

EFFECT OF ARSENIC (As) ON GROWTH AND PHYSIOLOGY OF MAIZE (*ZEAMAYS*) IN VARYING SOILS

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ABSTRACT

Maize is one of the main fodders and food source and even staple food in different countries. The presence of arsenic (As) in the soil has been reported to affect the growth and productivity of agricultural crops. The plant uptake of As from soil might depend on the composition and type of soil texture. In this study, we assessed the effect of four levels (0, 25, 50 and 100 mg As kg⁻¹ of soil) of As on As uptake as well as the growth and physiology of maize (*Zea mays*) grown in three soils varying in texture in a pot experiment conducted at Government college university Faisalabad in February-March, 2018. Results revealed that the growth parameters significantly decreased in response to increasing concentrations of As in all three types of soil. The most prominent effects of As treatments on maize were shown under sandy loam soil followed by loamy soil and then clayey soils. At highest level of As application, the maximum As concentration in plant shoot and root were found in sandy loam soil (45.1 and 93.4 mg kg⁻¹) followed by that in loamy soil (39.9 and 86.2 mg kg⁻¹) and clayey soils (34.9 and 81.1 mg kg⁻¹). Similarly, the maximum Diethylenetriamine Pentaacetic Acid (DTPA) extractable As (2.4 mg kg⁻¹) was detected in sandy loam soil, whereas, the lowest (2.1 mg kg⁻¹) was observed in clayey soil. Antioxidant enzyme activities and protein contents were maximum in the soils amended with 25 mg as kg⁻¹, and were significantly decreased in the soils containing higher levels of As. However, these values were relatively higher in the clayey soils followed by the loamy soil and least in the sandy loam soil. The reactive oxygen species (ROS) including hydrogen peroxide (H₂O₂), superoxide (O₂⁻) and melondialdehyde (MDA) were found to increase with increasing level of As in the soils. The effects of As on ROS were also maximum in sandy loam soil followed by the loamy soil and minimum in clayey soil. Conclusively we can say that the presence of As significantly affected the growth and physiological parameters of the maize crop. However, the effects of As were more severe in sandy loam soil as compared to the other ones.

Keywords: Arsenic, Maize, Soil texture, DTPA extractable As, Growth, Antioxidants.

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INTRODUCTION

Arsenic (As) is one of the oldest poisons in the world, metalloid in nature and was used in high profile murders in the Middle Ages and was famous as the "King of Poisons" as well as the "poison of kings" (Hughes *et al.*, 2011). It was discovered by Alberts in 1250 AD. In the order of abundance, it is 12th most abundant element in human body, 20th most abundant element in earth's crust and 14th most abundant element in sea water (Mandal and Suzuki, 2002). It occurs in inorganic forms [i.e As(-III), As (0), As (III), or As(V)] along with organic forms [for instance, monomethyl arsenic acid, arsenobetaine and arsenosugars]. Inorganic forms of Arsenic (As) are more mobile and toxic among which, As (III) is considered 60 times more lethal than As(V). As(V) is dominant in oxidized environments (Shakoor *et al.*, 2015).

Arsenic is considered one of the emerging very toxic contaminants worldwide. The addition of As in the

environment is mainly because of different natural phenomena including geological/geothermal/volcanic events and weathering of rocks and minerals as well as because of various anthropogenic activities including agricultural activities (i.e. fertilizer, pesticides, herbicides, and seed treatments), industrial processes (e.g. tanneries, electroplating etc) and mining and smelting activities. Among the anthropogenic sources, the mining and smelting of As-containing ores, combustion of fossil fuels especially coal, landfilling by pulp, paper, tannery, and textile industries, discharge of chemical warfare agents, petroleum refining and pharmaceutical manufacturing are very common sources of As contamination in soils (States, 2015).

The presence of As in the soil environment is harmful because it results in contamination of food crops, fruits and vegetables grown on arsenic (As) contaminated earth. The As can, therefore, enter into food chain as well as commercially used human food products even in non-contaminated areas (Zhao *et al.*, 2010). As per US

