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Nitric oxide production and antioxidant responses in maize under lead stress

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Abstract
Lead pollution is an important issue in the world. In this research, maize plants were treated with different concentrations of Pb (0, 0. 25, 0.75, 1, 1.5, 2.5 and 5 mM) as lead (II) nitrate for 10 days. The results showed that an increase in lead concentration leads to a reduction in growth, chlorophyll a and b content and an increase in oxidative damages. Application of Pb caused a progressive increase of hydrogen peroxide content, which was followed by a significant level of lipid peroxidation. These changes were accompanied by an increase in nitric oxide content at the low and its reduction at the high concentrations of Pb. The small increase in the generation of hydrogen peroxide and nitric oxide are envisaged as messengers in signaling pathways that may act in triggering defense functions to detoxify lead. Therefore, plant stress tolerance variables, including proline, ascorbic acid, flavonoids and anthocyanins content and activity of antioxidant enzymes, enhanced under different lead applications.

Keywords: Antioxidants; Lead stress; Nitric oxide; Oxidative stress; *Zea mays* L.

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Introduction

Lead (Pb) is a dangerous heavy metal contaminant that leads to inhibition of metabolic processes, growth and productivity of plants (Sharma and Dubey 2005). One of the detrimental effects of Pb is the balanced cellular redox status disruption and oxidative stress induction (Verma and Dubey 2003). It is well characterized that H_2O_2 accumulation, as the most stable form of reactive oxygen species (ROS), has a harmful effect on plants, but can act as messengers involved in signal transduction pathways, especially at low amounts (Cuypers *et al*. 2016). Previous data have indicated the involvement of nitric oxide (NO) in plant acclimation and heavy metal tolerance (Yu *et al*. 2012). The activity of the signal transductor NO can cause changes in the antioxidant system and the activation of numerous defense mechanisms under stress conditions (Xiong *et al*. 2010). The interaction between NO and H_2O_2 could also be involved in response to abiotic stresses including Pb. At low Pb content, sometimes plants can show adaptive response. However, at high Pb concentration, plants cannot combat Pb-induced oxidative stress (Kaur *et al*. 2015).

Plants respond to heavy metal stress by activating multiple responses. Under metal stress conditions, metal ion chelation is one of the defense strategies in plants (Hassan and Aarts 2011). Proline is one of the chelating compounds, which contributes to metal chelation in the cytoplasm and is involved in the amelioration of detrimental effects owing to heavy metal excess (Kovacik *et al*. 2010). To alleviate oxidative stress, plants have enzymatic and non-enzymatic antioxidant defense mechanisms to quench ROS (Smirnoff 1993). So, this study aimed to evaluate lead stress effects on maize (*Zea mays* L.) plants by evaluating chlorophyll a and b, lipid

peroxidation, endogenous signaling molecules content (NO and H_2O_2), non-enzymatic antioxidants and enzymatic antioxidants activity after exposure to different lead concentrations.

Materials and Methods

Experiment preparation

Seeds of maize (cv. 704) were provided from the Agriculture Research Center of Kerman, Iran. Uniform-sized seeds were surface sterilized in 10% (w/v) sodium hypochlorite, washed several times with sterilized distilled water and dipped into distilled water for 12 h. Thereafter, healthy seeds were germinated for three days. Then, seedlings were planted into plastic pots filled with sterilized sand and perlite (2:1 ratio). Growth chamber conditions for growth were set to day/night temperatures of 29/20 °C with a 16-h photoperiod, the relative humidity of 60/80% and light intensity of 150 µmol m^{-2}.s. Six-days-old seedlings were used for exposure to different Pb concentrations (0, 0. 25, 0.75, 1, 1.5, 2.5 and 5 mM) for 10 days. Finally, all plants in each pot were harvested and the shoots and roots were separated and stored at -80 ˚C until use.

Measurement of variables

Chlorophyll content was assayed using the methods of Lichtenthaler (1987). Anthocyanin content of leaves was determined according to Fulcki and Francis (1968). The determination of total flavonoid content was performed after reaction with the aluminum chloride according to the method of Chang *et al*. (2002) and catechin as the standard.

