Journal of Environmental Biology **September 2009,** 30(5) 677-684 (2009)
©Triveni Enterprises, Lucknow (India)
For personal use only
Free paper downloaded from: www.jeb.co.in
Commercial distribution of this copy is illegal

Response of ultraviolet-B and nickel on pigments, metabolites and antioxidants of *Pisum sativum* L.

Suruchi Singh, Shweta Mishra, Rima Kumari and S.B. Agrawal*

Ecology Research Circle, Department of Botany, Banaras Hindu University, Varanasi - 221 005, India

(Received: August 03, 2008; Revised received: September 23, 2008; Accepted: September 25, 2008)

Abstract: *Ultraviolet radiation (UV) though harmful but is an important and unavoidable component of terrestrial ecosystem to which plants have been exposed since their migration from aquatic to land habitat. Incoming UV-B radiation and heavy metals abundance in contaminated soils are significant environmental threat affecting metabolic functions of plants through generation of reactive oxygen species. Plants have evolved mechanisms to counteract these reactive radicals and to repair the damage caused by UV-B and heavy metals. This study describes the impact of supplemental UV-B (sUV-B) and nickel (Ni) singly and in combination on photosynthetic pigments, flavonoids, enzymatic and non-enzymatic antioxidants, metabolites and lipid peroxidation of Pisum sativum L. (pea) plants. Compared to the controls, both the stresses individually and in combination led to reductions in photosynthetic pigments, ascorbic acid, protein and catalase (CAT) activity, whereas a reverse trend was observed for flavonoids, phenol, proline and thiol contents, superoxide dismutase (SOD) and peroxidase (POX) activities and lipid peroxidation (LPO). However, flavonoids increased significantly under individual exposure of sUV-B as compared to other treatments. An increase of LPO by 81% indicated the generation of reactive oxygen species under both the stress conditions. sUV-B and Ni in combination acted synergistically with stimulation of CAT activity by 51.6% , additively on SOD activity with increase of 16.4%, while other parameters showed antagonistic action of both the stresses.*

Key words: *sUV-B, Ni, Pigments, Lipid peroxidation, Antioxidants, Pisum sativum*

PDF of full length paper is available online

Online Copy

Introduction

Ultraviolet-B (UV-B) radiation (280-315 nm) influences various biological processes, and is strongly absorbed by stratospheric ozone. Reductions in stratospheric ozone because of anthropogenic activities are therefore important as it leads to corresponding increase in UV-B radiation reaching the Earth's surface (Yogamoorthi, 2007). It has been predicted that UV-B will continue to increase till 2050 (Weatherhead *et al*., 2005). Numerous studies have been done on different plants to evaluate the direct and indirect effects of supplemental and ambient UV-B radiation and showed that they can affect plant growth and metabolism with morphological, physiological and biochemical changes and modification in biomass production and reproductive processes (Krywult *et al*., 2008). The effect of UV-B on plants was different not only to species level but also among the cultivars of the same species (Agrawal *et al*., 2006).

Many plants are quite resistant to UV-B and possess a number of protection mechanisms by either accumulating flavonoids or related UV absorbing compounds in the leaf epidermis or other UV screening mechanisms working as selective UV-B filters (Schmelzer *et al*., 1998). Other mechanisms are enzymatic and non-enzymatic antioxidative defense systems to cope with reactive oxygen species (ROS) toxicity developed due to UV-B radiation. Cellular UV-B chromophores such as aromatic amino acids, NADPH and phenolic compounds could be activated by the absorption of UV-B light which reacts with oxygen to form singlet molecular oxygen and superoxide radicals (Caldwell *et al*., 1995). Plants metabolize ROS by invoking the antioxidant defense system of low molecular weight antioxidants such as ascorbate, glutathione, α-tocopherol and carotenoids (Alscher and Hess, 1993) as well as enzymes such as superoxide dismutase, catalase, peroxidase and glutathione reductase (Bowler *et al*., 1992).The accumulation of proline is well documented in plant cells against various stresses (Mishra and Agrawal, 2006).

Heavy metal contamination has become a wide spread problem world over (Sharma and Agrawal, 2005). Metal pollution is continuously increasing and the main cause is the anthropogenic activities which interferes with the environment activities and makes condition hazardous for living organism (Singh *et al*., 2007). Excess concentrations of some heavy metals in soils such as Cd (II), Cr (IV), Ni (II) and Zn (II) have caused disruption of natural aquatic and terrestrial ecosystems (Meagher, 2000). Toxic effects of Ni on plant growth and photosynthesis have been reported in higher plants as well as in algae (Sheoran *et al*., 1990). Higher concentrations of Ni are responsible for reductions in Hill activity and oxygen evolution (Singh and Singh, 1987), and impairment of PSII activity by modifying Q_B site of electron transport chain (Mohanty *et al*., 1989). Rai *et al*. (1998) have suggested that UV-B may affect heavy metals uptake by altered membrane permeability in cyanobacteria as a result of peroxidation of membrane lipids in UV-B exposed cells.

The assessment of multiple environmental effects on plants is therefore important in obtaining more meaningful and realistic

* **Corresponding author:** sbagrawal56@gmail.com

evaluation of current changes in the environment. However, only few studies have been made so far to study the interaction of sUV-B and heavy metals on terrestrial vascular plants. Therefore, the objective of this study was to assess some important biochemical changes including enzymatic and non-enzymatic antioxidants in pea (*Pisum sativum* cv Arkel) in response to supplemental UV-B (sUV-B) and Ni applied individually or in combination.

Materials and Methods

The pot experiment was carried out at Agricultural farm of Allahabad Agricultural Institute, Allahabad situated at 24° 97'N latitude, 82° 21'E longitude and 96 m altitude in the eastern Gangetic plains of India. During the experimental period, the mean temperature ranged from 12.9-32.6 °C and relative humidity was 59.8-68.4% and PAR ranged between 1100-1200 µmol m^{-2} s^{-1}.

Pea (*Pisum sativum* L. cv Arkel), chosen for the experimental work is an excellent and cheap source of high quality protein (25%). Pea is consumed as whole grain as well as pulse in a variety of ways. It is also a green manuring crop. Being a leguminous crop, it has the capacity to fix atmospheric nitrogen. After harvesting the pods, green plants are uprooted or cut from ground level and chopped into small pieces to feed the cattles.

