International Journal of Academic Multidisciplinary Research (IJAMR)
ISSN: 2643-9670
Vol. 5 Issue 8, August - 2021, Pages: 212-217

Response of safflower (*Carthamus tinctorius* L.) seedlings to cobalt toxicity

Muhittin Dogan, Serap Sahin Yigit*, Gulcan Cinar

Department of Biology, Faculty of Arts and Sciences, Gaziantep University, 27310, Gaziantep, Turkey

*Corresponding author e-mail: serap.syigit@gmail.com

Abstract: *The present study was carried out to determine the toxic effects of different Co concentrations on safflower seedlings. The study was conducted in a controlled climate cabinet and a hydroponic medium was preferred for Co application to the seedlings. After two weeks of Co application, Co contents, some physiological changes and nutrient levels were determined. Cobalt contents of safflower seedling parts were found to increase with applied Co concentration. In addition, it was determined that the Co content was ordered as root>stem>leaf. There was a decrease in photosynthetic pigment contents of seedling leaves. On the contrary, total carbohydrate content of the seedling parts generally increased in a dose-dependent manner. In addition, increases in phenolic compounds of the above-ground parts of the seedlings were determined. Positive relationships were found between Co contents of seedling organs and malondialdehyde (MDA) contents. This clearly showed that Co toxicity caused oxidative stress in seedling cells. While potassium content decreased in root and stem tissues, it increased in leaves. Calcium and Mg contents were generally increased. In addition, increases were found in the Zn contents of the seedling parts, but decreased in the Fe and Cu contents. These results showed that applied Co caused nutrient imbalance in the seedlings.*

Keywords— Safflower (*Carthamus tinctorius* L.); Co toxicity; physiological changes; nutrient levels

1. INTRODUCTION

Cobalt is in group 9 in the periodic table of elements and its concentration in the earth's crust is between 9 and 12 ppm. Its average concentration in soils around the world is estimated at 10 ppm. Higher Co concentrations, that average is 12 ppm, and are usually found in heavy loam soils. Cobalt contamination in soils has been encountered in many areas. Especially high Co pollution has been reported for some mining areas. It may also contain high Co pollution in areas around metal processing industries [1].

Generally, Co uptake by plant roots is transported to more above-ground parts via transpiration flow. However, Co, due to its relatively low mobility in plants, restricts its transport from the stem to the leaves. The beneficial effects of Co on plant metabolisms have not been fully understood yet. These effects may be cross-linked by various interactions with other chemical elements [1]. On the contrary, excess Co taken up by plants can have negative effects on biological processes. In the literature, there are studies on the effects of Co application on plants. Growth parameters, photosynthetic pigment content, biochemicals and mineral content increased under the influence of 50 ppm Co. On the contrary, further increase in Co concentrations had a negative effect on growth, biochemical parameters and mineral contents [2]. Cobalt inhibited the vegetative growth of *Lemna minor*. Also, unlike in other plants, Co accumulation did not reduce the Fe content in the fronds [3]. It was found that only Mn and Fe from macronutrients and micronutrients analyzed in root and leaf tissue of mung bean treated with 5 µM Co were not significantly affected [4]. Excessive Co application decreased biomass, P, S and Fe, chlorophyll a and b, sugars and protein content in tomato plant. Similarly, there was a change in antioxidant enzyme activities in tomato leaves treated with excessive Co [5]. It has been reported that 100 µM Co may cause oxidative damage by interfering with ROS production and chlorophyll metabolism by inducing oxidative stress in Indian mustard leaves in a time-dependent manner [6].

Safflower (*Carthamus tinctorius* L.) is an annual oil plant belonging to the Asteraceae family and its seeds are used for various purposes in human nutrition due to its high linoleic acid and vitamin E content [7].

This study was carried out to determine the effect of Co concentrations on physiological parameters and mineral contents of safflower seedlings.

