Indian Journal of Fundamental and Applied Life Sciences ISSN: 2231-6345 (Online)
An Open Access, Online International Journal Available at http://www.cibtech.org/jls.htm
2015 Vol. 5 (4) October-December, pp.88-99/Esfahani and Rezayatmand
Research Article

EVALUATION OF SOME PHYSIOLOGICAL AND BIOCHEMICAL PARAMETERS OF VARIETY OF SUNFLOWER SANBERO (*HELIANTHUS ANNUUS* L.) UNDER NICKEL TOXICITY

Hassanpour Esfahani S. and *Rezayatmand Z.
Department of Biology, Falavarjan Branch, Islamic Azad University, Isfahan, Iran
**Author for Correspondence*

ABSTRACT

Soil and water contamination by heavy metals such as nickel is one of the major environmental problems and in addition to reducing performance and product quality; it has jeopardized sustainability of agricultural production and public health. The present study was conducted to evaluate the effect of different concentrations of nickel on some physiological characteristics of sunflower (Sanebro) under conditions of irrigated farming. First, the seeds were planted in pots filled with perlite and after establishment of plants and getting to the multi-leaf stage, they were treated by the nickel chloride solution at concentrations of 0, 25, 50, 100 and 200 mg/l in 4 replicates. After 14 days the plants were harvested to evaluate some physiological and biochemical parameters. The results of these experiments showed that the amount of chlorophyll content (b, a) and carotenoids have decreased significantly with increasing concentrations of nickel in comparison with the control treatment. The amount of Malondialdehyde (MDA) as an indicator of lipid peroxidation has significantly increased compared to control treatment. This reflects the formation of oxidative stress caused by the concentration of nickel used in the experimental plant. Initially Proline content has decreased slightly with increasing concentration of nickel and then has increased; these changes were not significant compared to the control group. Also the amount of protein decreased and the amount of carbohydrates increased, both of which were significant compared to the control group. Nickel use did not create certain changes in the activity of the enzyme catalase (CAT), but initially it increased the amount of the enzyme peroxidase (POD) activity at a concentration of 25 milligrams per liter, and then reduced it that had a significant difference compared to the control treatment. Morphological symptoms of Ni toxicity were observable as necrosis of the leaves.

Keywords: *Sunflower, Nickel, Lipid Peroxidation, Antioxidant Enzymes*

INTRODUCTION

Deposition of heavy metals such as nickel in soil and its effect on vegetation can affect most of parameters related to the plant growth and development and prevent most of enzymatic and metabolic reactions in plants (Ezhilvannan *et al.,* 2012).

Nickel is a micronutrient element and as a cofactor for the enzyme urease plays a unique role in metabolism of nitrogen compounds during the process of breaking down the protein into amino acids in the processes of germination and fruit development (Gheibi *et al.,* 2009). Despite the nutritional role of nickel in very low concentrations, nickel as a heavy metal in high concentrations is toxic to plants, humans and animals (Ashmann and Zasoski, 1987).

High concentrations of nickel reduce the growth and leads to emergence of toxicity symptoms in plants. There are many reports about the negative effects of nickel on several physiological processes of plant such as photosynthesis, transfer of organic materials, mineral nutrition and water balance in the tissues (Samarakoon and Rauser, 1979; Pandey and Sharma, 2002; Parida *et al.,* 2003).

The nickel toxicity as an abiotic stress, with disrupting the balance between production and destruction, leads to accumulation of free radicals of oxygen (ROS) and creates oxidative damages such as membrane lipid oxidation and destroys proteins, enzymes, pigments, and DNA and with disrupting the performance of the membrane reduces the amount of water in the cellular tissue (Baccouh *et al.,* 2001; Bhatia *et al.,* 2007; McKenna *et al.,* 2008).

According to reports high concentration of nickel reduces seed germination, reduces plant growth, prevents the development of root systems, creates yellow leaf, destroys chlorophyll molecules and reduces photosynthesis and respiration of the plant (Asada, 1999).
Extensive studies have been conducted about the effects of heavy metals on the growth of plants, but little information is available about the mechanisms of Ni toxicity in plants. However, the obtained data suggest that at least a part of the effects of nickel toxicity is due to the NI-induced oxidative stress on the plant (Baccouch *et al.*, 2001; Gonneli *et al.*, 2001).
Antioxidant compounds eliminate free radicals of oxygen in the plant (Molassiotis *et al.*, 2005). Toxicity and deficiency of nickel both create oxidative stress in plants. Regarding deficiency of nickel, accumulation of toxic metabolites of urease and nitrate in plants and in terms of it toxicity the accumulation of reactive oxygen species cause oxidative damage.
Antioxidant response of plant to toxicity and deficiency of nickel and oxidative damages caused by it have an important role in the metabolic tolerance of plants to stress and this metabolic tolerance among different varieties of plant is different (Ashmann and Zasoski, 1987).
The concentration of nickel in certain areas and as a result human activities such as mining, using coal and oil as fuel, creating sewage and using phosphate fertilizers and pesticides will significantly increase (Gimeno *et al.*, 1996).
Therefore, it is likely that the agricultural plants in different regions of the world are exposed to the toxicity of nickel and as a result their performance reduces. Therefore the present study aims to investigate the changes in biochemical and physiological processes of sunflower (Sanebro) under different nickel concentrations.

