Effects of sodium nitroprussid and calcium silicate on the physiological and biochemical parameters of wheat (*Triticum aestivum* L.) under cadmium stress

Saeid Soltani*

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

Abstract

Cadmium is a heavy metal with high toxicity that causes oxidative stress in plants and animals. Heavy metal stress tolerance in plants with increasing levels of reactive oxygen species (ROS) require using antioxidative pathways. Hydroponic experiments were performed with thirteen treatments consisting of a control wheat plants not receiving heavy metal stress (CdSO4) and the ameliorative factors CaSiO3 and sodium nitroprussid (SNP) and also plants treated with CdSO4 at 25, 50, 75, and l00 µM concentrations with or without 1.5 mM SNP or CaSiO3. Leaf samples from 60-day-old plants were used for determination of activities of ascorbate peroxidase, peroxidase, and polyphenol oxidase enzymes and also chlorophyll a, chlorophyll b, total chlorophyll, carotenoids, MDA, and proline contents. Results indicated that cadmium decreased chlorophyll a, b, total chlorophyll, and carotenoid contents significantly while it increased ascorbate peroxidase, peroxidase, and polyphenol oxidase activities specially by adding CaSiO3. Also, polyphenol oxidase activity increased under Cd stress significantly, which further increased by adding the ameliorative factor CaSiO3. Moreover, MDA and proline contents increased under Cd stress significantly, which decreased by adding ameliorative factors especially CaSiO3. It was concluded that silicate enhances the Cd tolerance in *Triticum aestivum* L., which is attributed to its ameliorative effects as it suppresses CdSO4 uptake and root to shoot CdSO4 transport. In addition, the ameliorative effects of silicate enhance the plants' antioxidant defense mechanisms.

Keywords: antioxidant enzyme, cadmium, chlorophyll, enzyme activity, wheat

Soltani, S. 2023. 'Effects of sodium nitroprussid and calcium silicate on the physiological and biochemical parameters of wheat (*Triticum aestivum* L.) under cadmium stress'. *Iranian Journal of Plant Physiology 13* (1), 4401-4408.

Introduction

Wheat, *Triticum aestivum* (poaceae), is an adaptable cereal species that grows in large regions of the world with different weather conditions. It is an annual self-fertile plant that forms the main source of carbohydrate and human food. Containing gluten, wheat has a very high economical value in bakery industries.

Cadmium (Cd) is a ubiquitous toxic heavy metal, which has negative effects on plants including respiration and nitrogen assimilation (Naeem et al., 2016), root cell destruction and decreases in root elongation ((Rizwan et al., 2016b)), and inhibiting seed germination ((Gao and Song, 2019)and (Rizwan et al., 2016a)). This heavy metal

* *Corresponding Author*
E-mail Address: Ketaban@gmail.com
Received: June, 2022
Accepted: October, 2022.

which is poisonous to the living cells even in low concentrations, is accumulated mainly in soils through industrial processes such as mining activities and frequent sewage of phosphate fertilizers. It causes plant death through disruption of nutrient uptake and photosynthesis inhibition by activation of CO_2 fixing enzymes (Sandeep et al., 2019). The research has shown that cadmium prevents antioxidant activities in plants through lipid peroxidases and oxidized proteins aggregation, the signs of oxidative damage (Hasanuzzaman et al., 2018).

Silicon is part of micronutrient elements that improves plant's resistance to disease, water-use-efficiency, and photosynthesis, remediates nutrient imbalance in various plant species such as wheat, rice, maize and bamboo, alleviates heavy metal toxicity like arsenic (As), lead (Pb), cadmium (Cd), and chromium (Cr), and improves plants' resistance to salinity stress, increases the growth in many plants, reduces Cd uptake and transport from root to shoot, decreasing its distribution in the shoots (Imtiaz et al., 2016).

Nitric oxide (NO), a redox-related signaling molecule, is considered as a key regulator in plants growth and development as well as their response to abiotic stresses such as that of heavy metals in terms of the production of reactive oxygen species, which lead to oxidative stress. Plants in their attempts to decrease the toxic effects of heavy metal stress activate several mechanisms such as antioxidative enzymes. NO could regulate a variety of related genes expressions and protein activities in response to abiotic stress in plants. For example, sodium nitroprusside and nitric oxide (with sodium Nitroprusside as a donor) have an ameliorative role in the processes of physiological growth, germination, accessing to the iron, and mitigating the harmful effects of heavy metals (Wei et al., 2020).