Hydrogen peroxide content was measured after reaction with potassium iodide (KI) according to the method of Alexieva *et al*. (2001). Malondialdehyde (MDA) content was measured by the method proposed by Heath and Packer (1968). Proline was extracted and estimated according to Bates *et al*. (1973). The determination of ascorbic acid (AsA) content was performed by the method of Omaye *et al*. (1979) using DTC (2,4-dinitrophenylhydrazine/thiourea/copper) reagent and AsA as the standard. Endogenous NO content was estimated by the method described by Ding *et al*. (1998) and Hu *et al*. (2003) with small changes using Greiss reagent and standard curve of $NaNO_2$.

For antioxidant enzyme extraction, 500 mg of fresh samples were ground in 50 mM potassium phosphate buffer (pH 7.0) containing 1% soluble polyvinyl polypyrrolidone (PVP), 1 mM EDTA and 1 mM phenylmethylsulfonyl fluoride (PMSF). The centrifugation of homogenate was performed at 10000 g for 20 min, and the supernatant was stored for different analyses of the enzyme activity.

The method of Dhindsa *et al*. (1981) was used for the assay of Catalase (CAT) activity by determining the decrease in absorbance due to H_2O_2 oxidation at 240 nm using the extinction coefficient of 40 $mM^{-1}cm^{-1}$ for H_2O_2. Ascorbate peroxidase (APX) activity was determined according to Nakano and Asada (1981) by observing the decrease in absorbance due to ascorbic acid oxidation for 1 min at 290 nm using the extinction coefficient of 2.8 $mM^{-1}cm^{-1}$.

Statistical analysis

All data were subjected to one-way analysis of variance and the means were compared using Duncan's multiple range test. Statistical analyses were carried out with the SPSS 19 Program.

Results

The data in Table 1 display that lead stress decreased both root and shoot lengths significantly at all concentrations, except at 0.25 mM. Results showed that under lead stress, chlorophyll b content decreased significantly in maize plants at the three highest Pb concentrations, 1.5, 2.5 and 5 mM, and chlorophyll a content at the two highest Pb concentrations, 2.5 and 5 mM. While exposing maize plants to other concentrations of lead did not show any significant effect on the contents of chlorophyll (Table 1). Pb treatments significantly increased the level of anthocyanins in the maize leaves in comparison with non-stressed plants and the highest level was observed at 1.5 mM concentration (Table 3). A rise in the levels of total flavonoids was recorded in the Pb treatments as compared with the control in both roots and shoots of the maize plants. The increase descended at 5 mM concentration (Table 2).

Table 1. Effects of different concentrations of Pb on root and shoot length, chlorophyll a and b and anthocyanin content of maize.

Treatment	Tissue	Length (cm)	Chlorophyll a (mg/gFW)	Chlorophyll b (mg/gFW)	Anthocyanin (µg/gFW)
Control	Root	$26.33^{a}\pm0.88$	$1.395^{ab}\pm0.55$	$0.644^{a}\pm0.55$	$38.222^{c}\pm2.91$
	Shoot	$36.33^{a}\pm0.88$			
Pb0.25	Root	$25^{ab}\pm0.57$	$1.380^{ab}\pm0.94$	$0.553^{ab}\pm0.66$	$48.350^{b}\pm0.50$
	Shoot	$37.33^{a}\pm0.88$			
Pb0.5	Root	$23b^{c}\pm0.57$	$1.452^{a}\pm0.55$	$0.639^{ab}\pm0.25$	$49.330^{b}\pm1.19$
	Shoot	$30^{b}\pm0.57$			
Pb1	Root	$22^{cd}\pm0.57$	$1.321^{ab}\pm0.78$	$0.511^{abc}\pm0.29$	$54.130^{ab}\pm0.35$
	Shoot	$28.9^{b}\pm0.60$			
Pb1.5	Root	$20.8^{cd}\pm0.92$	$1.296^{abc}\pm0.12$	$0.451^{bc}\pm0.32$	$59.550^{a}\pm1.17$
	Shoot	$28.7^{b}\pm0.69$			
Pb2.5	Root	$19.7^{d}\pm0.73$	$1.210^{c}\pm0.53$	$0.383^{c}\pm0.53$	$54.440^{ab}\pm1.51$
	Shoot	$21.6^{c}\pm1.45$			
Pb5	Root	$16.8^{e}\pm1.58$	$1.102^{c}\pm0.58$	$0.361^{c}\pm0.19$	$48.400^{b}\pm1.80$
	Shoot	$18.5^{d}\pm0.86$			

