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Relative toxicity of various nickel species on seed germination and early seedling growth of *Vigna unguiculata* L.

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Cowpea (*Vigna unguiculata* L.) seedlings were raised in water culture and exposed to varying concentrations (1,10,100,1000µM) of nickel as Ni $(NO_3)_2$ $NiSO_4$ and $NiCl_2$ with a view to compare the effect of the above nickel species on seed germination, ultimate percentage germination, dry matter production, chlorophyll contents and soluble protein (leaf) contents. The ultimate germination was 100% (except in 1000 µM) in all the treatments of various nickel species. The germination speed was found maximum at 1µM nickel concentration. Lower concentration of nickel resulted in an enhancement while higher levels resulted decrease in fresh mass, dry matter production and chlorophyll contents of seedlings. Soluble leaf protein contents increased linearly with increasing application rates of nickel. In general, $NiCl_2$ was more inhibitory than $NiSO_4$ and Ni $(NO_3)_2$. Ni $(NO_3)_2$ was found to be least toxic. The extent of inhibition increased with enhanced levels of Ni_2^+ ions. The toxicity series was found to be $NiCl_2$ > $NiSO_4$ > Ni $(NO_3)_2$.

Key words : Nickel, *Vigna unguiculata* L. Toxicity, Early seedling growth.

INTRODUCTION

Heavy metal contaminants affect the biosphere in many places worldwide (Cunningham *et.al.*, 1997; Raskin and Ensley, 2000; Meagher, 2000). In the majority of natural environments the concentration of heavy metal in soil is low and does not cause any significant phytotoxic effects (Gratao *et al.*, 2005). Some heavy metals such as Cu, Zn, and Ni are essential micronutrient for plants, but are toxic to organism at high concentrations (Munzuroglu and Geckil, 2002). Although essential heavy metal ions, such as Ni^{+2}, are of major importance in different enzymatic reactions, excess cellular levels of such metals are toxic to all living cells. Nickel has one essential role in plants, which is to form the hexameric enzyme urease (E.C. 3.5.1.5.3) (Gerendas and Settelmacher, 1999). Nickel is not toxic at low concentration, but it becomes toxic at high concentrations (Poulik, 1997).

Seed is a developmental stage in plant life cycle that is highly protected against various external stresses. However, soon after imbition and subsequent vegetative developmental processes, they become stress sensitive in general. Therefore, according to Karssen, 1982; Pritchard *et al.*, 1993; Bungard *et al.*, 1997 seeds are thought to carefully monitor such external parameters as light, temperature and nutrient in order to maintain the protective state until external conditions become favourable for following developmental processes. Seed germination represents an important and initial phase in the life cycle of plants (Bishnoi *et al.*, 1993). Seed germination and early seedling growth responses of plants to adverse environmental conditions are critical for raising a successful agricultural crop stand density and establishment of resultant crop especially under stress conditions (Jagetiya and Aery, 1994a). A number of environmental factors together with the make up of seed affect germination phenomenon. The subject has attracted the attention of many workers right from the dawn of scientific research. Many treatises, review and proceedings have produced voluminous finding on germinability of seeds of several plants.

Several studies have been conducted in order to evaluate the effect of different heavy metal concentration on living plants (Thompson *et al.*, 1997). Most of these studies have been conducted using seedling or adult plants (Flores Tana *et al.*, 1999; Lee and Leustek, 1999; Chatterjee and Chatterjee 2000; Gratton *et al.*, 2000; Oneel *et al.*, 2000; Pichtel *et al.*, 2000). In a few study the seeds have been exposed to the contaminants (Clair *et al.*, 1991; Vajtechova and Leblova, 1991; Xiong, 1998).

Investigations on the effect of nickel on seed germination, growth and crop yield have been given conflicting results (Mishra and Kar, 1974). Das *et al.* (1978) responded that rice seeds when treated with nickel salts increased the germinations rates. It has been reported that at non-phytotoxic levels nickel stimulates germination and growth, promotes physiological activities

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and increased crop yield where as high concentrations prove to inhibitory (Jagetiya and Aery, 1994a). To understand how nickel affects the ability of seed germination and early seedling growth of cowpea, we have examined the comparative response of cowpea (*Vigna unguiculata* L.) under various nickel species.