MATERIALS AND METHODS

The experimental plant of this study was sunflower sanbero (*Helianthus annuus* L.) and the oil seeds were provided from Research Institute of Agricultural Jihad. First, the seeds were irrigated in pots containing perlite and after germination of the seeds; they were irrigated in Hoagland environment for two weeks and then after getting to the 4-leaf stage, they were under different treatments of nickel at concentrations of 0, 25, 50, 100 and 200 mg/l, every other day for 10 days. After 14 days the plants were harvested to evaluate the toxic effect of nickel on the chlorophyll content, carotenoids, carbohydrates, proline, protein, lipids peroxidation and antioxidant enzyme activities.
Measurements of photosynthetic pigments including chlorophyll a, b and carotenoids were estimated using Lichtenthaler method (Lichtenthaler, 1987).
In order to measure the amount of proline in plant tissues method of Bates (1973) was used.
In order to measure the amount of carbohydrates method of Kochert (1978) was used. The mount of glucose is evaluated using the standard curve based on mg dry weight. In order to evaluate lipids peroxidation of membrane and malondialdehyde concentration (MDA), the method of Heath and Parcher (1968) was used. To measure the peroxidase enzyme method of Koroi (1989) and catalase enzyme method of Chance (1955) were used. The protein was estimated by method of Lowry (1951).

Statistical Analysis

This research was carried out in randomized design with 4 treatments and a group as the control. In each treatment there were 4plants as 4 replications. Data analysis was performed using SPSS statistical software (version 13) and ANOVA. The means were compared using Duncan test at 5 percent level and plotting graphs were done using Excel software.

RESULTS AND DISCUSSION

The Effects of Nickel Toxicity on the Amount of Photosynthetic Pigments

Plant treatment with different concentrations of nickel reduced the amount of chlorophyll a that this level has been significant at the concentrations of 100 and 200 mg/l compared to control (Figure 1).
In treatment of variety of sunflower Sanbero with various nickel concentrations, according to Figure 2 it was observed that the amount of chlorophyll b has decreased with increasing concentration of nickel and

this reduction in concentrations of 100 and 200 mg/lit nickel has been statistically significant and meaningful compared to control.

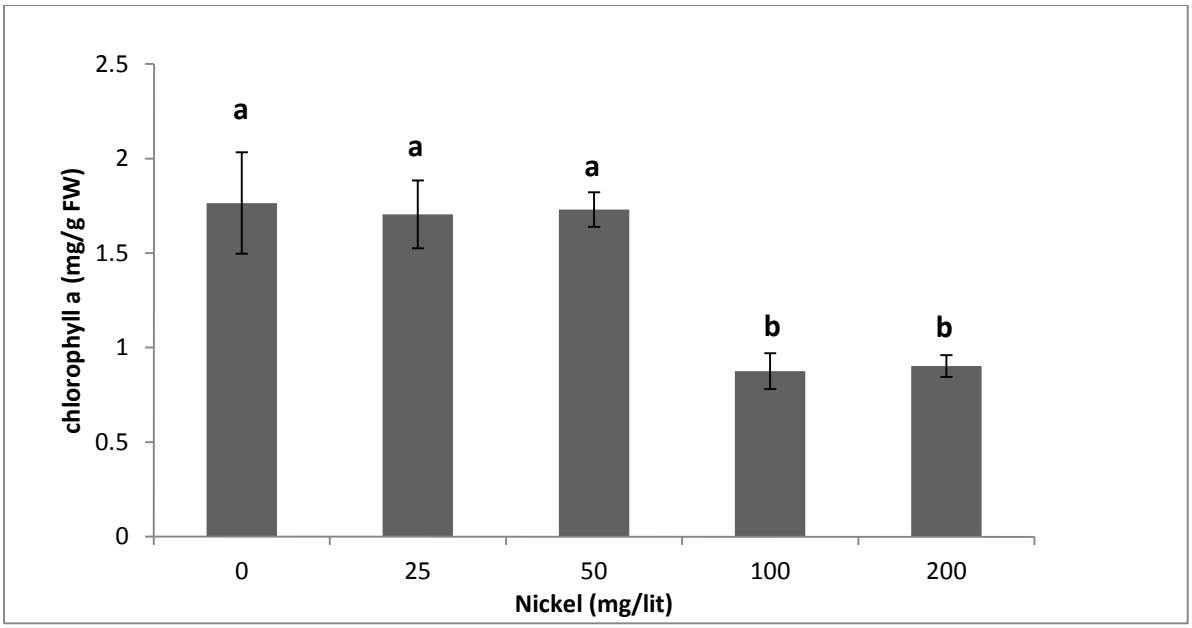

Figure 1: The effect of nickel toxicity on the chlorophylla amount of variety of sunflower Sanbero The data are the mean of 3 replicates ± Standard deviation (SE) and non-identical letters indicate significant differences according to Duncan test (P≤0. 05)

