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Physiological, nutritional, and biochemical responses under nickel toxicity in black poplar (*Populus nigra*)

ABSTRACT

Nickel (Ni) is an essential nutrient for plants and it has been identified as a component of a number of enzymes such as ureases. In this study, we have studied the long-term effects of nickel toxicity on black poplar (*Populus nigra*). The black poplars were exposed to Ni as $NiSO_4.6H_2O$ (200, 400, or 800 µM) for 28 days by using complete randomized design with three replications. In this context, Ni accumulation and biomass, photosynthetic pigments analyses [chlorophyll *a* and *b* (Chl *a* and *b*), carotenoid (Car)], malondialdehyde (MDA) content, antioxidant enzyme activities [catalase (CAT) and ascorbate peroxidase (APX)], and metallic ion accumulations were investigated. Ni concentrations significantly increased in root, bark, and leaves in all Ni treatments. Also, reductions were determined significantly in the photosynthetic pigments (Chl *a*, Chl *b*, Chl *a*+*b*, and Car) at all Ni treatments. The MDA content, CAT and APX activities significantly increased compared the control plants. According to element analyses, the concentration of metallic ion accumulations [potassium (K), calcium (Ca), magnesium (Mg), sodium (Na), iron (Fe), zinc (Zn), manganese (Mn), and copper (Cu)] were affected by Ni exposures, suggesting that Ni toxicity adversely affects physiological activities in *P. nigra*.

Key words: phytotoxicity, metallic ion accumulation, heavy metal, plant stress

Introduction

Nickel (Ni) that are found in soil, water and air samples within the biosphere is one of the trace metals emitted into the environment by anthropogenic and natural sources (WHO, 1991). Ni is found abundantly as a free metal or a complex with iron (Fe) in igneous rocks. Also, Ni is the 22nd most abundant elements in the earth's crust (Sunderman and Oskarsson, 1991). Ni is mostly present in the form of nickelous ion, Ni^{2+} in nature and hydrated form as Ni $(H_2O)_6^{2+}$ is common form observed in the soil solution (Yusuf et al., 2011). The uptake of Ni in plants is performed by the root system with passive diffusion and active transport (Seregin and Kozhevnikova, 2006). The general uptake of Ni by plants is affected by plant metabolism, the presence of other metals and organic matter composition, the acidity of soil or solution, and the concentration of Ni^{2+} (Chen et al., 2009). The absorption and translocation of Ni^{2+} from roots to shoots regulated by the inhibitory effect of various metal ions varied as $Fe^{3+}>Co^{2+}>Ca^{2+}>Mg^{2+}>NH^{4+}>K^{+}>Na^{+}$ (Temp 1991). Ni is one of the heavy metals and essential microelements for plant metabolism and a major component of plant enzymes such as urease (EC 3.5.1.5, urea amidohydrolase), Ni-dependent metalloenzyme (Sreekanth et al., 2013; Filiz et al., 2016). While Zn^{2+}, Cu^{2+}, Co^{2+}, Cd^{2+}, and Pb^{2+} inhibited Ni^{2+} influx in barley roots, Mn^{2+} did not. In addition, Zn^{2+} and Cu^{2+} were strongly competitive with Ni^{2+} (Körner et al., 1987). As with many heavy metals, Ni disturbs various physiological processes in plants, containing enzyme activities (Van Assche and Glijsters, 1990). Ni plays important roles in growth and development of plants such as seed germination, seedling, root, leaf, and stem growth, and total dry matter production. Also, the toxic effects of Ni were detected in various physiological processes, including

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photosynthesis, water relations, mineral nutrition, and effect on metabolites. The enzymes and other cellular processes were affected by Ni toxicity such as nitrate reductase (NR), nitrogen metabolism, plasma membrane H^+-ATPase, glutathione reductase (GR), and oxidative stress and antioxidant systems (Yusuf et al., 2011). In white birch (*Betula papyrifera*), Ni and copper (Cu) toxicity was evaluated by using physiological and genomics approaches (Theriault and Nkongolo, 2016). Fuentes et al. (2007) reported that heavy metal concentration increased and was always higher in roots than in shoots under Ni, Cu, and Zn exposure in *Pinus halepensis*, *Pistacia lentiscus*, *Juniperus oxycedrus*, and *Rhamnus alaternus*. In *P. nigra*, leaf Ni content was found as lower in mature than in developing leaves and Ni stress significantly decreased photosynthesis (Velikova et al., 2011). In this study, it was aimed to investigate the physiological and biochemical responses of *P. nigra* under excess Ni.