MATERIALS AND METHODS

Seeds of *Vigna unguiculata* L. were selected for uniformity, surface sterilized with 0.2% $HgCl_2$ solution for five minutes and thoroughly washed for one hour under running tap water. Ten seeds were placed on two layers of Whatman No. 1 filter paper discs in Petri plates which were moistenened with fixed amount of freshly prepared solution of various concentrations of different spices of nickel at regular intervals. A randomized block factorial design with four concentration of each metal species (1,10,100,1000 µM) was used. For nickel treatment $Ni(NO_3)_2$. $6H_2O$ [E. Merk (India) Limited]; $NiCl_2$. $6H_2O$ [E. Merk (India) Limited, Mumbai] and $NiSO_4$. xH_2O [E. Merk (India) Limited, Mumbai] were used. Control sets contained only distilled water. Three replicants for each treatment were kept in dark humid condition at a constant temperature. Experiment was conducted in month of July. Seed germination noted after every two hours. After seven days of treatment, plants were harvested and washed immediately after harvesting. Chlorophyll contents and soluble protein (leaf) contents were measure after Amron (1949) and Bradford (1976), respectively in one-week-old seedlings. Plants were dried in oven at 80°C for 48 hours and weighted for the dry matter production. Following indices were also calculated.

Seedling vigour index (SVI) = Percentage Germination x Hypocotyl length

$$\text{Grade of Growth Inhibition (GGI)} = \frac{\text{Dry weight of control plants-dry weight of metal treated plants}}{\text{Dry weight of control plants}} \times 100$$

RESULTS AND DISCUSSION

Some heavy metals at low doses are essential micronutrient for plants but at higher doses they may cause metabolic disorder and growth inhibition for most of the plant species (Fernandes and Henriques, 1991; Clair *et.al.*, 1991; Thompson *et al.*, 1997 ; Reeves and Baker, 2000; Raskin and Ensley, 2000). A limited number of publication demonstrated that nickel stimulates germination of some seeds where it is toxic to others. According to Kariev (1969) nickel actually increases the germinating power of the cottonseeds. Jagetiya and Aery (1994 a) and Jagetiya and Bhatt, 2005) observed that at low concentrations $NiSO_4$ enhances start of seed germination. $NiSO_4$ solutions at low concentration showed beneficial effect on the germination of lupine (Zanotti, 1938), Soybean (Wu and Yun Hsing, 1958) and barley seeds (Jagetiya and Bhatt, 2005). Certain other studies carried out on the effect of nickel on seed germination and early seedling growth are those of Thukral and Kaur (1987), Ormarod *et al.* (1986), Aggarwal *et al.* (1990), Slivinaskaya (1991), Pandolfini *et al.* (1992), Saradhi and Saradhi (1991), Broderick (1997), Gupta *et al.* (2001), Virginie *et al.* (2005) etc.

Gupta *et al.* (2001) studied the effect of Cu and nickel on seed germination and early seedling growth of *Raphanus sativus* var. Pusa Chetki. He observed that both heavy metals adversely affect seed germination and seedling growth at higher concentration (200 and 500 ppm).

Virginie *et al.* (2005) studied the effect of three-nickel salts ($NiCl_2$, $NiSO_4$ and $(CH_3Co_2)_2Ni.4H_2O$) on germinating seeds of *Gravillea exul* var. rubiginosa an endemic serpentine proteaceae of New Caledonia. They presumed during their investigation that $NiCl_2$ resulted in the greatest reduction in germination and root growth particularly at 500 mg L^{-1} followed by $NiSO_4$ and $(CH_3Co_2)_2Ni.4H_2O$.

$$NiCl_2 > NiSO_4 > NiCH_3COO^-$$

Germination speed and ultimate germination percentage:

In present investigation, in controls as well as in certain doses of nickel, the germination started after 17 hours of installation of the experiment and 100% germination was attained firstly over a 27 hours in 1 µM $Ni(NO_3)_2$ and $NiCl_2$ treatments while in others ($NiSO_4$), it could be achieved after 29 hours respectively. The higher concentration of different nickel species delayed the germination. After a period of 23 hours germination percentage at 1 µM nickel concentration was 85% in $Ni(NO_3)_2$; 70% in $NiSO_4$ and 80% in $NiCl_2$, which was higher than control (75%). Ultimate germination percentage was found to be 100%, except in 1000 µM nickel concentration in all (1,10,100) the treatment of all the three species of nickel. (Table 1).

