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ORIGINAL PAPER

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FATTY ACIDS AND PHYSIOLOGICAL RESPONSES OF CORN LEAVES EXPOSED TO HEAVY METALS

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ABSTRACT

Heavy metals affect biochemical pathway by changing the fatty acid composition in plant cells. The high concentration of heavy metals impresses biochemical pathway and changes fatty acid compositions of plant cells. Fatty acids participate in various biological processes and have the functional role in regulating membrane functions in plants. In the present study, heavy metal content was analyzed with ICP-MS, fatty acid composition was investigated with GC and physiological parameters were determined with spectrophotometrically in the leaves of tomato subjected to increasing doses of heavy metals. In this study, the treatment of heavy metals on the growth medium changed the fatty acid contents of corn. The application of Cu significantly increased the level of palmitic acid and oleic acid. The treatment of Pb raised the content of oleic acid, whereas it significantly decreased the content of α-linolenic acid and erucic acid at 20 and 50 mg kg^{-1}, respectively. The addition of Cd significantly increased the level of oleic acid and linoleic acid; however, it significantly decreased the content of α-linolenic acid and erucic acid. Cu and Pb significantly raised the proline content. The application of Cu and Cd showed similar effect on hydrogen peroxide and the higher doses of them increased the content of H_2O_2. The level of lipid peroxidation significantly increased in response to all applied concentration of Cu. The results obtained in this study show that the aapplication of heavy metals changed the content of fatty acids, particularly that of oleic acid significantly increased in response to them. The levels of proline and lipid peroxidation generally increased together with oleic acid and palmitic acid in the leaves in reply to copper.

Key words: fatty acid, heavy metal, lipid peroxidation, proline, *Zea mays*

INTRODUCTION

Contamination of the agricultural areas with heavy metals has caused various damages to plants. Heavy metals are the major environmental pollutants and their accumulation in plants impairs plant metabolism and defense systems. Heavy metals, such as Cd and Pb, do not have any function in plant life cycles and exert a toxic effect; some heavy metals, such as Cu, Fe, Mn and Zn are necessary for plant life. However, excessive accumulation of Cu exerts a toxic effect and decelerate of plant development. High levels of heavy metals in plant tissues alter the various physiological functions, such as protein metabolism, fatty acid com-

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position, membrane functions, and photosynthetic apparatus [Hasan et al. 2009].

Heavy metals lead to the formation of free radicals, such as H_2O_2, hydroxyl, and superoxide radicals, in plant tissues. They lead to cellular membrane damage through attacking the double bonds in the fatty acid and eventually membrane lipid peroxidation. Free radicals caused by toxic metals can induce compositional alterations of membrane lipids [Liu and Huang 2004]. The plant cell alters the membrane fatty acid composition of lipids to react to unfavorable conditions. The fatty acid composition and saturation of a plant membrane affects membrane fluidity, and proper composition of unsaturated fatty acids in cellular membranes is essential to overcome the heavy metal stress. However, plants have developed antioxidative defense and osmotic-adjustment systems under stress conditions such as catalase, peroxidase, superoxide dismutase, proline and soluble sugars, to protect themselves by regulating osmotic potential against heavy metal stress [Park et al. 2015]. The initial consequence of plants under this metal stress is the formation of reactive oxygen species (ROS). The levels of hydrogen peroxide (H_2O_2) and membrane lipid peroxidation level increase under the stress conditions such as drought, salinity and heavy metals. Plants in the presence of oxidative stress give various response to overcome by changing the levels of enzyme activities such as catalase, peroxidase, glutathione reductase and glutathione S-transferase, chlorophyll and carotenoids [Kisa 2018]. Heavy metals induce oxidative stress such as Cu and Fe via Haber-Weiss and Fenton reactions or by causing imbalances in the antioxidative system. The direct effects of oxidative stress are commonly determined by the level of H_2O_2 and malondialdehyde (MDA) [Gratao et al. 2008, Mourato et al. 2015].

Fatty acids are essential components of the plant cell membranes, suberin, and cutin wax which are structural barriers to the environmental agents. Fatty acids are not only prominent structural and metabolic constituents of the cell, but they also function as modulators of the signal transduction pathways induced by biotic/abiotic stress factors [Beisson et al. 2007]. Fatty acids contribute to inducible stress resistance through the remodeling of membrane fluidity. The cellular responses of the plants exposed to heavy metals are the changes in the fatty acid composition, and free linolenic acid is itself a stress signal. Polyunsaturated fatty acids (PUFA), which are essential components of the membrane lipids, are released from membrane in response to environmental stimuli [Iba 2002, Upchurch 2008, Walley et al. 2013].

