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Research Article

Effect of Excess Nickle on Induction of Oxidative Stress in *Zea mays* L. Plants Grown in Solution Culture.

Mishra S[1] , *Panjwani D[2], Mishra B[1], Sharma P.N[3]

[1]*Shree Krishna Institute of Pharmacy Shankhalpur, Bhechraji, Mehasana,Gujarat,India*
[2]*Faculty of Pharmacy, BBDNIT&M, Sector I, Faizabad Road, Lucknow, U.P., India*
[3]*Department of Botany Lucknow University,Lucknow,U.P,India*

ABSTRACT

Zea mays (cv. 777) seedlings precultured in hydroponic culture were treated with Excess (100 µ M) supply of Ni for 16 days with complete basal nutrient solution. The studies on fresh and dry matter yield, plant growth ,tissue concentration of chlorophylls,carotenoids,extent of lipid per oxidation and soluble proteins was done. Several metabolic parameters representative of oxidative damage and antioxidant activity in plants were regularly studied after the metal treatment.. Super oxide dismutase (SOD) activity ,catalase ,ascorbate peroxidase (APX) ,H_2O_2 and lipid per oxidation was measured. The results are discussed with regard to nickel induced oxidative stress. These results suggest that Ni reduces maize growth by a reduction in root mitotic activity, probably because of direct action on the meristem.Excess supply of nickel decreased the activity of catalase activity and other antioxidant enzymes : SOD and APX.The concentration of H_2O_2 and lipid per oxidation was increased.

Key words: Antioxidant activity , lipid per oxidation, nickel stress ,oxidative damage ,Zea mays.

INTRODUCTION

Heavy metals such as Cd, Cu , Ni, Pb , and Zn, are major environmental pollutants. Known symptoms of their toxic effects on plants include reduced growth and production. Among heavy metals, only Cu, Ni and Zn phytotoxicities occur frequently[1]. Most agricultural soils contain an average of 25 mg/kg soil dry wt. of Ni[2]. Cultivated plants may rapidly exhibit toxicity symptoms. They generally contain less than 5 pg of Ni/g dry matter[3], and symptoms of phytotoxicity often become apparent at Ni concentrations as low as 25-30 µg g-ls [4]. Growth inhibition, chlorosis and reduction of tissue-water content are commonly observed in plants exposed to phytotoxics are commonly observed in plants exposed to phototoxic amounts of Ni[5, 6]. The relationship between heavy metal toxicity and oxidative reactions in plant cells has been studied [5, 7, 8].

Such studies have reported that toxic levels of Cu, Ni and Zn result in a stimulation of lipid peroxidation leading to a loss of membrane function. Membrane destabilization appears to be due to an enhanced productionof highly toxic oxygen species such as superoxide anions, hydrogen peroxide and hydroxyl radicals.In the absence of any adopted protective mechanism, cell structure and function will be severely damaged [9,10]. Plants have an enzymatic protective mechanism which includes peroxidases, catalase (CAT), superoxide dismutase (SOD) and the enzymes involved in the synthesis and regeneration of low molecular mass antioxidants. SOD transforms superoxide radicals (O_2) into H_2O, a less destructive oxygen species, hence decreasing the risk of hydroxyl radical (OH) formation[11]. CAT and a wide group of peroxidases are involved in the destruction of H_2O_2.In leaf cells, H_2O_2 is destroyed in chloroplasts through the ascorbate dependent H_2O_2 scavenging pathway which involves successive oxidations and re-reductions of ascorbate, glutathione and NADPH. The purpose of the present study is to contribute to a better understanding of the biochemical responses of plants subjected to heavy metal stress. Little information is available in literature on the relationship between Ni-toxicity, oxygen free radicals formation and related membrane damage. We investigated the sequence of metabolic reactions representative of Ni-induced oxidative damage in maize shoots.

