Phytoremediation potential of *Brassica juncea* Ac. 1774 for mitigation of Cu (II) and As (V)

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Introduction

Phytoremediation is an emerging technology that employs the use of higher plants to clean up contaminated environments. Use of plants to extract toxic metals from contaminated soil and water, has emerged as a cost-effective, environment-friendly clean up alternative. In Sri Lanka large areas of soil and water contain high levels of heavy metals such as Cd, Cu, Co, Ni and Zn and other pollutants (Dissanayake *et al*., 2002). Members of the Brasicaceae are promising candidates for phytoextraction of metals (Kumar *et al*. (1995, Weerakoon and Somaratne, 2009).Sri Lanka has genetically diverse mustard (*B. juncea* (L.) Cazen) germplasm of over sixty accessions (PGRC Catalogue, 1999) and their true phytoextarctive potentials are yet to be determined. Most of the previously reported research on phytoremediation has conducted *in vivo* using soil to establish plants. Use of soil which is a highly heterogeneous medium could not unveil the true phytoremediation potential of plants due to micro-variations in the composition of soil. Hence, this study was performed *in vitro* and the plants were grown in well-defined plant tissue culture media where all other physical parameters such as light intensity and relative humidity were kept constant. The main objectives of this study was to investigate the maximum accumulation level of copper Cu (II) and Arsenic As (V) by *in vitro* raised plants of *Brassica juncea Ac:1774* which has already been identified as a hyper-accumulator of Cr (VI) and multiple metal bio-accumulator (Wijethunghe *et al*., 2010).

Methodology

Authenticated seed samples of *Brassica juncea* (Indian mustard) of AC: 1774 was obtained from the Plant Genetic Recourses Centre (PGRC) at Gannoruwa, Sri Lanka. Seeds were surface sterilized by rinsing with a 10% solution of commercial bleach for 5 min followed by rinsing with 70% ethanol solution for 2 min and three times through washings with sterilized distilled water. Sterilized seeds were briefly dried on filter papers and cultured on solid MS (Murashige and Skoog, 1962) media. One week old seedlings were transferred on to a wick placed in liquid MS media in 15 cm culture tubes supplemented with 0, 50, 150, 200 ppm Cu (II) and 0,5, 15, 25 ppm As (V) separately. Seedlings were allowed to grow for three weeks in this medium. After that plantlets were removed, washed with tap water and dried in an oven. Dried platelets were grounded separately to obtain a fine powder which was acid digested following dry-ash-method and the accumulated heavy metal concentration for each sample was determined using Atomic Absorption Spectrometer (Varian, Australia). Each treatment had three replicates within an experiment and each experimented was repeated three times.

Result and Discussion

Plants of *Brassica juncea Ac:1774* demonstrated relatively a high bioaccumulation of two heavy metals tested, As (V) and Cu (II). For As (V) the maximum tolerance level was 25 ppm while for Cu (V) it was 150 ppm (Fig 1 and 2). Plants showed a significantly ($p \leq 0.05$) higher Cu (II) toleranc e than As (V). At high concentrations plants showed signs of necrosis and retarded growth due to toxic effects of As (V). There was no significant effect by replication (Table 1 and 2) and this provides evidence that by growing plants *in vitro*, plant to plant variations as well as environmental effects could be kept at a minimum levels. Sings of toxicity was shown by plants after two to three days from transfer to MS media supplemented with high concentrations of heavy metals. The effect of AS (V) was prominent and physiological breakdown of plants is so fast. It has been reported that Arsenate replaces phosphate when taken up by plants that disrupts the production of ATP which results in sudden cell death. Also arsenic is inhibitory towards cell function because it reacts with sulfhydryl enzymes and disrupts their activity (Luongo and Ma, 2005).

