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# Responses of cucumber to deficient and toxic amounts of nickel in nutrient solution containing urea as nitrogen source

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## Abstract

Nickel (Ni) is an irreplaceable component of urease which reduces urea toxicity, but excess of Ni has detrimental effects on plant growth. The responses of cucumber (*Cucumis sativus* L. cvs. Negin and Dominus) plants supplied with urea as sole N source to four Ni concentrations (0, 50, 100 and 200 μM) were investigated. Nickel at a 50 μM concentration stimulated growth and reduced urea accumulation and lipid peroxidation in the leaves. However, the application of 100 and 200 μM Ni reduced a shoot dry mass and increased a malondialdehyde (MDA) content. An activity of catalase (CAT) was not affected by 50 μM Ni, whereas it was significantly increased by 200 μM Ni. The application of Ni resulted in an enhancement of a guaiacol peroxidase (GPX) activity in the leaves. An ascorbate peroxidase (APX) activity was reduced by 200 μM Ni in cv. Negin and by 100 μM Ni in cv. Dominus.

*Additional key words*: ascorbate peroxidase, catalase, *Cucumis sativus*, guaiacol peroxidase, malondialdehyde, Ni-induced oxidative stress.

## Introduction

Nickel is required at the trace amounts and plays a role as an irreplaceable component of metalloenzyme urease (Brown *et al*. 1987, Hansch and Mendel 2009) and so reduces urea toxicity (Bai *et al*. 2006). Therefore, Ni is considered to be an important micronutrient for plants fed with urea as nitrogen source (Follmer 2008, Witte 2011). The activity of urease and other enzymes responsible for nitrate reduction is completely inhibited by Ni deficiency (Follmer 2008). Ni deficiency causes an elevated accumulation of toxic metabolites and thereby induces oxidative stress in plant cells (Gomes *et al*. 2006).

Despite nutritional role at the trace amounts, Ni is toxic for plants at high concentrations (Gajewska and Skłodowska 2006, 2008). Leaf chlorosis, growth retardation, reduction of photosynthesis and respiration rates, yield depression, and impaired water and nutrient uptake are some consequences of Ni phytotoxicity (Llamas and Sanz 2008, Llamas *et al*. 2008). As abiotic stressor, nickel indirectly causes the production and accumulation of reactive oxygen species (ROS) and thereby induce oxidative damages, *e.g*., lipid peroxidation and breakdawn of proteins, enzymes, pigments, chlorophyll, and DNA (Gajewska and Skłodowska 2008). Lipid peroxidation is the first symptom of oxidative damage in plants in which cell membrane integrity is impaired and permeability is increased (Gomes *et al*. 2006). On the other hand, plants have different scavenging systems to cope with the formation of ROS. The scavenging system controlling ROS comprises both non-enzymatic antioxidants (*e.g.*, glutathione, ascorbic acid, and phenolic compounds) and antioxidative enzymes [*e.g*., superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (GTX), and glutathione reductase (GR)] (Gomes *et al*. 2006, Wang *et al*. 2009).

In comparison with some other metals, the biochemical responses of plants to nickel are not well understood. Although the antioxidant response of toxic amounts of Ni has been investigated in plants grown in soils and nutrient solutions containing nitrate and ammonium, less attention has been paid to the effect of Ni in plants fed with urea as sole nitrogen source. As far as it is known, this is the first time when the antioxidant response of cucumber cultivars supplied with urea as nitrogen source to both deficient and toxic amounts of Ni has been investigated. In regard with the requirement of

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*Abbreviations*: APX - ascorbate peroxidase, CAT - catalase, GPX - guaiacol peroxidase, MDA - malondialdehyde.
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Ni for the urease activity (Dixon *et al*. 1975), we hypothesized that Ni in low concentrations has beneficial effect on cucumber plants. In the previous study (Khoshgoftarmanesh and Bahmanziari 2012), 50 µM Ni stimulated growth and enhanced fruit yield of cucumber, whereas toxic effects of Ni were found at the concentrations 100 and 200 µM. Cucumber was chosen for this experiment because this crop has great economic importance.