Silicon and sodium nitroprusside might decrease the effects of cadmium heavy metal stress in wheats. This study investigated the ameliorating effects of silicate on *Triticum aestivum* L. grown in soils with Cd pollution.

Materials and Methods

Chemicals

Trichloroacetic acid (TCA), Thiobarbituric acid (TBA), Ascorbate, Pyrogallol, and Tris-HCl were purchased from Merck (Germany). All other chemicals were of analytical grade or purer.

Sampling, culture, and treatments

Wheat seeds (*Triticum aestivum* L.) were placed first in benomyl 2% fungicide for 10 minutes, then in alcohol 70% for 60 seconds and finally in Sodium Hypochlorid 10% for 10 minutes. At the end of each step, the seeds washed several times in distilled water and were put on wet filter papers for germination and transferred to Refrigerated incubator set at 25 ℃ for 3 days. Then, they were transferred to light for 2 days. Initially, the seedlings of equal size inside plastic pots (1000 ml volume) containing perlite were transferred to the culture chamber with the temperature set at 25±5 ℃, 80% humidity, and 16-8 hours lighting and darkness. The seedlings were irrigated up to 6 weeks with distilled water and fed with Arnon and Hoagland nutrient solution every other day. After that, treatments were applied for 2 weeks. In this experiment, cadmium heavy metal was used in the form of CdSO4. 5H2O, by 1 in 5 concentrations (0, 25, 50, 75, and 100 µM). Silicate was used as an ameliorative factor in form of $CaSiO_3$, by 2 in 1.5 mM concentration. Also, SNP was used as another ameliorative factor by 2 in 1.5 mM concentration. A total of 15 treatments were applied, each with three replicates. The control seedlings received no heavy metal stress or ameliorative factors. Treatments involved different concentrations of CdSO4.H2O with three levels, with or without ameliorative factor, CaSiO3 and SNP. In order to investigate the studied factors, the plant samples were harvested from aerial and vegetative parts and washed with distilled water at the end of week 8. Leaf samples were washed, weighed, and put in labeled sealed plastic bags, before they were transferred to a freezer set at -20 ℃. After washing several times with distilled water, the plant roots were also weighed and put into foils, labeled, and dried in an oven at 70 ℃ for 48 hours to measure dry weights.

Pigment analysis

Photosynthetic pigments were extracted from biomass in 20 ml acetone 80%. Then the extraction was separated from the sample residue by filtration through Whatman No.1 filter paper. Concentrations of chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids were determined spectrophotometrically using acetone 80% as a solvent. The pigment contents were calculated using the following equations (Lichtenthaler and Wellburn, 1983):

Chl a (mg ml^{-1}) = $12.25A_{663}$-$2.79A_{646}$

Chl b (mg ml^{-1}) = $21.50A_{646}$-$5.10A_{663}$

Tchl (mg ml^{-1}) = Chla + Chlb

Cx+c =$(1000A_{470}$-1.8Ca-85.02Cb)/198

where Chl a, Chl b, Tchl, and Cx+c denote Chlorophyll a, Chlorophyll b, total Chlorophyll, and carotenoids, respectively.

Enzyme Extraction and Assay

For antioxidant enzyme activity assays, fresh biomass samples (1 g) were ground in a mortar and suspended in 5 ml of Tris-HCl buffer (0.05 M, pH 7.5). The homogenates were centrifuged at 10,000 rpm for 25 min at 4 ℃, and the resulting supernatant was used for enzyme assays.

Enzymatic activities determination

The peroxidase activity was determined according to the method of Kar and Mishra (Kar and Mishra, 1976). The enzyme activity was calculated at 425 nm. Total ascorbate peroxidase (APX) was determined according to the method described by Nakano and Asada (Nakano and Asada, 1981). The reaction mixture consisted of phosphate buffer (pH 7), 5 mM ascorbate, 5 mM H_2O_2, and supernatant. Total ascorbate peroxidase activity was determined as the decrease in absorbance of ascorbate at 290 nm as ascorbate was oxidized. The polyphenol oxidase activity was determined according to the method of Nicoli et al(NICOLI et al., 1991). The enzyme activity was calculated at 420 nm.

