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Arsenic induced toxicity in *Pisum sativum* L. seedlings and its mitigation by exogenous silicon

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Abstract
Arsenic (As) is a rising threat for plant life and in the current work impacts of arsenate (AsV) on growth, lipid peroxidation and plasma membrane integrity have been evaluated. It was found that AsV deleteriously affected the growth, increased the lipid peroxidation and damage of membrane in *Pisum sativum* L. seedlings. Although, application of silicon (Si) ameliorates AsV toxicity by restoring growth as well as by decreasing membrane lipid peroxidation and damage.

Keywords: arsenic, silicon, growth, lipid peroxidation

Introduction
Arsenic (As) is a metalloid comprising the intermediate characteristics of nonmetals and metals (Zhao et al., 2010) [49]. Numerous anthropogenic activities like irrigation with contaminated water, exploration of groundwater having high quantity of As, improper mining, and mishandling of agrochemicals having As contribute to a rise in As concentration in agricultural soils (Van Geen et al., 2006; Lee et al., 2008) [22]. The two forms of As mainly found in soil are arsenate (AsV) and arsenite (AsIII) (Singh et al., 2018) [38]. From contaminated soil, As accumulates into the food and fodder crops which ultimately affecting the life of animals as well as human beings (Bakhat et al., 2017). Arsenic seriously affecting plants' life by obstructing plant growth, reducing transpiration intensity and by harming plant fertility and productivity (Stoeva and Bineva, 2003) [41]. Being a redox active metalloid, As enhances the oxidative stress by generating several reactive oxygen species (ROS) (Tripathi et al., 2012). Drastic impacts of AsV include inhibition in seed germination and growth (Requejo and Tena, 2012) [34]. AsV exposure is also responsible for reduced photosynthesis and modification in carbohydrate and protein metabolisms (Mishra and Dubey, 2006; Srivastava et al., 2013) [25, 40]. To survive under oxidative stress, plants consist antioxidant defence system (Liu et al., 2007) [23]. Various approaches have been used to decrease the uptake and toxicity of heavy metals in plants, which are humid substances (Noman et al., 2015) [28], osmoprotectants (Ali et al., 2015), soil amendments (Rehman et al., 2015) [33], and silicon (Rizwan et al., 2012) [35]. Silicon (Si) stands second in the list of most prevalent element of the earth's crust after oxygen (Epstein and Bloom, 2005) [9]. Plants uptake monosilicic acid from soil via the help of specific transporter (Epstein, 2009) [8]. After uptake when Si reaches to shoot, it concentrates and polymerizes into silica gel ($SiO_2 \cdot nH_2O$) due to the process of transpiration (Yoshida et al., 1962). Silicon positively affected the plant growth and development (Frew et al., 2018). It was reported by various studies that Si improve the tolerance ability of many plants during heavy metal toxicity (Rizwan et al., 2012; Bharwana et al., 2013; Kim et al., 2014) [35]. Diverse responses were shown by leguminous plants exposed to heavy metals, among all few of the leguminous species such as pea (Päivöke, 2003) [29], lentil (Ahmed et al., 2006) [1], lupines (Vázquez et al., 2008) [44], Vigna (Mandal et al., 2008) and chickpea (Gunes et al., 2009) [12] show drastic impacts on biomass, nutrient allocation and oxidative status under As stress. The information related to As stress in pea plant is inadequate. However, the current study was designed to examine the toxicity caused by As in *P. sativum* at morphological and histochemical aspects and here the detoxification agent applied is Si.

Materials and Methods
Plant material and growth conditions
Seeds of Pea (*Pisum sativum* L.) var. Aparna were procured from a registered supplier near Alopibagh, Prayagraj, Uttar Pradesh, India. Surface sterilization of seeds were done by using 2% of sodium hypochlorite solution only for 10 minutes. Later, washing of seeds were done by using distilled water and then the seeds were soaked overnight under dark conditions. Afterwards, seeds were covered in muslin cloth wetted by using half strength Hoagland's nutrient solution (Hoagland and Arnon, 1950) [17] and kept it in dark for 3 days in order to germinate. After germination, seeds were sown in properly sterilized sand and kept it in plant growth chamber having the photon flux density (PFD) of 250 μmol photons m^{-2} s^{-1}, relative humidity of 50-60% and a light/dark cycle of 12/12 h at 25±2˚C for 15 days. After 15 days of the growth, pea seedlings containing secondary leaves were uprooted and acclimatized in Hogland's solution.