Corresponding author: Dharamveer Panjwani
Lecturer, Faculty of Pharmacy, Babu Banarasi Das National Institute of Technology and Management, Dr. Akhilesh Das Nagar, Faizabad Road, Lucknow, U.P., India
Email id- divyansh0522@gmail.com

MATERIAL AND METHODS

Maize (*Zea mays* L.) cv. 777 was taken for experimental work. The seeds were washed thoroughly with deionized water to avoid surface contamination. Seeds were sown in purified sand contained in polythene trays provided with a central drainage hole. Thirteen days old seedlings were transplanted to plastic bucket filled with nutrient solution containing 2 mM KNO_3, 2.5 mM Ca $(NO_3)_2$, 1 mM KH_2PO4, I mM $MgSO_4$, 30 uM HBO_4, 50 pM Fe-K-EDTA, 10 pM $MnSO_4$,I uM $ZnSO_4$, and I pM $CuSO_4$. Four buckets were maintained for this experiment, 2 for control plants (Grown with complete nutrient supply) and 2 pots for Ni toxicity. Two plants were placed in one

Table 3 : Dry matter yield of maize

Treatment	*Dry matter Yield*			
	Shoot	**Root**	**Total yield**	**Shoot /Root**
Control	21.03 ±1.2	2.28 ±0.62	23.31	10.15 ±3.31
Ni	8.03 ±1.14	2.50 ±0.12	10.53	3.25 ±0.61

hole and thus ten plants per bucket were maintained. Buckets were filled with nutrient solution of desired composition and volume was made to 3 lit by deionized water. Water lost during transpiration was replaced by deionised water everyday .Nutrient solution was completely changed every 3rd day. The nutrient solution in the bucket was aerated using aquarium pumps. Plants were grown in a controlled-environment chamber (16 h light/8 h dark) ,a day/night temperature cycle of 26/22^{0}C and 65 (+ 5) % relative humidity. The hydroponic cultures were continuously aerated. Excess (100 µ M) supply of Ni was given for 16 days with complete basal nutrient solution.

Table 1: Effect of excess supply of Nickel on certain yield parameters of maize (Zea mays L.) var. 777 plants grown in solution culture.

Yield Parameters	Time	Control	Nickel
Plant	16 days	28.25±1.65	31.0±3.0
	30 days	37.43±2.04	37.0±2.0
	44 days	45.70±2.81	41.5±3.5
Lamina length	16 days	20.23±2.18	16.30±2.7
	30 days	32.85±2.52	29.5+3.3
	44 days	39.30±2.23	36.25±.75
Lamina width	16 days	1.53±0.25	1.75±0.25
	30 days	2.38±0.23	2.9±0.1
	44 days	2.78±0.18	3.2±0.10

Table 2 : Fresh matter yield of maize (Zea mays L.) cv. 777 plants grown in solution culture, supplied with excess Ni, (Data at 31 days of excess Ni treatment)

Treatment	Fresh matter yield (g plant $^{-1}$)			
	Shoot	**Root**	**Total yield**	**Shoot /Root**
Control	128.35 ±1.04	20.47 ±4.38	148.82	6.59 ±1.46
Ni	55.39 ±5.47	32.35 ±0.93	87.74	1.71 ±0.12

Fresh and Dry Matter Yield

After taking fresh weights of the plant samples, they were washed with tap water, followed by deionized water and glass distilled water to remove surface contamination before drying for the estimation of dry matter yield. Plants were separated into 2 parts, root and shoot. Fresh materials of different plant parts were kept for drying in an oven at 70^{0}C for 48 hours. The oven-dried materials was transferred to a desicator and then cooled to room temperature and weighed. The fresh and dry matter yield of plants has been expressed on per plant basis.

Enzyme Activities

Preparation of Enzyme extract

Activities of enzymes were assayed in crude leaf extracts made in 0.1 M potassium phosphate buffer pH 7. Finally, chopped leaf material was ground in cold potassium phosphate buffer in the proportion of 1g of tissues to 10 ml buffer with a pinch of acid washed silica sand in a clean ice chilled glass pestle and mortar kept in an ice bath. Before chopping, the mid rib of the leaves was removed. The temperature during the extraction procedure was maintained below 40^{0}C. The crude extracts were strained through 2-fold muslin cloth and stored at 40^{0}C in a refrigerator till the time of enzyme assay. The enzyme activity was expressed both on fresh weight and on mg protein basis termed as the specific activity.

Catalase

Catalase activity was assayed by the method of [12] .The reaction mixture consisted of 0.5 mM H_2O_2 and 0.10 mM potassium phosphate buffer pH 7, standardized against 0.1 N KMnO4 and diluted enzyme extract. The decomposition of H_2O_2 was followed by measuring the decrease in absorbance at 240 nm. Enzyme activity has been expressed as moles H_2O_2 decomposed per 5 minutes per 100 mg fresh weight and per mg protein.

Table 4: Effects of excess nickel supply on concentration of chlorophyll and carotenoids in the leaf tissue of maize (Zea mays L.) cv. 777, plants grown in solution culture.