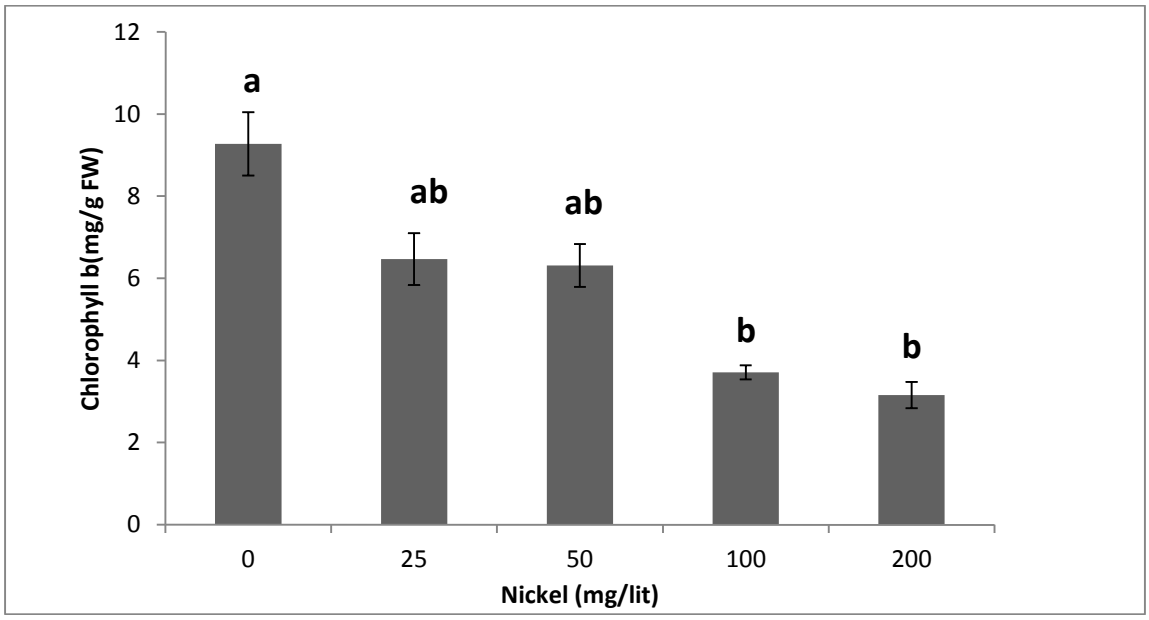

Figure 2: The effect of nickel toxicity on the chlorophyll-b amount of variety of sunflower Sanbero The data are the mean of 3 replicates ± Standard deviation (SE) and non-identical letters indicate significant differences according to Duncan test (P≤0. 05).

With the increase in nickel concentration with respect to Figure 3, the amount of carotenoids has decreased and this decrease in the concentrations of 100 and 200 mg/lit has been significant compared to the control treatment.

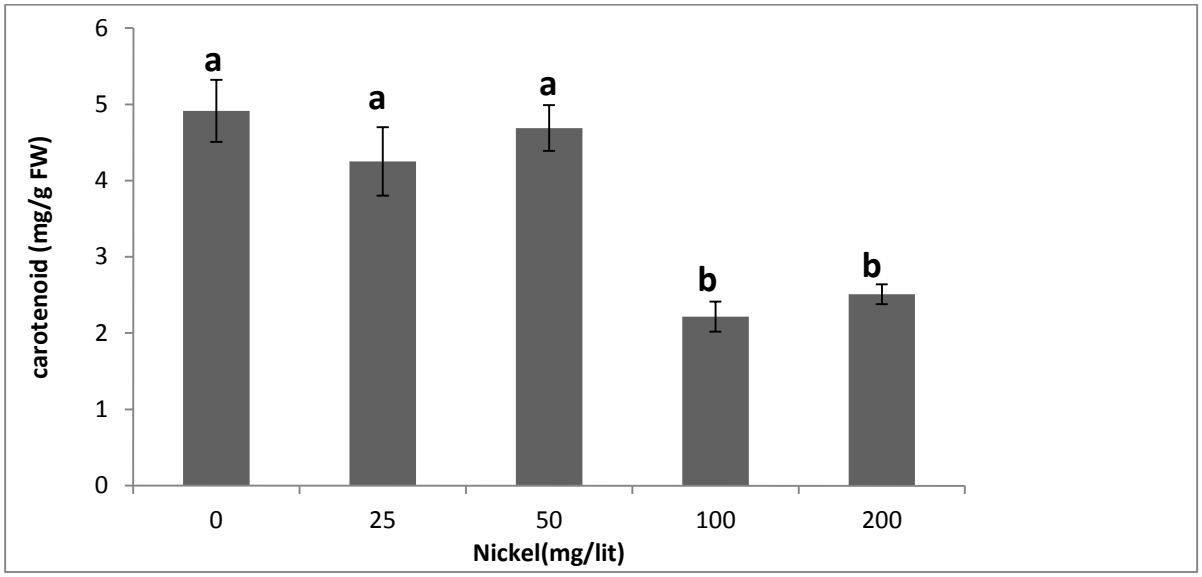

Figure 3: The effect of nickel toxicity on the carotenoids amount of variety of sunflower Sanbero The data are the mean of 3 replicates ± Standard deviation (SE) and non-identical letters indicate significant differences according to Duncan test (P≤0. 05).

