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Effects of different Concentrations of NI on Morphophysiological Characteristics of Maize (zea mays l.) Seedlings

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ABSTRACT

The responses of morphological characteristics of maize (Zea mays L.) seedlings to different concentrations of Ni were studied. Freshly germinated corn seedlings were transplanted in a mixture of sand and perlite (1:1) and fed by Hoagland's solution until they reached to the four-leave stage; afterwards Ni treatments (0, 50 and 100 µM) were applied for 10 days. Roots and shoots were detected after 4 and 7 days of the completion of seedling treatment and morphological; biochemical and physiological characteristics were examined. The results showed that Ni had significant effects on the most of morphological and physiological characteristics as well as chlorophyll a (in day 7) and Chlorophyll b (in day 4). Ni affected the plant height, shoot and root dry matter and wet weight negatively. On the other hand, the activity of antioxidant enzyme, Catalase and Peroxidase, declined and the concentration of malondialdehyde, which represents lipid peroxidation, rose in response to increase in Ni concentration.

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INTRODUCTION

Environmental pollution is one of the key troublesome of human life and may cause from several pollutants including heavy metals. Accumulation in soil and uptake by plants, heavy metals enter the food chain and causes toxicity in the flora and fauna [1]. Biological activity and fertility reduce in the heavy metal contaminated soil and eventually lower yield, quality of crop products and their concentration in agricultural products increase which endangered the health of consumers [9]. Heavy metals are known as plant oxidative stress inducers [41]. Oxidative stresses are accompanied by reactive oxygen species and free radicals production [42] and causes plant cell membrane damages and ultrastructural changes in cell compartments and metabolic impairments [35,42], as well as defects in biosynthesis of essential molecules of plant cells [41].

Even though, some of heavy metals such as nickel play an important role in many metabolic functions of plants, high cellular concentration of these ions are dangerous to living cells [29]. Besides, studies have shown that nickel has a negative impact on women's fertility and embryonic development [7]. Lower concentrations of Ni causes respiratory disorders, skin allergies and abortion in humans and animals and higher concentrations, cause arthrosclerosis [19].

Nickel concentration in soil and plants increases mainly through the weathering of maternal rocs, acidic lime and use of urban sludge and compost [24]. Based on Shimada *et al*. [40] incorporating cowpea nutrient solution with one molar Ni, removes necrotic spots on the leaves. Studies on tomato and soybean suggested that Ni has a basic role in nitrogen metabolism of plants [40]. Corn is one of the main forage crops in Iran since, 480000 ha of total field crops are under corn cultivation each year [12]. Soils of Iran are mostly Serpentine type with high concentrations of Ni and other heavy metals like Cr, Co as well as Mg and Fe [18]. Though, it is particularly important to study the effects of Ni on morphophysiological characters and resistance threshold of crop plants, to improve crop growth, development, productivity and efficiency and products quality. This experiment was

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conducted to study the effects of different concentrations of Ni on some of morphological and biochemical aspects of corn (Zea mays L.) seedlings in greenhouse condition.

MATERIALS AND METHODS

The study was carried out in the research green house of faculty of agriculture of Guilan University. To investigate the effect of different concentrations of nickel on the morpho-physiological characteristics of maize (Zea mays L.) seedlings a completely randomized design with three replications established in pots of mixed soil and perlite (1:1). Fresh corn seedlings of SC704 cultivar transplanted in pots of 10 cm height and a diameter containing the mixture of sand and perlite and nourished by Hoagland solution (table 1) until their four leaves appear. Afterwards pots were treated with different concentration of Ni (0 as control, 50 and 100 µM). Ni solutions were prepared from hydrated Nickel sulphate (NiSo4.6H2O). Control pots were irrigated by distilled water. Roots and shoots were sampled, washed out, fresh weighted, length measured and dry weighted and freeze dried in liquid nitrogen for biochemical analysis on day 4 and day7. Corn tissues were dried in 71°C for 72 hours.