Pigments	Time	Control	Ni
Chlorophyll	7 days	2.168 ±0.040	1.857 ±0.106
	14 days	2.657 ±0.024	2.282 ±0.003
Chlorophyll B	7 days	0.8 ±0.034	0.698 ±0.051
	14 days	1.179 ±0.011	0.995 ±0.001
Carotenoids	7 days	0.409 ±0.001	0.372 ±0.030
	14 days	0.380 ±0.007	0.336 ±0.000
Total Chl	7 days	2.964 ±0.073	2.56 ±0.157
	14 days	3.836 ±0.035	3.277 ±0.002
Chl a/b	7 days	2.712 ±0.064	2.664 ±0.042
	14 days	2.254 ±0.001	20293 ±0.005
Care/Chl.	7 days	0.138 ±0.003	0.145 ±0.003
	14 days	0.099 ±0.001	0.102 ±0.000

Peroxidase

Peroxidase was assayed by the modified method of Luck [13]. The reaction mixture consisted of 5 ml 0.1 M phosphate buffer pH 6, 1 ml 0.01% H_2O_2 (w/v) and 1 ml 0.5% (w/v) p-phenylenediamine and diluted enzyme extract. Reaction mixture was kept in refrigerator for 30 minutes and then centrifuged at 500 rpm for 10 min. The optical density of the supernatant was measured on a Chemito-2000 UV-Visible spectrophotometer at 85 nm.

Table 5: Effects of excess Ni supply on the activity of catalase, peroxidase, ascrobate peroxidase and superoxide dismutase in the leaf tissue of maize (Zea mays L.) cv. 777 plants grown in solution culture.

Enzymes	**Time**	**Control**		**Nickle**	
Catalase Dark (mmol H_2O_2 decomposed)	7 days	240±10	160±10	191.63±5.0	157.67±8.7
	14 days	180±0	130±10	156.91±1.33	114.11±10.39
Peroxidase (unit*)	7 days	40.60±3.80	41.40±0.60	32.41±2.62	40.64±0.85
	14 days	53.80±0.80	52.30±1.70	46.78±0.08	45.87±2.01
APX (mmol of ascrobate) oxidized min^{-1}	7 days	0.40±0.02	1.68±0.26	0.32±0.02	1.65±0.28
	14	0.53±0.0	0.54±0.03	0.46±0.02	0.47±0.04
SOD (Unit** min^{-1}	7 days	3.57±0.06	5.17±0.21	2.86±0.01	5.09±0.23
	14 days	5.49±0.20	5.51±0.27	4.79±0.22	4.82±0.17

Values given in the parentheses denote percent increase (+) or decrease (–) over control. Data are mean ± SE of two replicates in superscript indicate significant differences in mean value compare to corresponding control at $P<0.05$.
Unit* O.D. of 0.01 per minute.
Unit** Enzyme activity cousing 50% sub-pression in reduction of NBT to formazam.

The result has been expressed as unties per 100 mg fresh material and per mg protein. An enzyme unit is defined as ΔO.D. of 0.01 per minute of reaction time.

Superoxide dismutase

The activity of SOD was assayed by measuring its ability to inhibit the photochemical reaction of nitro-blue tetrazolium (NBT) (SD Fine or Loba Chemical, India) adopting the modified method of Beauchamp and Fridovich [14]. The 3.0 ml reaction mixture contained 50 mM potassium phosphate buffer (pH 7.8), 2.8 □M TEMED (Sigma), 1.17 □M riboflavin and 63 □M NBT and suitable enzyme extract. The absorbance of solution was measured at 560 nm against identical non-illuminated blanks. From the extract of control plant log A 560 was plotted as a function of fresh matter equivalent of enzyme extract used in the reaction mixture. From the resultant graph fresh matter equivalents of enzyme extract corresponding to 50% inhibition of the reaction was read and considered as one enzyme unit. The activity has been expressed as units per mg protein.

Ascorbate Peroxidase

The activity of ascorbate peroxidase (APX) was assayed by the method of Nakano and Asada [15] from the decrease in absorbance at 290 nm (an absorbance coefficient of 208 mM^{-1} cm^{-1}) as ascorbate was oxidized. The reaction mixture contained 50 mM K-phosphate buffer pH 7, 0.5 mM of ascorbate, 0.1 mM H_2O_2 and 0.1 mM EDTA in total volume of 4 ml. The reaction was started by adding the enzyme extract or H_2O_2 and absorbance decrease was recorded at each 30 second interval, in a Chemito-2000 UV-Visible spectrophotometer.

