

EFFECTS OF ARSENIC CONCENTRATIONS AND FORMS ON GROWTH AND ARSENIC UPTAKE AND ACCUMULATION BY INDIAN MUSTARD (BRASSICA JUNCEA L.) GENOTYPES

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ABSTRACT

By using two Brassica juncea genotypes (Varuna and DHR-9504) a green house experiment was carried out during crop cycle (2003-2004), at Agricultural Farm, Bilaspur, Chhattisgarh, India. In Indian mustard, arsenic extraction by plants increased significantly with increasing arsenic concentrations in soils. Uptake of arsenite by Indian mustard genotypes was higher than that of arsenate. Stunted growth of the plants was also observed in this study. This experiment clearly demonstrated the existence of genotypical variations in tolerance to As toxicity among Brassica juncea genotypes.

KEY WORDS: Arsenate, arsenite, Brassica juncea, genotypic variation, toxicity.

INTRODUCTION

Arsenic (As) is widely distributed in the environment, originating either from As in the soil parent material or from discharge of As onto land as a result of human activities. Consequently, people and livestock are being exposed to As via contamination of drinking water and consumption of food grown in As-contaminated soil or irrigated with As-contaminated water. Understanding how As is taken up by plants and subsequently transformed in plant tissue is therefore essential for estimating the risks posed to human and wildlife populations by As-contaminated soils (Meharg and Hartley-Whitaker, 2002).

Arsenic (As) is a widespread natural element, which is not a bioorganic element to plants (Stoeva et al., 2003). In terrestrial plants, both organic and inorganic As species have been found (Koch et al., 1999, 2000; Francesconi et al., 2002), with the inorganic species (Arsenate [As (V)] and arsenite [As (III)]) being the most dominant. Arsenate is the predominant As species in aerobic soils, whereas arsenite dominates under anaerobic conditions (Smith et al., 1998). Arsenic availability to plants is greatly influenced by its forms in soil. Agricultural application of arsenicals has introduced many different kinds of arsenic compounds to the soil environment. These arsenicals may influence arsenic mobility and plant uptake though they are subjected to oxidation–reduction transformation in soils.

As is a nonessential element for plants, and inorganic As species are generally highly phytotoxic. Biomass production and yields of a variety of crops are reduced significantly at elevated arsenic concentrations (Carbonell-Barrachina et al., 1997), with application of only 50 mg As kg⁻¹ to soil significantly decreasing the yields of barley (*Hordeum vulgare* L.) and ryegrass (*Lolium perenne* L.) (Jiang and Singh, 1994). Arsenic concentrations are generally low in plants (Matschullat, 2000). The limited accumulation of As by roots and its limited translocation to the shoots, is usually used by most plants such as carrot, tomato and grass. These plants contain relatively low arsenic and accumulate arsenic primarily in their root systems (O'Neill, 1995; Matschullat, 2000). In all plant species tested so far, it has been shown that arsenate is taken up via the phosphate transport systems (Asher and Reay, 1979; Ullrich-Eberius et al., 1989; Meharg and Macnair, 1992).

To our knowledge, there is no study in the literature dealing with As tolerance in Indian mustard genotypes. The objective of this study were to examine the growth and arsenic uptake and accumulation by Indian mustard plants in soils amended with different arsenic concentrations and forms and the concentrations of the

inorganic As species (arsenite and arsenate) found in soil at varying As (V) concentrations. Results would provide critical information regarding Indian mustard genotype's ability to tolerate and extract arsenic from soil and to translocate arsenic to its aboveground biomass.

MATERIALS AND METHODS

Soil Sampling

The soil used in this study was collected from Bilaspur (Chhattisgarh). Physico-chemical properties of the soil were measured by the standard methods of soil chemical analysis (NIAS, 1988). The soil had 0.68% organic carbon, 190 kg ha⁻¹ available nitrogen, 184 kg ha⁻¹ K₂O₅ and Zn, B and Mo 0.51, 0.37 and 0.05 mg kg⁻¹ soil respectively, available sulphate-sulphur 8.1 mg kg⁻¹ soil, available P 23.5 mg kg⁻¹ soil, and Cd 0.42 mg kg⁻¹ soil with pH 7.5. The mean arsenic concentration in the top soil layer (top 70 mm) was 0.69 mg kg⁻¹, while that at the bottom layer was 0.37 mg kg⁻¹.

