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Phytochemical analysis of *Nicotiana tabacaum* L. under heavy metal lead (Pb) stress

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Abstract
Nicotiana tabacum L is a well-known crop of Swabi and people called it the land of maize and tobacco. *Nicotiana tabacum* L play a vital role in the economy of a country because of its high price and demand over worldwide. My present study aimed to observed the effect of Salicylic acid on the phytochemical substances of *Nicotiana tabacum* L grown under Heavy Metal Lead Stress. After Propagation *Nicotiana tabacum* L treated with Pb stress at different concentration like 5 ppm, 5 ppm+ Sa, 9 ppm, 9 ppm +Sa and Control. Results revealed that those plants which have treated with Pb stress like 5 ppm and 9 ppm showed decline in the phytochemical substance like CAT, PO, ROS, GA3 AND ABA. While those plants which have treated with Pb as well as Sa like 5 ppm+ Sa and 9 ppm +Sa showed progress in the phytochemical substances of *Nicotiana tabacum* L. Therefore, I can say that salicylic acid is the key product to remove the heavy metal stress and promotes the primary and secondary growth of *Nicotiana tabacum* L.

Keywords: *Nicotiana tabacum* L, heavy metal lead, salicylic acid, sustainable agriculture

1. Introduction
Lead Pb is the most toxic and hefty metal belong to anthropogenic origin [1]. Pb is not environment friendly and accumulates in sediments like water and soil [2]. Different study finds out that Pb is very toxic for plants when present in soil and environment through any source [3]. Different diseases like Chlorosis and Necrosis are come and the symptom appears on leaf in which leaf become yellow and the root become black and brown [4]. Due to accumulation of lead the different biochemical compound like chlorophyll, sugar, protein flavonoids, alkaloids, proline are moving toward decline [5].
Catalases are enzymes found in plants and other living organisms that play a crucial role in the breakdown of hydrogen peroxide (H_2O_2) into water (H_2O) and oxygen (O_2). Hydrogen peroxide is a reactive oxygen species (ROS) that can be produced as a byproduct of various metabolic processes within cells. Catalases and peroxidases are both enzymes involved in managing hydrogen peroxide levels in plant cells, but they have different roles. Catalases directly break down hydrogen peroxide, while peroxidases use hydrogen peroxide in diverse enzymatic reactions, contributing to various physiological processes in plants but when Pb enter to plant cell it stops the production of CAT in which plant become destroyed [6].
Gibberellic acid (GA3) and Abscisic acid (ABA) are two important plant hormones that play distinct roles in the growth and development of plants and the main function of GA3 is elongation of stem and internodes of plants and breaking down the seeds and initiating the germination process as well as induction of flowering and development of fruits and the function of ABA in plants is response to environmental stresses and maintaining seeds dormancy and preventing premature seeds germination [7].
ROS are produced during normal cellular processes, such as respiration, photosynthesis, and metabolism. They are also generated in response to environmental stresses, including high light intensity, pathogens, and certain pollutants [8]. ROS can function as signaling molecules in various cellular processes, including plant growth, development, and responses to environmental stimuli. They participate in signaling pathways regulating processes like cell expansion, programmed cell death (apoptosis), and defense mechanisms against pathogens.

2. Material and Method
The experiment was conducted in the Department of Botany University of Swabi KPK Pakistan during the tabacum growing season of December 2023. Seeds of *Nicotiana tabacum* L were purchased from certified market of Swabi Khyber Pakhtunkhwa Pakistan.

Five seeds were sown in each pot at the depth of 0.5cm with 3 replicates, selected for each treatment and no of pots were 15. Plants were treated with heavy metals Pb in the form of solution at different concentration of 5ppm and 9ppm with the combination of salicylic acid plants growth promoter spray. 3 times treatment were performed after each seven days. After treatment Leaf was plucked for phytochemical analysis like CAT. PO, ROS, GA3 AND ABA. The method of [9] were used for growth parameters and heavy metals analysis.

2.1 Catalases (CAT) Determination
According to past work of [10] Crushed 0.5 g of plant sample in 10ml of 50 mM sodium phosphate buffer (pH 7.0), 2% polyvinylepyrrlidine-40 and 1ml EDTA-Na2. Sample was centrifuged at 11000 rpm for 15 minutes with 4 ℃. 3% (v/v) H_2O_2, 0.1 mM EDTA was added in 0.05 M Na-phosphate buffer (pH 7) to supernatant. Decline was indicating in H_2O_2 due to decrease in O.D at 240nm. Activity was estimated of µM H_2O_2 min-1.