Lipid peroxidation

The level of peroxidation in the leaf tissue was measured in terms of malondialdehyde (MDA, a product of lipid peroxidation) content determined by thiobarbituric acid (TBA) reaction according to the method of Heath and Packer [16]. 250 mg of leaf tissue was ground in 25 ml of 0.1% TCA. 2 ml of extract was mixed with 2 ml of 0.5% TBA (prepared in 20/ TCA) and the reaction mixture was heated for 30 min at 95°C. Samples were cooled immediately in ice bath and then centrifuged to obtain clear supernatant. The colour intensity of supernatant was measured spectrophotometrically at 532 nm and 600 nm in a Chemito-2000 UV Visible spectrophotometer using 1.0 cm cell with blank setting 0.00. Corrections were made for the colour development between A_{600} and A_{532} was then used to calculate n mol of MDA per 100 mg of fresh weight applying the extinction co-efficient of 155 mM-1.

Soluble proteins

For expressing the enzyme activities on protein basis, soluble proteins were precipitated from the same leaf tissue extract in which enzyme activity was measured. The leaf tissue extract was treated with equal volume of 20% chilled TCA and left over night in refrigerator. The precipitate so obtained was separated out by centrifugation. It was washed repeatedly with acetone to remove pigments. The precipitate was dissolved in 0.1 N NaOH and soluble protein content was measured by the method of Lowry *et al*., [17]. The colour intensity was measured on a Chemito-2000 UV-Visible spectrophotometer at 640 nm. Results have been expressed on percent fresh weight basis. The specific activity of each enzyme has been expressed on per mg protein basis.

Chlorophylls and Carotenoids

Concentration of chlorophylls and carotenoids were determined in the fresh leaf lamina by the method of Lichtenthaler [18]. Finally chopped fresh leaf lamina was homogenized in 85% acetone, using a glass pestle and mortar with a pinch of $CaCO_3$. The homogenate was filtered through Whatman no. 1 filter paper in a Buchner funnel. The residue was washed with small fraction of acetone till it became colourless. The extracts were finally pooled and made to a volume with 85% acetone. The colour intensity of the extract was measured in a Chemito-2000 UV-Visible spectrophotometer at 663 nm and 645 nm for chlorophyll a and chlorophyll b,respectively. Chlorophyll concentration was then calculated using the numerical expressions with specific absorption coefficients.

Chl a = [12.7(O.D.663.2)- 2.69 (O.D.646.8)]×V/ 1000 wt. in g

Chl b = [22.9 (O.D.646.8)- 4.68 (O.D.663.2)] × V/1000 wt. in g
V = volume of extract in ml
Wt. = fresh wt. of the plant material used
O.D. = optical density at the wave length indicated
Total carotenoid content (Xanthophylls+ β-carotene) was determined in leaf extract by measuring the absorbance at 470 nm using the following equation. Results have been expressed on mg/g fresh tissue weight basis.
Carotenoid = 1000 A 470- 1.82 chl a – 85.02 chl b /198 mg/l

Table 6 : Effects of excess Ni supply on concentration of H_2O_2 and lipid peroxidation in leaf tissue of maize (Zea mays L.) cv. 777 grown in solution culture.

H2O2	Time	Control	Ni
(Per 100mg fresh wt)	7 days	0.80±0.08	1.26±0.31
	14 days	0.93±0.09	2.07±0.03
Lipid peroxidation (%fresh wt.)	7 days	1.88±0.08	2.19±0.0
	14 days	0.74±0.06	1.48±0.01

Statistical analysis
Data are mean + SEM of two replicates superscript indicate significant differences in mean value in each data set ($P<0.05$) by one way ANOVA (Bonferroni t-test) while data value without superscript are not statistically different.

Table7 : Effects of excess of Ni supply on concentration of soluble proteins in leaf tissue of maize (Zea mays L.) cv. 777 grown in solution culture.

