JCHR (2020) 10(1), 57-65

Journal of Chemical Health Risks

www.jchr.org

ORIGINAL ARTICLE

Relationship of Sodium Nitroprusside with Growth and Antioxidant Enzymes of Canola under Lead Stress

Hossein Hamidi, Nahid Masoudian*, Mostafa Ebadi, Bostan Roudi

Department of Biology, Damghan Branch, Islamic Azad University, Damghan, Iran

(Received: 1 January 2019 Accepted: 21 May 2019)

KEYWORDS

Lead;
Weight;
Chlorophyll;
Antioxidant enzymes;
Sodium nitroprusside

ABSTRACT: Lead is a toxic heavy-metal pollutant which is hazardous to human health and the environment. Sodium nitroprusside is commonly used as a nitric oxide donor in plants. Nitric oxide is a bioactive molecule playing an important role in response to stress in plants. Weight, chlorophyll content, and the activity of catalase (EC 1.11.1. 6) and peroxidase (EC 1.11.1. 7) antioxidant enzymes of canola (*Brassica napus* L.) Hyola 401 in lead stress were investigated. This study tested the hypothesis that sodium nitroprusside plays an ameliorating role under lead-toxicity in canola. For seven days, thirteen-day plants were exposed to two levels of sodium nitroprusside (0 and 100 µM) and three levels of lead (0, 100, and 200 µM). Dry and fresh weight and chlorophyll content were decreased in lead stress, while sodium nitroprusside treatment increased weight and chlorophyll b in the same conditions. Lead stress increased the activity of antioxidant enzymes, and sodium nitroprusside treatment reduced their activity. The results showed that the use of sodium nitroprusside reduces lead toxicity.

INTRODUCTION

There are several varieties in the Brassicaceae species [1]. Canola (*Brassica napus* L.) can be cultivated in different regions of Iran and is used to produce plant oil [2]. Canola seeds contain 40% oil and 17 to 26 % protein. Most of its proteins include Napin, Cruciferin, and Oleosin. Therefore, canola oil is very useful for human and much effort is needed to cultivate it [3-4].

Heavy metals have a lot of negative effects on canola production [5]. The increasing pollution of the environment with hazardous chemicals, including lead, is extremely dangerous for animals and plants, and as the soil is contaminated, the production decreases [6-7]. Lead has a negative effect on plants, and plants do not need this heavy element. Lead toxicity causes to blockage of enzymes, disturbance in mineral nutrition, disturbance of water balance, changes in hormonal status and permeability of the membrane. The need for more crops, and the use of polluted environment is an important issue [8]. Reactive oxygen species (ROS) and hydrogen peroxide produced in lead stress cause oxidative damage [9-11]. ROS can be very harmful to organisms at high concentrations, although some of them may play a role in regulating gene expression. According to the research report of the geographic region of Golestan province in the Islamic Republic of Iran, heavy metals have an adverse effect and serious environmental consequences [12]. When the lead concentration increased in the shoot of rice, it increased oxidative stress and enhanced lead concentration and time, causing higher levels of stress [13].

High concentrations of heavy metals cause toxic effects such as the reduced height, shoot, and root growth, and

*Corresponding author: nahidmasoudian@yahoo.com (N. Masoudian)
DOI: 10.22034/jchr.2020.1863574.1020

even death of plants [14]. The dangerous concentration of lead in the soil will have serious consequences for plant life and growth [7]. Typically, measurements of chlorophyll content investigate the stress of heavy metals [15-16]. These metals can have a negative and dangerous effect on the plants' growth and the amount of photosynthesis [17]. Photosynthesis breakdown occurs in the stressed substitution of magnesium atoms in the center of chlorophyll by heavy metals such as lead, nickel and mercury, [18].

Under stress conditions, plants use their antioxidant system to neutralize and remove ROS in order to reduce the effects of oxidative stress, with its non-enzymatic defense system using compounds such as the reduced glutathione, vitamin C (ascorbate) and vitamin E (alpha-tocopherol), and the enzymatic portion includes superoxide dismutase (SOD, EC 1.15.1. 1), catalase (CAT, EC 1.11.1. 6) and peroxidases (POD, EC 1.11.1. 7) [19]. Catalase is an intracellular antioxidant enzyme, which is predominantly present in cell peroxisomes and to some extent, cytosol, and undergoes two stages of hydrogen peroxide reduction to water, and molecular oxygen and catalase are effective in high-intensity stresses. Another part of the hydrogen peroxide removal by peroxidases is located in the cytosol, vacuoles, and cell wall [20]. By increasing or decreasing the activity of antioxidant enzymes in different plants due to a variety of non-biological stresses the oxidative stress levels can be determined [21]. The increased activity of CAT and POD enzymes in lead stress has been reported [22].