2. MATERIALS AND METHODS

Safflower seeds were sterilized with 5% NaOCl in pre-experiment stage. The seeds were then sown in perlite medium under constant dark conditions at 26±1°C in a controlled climate cabinet (Snijders Scientific, Netherlands). The seeds were irrigated with distilled water in case of need. On the 14th day of the study, four seedlings in each container (2000 ml) were transferred to aerated water culture medium containing 10% nutrient solution (0.88 mM K_2SO_4, 2 mM Ca $(NO_3)_2$, 0.25 mM KH_2PO_4, 1 mM $MgSO_4$, 0.11 mM KCl, 100 µM Fe-EDTA, 10 µM H_3BO_3, 5 µM $MnSO_4$, 10 µM $ZnSO_4$, 2 µM $CuSO_4$ and 0.2 µM $(NH_4)_6Mo_7O_{24}$). After 2-week acclimation period under controlled conditions (~120 $\mu E.m^{-2}.s^{-1}$ light and 26±1 ºC temperature), the seedlings were treated with 0, 5, 25 and 50 mg/L Co concentrations as $CoCl_2$ for 2 weeks. The applied solutions were renewed every two days. The study was applied in three replications.

Fresh parts of seedling organs were used for physiological analyses. For photosynthetic pigment content, 0.1 gram of leaves were homogenized in 80% acetone. Samples prepared to determine the pigment contents were read in a UV/Vis spectrophotometer at 662, 645 and 470 nm (Cintra 202) [8]. Anthrone method was used to determine the total carbohydrate content [9]. Glucose was used as standard. Folin-Ciocalteu was used to determine the total phenolic content of seedling parts [10]. Malondialdehyde (MDA) analysis was performed to determine lipid peroxidation levels [11].

In order to determine the elemental contents of the seedling parts, the samples were dried at 80 ºC until constant weight. Wet digestion procedure (HNO_3-HCl) was then applied to these samples. Element contents were determined using flame atomic absorption spectrometry. (Perkin Elmer Aanalyst 400).

Bioconcentration factor (BCF) was calculated as follows [12]:

$$BCF = \frac{C_{Total\ Co\ contents\ of\ the\ seedling}}{C_{Co\ concentration\ added\ in\ solution}}$$

Transportation index (TI) was calculated as follows [13]:

$$TI = \frac{C_{aerial\ parts}}{C_{roots}}\ x\ 100$$

where C_{aerial} parts is Co content of in stems or leaves and C_{roots} is Co content of in roots.

Data analysis was performed by using SPSS (SPSS 11.0 for Windows). One-Way ANOVA LSD (Least Significant Difference) test, a multiple range test, was applied to determine the significance of differences between mean values. Pearson correlation analysis was performed to determine correlation between Co content and the analyzed parameters.

3. RESULTS AND DISCUSSION

Cobalt contents of safflower seedling organs increased in parallel with the applied Co concentrations. ($p<0.05$) (Fig. 1a). It was determined that the metal accumulation capacities of the seedling parts were also different. Accordingly, it was determined that the Co contents of the organs were ordered as root>stem>leaf. The maximum TIs of stem and leaf were calculated to be 43.3 and 22.3, respectively ($p<0.05$) (Fig. 1b). Thus, due to the low mobility of Co, it has restricted its transport to the aboveground parts. In general, transport of Co in higher plants is through the xylem sap [14]. Cobalt uptake by plants and its distribution among plant parts have been investigated before. Accordingly, consistent with our findings, it was reported that Co accumulated mostly in the roots [15]. In addition, the fact that the highest BCF was determined at 5 mg/L Co as 391.8 (Fig. 1c). This clearly indicated that the seedling had the potential to accumulate more Co at lower concentrations.

The increase in Co applications showed a marked depression of photosynthetic pigments of seedling leaves (Fig. 2a). Maximum reductions in Chl-a, Chl-b and carotenoid contents were 46.8%, 28.1% and 77.4% ($p<0.05$), respectively, compared to controls. The negative correlation between Co contents of leaves and Chl-a ($r=-0.634$; $p>0.05$), Chl-b ($r=-0.992$; $p<0.01$) and carotenoid ($r=-0.890$; $p>0.05$) contents may indicate that these decreases are due to Co toxicity. Cobalt toxicity caused reductions in photosynthetic pigment contents of different plants [5,16]. Cobalt stress probably blocked the synthesis and activities of enzyme proteins responsible for chlorophyll biosynthesis [2]. In addition, according to our findings, deterioration of nutrient uptake and transport, especially Fe, by Co may also reveal this result.

