

ARTICLE

The effect of nickel phytotoxicity on photosystem II activity and antioxidant enzymes in barley

Ali Dođru^{1*}, Hüseyin Altundađ², Mustafa Şahin Dünder²

¹Sakarya University Faculty of Arts and Science Department of Biology, Esentepe, 54187 Sakarya, Turkey

²Sakarya University Faculty of Arts and Science Department of Chemistry, Esentepe, 54187 Sakarya, Turkey

ABSTRACT In this study, the effect of mild (100 µM), moderate (300 µM) and severe (500 µM) nickel (NiSO₄·7H₂O) toxicity on the photosynthetic activity, photosynthetic pigment content and some antioxidant enzymes in the leaves of a barley cultivars (*Hordeum vulgare* L. cv. Tarm-92) was investigated. Moderate and severe nickel toxicity decreased root length while shoot length was not affected by nickel stress, probably due to over accumulation of nickel in roots. Similarly, biomass accumulation was declined by moderate and severe nickel toxicity as reflected by the lowered fresh and dry weight. Chlorophyll a, chlorophyll b and consequently total chlorophyll content decreased by all nickel applications, presumably because the reduced level of carotenoids. Chlorophyll a fluorescence measurements showed that nickel toxicity blocked electron movement in some specific points of the photosynthetic electron transport system. The constant F_o value indicated that PSII reaction centers was not damaged in the leaves of barley under nickel toxicity while the reduced F_m value showed that acceptor side of PSII was more sensitive to nickel toxicity as compared to donor side. Changes in JIP test parameters in the leaves of barley showed that primary photochemical reactions are reduced, and thermal dissipation of excess energy is increased. SOD and CAT activity is elevated in the leaves of barley under moderate and severe nickel toxicity which demonstrate an efficient superoxide dismutation. Severe nickel toxicity, however, did not affect SOD and CAT activity. The ascorbate-glutathione cycle was activated in the leaves of barley plants under nickel toxicity, probably indicating an efficient H₂O₂ detoxification. However, considerable H₂O₂ and MDA accumulation was observed in the leaves of barley under nickel stress. As a result, it may be concluded that the barley genotype Tarm-92 is moderately tolerant to nickel toxicity.

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*Corresponding author
E-mail: adogru@sakarya.edu.tr

Introduction

Any metal and metalloid that have a relatively higher density (above 5 g cm⁻³) is known as heavy metal (Gautam et al. 2017). Natural processes such as weathering and volcanic eruption as well as anthropogenic activities such as mining, waste disposal and intensive utilization of fertilizers and pesticides has been known to result in heavy metal accumulation in agricultural areas (Khan et al. 2016). Both the quality of agricultural soils and crop yield may be decreased by heavy metal contamination (Rizwan et al. 2016). Human, animal and plant health, on the other hand, is under a threatening risk because of contamination of environment by excess heavy metals. The heavy metal toxicity in plants has drawn the attention of many environmental scientists during the last decade because plants form the major source of heavy metal entry into the food chain. Dođru (2020) has reported that some heavy metals such as Cu, Zn, Ni and Co are essential for plant

growth and development, while some others (Cd, Cr, Pb and As) are not responsible for any physiological function. Plants require the essential heavy metals within physiological limits to fulfil their metabolic reactions because excess supply of heavy metals is known to be toxic for plants. Heavy metal toxicity affects various physiological pathways including water and mineral absorption from soil, photosynthesis, nitrogen metabolism and membrane functions all of which are result in the reduced crop yield and quality (Gopal and Rizvi 2008; Chen et al. 2009; Dođru 2020; Gajewska et al. 2009).

Ni forms about 0.008% of the earth's crust being 24th the most abundant element (Hedfi et al. 2007). Naturally, nickel is present in soils in the range of 3-100 ppm and in water 0-0.005 ppm, respectively (Sachan and Lal 2017). Ni, as the most recent element to be classified as essential, is required very small concentrations by plants for optimum growth and development. For example, Gajewska et al. (2006) have indicated that Ni at the concentration of 10 µM improved plant growth and increased biomass

accumulation. In the contaminated soils, on the other hand, Ni may exist in the range of 200 - 26 000 ppm which is 20-30-fold higher than the natural range (Izsimova 2005). Ahmad et al. (2009) and Ali et al. (2009) have reported that excessive nickel in the growth medium leads to the inhibition of germination and plant growth, degradation of chlorophyll pigments and reduction of photosynthetic activity. In addition, Ni toxicity also induces oxidative damage in some macromolecules such as lipids, proteins and nucleic acids as a result of the accelerated rate of reactive oxygen species (ROS) including hydrogen peroxide (H_2O_2), superoxide ($O_2^{\cdot-}$) and hydroxyl radical (OH^{\cdot}) (Gajewska and Sklodowska 2007). However, plants possess an efficient antioxidant system against oxidative stress which consist of enzymatic and non-enzymatic components (Bhaduri and Fulekar 2012).

Photosynthesis is an important metabolic process for plant growth and productivity. However, it has been well known that photosynthetic reactions are primary target for abiotic stress factors including Ni toxicity (Amari et al. 2017). Therefore, it is imperative to gain an improved understanding of the effect of Ni toxicity on photosynthetic processes to improve agricultural productivity. Today, chlorophyll a fluorescence has been reported to be the most modern and reliable technique for measuring photosynthetic activity (Maxwell and Johnson 2000; Doğru 2019; Doğru and Çakırlar 2020a and 2020b). The basic principle of chlorophyll a fluorescence kinetics is the redox state of quinone A (Q_A), which is the primary electron acceptor of photosystem II (PSII). Accordingly, the fluorescence yield is low when the Q_A is in the oxidised state, and the fluorescence yield is high when it is reduced. In this case, fluorescence yield is directly related to the net concentration of $Q_A^{\cdot-}$ (Govindjee 2004). In addition, at the minimum fluorescence (F_0), that is, at the "O" point, all Q_A molecules are in oxidised state, the reaction centers of PSII are open and primary photochemical processes are at maximum level. However, at the maximum fluorescence (F_m), that is, at the "P" point, all Q_A molecules are reduced, the reaction centers of PSII are closed and primary photochemical processes are at minimum level (Govindjee 2004). If the chlorophyll a fluorescence signals are plotted versus to the logarithm of time, the "OJIP" curve is obtained. The basic principle of OJIP curve can be explained as follows: When light is applied to the surface of a leaf adapted to dark, the fluorescence of chlorophyll a increases from the minimum level ("O") to the "J" level (F_j) within 2 ms due to reduction of Q_A molecules. It then rises to the "I" point (F_i) in about 30 ms due to the reduction of the entire plastoquinone pool. In the last stage, chlorophyll a fluorescence increases form the "I" point to the maximum level ("P" or F_m) due to the higher electron density in the acceptor side of photosystem I (PSI) (Go-

