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Responses of the Fatty Acid Composition of *Solanum lycopersicum* Exposed to Heavy Metal Stress

Ağır Metal Stresine Maruz Kalan Domatesin Yağ Asidi Dağılım Cevapları

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Abstract

The accumulation of heavy metals in the agricultural environments causes the oxidative stress in leading physiological and biochemical alterations in the plants. Regulation of fatty acids is considered as an adaptive mechanism for plants exposed to oxidative damages. In the present study, we investigated the changes of fatty acid composition with GC in the leaves of tomato subjected to increasing doses of heavy metals. The exposures of heavy metals changed the fatty acid compositions and α-linoleic acid, palmitic acid and linoleic acid were the main fatty acids in respect to percentage, respectively. The percentages of arachidic acid, behenic acid, lignoceric acid and docosahexaenoic acid clearly increased in leaves of tomato. The level of linoleic acid and palmitoleic acid significantly reduced in all application of heavy metals compared to control plants. The content of stearic acid and oleic acid methyl ester changed depending on heavy metal types and doses while the quantity of α-linolenic acid and palmitic acid remained unchanged by the treatment of Cu and Pb, but the application of Cd slightly increased the percentage of α-linolenic acid in tomato leaves. The content of lipid peroxidation significantly increased in all exposures of heavy metal. The exposures of heavy metal increased the content of saturated fatty acid like arachidic acid, behenic acid and lignoceric acid, while heavy metals decreased linoleic acid and palmitoleic acid which belong to unsaturated fatty acid compared to control plants. These changes in the fatty acid percentages may be related to the increases of lipid peroxidation.

Keywords: Arachidic acid, Heavy metal, Linoleic acid, Palmitic acid, Palmitoleic acid, Tomato

Öz

Tarımsal alanlarda ağır metallerin birikimin bitkilerde fizyolojik ve biyokimyasal değişimlere yol açarak oksidatif strese neden olur. Oksidatif hasara maruz kalan bitkiler için yağ asitlerinin düzenlenmesi adaptasyon mekanizması olarak düşünülür. Mevcut çalışmada, artan dozda ağır metale maruz kalan domates bitkisi yapraklarında yağ asidi kompozisyonundaki değişimleri inceledik. Ağır metal uygulaması yağ asidi dağılımını değiştirdi ve α-linoleik asit, palmitik asit ve linoleik asit yüzde dağılım bakımından sırasıyla temel yağ asitleridir. Araşidik asit, behenik asit, lignoserik asit ve docosaheksaenoik asit yüzdeleri domates yapraklarında açık şekilde artmıştır. Kontrol bitkilerine kıyasla linoleik asit ve palmitoleik asit seviyeleri ağır metal uygulamasında önemli derecede azalmıştır. Stearik asit ve oleik asit metil ester içeriği uygulanan ağır metal tip ve dozuna bağlı olarak değişirken, α-linolenik asit ve palmitik asit miktarı Cu ve Pb uygulamasında değişmemiştir. Fakat Cd uygulaması domates yapraklarında α-linolenik asit yüzdesini hafif şekilde artırmıştır. Lipid peroksidasyon içeriği tüm ağır metal maruziyetlerinde önemli şekilde artmıştır. Kontrol grubuyla karşılaştırıldığında, ağır metaller araşidik asit, behenik asit ve lignoserik asit gibi doymuş yağ asidi içeriğini artırırken, doymamış yağ asitlerinden olan linoleik asit ve palmitoleik asit azalmıştır. Yağ asidi yüzdelerindeki bu değişimler lipid peroksidasyonundaki artışla ilişkili olabilir.

Anahtar Kelimeler: Ağır metal, Araşidik asit, Domates, Linoleik asit, Palmitik asit, Palmitoleik asit

1. Introduction

The presence of heavy metals in the plant cultivated medium causes critical damages and becomes toxic for plant creating various symptoms of injuries such as reduction of plant growth, inhibition of enzyme activities, disruption of mineral distribution, water imbalance and chlorosis (Gajewska et al. 2012). The accumulation of heavy metal in plant tissues increases with the application of pesticides and fertilizers. Heavy metals bind to sulfhydryl groups in the protein causing the disruption of the cell structure. They give

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rise to generation of oxidative stress by producing reactive oxygen species (ROS) such as superoxide radicals (O_2^{-}) and hydroxyl radicals (OH). These radicals react with the main molecules in the plant cells such as DNA, protein and lipids (Sun et al. 2007; Tanyolaç et al. 2007). Heavy metals activate several genes encoding enzymes related with fatty acid peroxidases including a-dioxygenases and lipoxygenases. Accumulation of ROS induced by heavy metal and activities of enzymatic peroxidases may undergo peroxidation of the polyunsaturated fatty acids (USFA) in plant membrane lipids resulting in damage and loss of membrane integrity. Heavy metals sensitivity improves with increasing fatty acid unsaturation in plasma membranes (Upchurch 2008).