## Material and methods

**Plants and cultivation:** Seeds of cucumber (*Cucumis sativus* L. cvs. Dominus and Negin) obtained from the National Institute of Seed Preparation and Distribution, Iran were thoroughly rinsed with distilled water and germinated on moist filter paper in an incubator at 28 ºC. These two cultivars are grown in fields (Negin) and greenhouses (Dominus) in Iran. The germinated seeds were sown in pots filled with sterilized quartz sand and continuously irrigated with a nutrient solution (40 to 50 $cm^3$ per day). The nutrient solution contained 0.88 mM $K_2SO_4$, 2.0 mM $Ca(NO_3)_2$, 0.25 mM $KH_2PO_4$, 1.0 mM $MgSO_4$, 0.1 mM KCl, 5.0 mM urea, 1 µM $H_3BO_3$, 0.5 µM $MnSO_4$, 1.0 µM $ZnSO_4$, 0.2 µM $CuSO_4$, 0.02 µM $(NH_4)_6Mo_7O_{24}$, and 2 mM 2-(N-morpholino) ethanesulfonic acid (MES); it was adjusted to pH 6 with KOH. Six pots were used per treatment and four plants were planted in each pot. The photoperiod in a naturally lit greenhouse was 12 h, and daily maximum and minimum temperatures 30 and 18 °C, respectively. Nickel was supplied to the plants in the form of $NiCl_2$ and four concentrations of Ni (0, 50, 100, and 200 µM) were chosen according to a previous study (Khoshgoftarmanesh and Bahmanziari 2012). Nickel is a contaminant of almost all analytical grade transition metals and associated chelates and therefore can potentially influence treatments. In the present experiment, high-purity trace elements were obtained from *Merck* (Darmstadt, Germany). Trace nutrients were used without further purification and dissolved in water or in an acid as appropriate (Gerendas and Sattelmacher 1997). All solutions were renewed every day. Leaf samples were collected 50 and 100 d after the treatment for metal and enzyme analyses. The plants were harvested 100 d after the treatment (at reproductive stages) and were divided into shoot and roots. Shoot dry mass yields were determined for each pot.

**Leaf urea and Ni content:** Dried leaf samples (0.1 g) were placed in boiling water for 5 min and then centrifuged at 10 000 *g* for 60 min. The supernatant was passed through a *Whatman No. 42* filter paper. The content of urea was measured using the method of Cline and Fink (1956).

For the Ni analysis, the plant leaves were dried in a forced-air oven at 70 °C to a constant mass, ground to a fine powder in a *Wiley* mill to pass through a 20-mesh sieve. The samples (about 0.5 g) were digested in an *APCU-40 TFM Teflon* vessel of a microwave (*Milestone*, Sorisole, Italy) using 5 $cm^3$ of $HNO_3$ and 3 $cm^3$ of $H_2O_2$, and then filtered through *Whatman No. 42* filters, transferred to 50-$cm^3$ flasks, and diluted with deionized, distilled water. For quality control, reagent blanks and a *NIST* standard reference were included. The concentration of Ni in the digest solutions was determined by graphite furnace atomic absorption spectrometry (GFAAS) (*PerkinElmer 800*, Wellesley, MA, USA) (Chapman and Pratt 1961).

**Lipid peroxidation:** For the measurement of lipid peroxidation, the thiobarbituric acid (TBA) test was used (Hodges *et al*. 1999). Samples (500 mg) of leaves were homogenized in 4.0 $cm^3$ of a 1 % (m/v) trichloroacetic acid (TCA) solution and centrifuged at 10 000 *g* for 10 min. The supernatant was added to 1 $cm^3$ of 0.5 % (m/v) TBA in 20 % TCA. The mixture was incubated in boiling water for 30 min and the reaction was stopped by placing the tubes in an ice bath. The samples were centrifuged at 10 000 g for 5 min, and the absorbance of the supernatant was read at 532 nm on a spectro-photometer (*UV-1800*, *Rayelable*, China). The value for non-specific absorption at 600 nm was subtracted. The amount of MDA-TBA complex (red pigment) was calculated from the coefficient of absorbance (ε) 155 $mM^{-1}$ $cm^{-1}$.

**Leaf extraction and enzyme assays:** The leaf samples were homogenized in a mortar and pestle with a 100 mM TRIS-HCl buffer (pH 8; buffer volume : fresh mass, 3:1) containing 2 mM EDTA, 5 mM DL-dithiothreitol, 10 % (v/v) glycerol, 100 mM sodium borate, 4 % (m/v) insoluble polyvinylpyrrolidone (PVP), and 1 mM phenyl-methylsulphonylfluoride (PMSF). The homogenate was filtered through four layers of muslin cloth and centrifuged at 12 000 *g* for 40 min. The supernatant was stored in separate aliquots at -80 °C prior to enzyme analyses. Total protein was determined using the Bradford method (Bradford 1976).