Plant Growth

The experiment was carried out with two genotypes of Brassica juncea (Varuna and DHR-9504) at Agricultural Farm, Bilaspur Chhattisgarh, India during 2003-2004. Indian mustard (*Brassica juncea* L.) plants were grown under microbiologically controlled conditions such that their roots were maintained axenically. Seeds were surface-sterilized in 2.6% (w/v) sodium hypochlorite for 30 min, rinsed four times in autoclaved de-ionized water, and transferred onto sterile 1.2% (w/v) agarose plates. Plates were held vertically and the seeds allowed to germinate and grow in the dark at 22°C for 72 h. 5-d-old seedlings not showing microbial contamination on the agarose plates were transferred individually into small glass vials (29 × 65 mm) containing 23 mL of sterile nutrient solution. The nutrient solution consisted of 0.98 mM K₂SO₄, 3.0 mM Ca(NO₃)₂, 0.27 mM KH₂PO₄, 1 mM MgSO₄, 0.2 mM KCl, 100 μM Fe-EDTA, 1 μM H₃BO₃, 0.7 μM MnSO₄, 1 μM ZnSO₄, 0.4 μM CuSO₄ and 0.04 μM (NH₄)₆ Mo₇O₂₄. The water used for preparing the nutrient solution was deionized.

After 13 d, plants of Indian mustard (*Brassica juncea*) varieties were then transplanted in earthenware pots packed with 5 kg of air-dry soil. The experiment consisted of two parts. For Part I, the soil was amended with arsenic at different concentrations (0 (control treatment), 10, 20, 30, 40 and 50 mg As kg⁻¹ as Na₂HAsO₄ to examine the effect of different arsenic concentrations on mustard plants. For Part II, the soil was amended with different arsenic compounds at the rate of 50 mg As kg⁻¹ as inorganic arsenicals [Arsenite from NaAsO₂, arsenate

from Na_2HAsO_4]. Phosphorus as $\text{CaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ at $17.3 \text{ mg P kg}^{-1}$, K as KCl at $25.6 \text{ mg K kg}^{-1}$, and N as $\text{CO}(\text{NH}_2)_2$ at $70.5 \text{ mg N kg}^{-1}$ were supplied as solution (in distilled water) at the start of the experiment to ensure adequate mineral nutrition. Application of all nutrient solutions and first application of arsenate treatment to dry soil was conducted before transplantation of mustard seedlings. The experimental design was completely randomized with each treatment replicated three times. Plants were cultivated in a greenhouse with a 10-h light period, with light provided by fluorescent and incandescent lamps at an illuminance of 17,200 lux. All plants were maintained at a constant temperature of 27°C and a relative humidity of 50%, during both day and night. The plants were watered daily as needed. At harvest, the roots and stems were separated in order to determine dry weight and As concentration.

Determination of Arsenic in Plant

The plant tissue was dried at 72°C and then wet ashed using nitric and perchloric acids according to standard methods (Jones and Case, 1990). The resulting solution was analyzed for arsenic content by inductively coupled plasma spectrometry (Fisons Accuris, Fisons Instruments, Beverly, MA). Certified National Institute of Standards and Technology plant standards (peach leaves) were carried through the digestions and analyzed as part of the QA/QC protocol. Reagent blanks and internal standards were used where appropriate to ensure accuracy and precision in the analysis.