Lipid peroxidation determination

Concentration of MDA was measured according to the method described by Heath and Packer (Heath and Packer, 1968). In brief, the solution with an equal volume of 10% trichloroacetic acid (TCA) and 0.5% thiobarbituric acid (TBA) was put into a centrifuge tube; then, the mixture was heated at 95 ℃ for 15 min and quickly cooled in an ice bath. After centrifuging at 8,000 rpm for 5 min, the absorbance at 532 nm was measured and the value was subtracted at 600 nm. The concentration of MDA was calculated using an extinction coefficient of 155 $mM^{-1}cm^{-1}$.

Statistical Analysis

Experimental data were expressed as means ± SD of three replicates. The data were analyzed by analysis of variance ($P<0.05$), and the means were separated by Duncan's multiple range test.

Results

Under high cadmium heavy metal stress plant growth stopped and the biomass growth decreased, which led to plant death The typical symptoms of cadmium toxicity were observed in the medium culture such as development of reddish brown necrotic and leaf chlorosis, tubular leaves, brownish roots, and general decrease in root and shoot growth, especially in high concentrations. Adding $CaSiO_3$ and SNP to medium the culture in 1.5 mM concentration decreased the effects of cadmium toxicity. Under similar concentrations, the ameliorating effects of $CaSiO_3$ in decreasing cadmium toxicity symptoms were significantly different.

Results of photosynthetic pigments content measurement

Findings showed that maximum contents of photosynthetic pigments (Chlorophyll a, b, and total) belonged to the control plants (especially control with the ameliorative factor, $CaSiO_3$) so that with increasing cadmium concentration (cadmium sulfate without ameliorative factor) from 25 µM to 100 µM concentration, contents of chlorophylls decreased. However, with adding

ameliorative factors, $CaSiO_3$ and SNP (each 1.5 mM), specially $CaSiO_3$, the Chlorophyll contents increased in wheat plants (Fig. I. A, B, C).

Carotenoid contents

The results of carotenoid photosynthetic pigments suggested the destructive effects of cadmium sulfate on carotenoid contents of wheat plants. As with chlorophylls contents, different cadmium sulfate concentrations without ameliorative factors resulted in reduced carotenoids in comparison with other treatments, especially in high concentrations. The highest carotenoid contents were recorded in control plants. But with addition of ameliorative factors, $CaSiO_3$ and SNP (1.5 mM) especially $CaSiO_3$, the carotenoids contents increased in wheat plants (Fig. I. D).

Antioxidant enzyme contents

The effect of cadmium treatments and silicate on the wheat plants were examined and for assaying antioxidant enzymes activity, the activities of four enzymes, namely peroxidase, ascorbate peroxidase, and polyphenol oxidase, were measured.

Peroxidase

The peroxidase enzyme activity (Fig. I. A) increased with more stress from control to 75 µM $CdSO_4$ concentration without ameliorative factor; however, in 100 µM $CdSO_4$ concentration without ameliorative factor, the activity of this enzyme showed a decreasing trend. On the other hand, adding ameliorative factors, $CaSiO_3$ and SNP, had a positive effect and an increasing trend was observed in the activity of peroxidase. Meanwhile, the positive effect of ameliorative factor, $CaSiO_3$, in increasing the peroxidase enzyme activity was obvious.

