OPEN ACCESS Plant Health 03 (02) 2024. 219-230 DOI: 10.33687/planthealth.03.02.5505

Check for updates

Available Online at EScience Press

Plant Health

ISSN: 2305-6835

https://esciencepress.net/journals/planthealth

Effects of Lead (Pb) Contamination on Growth, Antioxidants and Osmo-Protectants in *Zea mays* (L.): Plants Strategy to Combat Metal Toxicity

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ARTICLE INFO

Article History
Received: May 13, 2024
Revised: July 29, 2024
Accepted: August 06, 2024

Keywords
Heavy metals
Growth
ROS
Biomarkers
Soluble sugar
Catalase
SOD
Chlorophyll

ABSTRACT

Abiotic stress includes the heavy metal stress which is consistently increasing and damaging agricultural crops productivity. The major cause of this issue is anthropogenic activities in the area. The present study was conducted to evaluate the phytotoxicity of lead (Pb) heavy metal on different morphological (plant height, leaf count, dry biomass) and biochemical (H_2O_2, MDA, activity of catalase & dismutase, free amino acids, total soluble proteins, soluble sugars, chlorophyll) attributes of two maize varieties (Pak Afgoi and Neelem Desi). Affected plant growth and accumulation of ROS-biomarkers have shown Pb toxicity in the test varieties. Although, the growth of maize plants was adversely affected by varying concentrations of Pb (30-300ppm) as Pb $(NO_3)_2$, yet plants were able to strive Pb-toxicity by inducing antioxidant enzymes and accumulation of some osmoprotectants. The activity of CAT and SOD was increased and showed a linear correlation with ROS-biomarkers. Both maize varieties showed a varying metal dose response regarding the accumulation of osmoprotectants that were less affected at lower metal doses than at higher doses. Accumulation of total free amino acids, soluble proteins and soluble sugars in Pb affected plants showed tolerance and adaptive trends specifically in Afgoi and Neelem maize varieties. Leaf chlorophyll also decreased due to Pb toxicity and might have a relation with plant photosynthetic and growth performance against Pb stress. However, we found that the Pak-Afgoi is highly tolerant maize against Pb polluted soils as it was less affected by Pb as compared to Neelem Desi and is suggested for the growers.

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INTRODUCTION

Heavy metal pollution is a major apprehension in various global ecosystems. Domination of widespread industrialization imparts the harmful effects of heavy metals on soil, and crop productivity due to amassing of heavy metals (Shahid *et al.,* 2015). These metals not only damage soil properties including pH, and soil nutrients, but also cause lessening of plant growth interfering with several physiological and molecular activities of plants (Hassan *et al.,* 2017; Tiwari and Lata, 2018).

Heavy metals are elements having a density greater than 5g/cm^3, and some of these act as essential elements (Zn, Cu, Mo, Mn, Co, Ni) for plant growth. However, many of them are harmful to the growing plants i.e., As, Al, Pb, Cd, Cr, and Hg (Salla *et al.,* 2011; Xiong *et al.,* 2014; Pierart *et al.,* 2015; Shahid *et al.,* 2015; Atta *et al.,* 2023b). Plants growing on metal enriched soils show varying symptoms like stunted growth, chlorosis, root browning, metabolic disorders, reduction in yield, and sometimes even death (Ozturk *et al.,* 2008; Ozturk *et al.,* 2015; Amari *et al.,* 2017). Moreover, the accumulation of heavy metals in plants adversely affects the plant physiological performance due to the excessive generation of reactive oxygen species (ROS) which target the key biological molecules.

In response to ROS generation, plants trigger their cellular defense system by activating antioxidants as a major strategy to overcome the adverse effects of heavy metal stress (Riyazuddin *et al.,* 2022). Chowardhara *et al.,* (2020) have reported an accumulation of non-enzymatic antioxidants with the increased activities of enzymatic antioxidants including superoxide dismutase (SOD), catalase (CAT), glutathione-s-transferase (GST), glutathione reductase (GR), ascorbate peroxidase (APX), and peroxidase (POX) under metal toxicity in *B. juncea*, representing a key role of these antioxidants in the HM-stress tolerance (Chowardhara *et al.,* 2020). In flax (*Linum usitatissimum* L.) and wheat (*Triticum aestivum*), plants under metal (Pb, As) stress showed excessive ROS accumulation, but in response these metal tolerance through activation of CAT (catalase) and SOD (superoxide dismutase) antioxidant enzymes (Pan *et al.,* 2020; Tyagi *et al.,* 2021).