The Effect of Nickel Toxicity on the Amount of Carbohydrates

According to the study it is observed that effect of nickel on plant has increased the amount of carbohydrate of the plant and this increase in the concentrations of 100 and 200 mg/lit has been significant compared to the control treatment.

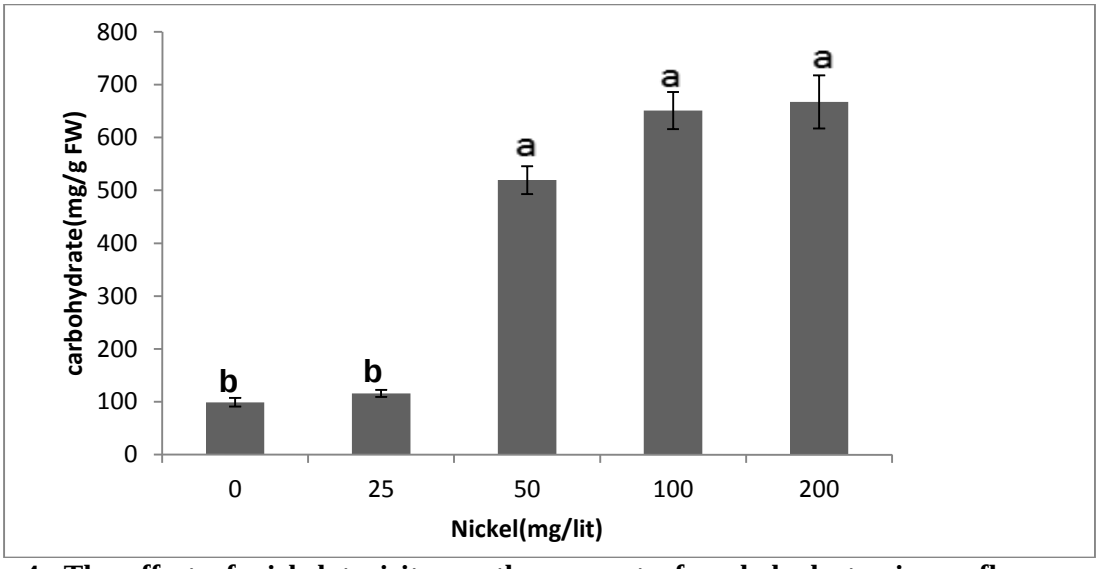

Figure 4: The effect of nickel toxicity on the amount of carbohydrates in sunflower sanebro The data are the mean of 3 replicates ± Standard deviation (SE) and non-identical letters indicate significant differences according to Duncan test (P≤0. 05).

The Effect of Nickel Toxicity on the Proline

The results show that with increasing concentrations of nickel, the amount of proline increased and this increase in the concentrations of 100 and 200 mg/lit has been statistically significant compared to control treatment (Figure 5).

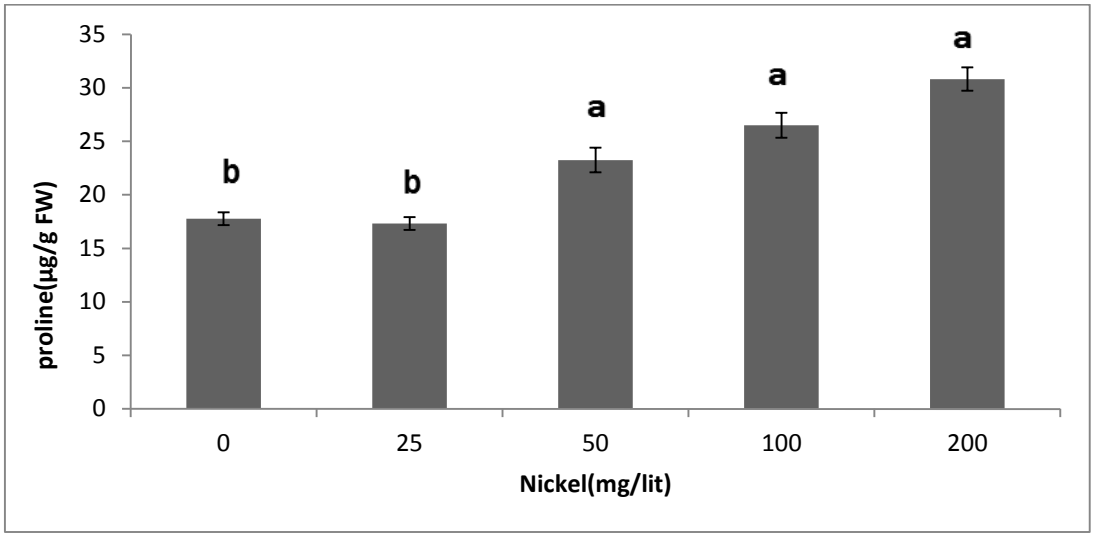

Figure 5: The effect of nickel toxicity on the amount of proline in sunflower sanebro The data are the mean of 3 replicates ± Standard deviation (SE) and non-identical letters indicate significant differences according to Duncan test (P≤0. 05).