Values followed by different letters within a column indicate a significant difference by Duncan's Multiple Range Test.

Proline concentration (Table 2) increased in roots and shoots of maize plants due to the Pb stress up to 2.5 mM, while the proline content at 5 mM concentration was not significantly different from the control. Generally, results showed that AsA content was increased with increasing Pb concentration. However, the changes in shoot AsA content at 0.25 and 5 mM concentrations and shoot and root AsA contents at 2.5 mM were not significant. Root AsA content decreased at the highest Pb concentration.

Table 2. Effects of different concentrations of Pb on proline, ASA, flavonoid and NO content of maize.

Treatment	Tissue	Proline (µg/gFW)	ASA (µg/gFW)	Flavonoid (mg/gFW)	NO (nmol/gDW)
Control	Root	$131.469^{d}\pm10.70$	$5.434^{b}\pm0.06$	$0.051^{c}\pm0.004$	$60.093^{b}\pm1.61$
	Shoot	$73.118^{e}\pm4.17$	$7.141^{b}\pm0.13$	$0.123^{c}\pm0.009$	$52.870^{b}\pm2.25$
Pb0.25	Root	$167.311^{c}\pm9.93$	$6.817^{a}\pm0.31$	$0.075^{b}\pm0.007$	$78.981^{a}\pm1.76$
	Shoot	$157.276^{d}\pm5.16$	$8.010^{ab}\pm0.50$	$0.222^{b}\pm0.01$	$62.500^{a}\pm3.20$
Pb0.75	Root	$218.207^{ab}\pm8.72$	$7.121^{a}\pm0.52$	$0.078^{b}\pm0.007$	$76.944^{a}\pm5.67$
	Soot	$190.967^{c}\pm4.47$	$8.194^{a}\pm0.23$	$0.225^{ab}\pm0.008$	$68.796^{a}\pm3.29$
Pb1	Root	$223.942^{ab}\pm13.62$	$7.296^{a}\pm0.50$	$0.077^{b}\pm0.007$	$77.685^{a}\pm1.61$
	Shoot	$214.623^{b}\pm8.95$	$8.472^{a}\pm0.15$	$0.228^{ab}\pm0.01$	$68.611^{a}\pm2.88$
Pb1.5	Root	$250.465^{a}\pm12.49$	$7.713^{a}\pm0.72$	$0.082^{b}\pm0.007$	$75.463^{a}\pm4.89$
	Shoot	$236.845^{a}\pm6.12$	$8.665^{a}\pm0.27$	$0.259^{a}\pm0.004$	$70.833^{a}\pm1.60$
Pb2.5	Root	$210.322^{b}\pm12.41$	$4.123^{bc}\pm0.26$	$0.107^{a}\pm0.002$	$50.463^{b}\pm3.33$
	Shoot	$197.491^{bc}\pm6.84$	$7.799^{ab}\pm0.26$	$0.244^{ab}\pm0.01$	$42.685^{c}\pm4.58$
Pb5	Root	$96.344^{d}\pm13.82$	$3.510^{c}\pm0.42$	$0.075^{b}\pm0.001$	$39.166^{c}\pm2.56$
	Shoot	$71.182^{e}\pm5.21$	$7.108^{b}\pm0.26$	$0.221^{b}\pm0.002$	$35.092^{c}\pm1.61$

Values followed by different letters within a column indicate a significant difference by Duncan's Multiple Range Test.