Treatment with AsV and Si
Healthy and uniform sized seedlings were treated with AsV and Si. The combinations were prepared as follows control (only half strength of Hoagland's nutrient solution), AsV (50μM), Si (10μM), AsV+Si (50μM+10μM). Treatment of seedlings were done for seven days and in each day solutions were aerated. Afterwards, control and treated seedlings were harvested and a variety of parameters were performed.

Growth parameters
To assess the changes in growth, plant height, root-shoot dry weight and root volume were measured. Plant height was

measured by using centimeter scale. For dry weight, first root and shoot were cut and then dried by using oven at 65-75 ºC for 48 h and then measured by using the digital weighing balance. Root volume was monitored by using water displacement method as described by Pang et al. (2011).

In vivo determination of lipid peroxidation and plasma membrane damage

In vivo examination of lipid peroxidation and plasma membrane damage was done by using Schiff's reagent and Evans blue as mentioned by Pompella et al. (1987) [32] and Yamamoto et al. (2001) [46]. respectively. First of all, roots were stained with respective dyes and photographed by using Olympus compound dark-field microscope attached with a digital camera.

Statistical analysis

Three experiments were performed independently to get the mean values as well as each of the experiment consists two replicates (n=6). Analysis of all obtained values were done with the help of one-way analysis of variance (ANOVA) to determine their significance. By using Duncan's multiple range test (DMRT) multiple comparison of mean values of control and treatment were performed at $p < 0.05$.

Results

Determination of growth

To observe the impacts of different treatments on growth of *P. sativum* seedlings various parameters viz; height, dry weight of root and shoot and root volume were measured (Figure 1-4). The results recommended that exposure of AsV at 50 µM concentration decreased the plant height by 31%. It was reported that dry weight of both root and shoot decreased at 50µM of AsV by 34 and 15% respectively. Si individual application enhanced the height by 15% and dry weight of root and shoot by 23 and 22%. However the exogenous supplementation of Si alleviates AsV induced toxicity in *P. sativum* seedlings. The combined AsV+Si treatment restored the plant height by 10% as well as dry weight of both root and shoot by 19 and 6% respectively.

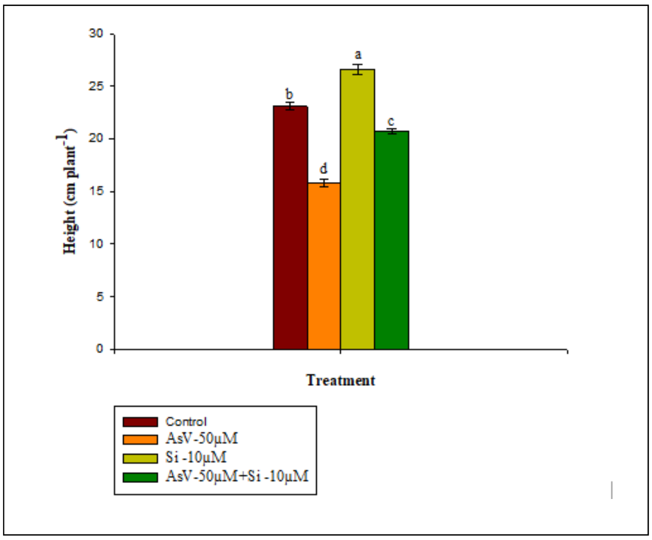

Fig 1: Impact of AsV and Si on height of *Pisum sativum* seedlings. Data are means ± standard error of three duplicates. Bars having different letters display significant differences (P<0.05) between treatments according to the Duncan's multiple range test.