The Effect of Nickel Toxicity on the Amount of Lipids Peroxidation (MDA)

The results obtained from the effect of nickel toxicity on the concentration of malondialdehyde (MDA) as marker of lipid peroxidation, in Figure 6, shows that with increasing concentrations of nickel, the amount of malondialdehyde also increased and this increase in the concentrations of 100 and 200 mg/lit compared to control treatment has been statistically significant.

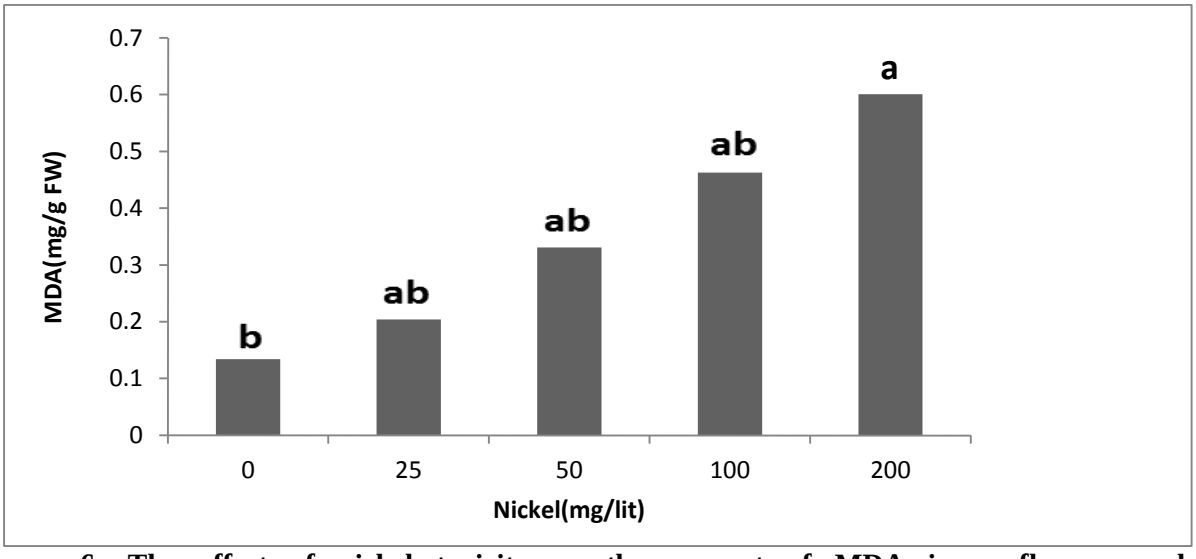

Figure 6: The effect of nickel toxicity on the amount of MDA in sunflower sanebro The data are the mean of 3 replicates ± Standard deviation (SE) and non-identical letters indicate significant differences according to Duncan test (P≤0. 05).

The Effect Nickel Toxicity of on the Amount of Protein

In treatment of variety of sunflower Sanbero with various nickel concentrations in Figure 7 it was observed that along with increasing concentrations of nickel, the amount of plant protein decreased and this reduction in concentrations of 100 and 200 mg/lit has been statistically significant compared to control treatment.

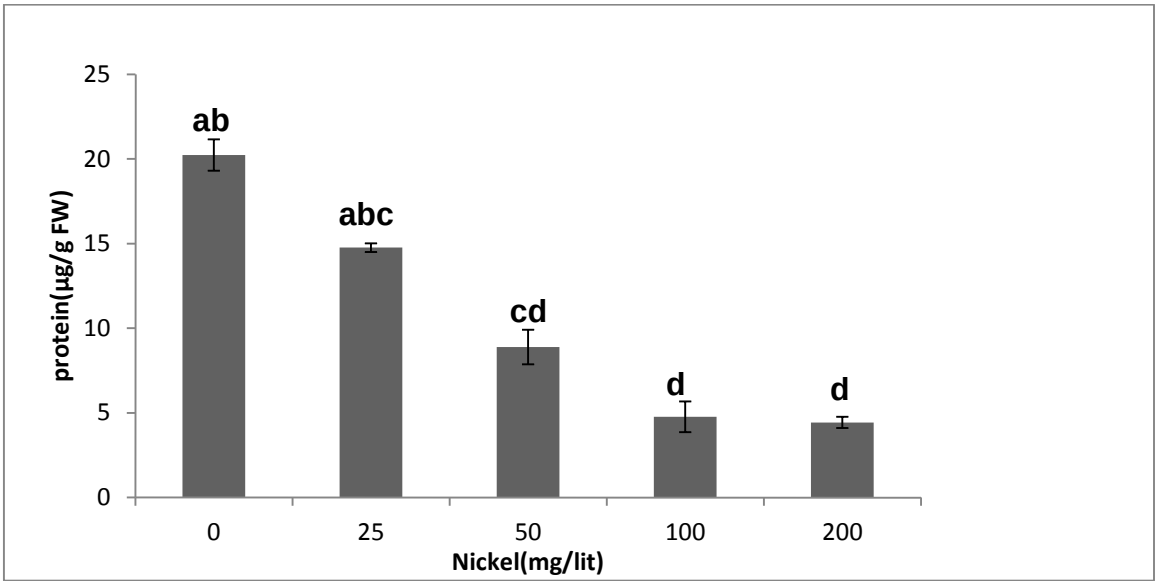

Figure 7: The effect of nickel toxicity on the amount of protein in sunflower sanebro The data are the mean of 3 replicates ± Standard deviation (SE) and non-identical letters indicate significant differences according to Duncan test (P≤0. 05).