Ascorbate peroxidase

The obtained data revealed that ascorbate peroxidase activity increased from control to 50 µM $CdSO_4$ concentration without applying ameliorators (Fig. II. B). But thereafter, the level of APX activity decreased until 100 µM $CdSO_4$ concentration, where adding $CaSiO_3$ and SNP

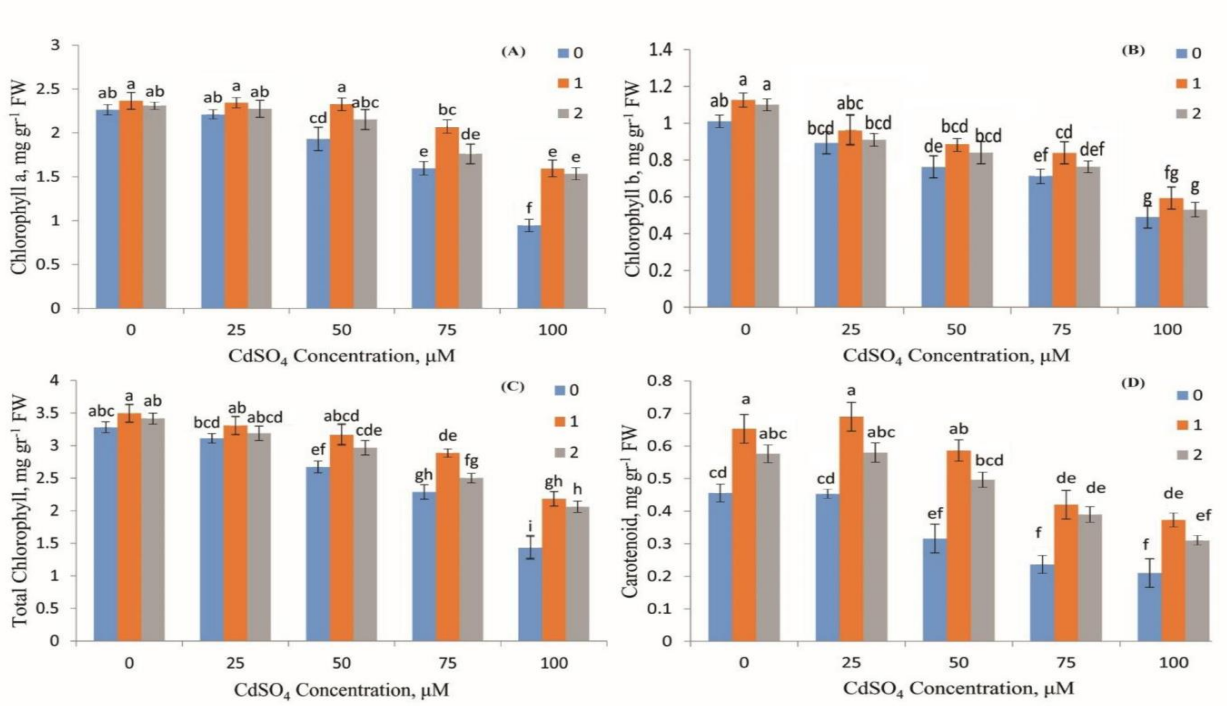

Fig. I. Effect of Cd in combination with Si or SNP on chlorophyll a (A), chlorophyll b (B), total chlorophyll (C), and carotenoid contents (D) of wheat plant; 0: without Si and SNP, 1: 0.2 mM Si, and 2: 0.2 mM SNP. Data represent means ± standard errors of three experiments with three replicates. Different letters indicate significant differences between treatments at P<0.05, according to Duncan's multiple range test.

treatments had considerable effects in increasing ascorbate peroxidase enzyme activities. The role of $CaSiO_3$ on APX activity was more prominent.

