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Original Research Article

Evaluation of potential of *Brassica juncea* for removal of arsenic from hydroponic solution

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A B S T R A C T

In the present scenario, heavy metals are an increasing ecological threat which commonly results from human activities. Mining, manufacturing and the use of synthetic products (e.g., pesticides, paints, batteries, industrial waste and land application of industrial or domestic sludge) can result in heavy metal contamination of urban and agricultural soil and water environment. Heavy metal pollution is a worldwide problem. Arsenic (As) is a potential environmental hazard. The present study discusses the heavy metal in particular As elimination by the phytoremediation technique. We have explored the potential of *Brassica juncea* for remediation of As. The young and healthy plants of *B. juncea* initially grown in liquid medium were transferred to Hoagland solution spiked with As -As $(NO_3)_2$ at various concentrations, viz. 0, 5, 10, 25 and 50 ppm separately. The As content in plant tissue was quantified using Atomic Absorption Spectroscopy. The result shows that most of As up to 26650 µg/g dry weight was located in roots while 5462 µg/g dry weight was translocated to shoots when exposed to 50 ppm concentration of As. The phytoremediation of As using *B. juncea* plant in hydroponic solution shows that during the period of experiment (i.e. 21 days) the plant was found to have potential to remove 80-86% of As.

Introduction

Human activities over the centuries have contaminated many areas of developing and developed countries. Soil –water contamination with heavy metals is one of the great problems of modern societies. Heavy metals periodically increase in the environment due to industrial activities. Increasing of these pollutants in the environment is considered as a serious threat to human and environmental activities.

Arsenic is highly toxic metal element that annually threatened the health of millions of people in the world. Inorganic Arsenic forms are more dangerous than other forms for human health. Arsenic-contaminated soils, sediments, and sludge are the major sources of arsenic contamination of the food chain, surface water, groundwater, and drinking water (Frankenberger and Arshad, 2002). Arsenic exposure can cause kidney and liver damage, erythrocyte haemolysis and cause death.

So, there is urgent need to remove Arsenic from the polluted environment. Phytoremediation is regarded as the cheapest and environmentally most friendly technology for cleaning up soil. Phytoremediation makes use of the natural ability of green plants to accumulate or degrade toxic heavy metals from contaminated environment (Baker, 1994; Kumar *et al.,* 1995; Reeves and Baker, 2000; Pulford and Watson, 2003; Lal and Srivastava, 2010). Topsoil would be preserved and the amount of hazardous materials reduced significantly (Ensley, 2000). The main factors controlling the ability of phytoremediation are plant species, metal availability to plant roots, metal uptake by roots, metal translocation from roots to shoots and plant tolerance to toxic metals. The plants which accumulate high amount of heavy metals are known as hyper accumulators. Hyper accumulators can be selected by growing the plants in hydroponic media supplemented with metals.

In the present study, Indian mustard plant has been screened and selected for the phytoremediation of heavy metal – Arsenic. Indian mustard (*Brassica juncea*) also known as mustard greens and leaf mustard, is a species of mustard plants and belongs to the family Brassicaceae. The plant was selected for the phytoremediation study based on their fast growth rate/high biomass along with the tolerance potential to thrive in heavy metals contaminated environment. *Brassica juncea* is best suited for phytoremediating arsenic contamination (Selvaraj et al 2013). The present study aimed to evaluate the uptake potential of arsenic by *B.juncea* plant grown in hydroponic media under *in vitro* condition.

Material and Methods

Plant materials

The seeds of *B. juncea* were obtained from local seed's market, Muzaffarpur, Bihar. Seeds were pre-soaked in soap and dettol solution for 15 minutes and thoroughly washed in running tap water. Then the seeds were sterilized with 70% ethanol for 30 seconds followed by sterilization with 0.1% mercuric chloride for 3–5 min. The seeds were thoroughly rinsed 5 times with sterile distilled water. These sterilized seeds were inoculated in test tubes containing MS (Murashige and Skoog, 1962) basal medium supplemented with 3% sucrose. Seedlings were allowed to grow for one month under *in vitro* condition.

