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ARSENIC OCCURRENCE IN GROUND WATER AND SOIL OF UTTAR PRADESH, INDIA AND ITS PHYTOTOXIC IMPACT ON CROP PLANTS

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ABSTRACT: The primary objective of the present study is to focus about the current arsenic contamination in soil and drinking water of Uttar Pradesh, India in order to make public aware about the health hazards of arsenic contaminated water and understand the need for immediate action. Agricultural soil and ground water samples of arsenic affected areas of district Lakhimpur Kheri and Unnao (Uttar Pradesh, India) were analyzed for presence of arsenic (As). Ground water samples collected from all the contaminated sites of both the districts contained As in the range of 23 to 140 $\mu g\ L^{-1}$ which was far above the permissible limit of WHO i.e. 10 $\mu g\ L^{-1}$. Surprisingly, the soil samples contain almost 40-45 times more arsenic than that found in the ground water samples of the same site. The seed germination of three selected crops (*Pisum sativum*, *Oryza sativa* and *Phaseolus vulgaris*) was also estimated in the presence of different concentrations (1-10 $mg\ L^{-1}$) of arsenate (AsV). Results showed a decrease in the percent seed germination, root length, shoot length and increase in phytotoxicity between 1.0 to 5 $mg\ L^{-1}$ level of arsenic, on all the three tested crops. The toxicity of arsenic was found in the order of *P. vulgaris* >*P. sativum* > *O. sativa*.

KEYWORDS: Arsenic; Germination Index; Health Hazard; Phytotoxicity; Uttar Pradesh

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1.INTRODUCTION

Arsenic contamination of environment is a global environmental concern because of its extravagant toxicity and wide abundance. This toxic metalloid generally exists in trivalent arsenite (As-III) and pentavalent arsenate (As-V) forms as well as in the form of arsine gas. Arsenic in terrestrial and aquatic ecosystems attracts worldwide attention primarily because of its adverse impact on human health [1]. WHO and USEPA recommend a provisional drinking water guideline of 10 $\mu g\ L^{-1}$[2]and 1.5 to 2 mg Kg^{-1} in soil [3]. The geogenic source of arsenic is reported in over 20 countries including Bangladesh and India where arsenic occurs naturally in aquifers [4], but anthropological activities like mining, burning of fossil fuels and uses of pesticides also cause arsenic contamination [5,6] The use of arsenic contaminated groundwater for irrigation purpose in crop fields may be responsible for elevated level of arsenic concentration in surface soil and in the plants grown in these areas [7]. Human exposure of arsenic is known to occur by ingestion of contaminated drinking water and food. Accumulation of arsenic by plants allows it to enter in the food chain and finally reaches to the body of human beings. Arsenic is known to cause skin lesions, blackening of foot, bladder, kidney, liver, lung and skin cancers [8,9]. Seed germination and early seedling growth are considered to be more sensitive to metal toxicity than the mature plants [10,11,12]. The present investigation was aimed to find out the level of arsenic contamination of soil and water in Lakhimpur Kheri and Unnao districts of Uttar Pradesh (India) and also to find out whether there is any relationship between arsenic contamination of soil and ground water. Further, efforts were made to study the phytotoxicity response of three important crops i.e., *Pisum sativum*, *Oryza sativa* and *Phaseolus vulgaris* against arsenic toxicity.

2. MATERIALS AND METHODS

2.1. Sampling

The ground water and soil samples were collected from five villages of Unnao district (26°32’0”N and 80°30’0”E) namely, Gaja Khera, Murtaza Nagar, Sultan Khera, Kuddu Khera, Shuklaganj and four villages of Lakhimpur Kheri district (27°57’0”N and 80°46’0”E) namely, Manjhara, Parasia, Pakharia and Trilokpur of Uttar Pradesh, India (Fig. 1). From each village two sites were selected randomly from where ground water (hand pump/bore well) and soil samples were collected. The soil samples were collected from a depth of 15 cm in sterile plastic bags.