Materials and Methods

Cultivation of plants and Ni treatment

The experiments were performed in the greenhouse under natural light conditions at an ambient temperature 70% average relative humidity and 25/18°C day/night average temperature in Cilimli Vocational School of Duzce University, Turkey (lat. 40°53′40″N, long. 31°02′55″E), in 2016 June. Cuttings (nearly 15 cm in length and 1 cm in diameter) of black poplar (*P. nigra* genotype Gazi) were taken from Poplar and Fast Growing Forest Trees Research Institute, Izmit, Turkey. One cutting with a sprout was planted and rooted in pots for 10 weeks. Later, these cuttings were transplanted at a rate of one plant per pot filled with 3 liters of perlite. The poplar cuttings were grown in two weeks by using quarter-strength, one week by half-strength and one week full-strength Hoagland solution for each day. The modified Hoagland solution consisted of 5 mM KNO_3, 5 mM $Ca(NO_3).4H_2O$, 2 mM $MgSO_4.7H_2O$, 1 mM KH_2PO_4, 45.5 µM H_3BO_3, 44.7 µM $FeSO_4.7H_2O$, 30.0 µM NaCl, 9.1 µM $MnSO_4.H_2O$, 0.77 µM $ZnSO_4.7H_2O$, 0.32 µM $CuSO_4.5H_2O$, 0.17 µM $NiSO_4.6H_2O$, 0.10 µM $(NH_4)_2Mo_7O_{24}.4H_2O$ and 54.8 µM $Na_2EDTA.2H_2O$ adjusted to pH 6.0 After an acclimatization period of 4 weeks, cuttings with a similar number of nodes and height were chosen for the experiment. For Ni treatments, four levels of Ni (0, 200, 400 and 800 µM) as $NiSO_4.6H_2O$ were applied to the perlite. The experiment was designed as a complete randomized design with three replications. Ni treatment to the plants continued for 28 days.

Determination of Ni and ion contents

After four weeks of Ni treatment, the shoots and roots were carefully harvested, washed with running tap water, and then rinsed three times with de-ionized water. The all shoots, barks, and roots were oven dried at 70°C for at least three days, and the dry weight (DW) was immediately measured. The shoots samples were ground to powder for nutrient ions analysis. Then, these samples were digested by using dry ash method for extractions in a muffle furnace at 500°C for 6 hours (Miller 1998). The Ni and nutrient ions were determined by ICP-OES (Perkin-Elmer Optima 2100 DV; Waltham, MA). The bio-concentration factor (BCF), translocation factor (TF), and total accumulation rate (TAR) were calculated according to the following formulas (Ait Ali et al., 2002; Shi et al 2010; Çikili et al., 2016):

BCF = [Cd]shoot or root / [total Cd]growth medium

TF (%) = 100 x [Cd]shoot / [Cd]root

TAR of Cd (µg/g DW /day) = ([Cd]shoot x DWshoot) + ([Cd]root x DWroot) / growth day x (DWshoot + DWroot)

Determination of photosynthetic pigments

The chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), and carotenoids (Car) concentration were determined spectrophotometrically (Shimadzu UV-1201; Tokyo) by using 500 mg fresh weight (FW) of leaf material ground in a homogenizer in the presence of 10 mL of acetone 90% (v/v). The absorbance of the extract was measured at 663, 645, and 470 nm and pigment concentrations were calculated according to Lichtenthaler (1987).