The seedling vigour index (SVI) was also calculated. The SVI value at 100 µM nickel treatment were 1924; 1867,1503 for $Ni(NO_3)_2$; $NiSO_4$ and $NiCl_2$, respectively, indicate that $NiCl_2$ was more toxic and $Ni(NO_3)_2$ was least toxic for cowpea, the toxicity of different nickel species in respect of seed germination are as follows.

Table 1 : Showing chlorophyll contents (mg g^{-1} fresh weight) and % increase/decrease over the control in *Glycine max* L. during different growth stages under different treatments of resorcinol

Resorcinol Concentration (ppm)	V_2 Chl'a'	V_2 Chl'b'	V_2 Total Chl	V_6 Chl'a'	V_6 Chl'b'	V_6 Total Chl	R_2 Chl'a'	R_2 Chl'b'	R_2 Total Chl	R_8 Chl'a'	R_8 Chl'b'	R_8 Total Chl
Control	0.887 ± 0.123	0.275 ± 0.078	1.30 ± 0.140	1.81 ± 0.091	0.395± 0.007	2.29 ± 0.087	1.93 ± 0.084	0.605 ± 0.021	2.66 ± 0.084	0.235 ± 0.007	0.03 ± 0.002	0.276 ± 0.005
1000	0.710± 0.014 (-19.95%)	0.255 ± 0.077 (-7.27%)	0.975 ± 0.007 (-25.00%)	1.59 ± 0.035 (-12.15%)	0.355± 0.021 (-10.12%)	2.04± 0.07 (-10.91%)	1.77 ± 0.007 (-8.2%)	0.585 ± 0.021 (-3.30%)	2.48 ± 0.05 (-6.76%)	0.215 ± 0.007 (-8.5%)	0.026 ± 2.82 (-13.33%)	0.265 ± 0.007 (-3.98%)
1500	0.595± 0.007 (-32.91%)	0.205± 0.021 (-28.00%)	0.795± 0.134 (-38.84%)	1.495± 0.007 (-17.40%)	0.330± 0.014 (-16.45%)	1.885± 0.02 (-17.68%)	1.61 ± 0.014 (-16.58%)	0.525± 0.007 (-13.22%)	2.265 ± 0.063 (-14.84%)	0.195 ± 0.007 (-17.02%)	0.019 ± 2.12 (-36.66%)	0.226 ± 0.006 (-18.11%)
2000	0.310± 0.014 (-65.05%)	0.160 ± 0.014 (-41.81%)	0.480± 0.028 (-63.07%)	1.05 ± 0.084 (-41.98%)	0.200± 0.014 (-49.36%)	1.33± 0.042 (-41.92%)	1.104± 0.149 (-42.79%)	0.430 ± 0.056 (-28.92%)	1.62 ± 0.042 (-39.09%)	0.130 ± 0.014 (-44.68%)	0.009 ± 0.0007 (-68.33%)	0.150 ± 0.014 (-45.65%)

$$NiCl_2 > NiSO_4 > Ni(NO_3)_2$$

Nickel chloride wasthe nickel treatment that resulted in the lowest germination. This can be explained by the toxicity of chloride ion, which has been demonstrated in studies of NaCl toxicity in plants (Britto *et al.*, 2004). Nickel sulphate was the least toxic with regard to germination. This may be due to the fact that, unlike the chloride ion, which is a micronutrient, sulphate is a macronutrient and is involved in the synthesis of cell detoxification molecules, such as metallothioneins (Virginie *et al.*, 2005). Curtin *et al.* (1993) found that sulphate salts induce a batter growth than chloride salt in barley. Higher concentration of nickel declined the activity of acid phosphatase. Lower concentration of nickel stimulates the activity of amylase, protease, acid phosphates and peroxidase in germination of pea, bean, wheat, castor bean, white lupine, soybean, timothy (Mishra and Kar, 1974) and rice (Das *et al.*, 1978). The beneficial effect of nickel on seed germination rates might be related to the activity of urease in germinating seeds and nitrogen economy of developing seedling (Matsumoto *et al.*, 1977 a, b; Sirko and Brodzick, 2000). Urease vis-à-vis nickel appears to have important function in the biochemical process utilizing nitrogen cycle through urea from anabolic reaction during seedling germination and growth (Eskew *et al.*, 1984). Dalton *et al.*, (1988) observed that urease plays an essential role in mobilization of nitrogenous compounds in plants, a process that is especially important during seed germination and fruit formation when protein reserves are degraded in to amino acids. According to urease might function coordinate with arginase in utilizing of seed protein reserve during germination.