vindjee 2004). The technique that enables chlorophyll a fluorescence signals to examine the changes exhibited at "J", "I" and "P" points of the OJIP curve and the interactions between these changes is called JIP test (Strasser et al. 2004). JIP test is used in the field of plant biology and agriculture in order to understand the reactions of the photosynthetic apparatus under many different environmental conditions (Yusuf et al. 2010). JIP test is based on energy flow theories in thylakoid membranes (Force et al. 2003). This test allows the investigation of the energy flow entering and leaving the PSII by means of parameters directly measured and calculated with the help of some equations.

As a result, the objective of this study is to investigate phytotoxic effects of mild, moderate and severe nickel toxicity in the leaves of barley plants through chlorophyll a fluorescence and some antioxidant enzymes.

Material and methods

Plant materials, growth conditions and experimental design

Barley genotype (*Hordeum vulgare* L. cv. Tarm-92) was grown in growth chamber in plastic pots containing Hoagland nutrient solution. The average temperature for day/night was 25/18 °C, respectively, relative humidity was 40-50%, the photoperiod for the day/night cycle was 16/8 h, respectively, and the maximum photosynthetically active radiation was about 200 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$. After 10 days of growth, nickel treatments were initiated by applying half-strength Hoagland nutrient solution containing 100, 300 and 500 μM nickel sulphate ($\text{NiSO}_4 \cdot 7 \text{H}_2\text{O}$) to seedlings. Control plants (no nickel sulphate treatment) and nickel-treated plants were grown in the growth chamber under the same physical conditions for another 6 days. The leaf tissue of 16 days of plants was used for chlorophyll fluorescence measurements and biochemical analysis.

Determination of root and shoot length

Measurement of root and shoot length were done with a millimetric ruler. The longest root was taken into consideration for measurement. Root and shoot length were expressed as cm plant^{-1} . After harvesting, barley seedlings were weighed for fresh weight (FW) determination. Dry weight (DW) of plants was measured after drying in hot-air oven at 70 °C for 2 days.

Determination of nickel content

For measuring nickel content plants were carefully harvested and divided into roots and shoots. The leaves were washed with double-distilled water. The samples

were acid-digested and analysed by inductively coupled plasma emission spectroscopy (ICP-OES) after digestion with concentrated HNO_3 .

Photosynthetic pigment analysis

Photosynthetic pigments were extracted from leaf segments in 3 ml 100% acetone. The absorbance of the extracts was measured at 644.8 and 661.6 nm using a Shimadzu mini 1240 UV visible spectrophotometer. The concentrations of chlorophyll a and chlorophyll b were calculated according to Lichtenthaler (1987).

Malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) analysis

MDA and H_2O_2 content were determined by the method of Heath and Packer (1968) and Ohkawa et al. (1979), respectively. Fresh leaf material (0.1 g) was homogenized in 6 ml of 5% TCA (4 °C) and centrifuged at 10,000 rpm for 15 min and the supernatant was used in the subsequent determination. To 0.5 ml of the supernatant were added 0.5 ml of 0.1 M Tris-HCl (pH 7.6) and 1 ml of TCA-TBA reagent. The mixture was heated at 95 °C for 60 min and then quickly cooled in an ice bath. After centrifugation at 10 000 g for 5 min to remove suspended turbidity, the absorbance of supernatant at 532 nm was recorded. Non-specific absorbance at 600 nm was measured and subtracted from the readings recorded at 532 nm. Concentration of MDA was calculated using its extinction coefficient of $155 \text{ mM}^{-1} \text{ cm}^{-1}$. For determination of hydrogen peroxide, 0.5 ml of 0.1 M Tris-HCl (pH 7.6) and 1 ml of 1 M KI were added to 0.5 ml of supernatant. After 90 min, the absorbance was read at 390 nm. A standard curve for hydrogen peroxide was prepared to determine hydrogen peroxide concentration in each sample.

Antioxidant enzyme activities

For determination of enzyme activities, 0.3 g fresh leaves material from non-acclimated and cold-acclimated leaves were powdered with liquid nitrogen and suspended in specific buffer with proper pH values for each enzyme. The homogenates were centrifuged at 14 000 rpm for 20 min at 4 °C and resulting supernatants were used for enzyme assay. The protein concentrations of leaf crude extracts were determined according to Bradford (1976), using BSA as a standard.

Superoxide dismutase (SOD; EC1.15.1.1) activity was determined by the method of Beyer and Fridovich (1987), based on the photo reduction of NBT (nitro blue tetrazolium). Extraction was performed in 1.5 ml homogenization buffer containing 10 mM K_2HPO_4 buffer (pH 7.0), 2% PVP and 1 mM Na_2EDTA . The reaction mixture consisted of 100 mM K_2HPO_4 buffer (pH 7.8), containing 9.9×10^{-3} M methionine, 5.7×10^{-5} M NBT, 1% Triton X-100 and

enzyme extract. Reaction was started by the addition of 0.9 μM riboflavin and mixture was exposed to light with an intensity of $375 \mu\text{mol m}^{-2} \text{ s}^{-1}$. After 15 min, reaction was stopped by switching off the light and absorbance was read at 560 nm. SOD activity was calculated by a standard graphic and expressed as unit mg^{-1} protein.