Under Pb treatments, the root and shoot NO content increased at the concentrations of 0.25 to 1.5 mM and decreased at higher concentrations as compared with the control (Table 2). A linear increase in the H_2O_2 level was observed with increasing Pb concentrations compared to the untreated plants in both roots and shoots of maize plants (Table 3). In the plants exposed to 0.25 mM of lead, no significant difference was observed between shoot MDA content and the control, whereas higher Pb concentrations significantly elevated MDA content in both roots and shoots. The highest MDA content was observed at the 5 mM Pb (Table 3).

Shoot APX activity was increased at 0.25 mM Pb concentration and decreased at 5 mM compared to control, while it did not exhibit significant variation in other treatments. In the case of roots, APX activity did not change significantly at 0.75, 1.5 and 5 Pb concentrations,

but increased significantly at 0.25, 1 and 2.5 mM treatments. A significant increase of CAT activity under Pb treatments was observed in both roots and shoots of the maize plants as compared to the control (Table 3).

Table 3. Effects of different concentrations of Pb on H_2O_2 and MDA content and APX and CAT activity of maize.

Treatment	Tissue	H_2O_2 (µg/gFW)	MDA (µg/gFW)	APX activity (µmol min^{-1} g^{-1} FW)	CAT activity (µmol min^{-1} g^{-1} FW)
Control	Root	0.163^{d}±0.06	0.951^{f}±0.02	64.508^{c}±3.46	24.500^{d}±0.90
	Shoot	0.942^{e}±0.05	1.038^{e}±0.03	76.116^{bc}±4.86	33.500^{d}±3.60
Pb0.25	Root	1.292^{c}±0.07	1.086^{e}±0.006	100.223^{a}±5.03	72.250^{a}±4.75
	Shoot	1.010^{e}±0.09	1.134^{ed}±0.03	104.463^{a}±4.35	79.000^{ab}±6.33
Pb0.75	Root	1.610^{bc}±0.08	1.122^{e}±0.02	70.982^{c}±2.78	46.625^{c}±1.62
	Soot	1.588^{d}±0.05	1.197^{d}±0.01	86.830^{bc}±1.94	80.000^{ab}±5.44
Pb1	Root	2.153^{bc}±0.23	1.379^{d}±0.03	83.035^{b}±0.38	77.750^{a}±1.95
	Shoot	2.041^{c}±0.13	1.567^{c}±0.01	84.375^{b}±2.78	83.750^{ab}±6.15
Pb1.5	Root	2.225^{bc}±0.44	1.590^{c}±0.01	71.428^{c}±1.18	71.750^{a}±2.88
	Shoot	2.368^{b}±0.03	1.623^{c}±0.01	85.715^{b}±2.04	53.500^{c}±2.53
Pb2.5	Root	2.543^{b}±0.33	1.828^{b}±0.06	89.062^{b}±2.78	78.500^{a}±4.11
	Shoot	2.624^{b}±0.06	1.746^{b}±0.05	74.107^{cd}±2.33	92.250^{a}±2.63
Pb5	Root	3.887^{a}±0.51	2.140^{a}±0.07	72.544^{c}±2.51	57.750^{b}±1.88
	Shoot	2.717^{a}±0.08	2.113^{a}±0.04	64.955^{d}±4.18	70.500^{bc}±2.17

Values followed by different letters within a column indicate a significant difference by Duncan's Multiple Range Test.

Discussion

The present study shows that Pb is more toxic to maize at high concentrations as shown by the toxicity symptoms such as decreases in the biomass and chlorophyll a and b content and elevation of hydrogen peroxide, which induced lipid peroxidation. One of the primary effects of lead stress on the plant is the aggressive reduction of plant growth. Such growth retardation is due to disturbance in various physiological and biochemical processes. John *et al*. (2012) also reported a notable decrease in early seedling growth and root and shoot length of *Brassica juncea* L. under Pb and Cd stress in a concentration-dependent manner.