Dry Matter Production :

In all the nickel species, lower concentration (1 µM) showed an enhancement in shoot- root DMP, which were 12.74 %, 11.11%; 9.80 %, 5.55% and 6.86%, 5.55% for $Ni(NO_3)_2$ and $NiSO_4$ respectively. At 100µM nickel treatment the reduction in DMP were maximum over controls. Shoot and root showed 11.76%, 30.0%; 14.70%, 47.22% and 16.66%, 48.33% reduction in DMP for $Ni(NO_3)_2$, $NiSO_4$ and $NiCl_2$, respectively. GGI values ranged from 12.74-11. 76, 9.80-14.70, 6.86- 48.33 in shoot and 38.88-30.00, 5.85- 47.22and 5.55-48.33 in root for $Ni(NO_3)_2$, $NiSO_4$ and $Nicl_2$, respectively. The effect of nickel on dry matter production is depicted in terms of Grade of Growth inhibition (GGI). On the basis of GGI following toxicity series could be established for cowpea.

$$NiCl_2 > NiSO_4 > Ni(NO_3)_2$$

Table 2 : Showing root- shoot dry matter production (mg $plant^{-1}$) and % increase/decrease over the control in *Glycine max* L. during different growth stages under different treatments of resorcinol

Resorcinol Concentration (ppm)	V_2		V_6		R_2		R_8	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
Control	159.05±1.343	45.10 ±1.55	226.40± 8.20	69.15±1.484	269.50±7.77	81.0 ±1.41	67.0 ±4.24	15.95±1.060
1000	154.50 ±0.707	42.80 ±0.282	215.0 ±7.07	65.50±0.707	257.0 ±4.24	75.50±0.707	61.0 ±1.41	13.95±0.07
	(-2.86%)	(-5.09%)	(-5.03%)	(-5.27%)	(-4.63%)	(-6.79%)	(-8.95%)	(-12.53%)
1500	135.0 ±7.07	37.10 ±1.27	197.0 ±4.24	59.00 ±4.24	239.50±3.53	69.0 ±1.41	49.15± 1.48	9.50±0.707
	(-15.12%)	(-17.73%)	(-12.98%)	(-1.46%)	(-11.13%)	(-14.81%)	(-26.64%)	(-40.43%)
2000	118.0±2.82	30.10 ±2.68	175.30±2.30	49.50±3.53	217.0±4.24	57.0±4.24	42.05±2.89	8.10±0.424
	(-25.80%)	(-33.25%)	(-22.61%)	(-28.41%)	(-19.48%)	(-29.62%)	(-37.23%)	(-49.2%)

Table 3 : Showing the effect of different nickel species on chlorophyll ('a' 'b' and total) contents (mg g^{-1}) of *Vigna unguiculata* L.

Nickel Concentrations ? M	$Ni(NO_3)_2$			$NiSO_4$			$NiCl_2$		
	Chl. 'a'	Chl. 'b'	Total chl.	Chl. 'a'	Chl. 'b'	Total chl.	Chl. 'a'	Chl. 'b'	Total chl.
Control	0.56 ± 0.014	0.252 ± 0.002	0.826 ± 0.014	0.56 ± 0.014	0.252 ± 0.002	0.826 ± 0.014	0.56 ± 0.014	0.252 ± 0.002	0.826 ± 0.014
1	0.584 ± 0.002	0.265 ± 0.004	0.847 ± 0.066	0.573 ± 0.002	0.264 ± 0.002	0.839 ± 0.001	0.571 ± 0.004	0.257 ± 0.004	0.842 ± 0.014
	(4.28%)	(5.15%)	(2.54%)	(2.32%)	(4.76%)	(1.57%)	(1.96%)	(1.98%)	(1.93%)
10	0.411 ± 0.001	0.191 ± 0.001	0.554 ± 0.002	0.388 ± 0.011	0.184 ± 0.002	0.53 ± 0.002	0.326 ± 0.001	0.151 ± 0.006	0.48 ± 0.006
	(-26.60%)	(-24.20%)	(-32.92%)	(-30.71%)	(-26.98%)	(-35.83)	(-41.78)	(-40.07)	(-41.88%)
100	0.288 ± 0.002	0.136 ± 0.005	0.436 ± 0.004	0.246 ± 0.005	0.133 ± 0.002	0.392 ± 0.005	0.238 ± 0.002	0.131 ± 0.002	0.37 ± 0.002
	(-48.57%)	(-46.03%)	(-47.21)	(-56.07%)	(-47.22%)	(-52.54%)	(-57.5%)	(-48.01%)	(-55.20%)
1000	-	-	-	-	-	-	-	-	-