Malondialdehyde content and membrane permeability

The level of lipid peroxidation in leaves was determined according to Hodges et al., (1999) by measuring malondialdehyde (MDA), routinely used as an indicator of membrane lipid peroxidation, using 250 mg of fresh tissue homogenize with 5% (w/v) trichloroacetic acid (TCA). Lipid peroxidation products were measured as the content of thiobarbituric acid (TBA)-reactive substances. Membrane permeability (MP) measurements using fresh matter were done before harvest. Membrane permeability was determined for the shoot disc samples by the electrical conductivity (EC%) method (Yan et al., 1996).

Antioxidant enzyme extraction and assay

For extraction and assay of enzymes, fully matured leaves (1.0 g) were homogenized (Heidolph, Diax 900) with 5 mL of extraction buffer (100 mM Na-phosphate buffer, pH 7.5) containing 0.5 mM EDTA-Na_2 at 4ºC. Also, 1 mM ascorbate

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was included to extraction buffer for ascorbate peroxidase due to the instability of APX in the absence of ascorbate. The homogenate was centrifuged at 10 000 g for 5 min. The supernatant was used for determining the enzymes activity analyses and a spectrophotometer (Shimadzu UV/VIS 1201, Japan) was used for all colorimetric measurements (including enzyme activities) at 25ºC. The activity of CAT (EC 1. 11.1.6) was determined by using a reaction solution containing 50 mM potassium dihydrogen phosphate (KH_2PO_4) and 1.5 mM H_2O_2 as a decrease in absorbance at 240 nm for 1 min following the decomposition of H_2O_2 and calculated using the extinction coefficient (E = 40 mM/cm) for H_2O_2 (Cakmak et al., 1993). The activity of APX (EC 1.11.1.11) was determined by using a reaction solution containing 50 mM KH_2PO_4, 0.05 mM ascorbic acid, 0.1 mM EDTA-Na_2, and 1.5 mM H_2O_2 as a decrease of ascorbate and measuring the change in absorbance at 290 nm for 1 min and calculated using the extinction coefficient (E = 2.8 mM/cm) for ascorbate (Nakano and Asada, 1981).

Statistical analyses

The experimental design was a completely randomized factorial design with three replicates and obtained data were analyzed by ANOVA. The differences were compared by the Tukey HSD test ($P \leq 0.05$) and performed by using the JMP package program (SAS Institute Inc., Cary, NC).

Results and Discussion

Ni accumulation and biomass

Ni accumulation in black poplar was determined four weeks after Ni exposure. The results indicated that Ni treatments significantly increased Ni contents in all parts of plant due to dose levels (Table 1). While the Ni concentration at 800 µM Ni as the highest level treatment was about 940, 272, and 578 folds higher in leaves, bark, and roots, respectively compared to control plants, it was seen about 304, 70, and 216 folds higher in leaves, bark, and roots, respectively at 200 µM Ni as the lowest level treatment. Velikova et al. (2011) reported that Ni contents dramatic increased 30 and 200 µM treatments in *P. nigra*. In Mediterranean woody seedlings (*P. halepensis, P. lentiscus, J. oxycedrus,* and *R. alaternus*), Ni concentration significantly increased under 25 and 50 µM of Ni exposures (Fuentes et al., 2007). These data are in agreement with our results. Also, Ni treatments inhibited the root, leaf, and bark dry weights in black poplar for all Ni levels but not significant (Table 1). Fuentes et al. (2007) indicated that intermediate application rates of Ni showed a positive trend on biomass accumulation in Mediterranean woody seedlings (*P. halepensis, P. lentiscus, J. oxycedrus,* and *R. alaternus*) at 25 and 50 µM. The total dry matter production and yield were significantly affected by nickel and Ni can also disturb some plant physiological processes, such as mineral nutrition, photosynthesis, and water relations (Sreekanth et al., 2013). In this study, the decrease of dry weights may be related to these adverse effects of Ni treatments at high doses on plant metabolism.