Environmental Protection Agency (EPA), arsenic (As) is carcinogenic in nature and, according to the IARC (International Agency for Research on Cancer), Arsenic and its composites are ranked as a group one human carcinogen (Shahid *et al.*, 2013). According to these reports, approximately 200 million people have been affected by As worldwide including Pakistan. The main health dangers include cancer of different body parts such as skin, lungs and bladder, and many other neurological, haematological, renal, and respiratory illnesses (Yadav *et al.*, 2017). Arsenic also has a hazardous impact on plants resulting in low biomass production, chlorosis, necrosis, leaf senescence, defoliation, stunted growth, chlorophyll degradation and low yield (Ansari *et al.*, 2013). The reduced plant growth is due to the production of reactive oxygen species (ROS) such as superoxide and hydrogen peroxide. These ROS result in oxidative stress toward biomolecules such as lipids and protein and reduction in antioxidant enzymes such as ATP, SOD etc. (Keshavkant *et al.*, 2012). These ROS not only have a negative impact on the metabolism of starch and sugars in plant cells but also affect the DNA by replacing phosphorous group and the protein functioning through binding of As (III) by sulfhydryl groups, ultimately resulting into the death of plant cell (Talukdar *et al.*, 2013; Shahid *et al.*, 2014; Singh *et al.*, 2016; Singh *et al.*, 2017; Shahid *et al.*, 2017). However, during the recent years, use of different amendments including the biochar and dicalcium phosphate has been reported to reduce the toxicity of different heavy metals including arsenic, copper, nickel and lead (Brennan *et al.*, 2014; Turan, 2019; Turan, 2020). Arsenic has also been reported as a contaminant in the most of the groundwater water resources existing in Pakistan (Shakoor *et al.*, 2015; Shahid *et al.*, 2017; Shahid *et al.*, 2018). Irrigating the agricultural crops with such contaminated groundwater contaminates the soils and might affect the growth, physiology and productivity of agricultural crops (Abbas *et al.*, 2018). Hence, there is need to study the effects of arsenic on agricultural crops sown in varying agricultural soils in Pakistan.

Maize is one of the major agricultural crops which is intensively cultivated in various countries including the United States of America, China and Pakistan (Ashraf *et al.*, 2016). In Pakistan, the maize is the 3rd largest crop and is grown on 1130 thousand hectares with annual yield of 4695 thousand tonnes (GOP, 2014-15). The arsenic might be affecting the growth and physiology of this crop in varying agricultural fields of Pakistan. Hence, the current study was designed to examine the effect of Arsenic (As) on physiological, biochemical, and morphological characteristics of maize grown on soils of varying texture i.e. clayey, loamy and sandy loam soils.

MATERIALS AND METHODS

Experimental details and treatments

Soil collection and characterization: Clayey, loamy and sandy loam soils were collected from Gujranwala (32.2439° N, 74.2975° E), Sahiwal (30.6522° N, 72.9691° E) and River Ravi near Sahiwal (30.9539° N, 73.2343° E), respectively. The soil samples were dried in air and sieved through 2.0 mm sieve in the research laboratory of the Department of Environmental Sciences & Engineering, Government College University, Faisalabad. Different physicochemical characteristics of soil were determined by adopting standard methods. The texture of all three soils was determined by the Technique of Gee and Bauder (1986) using the hydrometer. The soil pH and EC were measured by adopting procedure of Bigham (1996). The cation exchange capacity (CEC) and organic matter were measured by following the protocol described by Rhoades (1983) and Walkley-Black method (Jackson, 1962), respectively. The bioavailable As was determined through the method described by Mehlich (1984) and total As by the method of Hudson-Edwards *et al.*, (2004) and available and total phosphorus by following the protocol of Olsen (1954) and Sommers and Nelson (1972), respectively. The CaCO₃ contents in soil were determined by the method of Allison and Mooddie (1965). The Iron, Aluminium and Manganese were determined using DTPA method as described by Lindsay and Norvell, (1978). The measured physical and chemical characteristics of three type of soils are given in Table 1.

Soil spiking with As: Three-levels of Arsenic 25, 50 and 100 mg kg⁻¹ of soil were set in all three soils by using Na₂H₄AsO₅ salt. For this purpose, the dried and sieved soils were spiked with a solution of Na₂H₄AsO₅ to obtain three As treatments for each soil (Table 2). A small amount of soil was taken for each treatment and As solution was mixed with it thoroughly in a plastic container. The spiked soil was then thoroughly mixed with other soils in specific proportions to obtain the required levels.

Pot experiment: After spiking with As, the soils were kept in dark at 25 °C temperature for 7 days. Before incubation, soil was moistened to 65 % water holding capacity (WHC). After that 3 kg soil was put in each plastic pot. Total 36 pots were used for this study which comprised of four levels of arsenic (0, 25, 50 and 100 mg As kg⁻¹ of soil) and three types of soil (Sandy loam soil, loamy soil and clayey soil) with three replicate for each. The pots were arranged according to a completely randomized design in an open wire house of Government College University Faisalabad. The maize seeds of Monsanto 6103 hybrid variety were obtained from the market and 4 (four) maize seeds were sown in each pot. The balanced plant fertilizer [Grow Fertilizer (18-18-18)]

(Shahbaz *et al.*, 2018) was applied to plants after two weeks of germination. After 60 days of germination the plants were harvested. Before harvest, plants height was measured with the help of measuring tape. Other growth parameters including shoot fresh weight, root fresh weight was measured after the harvesting of the plants.

Measurements of physical and biological parameters of maize plant: The Hiscox and Israelstam (1979)

protocol was used to determine the chlorophyll a (Chl-a) and chlorophyll b (Chl-b) contents in the leaves of maize plant after 60 days of germination. In order to measure Chl-a and Chl-b, a homogenized mixture was prepared by using one-gram fresh leaf and methanol chloroform water in the ratio 12:5:3 and the contents of Chl-a (at 664.5 nm) and Chl-b (647.4 nm) were estimated using a UV/Visible Spectrophotometer (Hitachi, Berkshire, UK).