Plants expose to various environmental stress and they must adapt to unsuitable conditions for survival due to plants' lack of mobility. They respond to heavy metal stress in a series of ways by induction of general stress response pathways. These responses include antioxidant defense system which consists of enzymatic defense system such as catalase, peroxidase and superoxide dismutase, and antioxidant molecules like glutathione and aromatic compounds (Kisa et al. 2016). Also, plants response to abiotic stress including heavy metals toxicity immediately by changing the membrane lipid compositions. Free radical reactions have been considered to play a significant role in the degradation process of membrane lipids under the environmental stress (Djebali et al. 2005). The alterations in the composition of plasma membrane may change the membrane permeability and unsaturation of membrane may be closely related to heavy metal tolerance in plants (Nouairi et al. 2006a). Membranes are the main targets of the degradative processes induced by oxidative stress and it has been considered that a decrease in membrane lipid content is related to an inhibition of lipid biosynthesis (Bettaieb et al. 2009). The regulation of membrane lipid fluidity by modifying the content of USFA is a feature of stress acclimating plants and this modification provides a suitable environment for the function of critical integral proteins under the various kind of stress (Upchurch 2008). The regulation of fatty acid synthesis is essential to keep the membrane functions and it is considered as an adaptive mechanism for plant subjected to oxidative stress. Fatty acids (FAs) are the main structural constituent of the plasma membrane and they are particularly sensitive to heavy metal stress (Chaffai et al. 2007). The level of free FAs increases in response to different stress exposures and polyunsaturated fatty acids (PUFAs) are released from membranes by lipase in response stress. In case of the imbalance between the prooxidant/antioxidant systems, oxidative stress injures to biomolecules such as nucleic acids and lipids. The presence of ROS leads to fragmentation of lipids containing polyunsaturated fatty acids into diverse products, especially in the presence of transition metal ions. The membrane lipids containing PUFAs are sensitive to peroxidation and the presence of reduced metals, lipid hydroperoxide can break down to reactive aldehyde products such as malondialdehyde (MDA) and 4-hydroxy-2-nonenal (HNE). Lipid peroxidation is degenerative process affecting the PUFA which particularly susceptible to peroxidation and the level of lipid peroxidation products increases under the oxidative stress conditions. The content of lipid peroxidation may alter many metabolic processes which the degree of the fatty acid unsaturation of lipids (Nouairi et al. 2006a, Catalá 2009, Walley et al. 2013).

Studies investigating the antioxidant enzymes, total phenolic compound and the levels of H_2O_2, MDA and photosynthetic pigments in plants exposed to various stress factors have been intensively performed in different plant tissues (Gonçaalves et al. 2007, Skórzyńska-Polit et al. 2010, Li et al. 2013). However, there are relatively little information available on the effect of oxidative stress caused by heavy metals which is a significant issue understanding physiological changes because of the relationship between lipid composition and oxidative stress. The goal of the present study is to investigate the effect of heavy metals on fatty acid composition in the leaves of tomato exposed to different doses of Cd, Cu and Pb in relation to lipid peroxidation which is general index about stress conditions in plants.

2. Materials and Methods

2.1. Plant Material and Growth Conditions

Tomato (*Solanum lycopersicum* cv. ***çiko*** F1) seedlings were grown in plastic boxes containing the mixture of peat and garden soil (1:1) in unheated glasshouse conditions. The trial was carried as randomized plot design with three replications under the 16:8 light/dark and 24±3°C temperature. N, P and K were administered at 100 ppm doses, and Fe, B and Mg were applied in 20 ppm for plant growth and development. About three weeks after the acclimatization, the tomato plants were exposed to 10, 20 and 50 ppm doses of $CdCI_2$, $CuSO_4$ and $Pb(NO_3)_2$ salts as a heavy metal sources. The treatment of heavy metal solutions was done three times with an interval of 2 days and one time a day in the early hours of the morning. The leaves of tomato were harvested

two weeks after the heavy metal exposures and the samples were kept at -80°C until the fatty acid analysis.

2.2. Analysis of Fatty Acid by Gas Chromatography

The fresh leaves of tomato were air-dried in oven at 65°C constant weight. The dried samples were finely grinded into powders and lipids were extracted with chloroform-methanol (2:1) as described previously with minor modifications (Bligh and Dyer 1959). The homogenate was centrifuged at 4500x g for 10 min, and supernatant was added 1 % KCI and chloroform. The organic layer containing lipids was acidified with % 2 H_2SO_4, the mixtures were incubated for 12 hours and were added 5 % NaCI by vigorous shaking. The fatty acids methyl esters (FAMEs) were obtained by transmethylation. FAMEs were taken with 1 mL hexane into the vials for determining the fatty acid analysis. Fatty acid was analyzed by Gas Chromatography equipped Flame Ionization Detector (GC-FID), Perkin Elmer Precisely, Clarus 500 Series GC system, with an apolar capillary column (30 m×0.25 mm and 0.20 µm ID, Rtx-2330, USA) and helium was used as carrier gas. The temperature of the injector and detector were 250 and 260°C, respectively. The oven initial column temperature was held 100°C for 1 min, followed by a 10°C/min ramp to 180°C and a second ramp of 2°C/min to 230°C. The identification of fatty acid was accomplished based on the comparison of their retention times with the basic standards (FAME Mix, Bellefonte, USA). The peak area percentages of compounds were calculated based on the FID data.