Experimental setup

Seedlings (one month old) of similar size were transferred to Hoagland nutrient solution (Hoagland and Arnon, 1938), containing various plant nutrients spiked with different concentrations i.e. 0, 5, 10, 25 and 50 ppm of As separately. The experiments were carried out in triplicates for 21 days and average values are reported. Sample of 500 µl aliquots were withdrawn from each concentration at an intervals of $0^{th,}$ 1^{st}, 7^{th}, 14^{th} and 21^{st} day of treatment. These samples were used for the analysis of metal contents. The plants after treatment were harvested as roots and shoots, thoroughly washed three times with deionized water, and then dried at 60^oC to constant weight. The dry weight of roots and shoots were recorded.

Analytical methods

The dried root and shoot samples was digested with 10 ml of perchloric acid: nitric acid ($HClO_4$: HNO_3- 1:5 v/v) mixture separately. Acid digestion was carried out on hot plate at 70–100^{o} C. The digested sample was dissolved in distilled water, filtered for the removal of impurities (APHA, 1998) and made up to the desired volume. The samples were analyzed by Atomic Absorption Spectrophotometer using air-acetylene flame to estimate As content in the plant roots as well as in shoots.

Data analysis

The selected metal-As was taken up by the plants through their roots from the solution. Experimental data were analyzed for uptake of As by the plants of *B. juncea*. The experiment was carried out in triplicates and average values are reported. Data were analyzed for mean and standard deviation (X±S.D.) using standard statistical methods (Mahajan, 1997).

The ability of plants to translocate heavy metals from the roots to the harvestable aerial part is defined as translocation factor, TF (Mattina *et al.,* 2003). The phytoextraction rate or bioaccumulation coefficient was described as the heavy metal concentration in plant divided by heavy metal concentration in the solution (Kumar *et al.,* 1995).

Translocation factor and bioaccumulation coefficient were computed using the following formulae:

$$\text{Translocation factor (TF)} = \frac{\text{Shoot metal content}}{\text{Total plant metal content}} \times 100$$

$$\text{Bioaccumulation Coefficient (BC)} = \frac{\text{Arsenic content g}^{-1}\text{ Dry plant tissue}}{\text{Arsenic content ml}^{-1}\text{ nutrient solution}}$$

Results and Discussion

To observe the accumulation of heavy metals in the root and shoot biomass, experiments were performed in which well rooted plants of *Brassica juncea* were placed in Hoagland liquid medium supplemented with 4 different concentrations i.e. 5, 10, 25 and 50 ppm of As separately including control. After the experiment the plants were removed from the solution and harvested for the bioaccumulation study.

Dry biomass analysis

The accumulation study is based on the dry biomass of the roots and shoots of *B. juncea.* The dry biomass was determined after taking out the material from the solution, which was properly washed and weight dried at 60^{o}C and weighed. Finally the constant weight was taken. The data have been presented in the Table 1.

Perusal of the table indicates the dry biomass of the root was 0.21g and 0.098g for the shoot at 25 ppm of As. It was 0.016g and 0.078g for the roots and shoots dry weight, respectively at 10 ppm of As concentration; while at 5 ppm, it was 0.028g and 0.068g for roots and shoots, respectively (Figure 2). Similar observation was reported by Quartacci *et al.* (2005) in phytoextraction of cadmium by the Indian mustard. The data presented in the above table clearly indicates that the test plant is capable to grow in the presence of various concentrations of As, potential heavy metal, which is contaminated our soil and water bodies. These chemicals accumulate in the plant body even after this, if the plants are surviving, which means that they have the potential to tolerate the toxic effects of these metals and therefore can be used for phytoremediation because this technique is cheaper and can be easily practiced.

As removal from solution by *B. juncea*

Metal depletion was studied to understand the potential of plant- *B. juncea* at various exposure levels. Aliquots of 500 µl were withdrawn from each plant growth medium at the interval of 0, 1, 3, 7, 14 and 21 days. The depletion of As concentration in the solution is directly attributed with the removal of metal by *B. juncea.* Figure 1 demonstrates the removal of As from hydroponic solution after 21 days of exposure period. The research study highlights the metal uptake by *B. juncea* at each selected concentrations of As. *B. juncea* has remediated 80–86% of As from lower to higher concentration after 21 days of exposure. At lower concentrations of 5 and 10 ppm, the plants of *B. juncea* have shown As removal up to 81% after 21 days of experiment while at concentrations of 25 and 50 ppm uptake process was increased up to 86% of externally added As (Fig. 1) without the phenomenon of any toxicity. The results are similar with the report of Cd removal by *B. juncea* (Anamika *et al.*, 2009). As depletion from solution was found consistent throughout the experimental time, with the course of time the removal of As from solution was observed and increased with increasing concentrations.

