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Phytoaccumulation of Arsenic, Cadmium and Lead by *Brassica juncea* Parents and Their F1 Hybrids

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Abstract

Although *Brassica juncea* (Indian mustard) is reported to be a good accumulator of metals, little is known of the selected varieties of *B. juncea* (cvs. Rai and BARI-11). This paper investigates the phytoaccumulation of arsenic, cadmium and lead by *B. juncea* (cvs. Rai and BARI-11) parents and F1 hybrids. The experiment was conducted in the hydroponic media in the greenhouse of University of Southampton under a Randomised Block Design. Sodium arsenite, cadmium sulphate and lead nitrate with 0 ppm, 0.5 ppm and 1 ppm were used. The cadmium treated plants were analysed by Varian Atomic absorption spectrophotometer-200. The samples of arsenic and lead were analysed by Inductively Coupled Plasma Atomic Emission Spectrophotometer. The results suggest that arsenic was detected only in the root systems while cadmium and lead were detected both in the root and shoot systems. Significant differences in the uptake were observed for different concentrations. Accumulation of arsenic was detected only in the root systems of *B. juncea* (cvs. Rai and BARI-11) at lower concentrations. Hence, this can be used as an agriculturally viable and efficient phytoaccumulator in the arsenic affected areas where contamination level is low and the contamination occurs at the rooting level.

Keywords

Bioremediation, Phytoaccumulation, Heavy Metal, Arsenic, Cadmium, Lead, *B. juncea* (cvs. Rai and BARI-11)

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1. Introduction

The presence of Heavy metals in the environment is a natural phenomenon, although anthropogenic activities also add Heavy metals to the environment. Heavy metals as contaminants to the environment are causes of concern in many parts of the world. Among the potentially toxic metals, arsenic (As), cadmium (Cd) and lead (Pb) in particular, can cause adverse effects on agriculture [1]-[12]. The presence of high concentrations of metals in soil may pose a significant threat to agriculture, through bioaccumulation and consequently may affect the public health by contaminating the food chain [7] [11] [13]-[17].

Scientists have discovered physical and chemical remediation technologies to clean up contaminated sites [18]-[20] involving excavation, burial, *ex-situ* treatment, *in-situ* (chemistry-bioremediation), encapsulation, ion exchange reverse osmosis [18] and natural attenuation [21] [22]. These technologies can be expensive and impractical considering the notable area contaminated by metals around the world [15] [20] [23] [24].

Plants play a significant role in maintaining the environment, particularly through the management of soil. They have the ability to remediate contaminated land by uptaking metals from soil and can reduce the adverse impacts of anthropogenic air pollution by absorbing CO₂ emitted by combustion processes via photosynthesis [20] [25]. A plant-based technology ("phytoaccumulation") has been used to remediate metals from contaminated areas, and found to be cost effective and eco-friendly [12] [24] [26]-[28]. After plant uptake heavy metals the harvested plant parts can be classified as a hazardous waste and should be disposed of in such a way that the pollutants do not re-enter in to the environment. One way of doing this is to separate the heavy metal through precipitation and solidification for future industrial use. Macnair [29] provided a list of plant species, which are known to be phytoaccumulators for a range of potentially toxic metals and a number of them belong to the family *Brassicaceae*.

Several species of *B. juncea* (Indian mustard) can accumulate heavy metals (cadmium, arsenic and lead) from soil and aqueous solution through their root system and then can transport it to different parts of shoots [30]. However, the uptake behaviour of different varieties of *B. juncea* has been found to vary [31]. Although *B. juncea* is reported to be a good accumulator of metals [32], little is known of the selected varieties of *B. juncea* cv. Rai and *B. juncea* cv. BARI-11 and their ability to uptake metals. In addition, the effect of different concentrations of metals, such as arsenic, cadmium and lead on these two varieties has not been determined, although these are grown in most areas affected by these metals [32]. This paper investigates the phytoaccumulation of arsenic, cadmium and lead by *B. juncea* (cv. Rai and BARI-11) parents and F1 hybrids. The reason for considering F1 hybrids is that these are intermediate generation with unstable genetic characteristics, hence may show different result than the parents. Also in Bangladeshi context when farmer use their own seed stock these are likely to be the F1 hybrids of the two parents as the parents are cultivated in the adjacent fields.

