

EFFECTS OF SOME OF HEAVY METALS ON TOTAL PROTEIN AMOUNT AND PEROXIDASE ACTIVITY IN SOLANUM LYCOPERSICUM MILL

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ABSTRACT

In this research, different concentrations of cadmium, nickel, copper heavy metals applied on 10 weeks old Solanum lycopersicum Mill. cv. invictus seedlings by irrigation water. As a result of heavy metal applications total protein amount and peroxidase [EC 1.11.1.7] enzyme activity has been determined by spectrophotometrically. Changing in total protein amount and peroxidase activity in S. lycopersicum seedlings after heavy metal applications has been compared with control group. Depending the application of heavy metals on S. lycopersicum seedlings total protein amount decreased as 57% in 100 ppm of copper application and 10,9% increased in 10 ppm nickel application. The highest increases in peroxidase activity 100 ppm of copper applied and in proportion as 536,03% and treated group with 10 ppm nickel in proportion as 5.97% were determined.

Keywords: Solanum lycopersicum, cadmium, nickel, copper, total protein, peroxidase.

1. INTRODUCTION

Solanum lycopersicum Mill. is the member of Solanaceae family and one of the most important vegetable crop grown at the different part of the world (Manawadu et al., 2014). It contains a lot of nutrients and antioxidants (lycopene, phenol, flavonoid, vitamin C) which are quite important as food and for the human health (Periago et al., 2009). However, heavy metal impacts on food production and human health have become a global concern all over the world. The main reasons of heavy metal pollution are generally urbanisation and industrialisation with severe long-term consequences (Rai et al., 2019). The decline in environmental quality as a result of pollution is evidenced by loss of biological diversity, vegetation, harmful chemicals in food grains and atmosphere threats to life support systems (Rai, 2016). It is well known that, some of metals such as copper (Cu), iron (Fe), boron (B), nickel (Ni), manganese (Mn), zinc (Zn) and molybdenum (Mo) are needed in smaller amounts which are called micronutrients or trace elements (DalCorso et al., 2014). These metals can have adverse effect on crop agriculture and human health, when they reach high concentration (Wang et al., 2009; Chaves et al., 2011; DalCorso et al., 2014). Copper is the essential micronutrient and involved in numerous important for the physiological and biochemical functions (Sharma and Agrawal, 2005). Ni is recognized an essential micronutrient for plant growth and it is a component of the enzyme urease which is essential for nitrogen metabolism in higher plants (Bhalerao et al., 2015). Furthermore, some of metals (non essential elements) may potentially harmful effect such as cadmium which is the highest in terms of damage to human health and plant growth (Chaves et al., 2011). Heavy metal's effects can result in breaks on DNA strands, mutations of genetic materials, oxidative stress and damage by reactive oxygen species (ROS) and free radicals, structural and functional membrane disintegration (Rai et al., 2019). In many different plant species, oxidative stress has been involved in toxicity of heavy metals. Exposure to metal ions may increase the production of ROS such as hydroxyl radicals, superoxide radicals, or hydrogen peroxide which may react with proteins, lipids nucleic acids and resulted in lipid peroxidation, membrane damage and inactivation of enzyme. Therefore, heavy metals have adverse affect on many physiological processes and also cell viability. Plants have an important mechanism to maintain low ROS level and prevent from the harmful effects of excess level of ROS concentrations. This antioxidant mechanism consists of numerous soluble (glutathione, ascorbate) and membrane (tocopherol) compounds and enzymes to ROS scavenging such as catalase, ascorbate peroxidase, superoxide dismutase, peroxidase (Jomova and Morovic, 2009; Rucińska-Sobkowiak, 2010). Another important factor in plant defense system under stress conditions is variation in total protein level (Çördük et al., 2016). Exposure to heavy metal stress can cause protein degradation (Emamverdian et al., 2015).

In this study, specific aim was to investigate effects of different concentrations of cadmium, copper and nickel heavy metals on the peroxidase activity and total protein content in *S. lycopersicum*.