Total carbohydrate content of the seedling parts generally increased in a dose-dependent manner (Fig. 2b). In addition, a positive correlation was found between the Co contents of the seedling organs and their carbohydrate contents ($r=0,998$; $p<0.01$ for roots, $r=0.805$; $p>0.05$ for stems and $r=0.053$; $p>0.05$ for leaves). Increases were found in the phenolic compounds of the above-ground parts of the seedlings (Fig. 2c). Accordingly, the content increased up to 94.9% on stems and 31.4% on leaves. In contrast, there was no notable change in the phenolic compound content of the roots. Likewise, a positive correlation was found between the Co contents of stems and leaves and phenolic compound contents ($r=0.093$; $p>0.05$ for stems, $r=0.943$; $p<0.05$ for leaves), while a negative correlation was found in the roots ($r=-0.166$; $p>0.05$). When plants are exposed to biotic and abiotic stress, there may be changes in their phenolic compound content. The secondary metabolites have metal chelating properties. It also has antioxidant roles against oxidative stress [17-19]. As a result, increase in phenolic compound contents in seedling parts under Co stress may indicate that they have a role in Co toxicity. The positive relationship between cobalt and phenolic contents also supports this conclusion.

It was determined that the MDA contents of the seedling tissues increased except for leaf at 5 mg/L Co (Fig. 2d). In addition, it is understood from the positive relationship between Co and MDA contents of the seedling parts that these increases may be due to Co toxicity ($r=0.760$; $p>0.05$ for roots, $r=0.807$; $p>0.05$ for stems and $r=0.925$; $p<0.05$ for leaves). As a result, Co application caused the peroxidation of membrane lipids, as evidenced by the increase in MDA content. As with other heavy metals, excess Co triggered oxidative stress in seedling cells, causing an increase in MDA content. Similarly, it has been reported in many studies that Co toxicity triggers oxidative stress in plants and causes lipid peroxidation [20,21].