2. Methodology

The experiment was conducted in the hydroponic media in the greenhouse of University of Southampton under a Randomised Block Design (RBD) [33] involving *B. juncea* parents and their F1 hybrids. A hydroponic system is the cultivation of plants without (or minimal) soil to support the root system. In comparison to a conventional method, a hydroponic system permits better characterisation of pollutant uptake because of potential interaction between the soil matrix and the elements of interest are minimized [34] [35]. The hydroponic growing media allows adequate root distribution of plants and maintains homogeneous conditions of growing media, which is restricted in pot plant experiments [27] [36]-[38]. Furthermore, plants can uptake and translocate the soluble forms of metals easily in the hydroponic systems. It should be noted that a modified hydroponic system was used in this study where individual plastic containers were used to grow plants and nutrient solutions and metal concentrations were maintained manually.

The chemical forms of metals used in this experiment were arsenic as sodium arsenite, cadmium as cadmium sulphate and lead as lead nitrate. All of these chemical forms are soluble and were used in other studies conducted by Hossain [6], Kumar *et al.* [39] and Cox *et al.* [40].

The literature suggests that most of the studies involving *Brassica* species were conducted in the hydroponic conditions with concentrations of arsenic, cadmium and lead ranging from 0 - 1 ppm [6] [39] [40]. In the current study, 0.5 ppm and 1 ppm of arsenic, cadmium and lead were used.

2.1. Design of Experiment

Randomized Block Design (RBD) [33] was used with the aim of studying the variation in selected morphologi-

cal characteristics of two varieties, cv. Rai (P1) and cv. BARI-11 (P2) of *B. juncea*, and their reciprocal F1hybrids in accumulating arsenic, cadmium and lead. Parents and hybrids were considered as blocks and different concentrations of metals were considered as treatments.

Three treatments of the experiment were $R_1 = 0.0$ ppm (control treatment), $R_2 = 0.5$ ppm and $R_3 = 1.0$ ppm. Treatments were allocated randomly in each block. The RBD in this experiment consisted of multiple observations (r) per cell, where the number was 4 per cell. As the RBD conducted in this experiment had multiple observations per cell the analyses allowed an investigation of the combined effect, namely the interaction effects of concentrations of metals and parents and hybrids [41].

2.2. Growing Plants for Experiment

The experiment was carried out in a greenhouse at the University of Southampton, UK, during April-June 2006. The recorded temperature ranged between 14°C - 35°C (SD = $\pm 5^{\circ}\text{C}$), light intensity was 750 - 1980 lux and day length was 13 - 16 hours per day. Transparent plastic containers (1.5 feet \times 1 feet \times 1 feet) were used to grow plants and these enabled the maintenance of nutrient levels and the observation of plant root systems.

Each container was filled with twenty litres of full strength Hoagland solution, which were prepared following [34]. Iron was avoided in preparing the Hoagland solution as it has been reported to interfere with uptake of metals [42]. For example, Brown [43], Cohen *et al.* [42] and, Bao and Sun [44] used Hoagland solution in their experiments without iron to avoid its influence on uptake of metals. Twenty litres of nutrient solutions were made using stock solutions of 20 ml of ammonium di-hydrogen phosphate, 120 ml potassium nitrate, 80 ml calcium nitrate, 40 ml magnesium sulphate and 20 ml micronutrient solution and were added into 1720 ml of water.

Seeds of *B. juncea* cv. Rai (P1), *B. juncea* cv. BARI-11 (P2) and their F1 hybrids were germinated in the mixture of perlite and soil (3:1volume) in the greenhouse. Plants were allowed to grow for two weeks so that they achieved sufficient growth to be suitable for transplantation. Two week old seedlings were transferred to the experimental containers. Metals were added to the growing media after one week of transplantation of the seedlings. All containers were covered with black polythene sheets to avoid possible growth of algae due to exposure to sunlight. Each container was marked at twenty litres and this level was maintained daily by topping up with the same concentration of plant nutrients and metals as originally used [40]. The pH level of growing media was estimated using "pHtesting colour strip" (pH-Fix 0-14, Fisherbrand; EU code: FB33003). Aeration was maintained by continuous air bubbling (210 L/hr air flow and 0.17 mbar pressure) in the growing media by air pumps.