Chlorophyll and Carotenoid content determination

Leaf chlorophyll (chl a and chl b) and carotenoids were extracted and their contents measured based on Lichtenthaler and Wellburn method [28] and chlorometrically determined by model PG Instruments ItdT80+UV/VIS spectrophotometer. The spectrophotometer was setup in spectrum mode to scan between 600nm and 700 nm. Absorbance spectra for chlorophyll scanned in wavelengths 646.2 and 663.2 nm and 470 nm for carotenoids) and their contents calculated by use of following equations; where A646.2, A663.2 and A470 are respectively absorbance in wavelengths 646.2, 663.2 and 470 nm:

$$\text{chl a}\left(\frac{\text{mg}}{\text{ml}}\right) = [12.25 \times (\text{A663.2}) - 2.798 \times (\text{A466.2})] \quad \text{Equation (1)}$$

$$\text{chl b}\left(\frac{\text{mg}}{\text{ml}}\right) = [21.5 \times (\text{A466.2}) - 5.1 \times (\text{A663.2})] \quad \text{Equation (2)}$$

$$\text{chl T}\left(\frac{\text{mg}}{\text{ml}}\right) = \text{chl a} + \text{chl b} \quad \text{Equation (3)}$$

$$\text{Carotenoids}\left(\frac{\mu\text{g}}{\text{ml}}\right) = \frac{\left[1000 \times (\text{A470}) - 1.82 \times \left(\text{chl}\frac{\text{a}}{1000}\right) - 85.02\left(\text{chl}\frac{\text{b}}{1000}\right)\right]}{198} \quad \text{Equation (4)}$$

Malonaldehyde content determination:

Malonaldehyde as the byproduct of fatty acid peroxidation was extracted by phosphate buffer and its concentration determined according to Heath and Packer [22]. The red produced solution of thiobarbituric acid Malondialdehyde (MDA-TAB) spectrum absorbance was scanned in wavelengths 600 and 532nm and the MDA concentration calculated by use of following equation; where A532 and A600 are absorption in wavelengths 532 and 600.

$$\text{MDA}\left(\frac{\text{nmol}}{\text{gFW}}\right) = \left[\frac{(\text{A532} - \text{A600})}{155} \times 1000\right] \quad \text{Equation (5)}$$

Determination of Antioxidant enzymes activity:

Enzyme extraction from 0.5g of fresh leaves in 1ml phosphate buffer (pH=7) was conducted according to Beauchamp and Fridovich [3]. Upper extract was detected for antioxidant enzyme activity and lipid peroxidation assays.

Analysis of Catalase activity: Catalase activity was assayed based on Chance and Maehly [6]. Two phosphate buffer 25 and 50 mM were used to measure the enzyme activity. The assay was performed spectrometrically through hydrogen peroxide analyze enzyme activity. Enzymatic reaction rate was recorded as absorbance changes over time (OD / min) at wavelength of 240 nm for one minute. Dividing the timescale of absorbance changes on extinction coefficient 40 Mm/cm, enzymatic activity was calculated by use of the following equation.

$$\text{Cat. activity}\left(\frac{\mu\text{mol}}{\text{gFW} \times \text{min}}\right) = \left[\frac{\text{OD per min}}{40}\right] \quad \text{Equation (6)}$$

Analysis of Peroxidase activity: Peroxidase activity was assayed according to Cesar *et al*. [5]. Two buffers were used to measure the enzyme activity; one hydrogen peroxide 225mM buffer and the other Guaiacol 45mM buffer. Absorbance curve scanned spectrometrically in wavelength 470nm and eventually enzyme activity was calculated by use of beer & Lambert equation and extinction coefficient of catalyze of the guaiacol peroxidase in µmol/g FW.min [14]

$$\text{Peroxidase } \textit{activity} \left(\frac{\mu\text{mol}}{\text{gFW per min}}\right) = \left[\frac{\text{OD per min}}{26}.6\right] \quad \text{Equation (7)}$$

Data analysis carried out by use of SAS software version 9, statistical comparison was performed with the Tukey test at 1% and graphs were plotted by Microsoft Excel.

RESULT AND DISCUSSION

Effects on Shoot and rootlets length: Data analysis demonstrated that Nickel treatment levels had a significant effect on the length of the shoots and rootlets (table 1). Mean comparison indicated that higher concentrations of nickel had a significant effect on seedling and rootlet length, so that shoot height and root length reduced with increase in Ni (Fig 1 & 2). This results corresponded with reports of Yang *et al*. [45], Fayiga *et al*. [13], Arduini *et al*. [2], Hartley *et al*. [21] and Papazoglou *et al*. [36] on barley and rice. L'Huillier *et al*. [27] suggested that decrease in corn root length with increase in Ni may be of two main reasons; the direct effect of nickel on root meristematic cells or the consequence of decrease in root carbohydrate availability. Greger *et al*. [20] showed that the lack of root carbohydrates cause by starch breakdown restriction which ultimately reduced sucrose transmission into root. This has been also reported in other crops. On the other hand, Peralta-Videa *et al*. [39] stated that the main reason of shoot growth restriction might be root damages causes by heavy metals, chlorophyll decline and PSI disorders.

