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Study on individual and interactive effects of supplemental UV-B radiation and heavy metals on *Spinacea oleracea*

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Abstract

The effects of supplemental ultraviolet-B (sUV-B) irradiation and heavy metals (Cd and Ni) treatment alone and in combination were evaluated on the growth, biomass and yield of spinach plants. All the stresses caused reduction in biomass yield vis-a-vis alteration in its distribution pattern with more retention in below ground parts leading to higher root shoot ratio. Absolute growth rate (AGR) decreased in all treated plants due to reduction in their height at successive growth stages. Decrease in leaf area and number of leaves due to various stresses was responsible for decline in net assimilation rate (NAR), an index of photosynthetic assimilatory capacity of the plant. Supplemental UV-B increased the bioaccumulation of Cd and Ni in the root and shoot of exposed plants as compared to the control ones. The present study suggested that soil contaminated with Cd or Ni had a more negative impact on yield with higher retention of heavy metals in spinach growing under natural field conditions and exposed with elevated UV-B.

Key words

Bioaccumulation, Biomass, Heavy metals, *Spinacea oleracea,* UV-B radiation

Introduction

Ultraviolet-B (UV-B) radiation (280-315 nm) influences various biological processes, and is strongly absorbed by stratospheric ozone (O_3). Reduction in stratospheric O_3 concentration because of anthropogenic activities is therefore, important as it leads to corresponding increase in UV-B radiation reaching the Earth's surface (Yogamoorthi, 2007). However, even though the majority of these compounds are not currently used, it has been predicted that UV-B will continue to increase till 2050 (Weatherhead *et al.,* 2005). The increase in UV-B radiation causes damage in plants by negatively affecting photosynthesis and changing pigment content, affecting leaf area and plant height, biomass distribution, flowering time and crop yield (Kakani *et al.,* 2003). Biomass translocation pattern in underground parts were altered by UV-B leading to an increment in root to shoot biomass ratio (Singh *et al.,* 2011).

Bioaccumulation of toxic metals in humans can result in several critical symptoms in gastrointestinal, neurological, and immunological systems (Licata *et al.,* 2010). The most dangerous metals include the so-called "toxic trio": Cd, Pb, and Hg, for which no biological function has been found so far (Benavides *et al.,* 2005). Besides these, there is a long list of other metals which although essential in low doses, become toxic at high doses, such as Zn, Cu, Ni and Mn (Licata *et al.,* 2010). Soil is the principal source of Cd accumulated by plants. Many physico-chemical characteristics of soil and fertilization practices have been recognized as major factors which determine the bioavailability of Cd in the soil (Smolders *et al.,* 1998). Excessive use of phosphate fertilizers for the last 35 years in salt affected soils of Allahabad region of Uttar Pradesh, India have increased the Cd concentration in soils of this region up to 2.83 mg kg^{-1} of soil (Dar *et al.* 2011). Cadmium applied with phosphate fertilizer is highly available to plants and adversely affects the food quality. As yet, the problem is not only the presence of various toxic metals that might enlarge toxicity, but also the influence of one heavy metal in facilitating or limiting the accumulation of others (Liu *et al.,* 2008). Therefore to study the interaction of metals for bioaccumulation, Ni has been selected as another metal because it is a essential micronutrient for plants; and Cd and Ni are reported to use same membrane carrier for plant uptake.

India lies in a low stratospheric O_3 belt and receives high flux of UV-B radiation, which on increase may be damaging to the plants. Recently, Tandon and Attri (2011) observed significant decline of 3.6% in total ozone column (TOC) over numerous sampling stations lying in the northern part of India. Supplemental UV-B radiation and Cd in irrigated soils due to anthropogenic activities are 'new' man made stress and when present together may considerably affect the plants in various ways. Not much work has been done so far to assess the interactive effects of sUV-B, Cd and Ni on vegetables crops, and less has been focused on the effects of Cd contamination on the accumulation of other essential metals, such as Cu, Zn and Ni under natural field conditions. Therefore, objectives of the present study were to characterize the individual and interactive effects of sUV-B, Cd and Ni on the growth of spinach plants and to understand the pattern of accumulation of Cd and Ni under sUV-B to understand the adaptation of test plant against applied stresses. So, this study provides first hand data on the accumulation of Cd and Ni in different parts of spinach from soil under the influence of supplemental level of UV-B.

Materials and Methods

Experimental site : The present experiment was carried out at Agricultural Farm of Allahabad Agricultural Institute, Allahabad situated at 24º97'N latitude and 82°21'E longitude, at an elevation of about 96 m above sea level in the eastern Gangetic plains of India. The region has a moist, sub humid climate dominated by tropical monsoons. Physico-chemical properties of the experimental soil are given in Table 1.