2.3. Determination of Metals

Plants treated with arsenic, cadmium and lead were harvested and rinsed with deionised water to remove any nutrient solution and dried for 24 hours at 65°C [40]. Dried samples were homogenised using a Cyclotech Mill and stored in small plastic containers. A microwave digestion [45] procedure was followed to digest the plant material. The cadmium treated plants were analysed by Varian Atomic absorption spectrophotometer-200. The samples of arsenic and lead were analysed by Inductively Coupled Plasma Atomic Emission Spectrophotometer (ICP-AES).

2.4. Statistical Analysis

The experimental data were analysed using two-way analysis of variance (ANOVA). It should be noted that every characteristic under study was subject to testing of the same set of hypotheses (null and alternative). Hence, a separate ANOVA table for each characteristic was produced. The normality of the data was tested using the Kolmogorov-Smirnov test. The analysis of variance and the *Post hoc* tests were conducted using MINITAB 15 software.

3. Results

3.1. Effect of Arsenic Concentrations

The result suggests that arsenic was detected only in the root systems of *B. juncea* parents and their hybrids. **Table 1** shows the mean and standard error of root uptake of P1 (*B. juncea* cv. Rai) and P2 (*B. juncea* cv. BARI-11)

Table 1. Mean (standard error) of root uptake and shoot uptake of parents *B. juncea* cv. Rai (P1), *B. juncea* cv. BARI-11 (P2) and their F1 hybrids $[(Rai \supseteq X BARI-11 \varnothing) \& (BARI-11 \supseteq X Rai \varnothing)]$ with control, 0.5 ppm and 1 ppm arsenic, cadmium and lead treatments (n = 4).

Parents and F1 hybrids	Concentrations	Arsenic	Cadı	mium	Lead	
		Root uptake (mg/g)	Root uptake (mg/g)	Shoot uptake (mg/g)	Root uptake (mg/g)	Shoot uptake (mg/g)
P1	Control	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
	0.5 ppm	0.6 (0.0)	1.9 (0.1)	0.3 (0.0)	2.2 (0.6)	0.05 (0.0)
	1.0 ppm	0.7 (0.1)	2.3 (0.2)	0.4 (0.1)	2.4 (0.3)	0.07 (0.0)
P2	Control	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
	0.5 ppm	0.4 (0.1)	1.4 (0.2)	0.3 (0.0)	1.7 (0.3)	0.03 (0.0)
	1.0 ppm	0.7 (0.1)	2.2 (0.3)	0.5 (0.0)	2.5 (0.1)	0.08 (0.0)
F1	Control	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
	0.5 ppm	0.3 (0.6)	0.7 (0.5)	0.3 (0.0)	1.1 (1.0)	0.07 (0.0)
	1.0 ppm	1.0 (0.1)	1.9 (0.5)	0.6 (0.0)	1.1 (0.3)	0.06 (0.0)
RF1	Control	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
	0.5 ppm	0.4 (0.1)	0.8 (0.5)	0.5 (0.0)	0.1 (0.0)	0.06 (0.0)
	1.0 ppm	1.0 (0.2)	3.1 (0.2)	0.7 (0.1)	1.9 (1.0)	0.05 (0.0)

3.2. Effect of Cadmium Concentrations

The results suggested that cadmium was detected both in the root and shoot systems of two *B. juncea* parents and their hybrids. The mean and standard error of root uptake and shoot uptake of parents *B. juncea* cv. Rai (P1), *B. juncea* cv. BARI-11 (P2) and their F1 (Rai ? X BARI-11 ?) and RF1 (BARI-11 ? X Rai ?) hybrids are presented in **Table 1**.