Catalase (EC 1.11.1.6) activity was determined according to Cakmak and Marschner (1992). The reaction mixture contained a 25 mM sodium phosphate buffer (pH 7.0) plus 10 mM $H_2O_2$ in a total volume of 3 $cm^3$. The reaction was initiated by the addition of 0.1 $cm^3$ of the leaf extract to the reaction mixture, and the enzyme activity was determined by measuring the initial rate of dis-appearance of $H_2O_2$ at 240 nm for 30 s ($\varepsilon = 39.4$ $mM^{-1}$ $cm^{-1}$).

Guaiacol peroxidase (GPX) activity was measured using a modification of the procedure of Egley *et al*. (1983). The reaction mixture in a total volume of 2 $cm^3$ contained a 25 mM sodium phosphate buffer (pH 7.0), 0.1 mM EDTA, 0.05 % guaiacol (2-metho-xyphenol),

1.0 mM $H_2O_2$, and 0.1 $cm^3$ of the leaf extract. The increase of absorbance due to oxidation of guaiacol was measured at 470 nm ($\varepsilon = 26.6\ mM^{-1}\ cm^{-1}$).

Ascorbate peroxidase (APX) activity was determined according to Nakano and Asada (1981). The reaction mixture with a total volume of 3 $cm^3$ consisted of a 25 mM sodium phosphate buffer (pH 7.0), 0.1 mM EDTA, 0.25 mM ascorbate, 1.0 mM $H_2O_2$, and 0.1 $cm^3$ of the leaf extract. $H_2O_2$-dependent oxidation of ascorbate was followed by a decrease in the absorbance at 290 nm ($\varepsilon = 2.8\ mM^{-1}\ cm^{-1}$).

**Statistical analysis:** The experiment was set up in a completely randomized factorial design; each treatment contained four replicates. All values reported in this work are means of three independent experiments. There was homogeneity of variances and no significant difference was found between the experiments in the measured parameters. Therefore, the combined analysis of three experiments was performed. Treatment effects were analyzed by the analysis of variance using the GLM procedure. Means were compared using least significant differences (LSD) at $\alpha = 0.05$ (*SAS* Institute 2000).

## Results

The addition of 50 μM Ni stimulated shoot growth of Negin but it had no significant effect on the shoot dry mass of Dominus after 100 d (Table 1). The increasing Ni concentration in the nutrient solution caused a significant decrease of the cucumber shoot dry mass. The exposure to 100 and 200 μM Ni resulted in a shoot growth reduction of Negin, whereas in Dominus, a shoot growth reduction was significant only after 100 d.

Regardless of cucumber cultivar and growth stage, the addition of 50 μM Ni significantly reduced a leaf urea accumulation (Table 1). In Dominus, a leaf urea content was significantly reduced also by application of 100 and 200 μM Ni after both the 50 d and 100 d treatments. In Negin, a leaf urea content was decreased by 50 μM Ni but it was unaffected by 100 and 200 μM Ni after 50 d.

The increasing Ni concentration in the nutrient solution significantly increased the accumulation of Ni in cucumber leaves (Table 1). In general, Negin had a higher Ni content in shoots compared with Dominus.

The application of 50 μM Ni reduced lipid peroxidation (MDA production) in both the cultivars and treatment durations (Table 1). The application of 100 μM Ni significantly increased an MDA content in Negin after 50 d and in Dominus after 100 d. The addition of 200 μM Ni caused a significant increase of the MDA content in both the cultivars.

The effect of Ni addition on the leaf CAT activity was dependent on the cucumber cultivar (Table 2). After 100 d, the addition of 50 μM Ni resulted in a significant decrease of the CAT activity in the leaves of Negin. In contrast, at both the exposure periods, the addition of 100 and 200 μM Ni increased the CAT activity in this cultivar (Table 2). After the 50 d treatment, the CAT activity in Dominus was significantly enhanced by the application of 200 μM Ni, it remained unchanged in the presence of 50 μM Ni, and it was reduced at the 100 μM Ni. After the 100 d exposure to 100 μM Ni, the activity of CAT in Dominus was greater than at other Ni concentrations.