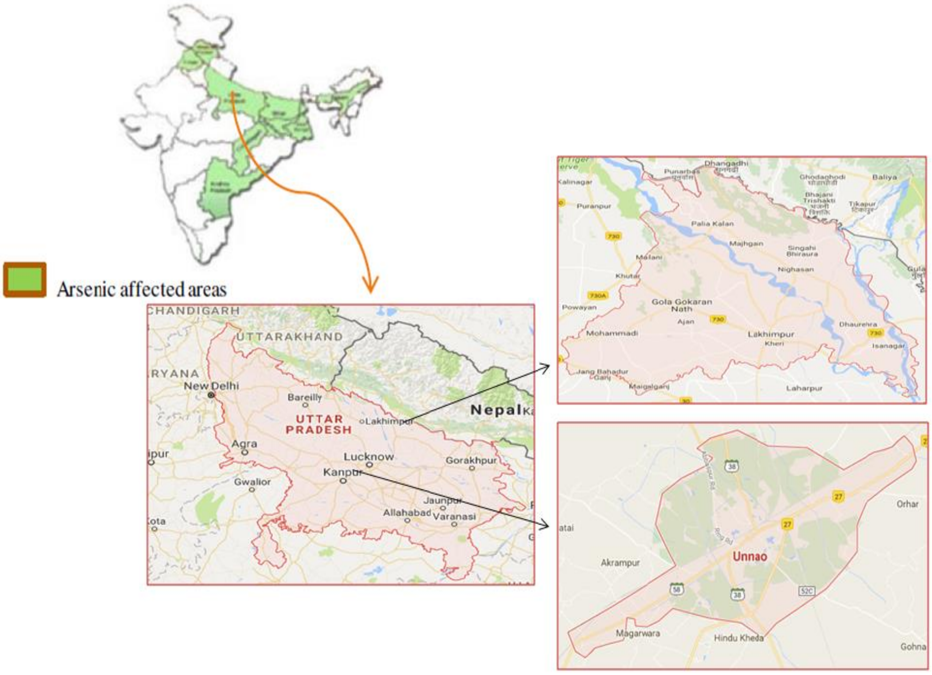

Fig 1: Arsenic affected surveyed areas including Lakhimpur kheri and Unnao districts of Uttar Pradesh, India.

2.2. Determination of Arsenic In Water And Soil Samples

Water samples were digested by following the standard method for examination of water [13]. The soil samples were digested by the method given by USEPA (3050 B)[14]. The arsenic concentration in soil and water samples was estimated by using Hydride Generator Double-Beam Atomic Absorption Spectrophotometer (Varian AA 240 FS, Australia) at 193.7 nm.

2.3. Phytotoxicity Assay

The stock solution of arsenic was prepared by dissolving Sodium Arsenate in Milli-Q water in calculated amount. Seeds of *P. sativum*, *O. sativa* and *P. vulgaris* were selected for testing the toxicity of As-V. Seeds were surface sterilized with 0.1% $HgCl_2$ solution and germinated in sterilized petri plates with different arsenic concentration (1-10 mg L^{-1}) in laboratory conditions (25±2ºC). At different time interval, radicle length was recorded. Emergence of radicles of more than 1.0 mm size was taken as a criterion for seed germination as well as calculation of different phytotoxicity parameters. Percent Phytotoxicity was calculated by the formula developed by Chou and Lin[15] and modified by Ray and Banerjee[16]

$$\text{Phytotoxicity (\%)} = \frac{\text{Radicle length of control (cm)} - \text{radicle length of test (cm)}}{\text{Radicle length of Control (cm)}} X100$$

Germination Index was calculated by the equation given by IRSA [17].

$$\text{Germination index (GI)} = \frac{Gs\ \ Ls}{Gc\ \ Lc}$$

Where Gs and Ls are seed germination percentage and root elongation (for the sample). Gc and Lc are corresponding control values.

Metal Tolerance Index was calculated from radicle length data using following formulae [18].

$$\text{MTI} \ (\%) = \frac{\text{radicle length of seedling in test (cm)}}{\text{radicle length of seedling in control (cm)}} \text{X } 100$$

3. RESULTS AND DISCUSSION

3.1. Concentration of as in Soil and Water

Maximum arsenic contamination of both soil (5.679 mg Kg^{-1} As) and water (139.5 µg L^{-1} As) was found at Shuklaganj (Unnao) among all the investigated sites (Table 1), followed by Trilokpur (4.901 mg Kg^{-1} As in soil and 100.15 µg L^{-1} As in water) and Parasia villages (3.515 mg Kg^{-1} As in soil and 83.5 µg L^{-1} As in water) (Lakhimpur Kheri). It was found that the arsenic level was higher only in those soil samples where ground water contains relatively higher level of arsenic. The soil samples contained almost 40-45 times more arsenic than that is present in the ground water samples of the same site. It can be argued that accumulation of arsenic in soil may be due to regular use of ground water for irrigation as soil arsenic level was higher in those areas where ground water was highly contaminated. Similar results were also obtained by Meharg and Rahman [7] who observed the elevated level of arsenic concentration in plants and surface soil where arsenic contaminated water was used for irrigation purpose. All the drinking water samples contain arsenic content beyond the permissible limit i.e.10 µg L^{-1} as prescribed by USEPA.