Root uptake of P1 was significantly higher than that of P2 at 0.5 ppm concentration of cadmium (**Table 1**). At 0.5 ppm, both the F1 and RF1 had significantly less root uptake than their parents. At 1 ppm, all the parents and hybrids had significantly increased root uptake compared to 0.5 ppm. Root uptake was significantly (p < 0.05) different between cadmium concentrations (**Table 2**). At 1 ppm concentration of cadmium root uptake of parents and hybrids was higher compared to that at 0.5 ppm, though the differences were not statistically significant.

At 0.5 ppm concentration of cadmium P1, P2 and F1 had more or less similar shoot uptake (0.3 mg/g), which was almost twice for RF1 (0.5 mg/g) (**Table 1**). At1 ppm, all the parents and hybrids had significantly increased shoot uptake compared to 0.5 ppm. In general, shoot uptake was much less than the root uptake for both the cadmium concentrations. Shoot uptake was significantly (p < 0.01) different between cadmium concentrations. Furthermore, shoot uptake was significantly (p < 0.01) different between parents and their F1 hybrids (**Table 2**). Significant interaction effect (between concentrations and parents and hybrids) was found for shoot uptake (p < 0.01).

Table 2. Two-way ANOVA for root and shoot uptake of parents *B. juncea* cv. Rai (P1), *B. juncea* cv. BARI-11 (P2) and their F1 hybrids [(Rai♀ X BARI-11♂) & (BARI-11♀ X Rai♂)] investigated in experiment with control, 0.5 ppm and 1 ppm arsenic, cadmium and lead concentrations.

C	Arsenic	Cadmium		Lead	
Sources of variation	Root uptake	Root uptake	Shoot uptake	Root uptake	Shoot uptake
Concentrations	103.3***	55.5***	273.5***	16.9***	127.1***
Parents and hybrids	0.6	1.5	14.6***	2.4	0.5
Concentrations X Parents and hybrids	3.3***	2.0	5.1***	1.3	5.9***

Note: $^{***}p < 0.01$, $^{**}p < 0.05$; Values represent F-values from ANOVA. Degrees of freedom for treatment, parents and hybrids, interaction and error were 2, 3, 6 and 36 respectively.

3.3. Effect of Lead Concentrations

Results revealed that lead was detected in both the root and shoot systems of parents and F1 hybrids. **Table 1** shows the mean and standard error of root uptake and shoot uptake of parents *B. Juncea* cv. Rai (P1), *B. Juncea* cv. BARI-11 (P2) and their F1 (Rai \supseteq X BARI-11 \supseteq) and RF1 (BARI-11 \supseteq X Rai \supseteq) hybrids.

P1 had higher (insignificant) mean root uptake than P2 at 0.5 ppm concentrations of lead (**Table 1**). Both the F1 and RF1 had significantly lower root uptake compared to their parents at 0.5 ppm. At 1 ppm, all parents and hybrids had increased root uptake compared to 0.5 ppm. The increase was significant in P2.Root uptake was significantly (p < 0.01) different between concentrations of lead (**Table 2**). Even though, root uptake of lead showed notable difference between parents and hybrids these were not statistically significant.

Shoot uptake of all parents and hybrids were negligible at both the concentrations (0.5 ppm and 1 ppm) of lead (**Table 1**). At 0.5 ppm concentrations of lead P1 and P2 had shoot uptake as 0.05 mg/g and 0.03 mg/g respectively, which were 0.07 mg/g and 0.06 mg/g for F1 and RF1 respectively. At 1 ppm, P1 and P2 had increase while F1 and RF1 had little decrease in shoot uptake compared to 0.5 ppm. Generally, shoot uptake for lead concentrations (0.5 ppm and 1 ppm) was much less than the root uptake for all parents and hybrids. Shoot uptake was significantly different (p < 0.01) between concentrations of lead (**Table 2**). Significant interaction effect (between concentrations and parents and hybrids) was found for shoot uptake (p < 0.01).