Lead (Pb) is one of the most extensively distributed trace heavy metals and exists in different forms in nature. Pb can potentially affect soil, flora, and fauna by contaminating them through leaded fuels, dust, old lead plumbing pipes, different industrial sites, or even old orchard sites in production where lead arsenate is used (Tangahu *et al.,* 2011). According to ATSDR (2017), Pb is the 2nd most hazardous element that has negative impacts on the environment. It has various sources to contaminate the environment i.e., paint industry, fertilizers, pesticides, automobiles, and smelting operations (Atta *et al.,* 2023a). Pb damages several biological processes in plants including seed germination, seedling development, root elongation, transpiration, chlorophyll biosynthesis, and cell division (Kumar *et al.,* 2017). Cell membrane permeability is also affected by Pb interaction with the active groups of different metabolic enzymes and disrupts phosphate groups of ADP or ATP. Lead metal replaces essential ions in plants, and ultimately causes severe phytotoxicity (Pourrut *et al.,* 2011; Kumar *et al.,* 2017). At cellular level, Pb toxicity induces ROS through lipid peroxidation and damages DNA at large (Tiwari and Lata, 2018).

MATERIALS AND METHODS

Experimental design

To evaluate Pb toxicity in maize varieties Pak-Afgoi and Neelem, a pot experiment was designed. Different treatments of Pb $(NO_3)_2$ *viz.* 30, 60, 100, 150, and 300 mg L^{-1} were prepared. The fraction of Pb (0.6255) was obtained by dividing the atomic mass (207.2 g/mol) of Pb by 331.2 g/mol of Pb $(NO_3)_2$. This fraction value was used to compute the required amount of Pb salt to prepare ppm solution as Pb dose. Each treatment including control (T0) was comprised of eight replicates and was arranged in a complete randomized design (CRD). Plastic pots of different colors were washed, cleaned, air-dried, and labeled [dimension (cm): 30.5 Dia. × 46 deep] followed by smooth filling with 13 kg of sieved garden soil. The soil was also mixed with humus (leaf material + cow dung) in a 3:1 ratio to keep the soil fertile. For each variety, healthy uniform size seeds were used. Ten seeds per pot were sown one inch deep in the soil of each pot. Tap watering started on the same day. On the 70th day of mature growth below given parameters were studied.

Plant growth measurement

The maize plants were harvested out of the pots and measured (cm) for total plant length (TPL) including stem (SL) and root length (RL) with the help of a measuring tape. Likewise, for dry weight measurement, maize plants were subjected to an electric oven at 70 °C for 72 hours (Veckstar- Germany). Dry weight biomass (g) of roots, stems, and leaves was taken by using a digital balance (SKU-2534). From each treatment, leaf count or number per plant was counted and recorded to assess the effect of Pb treatment on maize leaf count.

Assessment of biochemical attributes

Determination of Hydrogen peroxide (H_2O_2) and Malondialdehyde (MDA)content

Oxidative stress in maize was determined by measuring H_2O_2 by the method of Velikova *et al.,* (2000). About 0.5g fresh plant leaf sample was treated with 1ml of TCA (0.1% trichloroacetic acid). This solution was then centrifuged. From this material, 0.5 ml supernatant was added to 10 ml potassium phosphate buffer (pH 7) and finally 1 ml KI_2 (1M) was added to it. The absorbance of the sample was noted at 390 nm by UV-Vis spectrophotometer (Model-502) and was compared with a standard curve of H_2O_2 (35%) solution.

MDA (malondialdehyde) was determined by the method of Heath and Packer (1968). About 0.5g of fresh and frozen plant leaf sample was minced in 3 ml of TCA. This material was then centrifuged at 13000 rpm. The supernatant (2 ml) obtained was again added with 2 ml of 0.67% TCA. The solution was heated at 100 ˚C for 30 minutes, cooled, and again centrifuged at 12000 rpm. The absorbance of the sample was noted at 532 and 600 nm wavelengths by UV-Vis spectrophotometer (Model-502). For standardization, a blank sample of TBA (0.25% thiobarbituric acid) in 10% TCA was used. The calculation was computed using the given formula:

MDA (nmol/ml) = $(Abs_{532} - Abs_{600})/(155000) \times 10^6$

Determination of Catalase (CAT) activity

CAT activity was estimated by following the method as defined by Ghasemi *et al.* (2013). Fresh plant leaf material (0.50g) was used to extract enzyme, standardized in pre-chilled pestle and mortar using 3 ml Tris-HCl buffer solution (pH 7) including EDTA (1 mM) and $MgCl_2$ (3 mM). For enzyme extract, 1 ml of potassium phosphate buffer (pH 7: 50 mM), and 0.4 ml of H_2O_2 30 mM were added in 1ml of reaction mixture. Decomposition of H_2O_2 was observed by measuring a decrease in absorbance at 240 nm by UV-Vis spectrophotometer (Model-502).

Determination of Superoxide dismutase (SOD) activity

SOD activity was estimated following the method of Dhindsa and Matowe (1981). 200 mg fresh plant leaf sample was crushed in a pre-chilled pestle and mortar containing 100 ml of potassium phosphate buffer solution (100 mM, pH 7), and 100 ml of 1% polyvinyl pyrrolidone. This mixture was ground to obtain a crude extract. The crude extract was later centrifuged at 12000 rpm, and from obtained supernatant SOD activity was measured at 560 nm UV-Vis spectrophotometer (Model-502).

