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Alterations in Lipid Peroxidation and Some Antioxidant Enzymes in Germinating Beans (*Vigna unguiculata*) and Maize (*Zea mays*) Exposed to Nickel

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Abstract: The growth pattern, chlorophyll content, lipid peroxidation as well as the activities of superoxide dismutase (SOD), Glutathione Peroxidases (GP) and catalase (CAT) were studied in the radicles, roots and shoots of *Vigna unguiculata* (the common beans) and *Zea mays* (maize) grown in soils contaminated with two nickel concentrations {0.1% (65 μM) and 0.2% (130 μM)}. Two hundred and seventy of either bean or maize seeds were germinated in these contaminated soils and 10 of each germinated plants were harvested after either the 7th, 14th and 21st days. The study observed chlorosis and stunted growth in the nickel-treated plants. Nickel accumulated in the tissues in increasing order of the radicle, root and the shoot. Treatment of the plants with 0.1% or 0.2% nickel did significantly ($p<0.05$) affected the activities of SOD, CAT and GP in the tissues studied throughout the experimental periods. Lipid peroxidation as measured by the production of malondialdehyde was significantly increased ($p<0.05$) in the plants grown on nickel-contaminated soils compared with the controls. These results indicate that nickel cause oxidative damage in *Vigna unguiculata* and *Zea mays* and as an adaptive feature, they increase the activities of antioxidant enzymes of the radicle, root and shoot.

Key words: Nickel, *Vigna unguiculata*, *Zea mays*, lipid peroxidation, antioxidant enzymes

INTRODUCTION

Nickel is present ubiquitously in the environment (ATSDR, 1995) and is an essential cofactor required by some enzymes particularly urease. The metal is readily taken up by plant roots and subsequently distributed throughout the plant (Giordani *et al*., 2005). Available evidence shows that nickel accumulation by the root system of plants is carried more efficiently, than by leaves (Syshchykov and Hryshko, 2003). In beans, the roots have improved ability to store the metal compared with the other parts of the plant (Giordani *et al*., 2005). This metal can accumulate in the plant and can affect many biochemical processes. Seregin and Kozhevnikova (2006) have reported that a high nickel content in the endoderm and pericycle cells blocks cell divisions in the pericycle and results in the inhibition of root branching. The metal can interfere with Mg^{2+} homeostasis and replace zinc, cobalt or many other metals present at the active site of metallo-enzymes and disrupt their functioning (Pane *et al*., 2003).

Studies reveal that the resultant effect of nickel on plants arise from their ability to offset the balance between the production and quenching of reactive oxygen species as superoxide radical, singlet oxygen and peroxide is disturbed (Schutzendubel and Polle, 2002). This results in the generation of $HO^{\cdot}$ radicals causing extensive damages of membranes by peroxidation of their constituent lipids (Gupta *et al*., 1991). Exposure to nickel has been shown to result in a severe depletion of reduced glutathione (Madhava and Sresty, 2004), which is believed to be a critical step in nickel-induced active oxygen species (Schutzendubel and Polle, 2002).

Plants adopt several strategies to mitigate the effects of high concentrations of heavy metals. These include increase in the activity of peroxisomal H_2O_2 scavenging enzymes (Gonnelli *et al*., 2001); increase in the intracellular concentration of cysteine and O-acetyl-L-serine (OAS), in shoot tissue, are strongly correlated with the ability to accumulate nickel (Freeman *et al*., 2004). It has been shown that antioxidant enzyme activities were either enhanced or reduced by nickel ions in plants (Boominathan and Doran, 2002; Gonnelli *et al*., 2001; Madhava and Sresty, 2004; Wang *et al*., 2001). While studies by Syshchykov and Hryshko (2003) has reported an increase in antioxidant enzymes in the shoot of maize with increasing concentration of nickel, those of Wang *et al*. (2009) has reported that in maize (*Zea mays* L.) plants grown in a nickel (Ni)-contaminated soil, there is decrease in superoxide dismutase in the chloroplast.

Studies on the role of nickel on antioxidant enzymes is still ongoing and this study presents data for the effect

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of different concentrations of the metal on some antioxidant enzymes in sections of *Vigna unguiculata* (common beans) and *Zea mays* (maize).