In plants, there is a signaling molecule called nitric oxide (NO), which causes various biological functions due to its high diffusibility [23]. NO is a gas-free radical, and its chemistry is intermediate between three species. Sodium nitroprusside (SNP) is used as an antioxidant and plant growth regulator in order to reduce the negative effects caused by various stresses. The effects of SNP (nitric oxide donor) in a variety of abiotic stress in plants, such as salinity and heavy metals, have been shown [24-25].

NO treatment in heavy-metal stress conditions protects the destruction of DNA, prevents cell death, and reduces the effect of oxidative stress [26]. It has been reported that the use of NO causes the plant to be more resistant to lead stress [27].

Considering that even low levels of lead are toxic and dangerous, conducting research in this area is important and necessary [28]. This study was carried out due to the positive effect of SNP on proving the usefulness of SNP in lead contamination conditions to improve chlorophyll content and plant growth by decreasing the activity of antioxidant enzymes.

MATERIAL AND METHODS

Plant growth conditions

Canola seeds of Hyola 401 cultivars used in this study, were obtained from Golestan University of Gorgan, Islamic Republic of Iran. A total of 2.5% sodium hypochlorite solution was used for sterilization of the selected seeds for 20 minutes. The seeds were then washed with distilled water 5-8 times to remove the solution. Seed germination of petri dishes was carried out on a double layer of wet filter paper and inside the incubator in the dark at 25 ° C. The seven-day seedlings were transferred to Hoagland solution containers of 0.5%. The seedlings were exposed to 14-hour light and 10-hour darkness per day and night in the growth chamber in the containers. The temperature within the chamber was 25 ° C, and its humidity was 55% at day and 20 ° C and 70% humidity at night. The reconstitution of the nutrient solution was performed after six days, so that the Hoagland solution with different concentrations of NO from the source of SNP (control and 100 micromoles per liter) and lead from the lead nitrate source ($Pb(NO_3)_2$) (control, 100 and 200 micromoles per liter) [29] were added to the containers. The collection was done one week later and then the samples were transferred to the laboratory.

Morphological Parameters

Aerial parts and roots were separated and washed. It was kept at 90 ° C for one day and night, and the weight of dried samples was determined.

The leaves of plants exposed to different concentrations of lead, and NO were carefully separated to be used for research. The samples were mixed and crushed with 80% acetone, kept in the dark overnight and then passed through the filter. Using the spectrophotometer, the absorbance was read at 645, 652, and 663 nanometer

wavelengths. Finally, the levels of chlorophyll a and b was measured under different treatments using the following formula [30]:

$$\frac{C \times a \times v}{FW}\ Mg.g^{1-}Fw$$

$C = Concentration\ of\ chlorophyll$

$a = Dilution\ factor$

$V =$ The volume of chlorophyll extracts

$Fw = Fresh\ weight$

Enzyme Assay

Enzyme extraction

First, extraction solution was prepared with the necessary compounds, including 50 g polyethylene glycol 2000, 2g ascorbic acid, 2 g EDTA-Na_2, 3.8 g borax and 1.2 g tris, and then, distilled water was added to reach a final volume of 100 ml. In the next step, 1g of fresh organ was weighed separately, and the mixture was ground for 30 minutes with the extracted solution. The resulting solution was maintained for one day and night at 4°C. After that, the solution was centrifuged at 4000 g for half an hour. Then the upper clear solution (enzymatic extract) was maintained at 4°C to measure antioxidant enzyme's activity.

Activity of POD

To measure the enzymatic activity, one ml of acetate buffer 0.2 molar at pH = 5 with 0.2 ml of benzidine, soluble in 50 % alcohol 0.01 molar and 0.4 ml of hydrogen peroxide 3% was mixed. 0.1 ml enzyme extracts were added to the mixture. The absorbance changes were measured at 530 nanometer wavelength against the control device, and the enzyme activity was also calculated based on the unit (OD g^{-1} Fw min^{-1}) [31].

Activity of CAT

Five ml of the mixture containing 1ml of enzymatic extracts was diluted twice for 60 s at 25ºC. 300 µM phosphate buffer and 100 µM hydrogen peroxide was incubated. To stop the reaction, 10 ml sulfuric acid 2% was added, and then, the remaining hydrogen peroxide was titrated with potassium permanganate solution (0.01 N) for 15 seconds until turned pale purple. Enzyme activity was stopped during control. The enzyme activity was 1 µM degradation of hydrogen peroxide in 1 minute [32].

