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Toxicity of As(III) and As(V) on morphological traits and pigments of Gram Seed (*Cicer arietinum*) during germination and early seedling growth

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ABSTRACT

Arsenic (As) contamination is an important environmental consequence in some part of India and other countries. In this study, we investigated that individual phytotoxicity of both As(III) and As(V) on *Cicer arietinum*. Total five different concentrations (0, 2, 4, 6, 8, and 10 mg/L) of both As(III) and As(V) have been considered for this study. Results indicate that percent germination significantly ($p < 0.05$) reduced with increasing concentration of both As(III) and As(V). However, As(III) showed pronounced effects than As(V). The variation of root and shoot length was equally affected by arsenite, where as arsenate showed higher negative impact on shoot length than root length up to the concentration 6 mg/L. However, arsenate concentration greater than 6 mg/L showed opposite trend of variation in root and shoot length. The pigment (Chlorophyll 'a', 'b' and total chlorophylls) and carotenoids level also gradually decrease with increasing concentration of both forms of arsenic.

Keywords: Arsenic, Morphophysiology, Germination, Root and shoot length, Chlorophyll content.

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INTRODUCTION

Pulses are major sources of proteins among the vegetarians in India, and complement the staple cereals in the diets with proteins, essential amino acids, vitamins and minerals. They contain 22-24% protein, which is almost twice the protein in wheat and thrice that of rice. Pulses provide significant nutritional and health benefits, and are known to reduce several non-communicable diseases such as colon cancer and cardiovascular diseases (Yude et al. 1993, Jukanti et al. 2012).

Ground water contamination with arsenic (As) is a matter of great concern worldwide (Chakrabarty et al. 2009). The inorganic form of arsenic may present as As(III) and As(V) in soil, where as organic form of arsenic present as monomethylarsonic acid and dimethylarsonic acid (Tripathi et al. 2007).From the previous literature (Chun-Xi et al. 2007) it is clear that the removal of arsenic from the environment is extremely difficult task. Arsenic contamination of soil, streams, and underground water causes a major environmental and human health risk. The tremendous threat of arsenic towards health of human and animal is due to its (As) long-term persistence in the environment (Mercedes et al. 2002). Recent literature (Roy et al. 2014) highlighted that as contaminated groundwater is used for irrigation as well as for drinking water. The World Health Organization (WHO) provisional guideline value for drinking water is 0.01 mg/L. There are concern that arsenic may be absorbed by plants, particularly cereals, entering the grains and thus the food chain (Chun-Xi et al. 2007).

Pertinent literature highlighted that low concentrations of As stimulated the growth of plants; but excessive As did harm to plants (Han et al. 2002, Imran et al. 2013). In addition, arsenic stimulates the formation of free radicals and relative oxygen species, resulting in oxidative stress (Requejo and Tena 2005). However, Plants differently response against As(III) and As(V) stresses by stimulating the antioxidant system and thiol metabolism, respectively (Mishra et al. 2008). However, the entire mechanism for accumulation of arsenic by plants is different for As(III) and As(V) (Chakrabarty et al. 2009). Generally As(V) is accumulated through phosphate uptake system (Ullrich-Eberius et al. 1989), whereas As(III)uptake is thought to be accomplished through the aquaporins of the roots (Meharg and Jardive 2003).

Keeping in mind the above fact, present work highlighted the arsenic (As(III) and As(V) toxicity on gram (*Cicer arietinum*) during early seedling growth with respect to growth morphology and pigment concentration.

MATERIAL AND METHODS

Germination assay. The effects of different concentrations of arsenite and arsenate on the seed germination of *Cicer arietnum* was evaluated. The concentrations of the two arsenic species (As^{3+} and As^{5+}) were 0, 2.0, 4.0, 6.0, 8.0, and 10.0 mg/L. Germination of seeds was tested on moist filter paper (Whatman No. 1). Two pieces of filter paper (8.0 cm disks) were placed on10 cm diam Petri plate (plastic made) and moistened with aqueous solution (10.0 mL) of arsenite and arsenate. Twenty seeds were placed in each Petri plate, covered by the lid, and incubated at 25 ºC. Controls were maintained by moistening the filter paper with distilled water. Prior to germination, all the seeds were surface sterilized by soaking them in 0.1% mercuric chloride solution for 30 seconds and washed thoroughly with distilled water. The number of germinated seeds was counted 5 days after initiation, and the results were expressed as percentage over control. Seeds were considered germinated when both the plumule and radical were extended to more than 2 mm from their junction.