Ascorbate peroxidase (APX; EC1.11.1.11) activity was determined according to Wang et al. (1991) by estimating the decreasing rate of ascorbate oxidation at 290 nm. APX extraction was performed in 50 mM Tris-HCl (pH 7.2), 2% PVP, 1 mM Na_2EDTA , and 2 mM ascorbate. The reaction mixture consisted of 50 mM KH_2PO_4 buffer (pH 6.6), 2.5 mM ascorbate, 10 mM H_2O_2 and enzyme, containing 100 μg proteins in a final volume of 1 ml. The enzyme activity was calculated from initial rate of the reaction using the extinction coefficient of ascorbate ($E = 2.8 \text{ mM cm}^{-1}$ at 290 nm).

Glutathione reductase (GR; EC 1. 6. 4. 2) activity was measured with the method of Sgherri et al. (1994). Extraction was performed in 1.5 ml of suspension solution, containing 100 mM KH_2PO_4 buffer (pH 7.0), 1 mM Na_2EDTA , and 2% PVP. The reaction mixture (total volume of 1 ml) contained 100 mM KH_2PO_4 buffer (pH 7.8), 2 mM Na_2EDTA , 0.5 mM oxidised glutathione (GSSG), 0.2 mM NADPH and enzyme extract containing 100 μg protein. Decrease in absorbance at 340 nm was recorded. Correction was made for the non-enzymatic oxidation of NADPH by recording the decrease at 340 nm without adding GSSG to assay mixture. The enzyme activity was calculated from the initial rate of the reaction after subtracting the non-enzymatic oxidation using the extinction coefficient of NADPH ($E = 6.2 \text{ mM cm}^{-1}$ at 340 nm).

Catalase activity was measured with the method of Aebi (1984). Extraction was performed in 1.5 ml of suspension solution, containing 100 mM KH_2PO_4 buffer (pH 7.5) and 1 mM Na_2EDTA . The homogenates were centrifuged at 14 000 rpm for 20 min at 4 °C and resulting supernatants were used for enzyme assay. The reaction mixture (total volume of 3 ml) contained 1.5 ml phosphate buffer (100 mM, pH 7), 0.95 ml distilled water, 0.5 ml H_2O_2 and 0.05 ml supernatant. Decrease in absorbance at 240 nm was recorded. The enzyme activity was calculated from the initial rate of the reaction using the extinction coefficient of H_2O_2 ($E = 43.6 \text{ mM cm}^{-1}$ at 240 nm).

Chlorophyll a fluorescence measurement

Barley seedlings were pre-darkened for 45-60 min at room temperature. Chlorophyll a fluorescence measurements were performed with the Handy PEA fluorimeter (Hansatech Instruments, King's Lynn, Norfolk, UK). Red actinic light (wavelength at peak 650 nm; spectral line half-width 22 nm) with the intensity of $3500 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ was used for the induction of fluorescence and 1

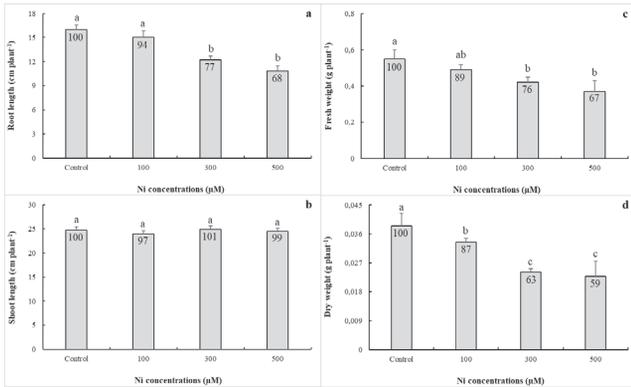


Figure 1. The effect of nickel toxicity on (a) root length, (b) shoot length, (c) fresh weight and (d) dry weight in barley seedlings. (Columns with different letters mean significant differences between the treatments according to Duncan's multiple range test ($P < 0.05$) and numbers on the columns indicate % change relative to control, control = 100).

s of transient fluorescence was recorded. The data were analysed, and the JIP test was conducted using Biolyzer software (Strasser et al. 2000). Measurements of chlorophyll fluorescence were done on 10 plants from each treatment and we had 3 replicates for each plant ($n = 30$).

Statistical analysis

Experiments were a randomised complete block design with three independent replicates. Analysis of variance (ANOVA) was performed using SPSS 20.0 statistical software for Windows. To separate significant differences between means, Duncan (least significant difference) test was used at $P = 0.05$.

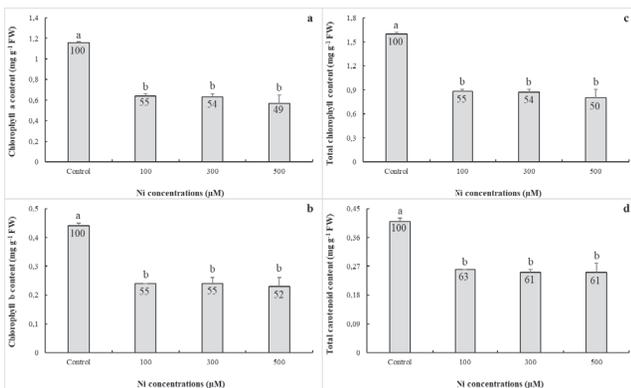


Figure 3. The effect of nickel toxicity on (a) chlorophyll a, (b) chlorophyll b, (c) total chlorophyll and (d) total carotenoid content in barley seedlings. (Columns with different letters mean significant differences between the treatments according to Duncan's multiple range test ($P < 0.05$) and numbers on the columns indicate % change relative to control, control = 100).