2. MATERIAL AND METHODS

2.1. Experimental Plant Material

Seeds were used in this study were obtained from agricultural store. Seeds of *S. lycopersicum* were planted in plastic pots which contain mixture of perlite and torf (1:3). Seedlings were grown in a growth chamber condition at 25±2 °C and 16/8 h photoperiod.

2.2. Heavy Metal Treatment

Ten weeks old seedlings were treated with CuCl₂ (12.5 ppm, 25 ppm, 50 ppm, 100 ppm), Cd (NO₃)₂·4H₂O and NiCl₂·6H₂O (10 ppm, 20 ppm, 30 ppm, 40 ppm) by irrigating with water.

All of the experiments have been repeated for three times. Each group has ten tomato seedlings and one group was selected as a control group in each treatment.

2.3. Preperation of Leaf Extracts

Approximately 0.5 g healthy fresh tomato leaves were harvested and grinded in cold 0.05 M sodium acetate buffer (pH 6.5). Homogenates transferred to the eppendorf tubes which were centrifugated at 13.000 rpm for 15 min at 4°C. The supernatants were used for determination of the enzyme activity and total protein content.

2.4. Determination of Total Protein Amount and Peroxidase Enzyme Activity

Total protein content has been analyzed according to Bradford (1976) using BSA as a standard. Amount of total protein was measured spectrophotometrically at 595 nm. Peroxidase activity in the leaf extracts were assayed spectrophotometrically at 300nm according to Kanner and Kinsella (1983). The kinetic enzyme reaction was monitored over 120 sec. and peroxidase measurements were taken in every approximately between 10-15 s. Peroxidase enzyme activity was defined as $\mu\text{mol/mgprot/min}$.

3. RESULTS AND DISCUSSION

In this research, different concentrations of cadmium, nickel, copper heavy metals applied on ten weeks old *S. lycopersicum* cv. invictus seedlings with irrigation water that is being growth under in vivo conditions. Physiological responses were determined as total protein and POX enzyme activity by spectrophotometrically. After exposing to different heavy metals and concentrations, changing of peroxidase activity levels and total protein content in *S. lycopersicum* cv. invictus seedlings were compared with control group.

3.1. Total Protein Amount Results

Our research results showed that total protein content decreased with the increasing of heavy metal concentration comparing with the control group (Fig. 1,2,3), except 10 ppm nickel concentration (Fig 1). The most important reduction as 57% was observed in plants which were treated with 100 ppm CuCl_2 (Fig. 3).