The experimental soil collected from the agricultural farm was prepared using farmyard manure (30 kg ha^{-1}) according to common agronomic practices. Soil of the study pot was sandy loam in texture (sand 45, silt 28 and clay 27 %, respectively). The recommended doses of N, P and K were given at the rate of 44.8, 56 and 56 kg ha^{-1} in the form of urea, single superphosphate and muriate of potash, respectively. Each pot was filled with 8 kg of air - dried soil. The experiment had three individual treatments: supplemental UV-B radiation, Cd and Ni and their interaction namely sUV-B + Cd, sUV-B + Ni, Cd + Ni, sUV-B + Cd + Ni and control. For each treatment 20 pots were prepared.

Table 1 : Physico-chemical properties of experimental soil at the time of initial sampling

Parameters	Units	Values
pH		6.27 ± 0.01
Moisture	%	4.04 ± 0.80
SO_4^{2-} -S	%	6.80 ± 0.94
Bulk density	g cm^{-3}	0.70 ± 0.34
Porosity	%	75.8 ± 0.94
Organic carbon	%	1.85 ± 0.08
Organic matter	%	3.20 ± 0.09
Available P	mg 100 g^{-1}	12.8 ± 1.28
Total N	mg 100 g^{-1}	690 ± 20.4
Exchangable Na	mg 100 g^{-1}	34.0 ± 4.20
Exchangable K	mg 100 g^{-1}	98.0 ± 6.42
Exchangable Ca	mg 100 g^{-1}	20.4 ± 1.62
Background Cd	mg 100 g^{-1}	ND
Background Ni	mg 100 g^{-1}	ND

ND : Not detected; Values are mean of replicates ± SE

For heavy metal treatment, Cd and Ni were applied at the rate of 4 mg kg^{-1} soil as $CdCl_2$ and $NiCl_2$, respectively and mixed thoroughly with soil. For Cd + Ni treatment each heavy metal was applied at the rate of 4 mg kg^{-1} soil. Ten pots from each treatment namely Cd, Ni, Cd + Ni and control were randomly selected for sUV-B exposure. Uniform soil moisture was maintained in all pots throughout the experimental period.

Set up and standardization of UV-B treatment system under field conditions : Supplemental UV-B radiation was provided artificially by UV-B 313 fluorescent lamps (Q panel, Cleveland, OH, USA) fitted 30 cm apart on adjustable steel frame. Plants were irradiated after germination for 2 hr day^{-1} (10.00-12.00 a.m.) for 45 days. The lamps were covered by either 0.13 mm cellulose diacetate filters (Cadillac Plastics, Baltimore, USA) which absorbed radiation emitted by lamps below 280 nm (to exclude UV-C radiation) for sUV-B radiation or covered with 0.13 mm polyester filters (absorbed radiation below 320 nm) for the control. Aluminium reflecting strip was used to avoid scattering of UV rays from the upper side of the lamps. Filters were changed twice a week to avoid aging effects. The sUV-B intensity at the top of plant canopy under the lamps was measured with an UV intensity meter (UP Inc., San Gabriel, USA). The plant beneath cellulose diacetate film received UV-B$_{BE}$ (ambient +7.1 KJ m^{-2}) that mimicked 20 % reduction in stratospheric ozone at Allahabad (20° 47'N) during the clear sky condition (Agrawal and Mishra, 2009).

Growth analysis : Plants were sampled randomly in triplicates from respective treatments at 15, 30 and 45 days after sowing (DAS) for the analysis of growth (number of leaves, leaf area, root and shoot length, and component wise biomass accumulation). Intact roots were carefully dug out at random from each pot and carefully washed by placing them on 1-mm mesh sieves under running tap water to remove soil particles. Leaves were counted and leaf area was measured using a portable leaf area meter (Model LI-3000, LICOR, Inc. USA). Root and shoot length were separately measured and added to obtain total plant length. The plant roots and shoots were separated and oven dried at 80°C until constant weight was achieved. To obtain total plant biomass root and shoot dry weights were added. Final harvesting of the plants was done at 45 days and yield (fresh weight of leaves per pot) was measured.

For understanding the dry matter production and allocation pattern growth indices were calculated by formulae given by Hunt (1982). Yield response to stress (YRS) was calculated by the formulae given by Yu *et al.* (2006).

Analysis of heavy metals : Soil and plant samples (1 g) were digested by adding tri-acid mixture (HNO_3, H_2SO_4, and $HClO_4$ in 5:1:1 ratio) at 80 ºC until a transparent solution was obtained (Allen *et al.*, 1986). After cooling, the digested sample was filtered using Whatman no. 42 filter paper, and brought to a volume of 50 ml with deionised water. The concentration of Cd and Ni were determined by Atomic Absorption Spectrophotometer (Model 2380, Perkin-Elmer, Inc., Norwalk, CT, USA) fitted with specific lamp of particular metal using appropriate drift blank.