2.3. The Determination of Lipid Peroxidation

The content of MDA was used to determine the lipid peroxidation level of samples by using thiobarbituric acid (TBA) method (Sreenivasulu et al. 1999). The leaves were crushed into liquid N_2, homogenized in 4 ml of 0.1 (w/v) TCA and then centrifuged at 10000x g for 20 min. The 0.5 ml of the supernatant was added to 1 ml of 20 % TCA containing 0.5 % (w/v) TBA. The mixture was incubated at 95°C hot water bath for 30 min and the reaction stopped by cooling the tubes in an ice bath. They were centrifuged at 10000x g for 5 min. The absorbance of the reaction was measured at 532 nm and the value for non-specific at 660 nm was subtracted by using spectrophotometer (Carry 50 UV/VIS, Japan). The content of MDA was calculated using the absorption coefficient of 155 nM^{-1} cm^{-1}.

2.4. Statistical Analyses

The experimental results were analyzed with SPSS software 20.0 by using Duncan multiple tests under the one-way ANOVA program. The numerical data of samples are indicated as mean ± S.D., $P < 0.05$ was regarded as significant differences in connection with the control groups.

3. Results

3.1. The Effect of Heavy Metals on the Fatty Acid Compositions

The content of arachidic acid (20:0), behenic acid (22:0), lignoceric acid (24:0), docosahexaenoic acid (22:6n3), linoleic acid (18:2n6), palmitoleic acid (16:1), α-linolenic acid (18:3n3), palmitic acid (16:0), stearic acid (18:0) and oleic acid methyl ester (18:1n9c) were determined in the leaves of tomato subjected to different doses of Cd, Cu and Pb. The application of them changed the content of fatty acids. α-linoleic acid, palmitic acid and linoleic acid were the main fatty acids, and they have a high proportion in regard of percentage, respectively. However, arachidic acid, behenic acid, lignoceric acid, docosahexaenoic acid, palmitoleic acid, stearic acid and oleic acid methyl ester have comparatively low percentages in all leaves of tomato. The level of arachidic acid and behenic acid significantly increased in application of heavy metals compared to control plants and these changes are shown Figure 1.

The application of heavy metals significantly raised the content of lignoceric acid, and docosahexaenoic acid except for 10 and 20 ppm of Cu, and the results are shown in Figure 2. The percentage of linoleic acid and palmitoleic acid significantly reduced in all treatment of heavy metals compared to control plants ($p<0.05$) and they are shown in Figure 3.

The levels of α-linolenic acid and palmitic acid remained unchanged by the treatment of Cu and Pb, but Cd slightly increased the percentage of α-linolenic acid in leaves and all results are shown in Figure 4. The quantity of stearic acid and oleic acid methyl ester in leaves of tomato growth in heavy metal contaminated boxes changed depending on heavy metal types and doses. The addition of Pb on tomato cultivated soil increased the content of stearic acid by the all application compared to control groups. Also, the application of Cd and Pb at high doses (50 ppm) increased the level of oleic acid methyl ester ($p<0.05$). The results of stearic acid and oleic acid methyl ester are shown in Figure 5.

3.2. The Effect of Heavy Metals on the Lipid Peroxidation

The application of heavy metals on tomato cultivated medium significantly increased the level of lipid peroxidation