MATERIALS AND METHODS

Experimental design: Viable bean (*Vigna unguiculata*) and maize (*Zea mays*) seeds from a local market in Benin City, Nigeria. The soil which was also obtained from the Department of Soil Science, of the University of Benin, Benin City, Nigeria was loose and reddish brown (pH 6, sand 88%, silt 4% and clay 8%) and is commonly referred to as the Benin Fasc. The viability of the plant seeds was determined by the floatation method. A total of 270 seeds of either beans or maize were used in this study. Three seeds of either the beans or maize were planted in horticultural black polythene bags containing 400 g of the soil. For each plant experiment, ninety bags were used such that they were divided into three groups of 30 bags each. One group was treated with deionised water and served as control, while the other two groups were treated with either 0.1 or 0.2% Nickel (0.1 g or 0.2 g $NiSO_4$ dissolved in 100 mL distilled water. 0.2 mL of the stock solution was taken and made up to 20 mL with distilled water) and served as the test groups. After germination, plants in 10 bags of each group were harvested after every week for three weeks for assays. From the harvested plants, the radicle, other portions of the root and the shoot were recovered and used for the assays. All the seeds planted in the nickel-free soil germinated 2 and 3 days after planting while the test plants germinated after between 4 and 6 days. Harvested plants from 3 bags were used for nickel determine, while the other plants were used to assess lipid peroxidation and antioxidant enzyme activities. Prior to the harvest, the following physical observations were made: colour of leaves, number of leaves and shoot length

Heavy metal analysis: Samples (root, shoot and leaves) of the germinated beans (*Vigna unguiculata*) or the maize (*Zea mays*) was each homogenized by the blender into the powder. A known weight of the sample was digested with 20 mL HNO_3-$HClO_4$ mixture (4:1v/v) at100°C. After diluting each sample to 100 mL with distilled deionised water, nickel were analyzed by atomic absorption spectroscopy. A standard solution for nickel ions (1000 μg L^{-1}) was prepared from analytical grade of $NiSO_4$ manufactured by May and Baker Limited, Dagenham, England. The working standards were prepared by serial dilution of the standard stock solutions and were used for the calibration of the instrument.

Thiobarbituric acid reactive substances (TBARS) assay: Ten percent homogenate of the plant radicle was homogenized under cold conditions with Tris-HCl buffer (pH 7.4) containing 1.5% Polyclar-AT [w/v] which eliminated polyphenols, it was centrifuged at 3,000 g for 30 min and the supernatant obtained was used for the assay of thiobarbituric acid reactive substances according to the method of Gutteridge and Wilkins (1980). Values for TBARS are quantified using a molar extinction coefficient of 1.56×105 M cm^{-1} and expressed in terms of malondialdehyde (MDA) units g^{-1} tissue and each unit represents one micromole of MDA.

Determination of chlorophyll content of leaves: This was assayed by the method of Dere *et al.* (1998). In this method, the absorbance properties of chlorophyll a (662 nm) and b (646 nm) were quantitative analyzed and the amount of these pigments was calculated according to the formulas of Lichtenthaler and Wellburn (1985). Antioxidant enzymes activities determination: Superoxide dismutase activity was assayed according to the method described by Misra and Fridovich (1972) and was expressed as units/g tissue weight. One unit of the enzyme was defined as the amount of the enzyme required for 50% inhibition of oxidation of epinephrine to adrenochrome in one minute. Glutathione peroxidase activity was assayed by the method of Chance and Maehly (1955) as modified by Addy and Goodman (1972). Catalase activity was determined by the method of Cohen *et al.* (1970). The protein content of the samples were analyzed by the method of Lowry *et al.* (1951).

Statistical analysis: The results of the study are expressed as Means±SEM. Analysis of variance was used to test for differences in the groups. Duncan's multiple range test was employed to determine significant differences between the means (Sokal and Rohlf, 1969).