Studies on plant abiotic stresses have been intensively carried out by plant physiologists, their efforts have aimed at gaining a better understanding of the adaptive mechanisms in plants in terms of changes in enzyme activity in response to stress parameters [Verma and Dubey 2003, Gonçaalves et al. 2007, Hassan and Mansoor 2014, Kısa 2017]. Fatty acids are essential components of the plant cell, they are considered to be a barrier against activated oxygen species [Upchurch 2008]. Although there are few studies on fatty acids, no general conclusion about the fatty acid composition of the plant has been reached in the context of plant defense, and various aspects related to fatty acids remain poorly known in the plant exposed to abiotic stress. Hence, we herein investigated the corn leaf fatty acids and some physiological parameters. The main goal of the present study is to reveal the pattern of fatty acid composition of the leaves of corn exposed to the different concentrations of copper, leads and cadmium. Moreover, lipid peroxidation, H_2O_2, and proline contents were associated with the changes of the fatty acid composition in plants exposed to heavy metals.

MATERIALS AND METHODS

Plant material and growth conditions. Corn (*Zea mays*) seedlings were planted in plastic boxes containing a equal mix of peat and garden soil (10 kg), and the experiment was carried out under greenhouse conditions with 16 h photoperiods with day/night temperature of 25/12 ±3°C. After two weeks of acclimatization, $CuSO_4$, $Pb(NO_3)_2$, and $CdCl_2$ were added to the pots containing soil in 10, 20, and 50 mg kg^{-1} in, respectively. The addition of heavy metals was carried out three times with a two-day interval. The corn leaves were harvested two weeks after the application, and all samples were kept at –80°C until analysis. Three individuals replicate per treatment were used for analysis.

Analysis of heavy metal contents. Corn leaves were washed in distilled water, and dried at 65°C in the oven until they had reached a constant weight. The tissues were ground, and they were burned in oven. The temperature was gradually increased to 550 ±50°C for 6 h. The samples were cooled and reacted with concentrated HCI, and 2 mol L^{-1} HNO_3. Then, they were allowed to cool to room temperature and pure water was added to the filter. Cu, Pb and Cd were determined by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) (7700; Agilent, Tokyo, Japan) by using external calibration method.

Analysis of fatty acid by gas chromatography. Extraction of lipids was performed as previously described with minor modifications [Bligh and Dyer 1959]. Fresh leaves were dried at 65°C in the oven; the dried samples were ground, and lipids were extracted with chloroform-methanol (2 : 1). The homogenate was filtered with Whatman paper (Grade No: 1), and was then added chloroform-methanol (2 : 1). The filtrate was centrifuged at 4500 rpm for 10 min, and 1% KCI and chloroform was added. The lipid-containing organic layer was acidified with 2% H_2SO_4, the mixtures were incubated for 12 h, and then, 5% NaCI was added by vigorous shaking. Then, fatty acid methyl esters (FAMEs) were recovered by adding 1 mL hexane into the vials for fatty acid analysis. FAMEs were analyzed by gas chromatography (Perkin-Elmer Precisely, Clarus 500-USA) using a capillary column (30 m × 0.25 mm; df: 0.20 µm, Rtx-2330) with flame ionization detection and hydrogen gas as a carrier, the flow ratio was 50 mm min^{-1}, and the split ratio was 20/1. Initially, the oven temperature was held 100°C for 1 min, followed by a 10°C min^{-1} ramp to 180°C and a second ramp of 2°C min^{-1} to 230°C.

The content of lipid peroxidation. The level of lipid peroxidation was detected by measuring the MDA level using the thiobarbituric acid (TBA) method [Sreenivasulu et al. 1999]. The leaves of corn were ground into liquid N_2, and homogenized in 4 mL of 0.1% (w/v) trichloroacetic acid (TCA). The suspension was centrifuged at 10000 g for 20 min and 0.5 mL of the supernatant was added to 1 mL of 20% TCA containing 0.5% TBA (w/v). The reaction tubes were incubated at 95°C hot water bath for 30 min, and the reaction was stopped by cooling the tubes in an ice bath. The absorbance of the reaction was measured by spectrophotometer at 532 nm and non-specific absorbance at 660 nm was subtracted (Carry 50 UV/VIS, Japan). The content of MDA was calculated using the absorption coefficient of 155 nM^{-1} cm^{-1}.

The content of H_2O_2. The H_2O_2 content was determined using the potassium iodide method [Velikova et al. 2000]. The leaves were ground into liquid N_2 and, homogenized with 0.1% TCA (w/v). The homogenate was centrifuged at 12 000 g for 15 min, and the obtained supernatant was added to potassium phosphate buffer (10 mM, pH 7.0) and 1 M KI solution. The absorbance of the reaction was read at 390 nm. The level of H_2O_2 was determined using a calibration curve (µmol mL^{-1}).