Table 1: Physicochemical properties of soils used in study.

Soil properties	Soil texture		
	Sandy Loam	Loamy	Clayey
Physical characteristics			
Sand (%)	67.1	36.9	13.9
Silt (%)	20.2	35.7	15.7
clay (%)	12.7	27.4	70.4
Saturation percentage	22.1	29.9	36.1
Chemical characteristics			
Soil organic matter (%)	0.19	0.58	0.69
Soil pH	7.23	7.85	7.90
Cation Exchange capacity [Cmol (+) kg ⁻¹]	5.90	21.9	39.9
Electrical conductivity (dS m ⁻¹)	0.22	0.49	0.59
Iron (g kg ⁻¹)	7.23	11.4	15.9
Aluminium (g kg ⁻¹)	0.39	0.69	0.97
Manganese (g kg ⁻¹)	0.13	0.19	0.25
Calcium carbonate (%)	8.9	13.2	20.8
Available Phosphorus (mg kg ⁻¹)	2.99	4.85	7.94
Total Arsenic (mg kg ⁻¹)	1.15	1.95	2.95
Water soluble Arsenic (mg kg ⁻¹)	0.31	0.15	0.07

Table 2: Three level of As 25, 50 and 100 mg kg⁻¹ of soil set in all three soils by using Na₂H₄AsO₅ salt.

Treatments	Clay	Loam	Sandy Loam
Control	NAC	NAC	NAC
25 mg Kg ⁻¹ of Soil	LAC	LAC	LAC
50 mg Kg ⁻¹ of Soil	MAC	MAC	MAC
100 mg Kg ⁻¹ of Soil	HAC	HAC	HAC

Treatments realized in the pot experiment. NAC=CONTROL, LAC =25mg Kg⁻¹, MAC =50 mg Kg⁻¹ and HAC =100 mg Kg⁻¹

Soil & plant analyses: A sharp scissor was used to harvest the maize plants near the soil surface after 60 days of germination. The plants were divided to shoots and roots biomass. Soil was taken from each pot and it was air dried. The pH of the soil samples was measured with a standardized pH meter by using a soil suspension (1:5 soil/de-ionized water) well shaken for 1 hour before measuring the pH. The Atomic Absorption Spectrophotometer (AAS) was used to measure the DTPA extractable As of the soils by following the standard procedure as already described by Hudson-Edward *et al.*, (2004).

The harvested plant shoots and roots biomass were rinsed with tap water to remove any dust particles followed by oven drying at 80°C for 48 hours. After

getting constant weight, dry shoot weights and dry root weights of the samples were determined. The plant biomass was ground to 0.5 mm by using grinding mill (IKAWerke, MF 10 Basic, Staufen, Germany). After grinding, a diacid mixture of HNO₃ and HClO₄ at the ratio 2:1 was used to digest the plant biomass as prescribed by Jones and Case (1990) and this digested plant biomass was used on AAS to measure the As contents.

Determination of reactive oxygen species such as malondialdehyde (MDA), hydrogen peroxide (H₂O₂) and O₂⁻ in maize leaves was carried out by using standard protocols of Jambunathan (2010), Velikova *et al.*, (2000) and Auclair and Voisin (1985) respectively. The MDA contents were determined by using a sample of fresh leaf

measuring 500 mg following the procedure as described in Shahbaz *et al.* (2018) after 60 days of germination.

Similarly, the method recommended by Velikova *et al.* (2000) was used to determine the contents of H₂O₂ on spectrophotometer by adopting the protocols as described in Shahbaz *et al.* (2018). Similarly, the Auclair and Voisin (1985) procedure was followed to determine the concentrations of O₂⁻ in maize leaves at 25 °C and 7.0 pH by following the procedure as mentioned in Shahbaz *et al.* (2018).

The antioxidants enzymes activities were determined in 500 mg fresh maize leaves. A supernatant solution was obtained from the maize leaves and the activities of antioxidant enzymes were determined as already described by Shahbaz *et al.* (2018). The procedure reported by Roth and Gilbert (1984) was followed to measure the activity of superoxide dismutase (SOD) in maize leaves on spectrophotometer at 420 nm by using one mL reaction mixture. The reaction mixture was prepared adopting the method as described in Shahbaz *et al.* (2018). Similarly, the activities of CAT and APX were estimated following the procedures of Cakmak and Marschner (1992) and Nakano and Asada (1981), respectively, as described in Shahbaz *et al.* (2018). An extraction coefficient at 40 mM⁻¹ cm⁻¹ was used for the determination of specific activity of APX in the reaction mixture.

A protein-dye binding procedure as recommended by Bradford (1976) was adopted to measure the protein contents as described in Shahbaz *et al.* (2018).