RESULTS

The growth of both plants (beans and maize) in the 0.1 and 0.2% nickel contaminated soils was significantly decreased ($p<0.05$) compared with their respective controls throughout the period of this study. After 7 days of germination, there was a 59 and 73% decrease in the shoot length of bean seedlings grown in 0.1 and 0.2% nickel contaminated soils respectively. Also, 0.1 and 0.2% nickel contamination respectively induced 48 and 71% decrease in the shoot length of maize after 7 days of germination. After 14 days of germination, the 0.1% nickel contaminated soil caused a 47% decrease in the shoot length of bean seedlings while a 70% decrease was

recorded for the 0.2% contaminated soils compared with the controls. The trend continued after 21 days of germination. We also observed a consistent reduction in the number of leaves of both plants grown in 0.1 and 0.2% nickel contaminated soils compared with their respective controls. A significant discolouration of the leaves of both plants grown in 0.1 and 0.2% nickel contaminated soils was also observed. This variegation also worsened throughout the period of this study. We observed that nickel was mobilized from the radicle to other parts of the roots and then the shoot since there was increased concentration of the metal in that order. Throughout the duration of this study, the chlorophyll content of the leaves of both plants grown in 0.1 and 0.2% nickel-contaminated soils was significantly decreased ($p<0.05$) compared with their respective controls. This effect of nickel was dependent on the duration of the exposure, with the 21 days exposure having the most effect. These data are presented in Table 1 and 2 for bean and maize, respectively.

There were observed significant ($p<0.05$) increases in the activities of glutathione peroxidase, superoxide dismutase and catalase in the radicle of beans grown in soils contaminated with different concentrations of nickel (Table 3). There was also a significant increase ($p<0.05$) in the level of MDA in this section of the beans root in the 0.1 and 0.2% nickel-contaminated soils compared with the controls (Table 3). The 0.2% nickel-contaminated soil caused the highest increase in MDA compared with the 0.1% contaminated and nickel-free soils. A similar trend in the MDA level and the activities of the antioxidant enzymes studied was observed in the other sections of the root of the beans compared with those observed in the radicle only that higher value were recorded in the other sections of the root compared with the radicle. In the shoot the activities of these antioxidant enzymes were even higher still compared with those observed in the roots and radicle (Table 3). Thus there appears to be an increase in the levels of MDA and antioxidant enzymes from the radicle to the shoot of beans grown in nickel contaminated soils.

We also observed that there was an increase in the level of MDA in response to nickel contamination associated with a corresponding significant increase ($p<0.05$) in the activities of SOD, GP and CAT in the radicle, other parts of the root and the shoot of maize

Table 1: Shoot length, colour, number of leaves, nikel and chlorophyll content of bean seedlings after 7, 14 and 21 days of germination in 0.1 and 0.2% nickel-contaminated soils

	Treatment		
Parameters	Control	0.1% Nickel	0.2% Nickel
7 days			
Shoot length	14.72±0.19^a	6.09±0.26^b	3.96±0.23^c
Number of leaves	21.00±2.10^a	12.00±1.08^b	8.00±0.99^b
Colour	10G	8G; 2V	6G; 4V
Radicle nickel content	ND	1.0±0.1^a	2.0±0.1^b
Root nickel content	ND	1.6±0.1^a	2.5±0.1^b
Shoot nickel content	ND	7.4±0.9^a	13.1±1.3^b
Chlorophyll content	53.14±0.95^a	37.27±0.63^b	22.10±0.35^c
14 days			
Shoot length	20.85±0.36^a	11.01±0.28^b	6.21±0.18^c
Number of leaves	42.00±3.33^a	20.00±1.87^b	12.00±2.01^c
Colour	8G; 2V	6G; 4V	4G; 6V
Radicle nickel content	ND	1.5±0.1^a	3.0±0.1^b
Root nickel content	ND	2.4±0.2^a	4.1±0.3^b
Shoot nickel content	ND	10.5±1.2^a	17.1±1.5^b
Chlorophyll content	72.11±0.71^a	50.65±0.69^b	34.19±0.87^c
21 days			
Shoot length	35.11±0.83^a	12.61±0.46^b	11.06±0.40^b
Number of leaves	50.00±5.06^a	24.00±1.00^b	15.00±0.05^c
Colour	6G; 4V	4G; 6V	4G; 6V
Radicle nickel content	ND	2.6±0.2^a	4.0±0.3^b
Root nickel content	ND	5.0±0.2^a	9.2±0.4^b
Shoot nickel content	ND	17.4±3.2^a	28.1±4.6^b
Chlorophyll content	83.34±0.74^a	60.78±0.66^b	40.26±0.56^c