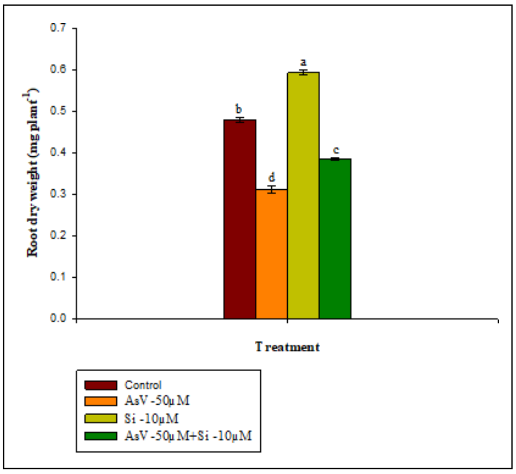

Fig 2: Impact of AsV and Si on dry weight of root of *Pisum sativum* seedlings. Data are means ± standard error of three duplicates. Bars having different letters display significant differences (P<0.05) between treatments according to the Duncan's multiple range test.

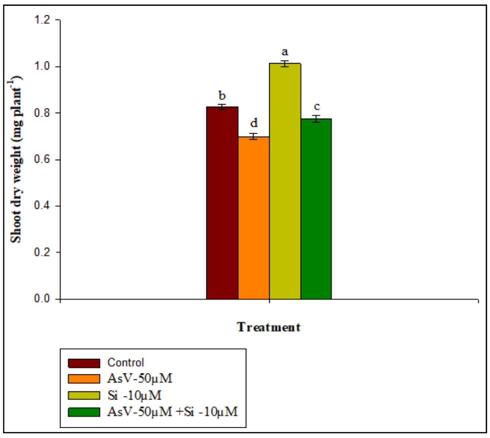

Fig 3: Impact of AsV and Si on dry weight of shoot of *Pisum sativum* seedlings. Data are means ±standard error of three duplicates. Bars having different letters display significant differences (P<0.05) between treatments according to the Duncan's multiple range test.

Application of AsV on pea seedlings declined the level of root volume by 11%. Si individual treatment increased the root volume by 6%. AsV+Si combination restored the root volume by 4% (Figure 4).

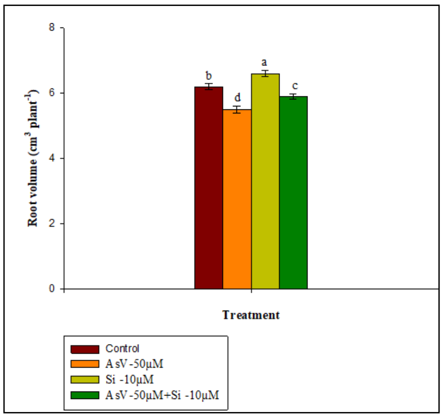

Fig 4: Impact of AsV and Si on root volume of *Pisum sativum* seedlings. Data are means ± standard error of three duplicates. Bars having different letters display significant differences (P<0.05) between treatments according to the Duncan's multiple range test.

In vivo determination of lipid peroxidation and plasma membrane damage

Lipid peroxidation and plasma membrane damage were assayed by using Schiff's reagent and Evans blue dye respectively. Schiff's reagent reacts with aldehyde group i.e. malondialdehyde (MDA), a product of lipid peroxidation and gives pink colour. Evans blue is an azo dye which penetrates the plasma membrane of only dead and damage cell. Pink colour deposition were appear more during AsV treatment. Least appearance were observed in Si solely treatment whilst in AsV+Si combination less accumulation were found. Blue colour deposits were observed more in AsV treatment. Minimum accumulation were observed in Si and in AsV+Si deposition were less (Figure 5-6).

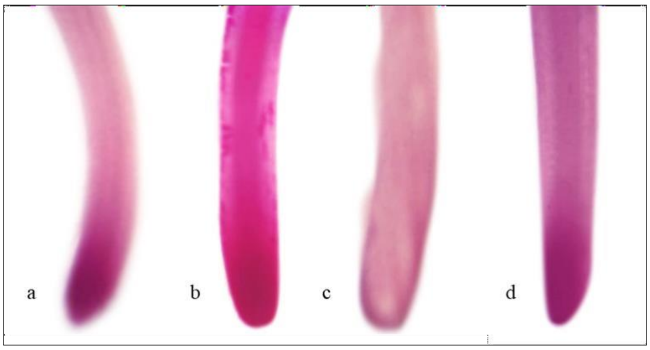

Fig 5: Detection of lipid peroxidation under AsV and Si treatments in roots of *Pisum sativum* seedlings by Schiff's staining. a- Control, b- AsV, c- Si, d- AsV+Si