Table 1. Quantification of Arsenic concentration in contaminated soil and water samples. Data are the mean of three replicates ±SD.U and L denote Unnao and Lakhimpur Kheri district respectively, and number 1, 2 denotes different sampling sites of same village.

Selected District	*Selected Village*	*Sampling Sites*	*Soil Arsenic concentration (mg Kg^{-1})*	*Water Arsenic concentration (µg L^{-1})*
Unnao District	Gaja Khera	UA_1	0.272±0.001	48.0±1.5
		UA_2	0.269±0.001	52.0±1.5
	Murtaza Nagar	UB_1	0.533±0.002	65.0±1.0
		UB_2	0.553±0.001	70.0±1.2
	Sultan Khera	UC_1	0.641±0.003	23.0±0.3
		UC_2	0.622±0.002	27.0±0.3
	Kuddhu Khera	UD_1	0.782±0.003	34.0±0.2
		UD_2	0.739±0.002	46.3±0.3

	Shukla Ganj	UE_1	**5.668±0.01**	**146.0±2.4**
		UE_2	**5.691±0.01**	**133.0±2.5**
Lakhimpur Kheri District	Manjhra	LF_1	0.683±0.001	60.0±1.0
		LF_2	0.710±0.002	55.0±1.0
	Parasia	LG_1	**3.497±0.02**	**85.0±0.7**
		LG_2	**3.533±0.02**	**82.6±0.6**
	Pakharia	LH_1	0.276±0.001	22.0±0.1
		LH_2	0.335±0.001	24.0±0.2
	Trilokpur	LI_1	**4.916±0.05**	**98.3±1.0**
		LI_2	**4.886±0.03**	**102.0±1.0**

3.2. Phytotoxicity assay of Arsenic

When seeds of all the three crops (*P. sativum, O. sativa* and *P. vulgaris*) were treated with varying concentration (1-10 mg L^{-1}) of arsenic (As V) it was found that seed germination of all the three crops decreased gradually with increasing concentration of arsenic up to 10 mg L^{-1} (Fig. 2A). At 10 mg L^{-1} arsenic concentration, the seed germination in *P. sativum* and *P. vulgaris* was found only 29.5 and 35.7%, respectively, whereas germination percentage in *O. sativa* was relatively less affected (70%) as compared to their respective control (without arsenic). It is well known that seed germination is an important indicator used to evaluate the toxicity potential of metals [10,12,19]. The results of the present investigation revealed that percent seed germination decreased gradually with increasing concentration of arsenic, as also reported by different workers [20,21]. Complete cessation of seed germination in different crops was also reported when treated with 50 mg L^{-1} arsenic [22]. Li et al. [23] reported stimulated seed germination and root length in crop plants when treated with lower concentrations of arsenic. Among the three tested crops, it was observed that percent germination was highest in case of *O. sativa*, followed by *P. vulgaris* and*P. sativum.* These results indicated that arsenic was less toxic for the germination of carbohydrate rich *O. sativa* seeds, when compared with protein rich seeds of *P. sativum* and *P. vulgaris*. Earlier it has been suggested that difference in the germination percentage may also be due to interspecific variation in the morphology of seed coats and different concentration of metals [24]. The radicle length of seedlings was taken after 24, 48 and 72 h of germination (Table 2) and it was observed that radicle length decreased with gradual increase in arsenic concentration in all the three crops as compare to control set. About 50% decrease in radicle length after 72 h of germination was found at 6, 5 and 2 mg L^{-1} arsenic concentration in case of*P. sativum, O. sativa* and *P. vulgaris,* respectively. The toxicity of arsenic was found in the order of *P. vulgaris*>*O. sativa*>*P. sativum*. Reduction in root length is possibly due to the accumulation of heavy metals in plant tissues and its interaction with the minerals as roots are the first contact point for the toxicants [20]. The germination index decreased with gradual increase in arsenic concentration (1-10 mg L^{-1}) for all the three crops (Fig. 2B). Among the three tested crops it was observed that germination index at 10 mg L^{-1} arsenic was maximum (17.08%) in case of *O. sativa*, followed by *P. sativum* (9.44%) and *P. vulgaris* (4.87%). It was found that germination index decreased with increasing concentration of arsenic for all the tested crops. Similar results were also observed by various workers [21,23]. Results on percent phytotoxicity (Fig. 2C) showed that in all the three tested crops percent phytotoxicity increased with increasing concentration of arsenic up to 10 mg L^{-1} and about 68, 75.6 and 85.3% phytotoxicity was observed in *P. sativum,O. sativa* and *P. vulgaris,* respectively. Metal tolerance index of all the three crops showed a gradual decrease with increasing concentration of arsenic 10 mg L^{-1} (Fig. 2D). This decrease was more prominent in case of *P. vulgaris* as compare to *P. sativum* and *O. sativa*. At 10 mg L^{-1} concentration of arsenic, percent metal tolerance index was found to be 14.7, 24.4 and 32% in *P. vulgaris, O. sativa* and *P. sativum*, respectively. These results also corroborated the results on phytotoxicity and suggested that arsenic was more toxic to *P. vulgaris* as compared to *O. sativa* and *P. sativum.* Results on the metal tolerance index and phytotoxicity parameters in all the three test crops showed that metal tolerance index decreased and phytotoxicity increased with increase in the arsenic concentration.