Total free amino acid (FAA)

The method of Hamilton and Van Slyke (1943) was used to determine total free amino acids. Fresh plant leaf was frozen, and 0.5 g material was standardized with 1 ml of phosphate buffer solution (pH 7). In this material, 1 ml of 10 % pyridine and 1 ml of 2% ninhydrin solution was supplemented. The mixture was heated for up to 30 minutes. Distilled water was used to make a volume of up to 50 ml. The absorbance of the sample was noted at 570 nm. For the standard curve, lucien was used and FAA was quantified from a standard curve using the formula:

FAA (mg/g) = value of graph × dilution factor/sample wt × 1000

Total soluble proteins (TSP)

Total soluble proteins were assessed by the method of Lowery *et al.* (1951) as described by Ghasemi *et al.* (2013). Bovine serum albumin standards series was used to estimate protein content in unknown plant samples at 650 nm absorbance.

Total soluble sugars

Total soluble sugars and starch were determined by Yoshida *et al.* (1976). 0.1g plant leaf sample was taken and crushed very fine. This material was then added to centrifuge tubes with 10 ml of 80% ethanol. After due process, the soluble sugar content was determined by anthrone reagent (by dissolving 0.2 g of anthrone in 100 ml of concentrated sulfuric acid) as devised by Yoshida *et al.* (1976) at 630 nm absorbance using a UV-vis spectrophotometer-502. A standard curve of glucose was prepared to know the concentration of sugars in the plant samples.

Leaf chlorophyll value

Leaf chlorophyll value was measured by using a digital leaf chlorophyll meter (SPAD chlorophyll meter *CL-01 UK*; SPAD: accuracy ±1). Readings were recorded in sunny morning time (8:00 AM – 10:00 AM). Sample leaf was placed in the leaf chamber trailed by a gentle press and chlorophyll reading was noted on screen.

Data analysis

Results from a completely randomized design (CRD) experiment were analyzed with the least significant difference (LSD 5%) to assess the difference between the treatment means by using SPSS V. 20, whereas bar graphs with error bars were prepared in MS-Excel. The significance of the data was represented by alphabets on the bars. Bars with the same letters were shown to be insignificant against metal treatment in the experiment.

RESULTS

Pb treatments showed a significant phytotoxic effect on plant growth and biochemical attributes in maize. We observed an increasing effect of Pb on these studied parameters from lower to higher Pb treatment (30-300 ppm). In contrast to control treatment, plant height (TPH) and its components; shoot length (SL) and root length (RL) presented a variable but a significant declining trend in two test varieties (Afgoi and Neelem) due to Pb toxicity. The decrease in TPH was 186.6 - 105cm in Afgoi, and 172.7 - 93.4cm in Neelem, respectively. Mean reduction in Afgoi maize for TPH, SL, and RL due to Pb-toxicity was observed by 25.4%, 24.7%, and 26.9%, respectively. Comparable declining trend was observed in Neelem maize i.e., 26.7%, 26.4%, and 28.8%, respectively. However, at the maximum metal concentration (300 ppm), both varieties presented the highest decrease in height components. The present study has also shown that SL was more affected by Pb metal toxicity than RL in test varieties (Figure 1-A).

Dry weight (DW) biomass in both varieties also showed similar responses under Pb application i.e., in Afgoi mean reduction (30-300 ppm) for total plant dry weight, leaf DW, stem DW, and root DW was up to 28.66%, 28.85%, 26.04%, and 30.8%, respectively. The decrease in dry weight in Neelem maize was 34.03%, 33.91%, 29.2%, and 39.4 percent. Assessment among dry weight components projected a high decreasing rate due to Pb in an order of RDW > LDW > SDW in the test varieties (Figure 1-B, C).

Pb toxicity also significantly affected the number of leaves in maize (Figure 1-D). Mean values for this agronomic trait have revealed a decline due to Pb these were affected by 35.7% & 36%, respectively in Afgoi and Neelem. At 30-60 ppm, no effect of Pb was observed in Afgoi, whereas Neelem also showed the same pattern having a minimal effect of 4% on leaf count. At 150 ppm dose, both varieties were much declining up to 28.6% and 36%, respectively. This declining effect was more adverse at 300 ppm level (Figure 1-D).

Pb is an identified phytotoxic metal, and in response, plants imply varying stress symptoms at the cellular level and generate reactive oxygen species (ROS) that harm the membranous system. However, plants also show their possible effort to overcome (tolerance) such harm through the regulation of certain antioxidant enzymes (CAT, SOD) or some non-enzymatic substances like osmoprotectants.