The content of proline. The fresh leaves of corn were ground into liquid N_2 and, homogenized in 4% sulfhosalicylic acid; then, the solution was filtered with a filter paper. The obtained extract reacted with the acid ninhydrin; then it was incubated for 1 h at 100°C, and the reaction was stopped using an ice bath. After the addition of toluene, the absorbance of the upper fraction was read at 520 nm. Free proline content was calculated using a calibration graph obtained from pure proline; this was expressed in mg g^{-1} FW [Bates et al. 1973].

Statistical analysis. Statistical analysis of the results was done with one-way ANOVA by using Duncan multiple range tests. The values of samples are presented a mean ±standard deviation. Significant differences in relation to the sample groups were indicated at $P < 0.05$.

RESULTS

Heavy metal levels in leaves of corn. Heavy metals concentration of leaves increased and the contents of Cu, Pb and Cd are shown in Figure 1. The level of Cd significantly raised with elevating concentration of it. The content of Cu and Pb changed depending on the applied concentration of them, and especially increased the highest doses of them.

The level of fatty acid in the corn leaves. The application of Cu, Pb and Cd on the plant growth soil changed the fatty acid contents, and the levels of palmitic acid, palmitoleic acid, oleic acid, linoleic acid, α-linolenic acid, and erucic acid depended on the types and doses of heavy metals applied. The application of Cu significantly increased the level of palmitic acid and oleic acid in the corn leaves except for 50 mg kg^{-1}.

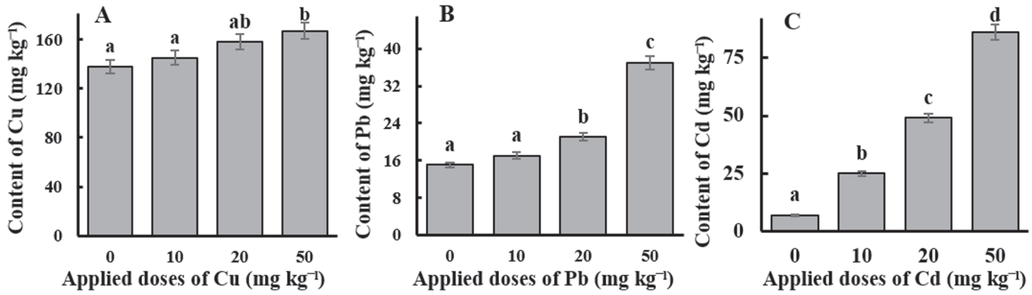

Fig. 1. The level of Cu, Pb and Cd in the leaves of corn subjected to 0, 10, 20, 50 mg kg^{-1} of $CuSO_4$, $Pb(NO_3)_2$ and $CdCl_2$, respectively. The data in the y-axis shows heavy metal concentrations. Values are the means ±SD and bars marked with different letters indicate significantly differences at p < 0.05 (Duncan test)

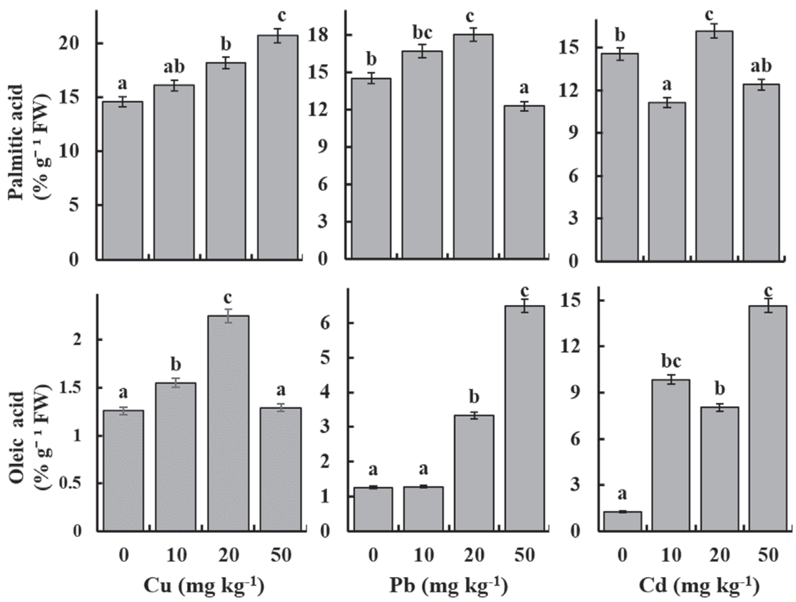

Fig. 2. The effect of heavy metals on palmitic acid and oleic acid in the leaves of corn subjected to 0, 10, 20, 50 mg kg^{-1} of $CuSO_4$, $Pb(NO_3)_2$ and $CdCl_2$, respectively. The data in the y-axis shows the percentage of fatty acids and different letters show significant difference at $P \leq 0.05$. Value expressed as percentage of analyzed fatty acids per gram fresh weight