4. Discussion

4.1. Arsenic Concentrations

In this experiment, an inorganic form of arsenic (sodium arsenite) was used. As an inorganic substance exists as ions, it is generally taken up via the membrane transporter proteins [24]. When inorganic pollutants are accumulated in tissues they often cause toxicity by inducing oxygen free radicals, which is called the oxidative stress [46] [47]. The oxidative stress is harmful to tissues causing damage to cell structure [24]. Moreover, it was reported that plants can generate a mechanism, known as detoxification, by producing phytochelatin that detoxifies arsenic after certain periods of oxidative stress [47]-[49]. All of these three mechanisms, e.g., transporting of arsenic, toxification and detoxification of arsenic may have different impacts on morphological characteristics expressing different association with uptake behaviour. Furthermore, the same mechanism may act differently between root and shoot systems due to the difference between cell structures of root and shoot. It should be noted that as F1 hybrids are expected to have different genetic combinations from parents, they may show different responses to the above mechanisms. The mechanisms mentioned above are likely to be present for other metals, e.g., cadmium and lead, as inorganic forms of the metals were used in this experiment, which are discussed in appropriate sections. As the current study was not intended to investigate the existing mechanisms reported in different literature, any specific interpretations of the results may not be possible. However, attempts are made to relate the findings that are observed elsewhere.

It is apparent from the results that arsenic was detected in the root systems of *B. juncea* parents and their hybrids but not in the shoot systems. A study by Pickering *et al.* [50] on *B. juncea* var. 426,308 with 250 µM of arsenic (arsenate V) reported that arsenic uptake was concentrated mostly in the root system with a small fraction exported into the shoot system. The reason for no arsenic accumulation in the shoot system may be that arsenite

[As (III)] form of arsenic was used in the present study. Generally, arsenite is more toxic than arsenate for plants because it reacts with sulfyhydryl groups (-SH) in enzymes and tissue proteins of the root [51]. Consequently, it leads to inhibition of cellular function and death [52], resulting in failure of transportation of arsenic into the shoot [31].

The mean root uptake of P1 was slightly higher than that of P2, while root uptake of hybrids was less than their parents at 0.5 ppm concentration of arsenic. At 1 ppm concentration of arsenic all parents and hybrids showed increase in root uptake; the increase was much higher in hybrids than the parents. Generally, with increase in concentrations of arsenic the uptake seemed to increase in *B. juncea* L. [31] [50] until certain level of toxicity [3]. Hybrids are made up with combination of characteristics from both parents and can sometimes behave extremely differently [53]. More precisely, F1 hybrids are intermediate generation and may possess some genetic advantage leading to higher uptake of arsenic than their parents.

The analysis further suggested that root uptake differed significantly (p<0.01) between different concentrations of arsenic. This may suggest that plants respond to different concentrations (toxicity) differently which finally results in different level of uptake. A significant interaction effect (combined effect of parent and hybrids and concentration) was also found for root uptake. This means that the action of the same concentration may impact differently for parents and their hybrids. Genotypic differences between parents and hybrids and their response to concentrations may be one of the reasons behind such phenomenon. However, this requires further investigation.

4.2. Cadmium Concentrations

For cadmium concentrations both root and shoot uptake were detected in the experiment. Similar was reported by Salt *et al.* [54], Fujimura *et al.* [55] in *B. juncea* var. 426308 and by Vatehova *et al.* [56] in *Brassica juncea* (L.) Czern. cv. Vitasso and *B. napus* L. cv. Atlantic, and Wue *et al.* [57] in *H. vulgare*. Both F1 and RF1 had less root uptake compared to parents though, P1 had slightly higher root uptake than that of P2 at 0.5 ppm concentration of cadmium. When concentration of cadmium was increased to 1 ppm all the parents and hybrids had much higher amount of root uptake compared to that achieved at 0.5 ppm. Root uptake was significantly different between cadmium concentrations.

At 0.5 ppm concentration of cadmium both the parents and F1had almost similar shoot uptake. Shoot uptake of RF1 was almost twice compared to parents. At 1 ppm, all the parents and hybrids experienced increase in shoot uptake. In general, shoot uptake was much less than the root uptake for both the cadmium concentrations. Significant difference was observed for shoot uptake between cadmium concentrations and between parents and their F1 hybrids. Also a significant interaction effect (between concentrations and parents and hybrids) was found.