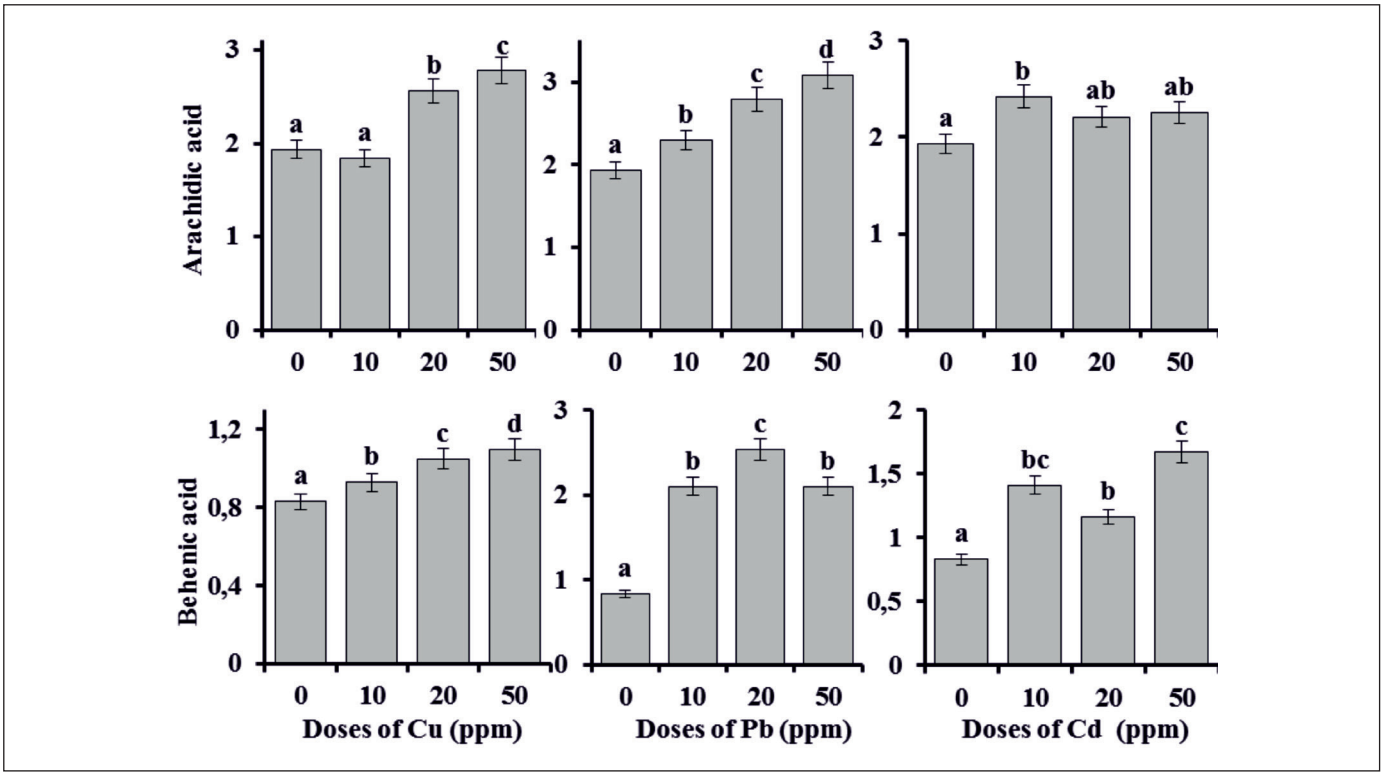

Figure 1. The effect of heavy metals on arachidic acid and behenic acid in the leaves of tomato. Mean values (The data in the y-axis shows the percentage of fatty acids) with different letter are significantly different at p ≤0.05 (Duncan test). Data expressed as a percentage of fatty acids per gram fresh weight.

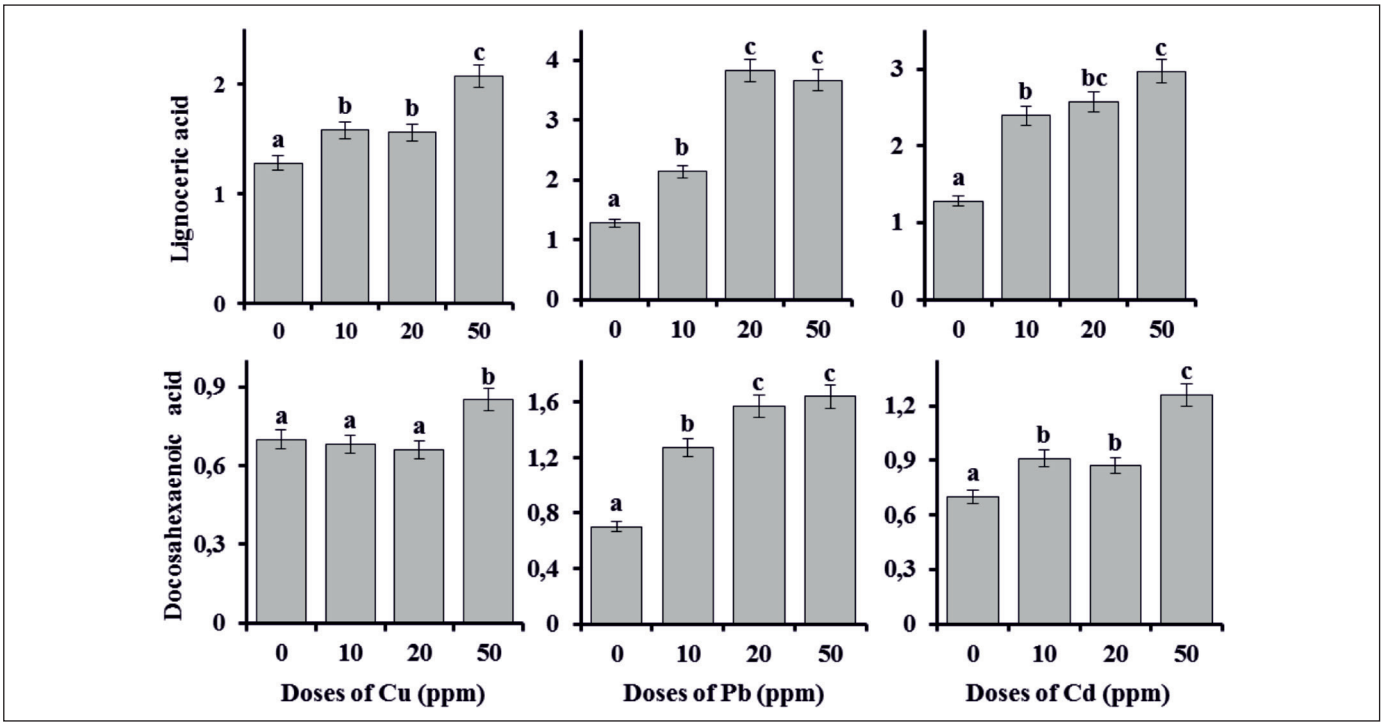

Figure 2. The effect of heavy metals on lignoceric acid and docosahexaenoic acid in the leaves of tomato. Mean values (The data in the y-axis shows the percentage of fatty acids) with different letter are significantly different at p ≤0.05 (Duncan test). Data expressed as a percentage of fatty acids per gram fresh weight.