The treatment of Pb significantly raised the content of oleic acid and palmitic acid excluding of 10 and 50 mg kg^{-1} of it, respectively. The level of oleic acid significantly increased by the exposure to Cd, but the percentage of palmitic acid changed according to the applied doses of Cd compared to control plants. The results for palmitic acid and oleic acid are shown in Figure 2. The level of α-linolenic acid significantly decreased by the application of all heavy metals compared with control plant leaves. The percentage of erucic acid significantly decreased by the addition of Cd at any concentration; however, low doses (10 mg kg^{-1}) of Cu and Pb raised the level of erucic acid. In addition, compared with control plant leaves, Cu and Pb treatments in the plant growth soil at 20 and 50 mg kg^{-1} doses reduced the erucic acid content in corn leaves ($P < 0.05$). The results for α-linolenic acid and erucic acid are shown in Figure 3. The level of linoleic acid and palmitoleic

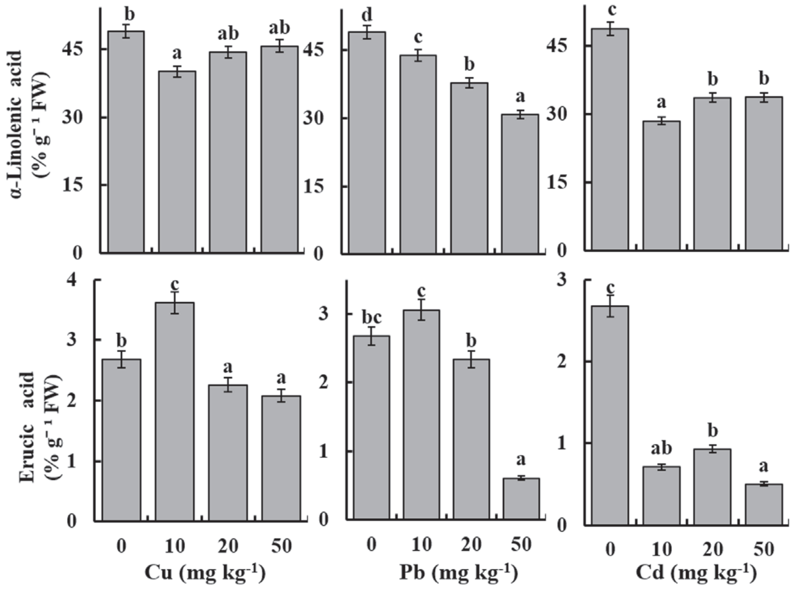

Fig. 3. The effect of heavy metals on α-linolenic and erucic acid in the leaves of corn subjected to 0, 10, 20, 50 mg kg^{-1} of $CuSO_4$, $Pb(NO_3)_2$ and $CdCl_2$, respectively. The data in the y-axis shows the percentage of fatty acids and different letters show significant difference at $P \leq 0.05$. Value expressed as percentage of analyzed fatty acids per gram fresh weight