Ni effects on photosynthetic pigments

The photosynthetic pigments content was significantly decreased in leaves of treated plants (Table 2). The content of Chl *a*, Chl *b*, Chl *a+b* and Car decreased dramatically in leaves at all levels of Ni treatments. At the highest dose as

Table 1. *Total Ni concentration and dried weights of roots, leaves, and bark in P. nigra exposed to $NiSO_4.6H_2O$ for four weeks.*

Ni treatments (µM)	Leaf Ni concentration (µg/g DW)	Bark Ni concentration (µg/g DW)	Root Ni concentration (µg/g DW)	Leaf DW (g/plant)	Bark DW (g/plant)	Root DW (g/plant)
0	$0.2^{\pm 0.04}$ d	$0.7^{\pm 0.12}$ d	$4.8^{\pm 0.06}$ c	$5.59^{\pm 0.38}$	$1.79^{\pm 0.26}$	$1.90^{\pm 0.27}$
200	$67.1^{\pm 6.15}$ c	$54.4^{\pm 2.03}$ c	$1032.8^{\pm 13.5}$ b	$4.69^{\pm 0.41}$	$1.66^{\pm 0.13}$	$1.57^{\pm 0.22}$
400	$158.6^{\pm 0.55}$ b	$125.4^{\pm 3.47}$ b	$2320.1^{\pm 268}$ a	$5.25^{\pm 0.22}$	$1.76^{\pm 0.18}$	$1.83^{\pm 0.01}$
800	$188.2^{\pm 1.20}$ a	$193.3^{\pm 6.50}$ a	$2758.5^{\pm 183}$ a	$4.54^{\pm 0.29}$	$1.39^{\pm 0.15}$	$1.44^{\pm 0.04}$
Tukey $HSD_{0.05}$	*14.2*	*17.3*	735.9	*ns*	*ns*	*ns*

Data are means of three replicates with standard errors (means ± SE, *n*=3). Different letters on the bars indicate statistically significant differences ($P \leq 0.05$) between the treatments according to Tukey's HSD test. ns: not significant

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Table 2. *The content of photosynthetic pigments under Ni exposure at different concentrations.*

Ni treatments (µM)	Chl *a*	Chl *b*	Chl *a+b*	Car	Chl *a/b*	Car/Chl
	(µg/g FW)					
0	$1069^{\pm 53}$ a	$267^{\pm 6}$ a	$1336^{\pm 49}$ a	$665^{\pm 13}$ a	$4.01^{\pm 0.28}$	$0.499^{\pm 0.01}$ b
200	$963^{\pm 26}$ a	$249^{\pm 13}$ a	$1212^{\pm 38}$ a	$625^{\pm 21}$ a	$3.88^{\pm 0.10}$	$0.516^{\pm 0.01}$ b
400	$484^{\pm 59}$ b	$112^{\pm 13}$ b	$595^{\pm 71}$ b	$321^{\pm 36}$ b	$4.33^{\pm 0.17}$	$0.541^{\pm 0.01}$ ab
800	$358^{\pm 8}$ b	$102^{\pm 15}$ b	$460^{\pm 14}$ b	$268^{\pm 31}$ b	$3.66^{\pm 0.50}$	$0.583^{\pm 0.02}$ a
Tukey HSD$_{0.05}$	*190*	*60*	*220*	*70*	*ns*	*0.06*

Data are means of three replicates with standard errors (means ± SE, *n*=3). Different letters on the bars indicate statistically significant differences ($P \leq 0.05$) between the treatments according to Tukey's HSD test. ns: not significant

800 µM Ni treatment, Chl *a*, Chl *b* and Car declined by nearly 66%, 63%, 65% and 59%, respectively. Nevertheless, Car/Chl was remarkably increased with Ni treatments. In four white poplar (*P. alba*) clones (Villafranca, L-12, L-80, and LBM), higher concentrations of Ni in the growth medium showed significant inhibitory effects on plant fresh mass and especially on the photosynthetic pigments content (Katanić et al., 2015). Heavy metals such as nickel affect the structure and function of the chlorophyll molecule by displacing the Mg atom in the center of chlorophyll molecule (Van Assche and Clijsters, 1986; Kupper et al., 1996). Seregin and Ivanov (2001) reported that heavy metals disrupted chloroplast structure, blocked chlorophyll synthesis, and disordered electron transport. Thus, our results are in agreement with these data.