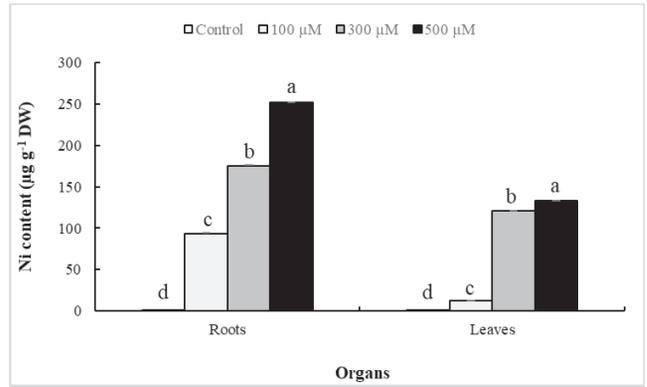


Figure 2. The effect of nickel toxicity on nickel content in the roots and leaves of barley seedlings.

Results

Mild nickel stress (100 μM) did not affect root growth in barley seedlings while moderate (300 μM) and severe nickel stress (500 μM) inhibited root growth to a certain extent (Fig. 1a) in comparison with control. Shoot growth, on the other hand, was not remarkably affected by nickel treatments (Fig. 1b). Like root growth, the fresh weight of barley seedlings under mild nickel toxicity were not affected, but moderate and severe nickel stress decreased the fresh weight considerably as compared to control (Fig.

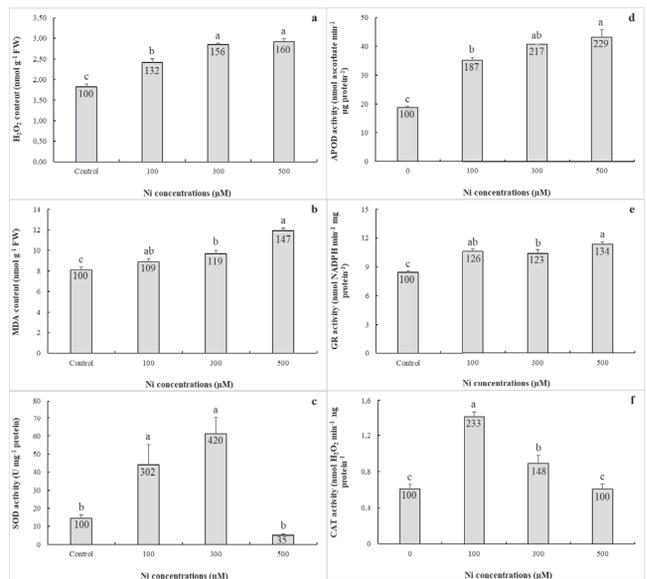


Figure 4. The effect of nickel toxicity on (a) H₂O₂ content, (b) MDA content, (c) SOD activity, (d) APOD activity, (e) GR activity and (f) CAT activity in barley seedlings. (Columns with different letters mean significant differences between the treatments according to Duncan's multiple range test ($P < 0.05$) and numbers on the columns indicate % change relative to control, control = 100).

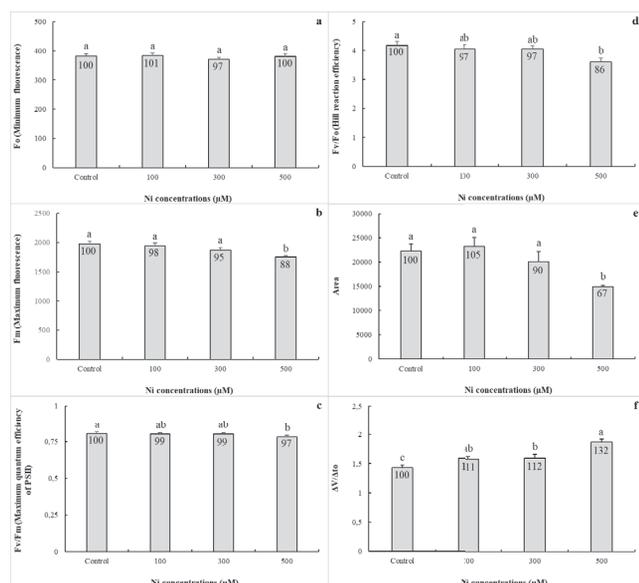


Figure 5. The effect of nickel toxicity on (a) F_o , (b) F_m , (c) F_v/F_m , (d) F_v/F_o , (e) Area and (f) $\Delta V/\Delta t_o$ in barley seedlings. (Columns with different letters mean significant differences between the treatments according to Duncan's multiple range test ($P < 0.05$) and numbers on the columns indicate % change relative to control, control = 100).

1c). All nickel treatments led to the significantly reduced dry mass accumulation than control plants in barley seedlings (Fig. 1d). Nickel accumulation in the roots and leaves of barley seedlings represented a linear increase as the nickel concentration applied increased (Fig. 2). Chlorophyll a, chlorophyll b, total chlorophyll and total carotenoid content in the leaves of barley seedlings were significantly decreased by mild, moderate and severe nickel toxicity as compared to the respective controls (Fig. 3a, b, c and d). In the leaves of barley seedlings, mild, moderate and severe nickel stress led to the increased H_2O_2 and MDA content as compared to the controls (Fig. 4a and b). SOD and CAT activity in the leaves of barley seedlings was significantly elevated by mild and moderate nickel stress, but it was not affected by severe nickel stress as compared to control (Fig. 4c and f). APOD and GR activity in the leaves of barley seedlings was remarkably increased as a result of mild, moderate and severe nickel stress applications in comparison with the respective controls (Fig. 4d and e).