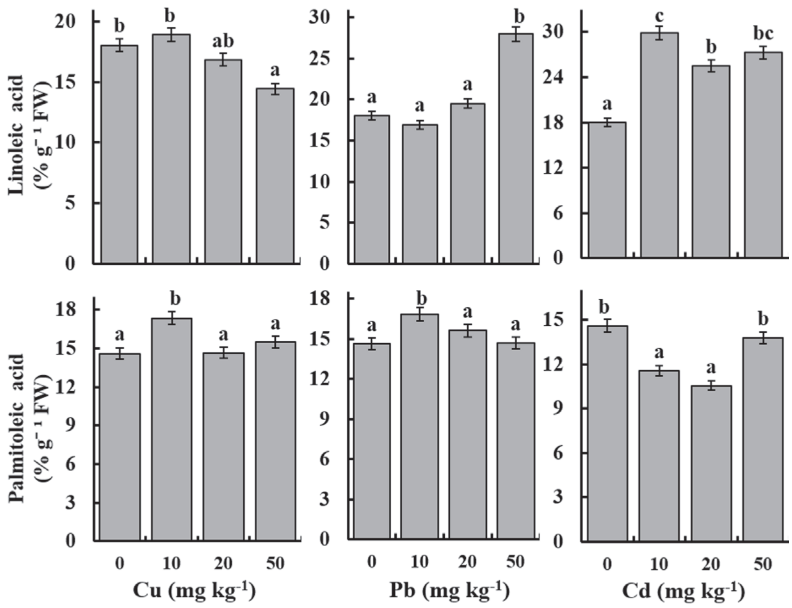

Fig. 4. The effect of heavy metals on linoleic and palmitoleic acid in the leaves of corn subjected to 0, 10, 20, 50 mg kg^{-1} of $CuSO_4$, $Pb(NO_3)_2$ and $CdCl_2$, respectively. The data in the y-axis shows the percentage of fatty acids and different letters show significant difference at $P \leq 0.05$. Value expressed as percentage of analyzed fatty acids per gram fresh weight

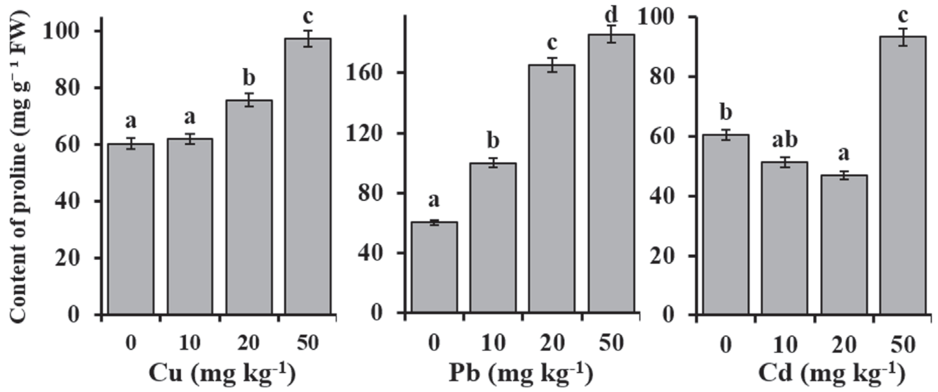

Fig. 5. The effect of heavy metals on the content of proline in the leaves of corn subjected to 0, 10, 20, 50 mg kg^{-1} of $CuSO_4$, $Pb(NO_3)_2$ and $CdCl_2$, respectively. Mean values with different letter show significant difference at $P \leq 0.05$. Data expressed as a mg g^{-1} fresh weight

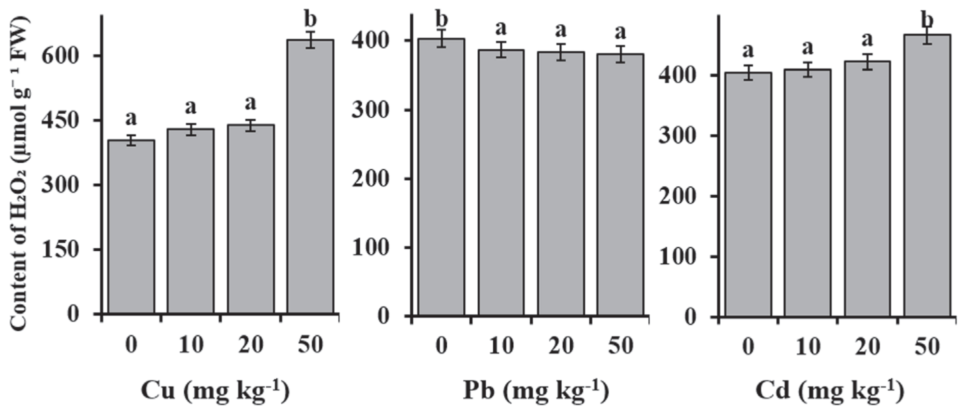

Fig. 6. The effect of heavy metals on the content of H_2O_2 in the leaves of corn subjected to 0, 10, 20, 50 mg kg^{-1} of $CuSO_4$, $Pb(NO_3)_2$ and $CdCl_2$, respectively. Mean values with different letter show significant difference at $P \leq 0.05$. Data expressed as a µmol g^{-1} per fresh weight