The Effect of Nickel Toxicity on the Peroxidase Enzymes Activity

The results showed that the amount of peroxidase enzymes activity has increased with increasing the concentrations of nickel, and according to Figure 8 in concentrations of 100 and 200 mg/lit, it has been statistically significant compared to the control treatment.

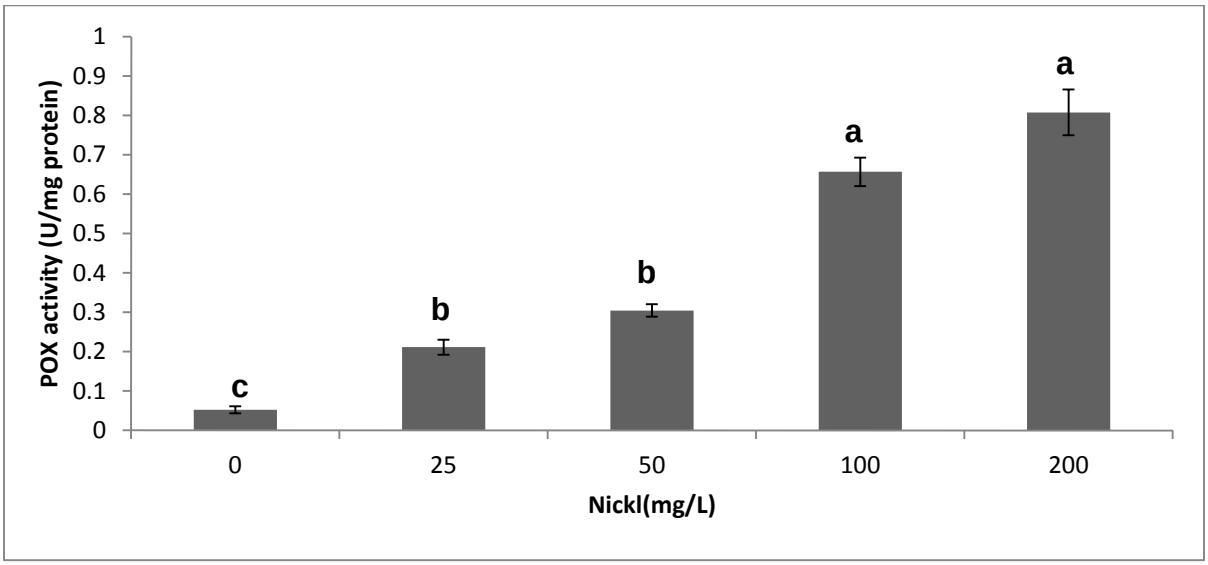

Figure 8: The effect of nickel toxicity on the amount of peroxidase enzymes in sunflower sanebro The data are the mean of 3 replicates ± Standard deviation (SE) and non-identical letters indicate significant differences according to Duncantest (P≤0. 05).

The Effect Nickel Toxicity of on the Amount of Catalase Activity

According to the results with increasing concentrations of nickel, the amount of catalase activity also increased. As can be seen in Figure 9 in the concentrations of 100 and 200 mg/lit, the amount of enzyme activity has been statistically significant compared to control treatment.