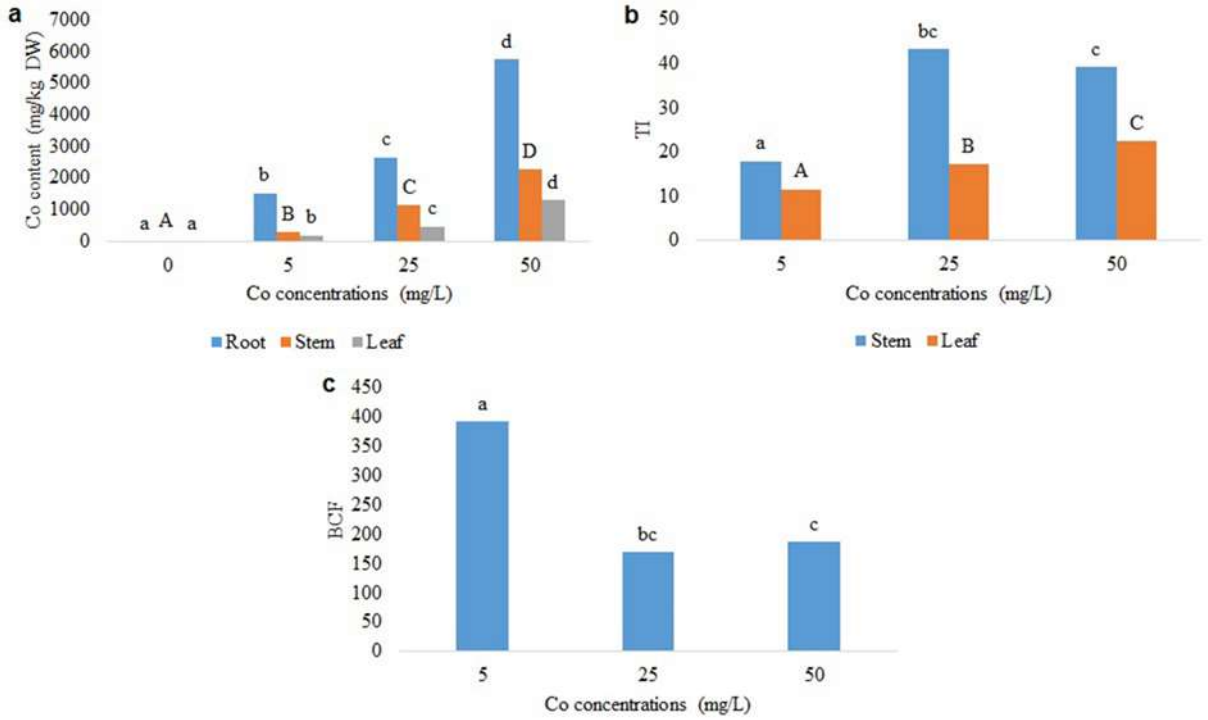

Fig. 1. Cobalt contents (a), Transportation index (TI) (b) and BCF (c) of safflower seedlings after Co application. Means with different letters are significantly different from one another according to LSD test ($p<0.05$).

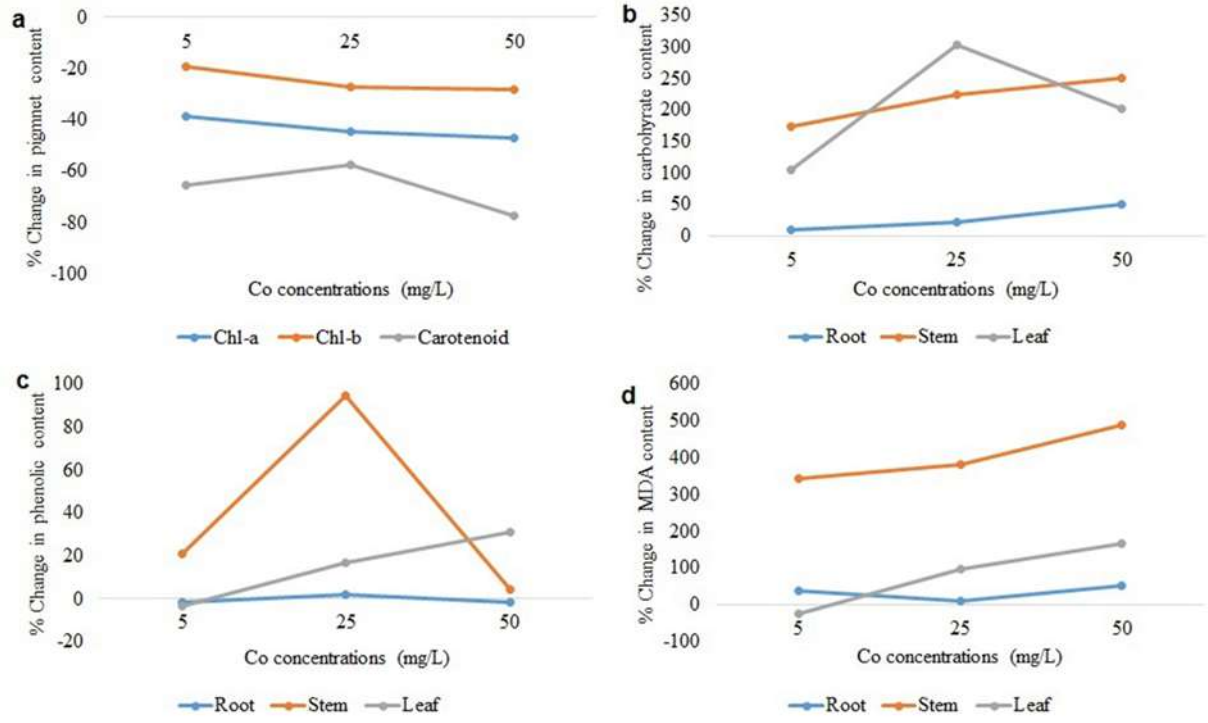

Fig. 2. Percentage change of some physiological parameters of safflower seedling parts after Co application with respect to control group.