Table 1. The shoot dry mass and content of urea, Ni, and malondialdehyde (MDA) in the leaves of cucumber cultivars exposed to different concentrations of Ni for 50 and 100 d. Means ± SE, $n = 4$. For each exposure period, means with the same letter are not significantly different at $P > 0.05$ according to the Fisher's protected LSD test.

| Parameter | Cultivar | Exposure [d] | 0 μM Ni | 50 μM Ni | 100 μM Ni | 200 μM Ni |
|---|---|---|---|---|---|---|
| Shoot d.m. [mg plant$^{-1}$] | Negin | 50 | 470 ± 20 b | 517 ± 30 a | 342 ± 10 c | 241 ± 20 d |
| | | 100 | 627 ± 30 b | 955 ± 50 a | 480 ± 10 c | 315 ± 20 d |
| | Dominus | 50 | 292 ± 10 bcd | 327 ± 40 a | 307 ± 20 b | 287 ± 30 cd |
| | | 100 | 550 ± 40 ab | 585 ± 20 a | 485 ± 20 c | 407 ± 10 d |
| Urea [μmol g$^{-1}$(f.m.)] | Negin | 50 | 10.10 ± 1.01 bc | 7.90 ± 0.88 de | 8.60 ± 0.55 cde | 9.00 ± 0.79 cd |
| | | 100 | 13.83 ± 0.93 a | 9.33 ± 1.33 f | 11.83 ± 0.89 cd | 12.67 ± 0.99 b |
| | Dominus | 50 | 11.00 ± 0.88 a | 6.00 ± 1.07 f | 9.00 ± 1.11 cd | 9.80 ± 1.02 bc |
| | | 100 | 12.16 ± 1.00 bc | 8.00 ± 0.99 gh | 10.00 ± 0.93 ef | 11.33 ± 0.81 d |
| Nickel [μg g$^{-1}$(d.m.)] | Negin | 50 | 6 ± 0.6 e | 68 ± 12.3 b | 121 ± 17.9 b | 184 ± 13.5 a |
| | | 100 | 7 ± 0.4 f | 114 ± 12.2 d | 158 ± 22.3 c | 216 ± 23.1 a |
| | Dominus | 50 | 5 ± 1.01 e | 64 ± 9.1 d | 81 ± 12.1 c | 126 ± 12.6 b |
| | | 100 | 3 ± 1.01 f | 83 ± 11.0 e | 108 ± 10.8 d | 170 ± 18.4 b |
| MDA [μmol g$^{-1}$(f.m.)] | Negin | 50 | 72 ± 13 e | 42 ± 8 f | 117 ± 19 cd | 152 ± 21 bc |
| | | 100 | 316 ± 34 b | 265 ± 18 c | 322 ± 23 b | 366 ± 28 a |
| | Dominus | 50 | 130 ± 12 cd | 111 ± 27.1 d | 124 ± 15 cd | 166 ± 22 a |
| | | 100 | 170 ± 19 de | 160 ± 21 ef | 318 ± 36 b | 368 ± 29 a |

Table 2. Catalase (CAT), guaiacol peroxidase (GPX), and ascorbate peroxidase (APX) activities [μmol(substrate) $mg^{-1}$(prot.) $min^{-1}$] in the leaves of cucumber cultivars exposed to different Ni concentrations for 50 and 100 d. Means ± SE, $n = 4$. For each exposure period, means with the same letter are not significantly different at $P > 0.05$ according to the Fisher's protected LSD test.

| Parameter | Cultivar | Exposure [d] | 0 μM Ni | 50 μM Ni | 100 μM Ni | 200 μM Ni |
|---|---|---|---|---|---|---|
| CAT | Negin | 50 | 0.63 ± 0.09 de | 0.75 ± 0.05 cd | 1.24 ± 0.14 b | 1.31 ± 0.10 a |
| | | 100 | 0.32 ± 0.03 b | 0.10 ± 0.03 d | 0.54 ± 0.09 a | 0.57 ± 0.11 a |
| | Dominus | 50 | 0.26 ± 0.04 g | 0.25 ± 0.02 g | 0.13 ± 0.03 h | 0.41 ± 0.05 f |
| | | 100 | 0.11 ± 0.02 cd | 0.33 ± 0.05 b | 0.56 ± 0.06 a | 0.35 ± 0.08 b |
| GPX | Negin | 50 | 2.31 ± 0.49 de | 4.22 ± 0.87 c | 4.63 ± 0.68 bc | 6.21 ± 1.02 a |
| | | 100 | 1.90 ± 0.41 c | 4.33 ± 0.64 ab | 5.00 ± 0.97 a | 5.20 ± 0.86 a |
| | Dominus | 50 | 1.22 ± 0.43 f | 1.74 ± 0.32 e | 2.87 ± 0.44 d | 4.70 ± 0.96 bc |
| | | 100 | 0.89 ± 0.07 d | 1.10 ± 0.14 d | 1.88 ± 0.23 c | 5.30 ± 1.69 a |
| APX | Negin | 50 | 0.24 ± 0.04 c | 0.26 ± 0.05 c | 0.24 ± 0.05 c | 0.12 ± 0.02 d |
| | | 100 | 0.33 ± 0.03 b | 0.36 ± 0.06 b | 0.33 ± 0.03 b | 0.09 ± 0.01 de |
| | Dominus | 50 | 0.46 ± 0.03 b | 0.59 ± 0.03 a | 0.08 ± 0.01 d | 0.09 ± 0.01 de |
| | | 100 | 0.36 ± 0.02 b | 0.60 ± 0.02 a | 0.11 ± 0.03 de | 0.08 ± 0.02 e |

In both the cucumber cultivars, the addition of Ni increased the activity of GPX in the leaves except in Dominus after the 100 d treatment with 50 μM Ni (Table 2). The highest leaf activity of GPX was found at the 200 μM Ni treatment for both the cultivars.