Table 2. Effect of Arsenic on Radicle length (cm) of *Oryza sativa, Pisum sativum* and *Phaseolus vulgaris*. Data are the mean of three replicates ± SD. Data was analyzed by one way analysis of variance (Duncan Multiple Range Test) at $p<0.05$. Different alphabets show significant differences between the treatments.

Conc. of As mgL^{-1})	*Oryza sativa* (Pusa Basmati)			*Pisum sativum* (Arkel)			*Phaseolus vulgaris* (Pusa Baisakhi)		
	24 h	*48 h*	*72 h*	*24 h*	*48 h*	*72 h*	*24 h*	*48 h*	*72 h*
0	1.05±0.14^{a}	3.15±0.24^{a}	4.63±0.33^{a}	1.02±0.2^{a}	1.35±0.3^{a}	1.78±0.2^{a}	0.83±0.01 ^{a}	1.21±0.12^{a}	1.50±0.3^{a}
1	0.88±0.06^{b}	2.85±0.14^{ab}	4.21±0.47^{ab}	0.90±0.01^{a}	1.20±0.01^{b}	1.51±0.1^{b}	0.51±0.02^{ab}	0.78±0.02^{b}	0.85±0.03^{b}
2	0.71±0.02^{c}	2.0±0.14^{b}	3.48±0.26^{b}	0.74±0.01^{b}	1.12±0.1^{c}	1.37±0.1^{c}	0.43±0.02 ^{b}	0.62±0.03^{c}	0.72±0.06 ^{b}
3	0.43±0.04^{d}	1.78±0.06^{b}	3.36±0.21^{c}	0.56±0.02^{c}	1.03±0.03^{c}	1.26±0.3^{d}	0.21±0.01 ^{c}	0.42±0.01^{c}	0.53±0.04^{c}
5	0.32±0.02^{e}	1.21±0.03^{c}	1.98±0.09^{d}	0.41±0.03^{d}	0.89±0.01^{c}	1.06±0.01^{e}	0.11±0.01 ^{d}	0.31±0.03^{c}	0.48±0.03 ^{c}
8	0.21±0.01^{f}	0.82±0.02^{d}	1.42±0.02^{e}	0.30±0.01^{e}	0.54±0.02^{d}	0.72±0.01^{d}	0.11±0.01 ^{d}	0.23±0.02^{d}	0.31±0.02^{c}
10	-	0.44±0.01^{e}	1.13±0.03^{ef}	-	0.35±0.01^{e}	0.57±0.02^{f}	0.09±0.01 ^{d}	0.12±0.01^{e}	0.22±0.01 ^{d}