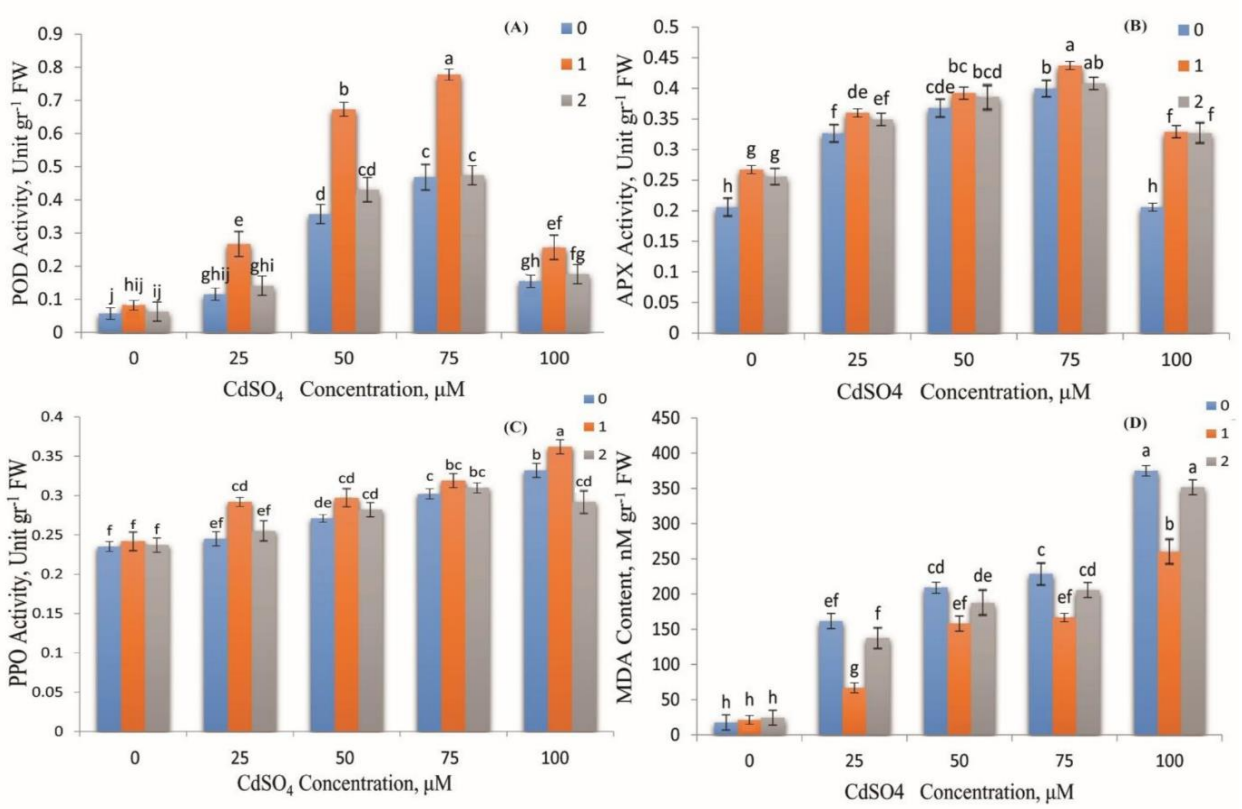

Fig. II. Effect of Cd in combination with Si or NO on peroxidase (POD) activity (A), ascorbate peroxidase (APX) activity (B), polyphenol (PPO) activity (C), and lipid peroxidase (MDA) activity (D) in wheat plant; 0: without Si and SNP, 1: 0.2 Mm Si, 2: 0.2 Mm SNP; data represent means ± standard errors of three experiments with three replicates. Different letters indicate significant difference between treatments at P<0.05, according to Duncan's multiple range test.

Polyphenol oxidase

The activities of polyphenol oxidase increased from control plants to 100 µM $CdSO_4$ concentration without applying ameliorative factors (Fig. II. C). Treatments with $CaSiO_3$ and SNP increased polyphenol peroxidase enzyme activity, with $CaSiO_3$ having a more pronounced effect. Generally, the lowest level of polyphenol oxidase activity was observed in 100 µM $CdSO_4$ concentration along with $CaSiO_3$.

Lipid peroxidation

The content of malondialdehyde increased with increasing $CdSO_4$ concentration from control to 100 µM, in the treatments without ameliorative factors (Fig. II. D). $CaSiO_3$ and SNP were able to decrease MDA contents with $CaSiO_3$ being more successful in decreasing MDA contents. The minimum and maximum MDA contents were recorded in control plants and those treated with 75 and 100 µM $CdSO_4$ without ameliorative factors, respectively.