Effects on Shoot and rootlets fresh weight: Analysis of variance showed that nickel had a significant effect on shoot and rootlet fresh weight (table1). Results of mean comparison indicated that high concentrations of nickel had a significant effect on shoot and rootlet fresh weights, so that, they significantly decreased with increase in Ni concentration (Fig 2 & 3). Maximum weight in both crop parts and for both sampling times was observed in 0 concentration of Ni (control). Many researchers have also confirm that heavy metals such as nickel may generally decrease the weight of roots, stems and leaves, root and shoot dry weight and plant biomass [37,38,36,15].

Effects on Shoot and rootlets dry weight: Analysis of variance showed that nickel had a highly significant effect on shoot and rootlet dry weight (table 1). Mean comparison results indicated that nickel had a significant effect on dry weight of shoots and rootlet, in the way that with increase in Ni concentration, root and shoot dry weight decreased (Fig 5 & 6). High nickel concentration affects the root cell membrane structure and reduces root water uptake, which leads to a decrease in plant water potential through physiological processes such as transpiration, respiration are negatively impacted and eventually end up to reduction in plant growth [15]. Molas and Baran [31] reported such effects in barley.

Effects on chlorophyll: Data analysis demonstrated that nickel had a very significant effect on chlorophyll (Table 1). Mean comparison of the data also showed that chlorophyll content decline with increase in Ni concentration in the nutrient solution (Fig 7, 8 & 9). Many Studies have shown that activity of 5-aminolevulinic acid dehydratase enzyme (ALAD) in the early stages of chlorophyll biosynthesis, which involve in the conversion of ALA to porphobilinogen, is the most heavy metal sensitive steps in chlorophyll biosynthesis. Heavy metals decrease chlorophyll accumulation through hindering this enzyme [44,33]. Manios *et al* [30] suggested that heavy metals interfere in different steps of chlorophyll biosynthesis and this might be the main reasons of decrease in chlorophyll content. Some researchers believe that one way to depress chlorophyll biosynthesis is the substitution of central Mg by heavy metals which disturb light interception and causes chlorosis and eventually photosynthesis reduction [26]. Gadallah [16] also reported a decrease in chlorophyll a as a result of increase in Ni concentration.

Effects on Malondialdehyde: Analysis of variance showed that nickel has a significant effect on Malondialdehyde content (Table 1). Results mean comparison showed that malondialdehyde augment due to increase in nickel concentration (Fig 10). It has been reported by Hegedüs *et al*. [23] that Malondialdehyde accumulate in roots and leaves under heavy metal stress which are known as oxidative stress inducers [41]. Oxidative stress are accompanied by active radicals and reactive oxygen species formation [42] that cause to defects in cell membranes structures and irreversible damages ultrastructural changes in plant cellular organelles as well as metabolic impaired in biosynthesis of essential plant cell molecules [35]. Disrupt in the biosynthesis of crucial plant cell molecules is also reports in oxidative stresses [41]. There are similar reports on wheat, rice and many other crops that lipid peroxidation occur as a result of reactive oxygen accumulation [25,34,35]. Generally lipid macromolecules, especially unsaturated lipids, are more susceptible to oxidation by reactive oxygen species; hence the presence of Malondialdehyde in high amounts, as a lipid peroxidation product, reveals a severe oxidative stress [4,8].

Effects on Antioxidant enzymes: The results of data analysis showed that the concentration of nickel in the plant nutrient solution cause a significant effect on the activity of antioxidant enzymes (Table 1). Mean comparison results indicated that the activity of antioxidant enzymes (catalase and peroxidase) declined

following the increase in nickel concentration (Fig 11 &12). The free oxygen radicals induced by heavy metal stresses attack the antioxidant enzymes and inhibit their oxidative damages [17]. While catalase activity decreases, H2O2 concentration increases and the process consequently inhibits catalase and peroxidase [10]. It is suggested that, catalase activity reduction in response to heavy metals may be the result of the effects of oxidative stress on suppressing protein synthesis [43] protease of peroxisomes are also capable to cease catalase [11]. The results of this study are in agreement with Gallego *et al*., [17] on sunflower and Nouri-Azad and Kafilzadeh [32] on safflower.