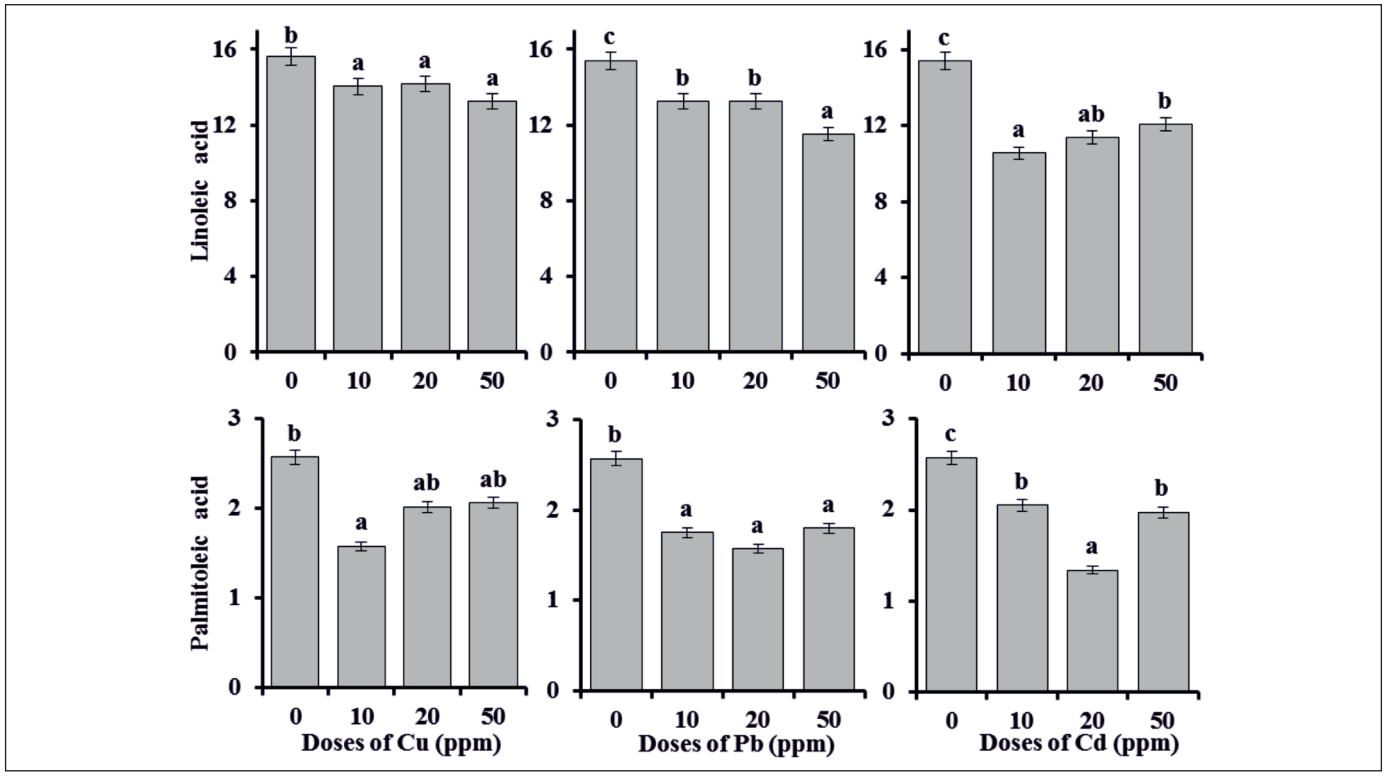

Figure 3. The effect of heavy metals on linoleic acid and palmitoleic acid in the leaves of tomato. Mean values (The data in the y-axis shows the percentage of fatty acids) with different letter are significantly different at p ≤0.05 (Duncan test). Data expressed as a percentage of fatty acids per gram fresh weight.

