Toxicology International, *30(4) : 469-474; 2023. DOI: 10.18311/ti/2023/v30i4/31022*

Online ISSN : 0976-5131; Print ISSN : 0971-6580

Toxicology International

Contents available at: www.informaticsjournals.com/index.php/toxi



Effect of Lead Toxicity on Wild Cannabis Species of Punjab Region

Dimpy Balgotra, Sabreen Bashir, Agrataben Vadhel, Madhuri Girdhar and Anand Mohan*

School of Bioengineering and Biosciences, Lovely Professional University, Phagwara - 144411, Punjab, India; anandmohan77@gmail.com

Abstract

Human activities are causing environmental pollution in many ways by contaminating air, water and soil by adding different types of pollutants. Among various pollutants, heavy metals are an emerging threat in today's world which are degrading our environment in a number of ways. Lead is the most widespread and evenly distributed poisonous element. Due to urbanization and growing human activities, lead emissions in different forms have increased, resulting in the contamination of soil and water. The lead transfers from environment to different forms of life, disrupting biological processes and causing various health issues. In this article, pot culture experiments were carried out to analyse the stress of Lead (Pb) and the capability of *Cannabis sp*. to tolerate the stress by studying the impact of different concentrations (0 mg/kg, 50 mg/kg, 100 mg/kg and 150 mg/kg of soil) on various biochemical aspects of the plant (Photosynthetic pigments, protein, antioxidant enzyme activity). Lead was given in the form of lead acetate. The results showed that the photosynthetic pigments-chlorophyll and carotenoid decreased with increasing lead concentration. Same effect was shown by the protein content in the leaves. On the other hand, Superoxide Dismutase (SOD), which is an antioxidant enzyme, increased with increasing concentration of lead.

Keywords: Contamination, *Cannabis sativa*, Heavy Metals, Phytoremediation, Toxicity

1. Introduction

A major threat to the wellbeing of humans in today's world is degradation of environment which is a result of expansion of industrial activities like mining, development of transportation, generation of power, intense agricultural practices etc. Among different pollutions, heavy metals have received great attention during the last few decades[1-3]. Heavy metals can be defined as those metals which have greater than 5 g/cm^3 density. Out of 90 elements which occur naturally, 53 elements are heavy metals and only few of them are biologically important. Heavy metal pollution increased during the late 19th century and early 20th century due to a number of anthropogenic activities. They are non-degradable and can put the health of humans and other animals in danger as they enter food chains readily[4]. The heavy metal pollution in soil is a chief problem for agricultural yield[5]. The present global mine production of lead, silver, cadmium and copper is considerable[6]. Since past 30 years, the topic of toxicity due to metals and plant tolerance has been broadly discussed[7].

Lead (Pb) is a bluish or silvery grey heavy metal with an atomic number 82 and atomic weight 207.19 at 327.5^{o}C melting point. There are four naturally occurring isotopes of lead with 208, 206, 207 and 204 atomic weights.

*Author for correspondence

Article Received on: 23.08.2022 Revised on: 19.07.2023 Accepted on: 07.08.2023

Globally lead exists in wide range of forms in nature. Although lead has a long history of being beneficial to mankind in various ways but there is no evidence of its biological function[8]. According to new Europe REACH regulations, lead is considered to be a metal of concern. In addition, on the basis of its toxicity, exposure potential and occurrence frequency, lead was reported to be the most dangerous substance after Arsenic by the ATSDR 2003. In perspective of quality of food, bio-testing, phytoremediation etc, the movement of lead from contaminated soil to plants was greatly studied[1,9]. Lead induces a wide range physiological, morphological and biochemical toxic effects on living organisms[10]. It causes negative impact on the plant growth, cell division, production of chlorophyll, germination of seeds, development of seedlings, elongation of roots etc[11-14]. But the degree of these effects depends on various factors such as concentration of the metal, exposure duration, developmental stage of plants etc. In human food, the maximum weekly acceptable intake of lead is around 25 μgkg^{-1} of weight[15]. Even though lead has no apparent biological function, it is readily absorbed by the plants as well as animals. When lead is absorbed by the plants with water and other minerals through roots or through shoots from the air, the metal then gets up taken and accumulated by the plants directly.