In comparison with control, all nickel treatments did not affect F_o (minimum fluorescence) in barley leaves (Fig. 5a). However, F_m (maximum fluorescence), F_v/F_m (maximum quantum efficiency of photosystem II), F_v/F_o (Hill reaction efficiency) and area (the area above the chlorophyll fluorescence curve between F_o and F_m) were remarkably decreased by only severe nickel stress as compared to controls (Fig. 5b, c, d and e). Mild, moderate

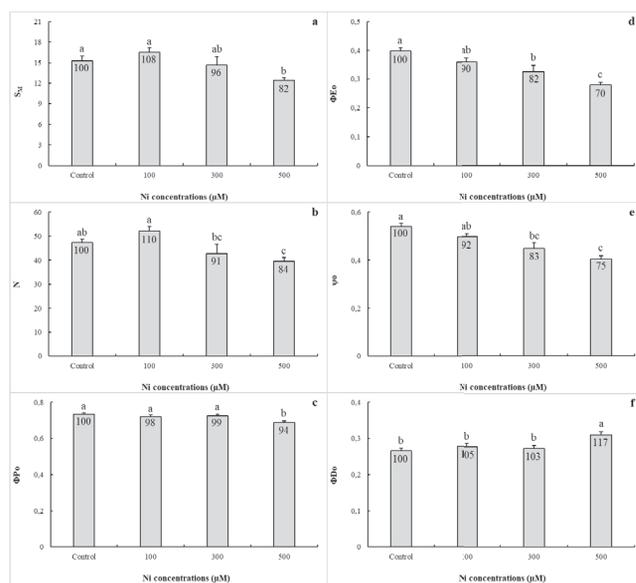


Figure 6. The effect of nickel toxicity on (a) S_M , (b) N, (c) ΦP_o , (d) ΦE_o , (e) ψ_o and (f) ΦD_o in barley seedlings. (Columns with different letters mean significant differences between the treatments according to Duncan's multiple range test ($P < 0.05$) and numbers on the columns indicate % change relative to control, control = 100). Submitted: 15 November 2020.

and severe nickel stress led to the significant reduction in $\Delta V/\Delta t_o$ (the accumulation rate of the closed reaction centers) in the leaves of barley seedlings as compared to control (Fig. 5f). S_M (energy necessary for the closure of all reaction centers), N (the number indicating how many times quinone A is reduced while fluorescence reaches its maximum value) and ΦP_o (maximum quantum yield of primary photochemistry) in the leaves of barley seedlings under severe nickel stress were significantly lower than the respective controls (Fig. 6a, b and c). Moderate and severe nickel stress led to the remarkably decreased ΦE_o (quantum yield of electron transport from quinone A to plastoquinone) and ψ_o (efficiency of a trapped exciton that moves an electron from quinone A to the electron transport system) values in the leaves of barley seedlings as compared to controls (Fig. 6d and e). ΦD_o (quantum yield of thermal dissipation) in the leaves of barley seedlings was significantly reduced by severe nickel stress as compared to control (Fig. 6f).

Discussion

The present study investigated the effect of mild (100 μM), moderate (300 μM) and severe nickel toxicity (500 μM) on some growth parameters, physiological and photochemical changes in a barley genotype, Tarm-92. The genotype Tarm-92, which is commonly grown in arid

and semi-arid parts of Central Anatolia and Transitional regions, is developed by Central Research Institute for Field Crops (Ankara) and registered in 1992. Tarm-92 (two-row feed barley) is known to be tolerant to drought, zinc and boron toxicity as well as moderately resistant to scald and barley leaf stripe. In addition, this barley genotype has facultative winter growth habit, high tillering capacity and good resistance to head loss, with the yield value of 350-450 kg da⁻¹ under rainfed conditions.

Our results showed that root length of barley genotype is reduced by moderate and severe nickel toxicity which is correlated with higher level of nickel accumulation in roots as compared to leaves. Also, the decreased fresh and dry weights of barley plants were observed under nickel toxicity conditions. The nickel-induced reduction in plant growth has also been found in other plant species such as tomato, cotton, brassica and pea (Mosa et al. 2016; Khaliq et al. 2016; Ansari et al. 2015; Sirhindi et al. 2015). It has been reported that Ni toxicity led to the damage in the cell nucleus and nucleolus in root tips of tomato. Similarly, Shi and Cai (2009) have explored that excessive nickel treatments change the plasticity of cell wall, which consequently impair cell division and/or elongation. Thus, higher nickel accumulation in root cells may be responsible for the decreased root growth rate by interfering mitotic activity in our study (Gautam et al. 2017). Another possible explanation for the reduced growth rate of roots and biomass accumulation in barley plants under nickel toxicity may be the decreased uptake and translocation rate of some essential elements such as Mg, Ca, Fe, Cu and Zn which have similar chemical character to Ni (Amari et al. 2017). Kidd et al. (2009) have indicated that nickel could compete with these elements in uptake and utilization in plants and lead to mineral element deficiency. It has been reported that nickel can easily be translocated from roots to the upper parts of the plants by xylem and phloem vessels (Ishtiaq and Mahmood 2012). On the other hand, it has been manifested that plants keeping higher amount of heavy metal in their roots have been considered as more tolerant to heavy metal (Rao and Sresty 2000). Therefore, the genotype Tarm-92, may be accepted as relatively tolerant to nickel toxicity as reflected by lower nickel content in the leaves.

Our results showed that chlorophyll a, chlorophyll b, total chlorophyll and total carotenoid content decreased under nickel toxicity. It has been well documented that decreases in chlorophyll content and photosynthetic efficiency are used to monitor the heavy metal-induced damage in leaves (Asopa et al. 2016). Kamran et al. (2016) have indicated that nickel toxicity leads to chlorophyll loss as a result of Mg and Fe deficiency in the leaves of plant species. In addition, it has been claimed that the reduced chlorophyll content in plants under nickel toxicity may

derive from either promotion of chlorophyllase activity or inhibition of chlorophyll-synthesizing enzymes (Abdel-Basset et al. 1995; Mysliwa-Kurczel and Strzalka 2002). Carotenoids are responsible for the dissipation of excess excitation energy and protection of chlorophyll pigments from light-induced damages as well light absorption for photosynthetic electron transport (Trebst 2003; Hashimoto et al. 2016). In this study, lower total carotenoid content observed in the leaves of barley plants may be associated with lower chlorophyll content due to photooxidation.