Table 01: Accumulation of As (V) (µg/g) in *in vitro* grown plants of *Brassica juncea Ac:1774* after 21 days

	0 ppm	5 ppm	15 ppm	25 ppm
R1	0.0	35.1	45.6	56.9
R2	0.0	26.6	49.5	64.6
R3	0.0	35.1	41.2	61.8

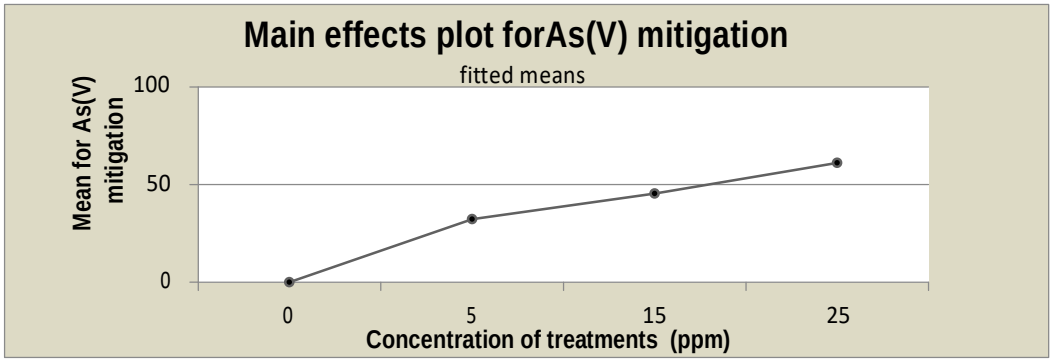

Figure 01. Comparison of means of accumulated As (V) (µg/g) after 21 days of Mitigation against As (V) concentration (ppm) of the different treatments.

Table 1: Accumulation of Cu (II) (µg/g) in *in vitro* grown plants of *Brassica juncea Ac:1774* after 21 days

	0 ppm	50 ppm	150ppm	200 ppm
R1	2	689	982	871
R2	8	600	1005	824
R3	6	702	1018	747

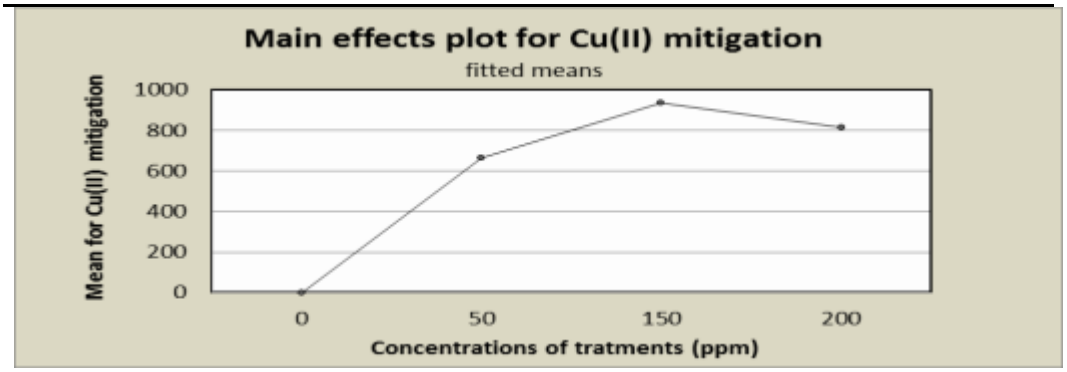

Figure 02. Comparison of means of accumulated Cu (II) (µg/g) after 21 days of Mitigation against Cu (II) concentration (ppm) of the different treatments.

The results indicate that plants of *Brassica juncea Ac:1774* could tolerate As (V) at levels higher than the maximum level (25 ppm) used in this study. The concentrations of As (V) in different treatments tried in this experiment were determined according to previously reported literature. The results of this study shows that *Brassica juncea Ac:1774* could tolerate even higher As (V) concentrations than previous studies for *Brassica juncea.* This study also demonstrates that *Brassica juncea* plants could even be used to treat not only contaminated soils as reported by Weerakoon and Somaratne (2009), but also to treat heavy metal contaminated ware water.

Conclusions

This study demonstrated that plants of *Brassica juncea Ac 1774* have hyper-accumulative ability of heavy metals such as As (V) and Cu (II). The amount of accumulation of AS (V) reported in this study by accession 1774 was higher than any previously reported amounts for other *Brassica juncea* varieties. Adaptation of *in vitro* technique has shown to reduce the replication error and environmental effects compared to the commonly adapted method of growing plants in soil which is highly heterozygous. The protocol adapted in this study is suitable for treatment of industrial waste water contaminated with heavy metals.

Acknowledgement

Authors acknowledge PGRC, Gannoruwa for supplying seed samples of *Brassica juncea Ac 1774* and the Department of Geology, Uuniversityof Peradeniya for their assistance in sample analysis.

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