Accumulation of As in the roots and shoots of *B. juncea*

Experiments were also performed for the removal of heavy metal – As from the solution which was spiked with 5 ppm, 10 ppm, 20 ppm and 50 ppm, respectively. From the table it is clear that roots of *B. juncea* at 5 ppm to 50 ppm accumulation of As was 3045 µg/gm and 26650 µg/gm, respectively. Similarly at this concentration the amount accumulated in shoot was 1075 µg/gm and 5462 µg/gm of the dry biomass of the stem. At 10 and 20 ppm the accumulation heavy metal As in roots was 4165 µg/gm and 20,850 µg/gm, respectively.

The study shows that As was efficiently taken at all concentrations using high biomass producing plant *B. juncea* grown in hydroponic solution. The results indicated increase in accumulation of As in biomass with increase in each selected concentration as well as time. Bioaccumulation was found more in Roots when compared to shoots. Similar observations were found in the investigation where *Brassica juncea* was used for the removal of copper from soil (Ariyakanon and Winaipanich, 2006).

More or less similar results have been reported in the accumulation pattern of heavy metals in *Amarantus* (Mellem *et al.*, 2009). Those authors suggested that accumulation potential of plants towards heavy metal depends on the availability of the metals in the soil/ growth media as well as on the plant genotype. However, there was no sign of As toxicity in this plant when testing in moderately contaminated solution (50 ppm). Thus, it could be classified as a As tolerant species. Phytoextraction efficiency is related to both plant metal concentration and dry matter yield. Thus, the ideal plant species to remedy a contaminated site should be a high yielding crop that can both tolerate and accumulate the target contaminants. Indian mustard demonstrated to accumulate moderate levels of Pb, Zn, Cu (Kumar *et al.* 1995, Ebbs and Kockhian 1997).

The bioaccumulation coefficient (BC) of As by *B. juncea* has also been determined and presented in Table 2. The result shows that BC of As 824 to 642 when exposed to minimum (5 ppm) to higher (50 ppm), respectively. Further the translocation of metals from roots to shoots was also studied, which showed that after 21 days of exposure period, only 26.1% of As was translocated from root to shoot at 5 ppm which was the maximum quantity.

Table.1 Dry biomass of *B. juncea* after 21 days of exposure to the different concentrations of As supplemented Hoagland solution

As Concentration (ppm)	Dry weight (g)	
	Roots	Shoots
0	0.034+_0.008	0.128+_0.012
5	0.028+_0.004	0.068+_0.014
10	0.016+_0.006	0.078+_0.016
25	0.021+_0.005	0.098+_0.027
50	0.036+_0.012	0.107+_0.016

Table.2 Bioaccumulation coefficient and transport index of *B. juncea*

Arsenic concentration (ppm)	**Bioaccumulation Coefficient**	**Transport index (%)**
5	824	26.1
10	617	25.3
25	933	10.6
50	642	17.2