Statistical analysis: Three replicates for each treatment were used in this pot study under complete randomized design (CRD). Two-way ANOVA, with As treatment levels and soil types as two factors, was used to interpret the results of study by using Statistic 8.1 ® (Analytical Software, Tallahassee, USA) and LSD test recommended by Steel *et al.*, 1997 was adopted to measure the significant variance among the treatments with means P < 0.5.

RESULTS

Treatment effects on Growth, Physiological parameters and Biological Compounds of maize plant:

The heights of plants ranged from 53.9 to 74.4 cm in clay, 75.0 to 50.5 cm in loam and 73.6 to 42.7 cm in sandy loam soil (Fig. 1). The plant height reduced by 8%, 17%, 30%; 13%, 20%, 33% and by 19%, 28% and 42% in clayey, loamy and sandy loam soil respectively as compared with control at low, medium and high level of As. The most significant stress of As on plant height was noted in sandy loam soil where the highest concentrations of As was used (Fig.1).

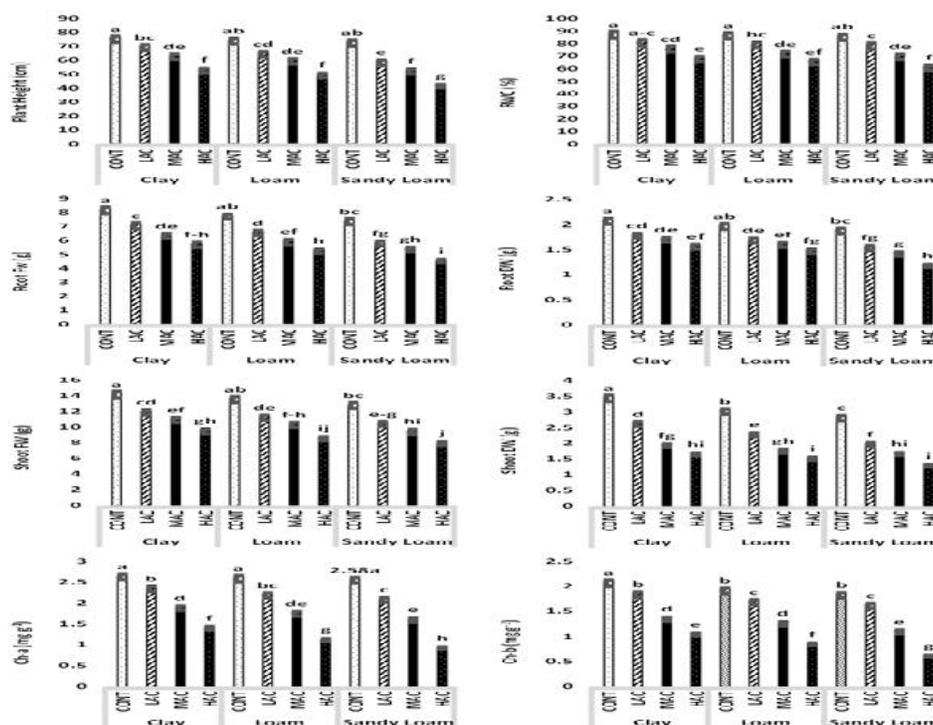


Fig. 1: The plant height (a), reactive water contents (RWC) (b), root fresh weight (c), root dry weight (d), shoot fresh weight (e), shoot dry weight (f), Chl-a contents (g) and Chl-b contents (h) in maize at various level of Arsenic. The means with same letters(s) are Non-Significant.

The shoot fresh and dry weights were in the range from 9.7 to 14.3 and 1.7 to 3.5, 8.7 to 13.7 and 1.6 to 3.6, 8.1 to 12.9 and 1.3 to 2.9 g pot⁻¹, respectively, in clayey soil, loamy soil and sandy loam soil (Fig. 1). The fresh and dry shoot weights reduced by 16%, 23% & 33%, and 24%, 43% & 51% in clayey soil, 17%, 24% & 36%, and 24%, 42% & 49%, respectively, in loamy soil and by 19%, 26% & 37%, and 30%, 40% & 53% in sandy loam soil as compared with control at low, medium and high levels of As, respectively.

The fresh and dry weights of roots were found in the range from 8.3 to 5.8 and 2.1 to 1.6 g pot⁻¹ in clay, 7.8 to 5.4 and 2.1 to 1.5 g pot⁻¹ in loam and 7.5 to 4.6 and 1.9 to 1.2 g pot⁻¹ in sandy loam (Fig. 1). The fresh and dry root weight reduced by 14%, 22% & 30%, and 14% 18% & 24% in clayey soil, 16%, 23% & 32%, and 14%, 19% & 24%, respectively, in loamy soil and by 21%, 27% & 36% and 18%, 24% & 37% respectively in sandy loam soil from control at low, medium and contents of As respectively.