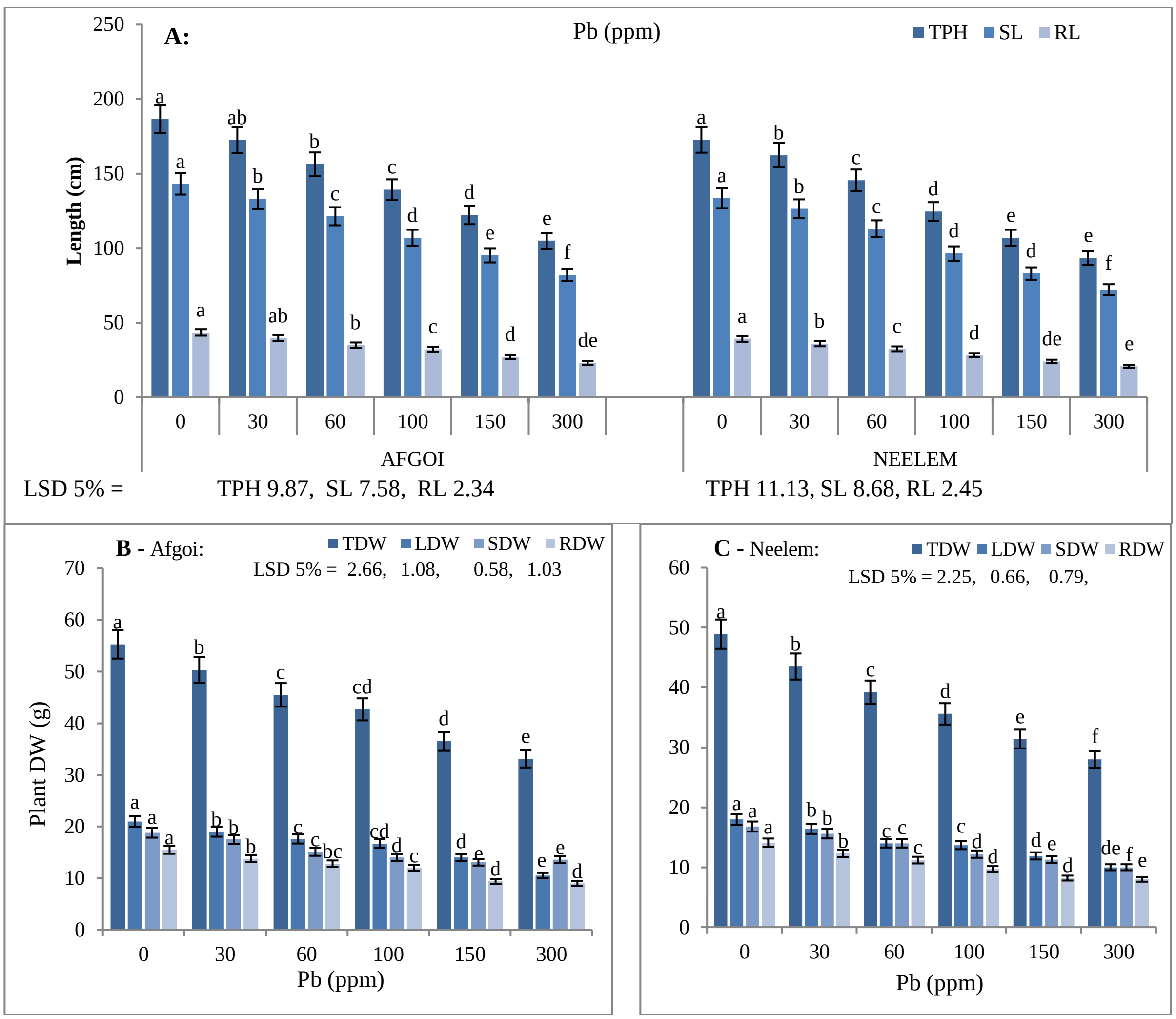
Figure 1 (A-D) showed a remarkable effect of Pb toxicity on the growth of maize plants that might be correlated with the understudy biochemical responses of maize. Analysis has revealed that H_2O_2 production increased as the Pb metal concentrations increased in the pot medium (Figure 2-A) increasing the level of H_2O_2 by 39% in Afgoi and 47% in Neelem, showing the production of ROS biomarker (H_2O_2) more in Neelem (sensitive) than in Afgoi (tolerant). By comparing plant-metal interaction, data significantly prevailed 300 ppm to be more toxic with the highest value of H_2O_2 (up to 60.6% in Afgoi and 66.1% in Neelem, as compared to control). Both maize varieties were resistant against ROS up to 60 ppm and then H_2O_2 increased due to elevated metal concentrations (Figure 2-A). A similar effect of Pb metal was observed in the accumulation of MDA content in test varieties. MDA contents were unaffected at lower metal doses than at higher. Afgoi showed 34.7% and 39% in Neelem maize. Accumulation of MDA content was slightly more in sensitive-Neelem than in tolerant-Afgoi (Figure 2-B).