Values are expressed as Mean±SEM (n = 10). Shoot length is in cm. Means of the same row followed by different letters differ significantly ($p<0.05$). 10G = 10 plants had leaves that were all green. 8G; 2V = 8 plants had leaves that were all green while 2 had variegated leaves. 6G; 4V = 6 plants had leaves that were all green while the other 4 had variegated leaves. 4G; 6V = 4 plants had leaves that were all green while the other 6 had variegated leaves

Table 2: Shoot length, colour, number of leaves, nikel and chlorophyll content of maize seedlings after 7, 14 and 21 days of germination in 0.1 and 0.2% nickel-contaminated soils

	Treatment		
Parameters	Control	0.1% Nickel	0.2% Nickel
7 days			
Shoot length	10.68±0.27^a	5.58±0.46^b	3.08±0.14^b
Number of leaves	22.00±1.00^a	16.00±1.01^b	10.00±1.03^b
Colour	10G	8G; 2V	6G; 4V
Radicle nickel content	ND	1.0±0.2^a	1.9±0.2^b
Root nickel content	ND	1.5±0.2^a	2.3±0.2^b
Shoot nickel content	ND	6.9±0.8^a	12.3±1.0^b
Chlorophyll content	62.79±0.78^a	42.17±0.56^b	22.42±0.65^c
14 days			
Shoot length	21.56±0.36^a	9.98±0.52^b	8.70±0.35^b
Number of leaves	35.00±0.07^a	22.00±0.07^b	15.00±0.65^b
Colour	10G	6G; 4V	4G; 6V
Radicle nickel content	ND	1.9±0.2^a	3.2±0.3^b
Root nickel content	ND	2.5±0.2^a	3.8±0.3^b
Shoot nickel content	ND	9.2±1.0^a	16.1±1.3^b
Chlorophyll content	72.25±0.34^a	46.57±0.86^b	30.65±0.98^c
21 days			
Shoot length	31.06±0.94^a	14.33±0.44^b	12.10±0.28^b
Number of leaves	45.00±0.25^a	28.00±2.08^b	20.00±0.95^c
Colour	8G; 2V	4G; 6V	4G; 6V
Radicle nickel content	ND	2.3±0.2^a	4.0±0.3^b
Root nickel content	ND	4.1±0.8^a	8.2±0.9^b
Shoot nickel content	ND	15.4±3.1^a	25.1±3.1^b
Chlorophyll content	74.74±0.43^a	55.01±0.29^b	35.49±0.34^c

Values are expressed as Mean±SEM (n = 10). Shoot length is in cm. Means of the same row followed by different letters differ significantly ($p<0.05$). 10G = 10 plants had leaves that were all green. 8G; 2V = 8 plants had leaves that were all green while 2 had variegated leaves. 6G; 4V = 6 plants had leaves that were all green while the other 4 had variegated leaves. 4G; 6V = 4 plants had leaves that were all green while the other 6 had variegated leaves

Table 3: Level of malondialdehyde (lipid peroxidation), glutathione peroxidase, superoxide dismutase and catalase activities in the radicle of bean seedlings grown in 0.1 and 0.2% nickel-contaminated soils