In the present study, we observed Pb effects on the photosynthetic pigments and decreased levels of chlorophyll a and chlorophyll b at the highest Pb concentrations (Table 1). One of the biochemical alterations occurring when plants are treated to Pb stress is the production of reactive oxygen species (Verma and Dubey 2003). The ability of ROS to cause photooxidative damage in organic molecules could probably explain the structural damages in the chloroplasts and the reduction of leaf chlorophyll. Similarly,

reductions in the level of photosynthetic pigments, including chlorophyll a and b, after exposure to heavy metals, including Pb, have been observed in many plant species (Sharma and Dubey 2005).

Generation and localization of anthocyanins cause plant resistance against abiotic stresses (Baek *et al*. 2012). Anthocyanin accumulation in stressed plants is believed to protect the plants from various types of stress (Baek *et al*. 2012). Other reports have also revealed that anthocyanins are accumulated in plants as a response to heavy metal stresses (Baek *et al*. 2012).

NO production has been well established to be an initial response of plants to multiple stresses. NO intercedes reactions to biotic and abiotic stresses (Yu *et al*. 2012). Low concentrations of NO act as a ROS inhibitor or scavenger and also improve plant resistance by inducing the expression of some genes (Xiong *et al*. 2010). Our results showed that long-term treatment with Pb significantly increased the endogenous level of NO (Table 2). A similar response to copper treatment (long-term) was previously noticed in two plants (Bartha *et al*. 2005). It is proposed that NO produced by plants challenged with low Pb concentrations could mediate signaling responses leading toward metal tolerance. But at higher concentrations, we observed a dose-dependent decrease in the production of NO. It has been reported that the effects of heavy metals on endogenous NO content depend on plant species, different metal concentrations and periods of metal treatment (Xiong *et al*. 2010). Similar results have been obtained by Lehotai *et al*. (2011) who showed that endogenous levels of NO decreased under high concentrations of Cu. They reported that the decrease in NO level could be attributed to the superoxide radicals by the reaction yielding peroxynitrite.

Our results demonstrated that accumulation of proline concentration (Table 2) increase in roots and shoots of the maize plants exposed to Pb concentrations up to 5 mM. Proline acts as an osmolyte, metal chelator, ROS scavenger, electron sink, protector of macromolecules and a component of the cell wall (Rejeb *et al*. 2014). Therefore, the increase in proline content might have a contribution to the improvement of plant defense. It has been reported that ROS and NO signaling induce proline accumulation in heavy metal stress (Zhang *et al*. 2008; Guzel and Terzi 2013). Therefore, H_2O_2 and NO signaling pathways and their interaction in a network cause accumulation of proline that increases plant's tolerance against stress. In this investigation, the lowest proline content was observed at the 5-mM Pb treatment. Similar results of proline content enhancement by Cd^{2+} were also shown by John *et al*. (2012) in *Brassica juncea* L. They found that accumulation of proline reduced with exposure to higher concentrations of Cd. The decrease in proline content may be due to the increase in degradation. The proline degradation pathway takes place in mitochondria by the consecutive action of proline dehydrogenase (ProDH) and pyrroline-5-carboxylate dehydrogenase. The activity of ProDH coincides with the accumulation of ROS (Rejeb *et al*. 2014). Therefore, proline catabolism promotes ROS levels.

AsA is a soluble antioxidant in plants, and the most substantial reducing/substrate for detoxification of H_2O_2 (Singh *et al*. 2005). In the present investigation, lead treatment increased the concentration of AsA (Table 2). The icreasing concentration of ascorbate in wheat seedlings caused by Pb stress was noticed by Genisel *et al*. (2015). It has been reported that NO might serve as downstream of physiological events (Wang *et al*. 2017). Wang *et al*. (2017) reported that NO accumulation could be necessary for endogenous AsA production. The increase of AsA content in this investigation under low lead concentration could be attributed to NO accumulation.