Cannabis sativa is one of the oldest plants to be cultivated by humans. Cannabis, also known as hemp or marijuana is an erect, annual herbaceous plant which belongs to the family Cannabaceae[16]. There are many arguments about the polytypic nature of Cannabis but it is still considered as monotypic plant i.e., it occurs as single species[17]. This plant is believed to be originated in Asia with many evidences suggesting Central Asia[18], and others suggesting lower latitudes of Asian continent[19]. There are two main subspecies of cannabis- *Cannabis indica* and *Cannabis sativa*, which differ in their physical characteristics from each other. *Cannabis indica* plants are short in height and have broad, dark green coloured leaves, while *Cannabis sativa* plants are taller in height with pale green coloured and thin leaves. Also, the cannabidiol content in *indica* strains is higher than the *sativa* strains in which the THC content is more. Cannabis sativa has been used for many purposes since ancient times like medicine, fibre, food, oil etc. Cannabis is famous for its cannabionoid compounds which have many benefits but also is the reason for its ban in many parts of the world. There are around 426 chemical compounds found in cannabis and 60 of them are cannabinoids. CBD, d-8-THC, d-9-THC and cannabinol are the four most researched compounds[20,21]. The first historical and archaeological evidences for cultivation of cannabis for fibre were found in China from the 4000 B.C. Cannabis is hyperaccumulator of heavy metals i.e., it absorbs heavy metals like lead from the contaminated sites.

Lead is highly poisonous and 2nd most toxic heavy metal following Arsenic (ATSDR 2015), which makes about 0.002% of the earth's crust. Lead is present in every ecological sphere because of its natural reserves on the earth[1] and contaminates the environment in various ways. A little portion of environmental pollution is contributed by the volcanic eruptions and mobilization of lead[22]. On contrary, the major sources of lead pollution include insecticides containing lead, making of lead acid batteries, mining, utilization of fuel containing lead etc[23]. In addition, lead arsenate pesticides, metal smelters, paints containing lead and fertilisers with phosphate are other chief sources of lead in the environment. The combustion of leaded gasoline releases metallic lead from automobiles along roadsides. Therefore, the plants which grow along the roadsides contribute to increase the lead concentration in environment either directly or indirectly because they contain lead in them. Watering the fields with sewage water or fertilizing with the sewage sludge[24], industrial processing of ores and minerals are also sources of lead pollution in the environment. Lead is also transported through fine particulate sediments present in mine. At last, the rock phosphate which is used in fertilizers contains lead as impurity.

Previous research suggest that lead induces oxidative stress as well as oxidative defence in various crop plants[25]. Furthermore, few studies suggest that nanosynthesized material like magnesium oxide mitigate lead induced stress[26].

2. Methodology

All the reagents used in the experiments were prepared from chemicals which were of analytical grade and purchased from Sigma Aldrich and LOBA. The chemicals were used without further purification. The aqueous solutions were prepared by using distilled water.

2.1 Plant Materials and Growth Conditions

The present research work was carried out in agricultural field of School of Agriculture, Lovely Professional

University, Punjab under controlled conditions at mean temperature of 27-32°C. The authenticated *Cannabis sativa* seeds were collected and sown after surface sterilization with sodium hypochloride and then washed with double distilled water. Pots were filled with 5 Kg of soil. The soil was taken at depth of 30 cm from the surface of nearby field and was dried and homogenized. After the emergence of plant seedling (30 days old) different concentration of lead was given. The metal salt was provided to the plant while watering. The experiment was conducted as complete randomized design with 12 trial pots including 1 control and 3 treatments-50 mg/kg, 100 mg/kg and 150 mg/kg of soil.