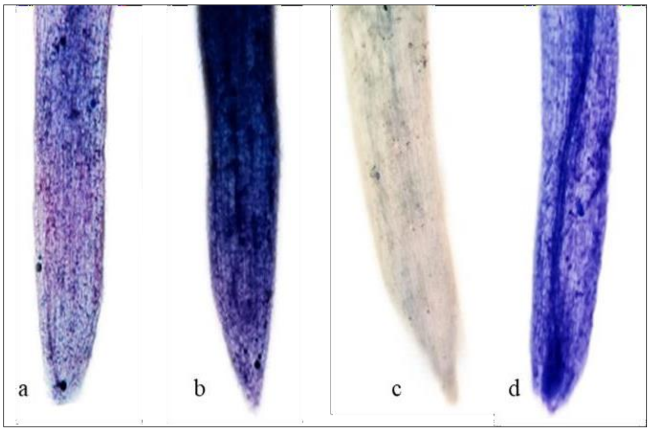

Fig 6: Detection of membrane damage under AsV and Si treatments in roots of *Pisum sativum* seedlings by Evans staining. a- Control, b- AsV, c- Si, d- AsV+Si

Discussion

Heavy metals affect the entire physiology and biochemistry of plant (Hossain and Komatsu, 2013) [18]. Arsenic is a toxic heavy metal for each forms of life including plants (Sil et al., 2019). Exposure of As causes reduction in root-shoot growth, chlorosis in leaves, necrosis in aerial organs of plant and also elevated the oxidative stress (Shri et al., 2009; Choudhury et al., 2011; Moreno-Jiménez et al., 2012; Hu et al., 2013). Exogenous supplementation of silicon could be an approach for providing enhanced tolerance to plants under unfavourable conditions. In this study, AsV at 50μM concentration causes reduction in height and dry weight of both root and shoot of *P. sativum* seedlings. The reduction in growth parameters might be correlated with increased accumulation of As. This kind of response due to AsV toxicity is well documented (Pandey and Gupta, 2015; Dixit et al., 2016). Exogenous supplementation of Si alleviates the toxicity of AsV resulting in improved growth because Si addition restricts the entry of As via roots and it also inhibits translocation of As from root to shoot (Guo et al., 2005, 2007). Similar to our findings, Gong et al. (2003) [11] reported the positive effects of Si on height of wheat plant during drought conditions. In a previous study, it was reported that supplementation of Si restored the dry weight under drought stress (Hattori et al., 2005) [16]. AsV exposure in pea seedlings resulted in reduced root volume. Heavy metals treatment usually slow down root development and suppression in root elongation is the first indication of any unfavorable conditions (Munzuroglu and Geckil, 2002). Likewise, Zhang et al. (2020) [48] observed that As application declined the root volume. However Si alone or along with AsV improved the root volume than AsV alone. Similarly, Kafi and Rahimi (2011) [20] observed that addition of Si in the medium induced the root volume and Hattori and co-workers in 2003 also noticed that Si application promoted the root elongation in sorghum. Induction in root elongation might be due to enhancement in cell wall extensibility in growing area of the roots caused by Si supplementation (Kafi and Rahimi, 2011) [20]. Staining with Schiff's reagent is for examination of lipid peroxidation whereas Evans blue staining detects plasma membrane damage. Roots of pea seedlings exposed to AsV showed high intensity of pink and blue coloured accumulation means more lipid peroxidation as well as membrane damage were noticed in AsV treatment. Although application of Si alone or in combination with AsV showed less intense pink and blue deposition i.e. less lipid peroxidation and membrane damage than AsV individual treatment. As stressed root tips of *Cajanus cajan* showed more intense pink staining than control (Yadu et al., 2019) [45]. Andrade et al. (2016) [3] observed that root tips of *Eichhornia crassipes* treated with As had more blue colour accumulation. Root tips of rice had less blue and pink colour staining exposed to Si (Song et al., 2011) [39].

Conclusions

AsV, a toxic form of arsenic is a critical problem for all the living beings, particularly plants. Our study also proved that how toxic AsV is for plants. AsV causes severe impacts on plant height, dry weight of root and shoot, root volume, enhanced lipid peroxidation as well as negatively affected plasma membrane integrity. The toxic behavior of AsV is here ameliorated by Si via improving the parameters related to growth and histochemistry of *P. sativum* seedlings. By restricting the entrance and translocation of heavy metals, Si help plants to recover and survive.