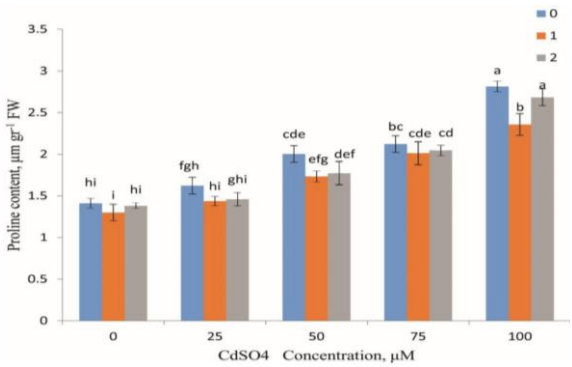

Fig. III. Effect of Cd in combination with Si or NO on proline content; 0: without Si and SNP, 1: 0.2 Mm Si, 2: 0.2 Mm SNP. Data represent means ± standard errors of three experiments with three replicates. Different letters indicate significant difference between treatments at P<0.05, according to Duncan's multiple range test.

Proline

The highest proline content was observed in 100 µM $CdSO_4$ concentration treatment without ameliorative factors while the lowest proline content was recorded in 50 µM $CdSO_4$ concentration treatment with $CaSiO_3$ treatment (Fig. III). Proline content increased from control and 25 µM $CdSO_4$ concentration treatments without ameliorative factor to 100 µM $CdSO_4$ concentration treatment without ameliorative factor. Treatments with $CaSiO_3$ and SNP had positive effects as they resulted in a decreasing trend in proline content with $CaSiO_3$ having a more prominent effect.

Discussion

Environmental toxicants including heavy metals seriously damage the function and structure of ecosystem (Alengebawy et al., 2021). Cadmium is toxic to any biological system including plants even at low concentrations, affecting the plants growth and human health (Shahid et al., 2017). The toxic effects of Cadmium on plants include inhibition of shoot and root growth, and reduction in photosynthesis, and further crop yield. Moreover, Cadmium could accumulate in different tissues of the plants such as fresh vegetables and transfer them into the human food chain, causing several health problems such as brain and kidney diseases and chromosomal disorders (Fattahi et al., 2019). The agricultural advantages of silicon on the soil ecosystems have been well proved. The obtained results from photosynthetic pigments (chlorophyll a, chlorophyll b, and total chlorophyll) are consistent with the results of previous studies(Feret et al., 2008). The photosynthetic apparatus appears to be especially sensitive to Cd negative effect with change of chlorophyll synthesis, transpiration, and respiration process, thus reducing photosynthesis. The responses of photosynthesis to Cd can be different and sometimes opposite according to the species. Chlorophyll fluorescence parameters were found to be strongly correlated with the whole-plant mortality following environmental stress and therefore can be considered as reliable indicators of the intensity of stress (Li et al., 2015). Plants have evolved enzymatic and non-enzymatic antioxidant mechanism to protect their cellular and sub-cellular components from the effects of reactive oxygen species. Antioxidant enzymes include superoxide dismutase, peroxidase, catalase, and ascorbate peroxidase, and antioxidant compounds include ascorbate, carotenoids, α-tocopherols, and glutathione. Carotenoids as a non-enzymatic mechanism against heavy metal stress have the ability to eliminate free oxygen radicals (Ulusu et al., 2017). These antioxidants protect photosynthetic tissues specially chlorophylls against oxidative stress in plants. Decrease in carotenoid levels is because of non-photochemical suppression of excited chlorophylls implemented by carotenoids that result in their disrupted structures. All types of active oxygen are reduced forms of atmospheric oxygen. There are many potential sources of active oxygen in plants, some of which are created in natural metabolism such as photosynthetic electron transport chain in chloroplasts and respiration. In the enzymatic defense mechanism of plans against ROS, antioxidant enzymes such as superoxide dismutase, ascorbate peroxidase, catalase, polyphenol oxidase, and ascorbate peroxidase have the main role (Ulusu et al., 2017). AlKahtani et al. (2021) reported the effects of silicon on some crops under drought stress, showing that it leads to an improved water absorption under drought stress. Activity of some antioxidant enzymes such as superoxide dismutase, catalase was shown to increase under stress condition when silicon, Si, and proline were added individually or in combination. Si and proline considerably increased root in sugar beet (AlKahtani et al., 2021)