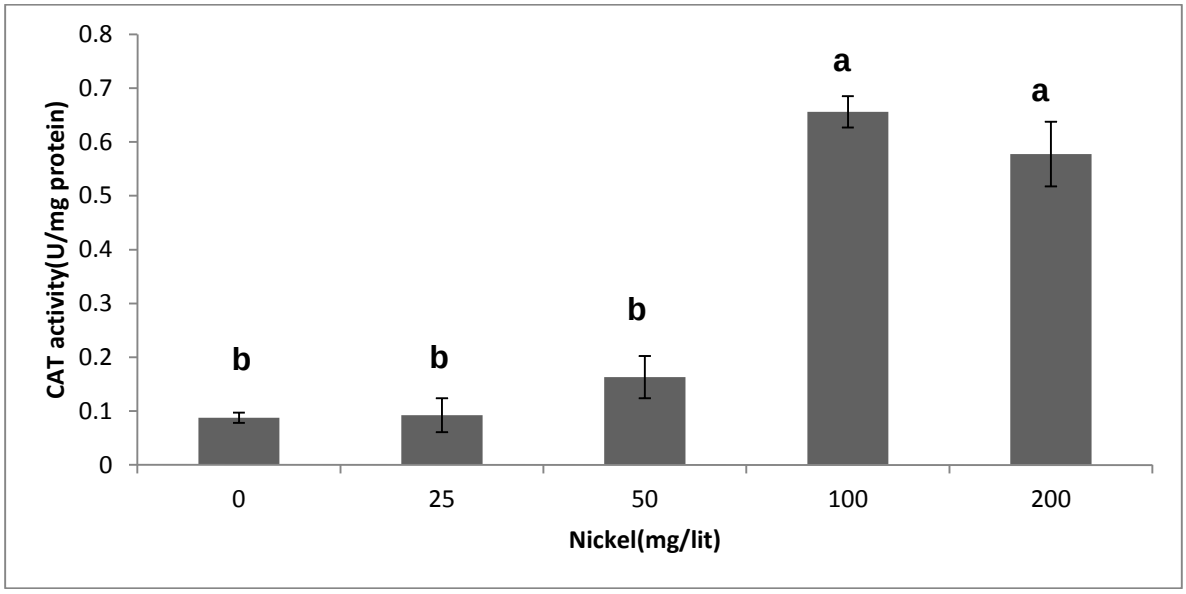

Figure 9: The effect of nickel toxicity on the amount of catalase activity in sunflower sanebro The data are the mean of 3 replicates ± Standard deviation (SE) and non-identical letters indicate significant differences according to Duncan test (P≤0. 05).

The Effect of Nickel Toxicity on the Amount of Carbohydrates

According to the results of this study, as a result of high concentrations of nickel in the environment the amount of carbohydrates in variety of sunflower Sanbero has increased. The results of the present study correspond with the reports from the treatment of nickel on the amount of carbohydrates in sunflower and canola (Yasin *et al.,* 2010; Ali *et al.,* 2009). An increase in glucose can be due to its accumulation. Accumulation in leaves could be due to the reduction in phloem loading and reduction in transmission capacity of osmolytes or slowing their use in parts of reservoir (Alaoui-sosse *et al.,* 2004). Accumulation of carbohydrates in leaves of under stress induces feedback inhibition of photosynthesis (Foyer, 1988). In six-day-old seedlings of rice that were under the stress of nickel and cadmium, an increase in carbohydrate content is accompanied by a decrease in net photosynthesis (Foyer, 1988). The amount of glucose production has an inverse relationship with relative water content (RWC) that shows when the relative water content is low, the possibility of glucose production increases. The plants for increasing the osmosis pressure in cells and increasing their resistance increase the amount of carbohydrates (Subbaro *et al.*, 2000).

Heavy metals reduce the transfer of water to leaves and therefore lead to disruption in leaf transpiration rate. This leads to ultra structural changes of the cell organelles and changes in the behavior of several key enzymes in metabolic pathways including glucose metabolic pathways. Following the heavy metal accumulation in the cell, transfer of water into glucose reduces and carbohydrate content in the plant increases. This phenomenon is probably the adaptive mechanism of plant in order to maintain osmotic potential for toxicity with heavy metal. Also it is thought with increase in glucose, under stress the plants can maintain their carbohydrates store at the desirable level in order to retain cellular basal metabolic rate (Verma and Dubey, 2001).

The Effect of Nickel Toxicity on the Amount of Proline

The results of this study showed that proline accumulation in plant tissues can result from, a decrease in proline degradation, an increase in proline biosynthesis, a decrease in protein synthesis or proline utilization, hydrolysis of proteins (Charest and Phan, 1990). In a report it has been stated that the effect of nickel on the plant increased the amount of proline (Kumar *et al.,* 2012). In another study on the alfalfa plant, toxicity of nickel increases the amount of proline (Bates *et al.,* 1973). Also in investigation of proline metabolism under nickel stress in oilseed rape it is stated that an increase in concentrations of

nickel increases the amount of proline (Ghorbanaly *et al.,* 2005). Studies conducted by Sanchez *et al.,* (2001 and 2002) showed the heavy metals on the metabolism of proline affected bean plant and green bean, and increased the amount of proline. The effect of heavy metals such as copper on sunflower also increased the amount of proline (Backor and Fhsel, 2004). Nickel increases free radicals, proline is highly sensitive to stress and acts as an antioxidant and decreases free radical (Chen *et al.,* 2009). The importance of proline accumulation in maintains the water status of the plant is more than other organic materials.

Also proline as the largest accumulated osmolite acts under stress (Shan *et al.,* 2001). An increase in the amount of proline can indicate the plant resistance to stress resulted from the use of nickel.

The Effect of Nickel Toxicity on the Amount of Malondialdehyde (MDA)

According to the results of this study, high concentrations of nickel significantly increased malondialdehyde (MDA) as marker of lipid peroxidation. Researches have demonstrated that MDA levels in wheat increase under treatment with nickel (Jafari *et al.,* 2009). In the study of Kumar *et al.,* (2012) on barley, at the concentration 400 µM, MDA level has increased significantly. Also in cabbage plant, under nickel stress, MDA levels have increased (Pandey and Sharma, 2002).

Also the effect of the heavy metals of chromium on sunflower has increased MDA levels (Kalantari *et al.,* 1391). Heavy metals such as nickel can cause oxidative stress and high production of ROS and subsequently result in non-enzymatic and enzymatic antioxidant responses and lipids peroxidation in plants (Yurekli and Porgali, 2006). Various forms of reactive oxygens (ROS) in case of activity can cause severe injuries to the macro molecules such as membrane lipids.

Lipid peroxidation with high free radicals is an indication of the presence of toxic substances in the environment that its end product is malondialdehyde (MDA). Increased MDA is an indicator of physiological stress (Pourakbar *et al.,* 2007).