2.2 Biochemical Estimation

2.2.1 Quantification of Photosynthetic Pigments

Chlorophyll and carotenoids are the main photosynthetic pigments in plants. The chlorophyll estimation was done by Arnon method[27], carotenoid content was found out by using Maclachlan and Zalik method[28]. 80% acetone extract of plant sample was centrifuged to measure absorbance at 663 nm, 645 nm (for chlorophyll) and 480 nm, 510 nm (for carotenoid) using uv-vis spectrophotometer (model- LI-2800 Ex). Following formulas were used for the estimation of photosynthetic pigments.

$$\text{Chlorophyll a (mg/g FW)} = (Abs_{663} \times 12.7) - (Abs_{645} \times 2.69) \times V/(1000 \times W)$$

$$\text{Chlorophyll b (mg/g FW)} = (Abs_{645} \times 22.9) - Abs_{663} \times 4.68) \times V/ (1000 \times W)$$

$$\text{Total carotenoid content (mg/g FW)} = 7.6\ (Abs_{480}) - 1.49\ (Abs_{510}) \times (V/d \times W \times 1000)$$

Where,
V = Volume of plant extract
W = Fresh Weight of sample

2.2.2 Protein Estimation

The total quantity of protein present in *Cannabis sativa* was determined by Lowry's method[29] of protein estimation. 0.1 g of fresh leaf sample was homogenized with PBS using ice cold mortar pestle, further sample was centrifuged (10,000 rpm, 20 min, 4°C). The supernatant was used to measure protein content by Lowry method using Bovine Serum Albumin (BSA) as standard.

2.2.3 Antioxidant Enzyme Activity

Superoxide Dismutase (SOD) activity was determined by Nitroblue Tetrazolium (NBT), using the method described by Dhindsa[30]. 0.1 g leaf sample was grounded and homogenised in Potassium Phosphate buffer, centrifuged (10,000 rpm, 10 min, 4°C) and the supernatant was taken to check the SOD activity. The reaction mixture (3 ml) contained plant extract, NBT (2.25 mM), methionine (200 mM), EDTA-Na (3 mM), Sodium carbonate (1.5 M), Riboflavin (60 µM), Potassium Phosphate buffer (0.05 M, pH 7). Absorbance at 560 nm was measured after incubating the mixture for 20 minutes.

2.2.4 Statistical Analysis

Each treatment was given in a set of triplicates and the data was obtained for the estimation of photosynthetic pigments, protein content, and antioxidant enzyme activity by using one-way ANOVA followed by Duncan's multiple comparison test ($p \leq 0.05$) using the statistical package SPSS Version 22.

3. Result and Discussion

By giving treatment of lead acetate to the cannabis plants, various morphological changes happened. After two days of treatment, plants started wilting. Within a week, plants showed chlorosis in which leaves become pale yellow due to lack of chlorophyll.

After lead stress was given to the *cannabis sp*., variabilities were observed in the pigments of photosynthesis. With increase in concentration of lead, the concentration of Chlorophyll *a* and chlorophyll *b* decreased, while Control possessed highest amount chlorophyll. Carotenoid content also decreased with increase in metal stress (Figure 1). Both chlorophyll and carotenoid in plants are responsible for imparting color to plants where chl *a* and *b* are responsible for green colour and carotenoid are responsible for yellow and red color. Decrease in concentration of these pigments will affect the process of photosynthesis. The inhibition of photosynthesis due to lead toxicity is most common symptom and has been proven in a number of studies[31-36]. The photosynthetic inhibition is believed to be result of indirect effect of lead rather than direct effect.