The bio-concentration and translocation factors, and total accumulation rate

BCFs, TFs, and TAR values were determined and shown in Table 3. The BCF is used for evaluating the metal accumulation efficiency in plants. The BCF values in leaves, bark, and root remarkably decreased in all Ni treatments compare to control plant. The present results showed that BCF value of black poplar in leaves, bark, and root was greater than critical level (BCF>1) for hyper-accumulator plants as accepted by Ma et al. (2001), and therefore black poplar could be a Ni hyper-accumulator plant. Hyper-accumulators have a greater absorbance capacity (50-500-fold more) than normal plant, because of having root-to-shoot transport system and increased detoxification capacity (McGrath and Zhao, 2003).

The TF is a crucial term to understand the ability of plants to translocate heavy metals from roots to shoot (Zacchini et

Table 3. *Effects of Ni treatments on bio-concentration and translocation factors and total accumulation rate of nickel in P. nigra.*

Ni treatments (µM)	Leaf BCF	Bark BCF	Root BCF	Leaf TF (%)	Bark TF (%)	TAR (µg/g DW/ day)
0	$21.93^{\pm 0.00}$ a	$70.70^{\pm 0.00}$ a	$476.9^{\pm 0.0}$ a	$4.59^{\pm 0.82}$	$14.87^{\pm 2.70}$ a	$3.9^{\pm 0.7}$ d
200	$5.71^{\pm 0.52}$ b	$4.63^{\pm 0.17}$ b	$88.0^{\pm 1.2}$ bc	$6.49^{\pm 0.60}$	$5.26^{\pm 0.22}$ b	$579.5^{\pm 85.8}$ c
400	$6.75^{\pm 0.02}$ b	$5.34^{\pm 0.15}$ b	$98.8^{\pm 11.1}$ b	$7.02^{\pm 0.82}$	$5.58^{\pm 0.78}$ b	$1660.0^{\pm 93.5}$ a
800	$4.01^{\pm 0.03}$ b	$4.12^{\pm 0.14}$ b	$58.7^{\pm 3.9}$ c	$6.88^{\pm 0.44}$	$7.05^{\pm 0.33}$ b	$1335.6^{\pm 49.1}$ b
Tukey HSD$_{0.05}$	*9.10*	*27.35*	*32.26*	*ns*	*6.40*	*308*

Data are means of three replicates with standard errors (means ± SE, *n*=3). Different letters on the bars indicate statistically significant differences ($P \leq 0.05$) between the treatments according to Tukey's HSD test. ns: not significant.

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al., 2011). In leaves, TF values increased but not significant, whereas TF values significantly decreased by about 65%, 63%, and 53% at 200, 400, and 800 µM Ni treatments, respectively in bark tissue. The TF in leaves and bark was much lower than the critical level (TF> 100%). Also, TAR values significantly increased depending on Ni treatment dose. While the highest TAR value was found at 400 µM Ni treatment (about 426 folds) as compared to control, the lowest value was identified at 200 µM Ni treatment (about 149 folds).

MDA and membrane permeability

MDA is widely used as an index for the status of lipid peroxidation. In this study, lipid peroxidation level in leaves was measured as MDA content, was given in Figure 1. At the highest Ni treatment (800 µM Ni), the highest increase in MDA content was identified as 18% compared to control plants. In 200 µM and 400 µM Ni treatments, increases of MDA content were found as about 6% and 7%, respectively, indicating that Ni toxicity induces oxidative stress. In membrane permeability, increases were detected in leaves for all levels of Ni treatments but not significant (Figure 1). The Ni2+ affected the sterol and phospholipid composition of the plasma membrane in rice and adversely affects the membrane permeability and ion homeostasis in the cytoplasm (Ros et al., 1990). In reviews of Ni metabolism in plants, Ni stress enhanced MDA concentration, disturbed membrane functionality, water balance, and ion balance in the cytoplasm in different plant species (Seregin and Kozhevnikova, 2006; Yusuf et al., 2011; Sreekanth et al., 2013). In the current study, these symptoms were detected in black poplar under Ni stress conditions.