Comparing both maize varieties, CAT activity significantly differed in the control treatment. CAT-activity was 25% increasing in tolerant-Afgoi, whilst sensitive-Neelem showed a mean increase of 14.6%, respectively. Data showed less CAT-activity in Neelem maize as it was affected more due to Pb metal. Moreover, by comparing control values, CAT-activity at the highest Pb dose (300 ppm) was increased in tolerant-Afgoi by 41.4%, while sensitive-Neelem maize revealed a sudden drop in CAT activity expecting this metal concentration toxic to enzyme activity. Noticeably, CAT-activity in Neelem was also observed to decrease at lower metal concentrations and then adaptively increased except at 300 ppm (Figure 2-C). Activity of SOD was found to increase under Pb toxicity in both the varieties revealing a significant effect of metal treatment (Figure 2-D). SOD activity was varying, at lower metal doses enzyme activity was lowering that later enhanced. The mean increase in enzyme activity in Afgoi was increased up to 46.1% showing much increase than Neelem. However, SOD activity decreased at elevated Pb doses (Figure 2-D).

Present study also revealed an accumulation of free amino acid content in test varieties due to the toxic effect of Pb metal. Although control values of FAA in test varieties showed significant differences between the two varieties, Neelem maize showed a greater accumulation of FAA under varying Pb doses. Comparative to the control treatment, Pb caused a slow accumulation of FAA in test varieties (23.4% Afgoi and 33% Neelem) up to 150 ppm concentration. This metal was found to be more adverse for the varieties as FAA quickly declined to 300 ppm. Data showed a greater accumulation of FAA in the sensitive maize variety Neelem than in the tolerant variety Afgoi (Figure 2-E). Total soluble protein (TSP) also tends to increase in the maize test varieties in a variable trend under varying concentrations of metals. Contrasting control values, TSP in both varieties increased up to 100 ppm level; and then decreased onward due to increased toxic levels of the metal. The increasing trend has expediently revealed the adaptive trend of the varieties. However, the mean increase in TSP was more in Afgoi than in Neelem (Figure 2-F).

Under Pb stress, both maize varieties showed a varying effect on the accumulation of content of soluble sugars in leaves. As compared to control, a significant effect of the treatments on the varieties was observed. Pb caused a slow accumulation of SS i.e., 27.9% in Afgoi and 26.1% in Neelem up to 150 ppm concentration. This heavy metal found to be toxic for the test varieties as SS was rapidly declined at 300 ppm as compared to SS increase up to 150 ppm of Pb (Figure 2-G).

Leaf chlorophyll content was assessed in the affected plants and compared with the control. Comparatively, the control values of chlorophyll for two varieties were not far different. However, the treatment effect (Figure 2-H) on chlorophyll content in both varieties was significant. In Afgoi and Neelem, Pb affected chlorophyll content up to 18.1% and 22.1%, respectively. Data showed a maximum decrease in leaf chlorophyll at 300 ppm (36.6% Afgoi, 39.1% Neelem), while a trifling effect of Pb was observed at 100-150 ppm. Afgoi was tolerant up to 60 ppm, whilst Neelem maize showed a gradual effect of Pb at lower treatment for chlorophyll decline (Figure 2-H).