	Time	Control	Ni
Soluble Protein (% fresh wt.)	7 days	1.25+0.01	1.02±0.01
	14 days	1.15+0.01	1.14±0.02

Results
Visible effects of Ni toxicity
The visual symptoms of Ni toxicity were initiated in young leaves after 5 days of treatment. The younger expanding leaves exhibited faint interveinal chlorosis. Later on shrinkage of apical margins of leaf lamina was observed in these leaves. The region showing shrinkage turned brown. In relatively mature leaves, off white lesions appeared on leaf lamina. Later on black dots developed in these lesions.Hastened senescence in the older leaves of plants receiving excess supply of Ni was observed. The older leaves developed brown spots and necrosis started from tip regions and extended along the lamina from apex to base, finally leaves turned brownish and were shed off.
The roots of maize plants were also affected by excess supply of Ni. After 5 days of initiating treatment, roots of Ni treated plants were without rootlets and turned brownish. After prolonged treatment, rootlets developed in Ni treated plants were thinner than that of controls. Roots became slimy in these plants.
Plant growth
Growths of Ni treated plants were slower than the growth of controlled plants. The observed increase in height of Ni treated plants was relatively lesser than that of controls. However the rate of expansion of leaf lamina was faster in Ni treated plants.
Fresh and Dry Matter Yield
Fresh matter yield, of Ni treated plants was decreased (61.08%). Fresh matter yield of shoots in Ni treated plants was decreased to a greater extent (56.85%). However, the fresh matter yield of roots did not decrease. The shoot/ratio in the fresh matter yield was significantly decreased (74.05%).
Moreover, similar to fresh matter yield, substantial reduction (50.33%) of dry matter yield of Ni treated plants were observed. Dry matter of roots of Ni treated plants, though increased mildly (9.57%). Shoot/ratio of dry matter decreased (68.01%) implies; this greater retardation in the growth of shoots.
Chloroplastic pigments
Excess supply of Ni decreased the concentration of chlorophylls and carotenoids. The changes observed at 7 days after excess Ni supply, were not significant. However, decreases in the concentration of chlorophylls (14.5% and carotenoids (11.6%) became significant at 14 days
The significant decrease in the concentration of both chl a and chl b were observed in plants receiving excess supply of Ni at 14 days of treatment. Chl a/b and carotenoid/chlorophyll were not affected significantly. However a mild decrease in chl a/b was observed at 7 days treatment and increase in caro/chl ratio was observed at 14 days of treatment.
Anti-oxidative Enzymes
The activities of anti oxidative enzymes were found to be altered by excess supply of Ni. The activity of CAT was decreased significantly both on fresh weight and soluble protein basis.
The activity of peroxidase was increased significantly (25.3%) after 7 days of excess Ni supply on soluble protein basis. On fresh weight basis the activity of peroxidase was not altered significantly . Activity of ascorbate peroxidase also increased at 7 days after excess Ni treatment both on fresh wt basis (324.7%) and protein basis (415.6%). The activity of APX did not alter after 14 days of excess Ni supply. Moreover, the activity of SOD increased significantly after 7 days of excess Ni supply which on advancement of duration of excess of Ni supply became non significant both on fresh weight and soluble protein basis.
The isoenzyme of CAT did not show much variation between Ni and control treated plants while isoenzymes of POD, APX and SOD increased their intensity in Ni treated plants .
Hydrogen peroxide and Lipid Peroxidation
The concentration of both hydrogen peroxide and malondialdehyde equivalents of thiobarbituric acid reactive substance (TBARS), an index of lipid peroxidation, were increased significantly after 14 days of excess Ni treatment. The data obtained after 7 days of excess Ni supply were not significant statistically.
Soluble proteins
The concentrations of soluble proteins in the young leaves of plants receiving excess supply of Ni were not altered significantly. A mild decrease (19.06%) in soluble protein after 7 days of excess Ni supply was estimated in the young leaves.

Discussion

The observed visible effects like the off- white lesions, chlorosis and necrosis of leaves of Ni excess maize plants were in consonance with earlier observations [19, 21]. The suppression in plant growth and reduced biomass production of plants subjected to excess of Ni may be attributed to decreased photosynthetic activity due to decreased Hill activity [22], inhibition of enzymes of Calvin cycle and/or loss of photosynthetic pigments. The decreased shoot/root ratio indicates that shoots were more prominently affected than roots. Moreover, suppression in growth of Ni excess plants may also have resulted from the inhibition in cell division and cellular expansion [23, 25]. Similar suppression in growth of excess Ni supplied plants has been reported earlier by several workers [26].

Decreased concentrations of chloroplastic pigments in the plants supplied excess Ni was in accordance of earlier observations of [21, 27, 28]. Decreased concentrations of chloroplastic pigments in excess Ni plants may be attributed to reduced synthesis and/or higher rate of degradation of chlorophyll and carotenoids as a consequence of enhanced photooxidative challenge resulting in chlorosis and necrosis of leaves. The increased ratio of carotenoid/chlorophyll in excess Ni plants shows that chlorophyll is more sensitive to the Ni stress. Increased carotenoid/chlorophyll ratio might be an adaptive response to increased ROS build-up in Ni stressed plants as carotenoids are known to be potent quenchers of ROS particularly of singlet oxygen.