The soil was prepared at one time by mixing farmyard manure in accordance to normal agronomic practices to avoid any changes in edaphic conditions. The recommended doses of nitrogen in form of urea, phosphorus as single super phosphate and potassium as muriate of potash were mixed at rates of 44.8, 56 and 56 kg ha^{-1}, respectively. Earthern pot of 14 inch diameter was filled with 8 kg of soil. For studying the effect of heavy metal, Ni was applied at the rate of 68 µmol kg^{-1} soil as $NiCl_2$ in 32 pots. The concentration of Ni selected for the experiment falls within Indian standards for heavy metals in soil (Awasthi, 2000). Sixteen pots kept without heavy metal amendment in soil served as controls. Sixteen pots of each treatment (heavy metal and without heavy metal) were randomly selected for sUV-B exposure. All pots were watered uniformly throughout the experiment period in order to maintain constant soil moisture. sUV-B was artificially provided by Q-panel UV-B 313 40W fluorescent lamps (Q panel Inc., Cleveland, OH, USA). Three lamps (120 cm long each) per bank were fitted at 30 cm distance apart from steel frame and were suspended perpendicular to the planted rows of each treatment. The lamps were covered by either 0.13 mm cellulose diacetate filter (transmission down to 280 nm) for sUV-B radiation or 0.13 mm polyester filters (absorbed radiation below 320 nm) for the control to provide ambient levels of UV-B. Lamps in frames were adjusted weekly to a distance of 30 cm to provide a mean supplemental UV-B (+7.1 KJ m^{-2} d^{-1}) for 3 h daily over the middle of photoperiod.

The intensity of UV-B at plant apices under the lamps was measured with an ultraviolet intensity meter (UVP Inc. San. Gabriel, (A), USA). Plants under polyester filter lamps received only ambient UV-B (8.6 KJ m^{-2} d^{-1} UVB_{BE}) on the summer solstice weighted against generalized plant response action spectrum of Caldwell (1971). The plants beneath cellulose diacetate film received UVB_{BE} (ambient +7.1KJm^{-2} d^{-1}) that mimicked 20% reduction in stratospheric O_3 at Allahabad during clear sky condition (Green *et al.*, 1980) normalized at 300 mm, the albedo 0 and the scatter 1.0.

Random samples of plants were taken in triplicate from respective treatments at 15, 30 and 45 days after sowing (days) for various analyses. Chlorophyll and carotenoids were extracted from the leaf disc with 80% acetone and quantified according to the methods given by Machlachan and Zalik (1963) and Duxbury and Yentsch (1956), respectively. Flavonoid content was determined by the method described by Flint *et al.* (1985). For protein extraction, fresh leaves were homogenized in tris buffer (0.1 M) followed by mixing of TCA (10%) and then dissolved into 0.1 N NaOH. Estimation of protein was done by the method of Lowry *et al.* (1951). For ascorbic acid, leaf samples were homogenized in oxalic acid and NaEDTA extraction solution. For developing colour, 2, 6-dichlorophenol-indophenol dye was used and the absorbance was taken at 520 nm. Ascorbic acid content was quantified using the method of Keller and Schwager (1977). Phenol content was estimated by homogenizing the leaf sample in acetone and then using Folin-ciocalteu reagent and Na_2CO_3 (Bray and Thorpe, 1954). For thiol determination, 100 mg of leaf sample was homogenized with 80% v/v ethanol and boiled for 15 min. Amount of 1ml Ellman's reagent (DTNB) was added and allowed to react for 5 min with thiol in plant extract. From the extract, thiol content was determined by taking the absorbance at 412 nm by using the method of Fahey *et al.* (1978). Determination of proline was performed by ninhydrin test as described by Bates *et al.* (1973).

Peroxidase (POX: EC 1.11.1.7) and catalase (CAT: EC 1.11.1.6) were extracted by homogenizing 100 mg fresh leaves at 4°C in 100 mM cold phosphate buffer (pH 7.0) containing 5mM cysteine, while superoxide dismutase (SOD: 1.15.1.1) was isolated in 100 mM EDTA-phosphate buffer (pH 7.8). For POX activity, enzyme extract was incubated for 5 min at 25°C with 125 mM phosphate buffer (pH 7.0), 50 µM H_2O_2. After 5 min. reaction was terminated by adding 0.5 ml of 5% H_2SO_4. The extraction of purpurogallin was carried out by adding diethyl ether and the amount was determined by taking absorbance at 430 nm with extraction coefficient of 2.47 by using the method of Britton and Mehley (1955). CAT activity was quantified by H_2O_2 decomposition. For this enzyme extract was incubated in 300 mM phosphate buffer and 100 µM H_2O_2 for 1 min at 25°C. Residual H_2O_2 was calculated by titration of reaction mixture against 0.02 N $KMnO_4$ following the method of Kar and Mishra (1976). SOD activity was assayed at 25°C according to the method of Giannopolitis and Ries (1977) using the reaction mixture containing 0.5 ml each of 3 µM riboflavin, 13µM methionine, 63 µM NBT, 0.05 M sodium carbonate, 0.9 ml of distilled water and 0.1 ml of enzyme extract incubated for 10 min at 25°C under illumination from fluorescent lamp. The increase in SOD activity was determined spectrophotometrically at 560 nm. Lipid peroxidation (LPO) in the leaf tissues was determined in terms of malondialdehyde (MDA) content by thiobarbituric acid (TBA) reaction as described by Heath and Packer (1968).

Online Copy

Effects of factors sUV-B, Ni, plant age and their interactions were determined by conducting three way analysis of variance test by using SPSS software (SPSS Inc., version 10.0).

Results and Discussion

Total chlorophyll and carotenoid contents decreased in all treated plants at all the ages as compared to the control, but increased with increasing age of plants (Fig. 1). ANOVA test showed that age and treatment significantly affected total chlorophyll and carotenoid contents (Table 1). Interactive effects of both the stresses showed antagonistic effect on chlorophyll. Reductions in total chlorophyll and carotenoid contents were 12.7 and 7.6%, 12.1 and 7.1%, 13.8 and 9.7% in treatments of sUV-B, Ni and sUV-B+ Ni, respectively when compared to their controls at 45 days (Fig. 1). Changes in the relative amount of photosynthetic pigments reflect the changes in the photosynthetic machinery. Ni and sUV-B induced reductions in chlorophyll content may be ascribed to inhibition of chlorophyll biosynthesis by inhibiting δ-aminolevulunic acid dehydrogenase and protochlorophyllide reductase activities, and breakdown of pigments or their precursors by heavy metal (Ouzounidou, 1995). UV-B alters mRNA turnover of the chlorophyll a/b binding proteins resulting in reduced chlorophyll content. Agrawal and Rathore (2007) also reported adverse effects of sUV-B on chlorophyll content in various cultivars of wheat and mungbean. Ni replaces central Mg from chlorophyll molecules (Kupper *et al.*, 1996). Carotenoids protect chlorophyll from photooxidative destruction (Middleton and Teramura, 1993) and therefore reduction in carotenoids could have serious consequence on chlorophyll pigments. Study of Barsig and Malz (2000) showed stability of carotenoid configuration after UV-B exposure of sugarcane and maize leaves. Panda and Khan (2003) also reported a sharp decrease in carotenoid content in rice under heavy metal treatment.