Table 1: Analysis of variation of morphophysiological characteristics of corn seedlings under different duration and concentration of Ni in nutrient solution.

		Mean square											
Source of variation	df	Seedling Height (cm)	Rootlet length (cm)	Shoot fresh weight (g)	Shoot dry weight (g)	Root fresh weight (g)	Root dry weight (g)	Chl a $\frac{mg}{(ml)}$	Chl b $\frac{mg}{(ml)}$	Total Chl $\frac{mg}{(ml)}$	MDA $(nmol/gFW)$	Catalase $((\mu mol)/(gFW.min))$	Peroxidase $\frac{\mu mol}{(gFW.min)}$
4 day Ni	2	145.23**	8.77**	9.22**	0.95**	2.32**	0.385**	49.31**	8.32*	94.022**	1.06**	0.2916**	13.29**
Error	6	2.20	0.36	0.17	0.086	0.019	0.003	2.015	1.424	1.863	0.037	0.0087	0.185
CV		6.58	6.41	14.64	3.59	9.8	7.17	7.79	5.86	6.33	6.57	20.538	12.2
7 day Ni	2	71.027**	10.19**	2.051**	6.63**	4.20**	1.142**	110.773*	2.516**	21.51**	1.755**	0.652**	1.1972**
Error	6	1.62	0.305	0.182	0.087	0.011	0.005	0.304	0.0482	0.534	0.2119	0.0145	1.0199
CV		4.55	5.85	6.21	12.14	5.17	5.43	9.30	12.81	9.14	13.51	12.68	8.07

- Chl a; Chlorophyll a, Chl b; Chlorophyll b, MDA; Malondialdehyde

- ns, * and ** respectively represent non-significant, significant in 5% probability and significant in 1% probability

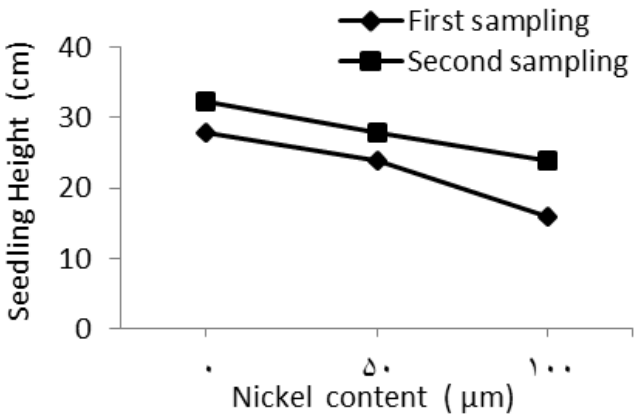

Fig. 1: Changes in plant height at different concentrations of nickel in the two-stage sampling.

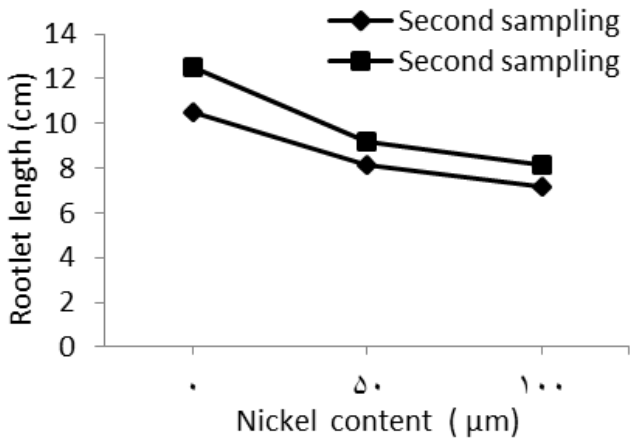

Fig. 2: Changes in rootlet length at different concentrations of nickel in the two-stage sampling.