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References

1. Ahmed FS, Killham K, Alexander I. Influences of arbuscular mycorrhizal fungus Glomus mosseae on growth and nutrition of lentil irrigated with arsenic contaminated water. *Plant and soil*,2006:*283*(1):33-41.
2. Ali S, Chaudhary A, Rizwan M, Anwar HT, Adrees M, Farid M, *et al*. Alleviation of chromium toxicity by glycine betaine is related to elevated antioxidant

enzymes and suppressed chromium uptake and oxidative stress in wheat (Triticum aestivum L.). *Environmental Science and Pollution Research*,2015:*22*(14):10669-10678.

3. Andrade HM, Oliveira JA, Farnese FS, Ribeiro C, Silva AA, Campos FV, et al. Arsenic toxicity: cell signalling and the attenuating effect of nitric oxide in Eichhornia crassipes. *Biologia plantarum*,2016;*60*(1):173-180.
4. Bakhat HF, Zia Z, Fahad S, Abbas S, Hammad HM, Shahzad AN, et al. Arsenic uptake, accumulation and toxicity in rice plants: possible remedies for its detoxification: a review. *Environmental Science and Pollution Research*,2017:*24*(10):9142-9158.
5. Bharwana SA, Ali S, Farooq MA, Iqbal N, Abbas F, Ahmad MSA. Alleviation of lead toxicity by silicon is related to elevated photosynthesis, antioxidant enzymes suppressed lead uptake and oxidative stress in cotton. *J. Bioremed. Biodeg*,2013:*4*(4):187.
6. Choudhury B, Chowdhury S, Biswas AK. Regulation of growth and metabolism in rice (Oryza sativa L.) by arsenic and its possible reversal by phosphate. *Journal of Plant Interactions*,2011:*6*(1):15-24.
7. Dixit G, Singh AP, Kumar A, Mishra S, Dwivedi S, Kumar S, et al. Reduced arsenic accumulation in rice (Oryza sativa L.) shoot involves sulfur mediated improved thiol metabolism, antioxidant system and altered arsenic transporters. *Plant Physiology and Biochemistry*,2016:*99*:86-96.
8. Epstein E. Silicon: its manifold roles in plants. *Annals of applied Biology*,2009:*155*(2):155-160.
9. Epstein E, Bloom AJ. Mineral nutrition of plants: principles and perspectives, 2nd edn. Sinauer Assoc. *Inc., Sunderland, UK, 2005*.
10. Frew A, Weston LA, Reynolds OL, Gurr GM. The role of silicon in plant biology: a paradigm shift in research approach. *Annals of botany*,2018:*121*(7):1265-1273.
11. Gong HJ, Chen KM, Chen GC, Wang SM, Zhang CL. Effects of silicon on growth of wheat under drought. *Journal of plant nutrition*,2003:*26*(5):1055-1063.
12. Gunes A, Pilbeam DJ, Inal A. Effect of arsenic–phosphorus interaction on arsenic-induced oxidative stress in chickpea plants. *Plant and Soil*,2009:*314*(1):211-220.
13. Guo W, Hou YL, Wang SG, Zhu YG. (2005).Effect of silicate on the growth and arsenate uptake by rice (Oryza sativa L.) seedlings in solution culture. *Plant and Soil*,2005:*272*(1):173-181.
14. Guo W, Zhu YG, Liu WJ, Liang YC, Geng CN, et al. Is the effect of silicon on rice uptake of arsenate (AsV) related to internal silicon concentrations, iron plaque and phosphate nutrition?.*Environmental Pollution*,2007:*148*(1):251-257.
15. Hattori T, Inanaga S, Araki H, An P, Morita S, Luxová M, et al. Application of silicon enhanced drought tolerance in Sorghum bicolor. *Physiologia Plantarum*,2005:*123*(4):459-466.
16. Hattori T, Inanaga S, Tanimoto E, Lux A, Luxová M, Sugimoto Y. Silicon-induced changes in visco elastic properties of sorghum root cell walls. *Plant and Cell Physiology*,2003:*44*(7):743-749.