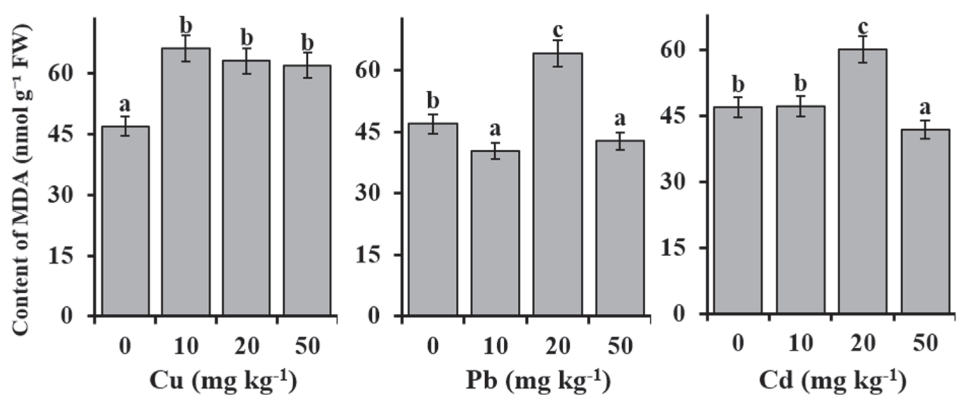

Fig. 7. The effect of heavy metals on the content of MDA in the leaves of corn subjected to 0, 10, 20, 50 mg kg^{-1} of $CuSO_4$, $Pb(NO_3)_2$ and $CdCl_2$, respectively. Mean values with different letter show significant difference at $P \leq 0.05$. Data expressed as a nmol g^{-1} per fresh weight

acid changed depending on heavy metal types and doses, and the results are shown in Figure 4. The treatment of Cd significantly increased the content of linoleic acid. In addition, the high dose (50 mg kg^{-1}) of Pb significantly raised the level of linoleic acid, but its other doses (10 and 20 mg kg^{-1}) did not show significant changes in the leaves of corn compared to controls. On the application 50 mg kg^{-1} of Cu, the content of linoleic acid was reduced; however, there was no significant change in content with 10 mg kg^{-1} of Cu in corn leaves compared with control leaves. There was no significant change in the percentage of palmitoleic acid by the treatment of Cu and Pb the 20 and 50 mg kg^{-1} doses, but a significant increase in its content was observed at the low dose of 10 mg kg^{-1} ($P < 0.05$).

The effect of heavy metals on the content of proline, H_2O_2, and MDA. The application of heavy metals changed the proline content in the leaves of corn. The level of proline significantly increased with increasing doses of Cu and Pb in the plant cultivation soil. However, the treatment of Cd decreased the level of proline at 10 and 20 mg kg^{-1} doses; and only increased it at the highest (50 mg kg^{-1}) dose in corn leaves compared with control groups. The results for proline contents are shown in Figure 5.

The content of H_2O_2 changed depending on the applied heavy metals and doses, and these results are shown in Figure 6. The exposures of the corn to 50 mg kg^{-1} dose of Cu and Cd increased the level of H_2O_2. However, the concentration of H_2O_2 remained constant in the leaves at other doses of Cu and Cd. The treatment of Pb slightly decreased the level of H_2O_2 in corn leaves at all concentrations compared to control groups.

The addition of heavy metals to the soil in the corn cultivated pots changed the level of lipid peroxidation depending the on applied concentration of heavy metals. The content of MDA significantly increased with Cu exposure at all concentrations, and with 20 mg kg^{-1} doses of Cd and Pb in corn leaves compared with control plants (P <0.05). The level of lipid peroxidation is shown in Figure 7.

DISCUSSION

Biological membranes are highly ordered structures consisting of mosaics of lipids and proteins [Niu and Xiang 2018]. Environmental stress causes a number of physiological and biochemical changes, such as membrane functions, enzymes activities, cell redox homeostasis, and osmotic adjustments. Plants possess several biochemical defense mechanisms to reduce the oxidative damage induced by heavy metals [Mithöfer et al. 2004, Tamás et al. 2008]. In this study, the effects of heavy metals are evaluated by measuring the levels of fatty acids, proline, H_2O_2 and MDA. The concentration of Cu, Pb and Cd increased in the leaves of corn cultivated plastic boxes containing heavy metals. High concentration of heavy metals affects the biochemical pathways by changing enzyme activities which are involved in these pathways and change fatty acid compositions in plant cells. Fatty acids are essential molecules that participate in various biochemical processes in plants. Primary site of plant cell interacting with heavy metals may be root cell membranes [Savchenko et al. 2010, Chalbi et al. 2013].