Activity of catalase was decreased in Ni excess plants particularly when assayed in the test tubes, though the intensity of isoform of catalase in Ni excess plants was near similar to that of control. The observed decrease in CAT is in accordance with some earlier observations [29, 30]. Presence of Ni in the vicinity of CAT protein appears to inhibit activity [31], and dissociation of Ni ion from enzyme proteins by electrophoresis might restore CAT activity on gel. Reduction in CAT activity might be due to enzyme inactivation either by metal ion interaction for metal-organic ligand formation [31], and/or by pre-transitional expression and post-transitional modification/disruption of enzyme protein.

Activity of peroxidase (POD), another H_2O_2 scavenging enzyme, increased in Ni excess plants. This observation is in accordance with earlier findings of [32]. Isoforms of peroxidase showed greater intensity of bands. The increase of POD activity has been shown to be strongly correlated with Ni ion concentration [33, 34], and also related with biomass production. Initially activity of APX was increased but after a prolonged Ni-excess it was not altered. Though observed increase in APX in Ni-excess maize plants is not in consonance with the earlier observations of Baccouch et al.[35]. Enhanced generation of H_2O_2 might have increased the expression and activity of APX, a key enzyme for H_2O_2 detoxification in both chloroplasts and cytosol[36]. An increase in APX activity by excess of Ni, suggesting its role in detoxification of H_2O_2 has also been reported earlier by Schickler and Caspi[37]. Toxic levels of other metals are also reported to increase APX activity viz. Cu[38, 40]; Zn[38, 41]; Mn[42]; Cd[43]; Fe[44] and Co[45]. Activity of SOD, enzyme responsible for dismutation of (O_2^-) radical into H_2O_2 , increased significantly in Ni treated plants but after a prolonged supply the effect became non-significant.[30] obtained similar results in Ni treated pigeon pea plants. Though initial increase in the activity of SOD is in aggrement with the observation made by [35] but later on they find decreased SOD activity. Moreover, Bomminathan and Doran et al.[29] also reported decrease of SOD activity in *Alyssum bertolonii* roots. Isoforms of SOD in plants with excess Ni supply showed greater intensity, suggesting enhanced SOD activity. Increased SOD activity has been suggested resulting from higher O_2^- accumulation in Ni excess plants. H_2O_2 , a product of SOD activity is a signal molecule and is known to induce a number of genes and proteins involved in stress defences. The concentration of H_2O_2 increased significantly in maize plants supplied with excess of Ni. Similar increase in concentration of H_2O_2 was also reported by Bomminathan and Doran et al.[29]. Increased accumulation of H_2O_2 in Ni excess plants might be due to reduced CAT activity, although it is not considered to be highly specific due to its low affinity for H_2O_2, but it detoxifies H_2O_2 at diffusion controlled rate[46]. Enhanced SOD activity may be another cause of greater content of H_2O_2 in the leaf tissue of Ni excess plants.

Excess supply of Ni induced lipid peroxidation was in accordance with those of Baccouch et al.[35]. Lipid peroxidation is known to be initiated by a Fe containing enzyme-lipoxygenase (LOX) [47]. As excess supply of Ni is known to increase tissue Fe level, this may possibly result in increased LOX in such plants and therefore increased lipid peroxidation in leaf tissue. Besides this, increased level of H_2O_2 also generates $OH^{\cdot}$ radicals in presence of transition metals (Fe^{2+}/ Cu^{2+}) that can further accentuate extent of lipid peroxidation. Therefore, lipid peroxidation is closely related to higher-level ROS under stress conditions[44]Lipid peroxidation induced K^+ efflux through cell membranes and reduced water status results in dehydration. This might be one of the reasons of retarded growth and reduced biomass production. The concentration of soluble proteins in these leaf tissues decreased. Decrease in protein concentration might be due to damage to proteins by different ROS.

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REFERENCES

1 Foy CD, Chaney RL, and White MC. The physiology of metal toxicity in plants. Annu. Rev. Plant Physiol. 1978; 29:511-566.

2 Holmgren GGS, Meyer MW, Chaney RL, Daniels RB. Cadmium, lead, zinc, copper and nickel in agricultural soils of the United States of America. J. Environ.Qual. 1993;22: 335-348.