Figure 1. Effects of Nickel on Total Protein Content in *S. lycopersicum*

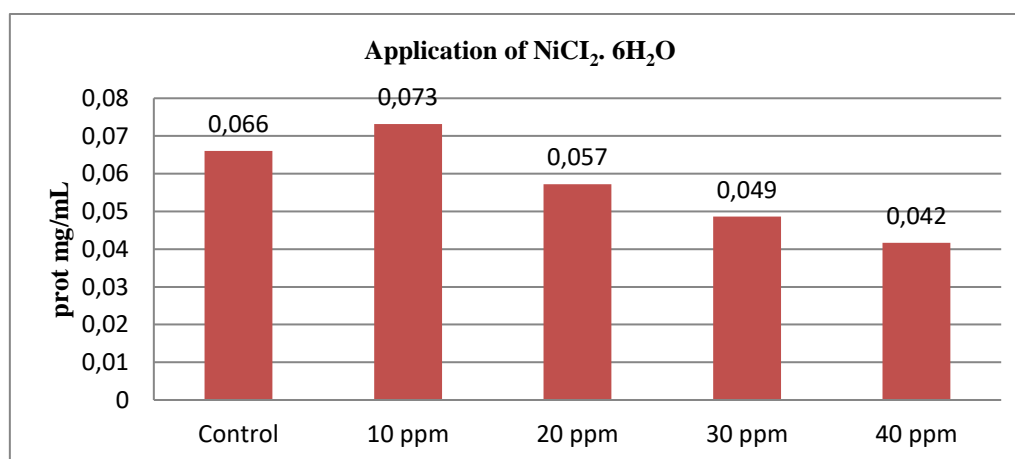


Figure 2. Effects of Cadmium on Total Protein Content in *S. lycopersicum*

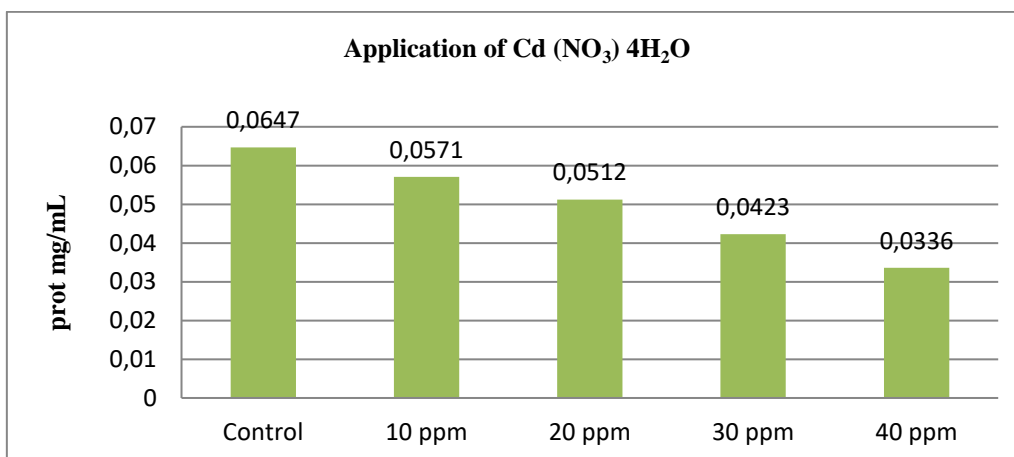
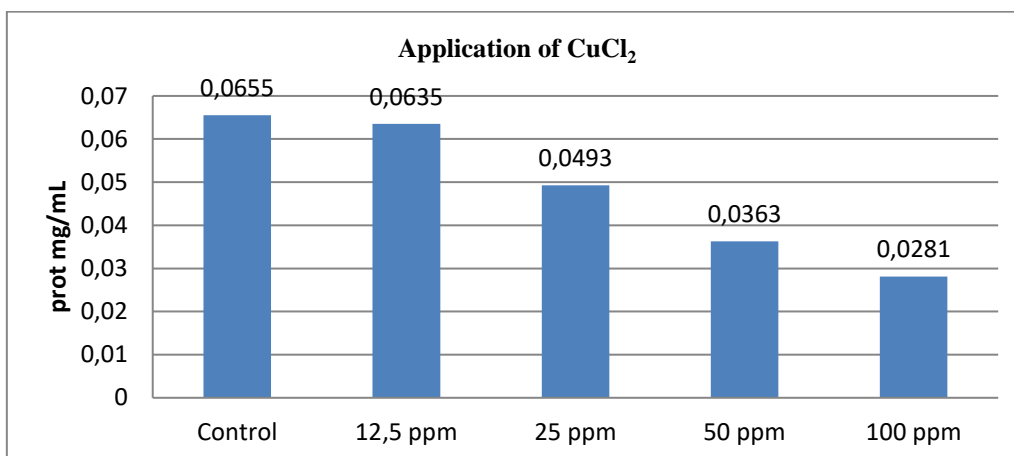


Figure 3. Effects of Copper on Total Protein Content in *S. lycopersicum*



3.2. Peroxidase Activity Results

In all treatments, peroxidase enzyme activities were increased in *S. lycopersicum* when compared with the control groups. Peroxidase activity increased with the increasing heavy metal concentration for three different heavy metals (Fig 4,5,6). The highest increase in peroxidase activity was measured as 536.03% in 100 ppm copper application (Fig. 6) and lowest increase was measured as 5.96% in 10 ppm nickel application (Fig. 4).