Statistical analysis

The canola plant with three replications and random sampling was studied. The mean data were compared using the Duncan method and SPSS software, and the graphs were plotted using Excel. Text results are probable (P), and a significance criterion is $p \leq 0.05$.

RESULTS AND DISCUSSION

The Earth and its soil, and the daily produced crops are threatened by the increased risk of heavy-metal inputs that endanger the health and life of creatures [33-34]. Lead reduces the quality of agricultural soils [35]. As ROS is caused by the contamination of land with heavy metals, which are harmful to the plant, the production of antioxidants can help the plant withstand oxidative stress [7]. NO has been introduced over the past two decades as an important contributor to plant resistance to toxic metals and other stresses [36]. The duality feature of NO is that it has different toxic or defensive behaviors under various stresses and is also ROS [37].

According to Tables 1 and 2, after changes in chlorophyll content, dry weight of the aerial parts, and the antioxidant enzyme activity were measured and recorded, no significant effect on SNP treatments was observed, but in other treatments, the results were significant.

Comparison of mean values of fresh and dry weight data (Figures 1-a, 1-b, 1-c, and 1-d) showed that lead toxicity had a high and significant effect on plant weight. As plants are not able to have their normal activity and cannot absorb soil nutrients, there is a decrease in the activity of root and various parts of the plant, which may be due to the toxicity of lead and its damaging effects on the root [5]. Investigating the effect of different concentrations of heavy metals has shown that a lower concentration has a positive effect on the plant and can even help to improve growth, but higher concentrations will damage the growth, and the plant's resistance to toxicity will be reduced [38]. It was observed that SNP could prevent the degradation of the canola plant during lead stress and disrupt its toxicity, and it was found that SNP could have inhibitory effects against the negative effects of lead; and the significant and potent role of NO in plant growth versus lead stress has been proven (Figures 1-a, 1-b, 1-c, and 1-d). Since the seed germinates

until it becomes a complete plant, NO has positive effects on plant growth [39].

As the figures 1-e and 1-f show, lead toxicity reduces chlorophyll content. Producing free radicals, lead stress may cause to chlorophyll degradation in the plant, and thus reduce its levels [40]. This reduction has a negative effect on carbon assimilation [41]. As shown in figure 1-e, chlorophyll a content is not increased in stresses with SNP treatment. In case of chlorophyll b, it was observed that NO treatment at a concentration of 100 µM lead causes an increase in its content (Figure 1-f). Therefore, the result was the positive effect of NO on biosynthesis and its beneficial role in preventing chlorophyll degradation in lead stress [42]. Usually, the low and high levels of chlorophyll content are directly associated with the amount of iron in the leaves [43]. Due to this relationship, NO increases the transfer of internal iron and homeostasis and thus increases the production of chlorophyll in the chloroplast [44].

It has been said that the activity of antioxidant enzyme increases in plants in lead stress [45]. It has been proved that when lead contamination affected the plant, it increased the activity of POD and higher activity of POD in the shoot control was observed (Figures 2-a and 2-b). One of the known mechanisms of plant protection against stress of heavy metals may be the induction of POD in contaminated plants [46]. It figures 2-a and 2-b shows that the SNP treatment has significantly reduced the POD activity of plants, which have been exposed to lead stress. The reduction of POD percentage to CAT in the root can be considered as one of the possible mechanisms for combating oxidative stress [47]. It is recommended that heavy-metal stress resistant cultivars are used to produce more and better canola and increase plant yield against contamination [48]. The results show that CAT activity of the plant has increased due to the effect of lead stress (Figures 2-c and 2-d). Lead stress significantly increased the activity of CAT compared to canola control. We found that the intensity of CAT activity in lead stress with a concentration of 200 µM was more than 100 µM, and a significant difference were observed. Previously, there has been a report on changes in the activity of CAT enzymes in the shoot rice of seedlings, so that high concentration inhibits and increases its activity [11]. To increase plant compatibility by keeping the concentration of hydrogen peroxide constant, CAT activity is a factor in increasing stress tolerance [9]. The intensity of stress is effective on plant resistance and antioxidant enzyme's performance. The more severe the stress, the lower the activity of the enzymes, and the more the stress is increased by the enzyme's activity, and the further enhance in the plant's adaptability and growth [38]. CAT activity in control plants did not change significantly with sodium nitroprusside treatment. However, in the shoot, plants under the stress of lead reduced the activity of this enzyme (Figures 2-c and 2-d). The application of SNP had no significant effect on the canola root. Recently, the effect of lead and SNP treatment on antioxidant enzyme's activity has been reported [42]. Considering the positive effect of NO on plant resistance after heavy-metal contamination and its protective role, it decreases the amount of hydrogen peroxide and hence decreases the enzymatic activity [49-51]. It is suggested that NO treatment be used to increase the resistance of the plant to the stress of lead and other heavy metals.