The protein content of leaves was also observed to decrease with increase in lead concentration (Figure 2), which is similar to other studies done by many authors. It

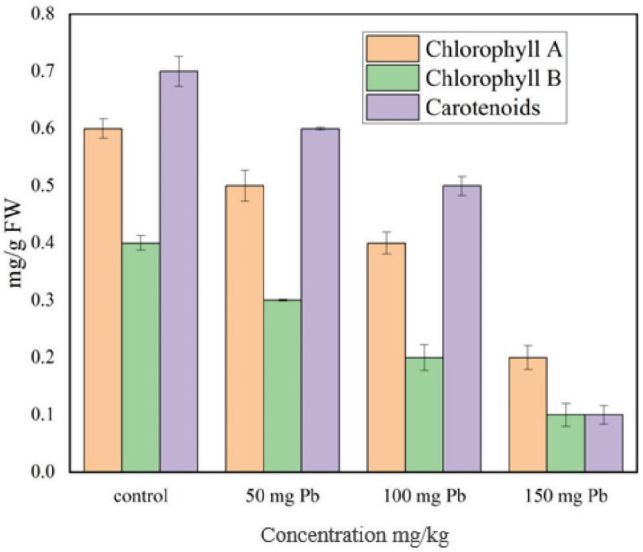


Figure 1. Effect of different concentrations of Lead (mg/kg) on photosynthetic pigment chlorophyll and carotenoid.

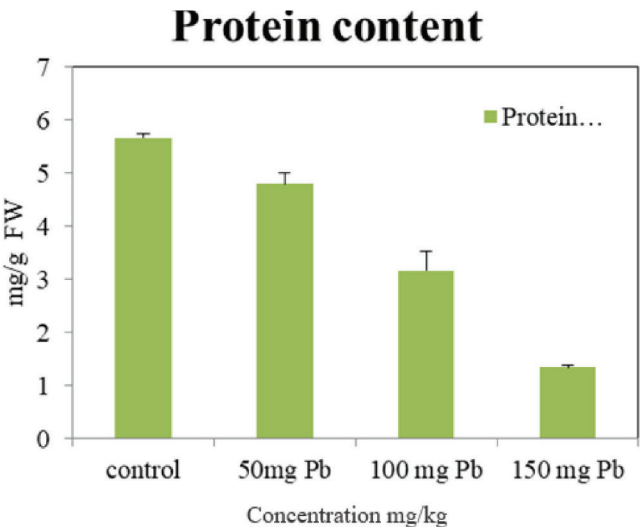


Figure 2. Effect of different concentration of Lead (mg/kg) on Protein content.

is believed that the decrease in protein content is because of a number of reasons like acute oxidative stress ROS[13], modified expression of genes[37], increase in the activity of ribonuclease activity[38], use of proteins by the plants to fight lead toxicity etc.

On contrary, the SOD was found to increase with increase in lead concentration (Figure 3). There have been many studies which show the same result that increase in lead toxicity results in increase in SOD activity. The same was found in other studies also[39,40].

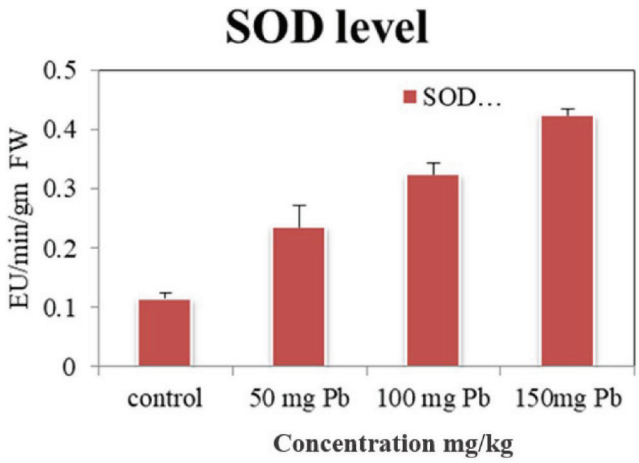


Figure 3. Effect of different concentration of lead (mg/kg) on SOD.