In general, uptake of cadmium by root system was found higher than that by shoot system in this study. Fujimura *et al.* [55] reported similar findings that cadmium concentration was always higher in roots than shoots for *Brassicas*. This finding also corroborates with the results of number of other investigations conducted by Salt *et al.* [54] in *B. juncea* var. 426308 and Wue *et al.* [57] in *H. vulgare*. This may be due to the barrier caused by endodermis that restricts the movement of cadmium into shoot system from root system. Such a mechanism has been reported for other metals, such as, lead in *O. sativa* [58]. At 1ppm, root uptake and shoot uptake were found to be increased compared to 0.5 ppm. Several other studies also reported that cadmium accumulation in roots and shoots increased with the increase in concentrations [8] [27] [57].

4.3. Lead Concentrations

Accumulation of lead was detected both in root and shoot systems in the experiment with different lead concentrations. Similar results were reported by others, for example, Liu *et al.* [59] in *B. juncea* var. Megarrhiza, Meyers *et al.* [60] and Kumar *et al.* [39] in *B. juncea*, Jones *et al.* [61] in rye grass and Lane and Martin [62] in *R. sativus*. Significant difference of root uptake was observed between concentrations of lead which may be due to the differences in responses to toxicity associated with different lead concentrations.

Shoot uptake of all parents and hybrids were very negligible at 0.5 ppm concentration of lead. At 1 ppm, shoot uptake was increased in P1 and P2 but it was decreased in F1 and RF1. Shoot uptake was significantly different between concentrations of lead. Again, significant interaction effect (between concentrations and parents and hybrids) was found for shoot uptake. It indicates that various concentrations of lead may interact differently with

parents and hybrids leading to differences in uptake.

The findings of this experiment with lead concentrations revealed that root uptake was much higher than shoot uptake for all parents and hybrids. Similar observations were made by other studies in *B. juncea* [39] [62]. Generally, lead moves into the root apoplast and finally across the cortex and accumulates near the endodermis [5] causing barrier for translocation of lead from root system to shoot system [58] [61]. This may lead to reduced accumulation of lead by shoot system than root system. The negligible amount of uptake of lead in shoot system observed in the experiment may be due to such mechanisms, which requires further investigation.

5. Conclusion

In this study, accumulation of arsenic was detected only in the root system of B. juncea (cvs. Rai and BARI-11) at lower concentrations. In many of the arsenic affected countries/regions like Bangladesh, West Bengal of India, Nepal, Vietnam, and southern China [14] [15] [63] B. juncea is a much cultivated oil seed crop [64] [65]. Among these arsenic affected areas where contamination level is low and the contamination occurs at the rooting level, B. juncea (cvs. Rai and BARI-11) can be used as an agriculturally viable and efficient phytoaccumulator. In such case only root parts of B. juncea will extract the arsenic and shoot system will not be affected, which will encourage the farmers to harvest oil seeds and consequently remediate the soil. However, the disposal of accumulated arsenic in the root system will be an important task for the farmers that should be handled carefully. Furthermore, the accumulated arsenic can be used for industrial purposes after extracting from the root system. If B. juncea (cvs. Rai and BARI-11) shows arsenic accumulation only in root system even at higher concentration, it will widen its potential as a sustainable phytoremediation tool for many arsenic affected areas of the world. For the other two potentially toxic metals (cadmium and lead) B. juncea (cvs. Rai and BARI-11) is found to be good accumulator too. However, as both root and shoot uptake is evident for the two metals (cadmium and lead) these are not agriculturally viable. For all the three metals (arsenic, cadmium and lead) further experiments may be conducted with higher concentrations. Finally, it should be kept in mind that the experiment of the study was performed in aqueous solution, hence the results may not exactly be the same in soil medium or in practical application. Future research should consider the presence and interaction of other soil based chemicals on phytoaccumulation.

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