The addition of 50 μM Ni had no effect (in Negin) or increased (in Dominus) the leaf APX activity (Table 2). The activity of APX was reduced by 200 μM Ni in Negin and by 100 and 200 μM Ni in Dominus.

## Discussion

At both exposure periods (50 and 100 d), Negin accumulated higher amounts of Ni in its leaves compared with Dominus. There are several mechanisms involved in genotypic variation of Ni content among plant genotypes, including differences in metal uptake by roots (Marschner 1995). Different responses of Negin and Dominus to various Ni concentrations have been reported previously (Khoshgoftarmanesh and Bahmanziari 2012) but further investigation is needed to fully understand the genotypic variations among cultivars in root Ni uptake.

The addition of 50 μM Ni had no significant effect on shoot growth of Dominus after 100 d. In contrast, the 50 μM Ni stimulated shoot growth of Negin. Beneficial effects of 10 - 50 μM Ni have been reported on coffee suspension cultures (Gomes *et al*. 2006), *Luffa cylindrica* seedlings (Wang *et al.* 2010), tea (Ghanati and Rahmati Ishka 2006), and wheat (Gajewska and Sklodowska 2006). The exposure of cucumber plants to 50 μM Ni resulted in a considerable accumulation of this metal in cucumber leaves depending on time of exposure and cultivar used. Although tissue Ni content in non-hyperaccumulator plants lies between 10 and 50 mg $kg^{-1}$(d.m.) (Hawkesford 2011), the range of Ni concentrations between critical deficiency on one side and toxicity on the other side is extremely wide as compared with other elements (Gerendás and Sattelmacher 1999, Brown and Bassil 2011). The critical toxicity of Ni is > 10 mg $kg^{-1}$(d.m.) in sensitive species (Kozlov 2005), > 50 mg $kg^{-1}$(d.m.) in moderately tolerant species (Asher 1991), and >1 000 mg $kg^{-1}$(d.m.) in Ni hyperaccumulators (Kupper *et al*. 2001, Pollard *et al*. 2002). Based on the results obtained from the present study, cucumber can be categorized as moderately tolerant species.

The positive effect of Ni on plants is genotype-dependent (Marschner 1995). The promoted shoot growth of the urea-fed Negin plants by 50 μM Ni seems to be as consequence of improved nitrogen metabolism. Although plants are able to use urea as N source for growth, this urea has to be broken down by urease (Wang *et al*. 2008). Urease content in vegetative tissues of Ni-deficient plants is very low (Wang *et al*. 2008) and urea accumulation causes toxicity and growth retardation. In the present study, the addition of 50 μM Ni increased a urease activity (data not shown) and as result, the reduced urea accumulation in the cucumber leaves, and this decrease was greater for Negin. In the Ni-free treatment, severe symptoms of leaf damage are observed on urea-fed cucumber plants (Khoshgoftarmanesh and Bahmanziari 2012). A lower urea accumulation was also accompanied with a less production of MDA in the leaves of cucumber plants supplied with 50 μM Ni compared with those grown at the Ni free treatment. The MDA accumulation indicates lipid peroxidation of cell membranes (Mittler 2002, Molassiotis *et al*. 2006). The positive effect of the 50 μM Ni treatment on ameliorating lipid peroxidation in cucumber might be due to a reduction of the urea accumulation in the plant leaves.

On the other hand, high concentrations of Ni in the nutrient solution resulted in a shoot growth depression of cucumber. A shoot growth reduction was observed at

100 μM Ni for Negin and at 200 μM Ni for Dominus. It was interesting that despite a greater positive effect of 50 μM Ni on shoot growth of Negin, the tolerance to higher Ni concentrations of this cultivar was less than that of Dominus. This might be due to a higher Ni accumulation in the leaves of Negin than of Dominus. Variations in tolerance to toxicity of Ni have been reported among plant species, and cultivars within a species (Kupper *et al*. 2001, Pollard *et al*. 2002, Kozlov 2005).