Absorption profile of flavonoids showed decline under individual treatment of Ni as well as its combined treatment with sUV-B (Fig. 2). However, flavonoids increased significantly under sUV-B treatment with maximum increase at wavelength of 310 nm. ANOVA test showed significant variations in flavonoid content due to plant age and sUV-B and their combination at all wavelengths (Table 2). Accumulation of UV-B absorbing pigments is one of the way by which plants avoid the harmful effects of sUV-B (Dillenburg *et al.*, 1995). The photoinduced accumulation of flavonoids was reported to precede by the induction of several enzymes involved in phenylpropanoid metabolism (Hahlbrock and Scheel, 1989). Many flavonols and flavones tend to accumulate at high concentrations in

Table - 1: F-ratios and levels of significance of two-way ANOVA test for various parameters of *Pisum sativum* L. plants

Parameters	Age (A)	Treatment (T)	A × T
Total Chlorophyll	56.1***	3.2*	0.209^{ns}
Carotenoids	15.3***	1.4^{ns}	0.107^{ns}
Protein	171.2***	16.1***	1.6^{ns}
Ascorbic Acid	101.4***	4.5*	0.713^{ns}
Phenol	157.7***	35.1***	0.904^{ns}
Thiol	32.3***	10.1***	0.708^{ns}
Proline	235.1***	53.2***	1.9^{ns}
SOD	19.8***	2.4^{ns}	0.042^{ns}
POX	6.4**	7.6***	0.056^{ns}
CAT	7858.1***	7404.9***	6260.1***
LPO	99.1***	28.3***	2.6*

* = p<0.05, ** = p<0.01, *** = p < 0.001, ns = Not significant

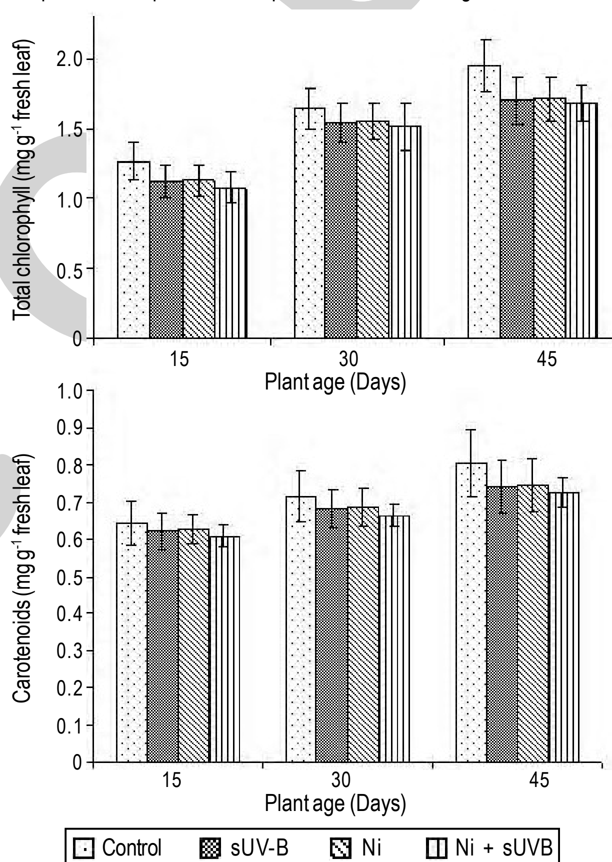

Fig. 1: Age wise changes in total chlorophyll and carotenoids due to sUV-B radiation and heavy metal (Ni) treatments given individually or in combination (bar represent mean ± 1SE)

Table - 2: F-ratios and level of significance of three way ANOVA test for flavonoid absorbance of *Pisum sativum* L. plants

Wave length (nm)	Plant age (A)	sUV-B (T)	Ni (N)	A × T	A × N	T × N	A × T × N
250	56.12***	84.12***	0.48^{ns}	18.70***	1.04^{ns}	0.32^{ns}	0.15^{ns}
270	64.20***	67.30***	1.40^{ns}	17.12***	6.23^{ns}	0.12^{ns}	0.09^{ns}
290	112.10***	52.60***	0.92^{ns}	16.10***	0.46^{ns}	1.56*	0.12^{ns}
310	145.20***	78.12***	1.32^{ns}	16.30**	1.12^{ns}	1.18*	0.18^{ns}
330	138.26***	114.60***	0.84^{ns}	9.12***	0.76^{ns}	0.32^{ns}	0.40^{ns}
350	84.12***	125.12***	1.16^{ns}	36.10***	0.72^{ns}	0.12^{ns}	0.45^{ns}

Level of significance: * = p<0.05, ** = p<0.01, *** = p<0.001, ns = Not significant