Catalase is one of the most important antioxidant enzymes that converts H_2O_2 to water in the peroxisome. In this organelle, H_2O_2 is produced from beta-oxidation of fatty acids and photorespiration (Ighodaro and Akinloye, 2018). The higher activity of catalase and ascorbate peroxidase decreased H_2O_2 level of the cells and increased cell membrane stability and stabilization of CO_2, because some of Calovin cycle enzymes in chloroplasts are very sensitive to H_2O_2. The high level of H_2O_2 inhibits CO_2 stabilization directly. Ighodaro and Akinloye (2018) reported that the ability of Catalase to effectively limit H_2O_2 concentration in cells underlines its importance in the aforementioned physiological processes as well as being the first line antioxidant defense

enzyme. Ascorbate peroxidase and catalase activities, representing the main enzymatic H_2O_2 scavenging mechanism in plants, are crucial for the suppression of toxic H_2O_2 levels in a cell (Sofo et al., 2015). (Małecka et al., 2019)reported that the presence of Pb, Cu, Cd, and Zn in *Brassica juncea* increased the activity of antioxidant enzymes such as superoxide dismutase, catalase, and ascorbate peroxidase, which increased uptake of these heavy metals. Zhang et al(Zhang et al., 2020)found that SOD, CAT, and POD were more active in responding to Cd stress in *Brassica napus* and high activity of enzymatic antioxidants at initial Cd stress stage was the main detoxification mechanism in Cd-tolerant rapeseed.

Proline accumulation is an adaptive strategy of plants to stressful environment which maintains the osmotic balance, scavenges excess free radicals, and stabilizes cell membrane structure under Cd stress (Khan et al., 2015).

Malondialdehyde is considered as a marker for the scale of lipid peroxidation or damage to the plasma and membrane of the organelle, which increases with environmental stresses. Concentrations of hydrogen peroxide and MDA was enhanced under Cd treatment in *Pisum sativum* (Sayed and Gadallah, 2019). The lipid peroxidation is related to the antioxidant enzyme activities, whereas with more superoxide dismutase, ascorbate peroxidase, glycol peroxidase, and catalase content, resistance to the oxidative stresses increased while the malondialdehyde contents decreased. cadmium treatment caused reduction in the growth parameters such as dry mass, chlorophyll, and carotenoid contents.

ACKNOWLEDGEMENTS

This research was partially supported by a grant from the Research Council of Islamic Azad University, Qaemshahr Branch, Qaemshahr, Iran.

References

Alengebawy, A., S. T. Abdelkhalek, S. R. Qureshi and M.-Q. Wang. 2021. Heavy metals and pesticides toxicity in agricultural soil and plants: Ecological risks and human health implications. *Toxics,* 9, (3) 42.

Alkahtani, M. D., Y. M. Hafez, K. Attia, E. Rashwan, L. A. Husnain, H. I. Algwaiz and K. A. Abdelaal. 2021. Evaluation of silicon and proline application on the oxidative machinery in drought-stressed sugar beet. *Antioxidants,* 10, (3) 398.

Fattahi, B., K. Arzani, M. K. Souri and M. Barzegar. 2019. Effects of cadmium and lead on seed germination, morphological traits, and essential oil composition of sweet basil (Ocimum basilicum L.). *Industrial Crops and Products,* 138, 111584.

Feret, J.-B., C. François, G. P. Asner, A. A. Gitelson, R. E. Martin, L. P. Bidel, S. L. Ustin, G. Le Maire and S. Jacquemoud. 2008. PROSPECT-4 and 5: Advances in the leaf optical properties model separating photosynthetic pigments. *Remote sensing of environment,* 112, (6) 3030-3043.

Gao, M. and Z. Song. 2019. Toxicity of cadmium to wheat seedling roots in the presence of graphene oxide. *Chemosphere,* 233, 9-16.

Heath, R. L. and L. Packer. 1968. Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. *Archives of biochemistry and biophysics,* 125, (1) 189-198.

Ighodaro, O. and O. Akinloye. 2018. First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. *Alexandria journal of medicine,* 54, (4) 287-293.

Imtiaz, M., M. S. Rizwan, M. A. Mushtaq, M. Ashraf, S. M. Shahzad, B. Yousaf, D. A. Saeed, M. Rizwan, M. A. Nawaz and S. Mehmood. 2016. Silicon occurrence, uptake, transport and mechanisms of heavy metals,

minerals and salinity enhanced tolerance in plants with future prospects: a review. *Journal of environmental management,* 183, 521-529.