The contents of chl-a, chl-b, were in the range of 2.7 to 1.4 and 2.1 to 1.1 mg g⁻¹ fresh weight, 2.6 to 1.1 and 1.9 to 0.9 mg g⁻¹ fresh weight and 2.4 to 0.9 and 1.9 to 0.6 mg g⁻¹ FW respectively in clayey soil, loamy soil and sandy loam soil in response to low, medium and high As application (Fig. 1). The chl-a, chl-b reduced by 11%, 28% & 46%, and 11%, 34% & 49% respectively in clayey soil, 15%, 32% & 56%, and 12%, 34% & 55%, respectively in loamy soil and by 8%, 37% & 63%, and 12%, 39% & 66%, respectively, in sandy loam soil as from control at low, medium and concentrations of As in maize leaves. Maximum reduction in chl-a and chl-b was noted in sandy loam soil at high level of As.

The low, medium and high As concentrations in three different textured soils significantly reduce the RWC as illustrated in Fig. 1. The RWC was in range from 88.2 to 69%, 87 to 66% and 86 to 62% in clayey soil, loamy soil and sandy loam soil, respectively. The

RWC contents reduced by 7%, 13% and 22% in clay soil, 8%, 17% and 24% in loamy soil and by 8%, 18% and 28% in sandy loam soil as compared to control at low, medium and high levels of As, respectively. The maximum reduction in RWC was detected in sandy loam soil where the higher As was applied in contrast to the plants grown in control without As.

As concentrations in plant tissues and DTPA-As:

Arsenic contents in plant shoots and roots of maize ranged from 0.03 to 34.9 and 0.05 to 81.0; 0.05 to 39.7 and 0.06 to 86.2; 0.06 to 45.0 and 0.08 to 93.3 mg kg⁻¹ of DW of the biomass of maize, in clayey soil, loamy soil and sandy loam soil respectively (Fig. 2). The DTPA-As was found in the ranges of 0.08 to 2.08; 0.03 to 2.21 and 0.03 to 2.38 mg Kg⁻¹ of soil (Fig. 2). The soil textures significantly influenced As uptake and retention in plant biomass. For example, the shoot As content in the plants grown in the soil having 25 mg As kg⁻¹ soil were found to be 7.6, 8.9 and 9.9 mg kg⁻¹ of the plant in clayey, loamy and sandy loam soils, respectively. The shoot As content in the plants grown in the soil containing 50 mg As kg⁻¹ soil were found to be 17.0, 18.9 and 20.5 mg kg⁻¹ of the plant in clayey, loamy and sandy loam soils, respectively. The shoot As content in the plants grown in the soil with 100 mg As kg⁻¹ soil were found to be 34.9, 39.7 and 45.0 mg kg⁻¹ of the plant in clayey, loamy and sandy loam soils, respectively. Similarly, the root As content in the plants grown in the soil containing 25 mg As kg⁻¹ soil were found to be 18.7, 20.5 and 21.9 mg kg⁻¹ of the plant in clayey, loamy and sandy loam soils, respectively. The root As content in the plants grown in the soil containing 50 mg As kg⁻¹ soil were found to be 37.3, 42.1 and 46.2 mg kg⁻¹ of the plant in clayey, loamy and sandy loam soils, respectively. The root As content in the plants grown in the soil containing maximum level of As was found to be 81.1, 86.2 and 93.4 mg kg⁻¹ of the plant in clayey soil, loamy soil and sandy loam soil, respectively.

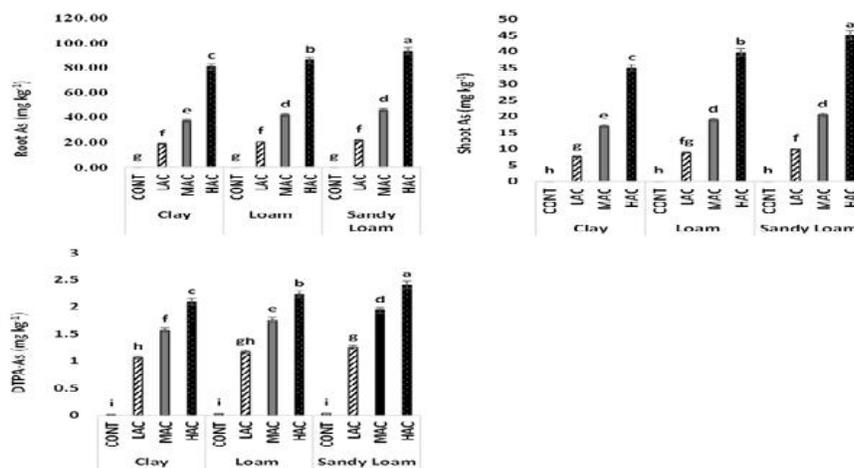


Fig. 2: The effect of various level of As on root As (a) and shoot As (b) in maize and DTPA As (c) in soil. Means having same letter(s) were non-significant $p < 0.05$.

The DTPA-extractable As contents in the soils spiked with low As concentration were found to be 1.06, 1.16 and 1.25 mg kg⁻¹, at medium As concentrations were found to be 1.56, 1.76 and 1.94 mg kg⁻¹ and at highest level were found to be 2.09, 2.23 and 2.41 mg kg⁻¹ of the soil in clayey soil, loamy soil and sandy loam soil, respectively.