Figure 4. Effects of Nickel on Peroxidase Enzyme Activity in *S. lycopersicum*

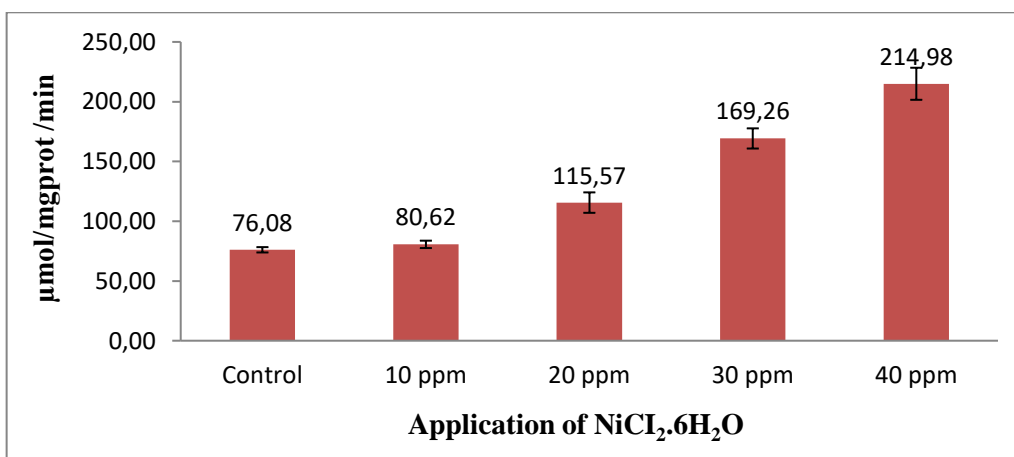


Figure 5. Effects of Cadmium on Peroxidase Enzyme Activity in *S. lycopersicum*

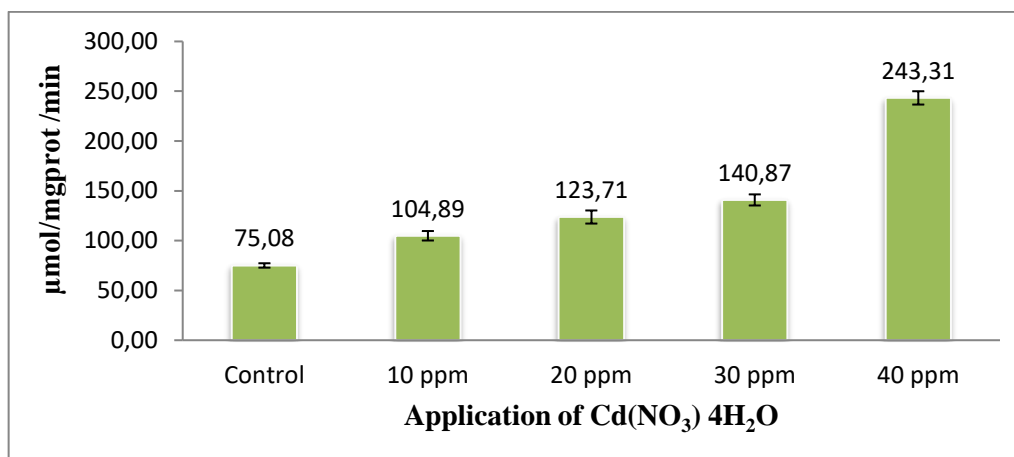
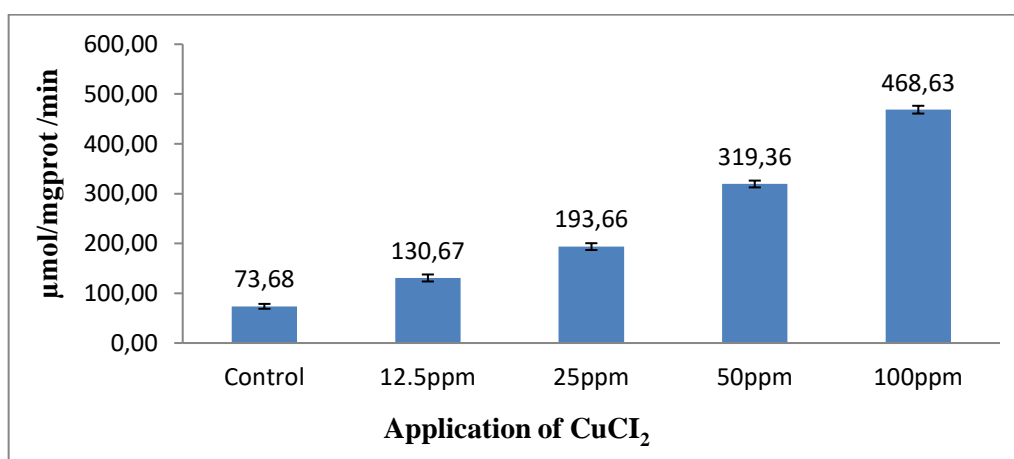