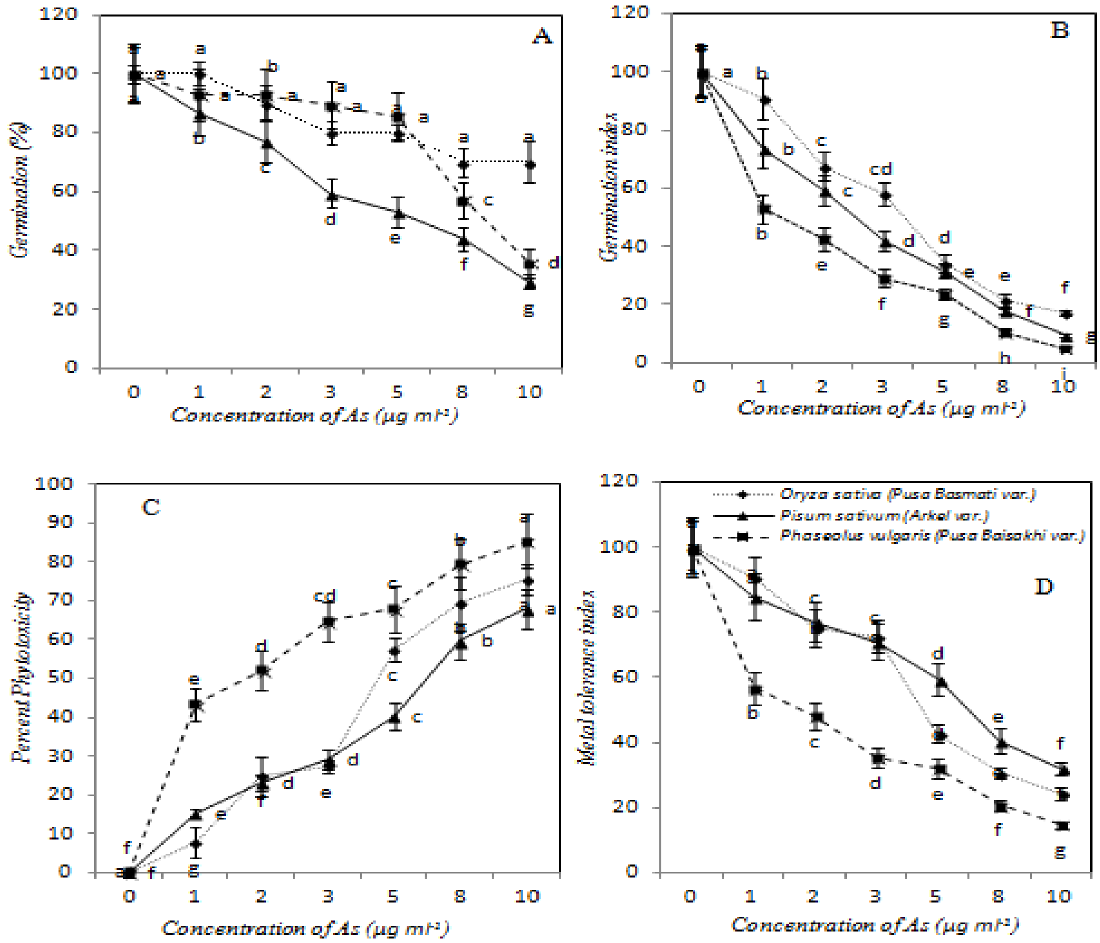

Fig 2: Effect of different concentration of arsenic (0-10 µg ml^{-1}) on the seed germination (A), Germination Index (B), phytotoxicity (C) and metal tolerance index (D) of *Oryza sativa, Pisum sativum* and *Phaseolus vulgaris*. Data was analyzed by one way analysis of variance (Duncan Multiple Range Test) at $p<0.05$. Different alphabets show significant differences between the treatments.

4. CONCLUSION

In the light of foregoing results, it was deduced that arsenic contamination of soil in both Lakhimpur kheri and Unnao districts were about 40-50 times higher than that of ground water, particularly at sites where ground water contamination was also found higher. The level of arsenic contamination of soil was found to be phytotoxic for all the three tested crops, though to varying extent, depending upon their sensitivity to arsenic. Further investigation on the accumulation of arsenic by these crops is expected to reflect the quantum of arsenic entering in the food chain. Elevated levels of arsenic in drinking water will pose health hazard to the mass consuming it. So awareness programmes should be conducted in order to spread information regarding consequences of arsenic toxicity. Drinking water supply contaminated with arsenic should be avoided and hygienic supply of drinking water should be provided to the people residing in vicinity of polluted areas.Soil mitigation practices should also be adopted in the As affected areas.

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6. CONFLICT OF INTEREST

The authors declare that they don't have any conflict of interest.

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