Reactive oxygen species (ROS) and antioxidants enzymes: The presence of As in the soil significantly affected the concentrations of reactive oxygen species in maize plants (Fig. 3). The level of ROS increased up to variable extents in different treatments. The levels of MDA, H₂O₂, and O₂⁻ were ranging from 11.7 to 43.8, 13.2 to 52.1 and 6.0 to 32.3 (nmol min⁻¹g⁻¹ FW) in the clay soil, 14.1 to 48.2, 13.5 to 55.9 and 7.1-36.6 (nmol⁻¹

min⁻¹g⁻¹ FW) in the loamy soil and 15.4 to 60.8, 13.2 to 52.1 and 9.5 to 45.2 (nmol min⁻¹ g⁻¹ FW) in the sandy loam soil in response to low, medium and high As application respectively (Fig. 3). The level of ROS was significantly increased in all the As containing treatments. The contents of MDA increased by 58%, 118%, 217%; 67%, 127%, 228% and 72%, 148% and 244%; H₂O₂ by 53%, 114%, 212%; 52%, 119%, 213% and 57%, 139% and 226 and O₂⁻ by 41%, 101%, 201%; 46%, 111%, 241% and 60%, 131% and 288 % in clay, loam and sandy loam soil by low, medium and high As level respectively. Generally, the lowest levels of ROS were observed in clayey soil at low As level and the highest levels were observed in the sandy loam soil at high level of As.

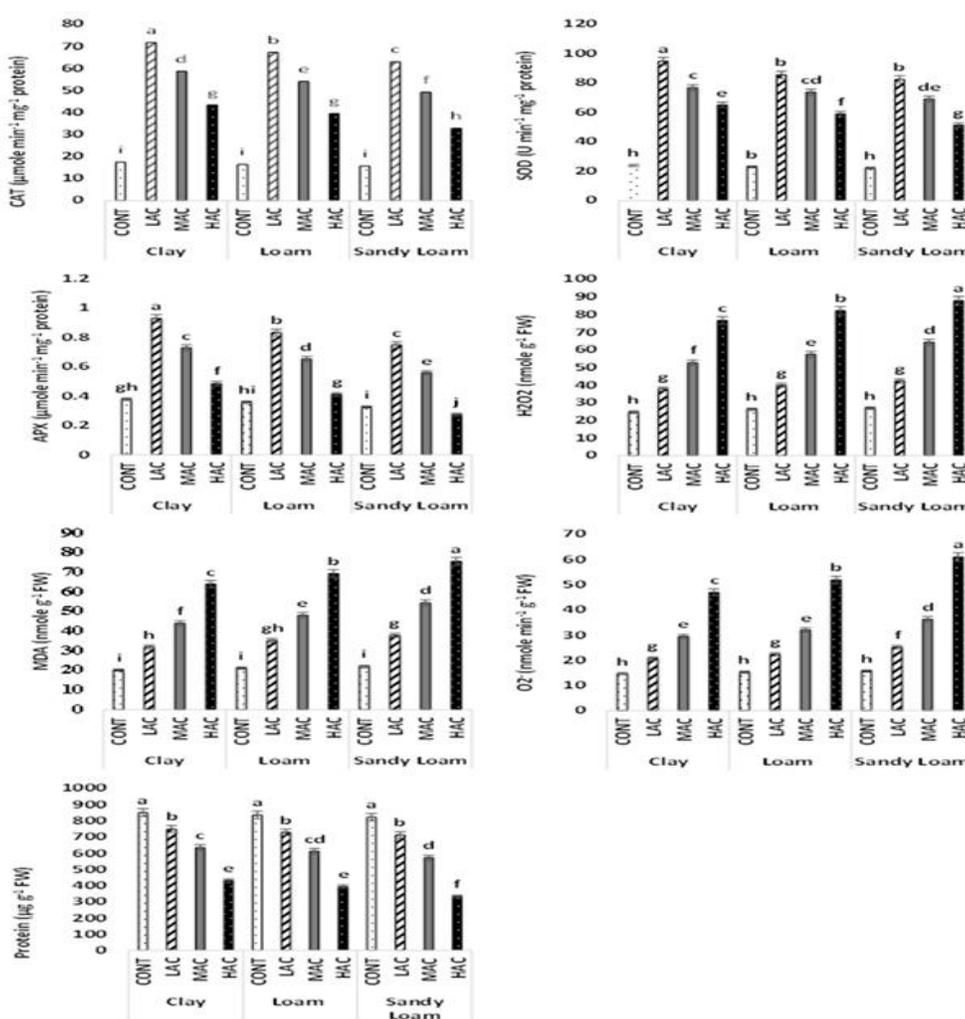


Fig. 3: The impact of different level of arsenic on concentrations of antioxidant enzymes [CAT (a), SOD (b), APX (c)], Reactive Oxygen Species (ROS) [H₂O₂ (d), MDA (e), O₂⁻ (f)], and protein (g) in maize.