Analysis of As Species in Soil

Concentrations of As species in soil were measured by HPLC-inductively coupled plasma-mass spectrometry. A Hamilton PRP X-100 (250 mm \times 4.1 mm, 10- μm column, Hamilton, Bonaduz, Switzerland) with a precolumn containing the same material was connected to a four-way Rheodyne valve (10- μL sample loop) and an HPLC pump. A solution of 30 mM H_3PO_4 set to pH 6.0 with NH_3 was used as a mobile phase with a flow rate of 1.0 mL min^{-1} , which allows a direct connection to a concentric nebulizer (Meriland C-Type) and a continuous transportation of the sample to the argon plasma of an ICP-mass spectrometer (Spectromass 2000, Spectro Analytical Instruments, Kleve, Germany). Standard plasma conditions were used. With a dwell time of 100 ms, the m/z 75 and 77 were monitored to check for possible ArCl interferences. Arsenite from NaAsO_2 and arsenate from Na_2HAsO_4 were preserved as stock solutions at $1,000 \text{ mg As L}^{-1}$. Standard solutions ($0\text{--}100 \mu\text{g L}^{-1}$) were prepared fresh from stocks for calibration.

Statistical Analysis

Results were expressed as a mean of three replicates and

analysis of variance was performed using SAS software (SAS Institute, 1987).

RESULTS AND DISCUSSION

Varuna appeared to have a higher susceptibility to As toxicity than DHR-9504 and this higher sensitivity was associated with corresponding decreases in stem and root growth (Table 1 and 2). The stem dry weight of Varuna and DHR-9504 were significantly reduced by 54.6 and 33.6% respectively over the control at the 30 mg kg^{-1} of As treated soil. Similar decreases were also noted for root dry weight. Our findings are consistent with other report (Simon et al., 1978).

Arsenic is generally considered phytotoxic and is expected to negatively affect plant growth (Kabata-Pendias and Pendias, 1991). As a result of many negative effects on plants, arsenic caused a reduced growth of plants. It has been demonstrated recently that catalase, Reactive oxygen species and Superoxide dismutase were all stimulated after exposure to arsenic. Reactive oxygen species can directly damage proteins, amino acids and nucleic acids and cause peroxidation of membrane lipids (Dat et al., 2000). Arsenic accumulated in the plant tissue stimulates peroxidase synthesis during the early phases of plant development, long before the appearance of visible changes (Stoeva et al., 2003).

Results showed that As concentration appearing toxicity was widely varied with plant genotypes. It might be because of varietal differences in As translocation and phytoextraction potential of the plants in Indian mustard varieties. It seems likely that the plant species and even genotypes differ greatly in their ability to take up, transport and accumulate As within the plants. This suggestion is also confirmed by Bernal et al. (1975).

The growth of Indian mustard plants was further confirmed in the experiment using varying arsenic forms. At 50 mg As kg^{-1} , both arsenic forms (Na_2HAsO_4 and NaAsO_2) significantly decreased the stem and root dry weight of mustard genotypes (Table 2). The stem dry weight of Varuna and DHR-9504 were significantly reduced by 67.5 and 68.9% and 59.2 and 60.4% respectively over the control when the soil was amended with arsenic compounds at the rate of 50 mg As kg^{-1} as Na_2HAsO_4 and NaAsO_2 . Both inorganic As species, As(V) and As(III), are highly toxic to plants. As(V) is a phosphate analog, and therefore it can compete with phosphate in the cytoplasm, replacing phosphate in ATP to form unstable complex ADP-As, which leads to the disruption of energy flows in cells (Ullrich-Eberius et al., 1989) whereas As(III) is highly toxic to plants because it reacts with sulfhydryl groups (-SH) in enzymes and tissue proteins

Table 1 Effect of increasing As supply on stem and root dry weight of *Brassica juncea* genotypes (Varuna and DHR-9504). Each point is an average of three replicates.

Total soil As† mg As kg ⁻¹	Varuna		DHR-9504	
	Stem	Root	Stem	Root
	Dry weight mg plant ⁻¹		Dry weight mg plant ⁻¹	
0.69 (control)	225d‡	80 d	250d	90 c
10	165c	59 cd	215c	77 b
20	115b	42 c	170b	60 ab
30	102ab	36 ab	166b	59 ab
40	96 a	34 ab	105a	36 a
50	70a	24 a	99 a	34 a
LSD (5%)	6	2	9	3

† Arsenic was added as Na₂HAsO₄.

‡ All results are the means of three replicates. Values followed by the same letter in a column are not significantly different ($p < 0.05$).