It has been stated that nickel toxicity interferes with photosynthetic electron transport reaction and some intermediates of cytochrome b6f and b559 in leaves (Shahzad et al. 2018). Several *in vitro* studies have showed that PSII is primary target for nickel toxicity in plants (Tripathy et al. 1981). Therefore, chlorophyll a fluorescence technique was used to investigate the performance of the photosynthetic apparatus in barley leaves under nickel toxicity. In our study, only severe nickel toxicity led to the decreased maximum quantum efficiency of PSII (as inferred Fv/Fm). This value is known to be close to 0.83 under optimum conditions and often proportional to photosynthetic efficiency (Kalaji et al. 2011). The lowered Fv/Fm values in the severe nickel-stressed leaves of barley plants clearly showed that large extent of photoinhibition has occurred in PSII units (Kalaji et al. 2011). On the other hand, relatively higher level of Fv/Fm ratio clearly demonstrated that electron transport reactions on PSII units remained almost intact under mild and moderate nickel toxicity. In the present study, Fo value was not affected by nickel toxicity treatments. It has been reported that several stress factors may result in the increase in Fo (Maxwell and Johnson 2000). Meravi and Prajapati (2018), for example, claimed that higher level of Fo may be associated with the accumulation of inactive PSII reaction centers. Kalaji et al. (2011) have shown that the reason for the higher Fo values is the slowing down of the transport of electrons from Q_A to Q_B and/or the decrease in the efficiency of PSII to capture light energy. Thus, it may be concluded that nickel stress did not lead to structural damage in PSII reaction centers in barley leaves. The lowered level of Fm value in the leaves of barley suggested that restrictive effect of severe nickel stress on the photosynthetic electron transport reactions in PSII acceptor side appeared, as indicated by Doğru and Çakırlar (2020a, 2020b). In addition, the results of this study clearly indicated that decline in Fv/Fm ratio in the severe nickel-stressed leaves of barley plants was mostly due to decrease in Fm. It has been well documented that water-splitting complex on the donor side of PSII is the most sensitive component in the photosynthetic electron transport system (Kalaji et al. 2011). Pereira et al. (2000)

have indicated that the decrease in Fv/Fo may be the result from the impairment in photosynthetic electron transport reactions. The efficiency of Hill reaction or water splitting complex on the donor side of PSII (as indicated by Fv/Fo) was declined in the leaves of barley under severe nickel stress. The reduced Fv/Fo in the leaves of barley plants may be the assumption that a lower number of electrons were transported from the oxygen-evolving complex (OEC) to the plastoquinone pool, which could be a result of severe nickel toxicity-induced damages in OEC. It has been revealed by previous studies that nickel can competitively replace calcium ion in the binding site of OEC (Kidd et al. 2009). In this study, the decreased value of the parameter area in the leaves of barley clearly confirmed that electron transport rate into PQ pool is reduced by severe nickel stress (Oukarroum et al. 2015). In addition, mild, moderate and severe nickel toxicity led to the increased level of the accumulation of the closed reaction centers, as confirmed by higher $\Delta V/\Delta t$ and lower S_M and N values in the leaves of barley (Gupta 2020). Under these conditions, it is clear that PSII reaction centers are already in the closed state (reduced) and small amount of Q_A molecules are reduced until Fm value is reached under severe nickel toxicity. ΦE_0 and ψ_0 , on the other hand, was declined by moderate and severe nickel toxicity in the leaves of barley seedlings, indicating that electron transport from Q_A to PQ is inhibited to a certain extent. As a result, severe nickel toxicity reduced ΦP_0 while ΦD_0 increased. This situation may be manifested that severe nickel toxicity led to thermal dissipation of absorbed light energy and primary photochemistry is reduced.

It has been well known that chloroplasts are the main site of ROS accumulation in plant cells because of the impairment of the photosynthetic electron transport reactions under stressful conditions (Doğru and Çakırlar 2020a, 2020b). Chloroplasts also possess enzymatic and non-enzymatic antioxidants to detoxify ROS. SOD, for example, is a metalloenzyme and responsible for the dismutation of superoxide radicals to H_2O_2 . Assche and Clijsters (1990) have reported that SOD is a key antioxidant enzyme to protect plants from heavy metal-induced oxidative stress. Induction of SOD is very important for plants to overcome oxidative stress (Alscher et al. 2002). In our study, SOD activity was increased by mild and moderate nickel toxicity, probably indicating an efficient superoxide dismutation and H_2O_2 production. In barley leaves under severe nickel toxicity, however, SOD activity remained unchanged. Hao et al. (2006) have stated that nickel toxicity interferes indirectly with several antioxidant enzymes by replacing the essential elements such as Fe, Zn, and Mg. It is also probable an interaction between nickel ions and ligand groups of SOD, resulting in the inhibition of enzyme activity (Amari et al.

2017). APOD and GR, which are the most important enzymes of ascorbate-glutathione cycle, are in charge of detoxification of H_2O_2 (Doğru and Çakırlar 2020a). In the present study, APOD and GR activity is almost increased by mild, moderate and severe nickel toxicity in a dose-dependent manner. This result clearly showed that ascorbate-glutathione cycle is active in the leaves of barley plants under nickel toxicity and detoxification of H_2O_2 occurs efficiently. However, our results indicated considerable H_2O_2 accumulation in the leaves of barley under mild, moderate and severe nickel stress. This result may be explained by negative correlation between nickel concentration applied and CAT activity, and a possible overproduction of H_2O_2 . Like H_2O_2 accumulation, MDA content in the leaves of barley was escalated with increasing nickel concentrations, indicating membrane damage. This results also explain the decreased level of chlorophyll pigments and photosynthetic activity in the leaves of barley under nickel toxicity.