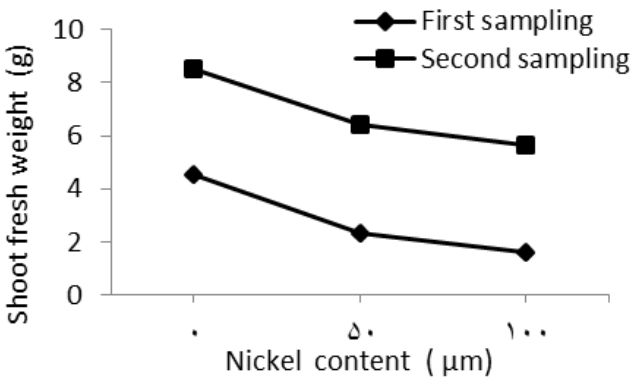

Fig. 3: Changes in fresh shoot weight at different concentrations of nickel in the two-stage sampling.

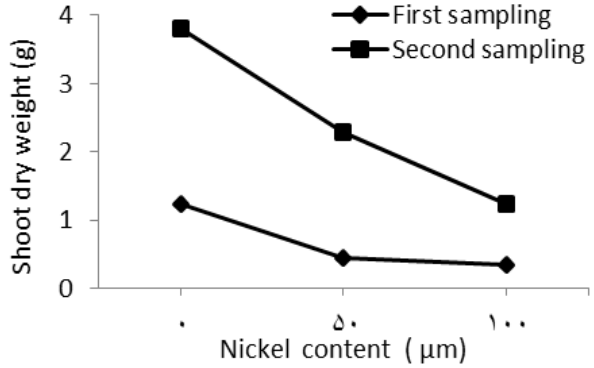

Fig. 4: Changes in dry shoot weight at different concentrations of nickel in the two-stage sampling.

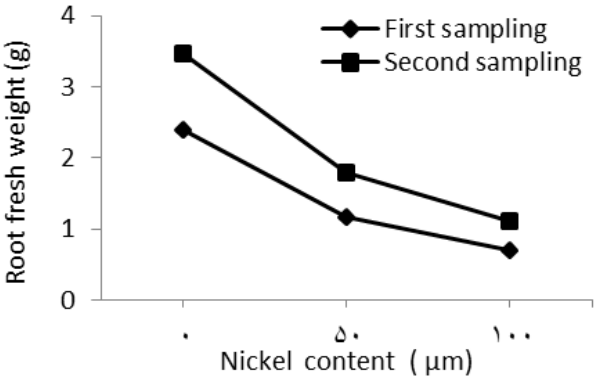

Fig. 5: Changes in fresh rootlet weight at different concentrations of nickel in the two-stage sampling.

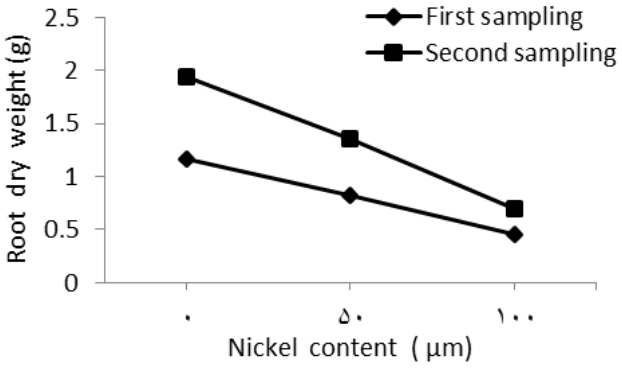

Fig. 6: Changes in dry rootlet weight at different concentrations of nickel in the two-stage sampling

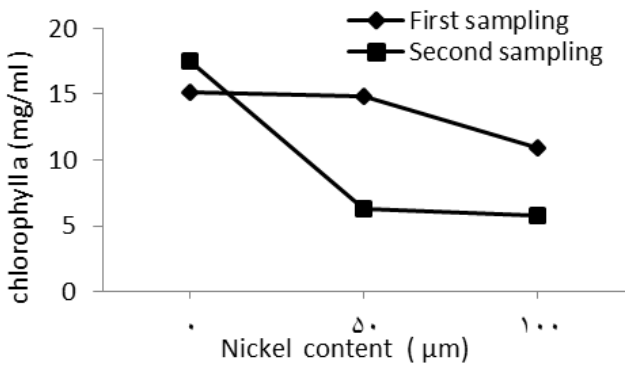

Fig. 7: Changes in Chlorophyll a content at different concentrations of nickel in the two-stage sampling.