Table 2 Effect of varying As forms (arsenite and arsenate) on stem and root dry weight of *Brassica juncea* genotypes (Varuna and DHR- 9504). Each point is an average of three replicates

Arsenic forms*	Varuna		DHR-9504	
	Stem	Root	Stem	Root
	Dry weight mg plant ⁻¹		Dry weight mg plant ⁻¹	
0.69 (control)	222d‡	81 cd	248d	92 d
Na ₂ HAsO ₄	72 a	26 a	101a	36 a
NaAsO ₂	69 a	25 a	98 a	35 a
LSD (5%)	5	1	6	2

* @50 mg As Kg⁻¹

‡ All results are the means of three replicates. Values followed by the same letter in a column are not significantly different ($p < 0.05$).

(Jocelyn, 1972), leading to inhibition of cellular function and death (Ullrich-Eberius et al., 1989). Arsenite has been considered at least twice as phytotoxic as arsenate either foliarly or root-applied (Sachs and Michael, 1971). However at 50 mg As kg⁻¹, such a difference was not observed for Indian mustard genotypes between treatments with Na₂HAsO₄ or NaAsO₂. This may be due to the reduction of the arsenate (As^V) to arsenite (As^{III}) in soil.

The inorganic As species found in soil were arsenite(As^{III}) and arsenate(As^V). In general, arsenite was the most predominant species, followed by arsenate (Fig. 1). Concentrations of arsenite and arsenate ranged between approximately 47.2%- 86.2% and 13.1%- 52.2% respectively. Arsenate accounts for 52.2% of the total As species for control treatments (0 mg As kg⁻¹). Thereafter, the proportion of arsenate was reduced to 13.1% at the highest arsenate treatment (50 mg As kg⁻¹). This reduction in the proportion of arsenate species in the highest arsenate treatment might be due to the conversion of arsenate to arsenite in arsenic-contaminated soil. The presence of arsenite in the soil solution at a larger proportion corroborates with the results of a number of investigators (Marin et al., 1993; Onken and Hossner, 1996) who found arsenite as the predominant species in the soil environment.

Arsenic concentration in Indian mustard plants was found to be directly proportional to soil arsenic concentration. Arsenic concentration in Chinese cabbage (*Brassica ampestris L.*), radish (*Raphanus sativus L.*) and lettuce (*Lactuca sativa L.*) was also found to be directly proportional to soil arsenic concentration (Kabata-Pendias and Pendias, 1984).

Arsenic is predominantly concentrated in the roots with less accumulated in the stem, especially in the case of DHR-9504 (Table 3 and 4). The As concentration in the root, in turn, was controlled by the rate of uptake into the root and the rate of translocation to the shoot. Arsenic concentration in the stem varied from 0.8 to 4.9 mg kg⁻¹ (with a mean of 2.83 mg kg⁻¹), while that in the root varied from 2.1 to 26.2 mg kg⁻¹ (with a mean of 16.0 mg kg⁻¹) at varying arsenic concentrations in the experiment. Marin et al. (1993) also reported similar trends for most plants. Pickering et al. (2000) also observed a large accumulation of As by mustard plants, in roots as compared to stems.

It appeared that arsenic forms (arsenite vs. arsenate) had little effect on arsenic concentrations in Indian mustard tissue, with no clear trends being observed (Table 4). This is possibly because the arsenate (As^V) could have transformed to As^{III}, during the experiment due to chemical oxidation–reduction. Carbonell et al. (1998) reported that As(III), and As(V) were stable for only 4 d

with respect to oxidation–reduction reactions. Pickering et al. (2000) also demonstrated that in Indian mustard roots, the arsenate (As^V) is reduced to As^{III}, and coordinated by three sulfur ligands, which can be modeled as the As^{III}-tris-glutathione complex. However, the transformation of As (V) in the plant tissue and the factors controlling the transformation and the subsequent translocation of As species from roots to shoots are not well understood.