2.2 Peroxidase assay (PO) Determination
This activity was done on the past method of [11]. Absorption was measured at 470nm in 3 minutes. PO activity was expressed as µmol min^{-1} mg^{-1} of protein (E= 26.6 mM^{-1} cm^{-1}).

2.3 GA3 and ABA Determination
According to the work of [12] 0.1 g leaf was crushed in distilled water in mortar. Centrifuged at 10000 rpm for 15 minutes with 4 ℃. Solution pH was adjusted between 1 and 2 by 0.1 ml HCL. 20 ml of ethyl acetate was added and shake for 6 sec. The aqueous phase was then transferred to a second separating funnel and the extraction procedure repeated by adding another 20ml ethyl acetate. The GA3 was extracted from ethyl acetate with portion of 20, 15 and 10ml phosphate buffer (pH 7.4), shaking each time for 60 seconds. A sample of 1ml and 1ml absolute ethanol were placed in 50ml volumetric flask. HCL 3.75 M was added to the flask and then mixed forcefully for 10 seconds. The absorbance was recorded at 254 nm for GA3 and 263 nm for ABA.

2.4 Reactive Oxygen Species (ROS) Determination
According to the work of [13] Antioxidant activity of crude extracts of *Nicotiana tabacum* L isolates was carried out by DPPH (2, 2-diphenyl-1-picrylhydrazyl) with minor modifications. Different concentrations of crude extracts (1000 µg/mL, 500 µg/mL, 250 µg/mL, 125 µg/mL, 62.5 µg/mL, 31.25 µg/mL, 15.62 µg/mL, and 7.81 µg/mL) were used to determine the scavenging of DPPH radicals.
DPPH scavenging activity (%): [(control absorbance − extract absorbance)/ (control absorbance) × 100].

3 Statistical Analysis
All experimental data were analyzed by the help of IBM SPSS Statistical software performed one-way analysis of variance (ANOVA) using T-test to determine the Pb stress in each treatment.

4 Results
4.1 Catalases (CAT) Determination
Pb expose plant showed sever effect on the enzyme level of *Nicotiana tabacum* L. In this study I have observed that the untreated plant control value is (0.3750±0.0512) showed optimistic effect on the enzymatic activity of *Nicotiana tabacum* L. While T_1 value is (0.1236±0.0127) and T_3 value is (0.0630±0.0205) show reduction in the enzymatic activity of *Nicotiana tabacum* L as compared to control. Similarly, In T_2 value is (0.1343±0.0239) and T_4 value is (0.2223±0.0973) showed progress in the enzyme level of *Nicotiana tabacum* L due to using of salicylic acid plant growth promoter spray as shown in the fig 1 and tab 1.