		Groups	
Parameters/Days	Control	0.10%	0.20%
7 days			
Radicle			
MDA	0.04±0.01^a	0.11±0.01^b	0.29±0.02^c
GP	0.16±0.01^a	0.37±0.02^b	0.61±0.01^c
SOD	0.17±0.02^a	0.44±0.02^b	0.80±0.02^c
CAT	2.40±0.01^a	2.89±0.02^b	3.00±0.02^c
Root			
MDA	0.05±0.01^a	0.17±0.01^b	0.29±0.01^c
GP	0.25±0.01^a	0.41±0.01^b	0.64±0.01^c
SOD	0.21±0.01^a	0.51±0.02^b	0.71±0.02^c
CAT	3.00±0.01^a	3.93±0.02^b	4.24±0.02^c
Shoot			
MDA	0.10±0.01^a	0.29±0.02^b	0.49±0.01^c
GP	0.34±0.02^a	0.67±0.02^b	0.98±0.02^c
SOD	0.36±0.02^a	0.77±0.02^b	0.99±0.01^c
CAT	2.98±0.04^a	3.24±0.02^b	3.67±0.03^c
14 days			
Radicle			
MDA	0.06±0.01^a	0.29±0.01^b	0.48±0.02^c
GP	0.17±0.02^a	0.67±0.01^b	0.88±0.02^c
SOD	0.21±0.02^a	0.78±0.02^b	0.99±0.01^c
CAT	2.49±0.03^a	3.11±0.02^b	3.31±0.03^c
Root			
MDA	0.08±0.01^a	0.22±0.01^b	0.49±0.01^c
GP	0.24±0.01^a	0.68±0.01^b	0.88±0.01^c
SOD	0.25±0.02^a	0.80±0.03^b	0.99±0.04^c
CAT	3.08±0.02^a	4.23±0.03^b	4.88±0.03^c
Shoot			
MDA	0.15±0.02^a	0.50±0.02^b	0.87±0.02^c
GP	0.39±0.02^a	0.91±0.01^b	1.08±0.01^c
SOD	0.32±0.02^a	1.00±0.02^b	1.41±0.02^c
CAT	3.01±0.04^a	3.67±0.05^b	3.92±0.02^c
21 days			
Radicle			
MDA	0.08±0.01^a	0.42±0.01^b	0.78±0.02^c
GP	0.22±0.01^a	0.91±0.02^b	1.10±0.02^c
SOD	0.24±0.02^a	0.98±0.01^b	1.23±0.02^c
CAT	2.50±0.01^a	3.77±0.02^b	4.11±0.04^c
Root			
MDA	0.11±0.01^a	0.39±0.01^b	0.68±0.02^c
GP	0.26±0.02^a	0.78±0.01^b	0.94±0.01^c
SOD	0.27±0.01^a	0.94±0.03^b	1.18±0.01^c
CAT	3.08±0.01^a	4.53±0.01^b	5.02±0.02^c
Shoot			
MDA	0.19±0.01^a	0.88±0.02^b	1.09±0.02^c
GP	0.45±0.02^a	1.18±0.03^b	1.49±0.03^c
SOD	0.37±0.03^a	1.50±0.02^b	1.91±0.02^c
CAT	3.12±0.02^a	4.03±0.01^b	4.68±0.02^c

Values are expressed as Mean±SEM (n = 10). Malondialdehyde (MDA) units are in mmoles MDA/g tissue, activities of glutathione peroxidases (GP), superoxide dismutase (SOD) and catalase (CAT) are in units/mg protein. Means of the same row followed by different letters differ significantly ($p<0.05$)

Table 4: Level of malondialdehyde (lipid peroxidation), glutathione peroxidase, superoxide dismutase and catalase activities in the radicle of bean seedlings grown in 0.1 and 0.2% nickel-contaminated soils

		Groups	
Parameters/Days	Control	0.10%	0.20%
7 days			
Radicle			
MDA	0.03±0.01^a	0.19±0.01^b	0.41±0.01^c
GP	0.13±0.02^a	0.49±0.01^b	0.67±0.02^c
SOD	0.23±0.01^a	0.39±0.01^b	0.64±0.01^c
CAT	2.11±0.01^a	2.67±0.02^b	2.98±0.02^c
Root			
MDA	0.04±0.01^a	0.18±0.01^b	0.34±0.02^c
GP	0.18±0.01^a	0.44±0.01^b	0.81±0.01^c
SOD	0.22±0.02^a	0.52±0.02^b	0.76±0.02^c
CAT	2.80±0.01^a	3.02±0.01^b	3.76±0.02^c
Shoot			
MDA	0.15±0.02^a	0.41±0.02^b	0.69±0.03^c
GP	0.29±0.03^a	0.57±0.02^b	0.88±0.01^c
SOD	0.28±0.02^a	0.67±0.02^b	0.91±0.02^c
CAT	2.90±0.03^a	3.11±0.02^b	3.67±0.01^c
14 days			
Radicle			
MDA	0.07±0.01^a	0.39±0.01^b	0.67±0.02^c
GP	0.16±0.02^a	0.69±0.01^b	0.81±0.01^c
SOD	0.20±0.01^a	0.51±0.02^b	0.89±0.02^c
CAT	2.10±0.02^a	2.79±0.02^b	2.99±0.03^c
Root			
MDA	0.05±0.01^a	0.38±0.02^b	0.59±0.01^c
GP	0.23±0.01^a	0.65±0.01^b	0.93±0.02^c
SOD	0.24±0.02^a	0.73±0.01^b	0.94±0.02^c
CAT	2.79±0.01^a	3.48±0.02^b	3.99±0.01^c
Shoot			
MDA	0.16±0.02^a	0.67±0.01^b	0.88±0.02^c
GP	0.35±0.01^a	0.91±0.02^b	1.19±0.01^c
SOD	0.27±0.01^a	0.88±0.02^b	1.21±0.01^c
CAT	2.95±0.02^a	3.39±0.01^b	4.12±0.02^c
21 days			
Radicle			
MDA	0.10±0.02^a	0.59±0.02^b	0.88±0.02^c
GP	0.19±0.02^a	0.87±0.03^b	1.00±0.03^c
SOD	0.25±0.03^a	0.67±0.03^b	0.99±0.02^c
CAT	1.98±0.02^a	2.90±0.02^b	3.15±0.05^c
Root			
MDA	0.08±0.01^a	0.53±0.01^b	0.77±0.01^c
GP	0.29±0.02^a	0.82±0.01^b	1.06±0.02^c
SOD	0.30±0.01^a	0.91±0.02^b	1.10±0.01^c
CAT	2.81±0.02^a	3.76±0.01^b	4.21±0.02^c
Shoot			
MDA	0.15±0.01^a	0.91±0.02^b	1.08±0.01^c
GP	0.42±0.02^a	1.13±0.01^b	1.39±0.02^c
SOD	0.30±0.03^a	1.09±0.02^b	1.58±0.02^c
CAT	3.00±0.02^a	3.68±0.0^b	4.56±0.02^c