Determination of photosynthetic pigments. Chlorophyll-'a', 'b' and total chlorophyll and carotenoid were estimated spectrophotometrically with slight modification (Arnon 1949). Leaf (0.25 g) was cut into small pieces leaving away the midribs, mixed thoroughly and ground finely using a mortar with 25 ml of 80% cold acetone. Then the solution was kept in refrigerator overnight. The solution was centrifuged with 5 ml 80% cold acetone. The optical density of each solution was measured at 480 nm, 645 nm, 652 nm, and 663 nm against 80 % cold acetone blank in 1.5 cm quartz cell.

Data Analysis. The experiment was laid out in a completely randomized design (CRI). Statistical analysis was performed with MINITAB version 16. Data were analyzed with the help of one way analysis of variance (ANOVA) to determine the significant effect of different treatments. The grouping was done at 5% level of significance and the treatments having the same letter are non-significant at the given level of significance.

RESULTS AND DISCUSSION

Percent germination. Germination of *Cicer arietinum* remarkably decreases with increasing concentration of arsenate salt solution (Figure 1). The percentage of germination with 2 mg/L As(III) solution was recorded 72%. However, the percentage of germination reduced to 54%, 37%, 20% and 12% with 4, 6, 7 and 10 mg/L

As(III) solution, respectively. However, As(V) showed much lower impact on germination. This is probably due to higher toxicity of As(III). Almost similar results reported by Winkel et al. (2008) and Imran et al. (2013). Results indicate that about 83% germination was recorded at 2 mg/L As(V) solution and it is reduced to 71 %, 65 %, 51 %, 35 %, and 25 % at 4, 6, 8, and with 10 mg/L As(V) solution, respectively (Figure 2). Therefore, in this study, germination of *Cicer arietinum* seeds was found to be affected considerably with arsenic species and their concentrations. The lower germination of *Cicer arietinum* seed at higher concentration of arsenite and arsenate could be an important consideration for *Cicer arietinum* cultivation because of presence of arsenite (Onken and Hossner 1996). It was reported in the earlier literature (BGS 2002) that under normal field conditions, application of contaminated irrigation water may have limited impact on rice germination as the highest reported concentration in Bangladesh groundwater was 2.4 mg As L^{-1}, and most of the added arsenic will be absorbed on soil particle surfaces leaving a relatively lower concentration in the soil solution (Onken and Hossner 1996). However, it is unwise to make a generalized conclusion, as there are significant variation among the varieties in tolerance to different concentration of arsenite and arsenate (Abedin and Meharg 2002). The reduction of root and shoot length is a typical response to toxic metals (Kabata-Pendias and Pendias 1984). Moreover, Abedin and Meharg (2002) highlighted that significant reduction of root length growth with increasing concentration of arsenic is due to the fact that plant roots were the first point of contact for these toxic arsenic species in the nutrient medium.

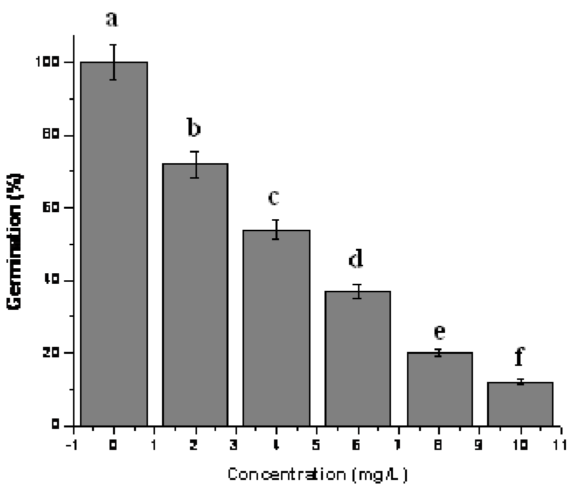

Figure 1. Percent germination of *Cicer arietinum* under different concentration of As(III).
Means followed by the same letter (S) within treatment are not significantly different at 5% using Duncan's multiple range test (DMRT). Means of three replicates are taken.