In conclusion, severe nickel stress was more effective in photochemical, growth and physiological parameters in barley plants. Root growth, biomass accumulation and photosynthetic pigment content in barley plants was decreased by mild, moderate and severe nickel toxicity. Chlorophyll a fluorescence measurements clearly showed that acceptor side of PSII was more sensitive to nickel toxicity as compared to donor side. Nickel toxicity did not damage PSII reaction centers but inhibited electron transport between intermediates of PSII units. Therefore, primary photochemistry was declined, and thermal energy dissipation is increased by nickel stress in the leaves of barley. In addition, it is obvious that nickel toxicity led to the oxidative stress in the leaves of barley, as reflected by higher H_2O_2 and MDA content. Higher APOD and GR activity confirmed higher efficiency of ascorbate-glutathione cycle in the leaves of barley plants under nickel toxicity. SOD and CAT activity, however, remained unchanged under severe nickel toxicity. As a result, it may be concluded that Tarm-92 is moderately tolerant to nickel toxicity.

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References

Abdel-Basset R, Issa AA, Adam MS (1995) Chlorophyllase activity: effects of heavy metals and calcium. *Photosynth*

- 31:421-425.
- Aebi H (1984) Catalase *in vitro*. Meth Enzymol 105:121-126.
- Ahmad MSA, Hussain M, Ashraf M, Ahmad R, Ashraf MY (2009) Effect of nickel on seed germinability of some elite sunflower (*Helianthus annuus* L.). Pak J Bot 41:1871-1882.
- Ali MA, Ashraf M, Athar HR (2009) Influence of nickel stress on growth and some important physiological/biochemical attributes in some diverse canola (*Brassica napus* L.) cultivars. J Hazard Mat 172:964-969.
- Alscher RG, Ertürk N, Heath LS (2002) Role of superoxide dismutases (SODs) in controlling oxidative stress. J Exp Bot 53:1331-1341.
- Amari T, Ghnaya T, Abdelly C (2017) Nickel, cadmium and lead phytotoxicity and potential of halophytic plants in heavy metal extraction. South Afr J Bot 111:99-110.
- Ansari MKA, Ahmad A, Umar S, Zia MH, Iqbal M, Owens G (2015) Genotypic variation in phytoremediation potential of Indian mustard exposed to nickel stress: a hydroponic study. Int J Phytorem 17:135-144.
- Asopa PP, Bhatt R, Sihag S, Kothari S, Kachhwaha S (2016) Effect of cadmium on physiological parameters of cereal and millet plants—a comparative study. Int J Phytorem 19:225-230.
- Assche FV, Clijsters H (1990) Effects of metals on enzyme activity in plants. Plant Cell Environ 13:195-206.
- Beyer WF, Fridovich I (1987) Assaying for superoxide dismutase activity: Some large consequences of minor changes in conditions. Anal Biochem 161:559-566.
- Bhaduri AM, Fulekar M (2012) Antioxidant enzyme responses of plants to heavy metal stress. Rev Environ Sci Biotechnol 11:55-69.
- Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248-254.
- Chen C, Huang D, Liu J (2009) Functions and toxicity of nickel in plants: recent advances and future prospects. Clean: Soil, Air, Water 37:304-313.
- Doğru A (2019) Evaluation of lead tolerance in some barley genotypes by means of chlorophyll a fluorescence. Bartın Uni Int J Nat App Sci 2:228-238.
- Doğru A (2020) Antioxidant responses of barley (*Hordeum vulgare* L.) genotypes to lead toxicity. Biologia 75:1265-1272.
- Doğru A, Çakırlar H (2020a) Effects of leaf age on chlorophyll fluorescence and antioxidant enzymes activity in winter rapeseed leaves under cold acclimation conditions. Braz J Bot 43:11-20.
- Doğru A, Çakırlar H (2020b) Is leaf age a predictor for cold tolerance in winter oilseed rape plants? Func Plant Biol 47(3):250-262.
- Force L, Critchley C, van Rensen JJS (2003) New fluorescence parameters for monitoring photosynthesis in plants. 1. The effect of illumination on the fluorescence parameters of the JIP test. Photosynt Res 78:17-33.
- Gajewska E, Skłodowska M, Słaba M, Mazur J (2006) Effect of nickel on antioxidative enzyme activities, proline and chlorophyll contents in wheat shoots. Biol Plant 50:653-659.
- Gajewska E, Skłodowska M (2007) Effect of nickel on ROS content and antioxidative enzyme activity in wheat leaves. Biometals 20:27-36.
- Gajewska E, Wielanek M, Bergier K, Skłodowska M (2009) Nickel induced depression of nitrogen assimilation in wheat roots. Acta Physiol Plant 31:1291-1300.
- Gautam S, Rathoure AK, Chhabra A, Pandey SN (2017) Effects of nickel and zinc on biochemical parameters in plants—A review. Octa J Environ Res 5(1):14-21.
- Gopal R, Rizvi AH (2008) Excess lead alters growth, metabolism and translocation of certain nutrients in radish. Chemosphere 70:1539-1544.
- Govindjee (2004) Chlorophyll a fluorescence: a bit of basics and history. In Papageorgiou G, Govindjee, Eds., Chlorophyll a Fluorescence A Signature of Photosynthesis. Springer, Dordrecht, pp 1-42.
- Gupta R (2020) Manganese repairs the oxygen-evolving complex (OEC) in maize (*Zea mays* L.) damage during seawater vulnerability. J Soil Sci Plant Nutr 20:1387-1396.
- Hao F, Wang X, Chen J (2006) Involvement of plasma-membrane NADPH oxidase in nickel-induced oxidative stress in roots of wheat seedlings. Plant Sci 170:151-158.
- Hashimoto H, Uragami C, Cogdell RJ (2016) Carotenoids and Photosynthesis. In Stange C, Ed., Carotenoids in Nature. Subcellular Biochemistry. Cham, Springer, pp 111-139.
- Heath RL, Packer L (1968) Photoperoxidation in isolated chloroplasts I. Kinetic and stoichiometry of fatty acid peroxidation. Arch Biochem Biophys 125:189-198.
- Hedfi A, Mahmoudi E, Boufahja F, Beyrem H, Aissa P (2007) Effects of increasing levels of nickel contamination on structure of offshore nematode communities in experimental microcosms. Bull Environ Contam Toxicol 79:345-349.
- Ishtiaq S, Mahmood S (2012) Phytotoxicity of nickel and its accumulation in tissues of three Vigna species at their early growth stages. J Appl Bot Food Qual 84:223-228.
- Izosimova A (2005) Modelling the interactions between calcium and nickel in the soil-plant system. FAL Agr Res 288:99.
- Kalaji MH, Govindjee, Bosa K, Kościelniak J, Gołaszewska KZ (2011) Effects of salt stress on photosystem II efficiency and CO₂ assimilation of two Syrian barley landraces. Env Exp Bot 73:64-72.
- Khaliq A, Ali S, Hameed, A, Farooq MA, Farid M, Shakoor MB, Mohmood K, Ishaque W, Rizwan M (2016) Silicon alleviates nickel toxicity in cotton seedlings through enhancing growth, photosynthesis and suppressing