Values are expressed as Mean±SEM (n = 10). Malondialdehyde (MDA) units are in mmoles MDA/g tissue, activities of glutathione peroxidases (GP), superoxide dismutase (SOD) and catalase (CAT) are in units/mg protein. Means of the same row followed by different letters differ significantly ($p<0.05$)

tissues studied throughout the duration of this study. Again the highest level of antioxidant enzymes was observed in the shoot, then the roots and then the radicle (Table 4). This study thus shows that beans and maize antioxidant enzymes are responsive to nickel toxicity.

DISCUSSION

This study investigated the effects of different doses of nickel (in the form of nickel sulphate) on lipid peroxidation and antioxidant enzymes in beans (*Vigna*

unguiculata) and (*Zea mays*). Plants have been shown to take up nickel easily (Giordani *et al*., 2005) and can accumulate it in their tissues. High levels of heavy metals have been reported to interfere adversely with the normal physiology of plants including reduction in photosynthesis, diminish water and nutrient uptake (Sanita di Toppi and Gabbrielli, 1999). They can also result in plant injuries such as morphological aberrations, reduction in biomass, stomatal abnormalities, chlorosis, growth inhibition, browning of root tips and death (Kahle, 1993; Alloway *et al*., 1990; Gajewska and Slodowska, 2007). The decreased shoot length and variegation in the leaves of both beans and maize (Table 1, 2) is therefore not surprising and it corroborates the findings of Gajewska *et al*. (2006) which also reported inhibition of shoot growth upon exposure to nickel.

We consistent observed a decline in the chlorophyll content of the leaves of both beans and maize with steady increase in the nickel content of the plants grown on nickel-contaminated soils (Table 3, 4). Nickel can displace manganese ion that is coordinated to the nitrogen (N) atoms of the *tetrapyrolles* of the chlorophyll molecule. This alteration in chlorophyll structure could lead to a loss of absorbing properties and hence the green coloration. It is worthy of note that this decline in chlorophyll content may account for the stunted growth. Therefore, yellowing of the leaves is an indication that the chlorophyll content is low. Other studies have shown show that nickel can cause chlorosis (Gajewska *et al*., 2006; Pandey and Sharma, 2002).

MDA is one of several low molecular weight end products formed via decomposition of certain primary and secondary lipid peroxidation products (Liu *et al*., 1997). Reactive oxygen species can react with polyunsaturated fatty acids to cause peroxidation of essential lipids in the membranes of intracellular organelles. Lipid peroxidation is associated with oxidative stress and contributes to the breakdown of the functional and structural integrity of biological membranes, resulting in increases in the permeability of the plasma membrane, which leads to leakage of K^+ ions and other solutes, amino acids oxidation and eventually may cause cell death (Gratao *et al*., 2005).