Flavonoids function as non-enzymatic antioxidants (Michalak 2006). Zafari *et al*. (2016) showed that NO together with H_2O_2 trigger signal transduction pathways to enhance the content of phenolic compounds. Results showed that treatment of Pb significantly increased total flavonoid content (Table 2). Therefore, we assume that low stimulation of NO at low Pb concentration can be the reason for flavonoid and anthocyanin enhancement. The production of antioxidant compounds is normally operated with heavy metal stress, but it can be prevented at certain stress levels (Michalak 2006). The enhancement of flavonoids content probably is in charge of metal ions binding, and also for their sequestration within the vacuole (Michalak 2006).

Oxidative damage in plants is generally experienced when Pb and availability other metals to plants is high (Sharma and Dubey 2005). H_2O_2 acts as a signaling molecule and has a dual role in the mechanism of plant defense. It improves tolerance and stress adaptation at lower content, whereas, it acts as a ROS at high intracellular levels and induces cellular damage resulting in cell death (Stone and Yang 2006). The present study demonstrated that H_2O_2 and MDA levels increased further with the enhancement in the concentration of the Pb (Table 3), which probably can be critical in growth inhibition and lipid peroxidation. Several studies showed that the level of H_2O_2 and MDA increased in response to heavy metal stresses (Kaur *et al*. 2015). At low concentrations, H_2O_2 acts as a secondary messenger and responds to environmental stress (acclimation) through crosstalk with signaling molecules such as NO (Cuypers *et al*. 2016) and possibly stress signal transduction by NO and H_2O_2 interceded defense responses at low Pb concentrations in this study. It has been reported that NO can ameliorate the toxic effects of heavy metal-induced oxidative stress by regulation of general mechanisms for cellular redox homeostasis and H_2O_2 accumulation (Mazid *et al*. 2011). Thus, the decrease in NO levels at high Pb concentrations can be the reason for higher H_2O_2 accumulation.

In our study, the maize plants treated with Pb exhibited an increase in CAT activity at all concentrations, however, an increase in the APX activity was only observed at some concentrations (Table 3). Enhanced activity of these enzymes in stress conditions is responsible for H_2O_2 scavenging. In many studies, it has been confirmed that Pb could stimulate and increase the production of ROS leading to enhancement in the antioxidant enzyme activities as a defense system (Verma and Dubey 2003; Sharma and Dubey 2005). The same results have been obtained by

Cho and Park (2000) in tomato plants grown under mercury stress. On the contrary, the decrease in shoot APX activity at higher Pb concentrations might be due to the down-regulation of gene expression or degradation and inactivation of these proteins (Lee *et al*. 2001).

Conclusions

The present results proved that the maize plants had a negative response to high Pb toxicity. The present study demonstrated that an increase in the concentration of lead caused a decrease in the NO content associated with more oxidative damage and membrane lipid peroxidation. Low concentrations of Pb resulted in a small stimulation of H_2O_2 and NO, which function as signal molecules implicated in the plant response to Pb stress.

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Conflict of Interest

The author declare that they have no conflict of interest with any organization in relation to the subject of the manuscript.

References

Alexieva V, Sergiev I, Mapelli S and Karanov E, 2001. The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat. Plant, Cell and Environment 24: 1337-1344.

Baek S, Han T, Ahn S, Kang H, Cho MR., Lee S and Im K, 2012. Effects of heavy metals on plant growths and pigment contents in *Arabidopsis thaliana*. Plant Pathology 28: 446-452.

Bartha B, Kolbert Z and Erdei L, 2005. Nitric oxide production induced by heavy metals in *Brassica juncea* L. Czern. and *Pisum sativum* L. Acta Biologica Szegediensis 49(1-2): 9-12.

Bates LS, Waldern RP and Tare ID, 1973. Rapid determination of free proline for water stress studies. Plant and Soil 29: 205-207.

Chang CC, Yang MH, Wen HM and Chern JC, 2002. Estimation of total favonoid content in Propolis by two complementary colorimetric methods. Journal of Food and Drug Analysis 10: 178-182.

Cho UH and Park JO, 2000. Mercury-induced oxidative stress in tomato seedlings. Plant Science 156(1): 1-9.