17. Hoagland DR, Arnon DI. The water-culture method for growing plants without soil. *Circular. California agricultural experiment station*, 1950, *347*(2).
18. Hossain Z, Komatsu S. Contribution of proteomic studies towards understanding plant heavy metal stress response. *Frontiers in Plant Science*, *3*, 310.
19. Hu H, Zhang J, Wang H, Li R, Pan F, Wu J, *et al*. Effect of silicate supplementation on the alleviation of arsenite toxicity in 93-11 (Oryza sativa L. indica). *Environmental Science and Pollution Research*,2013:*20*(12):8579-8589.
20. Kafi M, Rahimi Z. Effect of salinity and silicon on root characteristics, growth, water status, proline content and ion accumulation of purslane (Portulaca oleracea L.). *Soil Science and Plant Nutrition*,2011:*57*(2):341-347.
21. Kim YH, Khan AL, Waqas M, Shim JK, Kim D, Lee KY, et al. Silicon application to rice root zone influenced the phytohormonal and antioxidant responses under salinity stress. *Journal of Plant Growth Regulation*,2014:*33*(2):137-149.
22. Lee JS, Lee SW, Chon HT, Kim KW. Evaluation of human exposure to arsenic due to rice ingestion in the vicinity of abandoned Myung bong Au–Ag mine site, Korea. *Journal of Geochemical Exploration*,2008:*96*(2-3):231-235.
23. Liu X, Zhang S, Shan XQ, Christie P. Combined toxicity of cadmium and arsenate to wheat seedlings and plant uptake and antioxidative enzyme responses to cadmium and arsenate co-contamination. *Ecotoxicology and environmental safety*,2007:*68*(2):305-313.
24. Mandal SM, Pati BR, Das AK, Ghosh AK. Characterization of a symbiotically effective Rhizobium resistant to arsenic: isolated from the root nodules of Vigna mungo (L.) Hepper grown in an arsenic-contaminated field. *The Journal of general and applied microbiology*,2008:*54*(2):93-99.
25. Mishra S, Dubey RS. Inhibition of ribonuclease and protease activities in arsenic exposed rice seedlings: role of proline as enzyme protectant. *Journal of plant physiology*,2006:*163*(9):927-936.
26. Moreno-Jiménez E, Esteban E, Peñalosa JM. The fate of arsenic in soil-plant systems. *Reviews of environmental contamination and toxicology*, 2012, 1-37.
27. Munzuroglu O, Geckil HİKMET. Effects of metals on seed germination, root elongation, and coleoptile and hypocotyl growth in Triticum aestivum and Cucumis sativus. *Archives of Environmental Contamination and Toxicology*,2002:*43*(2):203-213.
28. Noman A, Ali S, Naheed F, Ali Q, Farid M, Rizwan M, *et al*. Foliar application of ascorbate enhances the physiological and biochemical attributes of maize (Zea mays L.) cultivars under drought stress. *Archives of Agronomy and Soil Science*,2015:*61*(12):1659-1672.
29. Päivöke AEA. Soil pollution alters ATP and chlorophyll contents in Pisum sativum seedlings. *Biologia plantarum*,2003:*46*(1):145-148.
30. Pandey C, Gupta M. Selenium and auxin mitigates arsenic stress in rice (Oryza sativa L.) by combining the role of stress indicators, modulators and genotoxicity assay. *Journal of hazardous materials*,2015:*287*:384-391.
31. Pang W, Crow WT, Luc JE, Mc Sorley R, Giblin-Davis RM, Kenworthy KE, et al. Comparison of water displacement and WinRHIZO software for plant root