Table 1. Variance analyses on the data of canola morphological parameters under different lead and SNP treatments as well as their interactions.

		Fresh weight		Dry weight		chlorophyll	
		Shoot	**Root**	**Shoot**	**Root**	**a**	**b**
Lead	df	2	2	2	2	2	2
	Mean squares	0.114^{**}	0.001^{**}	0.001^{**}	0.000^{**}	0.001^{**}	0.000^{**}
	CV	0.192	0.126	0.191	0.110	0.109	0.084
SNP	df	1	1	1	1	1	1
	Mean squares	0.001^{ns}	0.000^{ns}	0.029^{ns}	0.000^{*}	0.000^{ns}	0.000^{*}
	CV	0.097	0.017	0.068	0.046	0.089	0.063
Lead & SNP	df	2	2	2	2	2	2
	Mean squares	0.097^{**}	0.001^{*}	0.000^{**}	0.000^{**}	0.001^{**}	0.001^{**}
	CV	0.171	0.100	0.151	0.115	0.122	0.097

Notes: ns, not significant.
*P ≤ 0.05, **P ≤ 0.01.

Table 2. Variance analyses on the data of the activities of two antioxidant enzymes of canola under different lead and SNP treatments as well as their interactions.

		CAT		POD	
		Shoot	**Root**	**Shoot**	**Root**
Lead	df	2	2	2	2
	Mean squares	1799.389**	664.667**	0.000**	0.000**
	CV	0.197	0.295	0.112	0.217
SNP	df	1	1	1	1
	Mean squares	4.083ns	0.141ns	0.000ns	0.000ns
	CV	0.101	0.057	0.087	0.132
Lead	df	2	2	2	2
&	Mean squares	636.222**	658.667**	0.000**	0.000**
SNP	CV	0.138	0.286	0.102	0.171

Notes: ns, not significant.
*P ≤ 0.05, **P ≤ 0.01.