Table 4 : Effect of different nickel species on soluble protein (leaf) contents (mg g^{-1}) of *Vigna unguiculata* L.

Nickel concentrations ? M	$Ni(NO_3)_2$	$NiSO_4$	$NiCl_2$
Control	7.2 ± 0.494	7.2 ± 0.494	7.2 ± 0.494
1	7.5 ± 0.212 (3.19%)	7.8 ± 0.141 (3.55%)	8.00±0.358 (3.92%)
10	8.1 ± 0.282 (12.5%)	8.45 ± 0.494 (17.36%)	9.1 ± 0.141 (26.38%)
100	10.4 ± 1.31 (44.44%)	11.10 ± 0.707 (54.16%)	11.7 ± 0.141 (62.5%)
1000	-	-	-

Saleh (2002) found reduction in dry weight of *Chorcorus olitorius* seedling with increasing nickel concentration with respect to control. Decrease in dry weight at higher concentration of nickel due to the inhibitory effect of heavy metals on cell division and cell elongination and enzyme activity. Growth inhibition caused by nickel can be connected in addition to loss of cellular turgor (Powell *et al*., 1986; Gabbrielly *et al*., 1990), also to a reduced extensibility of the cell wall (Fry, 1986; Pandolfini *et al*., 1992) and it might be due to decreasing efficiency of certain enzymes involved in food and energy utilization (Jagetiya, 1998).

Chlorophyll contents:

Chlorophyll contents are key factor in determining the net plant production. Hence change in chlorophyll content has obvious implication in changes in plant biomass. The results presented in Table 3, indicated that lower level of nickel enhanced the chlorophyll contents while higher concentration proved retardatory. Lower 1µM nickel treatments enhanced the chlorophyll ('a', 'b', and total chl.) contents in all the three species of nickel. Among the three nickel salts the percentage increase over the controls were always higher in $Ni\ (NO_3)_2$ in comparison to sulphate and chloride salts. At 100µM nickel addition the decrease in chl.'a' contents over the control was 48.57%; 56.07 % and 57. 5 % for $Ni\ (NO_3)_2$, $NiSO_4$ and $NiCl_2$, respectively. The percentage decrease in chl. 'b' was lower than chl.'a' contents. The reduction in chlorophyll 'b' contents over the controls was 46.03; 47.22% and 48.01%, respectively.

According to Pandolfini *et al*. (1992) and Picini and Malvolta (1992), the application of excess levels of nickel caused a marked depression in the chlorophyll contents, whereas intermediate level of nickel it did not vary. These authors suggested that excessive nickel addition probably depress the chlorophyll contents of the leaves by inhibiting the incorporation of Mg in the protoporphyrin molecules. Heavy metals are known to interfere with chlorophyll synthesis either through direct inhibition of an enzymatic step or by inducing deficiency of an essential nutrient (van Assche and Clijesters, 1990) and reduction in Hill activity (Basak *et al*., 2001). Decreased chlorophyll contents associated with heavy metal stress may be the result of inhibition of enzymes responsible for chlorophyll biosynthesis (Zengin and Munzzuroglu, 2005).