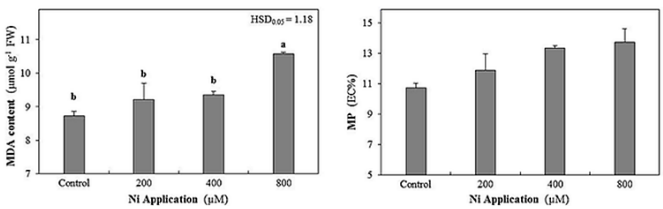

Figure 1. *Effects of different Ni concentrations on MDA content and MP in leaves. Bars indicate means of three replicates ± SE. Different letters on the bars indicate significant difference (P≤0.05) between the treatments according to Tukey's HSD test.*

Effect of Ni on antioxidative enzyme activities

Reactive oxygen species (ROS) are produced in plant cellular metabolism as a normal product. The different environmental stresses cause to excessive production of ROS resulting oxidative damage and ultimately cell death. Scavenging or detoxification of excess ROS are realized by an efficient antioxidative enzyme system, containing superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (GPX), ascorbate peroxidase (APX) etc. (Noctor and Foyer, 1998; Sharma et al., 2012). Hao et al., (2006) showed that excessive Ni causes significant increases in the concentration of hydroxyl radicals, superoxide anions, nitric oxide and hydrogen peroxide. In this study, the CAT and APX activities enhanced significantly in leaves under Ni treatments (Figure 2). While the CAT activity increased by about 21%, 34%, and 55% at 200, 400, and 800 µM treatments, respectively, the APX activity enhanced by about 17%, 34%, and 36% at 200, 400, and 800 µM Ni treatments, respectively. Thus, CAT activity was found as higher than APX activity in black poplar. It can be suggested that CAT activity may plays a more active role than APX to combat oxidative stress because under Ni stress in *P. nigra*. It has been previously reported that Ni can increase the activities of SOD, POD and APX (Gajewska and Skłodowska, 2008). Seregin and Kozhevnikova (2006) reported that most of enzyme activities were decreased, in contrast to some activities such as antioxidant enzymes increased at high Ni concentrations. These data are in agreement with our findings.

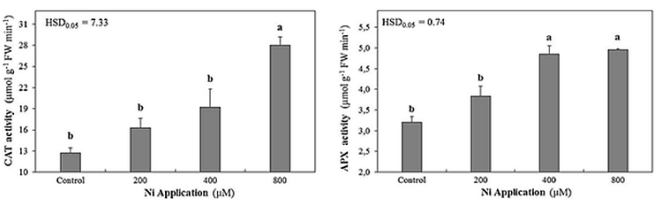

Figure 2. *Effects of different Ni concentrations on CAT and APX activity in leaves of P. nigra. Results are means ± SE of three independent replicates. Different letters are significant at P≤0.05 according to Tukey's HSD test.*

Metallic Cation Accumulations

The heavy metal toxicity can affect the many cellular processes and one of them is the reduction of cation and anion absorption by plant roots (Pallavi and Ram Shankar, 2005). The Ca, Mg, Mn, Fe, Cu, and Zn show the similar characteristics to Ni, thus Ni could compete with these minerals in absorption, uptake and utilization in the plant metabolism (Chen et al., 2009). Therefore, this could lead to perturbation in some physiological and biochemical processes, and finally concludes in phytotoxic damages