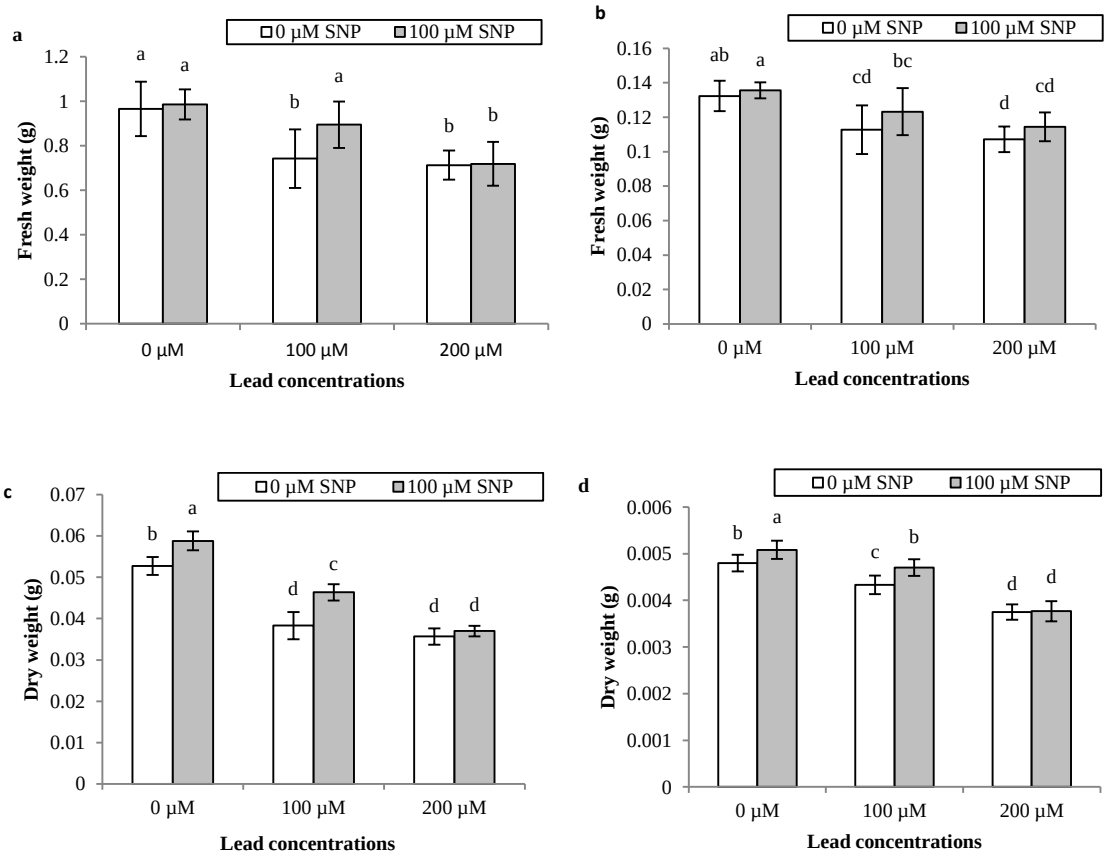

Figure 1. Shoots (a, c, e and f) and roots (b and d) morphological parameters of canola (*Brassica napus* L.) cultivar applied with SNP under lead stress conditions (mean ± S.E.). Letters (a–d) show the least significant difference between mean values.