Soluble Protein (Leaf) Contents:

Soluble protein (leaf) contents showed increasing trend with increased metal concentration (Table 4). The effect of $NiCl_2$ on soluble protein (leaf) contents was more pronounced than $NiSO_4$ and $Ni\ (NO_3)_2$. At 100µM nickel treatment the percentage increase in soluble protein (leaf) contents were 44.44% for $Ni\ (NO_3)_2$, 54.16% for $NiSO_4$ and 62.50% $NiCl_2$ (Table 4). Our results favour the findings of Jagetiya and Aery (1994a) and Jagetiya and Bhatt (2005). They all presumed in their investigation that new low molecular weight protein are synthesized that binds with nickel and accelerates breakdown of structural or insoluble proteins. This is a very sensitive and quick-change occurring in the plant system (Dutta, 1979).

REFERENCES

Aggarwal, N., Singh, J. and Sheoran, I.S. (1990). Effect of cadmium and nickel on germination, early seedling growth and photosynthesis in wheat and pigeon pea. *Int. J. Trop. Agric.*, **8(2) :** 141-147

Basak M., Sharma M. and Chakraborty, U. (2001). Biochemical responses of *Camellia sinensis* (L.) O. Kuntze to heavy metal stress. *J environ Biol*., **22 (1):** 37-41.

Bishnoi, N. R., Sheoran, I.S. and Singh, R. (1993). Effect of cadmium and nickel on mobilization of food reserves and activates of hydrolytic enzymes in germinating pigeon pea seeds. *Biologia Plantaarum.*, **35(4):** 585-589.

Britto, D.T., Ruth, T.J., Lapi, S. and Kronzucker, H.J. (2004). Cellular and whole plant chloride dynamics in barley: insights into chloride-nitrogen interactions and salinity responses. *Planta*, **218 :** 615-622.

Broderick, C.E. (1997). The effect of nickel on Germination, Seedling Development and Micropropogation of Limna Beans (*Phasiolus lunatus* L.). Eleventh Biennial research Symposium. October, **1-4 :** 1997.

Bungard, R.A., Mvneil, D. and Morton J.D. (1997). Effects of Chilling, light and nitrogen –containing compounds on germination, rate of germination and inhibition of *Clematis vitalba* L. *Ann. Bot*., **79 :** 643-645.

Chatterjee, J. and Chatterjee, C. (2000). Phytotoxicity of Cobalt, Chromium and Copper in Cauliflower. *Environmental Pollution*., **109:** 69-74.

Claire, L.C., Adriano, D.C., Sajwan, K.S., Abel, Thoma, D.P., and Driver, J.T. (1991). Effect of selected trace metals on germinating seeds of six plant species. *Water, Air, and Soil Pollution,* **59:** 231-240.

Cunningham, S.D., Shann, J.R.,Crowley, D.E. and Anderson, T.A. (1997). Phytoremediation of contaminated water and soil. Phytoremediation of soil and water contaminants, *American Chemical Society*, Washington, DC. 2-17.

Curtin, D., Steppuhn, H. and Selles, F. (1993). Plant responses to sulphate and chloride salinity : growth and ionic relations. *Soil Sciences Society of America Journal,* **57 :** 1304-1310.

Dalton, D.A., Russell, S.A. and Evans, H. J.(1988). Nickel as a micronutrient element for plants. *Boofactors*, **1(1):** 11-6

Das, P.K., Kar, M. and Mishra, D. (1978). Nickel nutrition of plants. I. Effect of Nickel and some oxidase activities during rice (*Oryza sativa* L.) seed germination. *Z.Pfanzenphysiol.Bd*., **90S:** 225-233.

Dutta, I. (1979). Lead effects on some aspects of growth and metabolism of forage sorghum *(Sorghum vulgare*). *Indian J. Exp. Biol.,* **18:** 197-201.

Eskew, D. L., Welch, R.M. and Norvell, W.A. (1984). Nickel in higher plants: further evidence for an essential role. *Plant Physiol*., **76:** 691-693.

Fernandes, J.C. and Henriques, F. S. (1991). Biochemical, physiological and structural effects of excess copper in plants. *The Biochemical Review,* **57:** 246 –273.

Flores Tana, F.J., Muñoz Salas, E.M. and Morquecho Buendia, O. (1999). Absorción de cromo y plomo por alfalfa y pasto ovillo, *Agroceincia,* **33 :** 381-388.

Fry, S.C. (1986). Polymer bound phenols as natural substrates of peroxidases. *In*: Molecular and Physiological Aspects of Plant Peroxidases (H. Greppin, C. Penel and th. Gaspar, eds), pp.169-182. University of Geneva, Geneva.