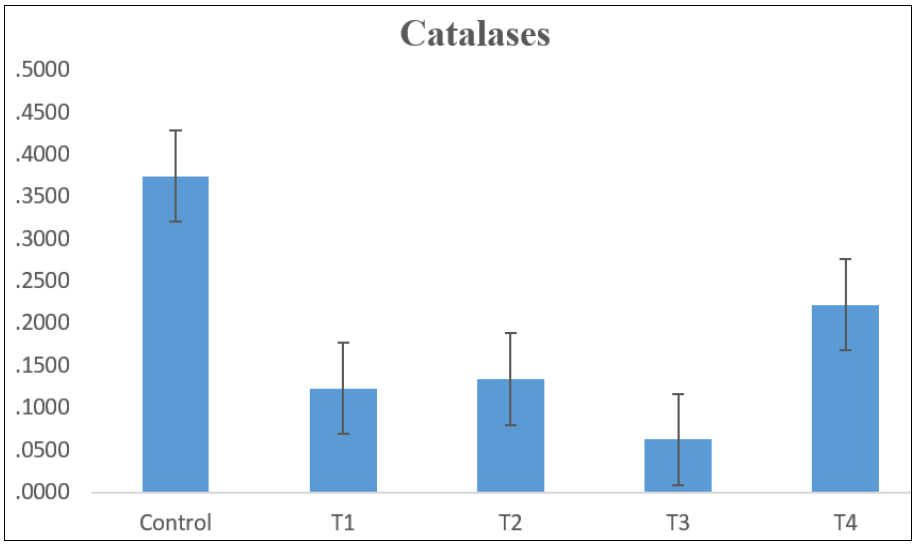

Fig 1: Catalases

4.2 Peroxidases (PO) Determination
Pb expose plant showed sever effect on the enzyme level of *Nicotiana tabacum* L. In this study I have observed that the untreated plant control value is (0.6336±0.0093) showed positive effect on the enzymatic activity of *Nicotiana tabacum* L. While T_1 value is (0.1730±0.0015) and T_3 value is (0.1483±0.0014) show reduction in the enzymatic activity of *Nicotiana tabacum* L as compared to control. Similarly, In T_2 value is (0.5663±0.0014) and T_4 value is (0.1890±0.0011) showed progress in the enzyme level of *Nicotiana tabacum* L due to using of salicylic acid plant growth promoter spray as shown in the fig 2 and tab 1.

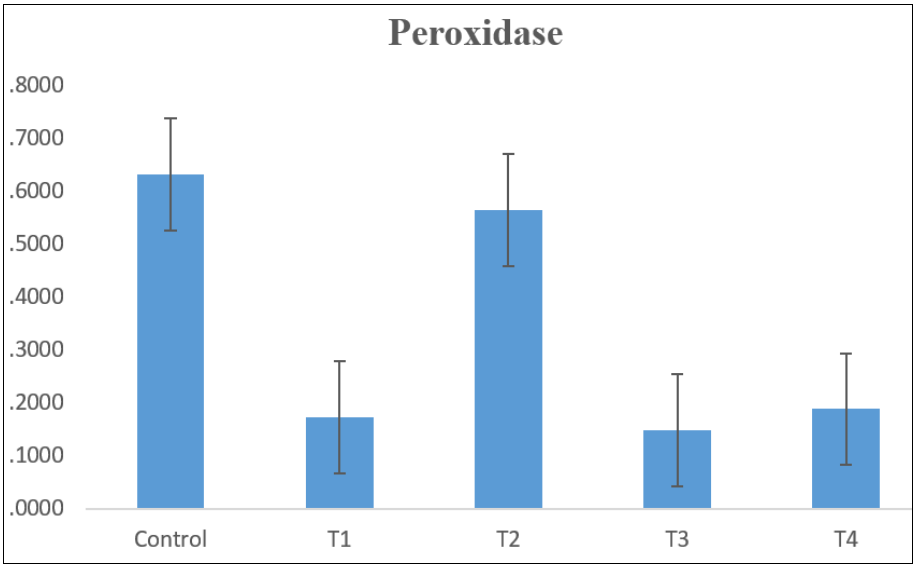

Fig 2: Peroxidase

4.3 Gibberellic Acid (GA3) Determination

Pb expose plant showed sever effect on the hormone level of *Nicotiana tabacum* L. In this study I have observed that the untreated plant control value is (0.0253±0.0037) showed positive effect on the hormonal activity of *Nicotiana tabacum* L. While T_1 value is (0.0173±0.0020) and T_3 value is (0.0176±0.0017) show reduction in the hormonal activity of *Nicotiana tabacum* L as compared to control. Similarly, In T_2 value is (0.0250±0.0036) and T_4 value is (0.0253±0.0037) showed progress in the hormone level of *Nicotiana tabacum* L due to using of salicylic acid plant growth promoter spray as shown in the fig 3 and tab 1.