Figure 6. Effects of Copper on Peroxidase Enzyme Activity in *S. lycopersicum*



In our research, peroxidase enzyme activity increased with the increasing heavy metal concentration whereas protein concentration mostly showed reduction. The change in the total protein content may be associated with inhibition of protein synthesis and protein degradation (Çördük et al., 2016).

In parallel with our work research reported that peroxidase activity and proline content increased with the increasing concentration for nickel in water lettuce whereas total protein amount and other carotenoids started to show reduction at higher concentrations (Singh and Pandey, 2011).

In another research was observed remarkably reduction in chlorophyll and total protein content as copper concentration increased in *Helianthus annuus* L. seedlings (Kirbag Zengin and Kirbag, 2007).

In pea plants, protein content decreased especially in the presence of higher cadmium concentrations (Bavi et al., 2011). Cd resulted in a decrease in total protein content which can be consequences of increase in protein degradation, a decrease in protein synthesis (Balestrasse et al., 2003).

Similar to our results it was reported that increasing concentrations of Cu treatments resulted in a reduction of total protein concentration and Cu stressed had the most detrimental effects while Ni stress showed less damaging in *Ocimum basilicum* L. following a combined, biochemical, analytical, and physiological approach (Georgiadou et al., 2018).

In another research, it was observed that APX, POD, and SOD were shown remarkable induction with the treatment of Cd, Cu (10, 20 and 50 ppm) in the leaves of tomato when compared to control group (Kısa, 2017). Although high CdCl₂ concentrations effect on plants defense mechanism was substantially high, peroxidase activity in both shoots and roots were higher at low CdCl₂ concentrations. Protein sythesis was reduced excessively and thus no peroxidase enzyme is synthesized (Bavi et al., 2011).

4. CONCLUSION

In conclusion, the present investigation showed that Cd, Cu and Ni at higher concentrations had adverse effect on *S. lycopersicum*. Our results generally showed that exposure to different concentrations of heavy metals caused increasing of POX acitivity and reduction of total protein level.