Online Copy

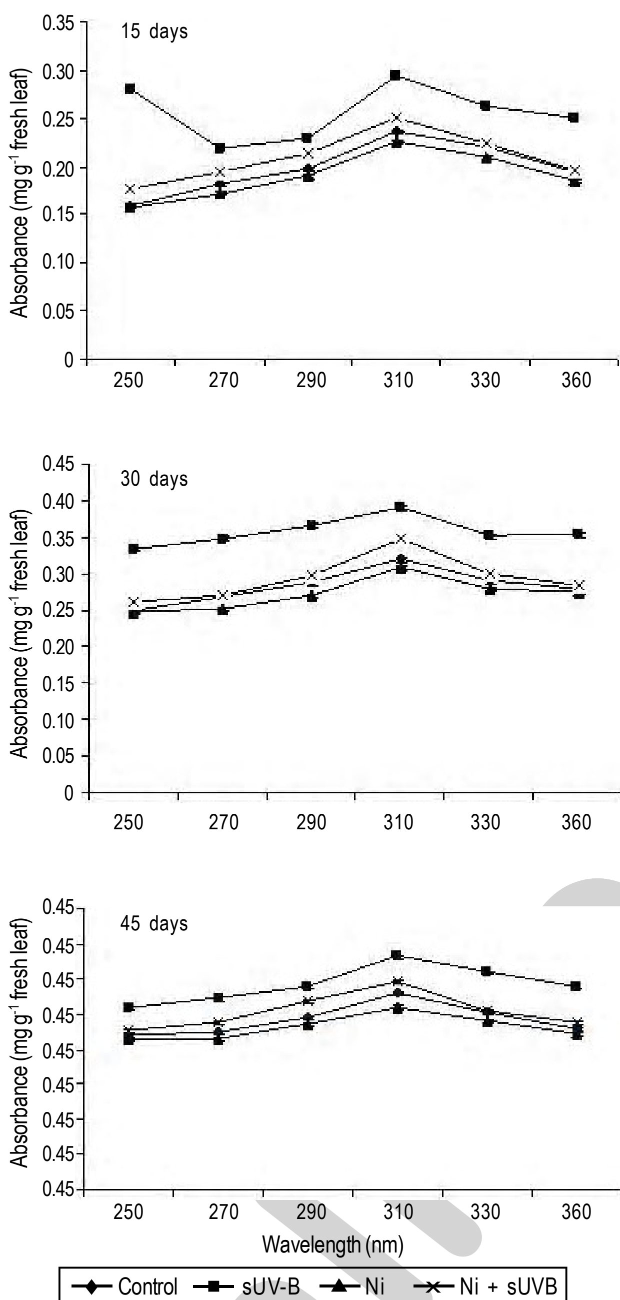

Fig. 2: Age wise changes in absorption profile of flavonoids of *Pisum sativum* L. due to sUV-B and Ni treatment given individually and in combination (bars represent mean ± 1SE)

the epidermal layer of leaves, making them well suited to screen harmful UV-B radiation and also reduce its doses penetrating in the leaf mesophyll tissue (Day, 2001). It seems therefore, that the protective mechanism against sUV-B radiation is impaired when plants are grown in an environment contaminated with Ni.

Foliar protein content was found to be significantly lower under all treatments applied singly. ANOVA test showed that combined treatment of sUV-B and Ni did not show significant effect on protein content of plants (Table 1). Combined treatment of sUV-B and Ni always resulted in more reduction in protein content as compared to their individual treatment. The reductions in protein content were found to be 25.9, 18.7 and 30.9% in treatments of sUV-B, Ni and sUV-B+Ni, respectively as compared to their control ones at 45 days (Fig. 3). UV radiation is not only known to cause modification or destruction of amino acid residues, but also known to inactivate the protein and enzymes (Prinsze *et al.*, 1990). Higher concentration of Ni also caused reduction of protein content in *Chorchorus olitorius* (Saleh, 2002). The decrease in protein content under heavy metal stress suggested that soluble proteins may be leaked or diffused out of the plants material, or possibly the induced catabolic enzymes have destroyed the protein (Sasadhar, 1987). Decrease in protein content was also well correlated with the increase in proline content of plant cells (Shukla *et al.*, 2001).

Foliar ascorbic acid content increased with successive growth stages in the test plants, but reduced significantly under sUV-B and Ni treatments. Reductions in ascorbic acid contents were 17.1, 12.2 and 20.5% in sUV-B, Ni and sUV-B+Ni exposed plants, respectively when compared to their controls at 45 days (Fig. 3). ANOVA test showed that both the treatments significantly affected ascorbic acid content of plants (Table 1). Reduction in ascorbic acid content of sUV-B treated plants was consistent with the results obtained by Agrawal and Rathore (2007) in wheat and mung bean plants. Ascorbic acid acts as in *vivo* biological antioxidant and functions as a co-substrate of plant peroxidases (Halliwell, 1982). Ascorbate can scavange many free radicals produced during oxidative stress such as $HO^{\cdot}$, $RO^{\cdot}$, $GO^{\cdot}$, urate and tocopheroxy radical ($TO^{\cdot}$) due to its low reduction potential. The reduction in ascorbic acid could be explained by the increase of ascorbate peroxidase (APX) activity after UV-B exposure which consumes more ascorbic acid for effective quenching of oxyradicals.

Phenol content increased under all the stress factors (Fig. 3). The percentage increments in phenol content were 41.9, 17.3 and 46.6 in sUV-B, Ni and sUV-B+Ni, respectively at 45 days as compared to their control ones (Fig. 3). There are several reports in a variety of plants showing increase in phenolic content in response to UV-B (Hada *et al.*, 2001) and heavy metal stress (Agrawal and Mishra, 2009). As an adaptation to UV-B, phenols can reduce the intensity of UV-B reaching to the photosynthetic tissues of the leaves (mesophyll) where it can be damaging. Rauser (1978) reported accumulation of apolar soluble phenolics as early effects of Ni toxicity in *Phaseolus vulgaris*. Heavy metals are known to induce oxidative stress (Panda and Patra, 2000), but a direct protective function of phenolics against heavy metal stress is yet to be determined (Suzuki, 2005).

Thiol content of pea also increased with successive growth stages of pea plants (Fig. 4). In case of combined treatments, interaction of sUV-B and Ni led to more increment in thiol content as compared to their individual treatment, but it was not significant (Fig. 4). The percent increments in thiol contents were 40.4, 13.2 and 49.4, respectively at 45 days in sUV-B, Ni and sUV-B+Ni treated plants (Fig. 4). ANOVA test showed that variations in thiol

Online Copy

contents were not significant due to interactions between Age×Ni and sUV-B×Ni (Table 1). Agrawal and Rathore (2007) reported increments of 27.8 and 22.4% in thiol contents of sUV-B exposed mung bean *Vigna radiata* cv. Malviya Jyoti and Malviya Janpriya, respectively at 65 days. Randhawa *et al.* (2001) also reported increase in thiol content by maintaining high GSH: GSSG ratio in Ni-tolerant B4 strain of a green alga *Scenedesmus acutus f. alternans*. Several studies suggest that GSH could be involved in protecting cells from the oxidative stress induced by heavy metals (Weckx and Clijsters, 1997) and is the direct precursor of phytochelatins. Phytochelatin is a cysteine rich peptide, synthesized from glutathione and chelates heavy metals on its thiol moieties (Zenk, 1996).

Foliar proline content also increased after treatment of sUV-B and Ni, singly and in combination. Variations in proline content were significant due to all the factors and their interactions (Table 1). At 45 days, proline accumulation increased by 32.7, 32.2 and 46.4%, respectively in sUV-B, Ni and sUV-B+Ni, treated plants (Fig. 4). The accumulation of free proline in plants is regarded as a general response to stress from different origin (Sumaryati *et al.*, 1992). Prasad *et al.* (2005) observed increments in proline by 38 and 11%, respectively in Ni and sUV-B treated soybean seedlings. Agrawal and Mishra (2009) reported 53.5% increment in proline content of sUV-B exposed pea plants. Proline accumulation is believed to protect plants from detoxifying free radicals by forming long-lived adducts with them (Alia *et al.*, 1997). Proline accumulation also caused considerable reduction in the time-dependent increase in the level of dienes conjugates and MDA during UV exposure of linolenic acid micelles. Proline participates in the reconstruction of chlorophyll, activates Krebs cycle and acts as an energy source (Mishra and Agrawal, 2006).