4. Conclusion

Heavy metal pollution is a rising threat worldwide. Due to anthropogenic activities, heavy metals are being added to both soil and water. Many strategies have been used to deal with the situation. Phytoremediation is the cost effective and environment friendly technique which can be used to remove heavy metals from contaminated soils. Cannabis is considered as weed but due to its wide range of benefits like fibre, which is used to make clothes, oil, which has many medicinal purposes etc., it is now recognized as a crop in various parts of the world. Cannabis is found to absorb heavy metals from the soil; however it impacts various biochemical contents of the plant. From this study, it was concluded that increase in lead concentration directly affects the chlorophyll content, carotenoid content as well as protein levels in the cannabis plant. The study reveals that lead stress lower the phytochemical concentrations. It was also found that due to increase in lead stress, the SOD levels increased with control having lowest levels.

5. Acknowledgement

The authors are very thankful to the authorities of Lovely Professional University (LPU), Punjab, India for the laboratory and infrastructural support.

6. References

1. Pourrut B, Shahid M, Dumat C, Winterton P, Pinelli E. Lead uptake, toxicity, and detoxification in plants. Rev

Environ Contam Toxicol. 2011; 213:113–36. https://doi.org/10.1007/978-1-4419-9860-6_4
2. Kaur N, Girdhar M, Mohan A. Toxic effects of hexavalent chromium on physiological and biochemical parameters of *Cyperus* iria (rice flatsedge) - A weed plant. Plant Cell Biotechnol Mol Biol. 2020; 20(3–4):67–73.
3. Girdhar. Comparative assessment for hyperaccumulatory and phytoremediation capability of three wild weeds. 3 Biotech. 2014; 4(6):579–89. https://doi.org/10.1007/s13205-014-0194-0
4. Sengar RS, Gautam M, Sengar RS, Garg SK, Sengar K, Chaudhary R. Lead stress effects on physiobiochemical activities of higher plants. Rev Environ Contam Toxicol. 2008; 196:73–93. https://doi.org/10.1007/978-0-387-78444-1_3
5. Zulfiqar U, Farooq M, Hussain S, Maqsood M, Hussain M, Ishfaq M, et al. Lead toxicity in plants: Impacts and remediation. J Environ Manage. 2019; 250. https://doi.org/10.1016/j.jenvman.2019.109557
6. Pinto AP, Mota AM, De Varennes A, Pinto FC. Influence of organic matter on the uptake of cadmium, zinc, copper and iron by sorghum plants. Sci Total Environ. 2004; 326(1–3):239–47. https://doi.org/10.1016/j.scitotenv.2004.01.004
7. Clemens S. Molecular mechanisms of plant metal tolerance and homeostasis. Planta. 2001; 212(4):475–86. https://doi.org/10.1007/s004250000458
8. Maestri E, Marmiroli M, Visioli G, Marmiroli N. Metal tolerance and hyperaccumulation: Costs and trade-offs between traits and environment. Environ Exp Bot. 2010; 68(1):1–13. https://doi.org/10.1016/j.envexpbot.2009.10.011
9. Uzu G, Sobanska S, Aliouane Y, Pradere P, Dumat C. Study of lead phytoavailability for atmospheric industrial micronic and sub-micronic particles in relation with lead speciation. Environ Pollut [Internet]. 2009; 157(4):1178–85. https://doi.org/10.1016/j.envpol.2008.09.053
10. Natasha, Shahid M, Khalid S, Saleem M. Unrevealing arsenic and lead toxicity and antioxidant response in spinach: A human health perspective. Environ Geochem Health. 2022; 44(2):487–96. https://doi.org/10.1007/s10653-021-00818-0