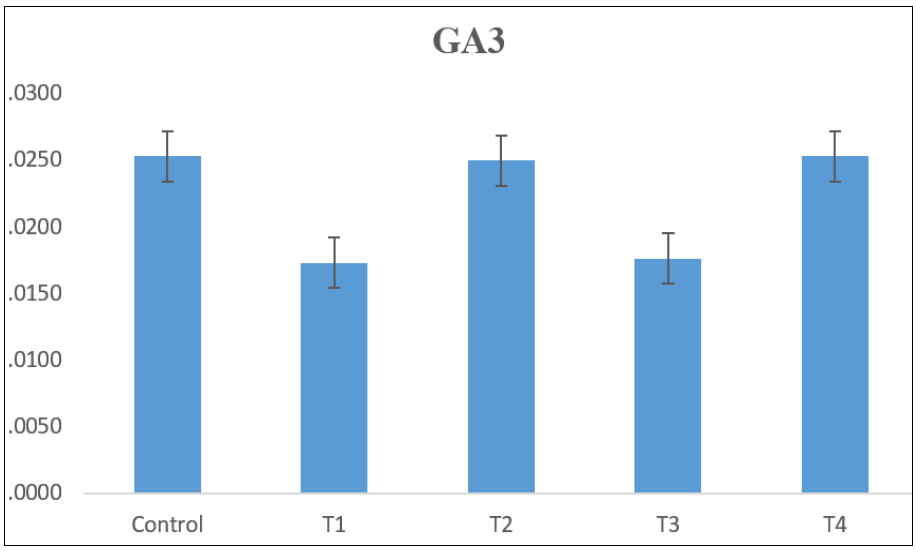

Fig 3: Gibberellic Acid

4.4 Abscisic acid (ABA) Determination

Pb expose plant showed sever effect on the hormone level of *Nicotiana tabacum* L. In this study I have observed that the untreated plant control value is (2.7037±0.4173) showed positive effect on the hormonal activity of *Nicotiana tabacum* L. While T_1 value is (1.8148±0.2252) and T_3 value is (1.5925±0.3031) show reduction in the hormonal activity of *Nicotiana tabacum* L. as compared to control. Similarly, In T_2 value is (1.8148±0.2252) and T_4 value is (1.8518±0.1959) showed progress in the hormone level of *Nicotiana tabacum* L. due to using of salicylic acid plant growth promoter spray as shown in the fig 4 and tab 1.

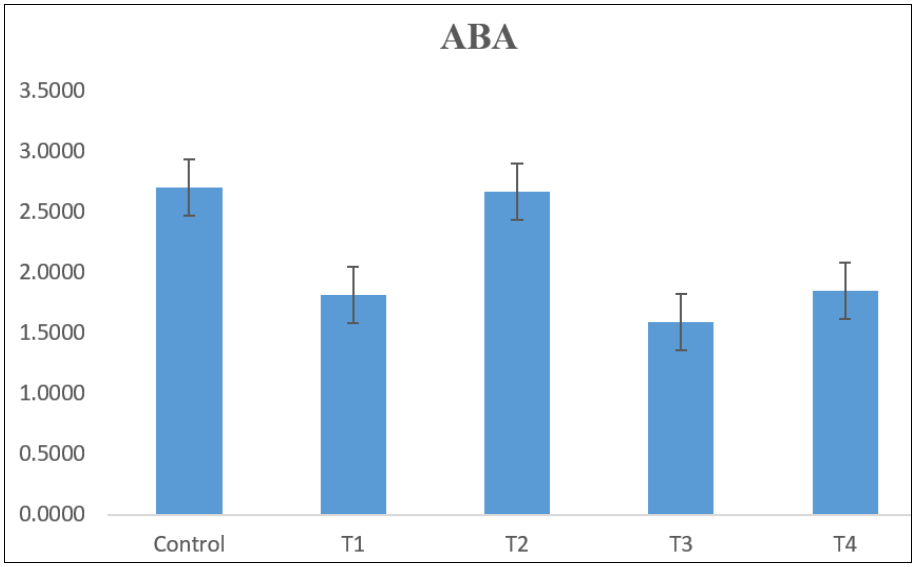

Fig 4: Abscisic acid

4.5 Reactive Oxygen Species (ROS) Determination

Pb expose plant showed sever effect on the ROS level of *Nicotiana tabacum* L. In this study I have observed that the untreated plant control value is (91.0065±0.0796) showed positive effect on the ROS level of *Nicotiana tabacum* L. While T_1 value is (78.8238±0.1785) and T_3 value is (67.2024±0.0584) show reduction in the ROS activity of *Nicotiana tabacum* L as compared to control. Similarly, In T_2 value is (87.4856±0.1668) and T_4 value is (87.6387±0.6392) showed progress in the ROS level of *Nicotiana tabacum* L due to using of salicylic acid plant growth promoter spray as shown in the fig 5 and tab 1.