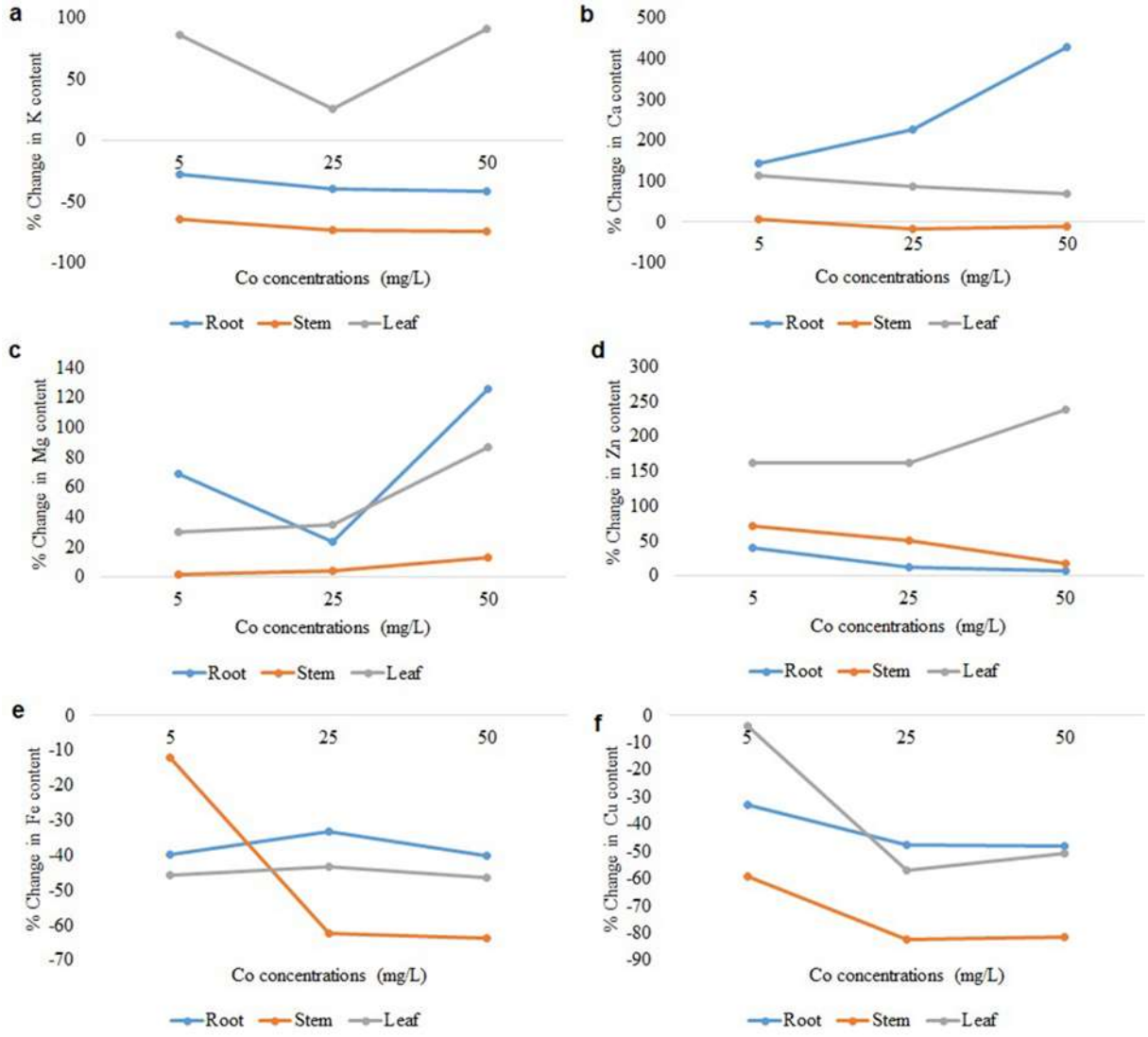

Fig. 3. Percentage change of element contents of safflower seedling parts after Co application with respect to control group.