Fig.1 % removal of As from hydroponic solution by *B. juncea*

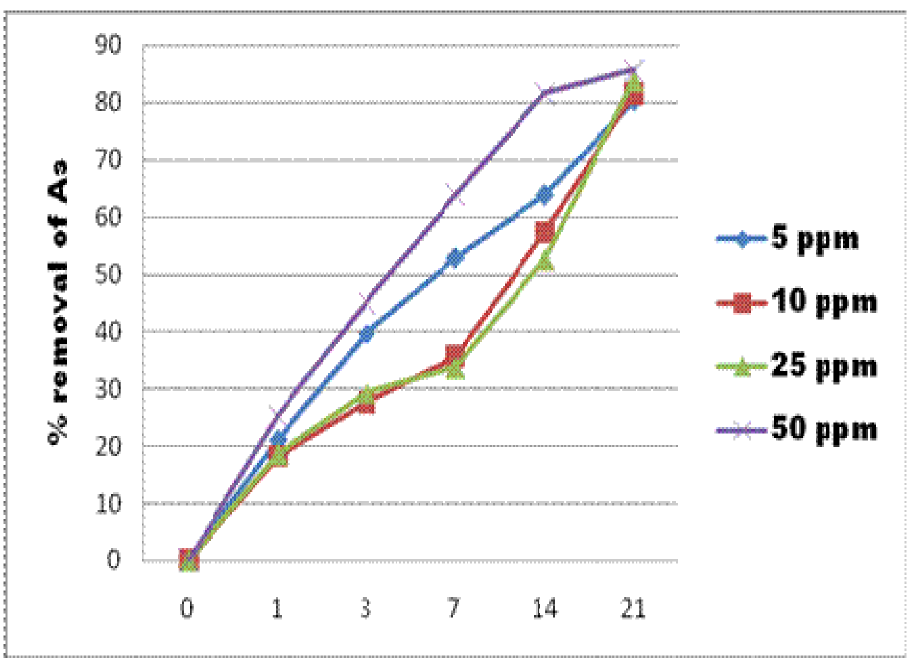
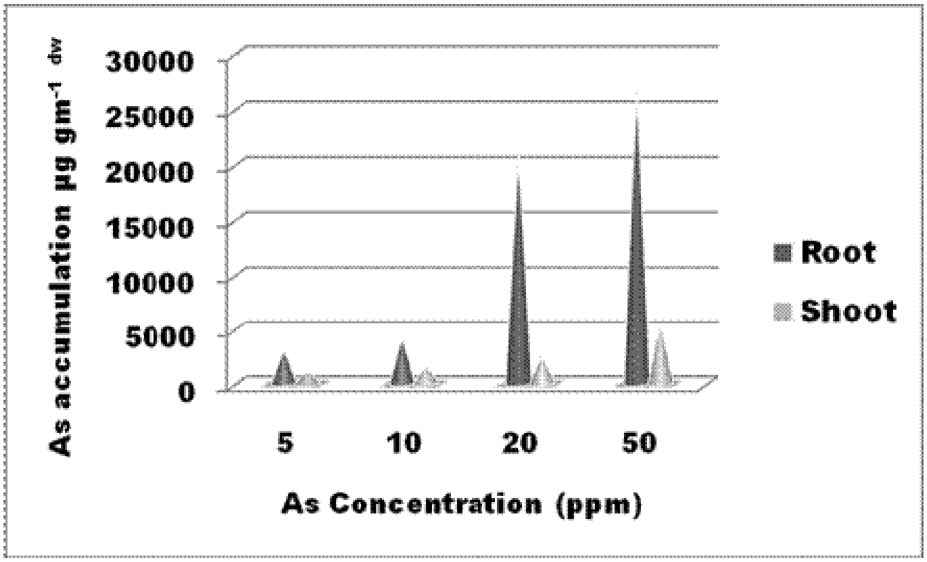


Fig.2 Accumulation in the dry biomass of roots and shoots of *B. juncea* cultivated in hydroponics media containing different concentrations of As



The TF values can describe the movement and distribution of heavy metals in the plants. Transport across root cellular membrane is an important process which initiates metal absorption in the plant tissues.

Uptake and accumulation of soil contaminants by the plant biomass is a well reported phenomenon. In plants, uptake of metals occurs primarily through the roots, so this is the primary site for regulating their accumulation (Rahman *et al.*, 2005). Many contaminants are also known to be broken down by plant enzymes into metabolites of parent compounds and these metabolites are feverally, though not always less toxic one than the parent compound (Arthur *et al.*, 2005).

In conclusion phytoremediation is widely considered as an emerging bio-based low cost and ecologically responsible alternative to the expensive physical-chemical methods currently practiced to clean up the contaminated environment. In present investigation higher As bioaccumulation was achieved in *Brassica juncea* indicates that this plant can be used in phytoremediation technology for remediation of As directly from the contamination sites.