Phytoavailable heavy metals in the soil samples were extracted by the method given by Quevauviller *et al.* (1997). 10 g of soil sample was mixed with 20 ml of 0.05 N EDTA solution (pH = 7) and incubated for one day before filtering. Phytoavailable concentration of Cd and Ni in the filtrate was determined by using atomic absorption spectrophotometer (Model 2380, Perkin-Elmer, Inc., Norwalk, CT, USA).

The translocation factor (TF) for Cd and Ni was calculated by dividing their concentrations in above and below ground parts, as described by Singh and Agrawal (2007).

Effects of factors, sUV-B treatment, Cd, Ni, plant age and their interactions were determined by using SPSS software (SPSS Inc., version 12). Multivariate Analysis of Variance (ANOVA) test was conducted to determine the significant effects of sUV-B, Cd and Ni treatments, plant age and their interactions on data recorded. The quantitative changes observed for various growth parameters due to sUV-B radiation, Cd and Ni treatments were tested at $P < 0.05$ level of significance by Duncan's Multiple Range Test (DMRT). Data presented in tables are Mean ± 1SE.

Results and Discussion

The physico-chemical properties of soil taken in present research work are shown in Table 1. The pH of the soil sample was 6.27. The pH of soil is a considerable factor in terms of the heavy metal mobilization since it affects the solubility of the heavy metals, its stability and cation bond ability of the colloids as well as on the activity of the microorganisms (Szalai, 2008). In the present study, acidic condition of soil caused effective mobilization of metals (68.9% phytoavailability of Cd and 71.8% phytoavailability of Ni). Soil amended with FYM and NPK showed higher concentrations of cations because decomposition products of FYM in soil help in fixation/adsorption of the applied fertilizer and resulted into higher availability of the nutrients (Reddy *et al.*, 1999).

Table 2 : Total and phytoavailable concentration of Cd and Ni in the soil at the time of initial sampling

Heavy metal	Control	Cd	Ni	Cd+Ni
		(mg kg^{-1} of soil)		
Total Cd	ND	3.28 ± 1.12	ND	3.26 ± 1.24
Phytoavailable Cd	ND	2.26 ± 0.80	ND	2.16 ± 0.84
Total Ni	ND	ND	3.54 ± 1.16	3.52 ± 1.18
Phytoavailable Ni	ND	ND	2.52 ± 0.86	2.48 ± 1.12

ND: Not detected; Values are mean of replicates ±SE

The total heavy metal contents and phytoavailable fraction of heavy metals are shown in Table 2. The success of risk assessment of heavy metals contaminated soils depend on bio-availability of heavy metals in soil and transfer to food chain. Therefore, it is necessary to evaluate the mobile and/or available fractions of Cd and Ni in the soil. EDTA has been reported to give a very good indication of deleterious effect of toxic elements in soil and a reliable extraction methodology to predict plant-available metals (Cajuste and Laird, 2000).

All growth parameters of spinach plants were adversely affected by all the stress factors namely sUV-B, Cd and Ni either applied individually or in combinations (Table 3). Both sUV-B exposure and heavy metals, individually and in combination, retarded root and shoot length at all the ages. In case of individual treatments, reduction in shoot length due to sUV-B was more in comparison to individual application of either heavy metals (Cd or Ni). Contrary to shoot length, root length was affected more severely by heavy metal treatment (Cd > Ni) as compared to sUV-B radiation. Multivariate analysis of variance showed significant variations in shoot and root lengths due to plant age, sUV-B exposure, heavy metal treatments and interactions between plant age × sUV-B and plant age × heavy metals in all the test plants (Table 4). Reduction in plant height is a characteristic of UV-B sensitive plants. Height reduction was ascribed to photo-oxidative destruction of the phytohormone indole acetic acid (IAA) followed by reduced cell wall extensibility as demonstrated in sunflower seedling (Ros and Tevini, 1995). Similarly reduction in both root and shoot lengths were observed in *Cicer arietinum* L. on treatment with $NiCl_2$ and effect was more pronounced in roots (Sood *et al.,* 2001). Reduction in root length was always greater as compared to shoot length under heavy metal treatment of plants. This may not be surprising since roots are the first to come in direct contact with the injurious heavy metals.