parameter assessment. *Plant Disease*,2011:*95*(10):1308-1310.
32. Pompella ALFONSO, Maellaro E, Casini AF, Comporti M. Histochemical detection of lipid peroxidation in the liver of bromobenzene-poisoned mice. *The American journal of pathology*,1987:*129*(2):295.
33. Rehman MZU, Rizwan M, Ghafoor A, Naeem A, Ali S, Sabir M, et al. Effect of inorganic amendments for in situ stabilization of cadmium in contaminated soils and its phyto-availability to wheat and rice under rotation. *Environmental Science and Pollution Research*,2015:*22*(21):16897-16906.
34. Requejo R, Tena M. Influence of glutathione chemical effectors in the response of maize to arsenic exposure. *Journal of plant physiology*,2012:*169*(7):649-656.
35. Rizwan M, Meunier JD, Miche H, Keller C. Effect of silicon on reducing cadmium toxicity in durum wheat (Triticum turgidum L. cv. Claudio W.) grown in a soil with aged contamination. *Journal of hazardous materials*,2012:*209*:326-334.
36. Shri M, Kumar S, Chakrabarty D, Trivedi PK, Mallick S, Misra P, et al. Effect of arsenic on growth, oxidative stress, and antioxidant system in rice seedlings. *Ecotoxicology and environmental safety*,2009:*72*(4):1102-1110.
37. Sil P, Das P, Biswas S, Mazumdar A, Biswas AK. (2019). Modulation of photosynthetic parameters, sugar metabolism, polyamine and ion contents by silicon amendments in wheat (Triticum aestivum L.) seedlings exposed to arsenic. *Environmental Science and Pollution Research*,2019:*26*(13):13630-13648.
38. Singh R, Parihar P, Prasad SM. Sulfur and calcium simultaneously regulate photosynthetic performance and nitrogen metabolism status in As-challenged Brassica juncea L. seedlings. *Frontiers in plant science*,2018:*9*:772.
39. Song A, Li P, Li Z, Fan F, Nikolic M, Liang Y. The alleviation of zinc toxicity by silicon is related to zinc transport and antioxidative reactions in rice. *Plant and Soil*,2011:*344*(1):319-333.
40. Srivastava S, Srivastava AK, Singh B, Suprasanna P, D’souzx SF. The effect of arsenic on pigment composition and photosynthesis in Hydrilla verticillata. *Biologia plantarum*,2013:*57*(2):385-389.
41. Stoeva N, Bineva T. Oxidative changes and photosynthesis in oat plants grown in As-contaminated soil. *Bulg J Plant Physiol*,2003:0*29*(1-2):87-95.
42. Tripathi P, Mishra A, Dwivedi S, Chakrabarty D, Trivedi PK, Singh RP. Differential response of oxidative stress and thiol metabolism in contrasting rice genotypes for arsenic tolerance. *Ecotoxicology and Environmental Safety*,2012:*79*:189-198.
43. Van Geen A, Zheng Y, Cheng Z, He Y, Dhar RK, Garnier JM, et al. Impact of irrigating rice paddies with groundwater containing arsenic in Bangladesh. *Science of the Total Environment*,2006:*367*(2-3):769-777.
44. Vázquez S, Esteban E, Carpena RO. Evolution of arsenate toxicity in nodulated white lupine in a long-term culture. *Journal of agricultural and food chemistry*,2008:*56*(18):8580-8587.
45. Yadu B, Chandrakar V, Tamboli R, Keshavkant S. Dimethylthiourea antagonizes oxidative responses by up-regulating expressions of pyrroline-5-carboxylate synthetase and antioxidant genes under arsenic stress. *International Journal of Environmental Science and Technology*,2019:*16*(12):8401-8410.
46. Yamamoto Y, Kobayashi Y, Matsumoto H. Lipid peroxidation is an early symptom triggered by aluminum, but not the primary cause of elongation inhibition in pea roots. *Plant Physiology*,2001:*125*(1):199-208.
47. Yoshida S, Ohnishi Y, Kitagishi K. Chemical forms, mobility and deposition of silicon in rice plant. *Soil Science and Plant Nutrition*,1962:*8*(3):15-21.
48. Zhang Q, Gong M, Liu K, Chen Y, Yuan J, Chang Q. Rhizoglomus intraradices Improves Plant Growth, Root Morphology and Phytohormone Balance of Robinia pseudoacacia in Arsenic-Contaminated Soils. *Frontiers in Microbiology*,2020:*11*:1428.
49. Zhao FJ, McGrath SP, Meharg AA. Arsenic as a food chain contaminant: mechanisms of plant uptake and metabolism and mitigation strategies. *Annual review of plant biology*,2010:*61*:535-559.
50. Ibrahim NA. An up-to-date review of digoxin toxicity and its management. Int J Res Pharm Pharm Sci. 2019;4(3):59-64.