Cuypers A, Hendrix S, Amaral dos Reis R, De Smet S, Deckers J, Gielen H, Jozefczak M, Loix C, Vercampt H, Vangronsveld J and Keunen E, 2016. Hydrogen peroxide, signaling in disguise during metal phytotoxicity. Frontiers in Plant Science 7: 1-25.

Dhindsa RS, Dhindsa P and Thorpe AT, 1981. Leaf senescence correlated with increased levels of membrane permeability and lipid peroxidation and decrease levels of superoxide dismutase and catalase. Journal of Experimental Botany 32: 93-101.

Ding AH, Nathan CF and Stuehr DJ, 1998. Release of reactive nitrogen intermediates and reactive oxygen intermediates from mouse peritoneal macrophages. Journal of Immunology 141: 2407-2412.

Fulcki T and Francis FJ, 1968. Quantitative method for anthocyanins extraction and determination of total anthocyanin in cranberries. Journal of Food Science 33: 72-77.

Genisel M, Turk H, Erdal S, Demir Y, Genc E and Terzi I, 2015. Ameliorative role of beta-estradiol against lead-induced oxidative stress and genotoxic damage in germinating wheat seedlings. Turkish Journal of Botany 39(6): 1052-1060.

Guzel S and Terzi R, 2013. Exogenous hydrogen peroxide increases dry matter production, mineral content and level of osmotic solutes in young maize leaves and alleviates deleterious effects of copper stress. Botanical Studies 54(1): 26. doi: 10.1186/1999-3110-54-26.

Hassan Z and Aarts MGM, 2011. Opportunities and feasibilities for biotechnological improvement of Zn, Cd or Ni tolerance and accumulation in plants. Environmental and Experimental Botany 72: 53-63.

Heath RL and Packer L, 1968. Photoperoxidation in isolated chloroplast, kinetics and satoichiometry of fatty acid peroxidation. Archives of Biochemistry Biophysics 125: 189-198.

Hu X, Fang J, Cai W and Tang Z, 2003. NO-mediated hypersensitive responses of rice suspension cultures induced by incompatible elicitor. Chinese Science Bulletin 48: 358-363.

John R, Ahmad P, Gadgil K and Sharma S, 2012. Heavy metal toxicity: effect on plant growth, biochemical parameters and metal accumulation by *Brassica juncea* L. International Journal of Plant Production 3(3): 65-76.

Kaur G, Kaur S, Singh HP, Batish DR, Kohli RK and Rishi V, 2015. Biochemical adaptations in *Zea mays* roots to short-term Pb^{2+} exposure: ROS generation and metabolism. Bulletin of Environmental Contamination and Toxicology 95(2): 246-253.

Kovacik J, Klejdus B, Hedbavny J and Backor M, 2010. Effect of copper and salicylic acid on phenolic metabolites and free amino acids in *Scenedesmus quadricauda* (Chlorophyceae). Plant Science 178: 307-311.

Lee DH, Kim YS and Lee CB, 2001. The inductive responses of the antioxidant enzymes by salt stress in the rice (*Oryza sativa* L.). Plant Physiology 158: 737-745.

Lehotai N, Peto A, Weisz M, Erdei L, and Kolbert Z, 2011. Generation of reactive oxygen and nitrogen species in pea cultivars under copper exposure. Acta Biologica Szegediensis 55(2): 273-278.

Lichtenthaler HK, 1987. Chlorophyll and carotenoids: pigments photosynthetic of biomembranes. Methods in Enzymology 148: 350-382.

Mazid M, Khan TA and Mohammad F, 2011. Role of nitric oxide in regulation of H_2O_2 mediating tolerance of plants to abiotic stress: a synergistic signaling approach. Journal of Stress Physiology and Biochemistry *7*(2): 34-74.

Michalak A, 2006. Phenolic compounds and their antioxidant activity in plants growing under heavy metal stress. Polish Journal of Environmental Studies 15: 523-530.

Nakano Y and Asada K, 1981. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplast. Plant and Cell Physiology 22: 867-880.