Percent increments in lipid peroxidation (LPO) expressed as MDA contents were 71.9, 48.5 and 81.0% in sUV-B, Ni and sUV-B+Ni treated plants, respectively at 45 days (Fig. 4). Under 8.7 KJ m^{-2} d^{-1} sUV-B irradiation, an increment of 77.7% in LPO was observed by Agrawal and Mishra (2009) in pea plants. UV-B is known to damage fatty acids in photosynthetic organisms or in reconstructed membrane systems. This could be achieved via the energetic cleavage of covalent bonds (Larson, 1988) by UV-B generated amino acid radicals that can oxidize fatty acids (Salmon *et al.*, 1990) or through the activation of ROS which can attack fatty acid chains. The increase in MDA content is an indicator of UV-B induced oxidative damage due to impairment of cell defense system. It has been observed that high concentration of metal increased lipid peroxidation in intact cells through the generation of free radicals (Severi, 1997).

Activity of super oxide dismutase (SOD) significantly increased after treatments of sUV-B and Ni (Fig. 4). However, interaction of sUV-B+Ni had additive effect on SOD activity of leaves. The activity of SOD was 13.6, 2.5 and 16.4% higher in treatments of sUV-B, Ni and sUV-B+Ni, respectively at 45 days as compared to their controls (Fig. 4). UV-B radiation produces oxidative stress by increasing the generation ROS such as singlet oxygen,

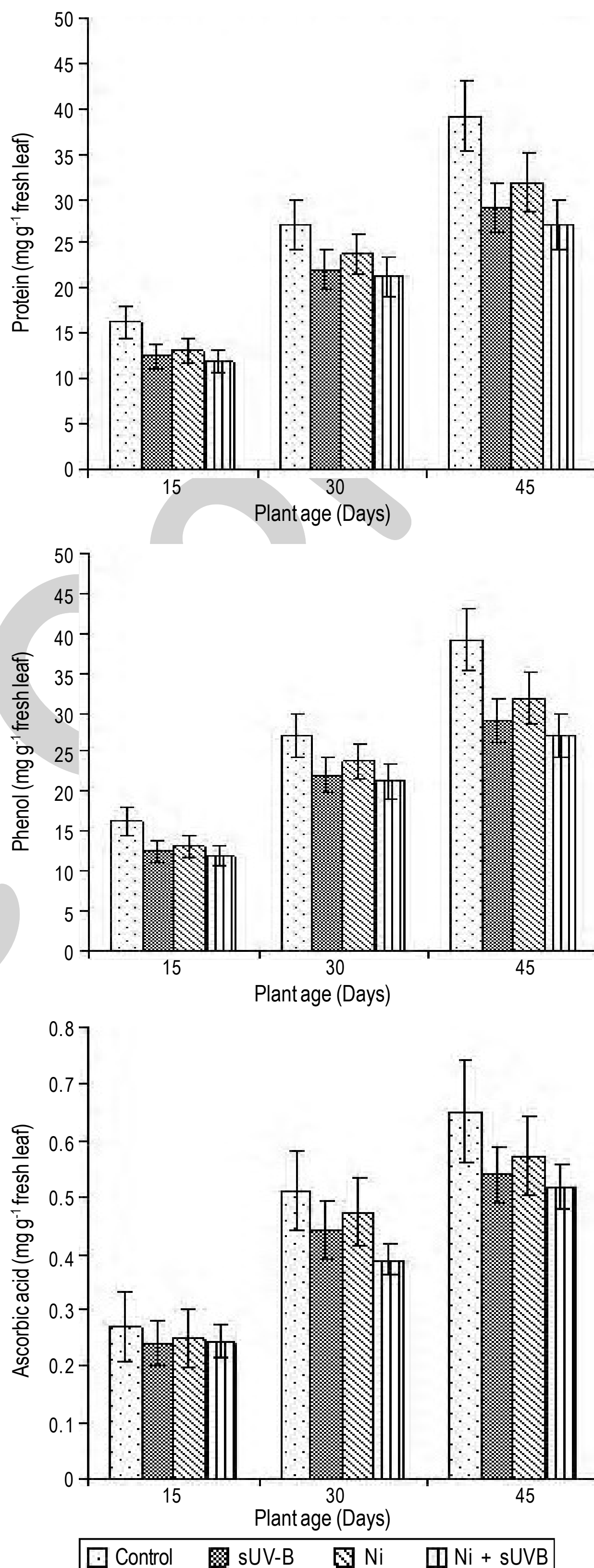

Fig. 3: Age wise changes in protein, phenol and ascorbic acid contents due to sUV-B radiation and heavy metal (Ni) treatments given individually or in combination (bar represent mean ± 1SE)