The increase in the level of MDA recorded in the root, radicle and shoot of both beans and maize throughout the duration of this study suggests that nickel induces lipid peroxidation, which may result in degrading membrane lipids and causing conformational changes in membrane proteins in plants. Though similar reports have been made earlier (Baccouch *et al*., 2001; Hao *et al*., 2006), the effect on the health of the tissues is still under debate. Cardoso *et al*. (2005) reported that the growth of *Crotalaria juncea* roots was essentially not affected by nickel. We reason that this may be related to the effect of the metal on antioxidant enzymes in the tissue.

As a defense against these oxidative damages engendered by the presence of nickel, it appears that the tissues of both plants in this our study increase their production of antioxidant enzymes such as SOD, GP and CAT. So the observed increase in the activities of these enzymes in the root, radicle and shoot of both beans and maize may be an improved attempt to mop up MDA produced by nickel-induced lipid peroxidative damage. Reports by Scandalios (2005) suggested that antioxidant enzymes are decreased in cases of high levels of MDA in plants.

Similarly, Pandey and Sharma (2002) reported decreases in catalase and peroxidases in the leaves of cabbage. Gajewska and Slodowska (2007) had reported a decrease in SOD action in leaves of wheat. Studies by Gajewska *et al*. (2006) have however reported increase in peroxidase activity in the shoot of wheat with nickel toxicity. Boominathan and Doran (2002) reported a 250% increase in the activity of SOD and catalase in the roots of *Alyssum bertolonii* treated nickel. It thus appears that the effect of nickel on antioxidant enzymes not only depends on the type of plant, its tissue but also on the type of enzyme. It is to be expected that the effect of the nickel on the plant tissues could have been worse off but for the increases observed in the antioxidant enzymes.

In conclusion, graded increases in the concentration of nickel cause significant oxidative damage to the tissues of *Zea mays* and *Vigna unguiculata* and as a strategy for survival, the plants increase their antioxidant enzymes.

REFERENCES

Addy, S.K. and R.N. Goodman, 1972. Polyphenol oxidase and peroxidases in apple leaves inoculated with a virulent or an avirulent strain of *Ervinia amylovora*. Indian Phytopath., 25: 575-579.

Alloway, B.J., A.P. Jackson and H. Morgan, 1990. The accumulation of cadmium by vegetables grown on soil contaminated from a variety of sources. Sci. Total Environ., 91: 329-336.

ATSDR, 1995. Toxicological profile for Nickel. Agency for Toxic Substances and Disease Registry/US. Public Health Service, ATSDR/TP 88/19.

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

Boominathan, R. and P. Doran, 2002. Nickel-induced oxidative stress in roots of the nickel hyperaccumulator, *Alyssum bertolonii*. New Phytol., 156: 205-215.

Cardoso, P.F., P.L. Gratão, R.A. Gomes-Junior, L.O. Medici and R.A. Azevedo, 2005. Response of *Crotalaria juncea* to nickel exposure. Braz. J. Plant Physiol., 17: 267-272.

Chance, B. and A.C. Maehly, 1955. Assay of catalases and peroxidases. Meth. Enzymol., 2: 764-775.

Cohen, G., D. Dembiec and J. Marcus, 1970. Measurement of catalase activity in tissue extracts. Ann. Biochem., 34: 30-37.

Dere, S., T. Günes and R. Sivaci, 1998. Spectrophotometric determination of chlorophyll A, B and total carotenoid contents of some algae species using different solvents. Turk. J. Bot., 22: 13-17.

Freeman, J.L., M.W. Persans, K. Nieman, C. Albrecht, W. Peer, I.J. Pickering and D.E. Salt, 2004. Increased glutathione biosynthesis plays a role in nickel tolerance in *Thlaspi* nickel hyperaccumulators. Plant Cell, 16: 2176-2191.

Gajewska, E., M. Sklodowska, M. Slaba and J. Mazur, 2006. Effect of nickel on antioxidant enzyme activities, praline and chlorophyll contents in wheat shoots. Biol. Plant., 50: 653-659.

Gajewska, E. and M. Slodowska, 2007. Effect of nickel on ROS content and antioxidant enzyme activities in wheat leaves. Biometals, 20: 27-36.