3 Vanselow AP. Nickel. In Diagnostic criteria for plantsand soils. *Edited by* H.D. Chapman. University of California, Riverside, Calif. 1966; 302-309.

4 Khalid BY, Tinsley J.. Some effects of nickel toxicity on rye grass. Plant Soil 1980 ; 55: 139-144.

5 Pandolfini T, Gabbrielli R, Comparini C.Nickel toxicity and peroxidase activity in seedlings of Triticum aestivum L., Plant Cell Environ. 1992; 15: 7 19-725.

6 Osmond CB, Ziegler H. Encyclopedia of plant physiology, physiological plant ecology III, 12C.Springer, Berlin, 1983; 245-300.

7 Weckx JEJ, Clijsters HMM. Oxidative damage and defense mechanisms in primary leaves of Pkaseolus vulgaris as a result of

root assimilation of toxic amounts of copper. Physiol. Plant. 1996; 96: 506-512.
8 Weckx JEJ, Clijsters HMM. Zn phyto toxicity induces oxidative stress in primary leaves of Pkaseolus vulgaris.Plant Physiol. Biochem. I 997; 35: 405-410.
9 Elstner EF. Oxygen activation and oxygen toxicity.Annu. Rev. Plant Physiol. 1982; 33: 73-96.
10 Salin ML. Toxic oxygen species and protective systems of the chloroplasts. Physiol. Plant. 1988; 72: 68l-689.
11 Foyer CH, Descourvibres P, Kunert K. Protection against oxygen radicals: an important defense mechanism studied in transgenic plants.Plant Cell Environ. 1994; 17: 507-523.
12 Euler H, Josephson K. Uber Katalase1. Leibigs Ann. 1927; 452**:** 158-187.
13 Luck M 1963. Peroxidase. In: Methods of Enzymic Analysis (H. V. Bergmeyer, Ed.) , pp. 895-897. Academic Press Inc. New York.
14 Beachamp C., Fridovich I. Superoxide dismutase: improved assay and an assay applicable to PAGE. Anal. Biochem.1971; 44: 276-287.
15 NakanoY. Hydrogen peroxide is scavenged by ascorbate specific peroxidase in spinach chloroplasts. Plant and Cell Physiology**.** 1981; 22: 867-880.
16 Lowry OH., Sebrough RQ, Farr AL.,Randall R L. Protein measurement with the folin phenol reagent. J Biol. chem**.** 1951; 193 : 265-275.
17 Lichtenthaler H K1987). Chorophylls and carotenoids : Pigments of photosynthetic biomembranes. In: *Methods* in *Enzymology, Plant Cel/Membranes.* (Lester Packer and Roland Douce. Eds.) Vol. 148, pp. 350-382.Academic Press Inc. New York.
18 Bisht SS, Sharma C P, Kumar A. Plant response to excess concentration of heavy metals. Geophytology. 1976; 5: 296-307.
19 Haselhoff E. Versuche Vber die Schedlick Wirkunj Von nickel haltigam. Wasser anf lP.flanyanLandw-Johrb. 1893; 22: 1962-1868.
20 Hewitt EJ. Relation of manganese and some other metals to the iron status of plants. Flature (London). 1948; 101: 489 - 490.
21 Austenfield FA**.** Effect of nickel, cobalt and chromium on net photosynthesis of primary and secondary leaves of Phaseolus vulgaris cv. Saxaphotosynthetia. 1979;13: 434- 438.
22 Gabbrielli R, Pandolfiini T, Vergnano O, Palandari MR. Comparison of two serpentine species with different nickel tolerance strategies. Plant Science and Soil. 1990; 122: 271-277.
23 L'Hullier L , D' Auzac J., Durand M.,Michoud FerN. . Nickel effects on two maize (Zea mays) cultivars; growth, structure, Ni concentration' ad location. Can. J. Bot. 1996; 74: 547-1554.
24 Robertosnt A I,Meakin M E R. The effect of nickel on cell division and growth of Brachystegia speciformis seedlings. Kirkica. 1980; 12: 115-125.
25 Bisht S S, Sharma C P, Kumar A. Plant response to excess concentration of heavy metals. Geophytology**.** 1976; 5: 296-307.
26 NichoIas D J D. Some effects of metal excess on crop plants grown in soil culture. III : Effect of cobalt, nickel and zinc on growth, metal and chlorophyll content of tomato. Long Aston Res. Sta. Annu. Report**.** 1950; 87-102.