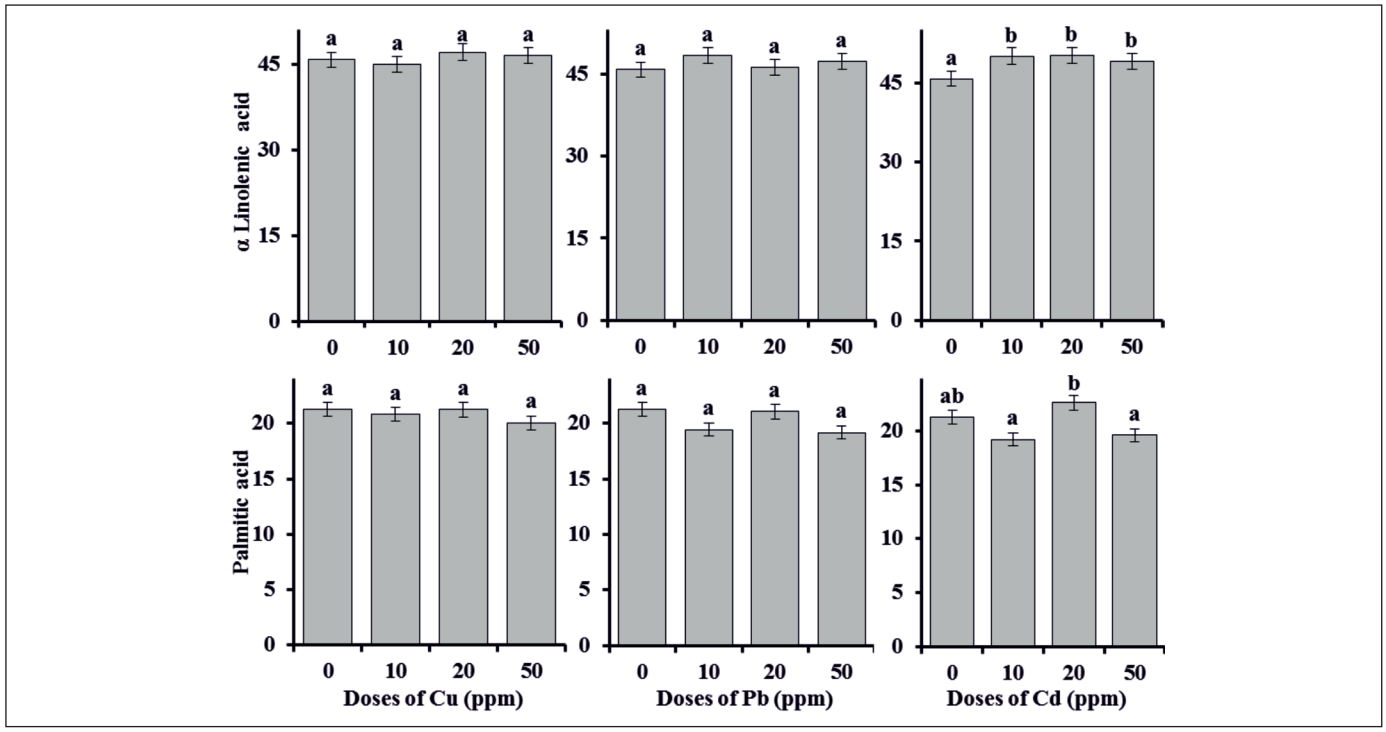

Figure 4. The effect of heavy metals on α-linolenic acid and palmitic acid in the leaves of tomato. Mean values (The data in the y-axis shows the percentage of fatty acids) with different letter are significantly different at p ≤0.05 (Duncan test). Data expressed as a percentage of fatty acids per gram fresh weight.