- Ni uptake and oxidative stress. *Arch Agron Soil Sci* 62:633-647.
- Khan MU, Shahbaz N, Waheed S, Mahmood A, Shinwari ZK, Malik RN (2016) Comparative health risk surveillance of heavy metals via dietary foodstuff consumption in different land-use types of Pakistan. *Hum Ecol Risk Assess Int J* 22:168-186.
- Kidd P, Barcelo J, Bernal MP, Navari-Izzo F, Poschenrieder C, Shilev S, Clemente R, Monterroso C (2009) Trace element behaviour at the root-soil interface: implications in phytoremediation. *Environ Exp Bot* 67:243-259.
- Lichtenthaler HK (1987) Chlorophylls and carotenoids: pigments of photosynthetic membranes. *Meth Enzymol* 148:350-382.
- Maxwell K, Johnson NG (2000) Chlorophyll fluorescence—a practical guide. *J Exp Bot* 51:659-668.
- Mosa A, El-Banna MF, Gao B (2016) Biochar filters reduced the toxic effects of nickel on tomato (*Lycopersicon esculentum* L.) grown in nutrient film technique hydroponic system. *Chemosphere* 149:254-262.
- Mysliwa-Kurdziel B, Strzalka K (2002) Influence of metals on biosynthesis of photosynthetic pigments. In: Prasad, M. N. V., Strzalka, K. (Eds.) *Physiology and Biochemistry of Metal Toxicity and Tolerance in Plants*. Kluwer Academic Publishers, Netherlands, pp.201-227.
- Ohkawa H, Ohishi N, Yagi NY (1979) Assay of lipid peroxides in animal tissue by thiobarbituric acid reaction. *Anal Biochem* 95:351-358.
- Oukarroum A, Bussotti F, Goltsev V, Hazem MH (2015) Correlation between reactive oxygen species production and photochemistry of photosystems I and II in *Lemna minor* L. plants under salt stress. *Env Exp Bot* 109:80-88.
- Pereira WE, de Siqueira DL, Martinez CA, Puiatti M (2000) Gas exchange and chlorophyll fluorescence in four citrus rootstocks under aluminium stress. *J Plant Physiol* 157:513-520.
- Rao KM, Sresty T (2000) Antioxidative parameters in the seedlings of pigeonpea (*Cajanus cajan* (L.) Millspaugh) in response to Zn and Ni stresses. *Plant Sci* 157:113-128.
- Rizwan M, Ali S, Qayyum MF, Ibrahim M, Rehman MZ, Abbas T, Ok YS (2016) Mechanisms of biochar-mediated alleviation of toxicity of trace elements in plants: a critical review. *Environ Sci Pollut Res* 23:2230-2248.
- Sachan P, Lal N (2017) An overview of nickel (Ni²⁺) essentiality, toxicity and tolerance strategies in plants. *Asian J Biol* 2:1-15.
- Sgherri CLM, Loggini B, Puliga S, Navari-Izzo F (1994) Antioxidant system in *Sporobolus stapfianus*: changes in response to desiccation and rehydration. *Phytochem* 35:561-565.
- Shahzad B, Tanveer M, Rehman A, Cheema SA, Fahad S, Rehman S, Sharma A (2018) Nickel; whether toxic or essential for plants and environment: a review. *Plant Physiol Biochem* 132:641-651.
- Sirhindi G, Mir MA, Sharma P, Gill SS, Kaur H, Mushtaq R (2015) Modulatory role of jasmonic acid on photosynthetic pigments, antioxidants and stress markers of *Glycine max* L. under nickel stress. *Physiol Mol Biol Plants* 21:559-565.
- Strasser RJ, Srivastava A, Tsimilli-Michael M (2000) The fluorescence as a tool to characterize and screen photosynthetic samples. In: Yunus, M., Pathre, U., Mohanty, P. (Eds.), *Probing Photosynthesis: Mechanisms, Regulation and Adaptation*. Taylor & Francis, London, pp.445-483.
- Strasser RJ, Tsimilli-Michael M, Srivastava A (2004) Analysis of chlorophyll fluorescence transient. (AIPH) Chlorophyll a Fluorescence 19:321-362.
- Trebst A (2003) Function of β -carotene and tocopherol in photosystem II. *Z Natur* 58:609-620.
- Tripathy BC, Bhatia B, Mohanty P (1981) Inactivation of chloroplast photosynthetic electron-transport activity by Ni²⁺. *Biochim Biophys Acta Bioenerg* 638:217-224.
- Wang SY, Jiao H, Faust M (1991) Changes in ascorbate, glutathione and related enzyme activity during thidiazuron-induced bud break of apple. *Plant Physiol* 82:231-236.
- Yusuf MA, Kumar D, Rajwanshi R, Strasser RJ, Tsimilli-Michael M, Govindjee, Sarin VB (2010) Overexpression of γ -tocopherol methyl transferase gene in transgenic *Brassica juncea* plants alleviates abiotic stress: physiological and chlorophyll fluorescence measurements. *Biochim Biophys Acta* 1797:1428-1438.