It has been reported by many researchers that Co application affects the uptake and transport of nutrients in different plants [1,22,23]. Similarly, macro- and micro-nutrient contents of safflower seedlings were affected by Co (Fig. 3). Potassium contents decreased in root and stem tissues compared to controls, while it increased in leaves (Fig. 3a). However, there was a negative correlation between the Co contents of the roots and stems and the K content ($r=-0.827$; $p>0.05$ for roots and $r=-0.693$; $p>0.05$ for stems), but a positive correlation was found in the leaf ($r=0.609$; $p>0.05$). Thus, it is understood that there is a relationship between the K contents of the seedling parts and the applied Co. It was determined that the Ca and Mg contents of the seedling organs increased with Co application, except for 25 and 50 mg/L Co in the stem for Ca (Fig. 3b and c). Correlations between the contents of these elements and Co were similarly found (relationships between Co and Ca: $r=0.995$; $p<0.05$ for root, $r=-0.681$; $p>0.05$ for stem, $r=0.250$; $p>0.05$ for leaf. relationships between Co and Mg: $r=0.863$; $p>0.05$ for root, $r=0.969$; $p<0.05$ for stem, $r=0.851$; $p>0.05$ for leaf). In a study with faba bean plant, it was found that the macronutrient content increased under the influence of Co [24].

Zinc contents, which is one of the micronutrients, increased up to 40.4%, 39.3% and 220.9% in root, stem and leaf compared to the control, respectively (Fig. 3d). It has been previously reported that there is a synergistic effect between Co and Zn [25]. The correlation between Co concentration and Zn was significant only in the leaf ($r=0.954$; $p<0.05$). In contrast, decreases were found in the Fe and Cu contents of the seedling parts (Fig. 3e and f). Correlation analyzes also clearly demonstrated these reductions (relationships between Co and Fe: $r=-0.707$; $p>0.05$ for root, $r=-0.900$; $p<0.05$ for stem, $r=-0.582$; $p>0.05$ for leaf. relationships between Co and Cu: $r=-0.810$; $p>0.05$ for root, $r=-0.756$; $p>0.05$ for stem, $r=-0.741$; $p>0.05$ for leaf). Similarly, Fe uptake was inhibited by Co in faba bean plant [24]. It has been reported that excess Co can replace some

micronutrients from physiologically important binding sites, thereby reducing the uptake and translocation of micronutrients [26]. The results showed that the applied Co affected the nutrient uptake by the roots and their transport to the stem and leaves, thus clearly causing nutrient imbalance.

In conclusion, toxic effects of Co application on safflower seedlings were determined in this study. Cobalt contents of the seedling organs increased with increasing Co concentrations. Cobalt accumulated mostly in the roots and its transport to above-ground parts was limited. There were some physiological changes in the seedlings under Co stress. Photosynthetic pigment contents were decreased, but total carbohydrate and phenolic compound contents were generally increased. In addition, the increase in the amount of MDA showed that Co toxicity caused oxidative stress in the seedling cells. The fact that the content of some elements increased and some decreased, showed that Co toxicity had an effect on the uptake of nutrients and their transport to the stem and leaves, so the applied Co caused nutrient imbalance.

4. REFERENCES

[1] Kabata-Pendias, A. (2011). Trace Elements in Soils and Plants. 4th ed. Boca Raton, FL: CRC Press.

[2] Jaleel, C.A., Jayakumar, K., Zhao, C.X., Iqbal, M. (2009). Low concentration of cobalt increases growth, biochemical constituents, mineral status and yield in *Zea mays*. J. Sci. Res., 1,128–137.

[3] Sree, K.S., Keresztes ,A., Mueller-Roeber, B., Brandt, R., Eberius, M., Fischer, W., Appenroth, K.J. (2015). Phytotoxicity of cobalt ions on the duckweed *L. minor* - morphology, ion uptake, and starch accumulation. Chemosphere, 131, 149-156.

[4] Liu, J., Reid, R.J., Smith, F.A. (2000). The mechanism of cobalt toxicity in mung beans. Physiologia Plantarum, 110, 104-110.

[5] Gopal, R., Dube, B.K., Sinha, P., Chatterjee, C. (2003). Cobalt toxicity effects on growth and metabolism of tomato. Commun. Soil Sci. Plant Anal. 34, 619-628.

[6] Karuppanapandian, T., Kim, W. (2013). Cobalt-induced oxidative stress causes growth inhibition associated with enhanced lipid peroxidation and activates antioxidant responses in Indian mustard (*Brassica juncea* L.) leaves. Acta Physiol. Plant, 35, 2429-2443.

[7] Kayacetin, F., Katar, D., Arslan, Y. (2012). Flower structure and biology of fertilization of safflower (*Carthamus tinctorius* L.). Journal of Central Research Institute for Field Crops, 21(2), 75-80.