Kar, M. and D. Mishra. 1976. Catalase, peroxidase, and polyphenoloxidase activities during rice leaf senescence. *Plant physiology,* 57, (2) 315-319.

Khan, M. I. R., F. Nazir, M. Asgher, T. S. Per and N. A. Khan. 2015. Selenium and sulfur influence ethylene formation and alleviate cadmium-induced oxidative stress by improving proline and glutathione production in wheat. *Journal of plant physiology,* 173, 9-18.

Li, S., W. Yang, T. Yang, Y. Chen and W. Ni. 2015. Effects of cadmium stress on leaf chlorophyll fluorescence and photosynthesis of Elsholtzia argyi—a cadmium accumulating plant. *International Journal of Phytoremediation,* 17, (1) 85-92.

Lichtenthaler, H. K. and A. R. Wellburn. 1983. Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. Portland Press Ltd.

Małecka, A., A. Konkolewska, A. Hanć, D. Barałkiewicz, L. Ciszewska, E. Ratajczak, A. M. Staszak, H. Kmita and W. Jarmuszkiewicz. 2019. Insight into the phytoremediation capability of Brassica juncea (v. Malopolska): Metal accumulation and antioxidant enzyme activity. *International Journal of Molecular Sciences,* 20, (18) 4355.

Nakano, Y. and K. Asada. 1981. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant and cell physiology,* 22, (5) 867-880.

Nicoli, M. C., B. E. Elizalde, A. Pitotti and C. R. Lerici. 1991. Effect of sugars and maillard reaction products on polyphenol oxidase and peroxidase activity in food. *Journal of Food Biochemistry,* 15, (3) 169-184.

Rizwan, M., S. Ali, T. Abbas, M. Zia-Ur-Rehman, F. Hannan, C. Keller, M. I. Al-Wabel and Y. S. Ok. 2016a. Cadmium minimization in wheat: a critical review. *Ecotoxicology and environmental safety,* 130, 43-53.

Rizwan, M., S. Ali, M. Adrees, H. Rizvi, M. Zia-Ur-Rehman, F. Hannan, M. F. Qayyum, F. Hafeez and Y. S. Ok. 2016b. Cadmium stress in rice: toxic effects, tolerance mechanisms, and management: a critical review. *Environmental Science and Pollution Research,* 23, 17859-17879.

Sandeep, G., K. Vijayalatha and T. Anitha. 2019. Heavy metals and its impact in vegetable crops. *Int J Chem Stud,* 7, (1) 1612-1621.

Sayed, S. and M. Gadallah. 2019. Hydrogen peroxide supplementation alleviates the deleterious effects of cadmium on photosynthetic pigments and oxidative stress and improves growth, yield and pods quality of pea (Pisum sativum L.) plants. *Acta Physiologiae Plantarum,* 41, 1-12.

Shahid, M., C. Dumat, S. Khalid, E. Schreck, T. Xiong and N. K. Niazi. 2017. Foliar heavy metal uptake, toxicity and detoxification in plants: A comparison of foliar and root metal uptake. *Journal of hazardous materials,* 325, 36-58.

Sofo, A., A. Scopa, M. Nuzzaci and A. Vitti. 2015. Ascorbate peroxidase and catalase activities and their genetic regulation in plants subjected to drought and salinity stresses. *International Journal of Molecular Sciences,* 16, (6) 13561-13578.

Ulusu, Y., L. Öztürk and M. Elmastaş. 2017. Antioxidant capacity and cadmium accumulation in parsley seedlings exposed to cadmium stress. *Russian journal of plant physiology,* 64, 883-888.

Wei, L., J. Zhang, C. Wang and W. Liao. 2020. Recent progress in the knowledge on the alleviating effect of nitric oxide on heavy metal stress in plants. *Plant Physiology and Biochemistry,* 147, 161-171.

Zhang, F., X. Xiao and X. Wu. 2020. Physiological and molecular mechanism of cadmium (Cd) tolerance at initial growth stage in rapeseed (Brassica napus L.). *Ecotoxicology and Environmental Safety,* 197, 110613.