11. Sharma, Dubey. Lead toxicity im plants. Brazilian J plant Physiol. 2005; 17:35–52. https://doi.org/10.1590/S1677-04202005000100004
12. Krzesłowska M, Lenartowska M, Mellerowicz EJ, Samardakiewicz S, Woźny A. Pectinous cell wall thickenings formation- A response of moss protonemata cells to lead. Environ Exp Bot. 2009; 65(1):119–31. https://doi.org/10.1016/j.envexpbot.2008.05.006
13. Gupta DK, Nicoloso FT, Schetinger MRC, Rossato LV, Pereira LB, Castro GY, et al. Antioxidant defense mechanism in hydroponically grown Zea mays seedlings under moderate lead stress. J Hazard Mater. 2009; 172(1):479–84. https://doi.org/10.1016/j.jhazmat.2009.06.141.
14. Gupta DK, Huang HG, Yang XE, Razafindrabe BHN, Inouhe M. The detoxification of lead in *Sedum alfredii* H. is not related to phytochelatins but the glutathione. J Hazard Mater. 2010; 177(1–3):437–44. https://doi.org/10.1016/j.jhazmat.2009.12.052
15. Fang Y, Sun X, Yang W, Ma N, Xin Z, Fu J, et al. Concentrations and health risks of lead, cadmium, arsenic, and mercury in rice and edible mushrooms in China. Food Chem. 2014; 147:147–51. https://doi.org/10.1016/j.foodchem.2013.09.116
16. McPartland JM. Cannabis systematics at the levels of Family, Genus, and Species. Cannabis Cannabinoid Res. 2018; 3(1):203–12. https://doi.org/10.1089/can.2018.0039
17. Hillig KW. Genetic evidence for speciation in Cannabis (Cannabaceae). Genet Resour Crop Evol. 2005; 52(2):161–80. https://doi.org/10.1007/s10722-003-4452-y
18. McPartland JM, Hegman W, Long T. Cannabis in Asia: Its center of origin and early cultivation, based on a synthesis of subfossil pollen and archaeobotanical studies. Veg Hist Archaeobot. 2019; 28(6):691–702. https://doi.org/10.1007/s00334-019-00731-8
19. Zhang Q, Chen X, Guo H, Trindade LM, Salentijn EMJ, Guo R, et al. Latitudinal adaptation and genetic insights into the origins of Cannabis sativa L. Front Plant Sci. 2018; 871:1–13. https://doi.org/10.3389/fpls.2018.01876
20. Pamplona FA, Takahashi RN. Psychopharmacology of the endocannabinoids: Far beyond anandamide. J Psychopharmacol. 2012; 26(1):7–22. https://doi.org/10.1177/0269881111405357
21. Girdhar M, Singh S, Rasool H, Srivastava V, Mohan A. Evaluating different weeds for phytoremediation potential available in tannery polluted area by conducting pot and hydroponic experiments. Curr World Environ J. 2014; 9(1):156–67. https://doi.org/10.12944/CWE.9.1.22
22. Yokel J, Delistraty DA. Arsenic, lead, and other trace elements in soils contaminated with pesticide residues at the Hanford site (USA). Environ Toxicol. 2003; 18(2):104–14. https://doi.org/10.1002/tox.10106
23. Gottesfeld P, Were FH, Adogame L, Gharbi S, San D, Nota MM, et al. Soil contamination from lead battery manufacturing and recycling in seven African countries. Environ Res. 2018; 609–14. https://doi.org/10.1016/j.envres.2017.11.055
24. Päivöke AEA. Soil lead alters phytase activity and mineral nutrient balance of Pisum sativum. Environ Exp Bot. 2002; 48(1):61–73. https://doi.org/10.1016/S0098-8472(02)00011-4
25. Navabpour S, Yamchi A, Bagherikia S, Kafi H. Lead-induced oxidative stress and role of antioxidant defense

in wheat (*Triticum aestivum* L.). Physiol Mol Biol Plants. 2020; 26(4):793–802. https://doi.org/10.1007/s12298-020-00777-3