The toxic effects of excess Ni (100 and 200 μM) were associated with oxidative damage, *i.e.*, the leaf MDA accumulation. The elevated production of MDA in plants exposed to adverse environmental conditions is an indicator of ROS formation in tissues (Baccouch *et al*. 2001). An increase in lipid peroxidation of cell membranes and a consequent modification of membrane integrity has been reported to be the initial sign of Ni toxicity in plant (Gajewska *et al*. 2012). In the present study, the lipid peroxidation increased with the increasing Ni concentration and exposure period. In agreement with our result, Gomes *et al*. (2006) and Maheshwari and Dubey (2009) reported that the production and accumulation of ROS and MDA increased over time of Ni treatment.

Several enzymes, *e.g*., SOD, CAT, APX, and GPX are involved in a defense system of plants against oxidative stress (Asada 1999, Ali *et al.* 2013). We hypothesized that both deficiency and toxicity of Ni could modify activities of antioxidant enzymes in plant cells. To test this hypothesis, the activities of CAT, APX, and GPX were measured in the cucumber leaves. The CAT activity in both the cultivarswas was not affected by the exposure to 50 μM Ni for 50 d but it was reduced after 100 d. The addition of 200 μM Ni enhanced the CAT activity. This might be due to the fact that the addition of 50 μM Ni improved urea hydrolysis and thus reduced the accumulation of this toxic metabolite in the plant tissues. On the other hand, 200 μM Ni caused oxidative stress and the activity of CAT increased to ameliorate damage. A close relationship between the Ni concentration, MDA content, and activity of CAT confirmed the antioxidative response of the cucumber cultivars to excess Ni. In line with our result, Yan *et al*. (2008) reported an increase in the CAT activity at toxic concentrations of Ni. In contrast, Madhava Rao and Sresty (2000) have found that a high Ni concentration reduces the activity of CAT in plant tissues. This contradictory results are probably due to the different plant species used as Zhang *et al*. (2007) reported that the activities of antioxidant enzymes may be enhanced, decreased, or remained unchanged in the presence of high concentrations of heavy metals depending on the metal type and concentration, enzyme, plant cultivar and plant tissue.

The activity of GPX is often used as biological index for cell damage induced by toxic concentrations of heavy metals (Radotic *et al*. 2000). Our results show that the addition of Ni enhanced the leaf GPX activity in both the cucumber cultivars. It seems that the elevated activity of GPX in the leaves of cucumber was a part of defense to excess Ni. In agreement with this result, Maheshwari and Dubey (2009) reported that the activity of GPX in rice leaves increases at 200 and 400 μM Ni. An increase in the activity of GPX under heavy metal toxicity has also been reported by Wang *et al*. (2009).

The activity of APX in the leaves of Negin was unaffected by 50 and 100 μM Ni but 200 μM Ni decreased the APX activity in both the cucumber cultivars. This decrease in the leaf APX activity seems to be due to a reduction of AXP synthesis under stress conditions (Asada 1999). Similar to this result, Gomes *et al*. (2006) indicated that 0.05 μM Ni increased the activity of APX in soybean cells, whereas 0.5 μM Ni decreased the APX activity. In another study, Duman and Oturk (2009) reported that the activity of APX in watercress is increased by the application of 1 mg $dm^{-3}$ Ni, whereas decreased at higher Ni concentrations. In contrast, Gajewska and Sklodowska (2008) showed that the activity of APX in wheat increases at 100 and 200 μM Ni in the media. Maheshwari and Dubey (2009) also reported that the activity of APX in rice tissues increases with increasing Ni concentration in the medium and with exposure. Baccouch *et al*. (2001) reported that the activity of APX was not affected by Ni toxicity.

In summary, the results show that the addition of 50 μM Ni reduced the urea accumulation and lipid peroxidation of leaf cells. On the other hand, high concentrations of Ni in the root media induced oxidative damage characterized by the production of MDA in the cucumber leaves. The activities of antioxidant enzymes were dependent on the plant cultivar, Ni concentration, and enzyme type. The increased CAT and GPX activities might represent a defense mechanism against oxidative stress induced by toxic concentrations of Ni. In contrast, the APX activity was inhibited by the excess of Ni.

## References

Ali, S., Farooq, M.A., Jahangir, M.M., Abbas, F., Bhardwana, S.A., Zhang, G.P.: Effect of chromium and nitrogen form on photosynthesis and anti-oxidative system in barley. - Biol. Plant. **57**: 758-763, 2013.