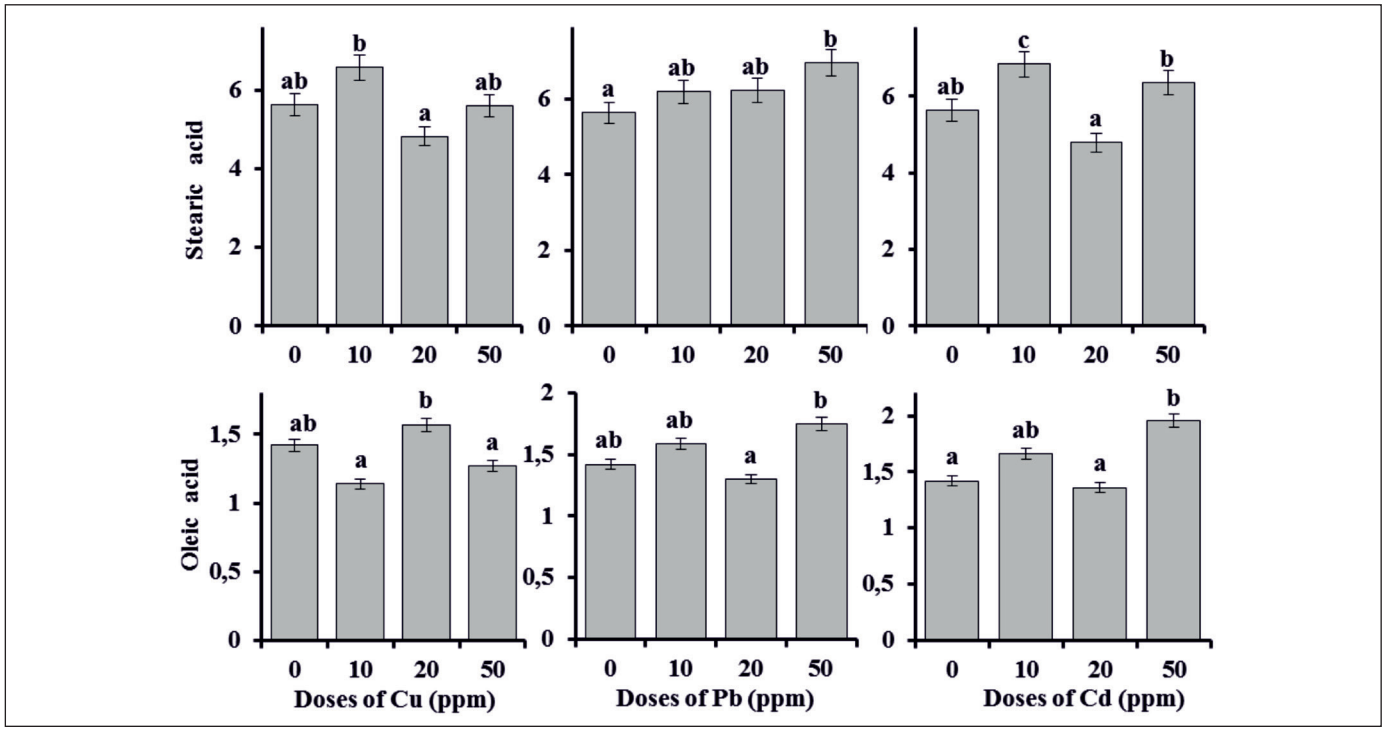

Figure 5. The effect of heavy metals on stearic acid and oleic acid methyl ester in the leaves of tomato. Mean values (The data in the y-axis shows the percentage of fatty acids) with different letter are significantly different at p ≤0.05 (Duncan test). Data expressed as a percentage of fatty acids per gram fresh weight.

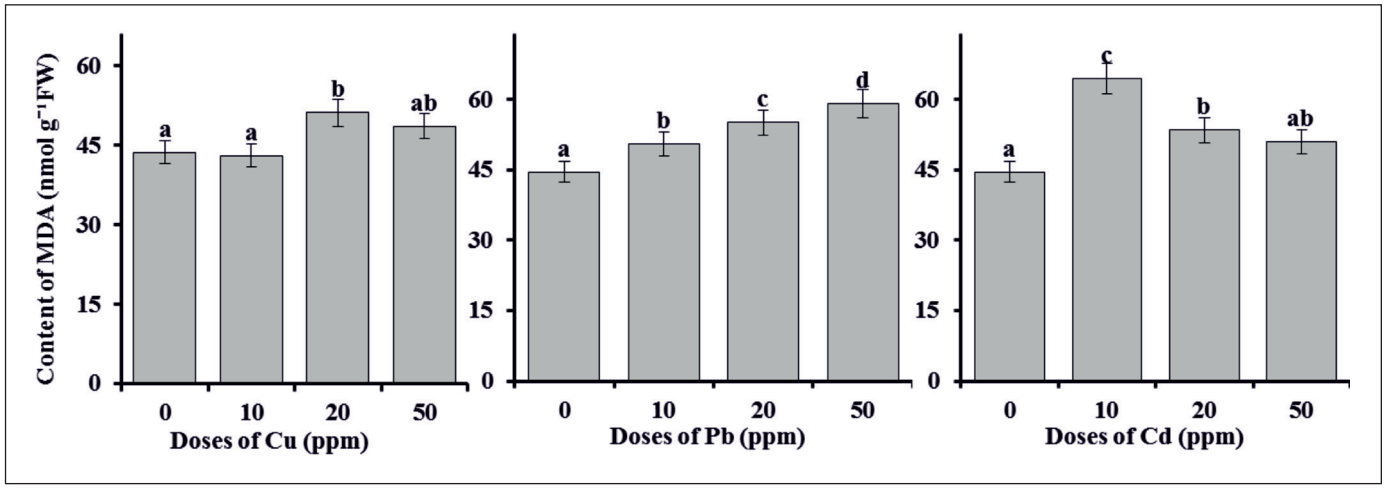

Figure 6. The effect of heavy metals on the content of MDA in the leaves of tomato. Mean values with different letter are significantly different at p ≤0.05 (Duncan test). Data expressed as a nmol g^{-1} per fresh weight, respectively.

in the leaves of tomato. However, the treatment of Cu at low doses (10 ppm) didn't show a significant change on the content of MDA in the leaves compared to control plants. The level of MDA increased with increasing doses of Pb and the treatment of Cd raised the level of lipid peroxidation, but it changed depending on applied doses. The results of lipid peroxidation are shown in Figure 6.

4. Discussion

The lipid composition of plant cell membranes changes with variations in the plant growth environment (Nouairi et al. 2006b). Lipids make up the key structure of biological membranes. The cell membrane is the first structure of plant cell and target of the oxidative stress. The properties

of membranes depend on the fatty acid composition and changes in the fatty acid profiles may cause unusual process. The peroxidation of unsaturated fatty acid is the prominent system of oxidative stress in plants (Djebali et al. 2005, Catalá 2009).