Gabbrielly, R., Pandolfini, T., Vergnano, O. and Polandari, M.R. (1990). Comparision of two serpentine species with different nickel tolerance strategies. *Plant and soil*., **122 :** 271-277.

Gerendas, J. and Settelmacher, B. (1999). Influence of nickel supply on growth and nitrogen metabolism of *Brassica napus* L. growth with NH_4NO_3 or urea as N source. *Annals of Botany.,* **83:** 65-71.

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

Gratton, W.S., Nkongolo, K.K. and Spiers, G.A.(2000). Heavy metal accumulation in Soil and Jack pine (*Pinus banksiana*) needles in Sudbury, Ontario, Canada, Bull. Environ. Cantam. *Toxicol*., **64 :** 550-557.

Gupta, R., Shashikala, K.S., Jain, U., Soni, D. (2001). Effects of copper and nickel on seed germination and seedling growth of *Raphanus sativus* var. Pusa Chetki. *Indian J. Environ. Sci.,* **5(1):** 93-96.

Jagetiya, B.L. and Aery, N.C. (1994a). Effects of low and toxic level of nickel on seed germination and early seedling growth of moong. *Bionature*., **14(10) :** 57-61.

Jagetiya B.L.(1998). Effect of nickel and cobalt on major Biochemical constituent, Physiology, Growth and Yield in *Vigna radiata* (L.) wilczeck and *Triticum aestivum* (L.). Ph. D. Thesis.M.L. Sukhadia University, Udaipur (Raj.).

Jagetiya, B.L. and Bhatt, K. (2005). Nickel induced Biochemical and physiological alteration in barley. *Bionature,* **25 (1&2) :** 75-81.

Kariev, A.A. (1969). Effect of zinc and nickel on the growth, development and some indices of redox process in cotton .In: Vop. Fiziol. Biochim. Kholp. (A.I. Irad Imamaliev,ed.). 92-98."Fan"uzb. SSSR. Tashkent, USSR.

Karssen, C.M. (1982). Seasonal patterns of dormancy in weed seeds. In: Khan A.A. (ed.), The Physiology and Biochemistry of Seed Development, Dormancy and Germination. Elsevier Biomedical Press, Amsterdam, The Netherlands, 243-270.

Lee, S. M. and Leustek, T. (1999). The effect of cadmium on sulphate assimilation enzymes in *Brassica juncea*. *Plant. Sci*., **1141:**201-207.

Matsumoto, T., Yatazawa, M. and Yamamoto, Y. (1977a). Distribution and change in the content of allantion and allantoic acid in developing nodulating and non-nodulating soybean plants. *Plant and Cell Physiol*., **18:** 353-359.

Matsumoto, T., Yatazawa, M. and Yamamoto, Y. (1977b). Effect of exogenous nitrogen –compounds on the concentration of allantoin and various constituents in several organs of soybean plants. *Plant and Cell Physiol*., **18:** 613-624.

Meagher, R.B. (2000). Phytoremediation of toxic elemental and organic pollutants. C. Op. in *Plant boil*., **3:** 153-162.

Mishra, D. and Kar, M. (1974). Nickel in plant growth and metabolism. *Bot. Rev.,* **40 :** 395-452.

Munzuroglu, O. and Geckil, H. (2002). Effects of metals on seed germination, root elongation and coleoptiles and hypocotyls growth in *Triticum aestivum* and *Cucumis sativus* Arch. *Environ. Cont. Tox.*, **433 :** 203-213.

Oncel, I., Kele, Y. and Ustun, A.S. (2000). Interactive effects of temperature and heavy metal stress on the growth and some biochemical compounds in wheat seedlings, *Environ. Pollut.,* **107:** 315-320.

Ormarod, D.P., Hale, J.C., Allen, O.B. and Laffey, P.J. (1986). Joint action of particulate fall out nickel and rooting medium nickel on soybean (*Glycin max.* cultivar Harcor) plants. Environ. Pollut. *Ser. A. Ecol. Boil.*, **41(3) :** 277-292.

Pandolfini, T. Gabbrielly, R. and Comparini, C. (1992). Nickel toxicity and peroxidase activity in seedlings of *Triticum aestivum* L. *Plant cell. Environ.*, **15:** 719-725.