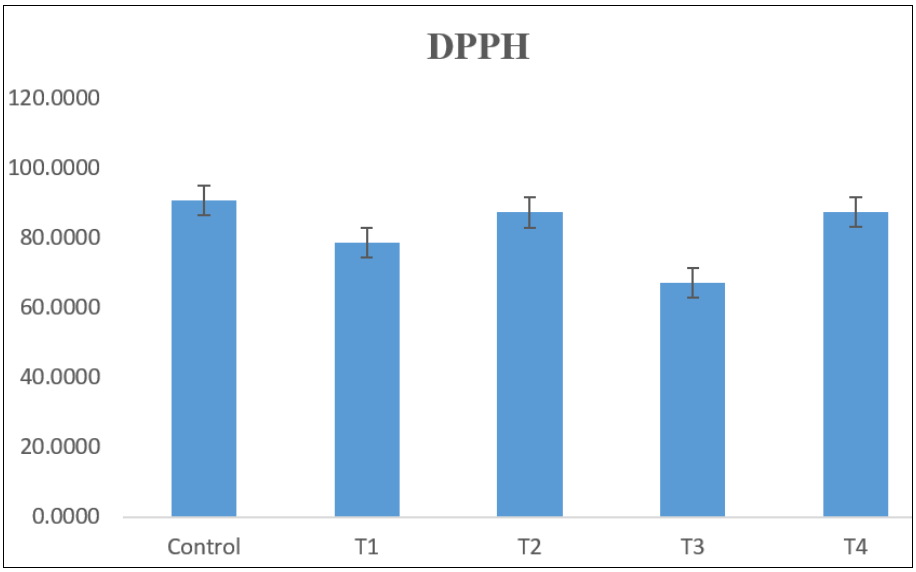

Fig 5: DPPH

Table 1: Treatment details

Treatment	Catalases	Peroxidases	GA3	ABA	DPPH
Control	0.3750±0.0512	0.6336±0.0093	0.0253±0.0037	2.7037±0.4173	91.0065±0.0796
T_1	0.1236±0.0127	0.1730±0.0015	0.0173±0.0020	1.8148±0.2252	78.8238±0.1785
T_2	0.1343±0.0239	0.5663±0.0014	0.0250±0.0036	2.6666±0.4006	87.4856±0.1668
T_3	0.0630±0.0205	0.1483±0.0014	0.0176±0.0017	1.5925±0.3031	67.2024±0.0584
T_4	0.2223±0.0973	0.1890±0.0011	0.0253±0.0037	1.8518±0.1959	87.6387±0.6392

Effect of Pb stress on Enzyme (catalases, Peroxidases) Hormone (GA3, ABA) and ROS. Results taken the Mean of three replicates + S.E

5 Discussion

Lead is considering one of the utmost poisonous heavy metals present everywhere in environment. Lead exposure experiment was performed in the presence of heavy meal stress. In this experimental study I have observed that untreated plant (control) show progress in the phytochemical substances of *Nicotiana tabacum* L. While Pb exposure plants showed reduction in the phytochemical substances of *Nicotiana tabacum* L [14]. Pb expose plants showed decreases in CAT level with increases of Pb stress but increases with salicylic acid growth promoter spray which show similarity with work of [15]. Old studies showed that Pb expose plant did not show any change in POD

activity in stress condition [16]. But in my study the POD activity was decreases with the increases of Pb stress but increases with salicylic acid plant growth promoter spray. Previous study showed that GA3 increased the uptake of ions and minerals in plants under stress condition [17]. But in my results GA3 level decreases in plant as increases Pb stress but showed progress due to salicylic acid growth promoter application. The study of [18] showed that Pb stress increased the ABA hormone. But in my study the level of ABA hormone decreases with the increases of Pb stress in *Nicotiana tabacum* L but increases with salicylic acid spray. My study concludes Pb expose plant showed little reduction in the production of ROS content but salicylic acid plant growth promoter spray increased the production of ROS content in *Nicotiana tabacum* L. which similarity with the work of [19].

Conclusion

In this study I have observed that plants which was treated with Pb stress show decline in the phytochemical substances of *Nicotiana tabacum* L. While the plants which was treated with Pb stress as well salicylic acid showed progress in the phytochemical substances like CAT, PO, GA3.ABA and ROS. Therefore, I can say that salicylic acid is the key product to remove heavy metal (Pb) stress in *Nicotiana tabacum* L.