In the current study, the treatment of heavy metals changed the level of fatty acids depending on the applied types and doses of heavy metal. The application of Cu generally increased the content of palmitic acid and oleic acid, and it significantly reduced the level of linoleic acid and α-linolenic acid in corn leaves compared to control groups. These decreases in the amount of these fatty acids maybe associated with an increased amount of MDA which degradation products of fatty acids, and the level of lipid peroxidation significantly increased in plants grown under the same conditions. It has been demonstrated that the alteration of fatty acid composition of modified low-density lipoproteins (LDL) when lipoproteins are oxidized have negative consequences such as the reduction of PUFAs levels and the increase of the peroxidation products of fatty acid [Deleanu et al. 2016]. The treatment of Pb in the plant cultivated medium raised the level of oleic acid and reduced the level of α-linolenic acid. These decreases of it was surprisingly correlated with a decrease in the amount of H_2O_2. The low dose (10 mg kg^{-1}) of Pb raised the content of palmitic acid, palmitoleic acid and erucic acid, and the high concentration of Pb significantly decreased the level of erucic acid. The level of linoleic acid was significantly increased when the corn was exposed to 50 mg kg^{-1} doses of Pb. It was reported that the content of linolenic acid is significantly reduced, and the content of linoleic, oleic, and stearic acids increased in the leaves of *Populous nigra* grown in fields contaminated with heavy metals such as Cd, Cr, Cu, Ni, Pb, and Zn [Guedard et al. 2012]. Biological membranes act synergistically to promote membrane structure and functions. Plants that survive under extreme conditions must first maintain constant membrane fluidity and integrity, which requires dynamic changes in the membrane composition [Gomez et al. 2018, Niu and Xiang 2018].

In the present study, the addition of Cd to the soil in plant cultivation pots significantly increased the content of oleic acid and linoleic acid, and significantly decreased the level of palmitoleic acid, α-linolenic acid, and erucic acid at the all concentrations; one exception was the content of palmitoleic acid in response to 50-mg kg^{-1} Cd treatment, which showed no significant changes. The quantity of palmitic acid changed depending on the applied concentrations. Fatty acids are necessary for formation and permeability of cell membrane, serving as precursors of important second messengers and they are the main components of lipids having an important role on biochemical and physiological response [Rabei et al. 2018]. A study conducted on tomato reported that the percentage of stearic acid, oleic acid, and linoleic acid increased 2- to 3-fold, and the percentage of hexadecatrienoic acid and linolenic acid decreased 2 to 3 fold. The percentage of other fatty acids remained generally unchanged when exposed to Cd [Djebali et al. 2005]. It was previously reported that the content of palmitic acid increased in the *Spinacia oleracea* with increasing Cd concentration [Zemanová et al. 2015]. It was declared that the percentages of linolenic, oleic, and linoleic acids significantly decreased, and that of palmitic acid significantly increased in sunflower plants exposed to $CdCl_2$ stress [Moradkhani et al. 2012]. The percentages of linolenic and hexadecatrienoic significantly decreased and that of palmitic, oleic and linoleic acids significantly increased in the leaves of tomato plants grown in the highly metal-contaminated soil [Verdoni et al. 2001]. Fatty acids are considered to have a functional role in regulating membrane functions. They have very important functions, particularly the poly unsaturated fatty acids-linoleic acid and linolenic acid. PUFA is used to maintain the structural parts of the membrane cells, and the main PUFAs represented in higher plants are linoleic acid and linolenic acid [Rahayu et al. 2014]. The ratio of C18:3/ (C18:0 + C18:1 + C18:2) is used as a tool to diagnose soil contamination to assess the ecotoxicity (called "lipid biomarker"), and this ratio decreases when plants are exposed to heavy metals [Le Guédard et al. 2012]. Linolenic acid (18:3) is a major fatty acid in photosynthetic tissues, and linoleic acid (18:2) is the main fatty acid in non-photosynthetic tissues in plants [Verdoni et al. 2001]. In the current study, we showed that the treatment of Cu increased the content of palmitic acid and oleic acid; Pb raised the level of oleic acid; and Cd enhanced the quantity of oleic acid and linoleic acid at all concentrations of them in the plant growth medium. However, it was observed that the addition of Cu and Pb in the plant growth medium decreased the level of linoleic acid and α-linolenic acid at all concentrations, respectively. Also, the amount of α-linolenic acid and erucic acid reduced at all concentrations of Cd. When plants exposed to stress factors, plant cells need to change the level of lipid composition of plasma membranes to keep optimum physical membrane properties. Decreases in fatty acid unsaturation of plasma membranes under heavy metal conditions has been also reported in *Zygophyllum* species whereas the saturated fatty acids increased in the same plants. Increases in the degree of fatty acid saturation is a typical consequences of plasma membranes to variations in the external environment [Morsy et al. 2012]. Stress factors can change properties of biological membranes, including their fluidity and permeability, through a holistic effect that involves changes in the lipid composition and/or interactions between lipids and specific membrane proteins. Membranes serve as a selectively permeable barrier, and they are primarily composed of proteins and lipids in moving mosaics [Niu and Xiang 2018]. Lipids form the physical boundary defning the boundaries of the cell itself as well as that of organelles within the cell based on their physicochemical properties [Gomez et al. 2018].