Leaf number and leaf area in spinach were adversely affected by the exposure to sUV-B and heavy metal treatments singly and in their combined treatments (Table 3). Statistical variation in number of leaves and leaf area were significant due to plant age, sUV-B and heavy metals and interactions between plant age x sUV-B, plant age x heavy metals for all the tested plants (Table 4). Reduction in leaf number and leaf area were frequently reported in several UV-B sensitive species and cultivars (Gao *et al.,* 2003; Santos *et al.,* 2004). Significant reduction in leaf size indicated UV-B induced inhibition of either cell division or cell expansion by changing turgor pressure or cell

Table 3 : Age wise change in various growth parameters of *Spinacea oleracea* L. due to s UV-B radiation and heavy metals (Cd and Ni) alone and in combination (mean ± 1 SE)

Age (DAS)	Treatments	Root length (cm)	Shoot length (cm)	RSR (g g^{-1})	No of leaves	Leaf area (cm^{2})	Biomass (g plant^{-1})	NPP (g plant^{-1} day^{-1})
15	Control	7.90±0.05^{a}	10.16±0.10^{ab}	0.22±0.027^{a}	6.80±0.03^{a}	22.20±0.12^{d}	145.3±22.2^{d}	9.68±0.06^{a}
	UV-B	7.66±0.07^{ab}	8.10±0.04^{c}	0.26±0.029^{bc}	5.20±0.02^{c}	17.80±0.13^{a}	122.5±21.2^{a}	8.16±0.05^{b}
	Cod	7.50±0.05^{ab}	9.35±0.04^{cd}	0.26±0.029^{bc}	5.80±O.O2^{b}	18.12±0.14^{b}	123.8±23.2^{a}	8.25±0.04^{ab}
	Cd+|UV-B	7.28±0.06^{d}	7.90±0.02^{ab}	0.28±0.031^{ab}	4.62±0.02^{c}	15.65±0.12^{c}	112.0±16.4^{bc}	7.46±0.04^{c}
	Ni	7.56±0.05^{ab}	9.48±0.03^{ab}	0.23±0.025^{g}	6.24±0.02^{d}	19.84±0.12^{ab}	135.1±21.6^{cd}	9.00±0.06^{a}
	Ni+UV-B	7.34±0.07^{cd}	7.96±0.03^{d}	0.26±0.028^{h}	5.14±0.02^{ab}	16.90±0.13^{bc}	114.8±16.8^{bc}	7.65±0.05^{bc}
	Cd+Ni	7.20±0.04^{cd}	8.86±0.02^{bc}	0.22±0.024^{d}	4.84±0.03^{c}	17.12±0.13^{a}	116.0±18.4^{bc}	7.73±0.05^{bc}
	Cd+Ni+UV-B	7.10±0.04^{d}	7.45±0.04^{ab}	0.25±0.027^{ab}	3.85±0.03^{d}	13.94±0.14^{e}	105.2±14.6	7.01±0.04^{e}
30	Control	10.75±0.12^{a}	16.05±0.12^{a}	0.31±0.037^{c}	15.80±0.08^{a}	45.45±0.18^{a}	442.4±35.6^{a}	14.74±0.16^{a}
	UV-B	9.66±0.06^{ab}	12.80±0.08^{c}	0.36±0.047^{b}	12.35±0.07^{cd}	34.50±0.16^{bc}	354.4±32.8^{c}	11.81±0.12^{c}
	Cd	9.15±0.07^{b}	14.45±0.12^{b}	0.33±0.044^{ab}	13.43±0.03^{c}	35.78±0.15^{bc}	357.4±34.2^{c}	11.91±0.12^{c}
	Cd+|UV-B	8.80±0.04^{c}	12.40±0.08^{cd}	0.38±0.049^{a}	11.60±0.05^{de}	30.35±0.16^{cd}	312.7±30.8^{cd}	10.42±0.12^{cd}
	Ni	9.18±0.05^{ab}	14.67±0.12^{b}	0.34±0.046^{b}	14.25±0.03^{ab}	38.98±0.18^{b}	392.3±35.6^{ab}	13.07±0.14^{b}
	Ni+UV-B	8.84±0.06^{c}	12.56±0.10^{cd}	0.37±0.047^{ab}	12.56±0.04^{bc}	31.70±0.15^{d}	326.0±31.6^{cd}	10.86±0.10^{cd}
	Cd+Ni	8.75±0.06^{c}	12.60±0.10^{cd}	0.33±0.045^{c}	12.82±0.04^{bc}	33.64±0.18^{cd}	327.3±31.5^{cd}	10.91±0.11^{cd}
	Cd+Ni+UV-B	8.54±0.04^{c}	11.85±0.12^{d}	0.37±0.048^{ab}	10.42±0.04^{de}	28.15±0.14^{de}	295.3±28.5^{e}	09.84±0.06^{de}
45	Control	14.20±0.12^{a}	22.90±0.15^{a}	0.29±0.034^{c}	24.35±0.14^{a}	67.46±0.17^{a}	571.3±45.8^{a}	12.69±0.14a
	UV-B	11.60±0.11^{bc}	18.15±0.12^{c}	0.35±0.039^{b}	19.25±0.12^{c}	45.06±0.18^{cd}	445.4±40.6^{c}	09.80±0.10^{bc}
	Cd	10.50±0.08^{c}	20.25±0.13^{b}	0.34±0.038^{bc}	20.24±0.14^{bc}	50.14±0.16^{c}	455.6±42.8^{c}	10.10±0.12^{b}
	Cd+|UV-B	9.75±0.08^{cd}	17.15±0.15^{cd}	0.39±0.043^{a}	18.45±0.15^{cd}	41.66±0.14^{cd}	401.5±40.0^{d}	08.91±0.06^{cd}
	Ni	10.70±0.13^{bc}	20.48±0.16^{b}	0.31±0.034^{bc}	21.15±0.15^{b}	55.05±0.16^{b}	496.5±44.2^{b}	11.00±0.12^{ab}
	Ni+UV-B	9.84±0.10^{cd}	17.35±0.14^{cd}	0.38±0.044^{a}	18.50±0.13^{cd}	43.50±0.14^{c}	415.6±40.8^{d}	09.23±0.08^{b}
	Cd+Ni	9.72±0.08^{d}	17.42±0.13^{cd}	0.37±0.048^{ab}	19.35±0.08^{c}	45.32±0.12^{c}	421.2±41.2^{cd}	09.36±0.07^{b}
	Cd+Ni+UV-B	9.50±0.06^{d}	16.4±0.12^{de}	0.41±0.044^{a}	16.50±0.06^{e}	41.15±0.14^{cd}	382.2±39.3^{e}	08.49±0.05^{cd}