26. Faiz S, Yasin NA, Khan WU, Shah AA, Akram W, Ahmad A, et al. Role of magnesium oxide nanoparticles in the mitigation of lead-induced stress in Daucus carota: Modulation in polyamines and antioxidant enzymes. Int J Phytoremediation. 2022; 24(4):364–72. https://doi.org/10.1080/15226514.2021.1949263
27. Arnon DI. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in Beta vulgaris. Plant Physiol. 1949; 24(1):1–15. https://doi.org/10.1104/pp.24.1.1
28. Maclachlan S, Zalik S. Plastid structure, chlorophyll concentration, and free amino acid composition of a chlorophyll mutant of barley. Can J Bot. 1963; 41(7):1053–62. https://doi.org/10.1139/b63-088
29. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem. 1951; 193(1):265–75. https://doi.org/10.1016/S0021-9258(19)52451-6
30. Dhindsa RS, Plumb-dhindsa P, Thorpe TA. Leaf senescence: Correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. J Exp Bot. 1981; 32(1):93–101. https://doi.org/10.1093/jxb/32.1.93
31. Xiong. Lead toxicity in *Brassica pekinensis* Rupr.: Effect on nitrate assimilation and growth Zhi-Ting. Environ Toxicol. 2006; 165. https://doi.org/10.1002/tox.20167
32. Hu JZ, Shi GX, Xu QS, Wang X, Yuan QH, Du KH. Effects of Pb^{2+} on the active oxygen-scavenging enzyme activities and ultrastructure in *Potamogeton crispus* leaves. Russ J Plant Physiol. 2007; 54(3):414–9. https://doi.org/10.1134/S1021443707030181
33. Liu D, Li TQ, Jin XF, Yang XE, Islam E, Mahmood Q. Lead induced changes in the growth and antioxidant metabolism of the lead accumulating and non-accumulating ecotypes of *Sedum alfredii*. J Integr Plant Biol. 2008; 50(2):129–40. https://doi.org/10.1111/j.1744-7909.2007.00608.x
34. Piotrowska A, Bajguz A, Godlewska-Zyłkiewicz B, Czerpak R, Kamińska M. Jasmonic acid as modulator of lead toxicity in aquatic plant *Wolffia arrhiza* (Lemnaceae). Environ Exp Bot. 2009; 66(3):507–13. https://doi.org/10.1016/j.envexpbot.2009.03.019
35. Singh R, Tripathi RD, Dwivedi S, Kumar A, Trivedi PK, Chakrabarty D. Lead bioaccumulation potential of an aquatic macrophyte *Najas indica* are related to antioxidant system. Bioresour Technol. 2010; 101(9):3025–32. https://doi.org/10.1016/j.biortech.2009.12.031
36. Cenkci S, Ciğerci IH, Yildiz M, Özay C, Bozdağ A, Terzi H. Lead contamination reduces chlorophyll biosynthesis and genomic template stability in Brassica rapa L. Environ Exp Bot. 2010; 67(3):467–73. https://doi.org/10.1016/j.envexpbot.2009.10.001
37. Kovalchuk I, Titov V, Hohn B, Kovalchuk O. Transcriptome profiling reveals similarities and differences in plant responses to cadmium and lead. Mutat Res - Fundam Mol Mech Mutagen. 2005; 570(2):149–61. https://doi.org/10.1016/j.mrfmmm.2004.10.004
38. Gopal R, Rizvi AH. Excess lead alters growth, metabolism and translocation of certain nutrients in radish. Chemosphere. 2008; 70(9):1539–44. https://doi.org/10.1016/j.chemosphere.2007.08.043
39. Malar S, Shivendra Vikram S, JC Favas P, Perumal V. Lead heavy metal toxicity induced changes on growth and antioxidative enzymes level in water hyacinths [*Eichhornia crassipes* (Mart.)]. Bot Stud. 2016; 55(1):1–11. https://doi.org/10.1186/s40529-014-0054-6
40. Usman K, Abu-Dieyeh MH, Zouari N, Al-Ghouti MA. Lead (Pb) bioaccumulation and antioxidative responses in Tetraena qataranse. Sci Rep. 2020; 10(1):1–10. https://doi.org/10.1038/s41598-020-73621-z