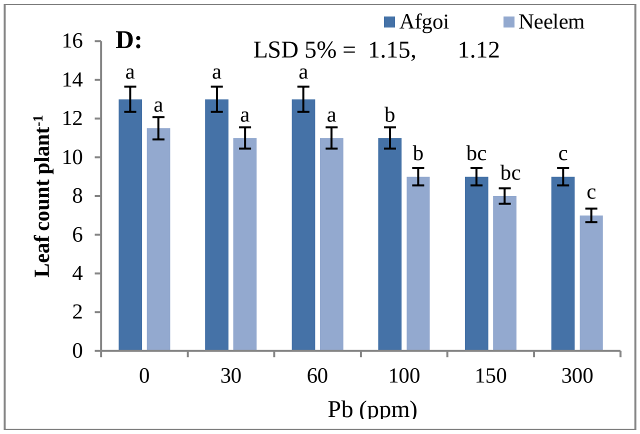
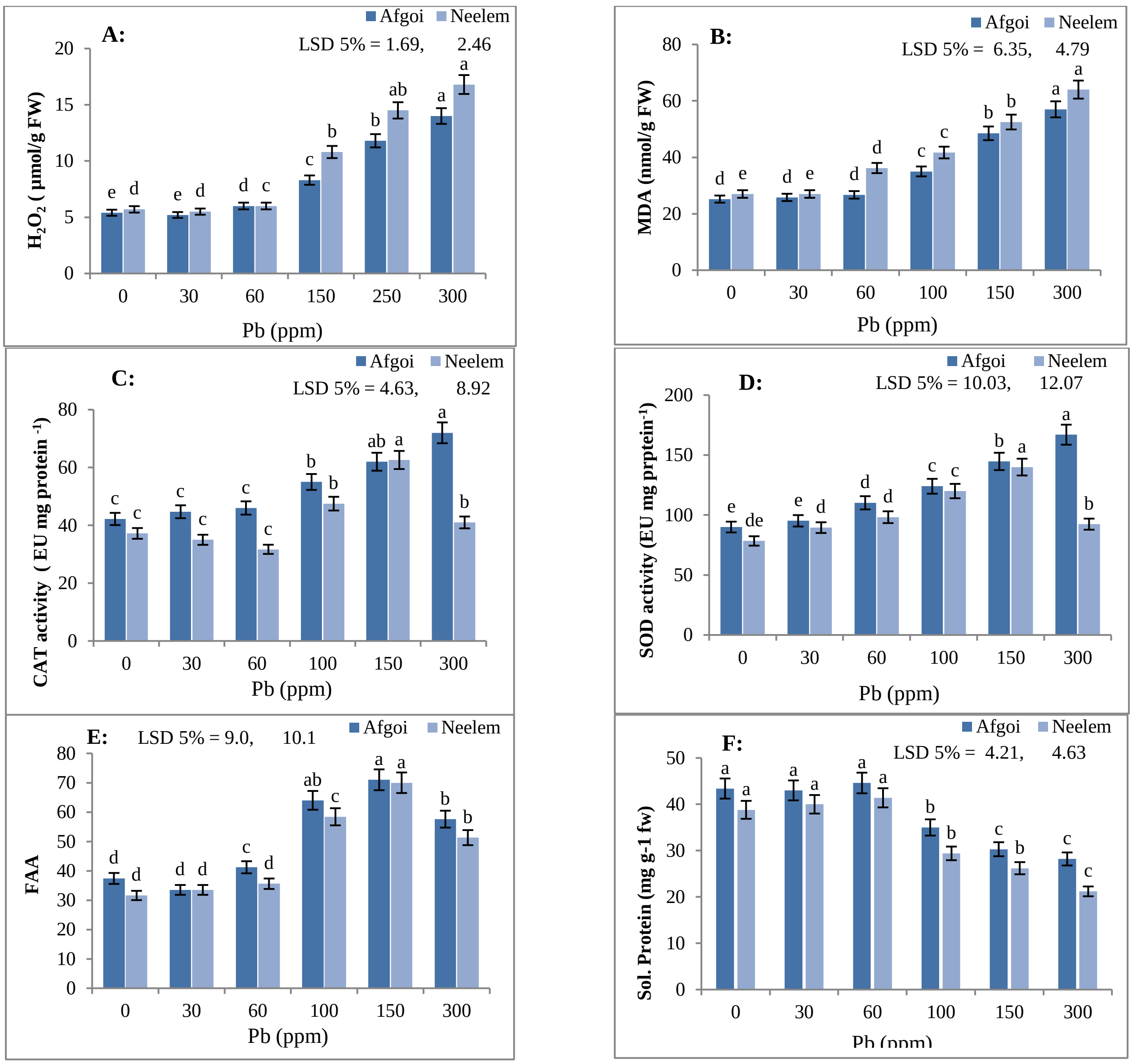
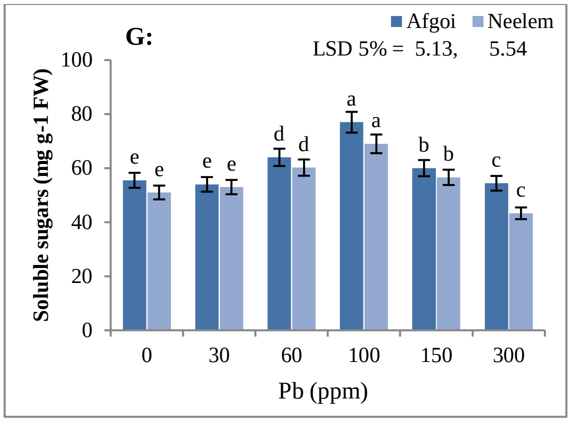


Figure 1 (A-D). Effect of Pb toxicity on plant length (A), plant dry weight in Afgoi (B), plant dry weight in Neelem (C), leaf count per plant (D).





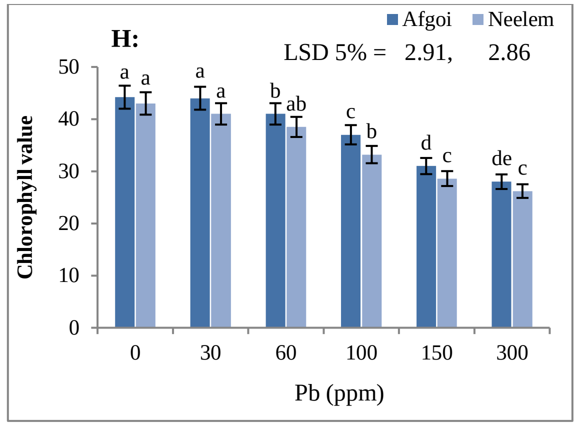


Figure 2 (A-H). Effect of Pb induced toxicity on ROS (A, B), catalase (C), superoxide dismutase (D), Osmo-protectants (E, F, G), and leaf chlorophyll (H) in maize plants.