Pichtel, J., Kuroiwa, K. and Sawyer, H.T. (2000). Distribution of Pb, Cd, and Ba in soils and plants of two contaminated sites, *Environ. Pollute*., **110:** 171-178.

Picini D.F. and Malavolta, E. (1992). Effects of nickel on two common bean cultivars.*Plant Nutr.*, **15:** 2343-2356.

Poulik Z. (1997). The danger of accumulation of nickel in cereals on contaminated soil. *Agric. Ecosyst. Environ*., **63 :** 25-29.

Powell, M.J., Davies, M.S. and Francis, D. (1986). The influence of zinc on the cell cycle in the root meristem of a zinc –tolerant and non tolerant cultivars of *Festuca rubra* L. *New Phytologist*, **102:** 419-428.

Pritchard, H.W., Wood, J.A. and Mjanger, K.R. (1993). Influence of temperature on seed germination and nutrional requirements for embryo growth in *Arum maculatum* L. *New Phytol*., **123:** 801-809.

Raskin, I., and Ensley, B.D. (Ed.), (2000). Phytoremediation of toxic metals: using plants to clean up the environment, John Wiley and Sons, N. York. 303.

Reeves, R.D. and Baker, A.J.M. (2000). Metal accumulating plants, In: I. Raskin and B.D. Ensley (Ed.) Phytoremediation of toxic metals: using plants to clen up the environment, john Wiley and Sons, Inc, Toranto, Canada.303.

Saleh, A. H. A. (2002). Response of anabolic capacities, proline, protein patterns and mineral Elements to nickel and EDTA stress in *Chorcorus olitorius*. *Pakistan Journal of Biological Sciences*, **5(4):** 455-460.

Saradhi, A. and Saradhi, P.S. (1991). Proline accumulation under heavy metal stress. *J. Plant Physiol*., **138(5):** 554-558.

Sirko,A. and Brodzik,R.(2000). Plant ureases roles and regulation. *Acta Biochimica Polonica*, **47(4):** 1189-1195.

Slivinaskayaa, R.B. (1991). Nickel effect on sunflower leaf cell membranes. *Acta Bot. Nerl.*, **40(2):** 133-138.

Thompson, E.S., Pick, F.R. and Bendell-Uoung, L.I. (1997). The accumulation of cadmium by the yellow pond lily, *Nuphar variegatum* in Ontario peatlands. *Arch. Environ. Contam. Toxicol*, **32:** 161-165.

Thukral, A.K. and Kaur, P. (1987). Effect of some trace elements of polluted waters on the germination of *Cyamopsis tetragonoloba* Taub. *Indian J.Ecol.*, **14(2):** 185-188.

Van Assche and Clijesters, H. (1990). Effects of metals on enzyme activity in plants. *Plant cell Evironment*, 13: 195-206.

Virginie, L., Jacques, R., Roger, N., Roxane, B., Xavier, M., Saliou, B.M., Josette,V. and Rene, P. (2005). Effects of three-nickel salts on Gemination seeds of *Gravillea exul* var. rubiginosa, an Endemic Serpentine Proteaceae. *Annals of Botany*, **95(4):** 609-618.

Vojtechova, M. and Leblova, S. (1991). Uptake of lead and cadmium by maize seedlings and the effect of heavy metals on the activity of phosphoenolpyruvate corboxilase isolated from maize. *Biologia Plantarum*, **33:** 386-394.

Wu, L. and Yun–Hsing, C. (1958). Effect of seed treatment with trace elements upon the growth, yield and Quality of soybean. *Nung Yeh Hsuch Pao*, **9:** 333-339.

Xiong, Z.T. (1998). Lead uptake and effect on seed germination and plant growth in a Pb hyperaccumulator *Brassica pekinensis* Rupr., Bull. *Environ. Contam. Toxicol.*, **60:** 285-291

Zanotti, V. (1938). Phytopharmacological. II. *Bol. Chim. Farm*., **77:** 309-312; 315-319.

Zengin, F.K. and Munzuroglu, O. (2005). Effects of some heavy metals on content of chlorophyll, proline and some antioxidant chemicals in Bean (*Phaseolus vulgaris* L.) seedlings. *Acta Biologica Cracoviensia series Botanica*, **47(2):** 157- 164.