Values are mean of 3 replicates ± SE; DAS: Days after sowing; RSR: Root shoot ratio; NPP: Net primary productivity; Values in each column followed by different superscripts are significantly different at $P < 0.05$ (DMRT)

wall extensibility. UV-B induced inhibition of cell expansion has been observed in barley leaves (Liu *et al.,* 1995). Rehmann *et al.* (2011) reported reduction in leaf number and leaf area due to Cd treatment in tomato plants. Combined treatment of UV-B and Cd also reduced the leaf area in *Brassica napus* (Mishra *et al.,* 2010).

Total biomass increased with increasing age of test plant and percentage reduction in biomass also increased with successive growth stages in all the treated plants, as compared to control (Table 3). Reduction in biomass at last sampling was 22.0, 20.3, 29.8, 15.3, 29.1, 26.3 and 33.1% in plants due to sUV-B, Cd, sUV-B+Cd, Ni, sUV-B+Ni, Cd+Ni and sUV-B+Cd+Ni treatments, respectively. Variations in biomass and NPP were significant due to all individual factors and their interactions (Table 4). Adaptive responses in biomass production and its allocation to different parts may be a primary mechanism by which the species can cope with the environmental characteristics of their respective habitats (Wu *et al.,* 2008). The exposure to sUV-B and heavy metal stress caused significant reductions in biomass and NPP. Similar reduction in biomass was observed by Shukla *et al* (2002) in *Triticum aestivum* seedlings and in cucumber (Mishra *et al.,* 2010) due to combined stress of UV-B and Cd. The total plant dry weight was also reported to be more sensitive to Cd than Ni (Agrawal and Mishra, 2009). Heavy metal contaminations of soil often reduced the availability of soil nutrients and limited the absorption ability of roots resulting in decreased in biomass production (Sun *et al.,* 2008). Decrease in NPP after exposure to UV-B and heavy metal treatments depicted a reflection of negative effects of these stress factors on biomass production.

Root shoot ratio (RSR) showed higher value in all the treated plant at all the ages. Increase in RSR was 13.6%, 19.4% and 41% due to sUV-B+Cd+Ni treatment at 15, 30 and 45 DAS, respectively. Variations in RSR were significant due to plant age, sUV-B and heavy metals (Table 3). Results of the present study suggested that sUV-B had more negative effect on shoot as compared to root, resulting in higher RSR. It remains unclear whether the UV-B induced changes in RSR resulted because of altered ontogeny or due to altered photosynthate partitioning priorities. RSR value increased at later stages of sampling showed that more photosynthate was translocated to underground parts to limit the growth of aerial parts as a defense

Table 4 : F- ratios and levels of significance of four way ANOVA test for various growth parameters of *Spinacea oleoracea* L. plants