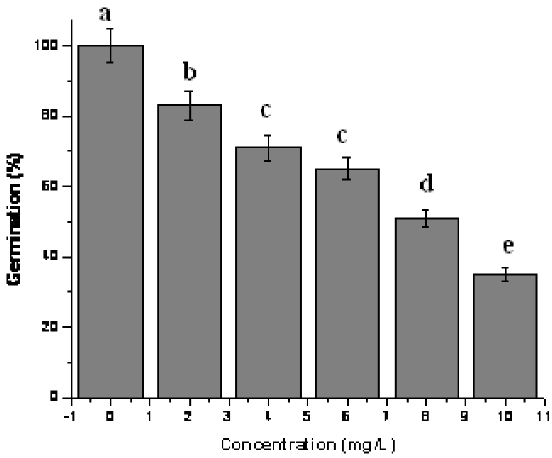

Figure 2. Percent germination of *Cicer arietinum* under different concentration of As(V).
Means followed by the same letter (S) within treatment are not significantly different at 5% using Duncan's multiple range test (DMRT). Means of three replicates are taken.

Effect of arsenic on growth parameters. As a preliminary experiment, *Cicer arietinum* seeds exposed to different concentrations of As(V) and As(III) to determine their effects on germination. It was recorded that arsenic was highly toxic to germination of *Cicer arietinum*. However, the relationships between soil As and growth of plants depends on the form and availability of the arsenic (Roychowdhury et al. 2005). The germination results clearly demonstrated that germination significantly decreased with increasing concentration of arsenic. The shoot and root growth in terms of root length and shoot length and number of lateral root (Figure 3) was greatly inhibited at 6 mg/L As(III) as well as 8 mg/L As(V). In addition, no lateral root formation was observed at concentration higher than 4 mg/L As(III). However, in case of As(V) all treated seeds coat and root surface turned black. The shoot and root growth of As(III) treated seeds of *Cicer arietinum* showed gradual reduction. However, reduction in shoot length is more prominent than root length (Figure 4). Almost similar results were recorded for As(V) (Figure 5). The variation of morpho-physiological parameters is due to the toxicity of different form and valency of arsenic. According to Wu and Xie (1990), the toxicity order of arsenic is AsH_3 > As(III) > As(V) > Organic As.

Variation of biomass. Biomass in the form of fresh and dry weight data indicate that the fresh weight of root gradually decrease with increasing concentration of both As(III) and As(V) (Table 1). On the other hand, both fresh and dry shoot biomass reduction was higher in case of As(III) than As(V). Data also suggest that both root and shoot (in fresh and dry) showed much higher reduction of biomass in higher As(V) salt solution than As(III) solution. Almost similar results reported by earlier research (Abedin and Meharg 2002).

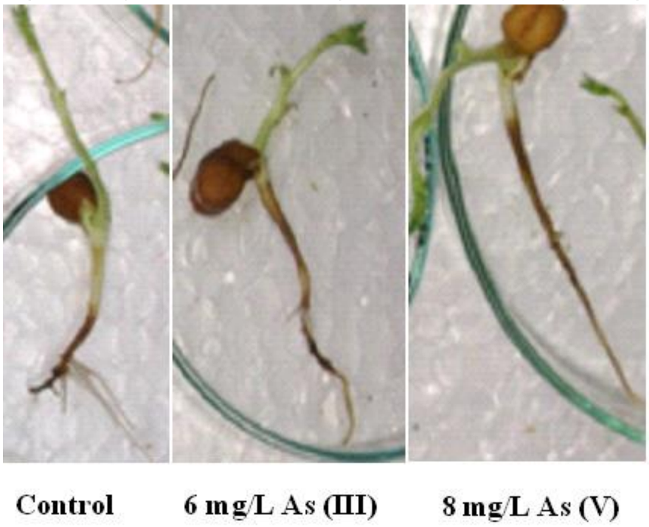

Figure 3. Drastic reduction of lateral root formation in 6 mg/L As(III) and 8 mg/L (V) with respect to control.