The activities of catalase, superoxide dismutase and ascorbate peroxidase and protein in fresh maize leaves ranged from 17.4 to 25.9, 23.9 to 70.1, 0.11 to

0.55 (μmole min⁻¹ mg⁻¹ protein) and 850.3 to 420.7 (μg g⁻¹ FW) respectively in clay soil. They were 16.3 to 50.9, 22.9–67.7, 0.05–0.47 (μmole min⁻¹ mg⁻¹ protein) and

835.7 to -422.7 ($\mu\text{g g}^{-1}$ FW) on loamy soil whereas they were 15.6 to 47.3, 22.0 to 60.4, 0.42 to 0.05 ($\mu\text{mole min}^{-1} \text{mg}^{-1}$ protein) and 822.7 to 490 ($\mu\text{g g}^{-1}$ FW) respectively in sandy loam soil. Application of all treatments in soil significantly reduced the activities of catalase, ascorbate peroxidase and superoxide dismutase and the contents of protein in maize leaves (Table 2). Interestingly, considerably the abnormal reduction in the activities of ascorbate peroxidase, superoxide dismutase and catalase and protein contents in maize leaves have been observed in sandy loam soil at the high level of As followed by loamy soil where the minimum activities were found in control treatment. Though, the As toxicity significantly affected the activities of antioxidant enzymes such as CAT by 311%, 237%, 149%; 312%, 230%, 141% and 303%, 210% and 110%; SOD by 296%, 221, 172%; 274%, 222%, 133% and 275%, 214% and 133%; APX by 146%, 93%, 28%; 133%, 82%, 15% and 131%, 72% and -5% and the contents of protein by -12%, -25%, -49%; -13%, -27%, -49% and -13%, -31% and -60% in Clayey, Loamy, and Sandy Loam soils respectively at low, medium and high level of As over control (Fig. 3).

DISCUSSION

In this study, the soil texture was found to significantly affect the impact of As on growth and physicochemical parameters of maize plant at all levels of As. Under the set conditions, the highest significant negative impacts of As on the maize plants were observed in sandy loam soil followed by the loam soil and then the clay soil. According to the results of this study, the maximum reduction in the plant height as well as the plant biomass of maize were recorded in the plants grown in sandy loam soil at highest level of As. This highest impact of As on plants in sandy loam soil might be due to the greater mobility and bioavailability of As in sandy loam soil which has relatively lower level of Fe and Al hydroxide(s), organic matter and clay contents as compared to loam and clayey soil (Inskeep *et al.*, 2001). The reduction in plant growth parameters in the presence of As stress is in line with the findings of different researchers who already studied the impact of As on the growth of different crops (Ansari *et al.*, 2013; Singh *et al.*, 2016; Niazi *et al.*, 2017). The As stress might have affected the metabolism of plant cells and the metabolic energy should have been used for the production of antioxidants and phytochelatin to cope with As stress resulting into a decrease in plant height (Ansari *et al.*, 2013; Singh *et al.*, 2016; Niazi *et al.*, 2017). The As stress has also been reported to result in tissue loss and penetrability, reduction in enzymatic activities, decrease in mitotic activity, reduced cell elongation and reduced turgor of plant cell at higher level of toxicity, which results in decreased plant biomass (Gomes *et al.*, 2013). The similar reasons for decrease in plant height and

biomass in response to As stress have also been invoked by other researchers in different plants such as wheat, rice, broad bean, velvet grass and *Arabidopsis thaliana* (Ansari *et al.*, 2013; Shahid *et al.*, 2015; Khalid *et al.*, 2017). The impact of As on the growth of the maize crop is also comparable with the impacts of different other metal ions on the growth and physiology of various other crops (Khan *et al.*, 2019; Shahbaz *et al.*, 2019). For example, Shahbaz *et al.*, (2019) reported the loss of wheat biomass due to nickel and Khan *et al.*, (2019) reported the loss of barley biomass due to Pb stress.

The elevated concentration of As significantly reduced the chl-a and chl-b and RWC contents in all three types of soils with maximum decrease in sandy loam soil at the high level of As. Our results appear to be well substantiated with the findings of other researchers who reported negative impact of As on chl-a and chl-b contents in other crops including *B. napus* and *B. juncea* (Niazi *et al.*, 2017), *Zea mays* L (Anjum *et al.*, 2017; Mehmood *et al.*, 2017), *Vigna mungo* L. (Srivastava *et al.*, 2017), *Boehmeria nivea* L (Mubarak *et al.*, 2016) and *Oryza sativa* L (Dwivedi *et al.*, 2012).