Giordani, C., S. Cecchi and C. Zanchi, 2005. Phytoremediation of soil polluted by nickel using agricultural crops. Environ. Manage., 36: 675-681.

Gonnelli, C., F. Galardi and R. Gabbrielli, 2001. Nickel and copper tolerance and toxicity in three Tuscan populations of *Silene paradoxa*. Physiol. Plant., 113: 507-514.

Gratao, P.L., A. Polle, P.J. Lea and R.A. Azevedo, 2005. Making the life of heavy metal-stressed plants a little easier. Funct. Plant Biol., 32: 481-494.

Gupta, S., M. Arhar, J.R. Behari and C. Srivastava, 1991. Cadmium mediated induction of cellular defence mechanisms: A novel example for the development of adaptive response against a toxicant. Ind. Health, 29: 1-9.

Gutteridge, J.M.C. and C. Wilkins, 1980. Copper dependent hydroxyl radical damage to ascorbic acid formation of a thiobarbituric acid reactive products. FEBS Lett., 137: 327-340.

Hao, F., X. Wang and J. Chen, 2006. Involvement of plasma-membrane NADPH oxidase in nickel-induced oxidative stress in roots of wheat seedlings. Plant Sci., 170: 151-158.

Kahle, H., 1993. Response of roots of trees to heavy metals. Environ. Exp. Bot., 33: 99-119.

Lichtenthaler, H.K. and A.R. Wellburn, 1985. Determination of total carotenoids and chlorophylls a and b of leaf in different solvents. Biol. Soc. Trans., 11: 591-592.

Liu, J., H.C. Yeo, S.J. Doniger and B.N. Ames, 1997. Heavy metal separation by functionalized mesoporous materials. Am. Chem., 214: 90-91.

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem., 193: 265-275.

Madhava, R.K.V. and T.V.S. Sresty, 2004. Antioxidative parameters in the seedlings of pigeonpea (*Cajanus cajan* (L.) Millspaugh) in response to Zn and Ni stresses. Plant Sci., 157: 113-118.

Misra, H.P. and I. Fridovich, 1972. The role of superoxide ion in the autooxidation of epinephrine and a simple assay for superoxide dismutase. J. Biol. Chem., 247: 3170-3175.

Pandey, N. and C.P. Sharma, 2002. Effect of heavy metal Co^{2+}, Ni^{2+} and Cd^{2+} on the growth and metabolism of cabbage. Plant Sci., 163: 753-758.

Pane, E.F., C. Smith, J.C. McGeer and C.M. Wood, 2003. Mechanisms of acute and chronic waterborne nickel toxicity in the freshwater cladoceran, *Daphnia magna*. Environ. Sci. Technol., 37: 4382-4389.

Sanita di Toppi, L. and R. Gabbrielli, 1999. Response to cadmium in higher plants. Environ. Exp. Bot., 41: 105-130.

Scandalios, J.G., 2005. Oxidative stress: Molecular perception and transduction of signal triggering antioxidant gene defenses. Braz. J. Med. Biol. Res., 38: 995-1014.

Schutzendubel, A. and A. Polle, 2002. Plant responses to abiotic stresses: Heavy metal-induced oxidative stress and protection by mycorrhization. J. Exp. Bot., 53: 1351-1365.

Seregin, I. and A. Kozhevnikova, 2006. Physiological role of Nickel and its toxic effects on higher plants. Russian J. Plant Physiol., 53: 257-277.

Sokal, R.R. and F.J. Rohlf, 1969. The Principles and Practice of Statistics in Biological Research. 1st Edn., Freeman and Co., San Francisco, pp: 469-484.

Syshchykov, D.V. and V.M. Hryshko, 2003. Effect of nickel compounds on glutathione-dependent antioxidant system of pea and maize shoots. Ukr. Biokhim. Zh., 75: 131-138.

Wang, H.H., J. Kang, F.H. Zeng and M.Y. Jiang, 2001. Effect of nickel at high concentractions on growth activities of enzymes of rice seedlings. Acta Agron. Sin., 27: 953-957.

Wang, H., T. Feng, X. Peng, M. Yan and X. Tang, 2009. Up-regulation of chloroplastic antioxidant capacity is involved in alleviation of nickel toxicity of *Zea mays* L. by exogenous salicylic acid. Ecotoxicol. Environ. Saf., 72: 1354-1362.