Parameter	Plant age (A)	sUV-B treatment (T)	Cadmium (C)	Nickel (N)	A × T	A × C	A × N	T × C	T × N	C × N	A×T×C	A×T×N	T×C×N	A×T×C×N
Plant height	185.4^{***}	332.90^{***}	140.12^{***}	160.32^{***}	12.30^{**}	11.80^{*}	12.30^{**}	14.80^{*}	11.60^{*}	10.42^{*}	0.48^{NS}	0.10^{NS}	6.12^{NS}	0.09^{NS}
Root length	178.45^{***}	32.20^{***}	42.90^{***}	36.12^{***}	10.80^{*}	14.40^{**}	16.70^{**}	1.48^{NS}	1.16^{NS}	1.14^{NS}	0.46^{NS}	0.89^{NS}	6.72^{NS}	0.03^{NS}
AGR	361.75^{***}	29.38^{***}	21.40^{***}	20.60^{***}	23.32^{**}	7.60^{*}	8.80	9.40^{*}	0.72^{NS}	1.18^{NS}	0.46^{NS}	1.62^{NS}	1.80^{NS}	1.41^{NS}
Biomass	460.8^{***}	609.0^{***}	124.12^{***}	120.60^{***}	24.13^{**}	11.24^{*}	14.67^{**}	6.72^{*}	7.32^{*}	1.12^{NS}	10.12^{*}	10.12^{*}	10.18^{*}	3.60^{NS}
NPP	218.70^{***}	48.60^{***}	36.12^{***}	30.14^{***}	11.69^{**}	1.27^{NS}	1.11^{NS}	1.30^{NS}	1.32^{NS}	1.46^{NS}	1.12^{NS}	7.61^{*}	7.14^{*}	1.41^{NS}
Leaf area	612.3^{***}	569.90^{***}	120.10^{***}	90.32^{***}	62.23^{***}	3.24^{NS}	2.46^{NS}	10.12^{*}	1.28^{NS}	6.29^{NS}	8.90^{*}	14.60^{**}	13.1^{**}	3.40^{NS}
Leaf No	120.60^{***}	21.40^{***}	23.60^{***}	24.12^{***}	3.16^{NS}	1.42^{NS}	1.30^{NS}	0.70^{NS}	0.92^{NS}	6.62^{NS}	0.15^{NS}	1.58^{NS}	1.40^{NS}	0.14^{NS}
NAR	150.20^{***}	29.60^{***}	24.12^{***}	32.60^{***}	10.15^{**}	2.10^{NS}	1.42^{NS}	1.30^{NS}	1.28^{NS}	0.80	0.47^{NS}	0.51^{NS}	0.42^{NS}	0.13^{NS}
RGR	370.42^{***}	48.60^{***}	44.112^{***}	40.18^{***}	16.60^{**}	2.10^{NS}	3.10^{NS}	1.44^{NS}	1.24^{NS}	1.12^{NS}	0.84^{NS}	1.32^{NS}	1.12^{NS}	0.09^{NS}
RSR	161.01^{***}	30.32^{***}	31.12^{***}	28.20^{***}	0.94^{NS}	1.18^{NS}	0.92^{NS}	0.84^{NS}	0.70^{NS}	0.64^{NS}	1.14^{NS}	1.75^{NS}	1.70^{NS}	0.21^{NS}

Level of significance: * = p<0.05, ** = p<0.01, *** = P<0.001. NS = Not significant

strategy. Hofmann *et al*. (2001) also reported increased RSR as defense strategy against UV-B stress in white clover plants.

Absolute growth rate (AGR) for plant height showed reduction in all treatments at all sampling intervals (Fig. 1) Reduction in AGR suggested less increment in plant height during two sampling dates. Maximum reduction in AGR was seen for plants treated with all the three stresses together i.e., sUV-B+Cd+Ni and it was 25.2 and 33.5% at 15-30 and 30-45 DAS intervals, respectively. AGR varied significantly with plant age, sUV-B and heavy metal treatments and for interactions between plant age × sUV-B, plant age × heavy metal and sUV-B × Cd (Table 4). AGR is the function of amount of growing material present. Reduction in AGR due to UV-B exposure and heavy metal stress reflects that growth of plants reduced with an increase in UV-B and heavy metal stress.

RGR and NAR were also decreased at successive plant age intervals in all the treated plants. Both RGR (7.7% and 7.6%) and NAR (2.9% and 5.6%) reduced maximally for combined treatment (sUV-B+Cd+Ni) at 15-30 and 30-45 DAS intervals, respectively (Fig. 1). Statistical variations in RGR and NAR were significant due to plant age, sUV-B and heavy metal treatments (Table 4). Lower RGR value due to combined treatments further suggested the relative influence of sUV-B radiation and heavy metal treatments on productivity. Inhibition of RGR was also reported by Han *et al*. (2008) in Cu-treated *Ulva* spp and Hofmann *et al*. (2001) in white clover plants due to UV-B radiation. Reduction in size and efficiency of assimilatory surface due to UV-B and heavy metal treatments was responsible for the decline in NAR. NAR is an index of the photosynthetic assimilatory capacity of the plant per unit of leaf area. Reduction in NAR further suggests that UV-B radiation and heavy metal treatments could induce inhibition of net photosynthesis and acceleration of respiration in plants. Comont *et al*. (2013) reported adverse affect of UV-B radiation on NAR in *Lolium perennae forage* crop.