DISCUSSION

We observed a very strong toxic effect of Pb on different growth and biochemical characteristics of maize plants. Toxicity of Pb treatment was variable, from low to high depending upon metal concentration in the growth medium. This study also revealed maize varieties put effort into activating the antioxidant system. Studies have uncovered that heavy metals (HMs) together with Pb are toxic chemical agents that impose harmful effects on growth and metabolism in a variety of plant species (Atta *et al.,* 2023b). Likewise, according to Anjum *et al.,* (2016), heavy metals cause a significant decrease in plant height in maize cultivars Wan Dan-30 and Run Nong-35. The effect of heavy metals was consistent together with its increasing level that decreased the shoot length (SL) of sunflower plants (Fozia *et al.,* 2008). Shiyab (2019) also revealed metal toxicity on plant height by a 39.3% decline in shoot length followed by a 90.4% decrease in SL at elevated levels in *Citrus aurantuim* (L.). An investigation by Singh *et al.,* (2015) documented suppressed SL by 19% under Pb stress. Stunted plant growth is due to the interface of heavy metals with the essential nutrients in the soil, which constrain mineral uptake by plants (del Real *et al.,* 2013; Ali *et al.,* 2014). Moreover, along with mineral disruption, heavy metals inhibit cell division and also affect plant height (Jasmine *et al.,* 2020). These reports exceedingly reinforced the findings of the current study. Plant height (TPH) and its components (SL, RL) have been affected significantly in both maize varieties (Figure 1-A). At a lower metal level having less toxicity, tolerant-Afgoi showed an adaptive mechanism than sensitive-Neelem, while Pb toxicity was pronounced at higher levels, and it was found to be a toxic metal. Overall, RL was found to be more sensitive than SL.

Leaf is an important photosynthetic organ of plants that plays a key role in the growth of plants. Pb adversely affects the growth and development of leaves in tomatoes and *Pisum sativum,* (NAS and Ali, 2017). Studies showed the inhibitory role of heavy metals (Pb) on leaf growth and development in rice plants through the generation of oxidative stress such as ROS (Yoon *et al.,* 2006; Xie *et al.,* 2018). These studies evidence the findings of the current study that leaf number in tested maize plants of maize significantly declined upon exposure to Pb treatment, and specifically a drastic decline was found at maximum Pb level indicating intolerance of varieties than at low metal level (Figure 1-D).

The toxic effects of HMs on plant biomass (dry weight) could be due to altered water potential, and mineral uptake through plant roots as metals become stuck in roots and contest water and mineral uptake from the soil (Ashfaque *et al.,* 2016; Singh *et al.,* 2016 and Kaya *et al.,* 2020). HMs decline the development of plant roots, decrease root surface area, and finally reduce the water absorption potential of the roots and its transport. Pb being a toxic metal affected dry biomass in wheat by up to 77% (Kanwal *et al.,* 2020), and in rice (cultivar Ediget) plants by 48.58% at 0.6mM - 1.2mM concentration. These observations also highlighted less toxicity at a lower Pb dose than at a higher level of Pb (Khan *et al.,* 2021). The findings of the present study are correlated with these documents. Data based on dry weight revealed the effect of metal ions on dry weight components of maize plants (Figure 1-B, C). Dry weight matter is the productivity of varieties under stressful conditions related to the chlorophyll and photosynthetic performance of treated plants. Disruption in this electron transport chain (ETC) process by HMs stress may result in affected plant growth and yield due to the inefficiency of plant enzymes that assimilate light and CO_2 (Fargasova, 2001; Taize and Zeiger, 2010). Our results regarding decreased plant biomass under Pb stressed environments are highly supported by these investigations and might be correlated with suppressed chlorophyll content in maize varieties. At a maximum level of 300 ppm Pb, a maximum decrease in leaf chlorophyll was noted by 36.6% in the Afgoi maize, following a decrease in sensitive Neelem maize by 39.1%, respectively. (Figures 1, 2).

Pb has diverse effects on plant efficiency, and in response to various abiotic stresses including HMs; plants like maize have been observed to induce certain reactive oxygen species (ROS) at the cellular level. ROS includes H_2O_2 and MDA that affect plant growth and development by degrading the cellular proteins and inactivation of enzymes. However, plants have the potential to overcome such oxidative damages and induce antioxidant defense mechanisms to counter ROS toxicity under metal stress. Superoxide-dismutase-SOD, catalases-CAT, and per-oxidases-POX etc. are activated in this way (Vanacker *et al.,* 1998; Borland *et al.,* 2006; Choudhury *et al.,* 2013; Shao *et al.,* 2019). Likewise, biosynthesis and accumulation of certain osmoprotectants (free amino acids, and soluble proteins and sugars) have also been reported as an adaptive approach in stressed plants (Ashraf and Foolad, 2007; Wahid *et al.,* 2009).