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REFERENCES

- BALESTRASSE, K.B., BENAVIDES M.P., GALLEGO S.M., TOMARO M.L., 2003, Effect of Cadmium Stress on Nitrogen Metabolism in Nodules and Roots of Soybean Plants, *Functional Plant Biology*, 30, 57-64.
- BAVI, K., KOHELDEBARIN B., MOREDSHAHI A., 2011, Effect of Cadmium On Growth, Protein Content and Peroxidase Activity in Pea Plants, *Pak. J. Bot.*, 43 (3), 1467-1470.
- BHALERAO, S.A., SHARMA, A.S., POOJARI, A.C., 2015, Toxicity of Nickel in Plants, *International Journal Of Pure & Applied Bioscience*, 3 (2), 345-355.
- BRADFORD, M.M., 1976, A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding, *Analytical Biochemistry*, 72, 248-254.
- CHAVES, L.H.G., ESTRELA, M.A., SOUZA, R.S., 2011, Effect on Plant Growth and Heavy Metal Accumulation by Sunflower, *Journal of Phytology*, 3 (12), 04-09.
- ÇÖRDÜK, N., AKINCI, N., KAYA, N., YÜCEL, G., AKI, C., 2016, Effects of Dodine on Total Protein Content and Peroxidase Activity in *Vicia faba* L., *SAÜ Fen Bil Der.*, 20 (3), 627-633.
- DALCORSO, G., MANARA, A., PIASENTIN, S., FURINI, A., 2014, Nutrient Metal Elements in Plants, *Metallomics*, 6 (10), 1770-88.
- EMAMVERDIAN, A., DING, Y., MOKHBERDORAN, F., XIE, Y., 2015, Heavy Metal Stress and Some Mechanisms of Plant Defense Response, *The Scientific World Journal*, Volume 2015, 1-18.
- GEORGIADOU, E.C., KOWALSKA, E., PATLA, K., KULBAT, K., SMOLISNKA, B., LESZCZYNSKA, J., FOTOPOULOS, V., 2018, Influence of Heavy Metals (Ni, Cu, and Zn) on Nitro-Oxidative Stress Responses, Proteome Regulation and Allergen Production in Basil (*Ocimum basilicum* L.) Plants, *Frontiers in Plant Science*, 9, 862.
- JOMOVA, K., and MOROVIC, M., 2009, Effect of Heavy Metal Treatment on Molecular Changes in Root Tips of *Lupinus luteus* L., *Czech J. Food Sci.*, 27, 386-389.
- KANNER, J., KINSELLA, J.E., 1983, Lipid Deterioration Initiated by Phagocytic Cells in Muscle Foods: β -Carotene Destruction by a Myeloperoxidase-Hydrogen Peroxide Halide System, *Journal of Agricultural and Food Chemistry*, 31, 370-376.
- KISA, D., 2017, The Responses of Antioxidant System against the Heavy Metal-Induced Stress in Tomato, *Süleyman Demirel University Journal of Natural and Applied Sciences*, 22 (1), 1-6.
- KİRBAĞ ZENGİN, F., KİRBAĞ, S., 2007, Effects of Copper on Chlorophyll, Proline, Protein and Abscisic Acid Level of Sunflower (*Helianthus annuus* L.) Seedlings, *Journal of Environmental Biology*, 28 (3), 561-566.
- MANAWADU, I.P., NILANTHI D., SENANAYAKE S.G.J.N, 2014, Callus Formation and Organogenesis of Tomato (*Lycopersicon esculentum* Mill. variety thilina), *Tropical Agricultural Research and Extension*, 17 (2), 87-94.
- PERIAGO, M.J., GARCIA-ALONSO, J., JACOB, K., OLIVARES, A.B., BERNAL, M.J., INIESTA, M.D., MARTINEZC., ROS G., 2009, Bioactive Compounds, Folates and Antioxidant Properties of Tomatoes (*Lycopersicum esculentum*) During Vine Ripening, *International Journal of Food Sciences and Nutrition*, 60 (8), 694–708.

- RAI, P.K., 2016, Particulate Matter and Its Size Fractionation, Biomagnetic Monitoring of Particulate Matter, Chapter 1, pages 1-13.
- RAI, P.K., LEES, S., ZHANG, M., TSANG, Y.F., KIM, K.H., 2019, Heavy Metals in Food Crops: Health Risks, Fate, Mechanisms and Management, *Environment International*, 125, 365–385.
- RUCINISKA-SOBKOWIAK, R., 2010, Oxidative Stress in Plants Exposed to Heavy Metals, *Postepy Biochem.*, 56 (2),191-200.
- SHARMA, R.K., AGRAWAL, M. 2005, Biological effects of heavy metals: An overview, *Journal of Environmental Biology*, 26 (2), 301-313.
- SINGH, K., PANDEY, S.N., 2011, Effect of Nickel-Stresses on Uptake, Pigments and Antioxidative Responses of Water Lettuce, *Pistia stratiotes* L. J., *Environ. Biol.* 32, 391-394.
- WANG, C., ZHANG, S.H.,WANG, P.F., HOU, J., ZHANG, W.J., LI, W., LIN, Z.P., 2009, The Effect of Excess Zn on Mineral Nutrition and Antioxidative Response in Rapeseed Seedlings., *Chemosphere*, 75 (11), 1468–1476.