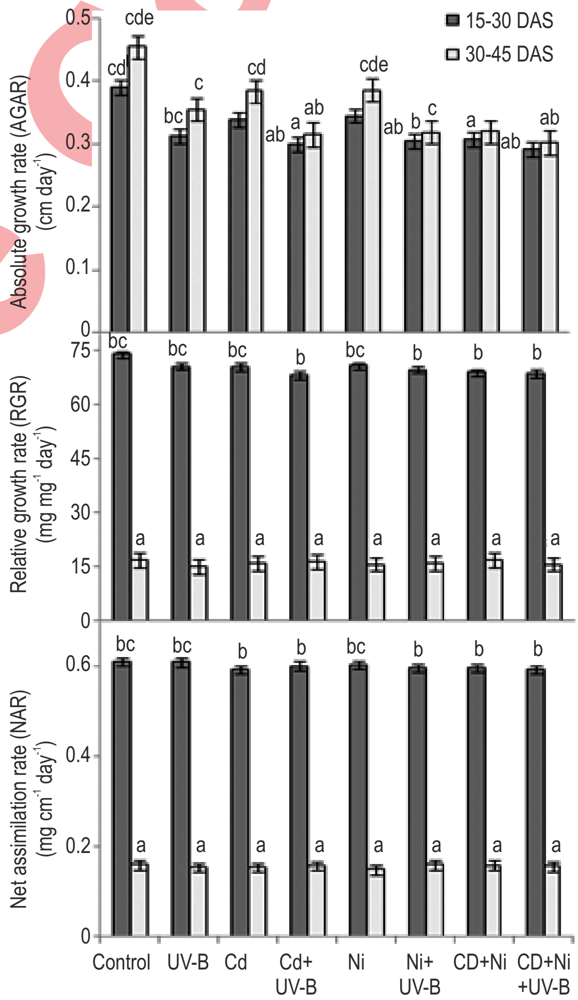

Fig. 1 : Yield responses to stress of *Spinacea oleracea* L. due to sUV-B radiation and heavy metals (Cd and Ni) alone and in combination; Values are mean of replicates ± SE. Level of significance: $^{***}p<0.001$; $^{**}p<0.01$; $^{*}p<0.05$)

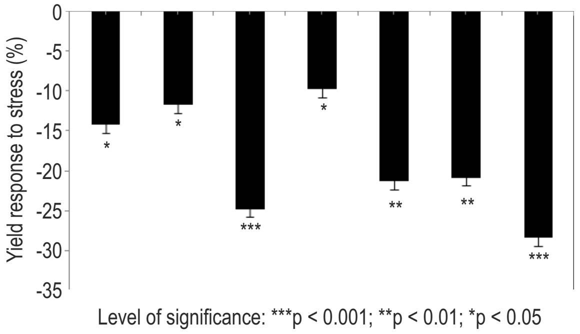

Fig. 2 : Yield responses to stress of *Spinacea oleracea* L. due to sUV-B radiation and heavy metals (Cd and Ni) alone and in combination (mean ± 1SE). (Level of significance: $^{***}p < 0.001$; $^{**}p < 0.01$; $^{*}p < 0.05$)

The yield of spinach decreased due to treatment with sUV-B and heavy metals, applied alone or in combination. Yield response to stress (YRS) measures reduction in yield as compared to control plants. It was reduced from 11.8 to 28.5% in response to various treatments. Multivariate analysis showed that YRS varied significantly due to various treatments and their interaction (Fig. 2). Reduction in yield is a typical index of sensitivity of plants to various stresses, as it represents the cumulative effects of damaged or inhibited physiological functions. In the present investigation, all the test plants showed reduction in yield with sUV-B and heavy metal stress applied either individually or in combinations. The dry matter and yield of many higher plants such as pea, wheat, rapeseed and maize have been reported to decrease under multiple heavy metal stresses (Sharma and Agrawal, 2005).