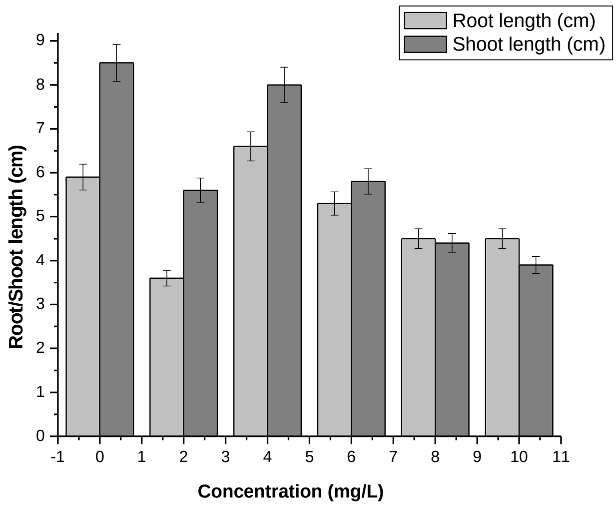

Figure 4. Variation of root and shoot length under different concentration of arsenite salt solution.
Vertical bars indicate the standard error of means.

Effect of arsenic on pigment content in *Cicer arietinum*. Photosynthesis by the green plant is the most important biochemical event on earth (Rahman et al. 2007). This particular biochemical reaction converts massive amount of sunlight into electrical energy and subsequently it convert to chemical energy (Hall and Rao 1999). The most import photosynthetic pigment in chloroplasts consist of two types of chlorophylls, chlorophyll-'a',-'b', and total chlorophyll and carotenoid. However, chemically they differ from each other and absorb light of different wavelengths to perform photosynthesis. Results also indicated that with increasing concentration of both As(III) and As(V) showed decreased in both chlorophyll-'a', and 'b' (Table 2). However, more drastic reduction was recorded for chlorophyll 'a' than chlorophyll 'b'. Rahman et al. (2007) reported that the shape of chloroplast along with rounding and shortening of the longitudinal axis of plant cell can be observed with increasing arsenic concentrations. Other observations such as concaving membrane, bending and partial destruction as well as changes in the accumulation and flow of assimilates were also reported under arsenic stress condition (Miteva and Merakchiyska 2002). Moreover, results also highlighted that decrease of carotenoid content is more prominent in arsenate than arsenite (Table 2).

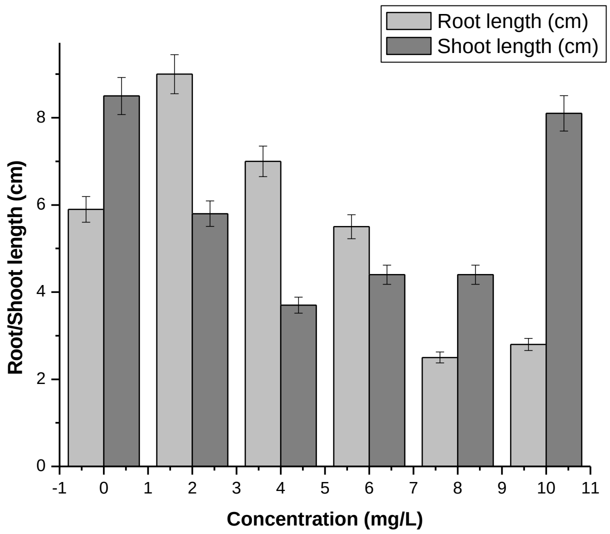

Figure 5. Variation of root and shoot length under different concentration of arsenate salt solution.
Vertical bars indicate the standard error of means.