The metals stress results in lipid and nucleic acid peroxidation and loss of oxidizing protein which increases the production of ROS in plant (Turan *et al.*, 2018). The application of As at various levels significantly increased the contents of ROS and negatively affected the activities of antioxidants in maize crop. Though the most significant impact was observed in sandy loam soil at the highest level of As treatment (Fig. 1). The content of ROS such as MDA, H_2O_2 , and O_2^- increased by 244, 226 and 288 %, respectively, as compared with control in sandy loam soil at highest level of As. Reportedly, the As toxicity enhanced the concentrations of ROS in different plants such as *Boehmeria nivea* L (Mubarak *et al.*, 2016), *Oryza sativa* L (Singh *et al.*, 2017). Similarly, increase in ROS in response to As addition has also been reported in many edible food crops such as wheat (Ghosh *et al.*, 2013), rice (Choudhury *et al.*, 2011), *Spinacia oleracea* (Shahid *et al.*, 2017) and *Pisum sativum* (Rafiq *et al.*, 2017). The cell functions of plants are distressed due to unnecessary increase in the contents of ROS in plants due to the damage of metals on nucleic acids, oxidizing proteins, and lipids peroxidation (Abderrahim *et al.*, 2015).

The oxidation processes of lipids and molecules are also significantly affected by metal stress in plants due to inhibition of oxidation chain reactions (Kumar *et al.*, 2012). The activities of antioxidant enzymes such as CAT, SOD, and APX were increased by applying As with maximum increase at As level of 25 mg kg^{-1} of As but significantly reduced by increasing the level of As in all three soils. However, the maximum change in activities of antioxidants was observed in sandy loam soil at the high contents of As. The protein contents were also reduced by 60% at the high level of As in sandy loam

soil. Similar results have already been reported in other crops such as in *Cicer arietinum* L (Tripathi *et al.*, 2017), *Vigna mungo* L (Srivastava *et al.*, 2017) and *Boehmeria nivea* L (Mubarak *et al.*, 2016), whereas, reduced antioxidant activities of enzymes have also been reported in rice (Upadhyay *et al.*, 2016) and *Brassica juncea* L (Kanwar *et al.*, 2015). Reduction in protein under As stress is due to the suppression of both nitrate and nitrite reductase enzymes and, ROS results in oxidation of protein and create free carbonyl groups, which results in hindering and altering the activities of protein (Rajjou *et al.*, 2008). Consequently, proteins become more vulnerable to proteolytic attacks (Moller *et al.*, 2007). Similar observations were also reported in other crops like rice (Singh *et al.*, 2009) and maize (Stoeva *et al.*, 2003).

The enhanced concentration of As in soil elevated the concentration of As in plant parts in all three types of soil as compared to control. The accumulation of As was more in the roots as compared to leaves in all types of soils. This order of accumulation of As in plant parts is mostly likely due to the fact that roots are directly exposed to As and As enters through the apoplastic tissue of cell and also due to reduction in transpiration and decreased As fluxes from root to shoot (Silva *et al.*, 2018). The maximum uptake of As in root (93.4 mg kg^{-1}) and shoot (44.03 mg kg^{-1}) as well as the DTPA extractable As in soil (2.41 mg kg^{-1}) was observed in sandy loam soil followed by the loam soil and the least was in clayey soil. This might be due to the maximum mobility and availability of As in the sandy loam soil as compared to other types of soil. Our observations are in line with the findings of other researchers (Quazi *et al.*, 2013; Piracha *et al.*, 2016) also reported the same order of As uptake in soil with different textures. The relatively lower DTPA extractable As in clayey soil was due to the fact that the clayey soil retained more As due to the high clay particle, organic matter, CEC and CaCO_3 which might have adsorbed the As making it less available to be extracted with DTPA (Quazi *et al.*, 2013; Masindi *et al.*, 2014; Piracha *et al.*, 2016; Uddin *et al.*, 2017). The soil characteristics such as texture, organic matter content, Fe and Al (hydro) oxides, CEC and clay particles have already been found to significantly alter the fate of As in soil (Taggart *et al.*, 2009; Simmler *et al.*, 2016; Azeem *et al.*, 2017; Strawn 2018).

Conclusions: On the basis of the results of the present study, it can be concluded that As significantly affects the growth and physiology of the maize crop. However, the toxic effects of As are influenced by the soil texture with the severe effects in the sandy loam soil as compared to the loamy and clayey soils with least effects in clayey soil. Moreover, the defence system of plant was also significantly affected by As in all textural soils but the plants grown on course textured soil (sandy loam)

showed highest reduction in the enzymatic activities and maximum increase in ROS contents and least impact was observed in fine texture soil (clayey soil). The maximum negative impact of As on plant growth, biomass, and physiological parameters were noted in plants grown in sandy loam soil and least was in the plants grown in clayey soil. The least concentrations of DTPA As was noted in clayey soil due to the greater retention sites. It was suggested that textures of soil are important factors to control the mobility, bioavailability and uptake of As by the plant.

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Acronyms:

As.	Arsenic.
LAC	Low arsenic level
MAC	Medium arsenic level
HAC	High arsenic level
ROS	Reactive Oxygen Species
ATP	Adenosine triphosphate
SOD.	Super Oxide Dismutase
CAT	Catalase
APX	Ascorbate peroxidase
MDA	Malondialdehyde
H_2O_2	Hydrogen peroxide

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