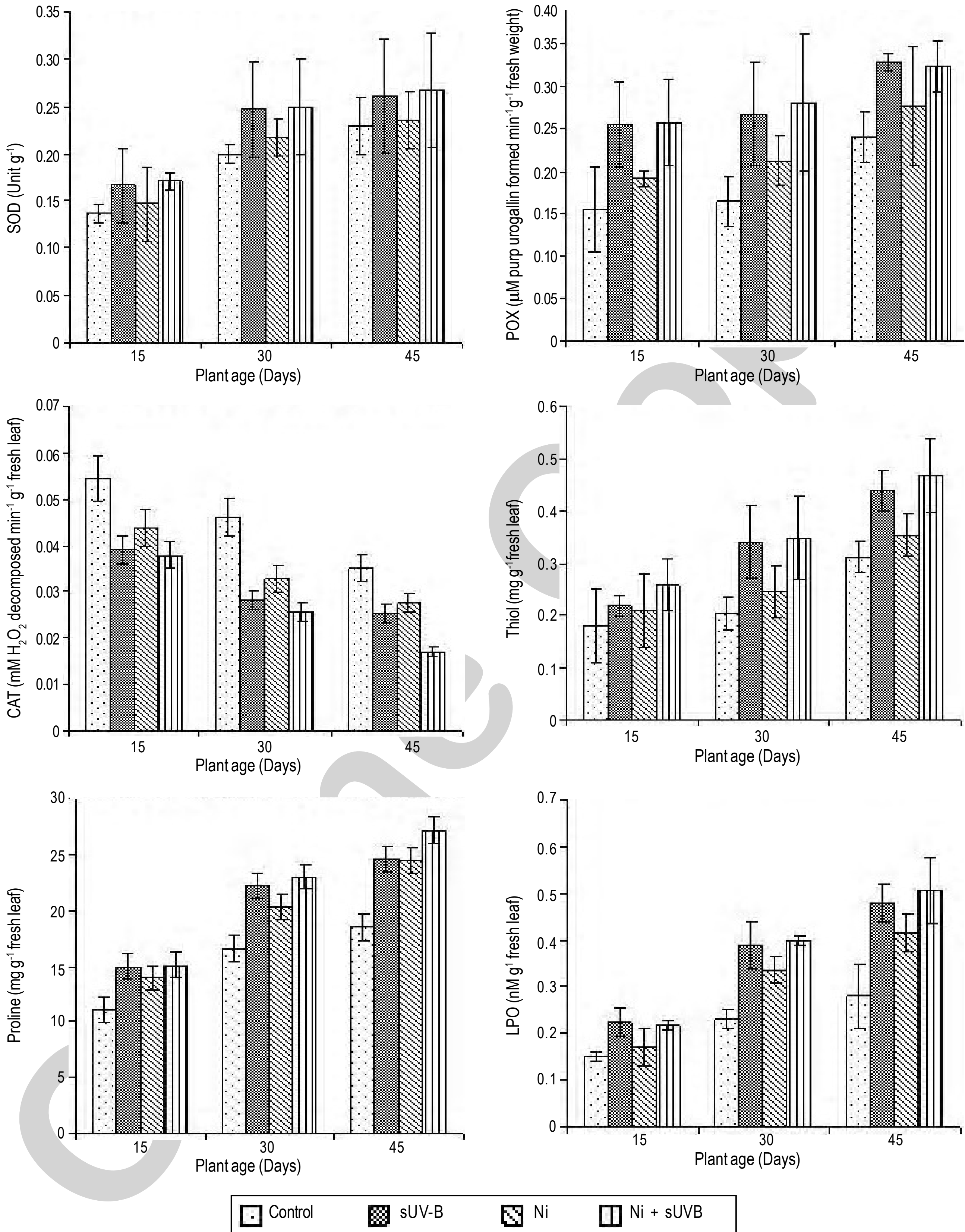

Fig. 4: Age wise changes in POX, SOD and CAT activities and LPO, proline and thiol contents due to sUV-B radiation and heavy metal (Ni) treatments given individually or in combination (bar represent mean ± 1SE)

superoxide anion, hydrogen peroxide and hydroxyl radicals. High ROS levels initiate signaling responses and include enzyme inactivation, gene expression, programmed cell death and cellular damage (Mackerness *et al*., 1998). SOD is ubiquitous, widely distributed multimeric metallic proteins, which catalyzes the dismutation of $\cdot O_2^-$ into H_2O_2. The increase in the activity of SOD, as observed in present study may be a consequence of the production of $\cdot O_2^-$ during sUV-B and Ni treatments. Agrawal and Mishra (2009) reported an increment of 17.4% in SOD activity under sUV-B stress at 45 days. SOD activity also increased significantly in *Alyssum mantinum* under Ni^{2+} treatments (Schickler and Caspi, 1999).

Peroxidase (POX) activity measured as purpurogallin formation was found to be higher under all the treatments at all the ages. At 45 days, increase in POX activities was 36.5, 14.9 and 34% under sUV-B, Ni and sUV-B+Ni treated plants, respectively as compared to controls (Fig. 4). In contrast to POX activity, catalase (CAT) activity decreased in treated plants at all ages as compared to the controls (Fig. 4). Reduction in CAT activity was always higher in individual treatment of sUV-B whereas interaction of both the stresses was synergistic causing more reduction in its activity. Reductions in CAT activity were 27.9, 21.4 and 51.5% in sUV-B, Ni and sUV-B+Ni, respectively when compared with their controls at 45 days (Fig. 4). Catalase and peroxidase are important enzymes of antioxidant defense system for scavenging H_2O_2 and free radicals (Gerbling *et al*., 1984). CAT is susceptible to photoinactivation and degradation. The inhibition of CAT activity may be ascribed to enzyme consumption to detoxify H_2O_2 or its inactivation under UV-B stress condition (Ambasht and Agrawal, 2003). Similar pattern of changes in activities of both enzymes were reported by Agrawal (2002) in *Vicia faba* under sUV-B radiation. Increased POX and reduced CAT activities were also reported in Ni treated *Helianthus annuus* and *Hyptis suaveolens* L plants (Pillay *et al*., 1996).

The study concludes that individual treatment of sUV-B and Ni stress adversely affected the primary and secondary metabolites with activation of antioxidative defense system. Among sUV-B and Ni, sUV-B leads to more increments in flavonoids, phenol, proline and thiol contents as well as in SOD, CAT and POX activities. sUV-B effect on SOD and CAT activites and proline, thiol and phenol contents was less than the combined effect of sUV-B and Ni. The interactive effects of both the stresses were, however, antagonistic for most of the parameters, synergistic for CAT while additive for SOD activity. The enzymes, CAT and SOD played the key role in triggering the antioxidant defense system under combination which resulted in less than additive effects on most of the parameters.

Acknowledgments

We thank to the Head Department of Botany, Allahabad Agricultural Institute, Deemed University (DU) Allahabad for necessary laboratory facilities and to CSIR (New Delhi) and UGC (New Delhi) for financial assistance.

References

Agrawal, S.B. and S. Mishra: Effects of supplemental ultraviolet-B and cadmium on growth, antioxidants and yield of *Pisum sativum* L. *Ecotoxicol. Environ. Saf.*, **72**, 610-618 (2009).

Agrawal, S.B. and D. Rathore: Changes in oxidative stress defense in wheat (*Triticum aestivum* L.) and mung bean (*Vigna radiata* L.) cultivars grown with or without mineral nutrients and irradiated by supplemental ultraviolet-B. *Environ. Exp. Bot.*, **59**, 21-23 (2007).

Agrawal, S.B., D. Rathore and A. Singh: Combined effects of enhanced ultraviolet-B radiation and mineral nutrients on growth, biomass accumulation and yield characteristics of two cultivars of *Vigna radiata* L. *J. Environ. Biol.*, **27**, 55-60 (2006).

Agrawal, S.B.: Physiological and Biochemical responses of field grown *Vicia faba* L. plants to supplemental ultraviolet-B radiation. *Nature Environ. Pollut. Tech.,* **1**, 67-72 (2002).