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References

1. Mehla N, Sindhi V, Josula D, Bisht P, Wani SH. An introduction to antioxidants and their roles in plant stress tolerance. In: Reactive Oxygen Species and Antioxidant Systems in Plants: Role and Regulation under Abiotic Stress. Springer, Singapore; c2017. p. 1-23. https://doi.org/10.1007/978-981-10-5254-5_1.
2. Alkhatib R, Alkhatib B, Abdo N, Al-Eitan L, Creamer R. Physio-biochemical and ultrastructural impact of (Fe3O4) nanoparticles on tobacco. BMC Plant Biol. 2019;19(1):1-12. https://doi.org/10.1186/s12870-019-1864-1.
3. Mahmood S, Malik SI, Hussain M. Heterosis and combining ability estimates for ear traits and grain yield in maize hybrids. Asian J Agric Biol. 2016;4(4):91-98.
4. Samreen S, Khan AA, Khan MR, Ansari SA, Khan A. Assessment of phytoremediation potential of seven weed plants growing in chromium- and nickel-contaminated soil. Water Air Soil Pollut. 2021;232(5):1-18. https://doi.org/10.1007/s11270-021-05124-0.
5. Zhou C, Huang M, Ren H, Yu J, Wu J, Ma X. Bioaccumulation and detoxification mechanisms for lead uptake identified in Rhus chinensis Mill. seedlings. Ecotoxicol Environ Saf. 2017;142:59-68. https://doi.org/10.1016/j.ecoenv.2017.03.052.
6. Iannone MF, Groppa MD, Benavides MP. Cadmium induces different biochemical responses in wild type and catalase-deficient tobacco plants. Environ Exp Bot. 2015;109:201-211.
7. Thorpe TA, Meier DD. Effects of gibberellic acid and abscisic acid on shoot formation in tobacco callus cultures. Physiologia Plantarum. 1973;29(1):121-124.
8. Huang H, Ullah F, Zhou DX, Yi M, Zhao Y. Mechanisms of ROS regulation of plant development and stress responses. Front Plant Sci. 2019;10:800.
9. Madiha I, Bakht J, Shafi M, Rafi U. Screening of different brassica species for phytoextraction of heavy metal from soil. Afr J Biotechnol. 2012;11:7649-7658.
10. Radhakrishnan R, Khan AL, Kang SM, Lee I-J. A comparative study of phosphate solubilization and the host plant growth promotion ability of Fusarium verticillioides RK01 and Humicola sp. KNU01 under salt stress. Ann Microbiol. 2015;65:585-593.
11. Chakraborty K, Joseph D, Praveen NK. Antioxidant activities and phenolic contents of three red seaweeds (Division: Rhodophyta) harvested from the Gulf of Mannar of Peninsular India. J Food Sci Technol. 2015;52:1924-1935.
12. Berríos J, Illanes A, Aroca GJ. Spectrophotometric method for determining gibberellic acid in fermentation broths. Braz Arch Biol Technol. 2004;26:67-70.
13. Khalil A, Abdelaziz A, Khaleil M, Hashem A. Fungal endophytes from leaves of *Avicennia marina* growing in semi-arid environment as a promising source for bioactive compounds. Lett Appl Microbiol. 2021;72:263–274.
14. Liu D, Jiang W, Liu C, Xin C, Hou W. Uptake and accumulation of lead by roots, hypocotyls and shoots of Indian mustard (*Brassica juncea* (L.)). Bioresour Technol. 2000;71(3):273-277.
15. Reddy AM, Kumar SG, Jyonthsnakumari G, Thimmanaik S, Sudhakar C. Pb induced changes in antioxidant metabolism of horsegram (*Macrotyloma uniflorum* (Lam.) Verdc.) and bengalgram (*Cicer arietinum* L.). Chemosphere. 2005;60:97–104.
16. Shaw BP. Effects of mercury and cadmium on the activities of antioxidative enzymes in the seedling of Phaseolus aureus. Biol Plant. 1995;37:587–596.
17. Uzal O, Yasar F. Effects of GA3 hormone treatments on ion uptake and growth of pepper plants under cadmium stress. Appl Ecol Environ Res. 2017, 15(4).
18. Atici Ö, Ağar G, Battal PEYAMI. Changes in phytohormone contents in chickpea seeds germinating under lead or zinc stress. Biologia plantarum. 2005;49:215-222.
19. Kaur G, Kaur S, Singh HP, Batish DR, Kohli RK, Rishi V. Biochemical adaptations in Zea mays roots to short-term Pb2+ exposure: ROS generation and metabolism. Bull Environ Contam Toxicol. 2015;95:246-253.