In the present study, , the effect of heavy metals on fatty acid composition and lipid peroxidation in the leaves of tomato exposed to heavy metals are investigated. The application of them significantly increased the level of arachidic acid, behenic acid and lignoceric acid which belong to saturated fatty acids. The treatment of Cu, Cd and Pb significantly decreased the percentage of unsaturated fatty acids (USFA) which are linoleic acid and palmitoleic acid compared to control groups. The level of docosahexaenoic acid significantly increased by the addition of heavy metals on plant growth medium except for 10 and 20 ppm of Cu. The exposures of tomato to the Cu and Pb didn't generally show a significant change at the percentage of α-linolenic acid and palmitic acid which are unsaturated and saturated fatty acid respectively, but the Cd slightly increased in the leaves of tomato compared to uncontaminated plants. The content of stearic acid increased by the addition of Pb, and the content of oleic acid methyl ester raised by the treatment of high doses of Cd and Pb (50 ppm) in the leaves compared to controls. In general, the percentage of stearic acid and oleic acid varied with the kind of heavy metal types and doses. A study carried on tomato reported that the percentage of stearic acid (18:0), oleic acid (18:1) and linoleic acid (18:2) increased while the percentage of hexadecatrienoic acid (16:3) and linolenic acid (18:3) decreased by the treatment of Cd (Djebali et al. 2005). It was previously reported that the level of linolenic acid (18:3) is significantly reduced, and the content of linoleic (C18:2), oleic (C18:1) and stearic (C18:0) acid increased in the leaves of *Populous nigra* cultivated in the contaminated soils with heavy metals such as Cd, Cr, Cu, Ni, Pb and Zn (Guedard et al. 2012). The exposure of wheat seedling to Ni decreased linolenic acid (18:3) in shoots and roots of plants (Gajewska et al. 2012). The content of saturated fatty acids mainly palmitic acid increased while the content of unsaturated fatty acid like hexadecatrienoic acid (16:3n-3) and α-linolenic acid (18:3n-3) declined in response to Cd contamination in spinach compared to control groups (Zemanová et al. 2015). It was noted that the level of linolenic, oleic and linoleic acid significantly decreased, and the percentage of the palmitic significantly increased in the sunflower exposed the $CdCI_2$ (Moradkhani et al. 2012). The study on tomato showed that the quantity of linolenic acid (18:3) and hexadecatrienoic acid (16:3) decreased while oleic acid (18:1) palmitic acid (16:0) and linoleic acid (18:2) increased in the leaves cultivated in the metal contaminated soils (Verdoni et al. 2001). The level of PUFAs mainly linolenic acid (C18:3) and hexadecatrienoic acid (C16:3) decreased in the young leaves of *Brassica napus* exposed to Cd (Nouairi et al. 2006a). The content of saturated fatty acid (SFA) like palmitic acid and stearic acid increased while USFAs like linoleic acid and linolenic acid decreased in response to heat stress in the leaves of *Bryophyllum pinnatum* (Ahmad et al. 2013). Another study reported that drought stress decreased significantly the foliar fatty acid content like oleic, linoleic and linolenic acids in the leaves of *Salvia officinalis* (Bettaieb et al. 2009).

The accumulation of heavy metals in the plant tissues can causes the lipid peroxidation. In the current work, the exposures of heavy metals significantly increased the level of lipid peroxidation in leaves of tomato, but the content of MDA remained unchanged when the tomato exposed to the low doses of Cu (10 ppm) compared to control groups. The study on tomato reported that the content of MDA significantly increased in chloroplast of the seedling leaves of tomato exposed to NaCI and Se (Diao et al. 2014). It was declared that the significant increases in the level of MDA were observed in both roots and leaves of barley after the plant exposed to stress environments under the combined toxicity of Al, Cu and Cd (Guo et al. 2007). Studies on maize plants stated that the application of Cd and Cr increased the content of lipid peroxidation in the leaves of maize (Pál et al. 2005, Maiti et al. 2012) 25 and 50 μM Cd (NO_3). The application of heavy metals affects the lipid compositions leading to significant changes in fatty acid contents. The decreases of the PUFA concentration like linoleic acid and palmitoleic acid may be related to direct reaction of free radicals with unsaturated fatty acids. It is considered that the degree of fatty acid saturation/unsaturation ratio is the important factor mediating in the keeping of membrane fluidity and provides the appropriate environment for membrane function. The fatty acid composition have the functional role in regulating membrane functions and provides the processes of plant adaptation under unsuitable conditions (Nouairi et al. 2006b, Bettaieb et al. 2009, Rahayu et al. 2014).

In conclusion, this study showed significant changes in the fatty acid composition of tomato leaves and alterations of fatty acids percentages may be related to the increases of

lipid peroxidation, which MDA content is considered as an indicator of oxidative damage to lipids. It is observed that α-linoleic acid, palmitic acid and linoleic acid were found to be the abundant fatty acids, while arachidic acid, behenic acid, lignoceric acid, docosahexaenoic acid, palmitoleic acid, stearic acid and oleic acid were present in the low concentrations. The study highlights that the content of SFAs like arachidic (20:0), behenic (22:0) and lignoceric (24:0) acids increased, but the percentages of USFA such as linoleic (18:2n6) and palmitoleic (16:1) acids decreased in the leaves of tomato exposed to different doses and types of heavy metals. The application of heavy metals generally increased the level of lipid peroxidation in the leaves of tomato. To further elucidate the responses of fatty acid composition to various environmental conditions, additional studies are needed to clearly define because ten fatty acids were investigated and next studies can be carried out by adding further fatty acids in the tissues of plant grown under the various stress conditions.

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