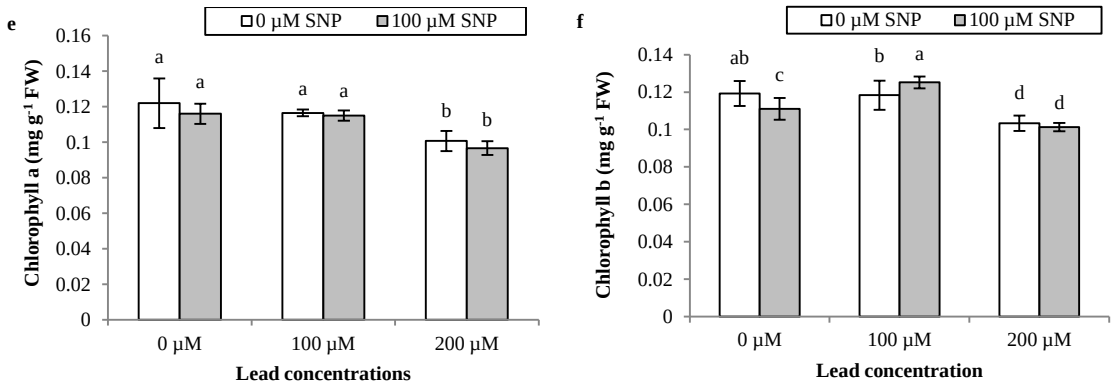

Figure 1. Continued

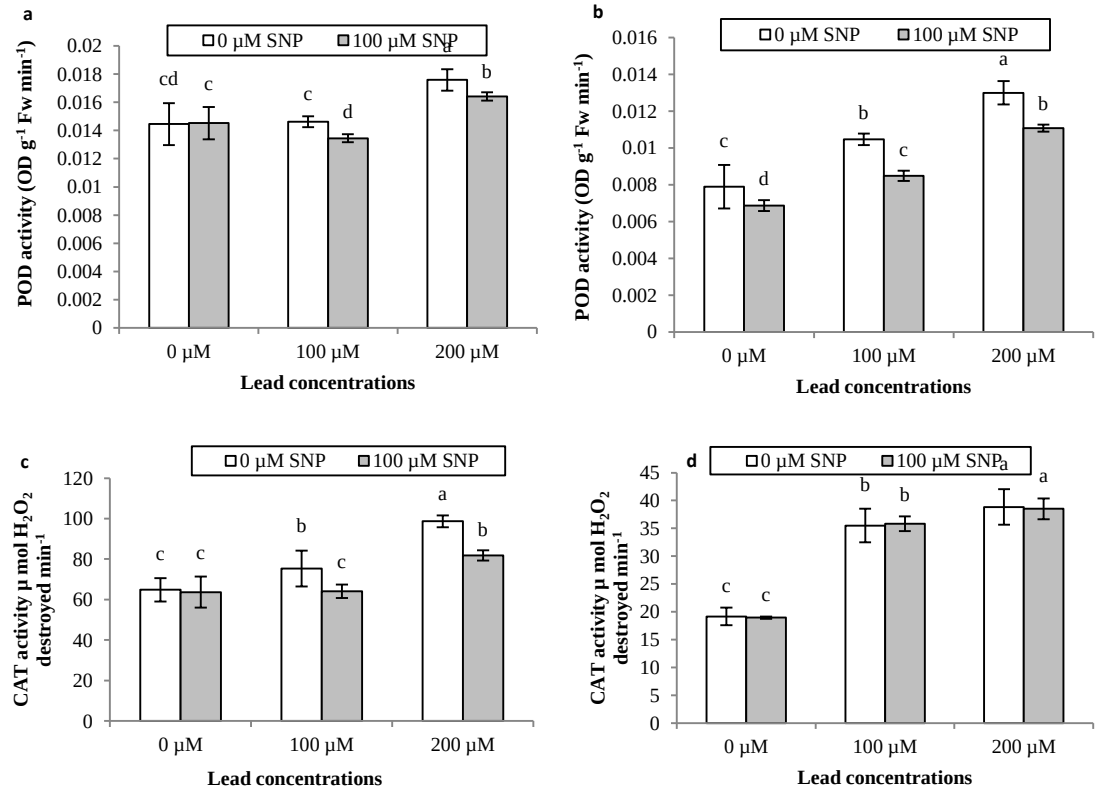

Figure 2. Shoots (a and c) and roots (b and d) the activities of two antioxidant enzymes of canola (*Brassica napus* L.) cultivar applied with SNP under lead stress conditions (mean ± S.E.). Letters (a–d) show the least significant difference between mean values.

CONCLUSIONS

Lead stress significantly decreased fresh and dry weights. The effects of SNP on canola grown under the lead stress conditions were positive as shown by the increased weights.

Chl a and Chl b content reduced in response to lead stress. The addition of SNP (100 µM), significantly enhanced the chlorophyll b contents in canola plants under 100 µM lead concentration.

Antioxidant enzyme activities were enhanced by the increase of the lead concentration, and the use of SNP caused a reduction in these activities.

Application of exogenous SNP increased the plant growth by decreasing antioxidative enzyme activities. Thus, it can be concluded that SNP has ameliorative effects on canola plants grown under lead stress conditions.

ACKNOWLEDGMENTS

Thanks to Islamic Azad University of Damghan and Journal of Chemical Health Risks.

REFERENCES

1. Rakow G., 2004. Species Origin and Economic Importance of *Brassica*. In *Brassica*; Pua E-C, Douglas CJ. Eds.; Springer Berlin Heidelberg: Berlin, Germany. 54, 3-11.

2. Hemmat A., 2009. Reduction in Primary Tillage Depth and Secondary Tillage Intensity for Irrigated Canola Production in a Loam Soil in Central Iran. Journal of Agricultural Science and Technology. 11(3), 275-288.

3. Rahimi S., Kamran Azad S., Karimi Torshizi M.A., 2011. Omega-3 Enrichment of Broiler Meat by Using Two Oilseeds. Journal of Agricultural Science and Technology. 13(3), 353-365.

4. Kowalska J., 2014. Organically grown *Brassica napus* – Use of border strips and *Trichoderma*. Acta Agriculturae Scandinavica, Section B — Soil & Plant Science. 64(6), 529-536.

5. Ali B., Mwamba T.M., Gill R.A., Yang C., Ali S., Daud M.K., Wu Y., Zhou W., 2014. Improvement of element uptake and antioxidative defense in *Brassica napus* under lead stress by application of hydrogen sulfide. Plant Growth Regulation. 74(3), 261-273.

6. Taghizadeh M., Kafi M., Fattahi Moghadam M.R., 2015. Breeding by *In vitro* Culture to Improve Tolerance and Accumulation of Lead in *Cynodon Dactylon* L. Journal of Agricultural Science and Technology. 17(20), 1851-1860.

7. Gubrelay U., Agnihotri R.K., Shrotriya S., Sharma R., 2015. Effect of Lead stress on phosphatase activity and reducing power assay of *Triticum aestivum*. Cellular and molecular biology. 61(3), 57-62.

8. He Z.L., Yang X.E., Stoffella P.J., 2005. Trace elements in agroecosystems and impacts on the environment. Journal of Trace Elements in Medicine and Biology. 19(2-3), 125-140.

9. Reddy A.M., Kumar S.G., Jyonthsnakumari G., Thimmanaik S., Sudhakar C., 2005. Lead induced changes in antioxidant metabolism of horsegram (*Macrotyloma uniflorum* (Lam.) Verdc.) and bengalgram (*Cicer arietinum* L.). Chemosphere. 60(1), 97-104.

10. Ruley A.T., Sharma N.C., Sahi S.V., 2004. Antioxidant defense in a lead accumulating plant, *Sesbania drummondii*. Plant Physiology and Biochemistry. 42(11), 899-906.

11. Verma S., Dubey R.S., 2003. Lead toxicity induces lipid peroxidation and alters the activities of antioxidant enzymes in growing rice plants. Plant Science. 164(4), 645-655.

12. Ghorbani H., Hafezi Moghads N., Kashi H., 2015. Effects of Land Use on the Concentrations of Some Heavy Metals in Soils of Golestan Province, Iran. Journal of Agricultural Science and Technology. 17(4), 1025-1040.