Significant differences among both genotypes were noted in As translocation at varying arsenic concentrations (Table 3). It seems that the amount of As accumulated and translocated in plants varies depending on the experimental conditions (species and soil properties etc.). Arsenic accumulation and translocation in plants are influenced by such factors as plant species (Bernal et al., 1975; Matschullat, 2000), soil arsenic concentration (Jiang and Singh, 1994), soil properties (Jiang and Singh, 1994; Matschullat, 2000), the presence of other ions (Khattak et al., 1991).

Compared with arsenite (NaAsO₂), arsenate (Na₂HAsO₄) resulted in less arsenic translocation from root to stem, that is, more arsenic was stored in the roots (Table 4). It might be because of different transport mechanisms for arsenite and arsenate uptakes by the roots, possibly arsenate uptake was suppressed in the presence of phosphate, whereas arsenite transport was not affected by phosphate. Cox et al. (1996), also demonstrated that arsenate uptake mechanism is inhibited by phosphate, suggesting that phosphate and arsenate are transported by the same uptake system.

In Indian mustard, arsenic extraction by plants increased significantly with increasing arsenic concentrations in soil (Table 5). Among the five arsenic concentrations tested, 50 mg As kg⁻¹ resulted in the greatest arsenic uptake by plants (1037 µg plant⁻¹ in Varuna and 959 µg plant⁻¹ in DHR-9504). The transfer of As from soil to plant is low for most plant species. This may be because of several reasons: i) low bioavailability of As in soil, ii) restricted uptake by plant roots, iii) limited translocation of As from roots to shoots, and iv) As phytotoxicity at relatively low concentrations in plant tissues. Indian mustard genotypes (DHR-9504 and Varuna), showed that uptake of As by DHR-9504 was less than that of Varuna. The difference in uptake rate of As in mustard genotypes might be because of varietal differences in root systems of both genotypes.

Uptake studies were conducted with arsenate and arsenite to observe how these species are taken up into the plants. Uptake of arsenate by both genotypes was less than that of arsenite (Table 6). High uptake rates of arsenite by Indian mustard is a matter concern because it is the dominant As species in the highly reduced soil

Table 3 Effect of increasing As supply on stem and root As concentrations of *Brassica juncea* genotypes (Varuna and DHR- 9504). Each point is an average of three replicates.

Total soil As†	Varuna		DHR-9504	
	As concentration			
	Stem	Root	Stem	Root
mg As kg ⁻¹	mg As kg ⁻¹		mg As kg ⁻¹	
0.69 (control)	N.D.*	2.1±0.1†	N.D.**	3.7±0.2
10	1.1±0.2	10.4±0.1	0.8±0.1	8.7±0.2
20	2.7±0.2	15.2±0.1	1.4±0.1	17.7±0.3
30	3.1±0.1	18.8±0.2	2.8±0.2	21.5±0.2
40	4.5±0.2	21.3±0.3	3.4±0.1	22.9±0.3
50	4.9±0.3	23.5±0.2	3.9±0.2	26.2±0.5
LSD (5%)	1.1	2.3	1.2	2.7

**could not be determined

† Mean ± standard error.

Table 4 Effect of varying As forms (arsenite and arsenate) on stem and root As concentrations of *Brassica juncea* genotypes (Varuna and DHR- 9504). Each point is an average of three replicates.

Arsenic forms*	Varuna		DHR-9504	
	As concentration			
	Stem	Root	Stem	Root
	mg As plant ⁻¹		mg As plant ⁻¹	
0.69 (control)	N.D.**	2.2±0.1†	N.D.**	3.5±0.1
NaAsO ₂	9.9±0.1	25.7±0.2	8.8±0.1	27.2±0.2
Na ₂ HAsO ₄	4.2±0.1	23.1±0.1	3.4±0.2	25.3±0.3
LSD (5%)	0.8	1.3	0.5	2.1

* @50 mg As Kg⁻¹

**could not be determined

† Mean ± standard error.