CONCLUSION

West Bengal is a state which is agriculturally advanced. This particular state has surplus food production and main crops are paddy, vegetables and cereals. Present research highlighted that *Cicer arietinum* is severely affected by both As(III) and As(V). Results are revealed that As(III) has pronounced effect on germination than As(V). The number of lateral root formation was greatly inhibited at 6 mg/L As(III) and 8 mg/L As(V) solution. Similarly, both fresh and dry shoot biomass reduction was higher in case of As(III) than As(V). On the other hand, the pigment contents in the form of Chl'a' and Chl'b' decreased by the application of both As(III) and As(V). Therefore, extensive use of groundwater should be restricted for the agricultural purposes.

References

Abedn MdJ, Meharg AA. 2002. Relative toxicity of arsenite and arsenate on germination and early deedling growth of rice (*Oryza sativa* L.). Plant Soil 243: 57–66.

Table 1. Variation of fresh weight and dry weight of *Cicer arietinum* under As^{3+} and As^{5+} treatment.

Metal salt	Treatment	Root		Shoot	
		Fresh weight (g)	Dry weight (g)	Fresh weight (g)	Dry weight (g)
	0	0.595	0.055	0.365	0.033
	2	0.311	0.031	0.411	0.044
	4	0.262	0.041	0.376	0.035
As^{3+}	6	0.243	0.033	0.341	0.031
	8	0.233	0.028	0.313	0.027
	10	0.222	0.018	0.301	0.020
	0	0.595	0.055	0.365	0.033
	2	0.250	0.033	0.344	0.031
	4	1.055	0.031	0.356	0.036
As^{5+}	6	0.208	0.030	0.037	0.021
	8	0.200	0.003	0.028	0.019
	10	0.176	0.030	0.044	0.034

Table 2. Variation of pigment content under As^{3+} and As^{5+} treatment.

Metal salt	Concentration (mg/L)	Chl 'a' (mg/g.f.w)	Chl 'b' (mg/g.f.w)	Total Chl (mg/g.f.w)	Chl 'a'/'b' ratio	Carotenoid (mg/g.f.w)
As^{3+}	Control (0)	0.004	0.004	0.009	1.000	$8x10^{-4}$
	2	0.005	0.006	0.012	0.830	$8x10^{-4}$
	4	0.003	0.001	0.008	3.000	$7x10^{-4}$
	6	0.003	0.004	0.007	0.750	$6x10^{-4}$
	8	0.003	0.003	0.006	1.000	$5x10^{-4}$
	10	0.002	0.003	0.005	0.670	$4x10^{-4}$
As^{5+}	2	0.001	0.001	0.002	1.000	$2x10^{-4}$
	4	0.002	0.0005	0.004	4.000	$4x10^{-4}$
	6	0.001	0.002	0.003	0.500	$4x10^{-4}$
	8	0.002	0.002	0.004	1.000	$5x10^{-4}$
	10	0.014	0.029	0.043	0.483	$0.3x10^{-4}$

Arnon DI. 1949. Copper enzyme polyphenoloxides in isolated chloroplast in *Beta vulgaris*. Plant Physiol 24: 1–15.

BGS – British Geological Survey. 2000. Executive summary of themain report of Phase I, Groundwater Studies of Arsenic Contaminationin Bangladesh, by British Geological Survey andMott MacDonald (UK) for the Government of Bangladesh,Ministry of Local Government, Rural Development and Cooperatives,Department of Public Health Engineering, and Departmentfor International Development (UK). (http://www.dainichiconsul.co.jp/engl ish/ article/DFID-sum.html).

Chun-Xi L et al. 2007. Effects of arsenic on seed germination and physiological activities of wheat seedlings. J Environ Sci 19: 725–732.

Chakrabarty D et al. 2009. Comparative transcriptome analysis of arsenate and arsenitestresses in rice seedlings. Chemosphere 74: 688–702.

Hall DO, Rao KK. 1999. Photosynthesis, 6th ed. Cambridge University Press, Cambridge, UK, ISBN-521-644976, p. 214.

Han ZX et al. 2002. Study on effects of As(III) in environment on wheat sprout and the original researcher of prevention and treatment of arsenic toxicant. Acta Bot BOS 18: 123–128.