Environmental stresses, such as those from heavy metals, cause oxidative damage by triggering H_2O_2 production. It is widely generated in plant biological systems as a common cellular metabolite [Niu and Liao 2016]. In the current study, the treatment of Cu and Cd have similar effect on H_2O_2 content, especially increased the amount of H_2O_2 at 50 mg kg^{-1} doses of them. The content of H_2O_2 remained unchanged when exposed to Pb. A study on the seedlings of barley indicated that the level of H_2O_2 did not change except for in the presence of 500 μM of $CdCl_2$ [Demirevska-Kepova et al. 2006]. Another study on two maize cultivars revealed that a significant increase in the level of H_2O_2 is seen in the leaves of both cultivars of maize [Maiti et al. 2012]. It is considered that H_2O_2 function as a signal in activating the antioxidant defence mechanism. The production of H_2O_2 could be considered as the early outcome of oxidative stress, and it could induce stress responsive molecules by protecting the chloroplast to maintain photosynthesis under stress conditions [Niu and Liao 2016].

The excessive accumulation of heavy metals in the plant growth medium can cause the lipid peroxidation. In our study, the application of Cu increased the MDA content at all doses of it compared to control plants. However, the level of MDA only increased at 20 mg kg^{-1} doses of Pb and Cd showing comparable results. The increase in the amount of MDA showed similar correlation with a reduction in the content of α-linolenic acid in the leaves of corn exposed to Cu. It was reported that the lipid peroxidation levels of Cd-stressed maize seedlings were similar to those of control groups [Pál et al. 2005]. The MDA content of *Lemna minor* fronds increased gradually with increasing concentrations of Cu and Cd, with both of them showing comparable results [Hou et al. 2007]. A study on the leaves showed significant increases in MDA content in barley exposed to Al, Cu, Cd, and their combinations. MDA level is commonly used as an indicator of lipid peroxidation because it is a result of the peroxidation of fatty acids of lipid membrane and accumulates when plants are exposed to environmental stress [Guo et al. 2007].

Heavy metals may induce proline accumulation, which is one of the most common stress metabolites in plants. Proline is believed to have an important protective role against heavy metal stresses [Sun et al. 2007]. In the present study, the application all concentrations of Cu and Pb significantly increased the content of proline, whereas low-dose treatments of Cd decreased the level of proline and significantly increased the content at the highest dose (50 mg kg^{-1}). The raises of proline content generally increased together with oleic acid and palmitic acid in the leaves of corn subjected to Cu and Pb; one exception was for palmitic acid in response to in reply to 50 mg kg^{-1} Pb treatment. A study on the non-tolerant *Silene vulgaris* revealed that the exposures of Cu, Cd, and Zn significantly induced the accumulation of proline in the leaves [Schat et al. 1997]. It was demonstrated using the leaves of *Lemna polyrrhiza* that the treatment of Pb and Cd generally increased the content of proline at ≤30 ppm doses and decreased it at >30 ppm doses compared to the control groups; in addition, the results have also shown changes depending on the time [John et al. 2008]. It was previously stated that Cd treatment significantly increased the free proline in the leaves of *Solanum nigrum* [Sun et al. 2007]. Its accumulation is a general physiological response in plants exposed to various environmental stresses, such as heavy metal toxicity and nutrient deficiency. Proline has diverse roles as a multifunctional amino acid under stress conditions and functions via stabilization of proteins, membranes and scavenging ROS [Kaur and Asthir 2015].

CONCLUSONS

The results presented in this study show that the treatment of heavy metals changed the content of fatty acids, particularly that of oleic acid significantly increased in response to the applications of Cu, Cd and Pb. Our results indicate that the content of oleic acid and palmitic acid was clearly associated with the increasing levels of proline, H_2O_2, and lipid peroxidation in response to copper concentrations in corn leaves. Moreover, the changes of fatty acid content may be linked with the alteration of MDA levels; because it is product of lipid peroxidation. Proportion of fatty acids in lipid molecules may be regulated to keep the integrity of the membrane structure, function and fluidity under the heavy metal stress conditions. We demonstrated six fatty acids in the present study; these findings could help improve our understanding of the relation between fatty acids and lipid peroxidation, but additional experiments can be performed by including other fatty acids and physiological parameters of plants that are affected on exposure to environmental stresses.

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CONFLICT OF INTEREST

All authors declare that they have no conflicts of interest.

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