Alia, P., S. Pradha and P. Mohanty: Involvement of proline in protecting thylakoid membranes against free radical induced photodamage. *J. Photochem. Photobiol.*, **38,** 253-257 (1997).

Alscher, R.G. and J.L. Hess: Antioxidants in higher plants (*Eds.*: R.G. Alscher and J.L. Hess). CRC Press, Boca Raton. pp. 73-189 (1993).

Ambasht, N.K. and M. Agrawal: Interactive effects of ozone and ultraviolet-B singly and in combination on physiological and biochemical characteristics of soybean plants. *J. Plant Biol.,* **30**, 37-45 (2003).

Awasthi, S.K.: Prevention and Food Adulteration Act No. 37 of 1954. Central and State Rules as Amended for 1999, 3rd Edn., Ashoka Law House, New Delhi (2000).

Barsig, M. and R. Malz: Fine structure, carbohydrates and photosynthetic pigments of sugar maize leaves under UV-B radiation. *Environ. Exp. Bot.*, **43**, 121-130 (2000).

Bates, L.S., R.P Waldre and I.D. Teare: Rapid determination of free proline for water stress studies. *Plant and Soil*, **39**, 205-207 (1973).

Bowler, C., M. van Moutage and D. Inze: Superoxide dismutase and stress tolerance. *Annu. Rev. Plant Physiol. Mol. Biol.,* **43**, 83-116 (1992).

Bray, H.G. and W.Y. Thorpe: Analysis of phenolic compounds of interest in metabolism. *In*: Methods of biochemical analysis (*Ed.*: D. Glick). Vol. 1, Interscience Pub. New York. pp. 27-52 (1954).

Britton, C. and A.C. Mehley: Assay of catalase and peroxidases. *In*: Methods in enzymology (*Eds.*: S.P. Colowick and N.O. Kaplan). Vol II,Academic Press, New York. pp. 764-775 (1955).

Caldwell, M.M., A.H. Teramura, M. Tevini, J.F. Bornman, L.O. Bjorn and G. Kulandaivelu: Effects of increased solar ultraviolet radiation on terrestrial plants. *Ambio.,* **24**, 166-173 (1995).

Caldwell, M.M.: Solar ultraviolet radiation and the growth and development of higher plants. *In*: Photophysiology (*Ed.*: A.C. Giese). Academic Press, New York. pp. 131-171 (1971).

Day, T.A.: Ultraviolet radiation and plant ecosystem. *In*: Ecosystem, evolution and ultraviolet radiation (*Eds.*: C.S. Cockell and A.R. Blaustein). Springer-Verlag. New York. pp. 80-117 (2001).

Dillenburg, L.R., J.H. Sullivan and A.H. Teramura: Leaf expansion and development of photosynthetic capacity and pigments in *Liquidambar styraciflua* (Hamamelidaceae) of UV-B radiation. *Am. J. Bot.*, **82**, 878-885 (1995).

Duxbury, A.C. and C.S. Yentsch: Plankton pigment monographs. *J. Mar. Res.,* **15**, 19-101 (1956).

Fahey, R.C., W.C. Brown, W.B. Adams and M.B. Warsham: Occurrence of glutathione in bacterial cell. *J. Bacteriol.,* **133**, 1126-1129 (1978).

Flint, S.D., P.W. Jordon and M.M. Caldwell: Plant protection response to enhanced UV-B radiation under field conditions: Leaf optical properties and photosynthesis. *Photochem. Photobiol.*, **41**, 95-99 (1985).

Gerbling, K.P., G.J. Kelly, K.H. Fisher and E. Latzko: Partial purification and properties of soluble ascorbate peroxidase from pea leaves. *J. Plant Physiol.,* **115**, 59-67 (1984).

Giannopolitis, C.N. and S.K. Ries: Superoxide dismutase. I Occurence in higher plants. *Plant Physiol.,* **59**, 309-314 (1977).

Online Copy

Green, A.E.S., K.R. Cross, L.A. Smith: Improved analytical characterization of ultraviolet skylight. *Photochem. Photobiol.*, **31**, 59-65 (1980).

Hada, M., K. Hino and Y.Takeuchi: Development of UV defense mechanisms during growth of spinach seedlings. *Plant Cell Physiol.,* **42**, 784-787 (2001).

Hahlbrock, K. and D. Scheel: Physiology and molecular biology of phenyl propanoid metabolism. *Ann. Rev. Plant Physiol. Plant Mol. Biol.,* **40**, 347-369 (1989).

Halliwell, B.: Ascorbic acid and the illuminated chloroplast. *In*: Ascorbic acid: Chemistry, metabolism and uses (*Eds.*: P.A. Seib and B.M. Tolbert). American Chemical Society, Washington. pp. 263-274 (1982).

Heath, R.L. and L. Packer: Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. *Arch. Biochem. Biophys.,* **125**, 189-198 (1968).

Kar, M. and D. Mishra: Catalase, peroxidase and polyphenol oxidase activities during rice leaf senescence. *Plant Physiol.,* **57**, 315-317 (1976).

Keller, T. and H.S Schwager: Air pollution and ascorbic acid. *Eur. J. Forest Pathol.,* **7**, 338-350 (1977).

Krywult, M., J. Smykla, H. Kinnunen, F. Martz, M.L Sutinen, K. Lakkala and M. Turunen: Influence of solar UV radiation on the nitrogen metabolism in needles of scots pine (*Pinus sylvestris* L.). *Environ. Pollut.*, **156**, 1105-1111 (2008).

Kupper, H., F. Kupper and M. Spiller: Environmental relevance of heavy metal substituted chlorophyll using the example of water plants. *J. Exp. Bot.,* **47**, 259-266 (1996).

Larson, R.A.: The antioxidants of higher plants. *Phytochem.,* **27,** 969-978 (1988).

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

Mackerness, A.H.S., S.L. Surplus, B.R. Jordan and B. Thomas: Effects of supplementary ultraviolet-B radiation on photosynthetic transcripts at different stages of leaves development and light levels in pea (*Pisum sativum* L.) role of active oxygen species and antioxidant enzymes. *Photochem. Photobiol.*, **68**, 88-96 (1998).

Maclachalan, S. and S. Zalik: Plastid structure, chlorophyll concentration and free amino acid composition of chlorophyll mutant barley. *Can. J. Bot.,* **4**, 1053-1063 (1963).

Meagher, R.B.: Phytoremediation of toxic elemental and organic pollutants. *Curr. Opinion Plant Biol.,* **3**, 153-162 (2000).