13. Thakur S., Singh L., Zularisam A.W., Sakinah M., Din M.F.M., 2017. Lead induced oxidative stress and alteration in the activities of antioxidative enzymes in rice shoots. Biologia Plantarum. 61(3), 595-598.

14. Adriano D.C., 2001. Trace Elements in Terrestrial Environments: Biogeochemistry, Bioavailability, and Risks of Metals, 2nd edition. Springer, New York.

15. Krishnaraj S., Dan T.V., Saxena P.K., 2000. A Fragrant Solution to Soil Remediation. International Journal of Phytoremediation. 2(2), 117-132.

16. Macfarlane G.R., 2003. Chlorophyll a Fluorescence as a Potential Biomarker of Zinc Stress in the Grey Mangrove, *Avicennia marina* (Forsk.) Vierh. Bulletin of Environmental Contamination and Toxicology. 70(1), 90-96.

17. Maksymiec W., Wójcik M., Krupa Z., 2007. Variation in oxidative stress and photochemical activity in *Arabidopsis thaliana* leaves subjected to cadmium and excess copper in the presence or absence of jasmonate and ascorbate. Chemosphere. 66(3), 421-427.

18. Küpper H., Küpper F., Spiller M., 1996. Environmental relevance of heavy metal-substituted chlorophylls using the example of water plants. Journal of Experimental Botany. 47(2), 259-266.

19. Shah K., Kumar R.G., Verma S., Dubey R.S., 2001. Effect of cadmium on lipid peroxidation, superoxide anion generation and activities of antioxidant enzymes in growing rice seedlings. Plant Science. 161(6), 1135-1144.

20. Mittler R., 2002. Oxidative stress, antioxidants and stress tolerance. Trends in Plant Science. 7(9), 405-410.

21. Geebelen W., Vangronsveld J., Adriano D.C., Van Poucke L.C., Clijsters H., 2002. Effects of Pb-EDTA and EDTA on oxidative stress reactions and mineral uptake in *Phaseolus vulgaris*. Physiologia Plantarum. 115(3), 377-384.

22. Malar S., Manikandan R., Favas P.J.C., Sahi S.V., Venkatachalam P., 2014. Effect of lead on phytotoxicity, growth, biochemical alterations and its role on genomic template stability in *Sesbania grandiflora*: A potential plant for phytoremediation. Ecotoxicology and Environmental Safety. 108, 249-257.

23. Qiao W., Li C., Fan L.M., 2014. Cross-talk between nitric oxide and hydrogen peroxide in plant responses to abiotic stresses. Environmental and Experimental Botany. 100, 84-93.

24. Lopez-Carrion A.I., Castellano R., Rosales M.A., Ruiz J.M., Romero L., 2008. Role of nitric oxide under saline stress: implications on proline metabolism. Biologia Plantarum. 52(3), 587-591.

25. Arasimowicz M., Floryszak-Wieczorek J., 2007. Nitric oxide as a bioactive signalling molecule in plant stress responses. Plant Science. 172(5), 876-887.

26. Xiong J., Fu G., Tao L., Zhu C., 2010. Roles of nitric oxide in alleviating heavy metal toxicity in plants. Archives of Biochemistry and Biophysics. 497(1-2), 13-20.

27. Phang I.C., Leung D.W.M., Taylor H.H., Burritt D.J., 2011. The protective effect of sodium nitroprusside (SNP) treatment on *Arabidopsis thaliana* seedlings exposed to toxic level of Pb is not linked to avoidance of Pb uptake. Ecotoxicology and Environmental Safety. 74(5), 1310-1315.

28. Veeramani K., Avudainayagam S., Doraisamy P., Chandrasekharan C.N., 2012. Chemical Immobilization of Lead (Pb) in Long Term Sewage Irrigated Soil. Journal of Agricultural Science and Technology. 14(2), 449-458.

29. Kaur G., Singh H.P., Batish D.R., Mahajan P., Kohli R.K., Rishi V., 2015. Exogenous nitric oxide (NO) interferes with lead (Pb)-induced toxicity by detoxifying reactive oxygen species in hydroponically grown wheat (*Triticum aestivum*) roots. PLoS One. 10(9): e0138713.

30. Jensen A., 1978. Chlorophylls and carotenoids. In Handbook of Phycological Methods: Physiological and Biochemical Methods (Hellebust J.A., Craigie J.S., editors), Cambridge University Press, Cambridge, 59-70.

31. Koroi S.A., 1989. Gel electrophoresis tissue and spectrophotometrscho unter uchungen zomeinfiuss der temperature auf struktur der amylase and peroxidase isoenzyme. Physiology Review. 20, 15-23.