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(Gajewska et al., 2006). In this study, metallic ion accumulations such as K, Ca, Mg, Na, Fe, Zn, Mn, and Cu were investigated under excessive Ni conditions in leaves, bark, and roots of *P. nigra* (Table 4 and 5). In roots, accumulations of K, Ca, Na, Fe, Zn, and Cu significantly decreased compared to control plants, except for Cu concentration at the highest Ni treatment, whereas Mn accumulation enhanced in all levels of Ni treatments. In leaves, K, Mg, and Na accumulations significantly increased, in contrast, declines of Zn accumulation was determined. Also, an increment in Ca and Fe accumulations and decreases in Mn and Cu accumulations were observed but not significant. In bark, Mg, Fe, Zn, Mn, and Cu accumulations significantly decreased whereas Na accumulation significantly enhanced at 800 µM Ni treatment. Petráš et al. (2012) reported that the nutrient reserves followed the sequence Ca>K>Mg>Si>Na in bark in poplar clones Robusta (*Populus x euramericana*) and I-214 (*Populus x euramericana*). In this study, the nutrient reserves were found as K>Ca>Mg>Na in the bark, suggesting that genetic background and environmental conditions may affect the differences in metal ion concentrations in *P. nigra*. The reduction in uptake of Mg and Fe is one of the major causes of chlorosis induced by excess of environmental Ni (Piccini and Malavolta 1992). At high Ni concentrations (about 0.1–1 mM), the contents of macro and micro-nutrients are negatively affected because of the perturbations in absorption and transport (Rubio et al., 1994). In barley, reductions in Ca, Fe, K, Mg, Mn, P, and Zn contents were determined under toxic concentration of Ni in leaves and roots (Brune and

Table 4. *The changes in K, Ca, Mg, and Na concentrations under Ni exposures in P. nigra*.

Ni treatments (µM)	K (mg/g DW)	Ca (mg/g DW)	Mg (mg/g DW)	Na (mg/g DW)
		Leaves		
0	$24.8^{\pm0.7}$ b	$4.55^{\pm0.06}$ a	$3.88^{\pm0.07}$ b	$0.31^{\pm0.03}$ b
200	$28.7^{\pm0.2}$ a	$4.86^{\pm0.07}$ a	$4.37^{\pm0.02}$ a	$0.33^{\pm0.02}$ b
400	$25.5^{\pm0.2}$ b	$5.16^{\pm0.21}$ a	$4.58^{\pm0.08}$ a	$0.43^{\pm0.04}$ ab
800	$25.9^{\pm0.4}$ b	$5.14^{\pm0.13}$ a	$4.34^{\pm0.05}$ a	$0.53^{\pm0.06}$ a
Tukey HSD$_{0.05}$	*1.87*	*0.61*	*0.25*	*0.16*
		Bark		
0	$32.3^{\pm0.6}$	$20.77^{\pm0.06}$ ab	$8.82^{\pm0.08}$ a	$0.90^{\pm0.10}$ b
200	$35.7^{\pm1.2}$	$21.61^{\pm0.07}$ a	$8.14^{\pm0.14}$ b	$0.88^{\pm0.01}$ b
400	$34.8^{\pm0.4}$	$20.67^{\pm0.21}$ ab	$8.08^{\pm0.07}$ bc	$0.96^{\pm0.01}$ ab
800	$34.9^{\pm1.0}$	$18.57^{\pm0.13}$ b	$7.66^{\pm0.04}$ c	$1.21^{\pm0.06}$ a
Tukey HSD$_{0.05}$	*ns*	*2.59*	*0.42*	*0.29*
		Root		
0	$26.8^{\pm0.4}$ a	$10.91^{\pm0.17}$ a	$6.81^{\pm0.05}$ ab	$10.32^{\pm0.14}$ b
200	$25.5^{\pm0.3}$ a	$8.49^{\pm0.23}$ b	$6.17^{\pm0.12}$ c	$11.06^{\pm0.33}$ ab
400	$21.6^{\pm0.5}$ b	$7.94^{\pm0.22}$ b	$7.14^{\pm0.02}$ a	$11.66^{\pm0.04}$ a
800	$17.7^{\pm0.3}$ c	$8.12^{\pm0.01}$ b	$6.55^{\pm0.16}$ bc	$7.42^{\pm0.21}$ c
Tukey HSD$_{0.05}$	*1.70*	*0.80*	*0.48*	*0.96*