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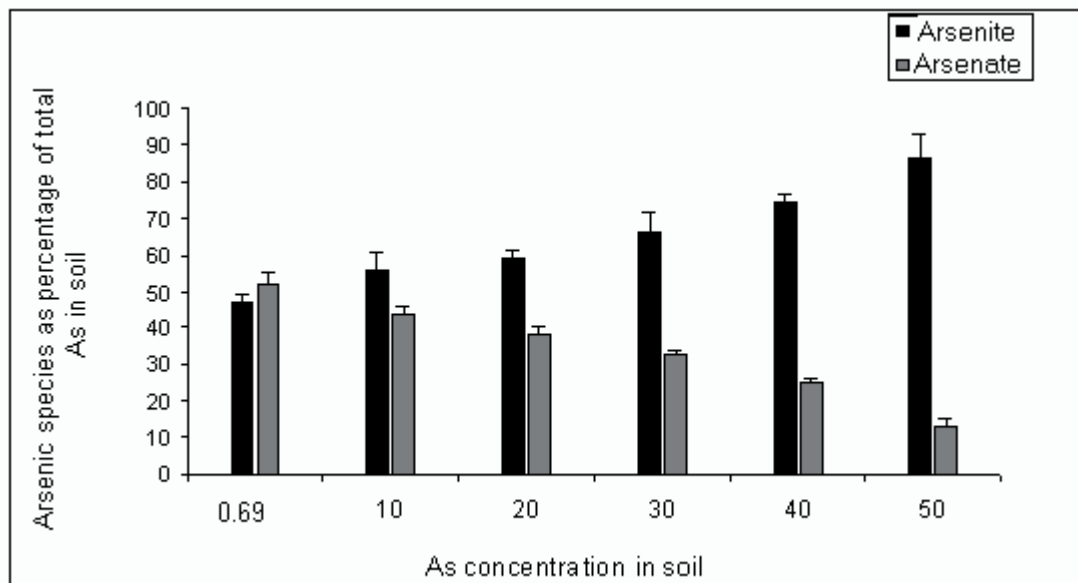


Fig-1 As species present in soil solution from a greenhouse experiment at different concentrations of arsenate. Error bars represent \pm SE of three replicates.

Table 5. Total As uptake in each plant of Varuna and DHR-9504 in soils of different As concentration. Each point is an average of three replicates.

As conc. (mg kg ⁻¹)	Absorbed As conc. (μ g plant ⁻¹)	
	Varuna	DHR-9504
0.69 (Control)	24 \pm 1.2†	ND*
10	207 \pm 4.2	175 \pm 5.2
20	377 \pm 7.1	244 \pm 7.0
30	425 \pm 6.5	299 \pm 7.2
40	667 \pm 8.1	594 \pm 9.7
50	1037 \pm 12	959 \pm 14
LSD (<0.05)	78	45

*could not be determined

† Mean \pm standard error.

LSD: minimum significant difference

Table 6 Total As uptake in each plant of Varuna and DHR-9504 in a soil amended with arsenic in varying forms at 50 mg kg⁻¹. Each point is an average of three replicates.

Arsenic forms*	Absorbed As conc. ($\mu\text{g plant}^{-1}$)	
	Varuna	DHR-9504
0.69 (control)	24 \pm 1.2†	N.D.**
NaAsO ₂	1378 \pm 12	1225 \pm 17
Na ₂ HAsO ₄	1029 \pm 10	950 \pm 7
LSD (<0.05)	78	45

* @50 mg As Kg⁻¹

**could not be determined

† Mean \pm standard error.

LSD: minimum significant difference

environment, as illustrated in the data presented in Fig. 1. There are a number of studies investigating the mechanism of arsenate uptake, both in higher and lower plants (Asher and Reay, 1979; Meharg and Macnair, 1992; Meharg et al., 1994).

Table 5 and 6

CONCLUSION

This experiment clearly demonstrated that Varuna appeared to have a higher susceptibility to As toxicity than DHR-9504 which might be because of varietal differences in translocation and uptake systems of both genotypes. Our study has shown that As (V) can be taken up by mustard roots, at a slow rate than that of As(III) in both Indian mustard genotypes (Varuna and DHR-9504) because the most predominant As species in the soil was arsenite. We conclude that As(V) is readily reduced to As(III) due to chemical oxidation–reduction. However, it is not clear whether this result would hold for other varieties of Indian mustard. More data are needed to ascertain the findings of this study.

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