[8] Lichtenthaler, H.K., Wellburn, A.R. (1985). Determination of total carotenoids and chlorophylls a and b of leaf in different solvents. Biochemical Society Transactions, 11, 591-592.

[9] Plummer, D.T. (1998). An introduction to practical biochemistry. Third edition. Tata McGraw-Hill Publishing Company Ltd., New Delhi.

[10] Ratkevicius, N., Correa, J.A., Moenne, A. (2003). Copper accumulation, synthesis of ascorbate and activation of ascorbate peroxidase in *Enteromorpha compressa* (L.) Grev. (Chlorophyta) from heavy metal-enriched environments in northern Chile. Plant, Cell and Environment, 26, 1599-1608.

[11] Zhou, Q. (2001). The measurement of malondialdehyde in plants. In: Zhou Q. (Ed.): Methods in Plant Physiology. China Agricultural Press, Beijing: pp. 173-174.

[12] Zayed, A., Gowthaman, S., Terry, N. (1998). Phytoaccumulation of trace elements by wetland plants: I, Duckweed. Journal of Environmental Quality, 27, 715-721.

[13] Ghosh, M., Singh S.P. (2005). Comparative uptake and phytoextraction study of soil induced chromium by accumulator and high biomass weed species. Applied Ecology and Environmental Research, 3, 67-79.

[14] Tiffin, L.O. (1967). Translocation of manganese, iron, cobalt and zinc in tomato. Plant Physiol., 42, 1427–1432.

[15] Young, R.S. (1979). Cobalt in biology and biochemistry, Academic Press, New York.

[16] Lwalaba, J.L., Gerald, Z., Mwamba, T.M., Mukobo, R.P.M., Zhang, G.P. (2017). The effect of cobalt stress on growth and physiological traits and its association with cobalt accumulation in barley genotypes differing in cobalt tolerance. J. Plant Nutr. 40(15), 2192-2199.

[17] Michalak, A. (2006) Phenolic compounds and their antioxidant activity in plants growing under heavy metal stress. Polish Journal of Environmental Studies, 15, 523-530.

[18] Marquez-Garcia, B., Fernandez-Angeles, M., Cordoba, F. (2009). Phenolics composition in *Erica* sp. differentially exposed to metal pollution in the Iberian Southwestern Pyritic Belt. Bioresour. Technol., 100(1), 446-451.

[19] Cervilla, L.M., Blasco, B., Rios, J.J., Rosales, M.A., Sanchez-Rodriguez, E., Rubio-Wilhelmi, M.M., Romero, L., Ruiz, J.M. (2012). Parameters symptomatic for boron toxicity in leaves of tomato plants. J Bot., doi:10.1155/2012/726206.

[20] Chatterjee, J., Chatterjee, C. (2000). Phytotoxicity of cobalt, chromium and copper in cauliflower. Environmental Pollution, 109, 69-74.

[21] Pandey, N., Pathak, G.C., Pandey, D.K., Pandey, R. (2009). Heavy metals, Co., Ni, Cu, Zn and Cd, produced oxidative damage and evoke differential antioxidative responses in spinach. Brazil Journal of Plant Physiology 21,103-111.

[22] Atta- Aly, M.A., Shehata, N.G., ElKobbia, T.M., (1991). Effect of cobalt on tomatoplant growth and mineral content. Annals Agric. Sci., Ain Shams Univ., Cairo., 36, 617-624.

[23] Blaylock, A.D., Davis, T.D., Juolly, V.D., Walser, R.H. (1995). Influence of cobalt and iron on photosynthesis, chlorophyll and nutrient in regreening chlorotic tomatoes and soybeans. J. Plant Nutr., 8, 823- 838.

[24] Kandil, H. (2007). Effect of cobalt fertilizer on growth, yield and nutrients status of faba bean (*Vicia faba* L.) Plants. Journal of Applied Sciences Research, 3(9), 867-872.

[25] Berry, W.L., Wallace A. (1981). Toxicity: The concept and relationship to the dose response curve, Journal of Plant Nutrition, 3, 13-19.

[26] Mengel, K., Kirkby, E.A. (1987). Copper, further elements of importance. In: Principles of Plant Nutrition, 4th Edition. International Potash Institute, Berne, Switzerland.