Middleton, E.M. and A.H. Teramura: The role of flavonoids glycosides and carotenoids in protecting soybean from UV-B damage. *Plant Physiol.,* **103**, 741-752 (1993).

Mishra, S. and S.B. Agrawal: Interactive effects between supplemental UV-B radiation and heavy metals on growth and biochemical characteristics of *Spinacia oleracea* L. *Braz. J. Plant Physiol.,* **18**, 1-8 (2006).

Mohanty, N., I. Vass and S. Demeter: Impairment of photosystem II activity at the level of secondary quinone acceptor in chloroplasts treated with cobalt, nickel and zinc ions. *Physiol. Plant,* **76**, 386-390 (1989).

Ouzounidou, G.: Cu-ions mediated changes in growth, chlorophyll and other ion contents in a Cu-tolerant *Koeleria splendens*. *Biol. Plant*, **37**, 71-78 (1995).

Panda, S.K. and H.K Patra: Does a Cr ion produce oxidative damage in excised wheat leaves? *J. Plant Biol.,* **27**, 56-57 (2000).

Panda, S.K. and M.H. Khan: Antioxidant efficiency in *Oryza sativa* leaves under heavy metal toxicity. *J. Plant Biol.,* **30**, 23-29 (2003).

Pillay, S.V., V.S. Rao and K.V.N. Rao: Effect of nickel toxicity in *Hyptis suaveolens* L. and *Helianthus annuus* L. *Ind. J. Plant Physiol.*, **1**, 153-156 (1996).

Prasad, S.M., R. Dwivedi and M. Zeeshan: Growth, photosynthetic electron transport, and antioxidant responses of young soybean seedlings to simultaneous exposure of nickel and UV-B stress. *Photosynthetica,* **43**, 177-185 (2005).

Prinsze, C., T.M.A.R. Dubbleman and J.V. Steveninck: Protein damage induced by small amounts of photodinamically generated singlet oxygen or hydroxyl radicals. *Biochem. Biophys. Acta,* **1038**, 152-157 (1990).

Rai, L.C., B. Tyagi, P.K. Rai and N. Mallick: Interaction effects of UV-B and heavy metals (Cu and Pb) on nitrogen and phosphorus metabolism of a N_2-fixing cyanobacterium *Anabaena doliolum*. *Environ. Expt. Bot*., **39**, 221-231 (1998).

Randhawa, V.K., F. Zhou, X. Jin, C. Nalewayko and D.J. Kushner: Role of oxidative stress and thiol antioxidant enzymes in nickel toxicity and resistance in strains of the green alga *Scenedesmus acutus f. alternans*. *Can. J. Microbiol.,* **47**, 987-993 (2001).

Rauser, W.E.: Early effects of phytotoxic burdens of calcium, cobalt, nickel and zinc in white beans. *Can. J. Bot.,* **56**, 1744-1749 (1978).

Saleh, A.H.: Response of anabolic capacities, proline, protein patterns and mineral elements of nickel and EDTA stress in *Chorcorus olitorius*. *Pakistan J. Biol. Sci.,* **5**, 455-460 (2002).

Salmon, S., J.C. Maziere, R. Santus, P. Morliere and N. Bouchemal: UV-B-induced photoperoxidation of lipids of human low and high density lipoproteins. A possible role of tryptophan residues. *Photochem. Photobiol.,* **52**, 541-545 (1990).

Sasadhar, J.: Effects and relative toxicity of heavy metals on *Cuscuta reflexa*. *Water, Air Soil Pollut.,* **33**, 23-27 (1987).

Schickler, H. and H. Caspi: Response of antioxidative enzymes to nickel and cadmium stress in hyperaccumulator plants of the genus *Alyssum*. *Physiol. Plant.,* **105**, 1-39 (1999).

Schmelzer, E., W. Jahnen and K Hahlbrock: *In situ* localization of light-induced chalchone synthase mRNA, chalcone synthase, and flavonoids end products in epidermal cells of parsley leaves. *Proc. Nat. Acad. Sci. USA,* **85**, 2989-2993 (1998).

Severi, A.: Aluminum toxicity in *Lemna minor* L.: Effects of citrate and kinetin. *Environ. Expt. Bot.,* **37**, 53-61 (1997).

Sharma, R.K. and M. Agrawal: Biological effects of heavy metals: An overview. *J. Environ. Biol.,* **26**, 301-313 (2005).

Sheoran, I.S., H.R. Singhal and R. Singh: Effect of Cd and Ni on photosynthesis and the enzymes of the photosynthetic carbon reduction cycle in pigeon pea (*Cajanus cajan* L.). *Phosynth. Res.,* **23**, 345-351 (1990).

Shukla, J.K., P.K. Kasera, S. Mohammed and D.D. Chawan: Proline accumulation in *Prosopis cineraria* (L) druce growing in the Indian Thar desert. *J. Ind. Bot. Soc.,* **80**, 85-90 (2001).

Singh, D., K. Nath and Y.K. Sharma: Response of wheat seed germination and seedling growth under copper stress. *J. Environ. Biol.,* **28**, 409-414 (2007).

Singh, D.P. and S.P. Singh: Action of heavy metals on Hill activity and O_2 evolution in *Anacystis nidulans*. *Plant Physiol.,* **83**, 12-14 (1987).

Sumaryati, S., I. Negruti and M. Jacobs: Salt and water stress resistant mutants isolated from protoplast of *Nicotiana plumbaginifolia* (Viviani). Mededelingen- van-de faculteit Landbouwwelenscappen. Universitiet-Gent. 57 4a: 1507-1516. Proceedings part I. Sixth forum for applied biotechnology, Bruges, Belgium 24-25 September (1992).

Suzuki, N: Alleviation by calcium of cadmium-induced root growth inhibition in *Arabidopsis* seedlings. *Plant Biotech.,* **22**, 19-25 (2005).

Weatherhead, B., A. Tanskanen and A. Steverner: Ozone and ultraviolet radiation. *In*: Arctic Climate Impact Assessment Scientific Report (*Eds.*: C. Symon, L. Arries and B. Heal). Cambridge University Press, New York. pp. 151-182 (2005).

Weckx, J.E.J. and H.M.M. Clijsters: Zn phytotoxicity induces oxidative stress in primary leaves of *Phaseolus vulgaris* plant. *Physiol. Biochem.,* **35**, 405-410 (1997).

Yogamoorthi, A.: Artificial UV-B induced changes in pigmentation of marine diatom *Coscinodiscus gigas*. *J. Environ. Biol.,* **28**, 327-330 (2007).

Zenk, M.H.: Heavy metal detoxification in higher plants - A review. *Gene.,* **179**, 21-30 (1996).

Online Copy