Asada, K.: The water cycle in chloroplasts: scavenging of oxygens and dissipation of excess photons. - Annu. Rev. Plant Physiol. Plant mol. Biol. **50**: 601-639, 1999.

Asher, C.J.: Beneficial elements, functional nutrients and possible new essential elements. - In Mortvedt, J.J., Cox, F.R., Shuman, L.M., Welch, R.M. (ed.): Micronutrients in Agriculture. Pp. 703-723. Soil Science Society of America, Madison 1991.

Baccouch, S., Chaoui, A., El Ferjani, E.: Ni toxicity induces oxidative damage in *Zea mays* roots. - J. Plant Nutr. **24**: 1085-1097, 2001.

Bai, C., Reilly, C.C., Wood, B.W.: Nickel deficiency disrupts

metabolism of ureides, amino acids, and organic acids of young pecan foliage. - Plant Physiol. **140**: 433-443, 2006.

Bradford, M.M.: 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, 1976.

Brown, P.H., Welch, R.M., Cary, E.E.: Nickel: a micronutrient essential for higher plants. - Plant Physiol. **85**: 801-803, 1987.

Brown, P.H., Bassil, E.: Overview of the acquisition and utilization of boron, chlorine, copper, manganese, molybdenum, and nickel by plants and prospects for improvement of micronutrient use efficiency. - In Hawkesford, M.J., Barraclough, P. (ed.): The Molecular and Physiological Basis of Nutrient Use Efficiency in Crops. Pp. 377-427. Wiley-Blackwell, Oxford 2011.

Cakmak, I., Marschner, H.: Magnesium deficiency and high light intensity enhance activities of superoxide dismutase, ascorbate peroxidase, and glutathione reductase in bean leaves. - Plant Physiol. **98**: 1222-1227, 1992.

Chapman, H.D., Pratt, P.F.: Methods of Analysis for Soil, Plant and Water. - Division of Agriculture Science, California University, Riverside 1961.

Cline, R.E., Fink, R.M.: Investigation of color reaction between *p*-dimethyl aminobenzaldehyde and urea or ureide acids. - Anal. Chem. **28**: 47-52. 1956.

Dixon, N.E., Gazzola, C., Blakeley, R.L., Zerner, B.: Jack bean urease (EC 3.5.1.5). A metalloenzyme. A simple biological role for nickel? - J. amer. chem. Soc. **97**: 4131-4133, 1975.

Duman, F., Oturk, F.: Nickel accumulation and its effect on biomass, protein content and antioxidative enzymes in roots and leaves of watercress. - J. environ. Sci. **24**: 526-532, 2009.

Egley, G.H., Paul, R.N., Vaughn, K.C., Duke, S.O.: Role of peroxidase in the development of water impermeable seed coats in *Sida spinosa* L. - Planta **157**: 224-232, 1983.

Follmer, C.: Insights into the role and structure of plant ureases. - Phytochemistry **69**: 18-28, 2008.

Gajewska, E., Bernat, P., Delugonski. J.: Effect of nickel on membrane integrity, lipid peroxidation and fatty acid composition in wheat seedlings. - J. Agron Crop Sci. **198**: 286-294, 2012.

Gajewska, E., Sklodowska, M.: Effect of nickel on ROS content and antioxidative enzyme activities in wheat leaves. - Biometals **20**: 27-36, 2006.

Gajewska, E., Sklodowska, M.: Differential biochemical responses of wheat shoots and roots to nickel stress: antioxidative reactions and proline accumulation. - Plant Growth Regul. **54**: 179-188. 2008.

Gerendas, J., Sattelmacher, B.: Significance of Ni supply for growth, urease activity and the contents of urea, amino acids and mineral nutrients of urea-grown plants. - Plant Soil **190**: 153-162, 1997.

Gerendas, J., Sattelmacher, B.: Influence of Ni supply on growth and nitrogen metabolism of *Brassica napus* L. grown with $NH_4NO_3$ or urea as N source. - Ann. Bot. **83**: 65-71, 1999.

Ghanati, F., Rahmati Ishka M.: Improvement of antioxidant system and decrease of lignin by nickel treatment in tea plant. - J. Plant Nutr. **29**: 1649-1661, 2006.

Gomes, R.A., Jr., Moldes, C.A., Delite, F.S., Gratao, P.L., Mazzafera, P., Lea, P.J., Azedevo, R.A.: Nickel elicits a fast antioxidant response in *Coffea arabica* cells. - Plant Physiol. Biochem. **44**: 420-429, 2006.

Hansch, R., Mendel, R.R.: Physiological functions of mineral micronutrients (Cu, Zn, Mn, Fe,Ni, Mo, B, Cl). - Curr. Opin. Plant Biol. **12**: 259-266, 2009.