It is reported that with the increase in lipid peroxidation which occurs due to toxicity with heavy metal and creation of free radicals, lipoxygenase activity increases (Sanita *et al.,* 1999). This enzyme catalyzes the oxygenation of long-chain unsaturated fatty acids.

The Effects of Nickel Toxicity on the Amount of Protein

Reduction in protein during the heavy metal stress can indicate protein-analyzing enzyme activity. In this respect it is stated that the produced free radicals change the position of the amino acid in proteins, as a result protein-analyzing enzyme can better analyze the protein (Rezayatmand, 2012). The results of the study showed that the increase in nickel concentration significantly reduced the amount of protein. Yasin Ashraf *et al.,* (2010) also reported that nickel has a negative descending impact on the amount of total proteins of sunflower; also, Kumar *et al.,* (2012) stated that nickel reduces the amount of total protein content in alfalfa. Nickel solution reduces the protease enzyme activity and protein content in rice plant (Verma and Dubey, 2001; Shah *et al.,* 2001).

The effect of other heavy metals such as copper chloride on the bean plant has reduced the amount of total protein content (Yurekli and Porgali, 2007).

The Effect of Nickel Toxicity on the Amount of Peroxidase and Catalase Enzymes Activity

The results showed that high nickel concentration in the environment has increased peroxidase enzyme activity (POX) in the sunflower Sanebro. Increase of nickel in plants leads to a significant increase in hydroxyl radicals concentration, superoxide anions, nitric oxide and hydrogen peroxide. Since nickel is not a redox-active metal, it cannot directly create reactive oxygen species (ROS); however, it indirectly interferes with the activity of some antioxidant enzymes (Chen *et al.,* 2007). These enzymes in plant defense mechanisms against reactive oxygen species have a fundamental tension; induction of peroxidase enzyme is a general response of higher plants in response to absorption of some toxic elements (Van Assche and Clijsters, 1999).

Also in investigating antioxidant enzymes activity under nickel stress in oilseed rape it is stated that increase in nickel concentrations increased the amount of peroxidase and catalase enzymes (Ghorbanaly *et al.,* 2005). These results are similar to studies by Yan *et al.,* (2008) and Kumar *et al.,* (2012) on the plant barley. In this study it was shown that high concentrations of nickel increased catalase activity in

variety of sunflower Sanbero. Jiang and Hung, (2001) reported that in high concentrations of heavy metals, antioxidant each enzyme activity can increase or decrease or remain unchanged. The process of change in each of the enzymes activities varies according to type of element causing tension, concentration of element in the culture medium, enzymes tested, and under study cultivars and organs (Sharma and Duby, 2007).

In another study an increase in catalase activity in terms of nickel toxicity has been reported (Yan *et al.,* 2008). In contrast, it is also showed that catalase activity has decreased at high levels of nickel in the tomato plant (Madhava and Sresty, 2000). Anyway, increase or decrease of catalase activity because of nickel deficiency which is due to excessive urease accumulation and nitrate in the plant and/or nickel toxicity and accumulation of reactive oxygen species can be created as a part of the plant's defense mechanism against oxidative damage.

Discussion

Amount of chlorophyll in live plants is one of the important factors in maintaining photosynthesis capacity (Jiang and Huang, 2001). Reduction in levels of photosynthetic pigments has been observed in many species as a result of using heavy metals such as nickel. Heavy metals prevent chlorophyll synthesis either by direct inhibition of an enzymatic step or by induction of a major nutrient (Yurekli and Porgali, 2006).

Magnesium in chlorophyll structure can be moved by heavy metals, such as nickel and this movement has been a major factor for chlorophyll degradation by heavy metals and decreased the photosynthetic activity (Chen *et al.,* 2009).

The results of this research suggested that the presence of high concentrations of nickel in the environment showed a significant reduction in photosynthetic pigments such as chlorophyll a, chlorophyll b and carotenoids in variety of sunflower Sanbero. Results of this research are consistent with other reported studies such as the reduction of chlorophyll a and b and carotenoids in barley under nickel stress by Kumar *et al.,* (2012) and with other similar studies (Singh *et al.,* 1989; Molas, 1997; Amini and Amirjani, 2011; Lahouti *et al.,* 2009).

Reduction in pigments can be in relation to the changes in the permeability of the membrane and chloroplast ultra structure can be a result of oxidation and degradation of pigments that occurs with an increase in peroxidase activity (Kumar *et al.,* 2012).

The reports concerning carotenoid content in heavy metal stress are different; however, most reports indicate reduction in carotenoids due to increase in heavy metal (Yasin *et al.,* 2010; Chen *et al.,* 2009) that are consistent with our results. Carotenoids are destroyed as a protection system against induced oxidative stress (Kumar *et al.,* 2012).

Conclusions

In general, the findings of this research suggest that presumably one of the mechanisms causing nickel's damage in variety of sunflower (sanbero) is the increase in the antioxidant enzymes activity is the increase in the antioxidant enzymes activity which is as a result of oxidative stress resulting from high concentrations of nickel in the environment. Our research findings suggest that sunflower affected by stress when it is confronting with nickel.

Nickel metal can act as an essential element at low concentrations and be useful for plants; however, at high concentrations it causes toxicity symptoms in plant and jeopardizes growth and the survival of the plant.

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