Imran MA et al. 2013. Toxicity of arsenic (As) on seed germination of sunflower (*Helianthus annuus* L.). Inter J Phy Sci 8: 840–884.

Jukanti AK et al. 2012. Nutritional quality and health benefits of chickpea (Cicer arietinum L.): a review. British J Nutr 108: S11–S26.

Meharg AA, Jardine L. 2003. Arsenite transport into paddy rice (Oryza sativa) roots. New Phytologist 157: 39–44.

Mishra S et al. 2008.Thiolmetabolism and antioxidant systems complement each other during arsenate detoxification in *Ceratophyllum demersum* L. AquatToxicol 86: 205–215.

Miteva E, Merakchiyska M. 2002. Response of chloroplasts and photosynthetic mechanism of bean plants to excess arsenic in soil. Bulg J Agri Sci 8: 151–156.

Mercedes DR et al. 2002. Heavy metals and arsenic uptake by wild vegetation in the Guadiamarriver area after the toxic spill of the Aznalcollar mine. J Biotechnol 98: 125–137.

Onken B, Mand Hossner LR. 1996. Determination of arsenic speciesin soil solution under flooded conditions. Soil Sci Soc A. J 60: 1385–1392.

Rahman MA et al. 2007. Effect of arsenic on photosynthesis, growth and yield of five widely cultivated rice (*Oryza sativa* L.) varieties in Bangladesh. Chemosphere 67: 1072–1107.

Requejo R, Tena, M. 2005. Proteome analysis of maize roots reveals that oxidativestress is a main contributing factor to plant arsenic toxicity. Phytochem 66: 1519–1528.

Roy P et al. 2014. Modeling of the adsorptive removal of arsenic: a statistical approach. J Environ Chem Eng 2: 585–597.

Roychowdhyry T et al. 2005. Effects of arsenic-contaminated irrigation water on agricultural land soil and plants in West Bengal, India. Chemosphere 58: 799–810.

Tripathi RD et al. 2007. Arsenic hazards: strategies for tolerance and remediation by plants. Trends Biotech 25: 158–165.

Ullrich-Eberius sanz CI et al. 1989. Evaluation of arsenate and vandate-associated changes of electrical membrane potential and phosphate transport in Lemna gibba G1. J Expt Bot 40: 119–128.

Winkel L et al. 2008. Predicting ground water arsenic contamination, in South east Asia from surface parameters. Nature Geosci 1: 536–542.

Wu ZH, Xie CG. 1990. Method for analysis of total As, inorganic As and organic As in soil and plant. Nongcun Shengtai Huanjing 2: 57–60.

Yude C et al. 1993. The potential and utilization prospects of kinds of wood fodder resources in Yunnan. Forestry Res 6: 346–350.

RESUMO

Toxicidade de As(III) e As(V) sobre características morfológicas e pigmentos de grão-de-bico (*Cicer arietinum*) durante a germinação e o crescimento inicial de plântula. A contaminação por Arsênio (As) é uma consequência ambiental importante em partes da Índia e de outros países. No presente estudo, investigou-se a fitotoxicidade de As(III) e As (V) em *Cicer arietinum*. Cinco concentrações de As(III) e As(V) (0, 2, 4, 6, 8 e 10 mg/L) foram testadas. Os resultados indicam que a porcentagem de germinação foi significativamente (p<0,05) reduzida com o aumento da concentração de As(III) e As (V). No entanto, As(III) evidenciou efeitos mais pronunciados que As(V). A variação do comprimento de raiz e comprimento da parte aérea foi igualmente afetada por arsenito, e como arseniato mostrou maior impacto negativo sobre o comprimento da parte aérea do que o comprimento das raízes até a concentração de 6 mg/L. No entanto, a concentração de arseniato superior a 6 mg/L mostrou tendência oposta de variação na raiz e comprimento de parte aérea. O teor de pigmento (clorofila 'a', 'b' e clorofilas totais) e nível de carotenoides também diminuiu gradualmente com o aumento da concentração de ambas as formas de arsénio.

Palavras-chave: Arsenico, Morfofisiologia; Germinação, Comprimento de raiz e parte aérea, teor de clorofila.