Data are means of three replicates with standard errors (means ± SE, n=3). Different letters on the bars indicate statistically significant differences (P≤0.05) between the treatments according to Tukey's HSD test. ns: not significant

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Table 5. *The changes in Fe, Zn, Mn, and Cu concentrations under Ni exposures in P. nigra.*

Ni treatments (µM)	Fe (µg/g DW)	Zn (µg/g DW)	Mn (µg/g DW)	Cu (µg/g DW)
		Leaves		
0	$84.10^{\pm 2.69}$	$25.66^{\pm 0.53}$ a	$30.66^{\pm 0.43}$	$5.08^{\pm 0.29}$
200	$91.10^{\pm 0.63}$	$15.86^{\pm 0.95}$ c	$28.72^{\pm 1.36}$	$4.21^{\pm 0.18}$
400	$91.18^{\pm 5.32}$	$17.43^{\pm 0.81}$ c	$28.30^{\pm 0.92}$	$4.59^{\pm 0.20}$
800	$92.12^{\pm 1.17}$	$22.16^{\pm 0.50}$ b	$28.98^{\pm 0.63}$	$4.32^{\pm 0.12}$
Tukey $HSD_{0.05}$	*ns*	*3.26*	*ns*	*ns*
		Bark		
0	$74.62^{\pm 0.87}$ a	$47.68^{\pm 4.21}$ a	$26.45^{\pm 0.85}$ a	$16.40^{\pm 1.89}$ a
200	$69.01^{\pm 1.18}$ a	$30.81^{\pm 2.33}$ b	$21.51^{\pm 1.76}$ b	$10.95^{\pm 1.37}$ ab
400	$54.48^{\pm 1.76}$ b	$26.92^{\pm 0.32}$ b	$15.98^{\pm 0.46}$ c	$9.22^{\pm 0.90}$ b
800	$58.85^{\pm 1.00}$ b	$29.05^{\pm 0.46}$ b	$15.27^{\pm 0.16}$ c	$11.22^{\pm 0.15}$ ab
Tukey $HSD_{0.05}$	*5.63*	*10.94*	*4.54*	*5.70*
		Root		
0	$461.32^{\pm 30.1}$ a	$28.09^{\pm 2.20}$ a	$21.03^{\pm 0.69}$ b	$15.06^{\pm 0.54}$ b
200	$238.15^{\pm 15.2}$ b	$21.51^{\pm 0.45}$ b	$26.55^{\pm 0.41}$ a	$12.36^{\pm 0.18}$ c
400	$182.15^{\pm 5.54}$ b	$17.85^{\pm 0.44}$ bc	$28.64^{\pm 0.68}$ a	$12.67^{\pm 0.47}$ c
800	$164.45^{\pm 7.72}$ b	$15.88^{\pm 0.26}$ c	$30.36^{\pm 2.08}$ a	$17.46^{\pm 0.33}$ a
Tukey $HSD_{0.05}$	*79.30*	*5.22*	*5.25*	*1.85*

Data are means of three replicates with standard errors (means ± SE, n=3). Different letters on the bars indicate statistically significant differences ($P \leq 0.05$) between the treatments according to Tukey's HSD test. ns: not significant

Deitz, 1995). Based on previous data, dramatic reductions in root ion contents may be explained Ni effects on cellular structures, ion uptakes and homeostasis in plant metabolism.

In conclusion, this study demonstrated the phytotoxic effects of excessive Ni on physiological and biochemical processes in *P. nigra*. The findings of this study revealed that excessive Ni attributed to prevention of growth and chlorophyll degradation. The antioxidant enzymes such as CAT and APX were induced by Ni exposure. Furthermore, mineral homeostasis (uptake and transport) under Ni stress was found to be adversely affected.

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