References

Anamika, S., Eapen, S., Fulekar, M.H. 2009. Potential of *Medicago sativa* for uptake of cadmium from contaminated environment. *Rom. Biotechno. Lett.*, 14(1): 4164–4169.

Ariyakanon, N., Winaipanich, B. 2006. Phytoremediation of Copper Contaminated Soil by *Brassica juncea* (L.) Czern and *Bidens alba* (L.) DC. var. *radiate. J. Sci. Res. Chula. Univ.*, 31(1): 49–56.

Arthur, E.L., Rice, P.J., Rice, P.J., Anderson, T.A., Baladi, S.M., Henderson, K.L.D., Coats, J.R. 2005. Phytoremedation – an overview. *Crit. Rev. Plant Sci.*, 24: 109–122.

Baker, A.J.M., *et al.* 1994. The possibility of in situ heavy metal decontamination of polluted soils using crops of metal-accumulating plants. *Res. Conserv. Recycl.*, 11: 41–49.

Ebbs, D.S., Kochian, L.V. 1997. Toxicity of zinc and copper to *Brassica* species: implications for phytoremediation. *J. Environ. Qual.*, 26: 776–719.

Ensley, B.D. 2000. Rationale for use of phytoremediation. In: Raskin, I., Ensley, B.D. (Eds.), Phytoremediation of toxic metals: Using plants to clean up the environment. John Wiley & Sons, Inc., New York. Pp. 3–11.

Frankenberger, Jr., W.T., Arshad, M. 2002. Volatilisation of arsenic. In: Frankenberger, Jr., W.T. (Ed.), Environmental chemistry of arsenic. Marcel Dekker, New York. Pp. 363–380.

Hoagland, D.R., Arnon, D.I. 1938. The water culture method for growing plants without soil. *Calif. Agri. Exp. Station Circ.*, 3: 346–347.

Kumar, P.B.A.N., Dushenkov, S., Motto, H., Raskin, I. 1995. Phytoextraction: the use of plants to remove heavy metals from soils. *Environ. Sci. Technol.,* 29: 1232–1238.

Lal, N., Srivastava, N. 2010. Phytoremediation of toxic explosives. In: Ashraf, M., Ozturk, M., Ahmad, M.S.A. (Eds.), Plant adaptation and phytoremediation. Springer Dordrecht, Heidelberg, London, New York. Pp. 383–397.

Mahajan, B.K. 1997. Methods in biostatics for medical students and research workers, 6th edn. Jaypee Brothers Medical Publishers (P) Ltd, New Delhi

Mattina, M.J.I., Lannucci-Berger, W., Musante, C., White, J.C. 2003. Concurrent plant uptake of heavy metals and persistent organic pollutants from soil. *Environ. Pollut.*, 124: 375–378.

Mellem, J., Baijanth, H., Odhav, B. 2009. Translocation and accumulation of C, Hg, As Pb, Cu and Ni by *Amaranthus dubius* (Amaranthaceae) from contaminated sites. *J. Environ. Sci. Health*, 44: 568–575.

Murashige, T., Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiol. Plant.*, 15: 473–497.

Pulford, I.D., Watson, C. 2003. Phytoremediation of heavy metal-contaminated land by trees—A review. *Environ. Int.,* 29: 529–540.

Quartacci, M.F., Baker, A.J.M., Navari-Izzo, F. 2005. Nitrilotriacetate and citric acid-assisted phytoextraction of cadmium by Indian mustard. *Chemosphere,* 59: 1249–1255.

Rahman, H., Sabreen, S., Alam, S., Kawai, S. 2005. Effects of nickel on growth and composition of metal micronutrients in barley plants grown in nutrient solution. *J. Plant Nutr.,* 28: 393–404.

Reeves, R.D., Baker, A.J. 2000. Metal-accumulating plants. In: Phytoremediation of toxic metals: Using plants to clean up the environment. Raskin, I., Ensley, B.D. (Eds.), John Wiley & Sons, Inc., New York. Pp. 193–229.

Selvaraj, K.A., Sevugaperumal, R., Subramanian, R. 2013. Phytoremediation of arsenic chloride by Indian mustard (*Brassica Juncea*). *Indian J. Fundam. Appl. Life Sci.,* 3(1): 184–191.