Omaye ST, Turnbull JD and Sauberilich HE, 1979. Selected methods for the determination of ascorbic acid in animal cells, tissues and fluids. Methods in Enzymology 62: 3-11.

Rejeb KB, Abdelly C and Savoure A, 2014. How reactive oxygen species and proline face stress together. Plant Physiology and Biochemistry 80: 278-284.

Sharma P and Dubey RSH, 2005. Lead toxicity in Plants. Brazilian Journal of Plant Physiology 17: 35-52.

Singh A, Agrawalsb A and Rathore D, 2005. Amelioration of Indian urban air pollution phytotoxicity in *Beta vulgaris* L. by modifying NPK nutrients. Environmental Pollution 134: 385-395.

Smirnoff N, 1993. The role of active oxygen in the response of plants to water deficit and desiccation. New Phytologist 125: 27-58.

Stone JR and Yang S, 2006. Hydrogen peroxide: a signaling messenger. Antioxidants and Redox Signaling 8: 243-270.

Verma S and Dubey RS, 2003. Lead toxicity induces lipid peroxidation and alters the activities of antioxidant enzymes in growing rice plants. Plant Science 164: 645-655.

Wang Z, Li Q, Wu W, Guo J and Yang Y, 2017. Cadmium stress tolerance in wheat seedlings induced by ascorbic acid was mediated by NO signaling pathways. Ecotoxicology and Environmental Safety 135: 75-81.

Xiong J, Fu G, Tao L and Zhu C, 2010. Roles of nitric oxide in alleviating heavy metal toxicity in plants. Archives of Biochemistry and Biophysics 497(1): 13-20.

Yu Q, Sun L, Jin H, Chen Q, Chen Z and Xu M, 2012. Lead-induced nitric oxide generation plays a critical role in lead uptake by *Pogonatherum crinitum* root cells. Plant and Cell Physiology 53(10): 1728-1736.

Zafari S, Sharifi M, Chashmi NA and Mur LA, 2016. Modulation of Pb-induced stress in Prosopis shoots through an interconnected network of signaling molecules, phenolic compounds and amino acids. Plant Physiology and Biochemistry 99: 11-20.

Zhang LP, Mehta SK, Liu ZP and Yang ZM, 2008. Copper-induced proline synthesis is associated with nitric oxide generation in *Chlamydomonas reinhardtii*. Plant and Cell Physiology 49(3): 411-419.

تولید نیتریک اکسید و پاسخ های آنتی اکسیدان ها در گیاه ذرت تحت تنش سرب

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چکیده

آلودگی سرب یک چالش مهم در جهان محسوب می شود. در این پژوهش گیاه ذرت با غلظت های مختلف سرب (صفر، ۰,۲۵، ۰,۷۵، ۱، ۱,۵، ۲,۵ و ۵ میلی مولار) به مدت ۱۰ روز تیمار شدند. نتایج نشان داد که افزایش در غلظت سرب منجر به کاهش رشد، محتوای کلروفیل a و b و افزایش در خسارات اکسیداتیو گردید. کاربرد سرب به طور معنی داری میزان پراکسید هیدروژن را افزایش داد که سطح قابل توجهی از پراکسیداسیون لیپیدها را به دنبال داشت. این تغییرات با افزایش مقدار اکسید نیتریک در غلظت های پایین و کاهش آن در غلظت های بالا همراه بود. کمی افزایش در تولید پراکسید هیدروژن و نیتریک اکسید در غلظت های کم به عنوان پیامبر در مسیرهای سیگنالینگ انتظار می رود که در تحریک عملکردهای دفاعی برای سم زدایی سرب عمل کنند. بنابراین متغیرهای تحمل به تنش گیاهان، از جمله پرولین، اسید اسکوربیک، فلاونوئید و آنتوسیانین ها و فعالیت آنزیم های آنتی اکسیدانی تحت تیمار غلظت های مختلف سرب افزایش می یابد.

واژه‌های کلیدی: آنتی اکسیدان‌ها؛ تنش سرب؛ تنش اکسیداتیو؛ گیاه ذرت؛ نیتریک اکسید.