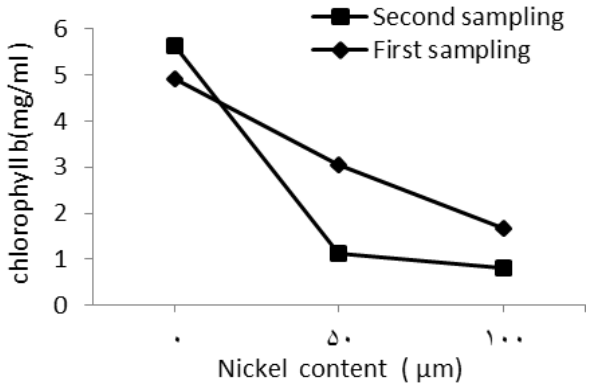

Fig. 8: Changes in Chlorophyll b content at different concentrations of nickel in the two-stage sampling.

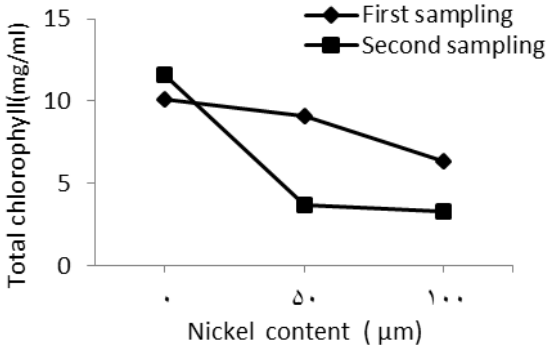

Fig. 9: Changes in total Chlorophyll content at different concentrations of nickel in the two-stage sampling.

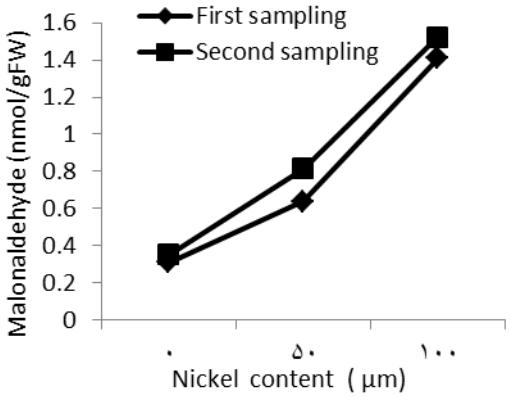

Fig. 10: Changes in MDA content at different concentrations of nickel in the two-stage sampling.

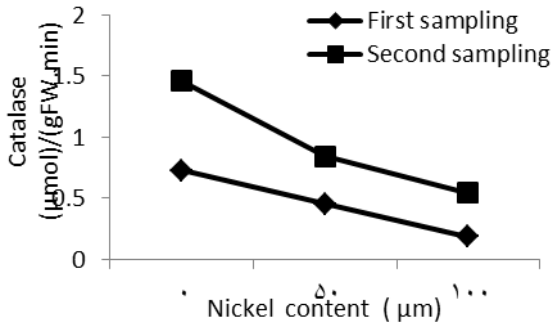

Fig. 11: Changes in Catalase activity at different concentrations of nickel in the two-stage sampling.

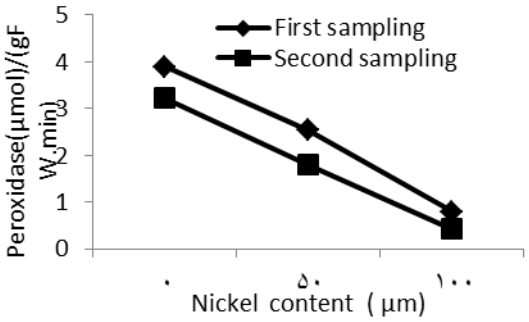

Fig. 12: Changes in Peroxidase activity at different concentrations of nickel in the two-stage sampling.

Conclusion:

The evidence presented in this study show that the concentration of nickel significantly affects the morphophysiological characteristics of crop roots and shoots. Reduction in fresh and dry weight of shoots and roots, decrease in the amount of photosynthetic pigments and slump of catalase and peroxidase activity suggest that corn seedlings were exposed to the toxic effects of nickel and free oxygen radicals and cause oxidative damages and reduced growth.

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