ± 33.84[b] |
| 1847 | 72.8 ± 6.19[b] | 179.2 ± 73.32[b] |
| *Ageratum* | 327.5 ± 47.70[a] | 411.5 ± 86.23[a] |

Mean Cd uptake with different letters along a column are significantly different.

**Table 2 - Root Cd uptake in *B. Juncea* and A. *conisoides* when exposed to 30 μM and 50 μM Cd. Values are means ± SE (n=3)**

| Genotype | Cadmium in solution | |
|---|---|---|
| | 30 μM[NS] | 50 μM |
| 1440[a] | 842.50 ± 76.61 | 1285.50 ± 213.21[a] |
| 9726[b] | 643.97 ± 69.84 | 779.97 ± 219.42[b] |
| 1847[ab] | 1049.47 ± 387.52 | 704.97 ± 102.00[b] |
| *Ageratum*[c] | 1048.43 ± 289.25 | 2364.50 ± 88.84[d] |

Mean Cd uptake with different letters along a column are significantly different.
[NS]Mean Cd uptake is not significant at P=0.05

**Table 3 - Cadmium concentrations in 30 µM Cd treated *B. juncea* accessions at 0.3 M and 0.4 M strength Hoagland solutions. Values are means ± SE (n=4)**

| Accession | Hoagland concentration | | | |
|---|---|---|---|---|
| | Shoot Cd concentration | | Root Cd concentration | |
| | 0.3M | 0.4M | 0.3M | 0.4M |
| 1440 | [a]174.05 ± 45.79 | [a]205.8 ± 37.58 | [a]1009.5 ± 284.11 | [a]845.22 ± 76.60 |
| 9726 | [b]120.25 ± 18.30 | [b]126.6 ± 72.76 | [a]665.42 ± 182.45 | [a]643.96 ± 643.96 |
| 1847 | [b]70.65 ± 53.64 | [b]72.8 ± 6.19 | [a]1162.9 ± 328.87 | [a]1049.49 ± 387.53 |

Mean Cd uptake with different letters along a column are significantly different.

The depth of soil that can be cleaned or stabilized is restricted to root zone of the plants being used (Schnoor *et al.*, 1995).

Thus, *A. conisoides* accumulated higher amount of Cd when grown in 0.2 M Hoagland nutrient solution, where half strength from the medium, *B. juncea* was grown. The reason for this high performance may be due to *A conisoides* being a hardy weed. Salt *et al.*, (1995) found Cd is preferentially accumulated in the trichomes on the leaf surface in *B. juncea*. Non significant shoot Cd accumulation by *A. conisoides* and 1440 *B. juncea* accession could be described by the presence of trichomes on the lower and upper leaf surfaces. *A. conisoides* has trichomes along the stem and petioles too.

Cd uptake and accumulation by *B. juncea* that grown in 0.3 M Hoagland solution was not significantly different from that of 0.4 M Hoagland solution (Table 3). However, at low nutrient medium, plants visualized more toxic symptoms compared to high nutrient medium. Cadmium untreated controls also showed deficiency symptoms like chlorosis.

Analysis of Cd untreated plants also showed small amount of Cd (data not shown). This may be due to cross contaminations.

## CONCLUSION

*Ageratum conisoides* is capable to uptake and accumulate high amount of Cd at a low nutrient level, 0.2 M Hoagland solution, though with some toxicity symptoms. At 50 µM Cd treatment *A conisoides* and the three *B. juncea* accessions were hyperaccumulators of Cd. There was no difference between Cd uptake and accumulation by the three accessions of *B. juncea* when exposed to 0.3 M and 0.4 M Hoagland nutrient solutions. In 0.4 M Hoagland solution 1440 accession uptakes and accumulates high amount of Cd than other two accessions from both 30 µM and 50 µM Cd solutions. Further experiments are required to confirm the results obtained.

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