32. Chance B., Maehly A.C., 1955. Assay of Catalase and Peroxidase. Methods in Enzymology. 2, 764-775.

33. Sun Y., 2017. Ecological Risk Evaluation of Heavy Metal Pollution in Soil Based on Simulation. Polish Journal of Environmental Studies. 26(4), 1693-1699.

34. Hang X.S., Wang H.Y., Zhou J.M., 2010. Soil heavy-metal distribution and transference to soybeans surrounding an electroplating factory. Acta Agriculturae Scandinavica, Section B — Soil & Plant Science. 60(2), 144-151.

35. Shahid M., Dumat C., Silvestre J., Pinelli E., 2012. Effect of fulvic acids on lead-induced oxidative stress to metal sensitive *Vicia faba* L. plant. Biology and Fertility of Soils. 48(6), 689-697.

36. Santolini J., Andre F., Jeandrozb S., Wendehenneb D., 2017. Nitric oxide synthase in plants: Where do we stand? Nitric Oxide. 63, 30-38.

37. Beligni M.V., Lamattina L., 1999. Is nitric oxide toxic or protective? Trends in Plant Science. 4(8), 299-300.

38. Emamverdian A., Ding Y., 2017. HMs Induced Changes on Growth, Antioxidant Enzyme's Activity, gas Exchange Parameters and Protein Structures in *Sasa Kongosanensis f. Aureo – Striatus*. Polish Journal of Environmental Studies. 26(2), 585-592.

39. Desikan R., Cheung M.K., Bright J., Henson D., Hancock J.T., Neill S.J., 2004. ABA, hydrogen peroxide and nitric oxide signalling in stomatal guard cells. Journal of Experimental Botany. 55(395), 205-212.

40. Kaur L., Gadgil K., Sharma S., 2018. Lead and nickel accumulation in *Brassica juncea arawali* growing in contaminated soil. Jornal of Chemical Health Risks. 8(2), 157-175.

41. Mahanty H.K., McWha J.A., 1976. Sensitivity of *Spirodela oligorrhiza* (Kurz) Hegelm. to a polychlorinated biphenyl (Aroclor 1242). New Zealand Journal of Botany. 14(1), 9-12.

42. Sadeghipour O., 2016. Pretreatment with nitric oxide reduces lead toxicity in cowpea (*Vigna unguiculata* [L.] Walp.). Archives of Biological Sciences. 68(1), 165-175.

43. Graziano M., Beligni M.V., Lamattina L., 2002. Nitric Oxide Improves Internal Iron Availability in Plants. Plant Physiology. 130, 1852-1859.

44. Graziano M., Lamattina L., 2005. Nitric oxide and iron in plants: an emerging and converging story. Trends in Plant Science. 10(1), 4-8.

45. Mishra S., Srivastava S., Tripathi R., Kumar R., Seth C., Gupta D., 2006. Lead detoxification by coontail (*Ceratophyllum demersum* L.) involves induction of phytochelatins and antioxidant system in response to its accumulation. Chemosphere. 65(6), 1027-1039.

46. Parmar N.G., Vithalani S.D., Chanda S.V., 2002. Alteration in growth and peroxidase activity by heavy metals in *Phaseolus* seedlings. Acta Physiologiae Plantarum. 24(1), 89-95.

47. Sedghi M., Seyed Sharifi R., Pirzad A.R., Amanpour-Balaneji B., 2012. Phytohormonal Regulation of Antioxidant Systems in Petals of Drought Stressed Pot Marigold (*Calendula officinalis* L.). Journal of Agricultural Science and Technology. 14(4), 869-878.

48. Ali B., Deng X., Hu X., Gill R.A., Ali S., Wang S., Zhou W., 2015. Deteriorative Effects of Cadmium Stress on Antioxidant System and Cellular Structure in Germinating Seeds of *Brassica napus* L. Journal of Agricultural Science and Technology. 17(1), 63-74.

49. Singh H.P., Batish D.R., Kaur G., Arora K., Kohli R.K., 2008. Nitric oxide (as sodium nitroprusside) supplementation ameliorates Cd toxicity in hydroponically grown wheat roots. Environmental and Experimental Botany. 63(1-3), 158-167.

50. Wang Y.S., Yang Z.M., 2005. Nitric Oxide Reduces Aluminum Toxicity by Preventing Oxidative Stress in the Roots of *Cassia tora* L. Plant and Cell Physiology. 46(12), 1915-1923.

51. Akram N.A., Iqbal M., Muhammad A., Ashraf M., Al-Qurainy F., Shafiq S., 2018. Aminolevulinic acid and nitric oxide regulate oxidative defense and secondary metabolisms in canola (*Brassica napus* L.) under drought stress. Protoplasma. 255(1), 163-174.