Figure 2(A, B) showed a significant increase in H_2O_2, and MDA content vides Pb stress in the tested maize varieties. Induction of oxidative stress increased gradually towards the maximum Pb level. Karkonen and Kuchitsu (2015) have revealed H_2O_2 signaling as a result of fatty acid and glycolate metabolism during photorespiration under abiotic stress, while SOD significantly plays a role in the dismutation of free oxygen radicals into hydrogen peroxide. Likewise other aldehydes, MDA is also produced during lipid peroxidation and is induced by the reaction of oxidants with fatty acids, and behaves as extremely mutagenic (Ayala *et al.,* 2014). Pb acts as redox metals and induces ROS, damaging the cell membrane. Pb with Cu metal stimulated H_2O_2 and MDA accumulation at 500 and 800 μM concentrations pinpointing this kind of lipid peroxidation with a decreased level of fatty acids in plants (Giannakoula *et al.,* 2021). As concerns, the present study, affected maize growth, decreased dry biomass and leaf count might have a strong correlation with the increased H_2O_2 and lipid peroxidation (Figures 1, 2-A, B).

Plants under stress regulate several protective enzymes (antioxidants) such as catalase (CAT) and superoxide dismutase (SOD) as a defense line (Rehman *et al.,* 2016). For example, maize plants struggling against Cd stress (100 μM & 200 μM) induced CAT and SOD with their elevated activities 23.5% - 64.2 % (Alam *et al.,* 2021). Activation of SOD prompts H_2O_2 from superoxide radicals, and CAT disrupts H_2O_2 into water and nascent oxygen and acts as biological scavenger of ROS (Ahmad *et al.,* 2010). In the current study, both Pak-Afgoi and Neelem produced CAT and SOD under Pb stress. As compared to Afgoi (tolerant) and SOD-activity, the activity of CAT in Neelem (sensitive) decreased up to 60 ppm of Pb, and they increased toward higher concentrations. However, SOD activity was suppressed at 300 ppm level in Neelem maize. It shows a high toxicity level of Pb for both of the varieties along with the tolerance pattern of the tested varieties (Figure 2-C, D).

Osmoprotectants are the metabolites produced by stressed plants and help in sustaining cellular redox homeostasis. They also shield the photosynthetic apparatus of the plants, and act as significant signaling molecules under stressed conditions (Ahmad *et al.,* 2020). Total free amino acids (FAA), soluble proteins (SP), and soluble sugars (SS) were measured as osmoprotectants under Pb stress. Maize varieties (Afgoi & Neelem) were successful in accumulating these as an adaptive strategy under Pb metal stress. Tolerant Afgoi maize was found as a successive variety in accumulation of FAA, SP, and SP than sensitive Neelem maize. However, for both varieties, Pb was found to be phytotoxic for these physiological traits as these traits progressively increased along metal concentration but adversely declined at 300 ppm level. Such a varying trend of these physiological traits uncovered the potency of varieties to withstand metal stress (Figure 2-E-G). The present study is supported by the findings of Duan *et al.,* (2020) who reported a negative impact of heavy metals on soluble sugars in *Glycine max*, while Dhir *et al.* (2012) investigated increased levels of osmolyte accumulation in *Salvinia natans* due to heavy metals including Pb.

The effect of Pb metal on the content of soluble proteins was also studied in maize plants. Results have shown a direct effect of Pb on the protein status of maize plants. The content of protein was increasingly at lower metal doses, and then decreased, remarkably (Figure 2-F). These results show the osmoregulatory habit of protein accumulation in maize plants but at lower metal treatment only. Such decreasing variation in protein contents has been reported due to the suppression of proteases under metal toxicity (Joshi, 2018). Moreover, decreased protein content strongly reveals the hydrolyzing/degradation effect of heavy metals both in the tolerant and sensitive maize varieties (Cherest *et al.,* 1990). Likewise, rice plants showed a drastic effect of Pb stress with a 44.93% decrease in total protein content (Khan *et al.,* 2021). These investigations are in agreement with our experimental evidence in this current study.

CONCLUSION

Pb metal found to be toxic for different agronomic characteristics of maize varieties. Plant growth pattern was adversely declined due to Pb treatment. However, plants have reflected varying biochemical responses against the metal stress. ROS-biomarkers (H_2O_2 & MDA) increased along with the increasing concentrations of Pb heavy metal. As a result, antioxidant enzymes (CAT, SOD) also increased to contest ROS-toxicity. Likewise, accumulation of FAA, soluble proteins and sugars was pronounced significantly. However, chlorophyll content showed a rapid decreasing trend due to Pb application and correlates with abrupted plants growth and photosynthetic performance of leaf. Maize varieties showed a tolerant behavior at lower metal doses toward these attributes. The declining trend was more prominent in sensitive Neelem than tolerant-Afgoi maize. On the basis of tolerance, Afgoi is recommended for the growers.

CONFLICT OF INTEREST

All authors have declared that there is no conflict of interest regarding the publication of this article.

AUTHOR CONTRIBUTIONS

The writing of this article was assisted and helped by all Authors.

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