Hawkesford, M.J., Barraclough, P.: The Molecular and Physiological Basis of Nutrient Use Efficiency in Crops. - Wiley-Blackwell, Oxford 2011.

Hodges, D.M., DeLong, J.M., Forney, C.F., Prange, R.K.: Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. - Planta **207**: 604-611, 1999.

Khoshgoftarmanesh, A.H., Bahmanziari, H.: Stimulating and toxicity effects of nickel on growth, yield, and fruit quality of cucumber supplied with different nitrogen sources. - J. Plant Nutr. Soil Sci. **175**: 474-481, 2012.

Kozlov, M.V.: Pollution resistance of mountain birch, *Betula pubescens* subsp. *czerepanovii*, near the copper-nickel smelter: natural selection or phenotypic acclimation?. - Chemosphere **59**: 189-197, 2005.

Kupper, H., Lombi, E., Zhao, F.J., Wieshammer, G., McGrath, S.P.: Cellular compartmentation of nickel in the hyperaccumulators *Alyssum lesbiacum*, *Alyssum bertolonii* and *Thlaspi goesingense*. - J. exp. Bot. **52**: 2291-2300, 2001.

Llamas, A., Sanz, A.: Organ-distinctive changes in respiration rates of rice plants under nickel stress. - Plant Growth Regul. **54**: 63-69, 2008.

Llamas, A., Ullrich, CI., Sanz, A.: $Ni^{2+}$ toxicity in rice: effect on membrane functionality and plant water content. - Plant Physiol. Biochem. **46**: 905-910, 2008.

Madhava Rao, K.V., Sresty, T.V.S.: Antioxidative parameters in the seedlings of pigeon pea (*Cajanus cajan* L.) in response to Zn and Ni stresses. - Plant Sci. **157**: 113-128, 2000.

Maheshwari, R., Dubey, R.S.: Nickel-induced oxidative stress and the role of antioxidant defense in rice seedlings. - Plant Growth Regul. **59**: 37-49, 2009.

Marschner, H.: Mineral Nutrition of Higher Plants. - Academic Press, London 1995.

Mittler, R.: Oxidative stress, antioxidants and stress tolerance. - Trends Plant Sci. **7**: 405-410, 2002.

Molassiotis, A., Sotipoulos, T., Tanou, G., Diamantidis, G., Therios, I.: Boron-induced oxidative damage and antioxidant and nucleolytic responses in shoot tips culture of the apple rootstock EM9 (*Malus domestica* Borkh). - Environ. exp. Bot. **55**: 54-62, 2006.

Nakano, Y., Asada, K.: Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. - Plant Cell Physiol. **22**: 867-880, 1981.

Pollard, A.J., Powell, K.D., Harper, F.A., Smith, J.A.C.: The genetic basis of metal hyperaccumulation in plants. - Crit. Rev. Plant Sci. **21**: 539-566, 2002.

Radotic, K., Ducic, T., Mutavdzic, D.: Changes in peroxidase activity and isoenzymes in spruce needles after exposure to different concentrations of cadmium. - Environ. exp. Bot. **44**: 105-113, 2000.

Wang, W.H., Kohler, B., Cao, F.Q.: Molecular and physiological aspects of urea transport in higher plants. - Plant Sci. **175**: 467-477, 2008.

Wang, C., Zhang, S.H., Wang, P.F., Hou, J., Zhang, W.J., Li, W., Lin, Z.P.: The effect of excess Zn on mineral nutrition and antioxidantive response in rapeseed seedlings. - Chemosphere **75**: 1468-1476, 2009.

Wang, S., He, X., An, R.: Responses of growth and antioxidant-metabolism to nickel toxicity in *Luffa cylindrica* seedlings. J. Anim. Plant Sci. **2**: 810-821, 2010.

Witte, C.: Urea metabolism in plants. - Plant Sci. **180**: 431-438. 2011.

Yan, R., Gao, S., Yang, W., Cao, M., Wang, S., Chen, F.: Nickel toxicity induced antioxidant enzyme and phenylalanine ammonia-lyase activities in *Jatropha curcas* L. cotyledons. - Plant Soil Environ. **54**: 294-300, 2008.

Zhang, F.Q., Wang, Y.S., Lou, Z.P., Dong, J.D.: Effect of heavy metal stress on antioxidative enzymes and lipid peroxidation in leaves and roots of two mangrove plant seedlings (*Kandelia candel* and *Bruguiera gymnorrhiza*) - Chemosphere **67**: 44-50. 2007.