27 Pukett K J. The effect of heavy metals on some aspects of lichen physiology. Can. J. Bot**.** 1976; 54: 2695-2703.
28 Boominathan R M, Dorsan. P. Ni-induced oxidative stress in roots of "-the Nt hyperaccumulator, Alyssum bertolonll. New Phytologist**.** 2002; 156: 205-215.
29 Rao K V M, Sresty T S. Antioxidative parameters in the seedlings of pigeonpea Cajanus cajan (L.) in response to Zn and Ni stresses. Plant Science**.** 2000; 157: 113-128.
30 Hewitt EJ. Essential nutrient elements: Requirements and interaction in plants. Plant Physiology Vol. III. Academic press, New York 1963, 137-360
31 Gabbrielli R, Pandolfiini T, Vergnano O. Peroxidase involement in tolerance mechanisms. G. Bot. Ita!. 1987; 21 : 200-201.
32 Mukherjii S ,Das B C. Characterisation of copper toxicity inlettuce seedlings. PlantPhysiol. 1972; 27: 126-129.
33 Van Assche F, Cardinalls C, Clijsters H. Induction of enzyme capacity in plants as a result of heavy metal toxicity: dose - response relations in Phaseolus Vulgaris L. treated with zinc and cadmium. Environ. Pollut. 1988;52: 103-113.
34 Baccouch S, Chaoui A, Ferjani E. Nickel - induced oxidative damage and antioxidant responses in Zea mays shoots. Plant Physiol. Biochem. 1998; 36: 689-694.
35 Halliwell B, Gutteridge J N C**.** Iron and free radical reactions: two aspects of antioxidant protection. Trends Biochemical Science. 1986; 11: 375.
36 Schickler H. and Caspi H.. Response of antioxidative enzymes to nickel and cadmium stress in hyperaccumulator plants of the genus Alyssum**.** Physiol. Plant1999; 105: 39-44.
37 Weekx J E J, Clijster, H M M. Oxidative damage and defense mechanisms in primary leaves of Phaseolus vulgaris as result of root assimilation of toxic amounts of copper. Physiologia Plantarum**.** 1996; 96: 506-512.
38 Navari- Izzo F, Avatacci F, Pinzino C, Vecchia F D, Sgherri C L M. Thylakoid bound and stromal antioxidative enzymes in wheat treated with copper. Plant Physiol**.** 1998; 104: 630-638.
39 Cuypers A, Vangronsveld J, Clijsters H. The chemical behaviour of heavy metal play a prominant role in induction of oxidative stress. Free Rad. Res. 1999; 31: 39-43.
40 Prasad K V S K, Saradhi P P , Sharmila P . Concerted action of anti oxidant enzymes and curtailed growth under zinc toxicity in Brassica juncea. Environ. Exp. Bot**.** 1999; 42: 1-10.
41 Gonzalez A, Steffen K L, Lyncj T P. Light and excess manganese: implcations for oxidative stress in common bean plant Physiol.1998; 118: 493-504.
42 Piquers A, Olmos E, Martinex-Solano J R, Hellin E.. Cd-induced oxidative burst in tobacco BY2 cells: time-.course. subcellular location and antioxidant response. Free Radical Research. 1999; 31 : 33-38.
43 Kampfenkel K, Van Montagu M, Inze D.. Effects of iron excess on Nicotiana plumbaginifolia plants. Implications to oxdative stress. Plant Physiol.1995; 107: 725-735.
44 Tiwari R K, Kumar P, Sharma P N, Bisht S S. Modulation of oxidative stress responsive enzymes by excess cobolt. Plant Sci**.** 2002162: 381-388.
45 Dalton D A, Post C J, Langenberg L. Effect of ambient oxygen and fixed nitrogen on concentrations of glutathione ascorbate and associated enzymes in soyabean root nodules. Plant Physrol. 1991; 96: 812-818.
46 Thompson J E, Legge R I, Barber R J**.** The role of free radicals in senescene and wounding. New Physiol. 1987; 105: 317 - 344.
47 Vasantha Pillay, S Rao, V S, Rao K V N. Nickel toxicity in Hyptis suaveolens and Helianthus annus. (i) Effect on plant growth and distribution of nickel with in plants. Indian J Plant Nutrition**.** 6: 23-29 (ii) Effect on mineral element compostion. Indian J Plant Nutrition**.** 1987; 6: 31-34.