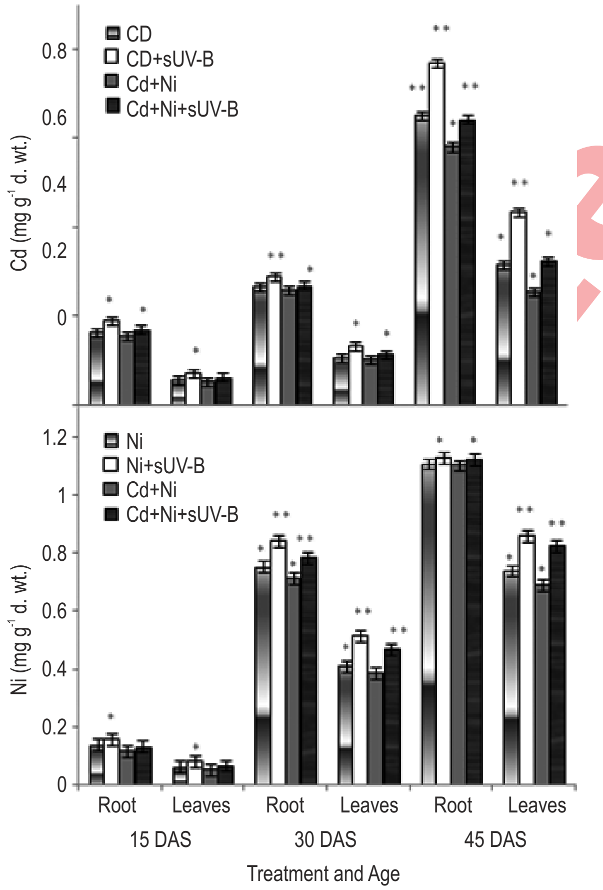

Fig. 3 : Age wise variation in Cd and Ni accumulation in root and shoot of *Spinacea oleracea* L. due to sUV-B radiation and heavy metals (Cd and Ni) alone and in combination (mean ± 1SE). (Level of significance: $^{***}p < 0.001$; $^{**}p < 0.01$; $^{*}p < 0.05$)

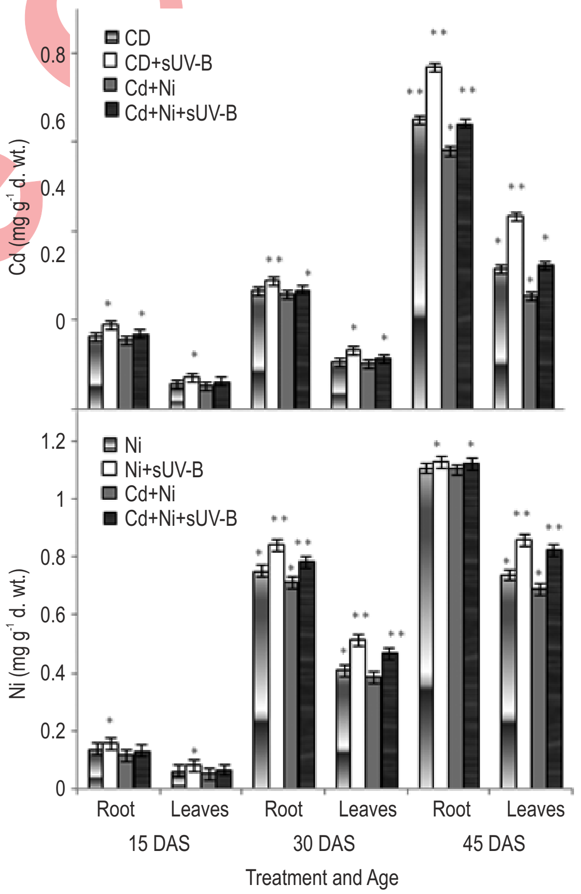

Fig. 4 : Age wise variation on translocation factor of Cd and Ni in *Spinacea oleracea* L. due to sUV-B radiation and heavy metals (Cd and Ni) alone and in combination (mean ± 1SE) (Level of significance: $^{***}p < 0.001$; $^{**}p < 0.01$; $^{*}p < 0.05$)

Bioaccumulation of heavy metals was always higher in plants exposed to the combined treatments of sUV-B+Ni and sUV-B+Cd, whereas it was lower in the case of Cd+Ni treatment, as compared to individual treatment with Cd or Ni (Fig. 3). The translocation factor (TF) was also observed to be less than a unit, both in the plants exposed to sUV-B and in those that were not. Maximum increase in TF values were 23.2% and 23.6% at 15-30 DAS and 30-45 DAS, respectively with combined treatment of sUV-B+Ni (Fig. 4). Roots generally show higher heavy metal contents than shoots because they are the first organ to come into contact with the toxic metals. This has been shown through the results of the present investigation, where both Cd and Ni contents were higher in roots than shoots in all the treated plants. Similarly, Dixit *et al.* (2001) and Saleh (2002) also showed maximum accumulation of Cd and Ni in roots than shoots in *Pisum sativum* and *Chorcorus olitoius*, respectively. In case of combined metal stress, interaction between heavy metals may be independent, antagonistic, additive or synergistic. Thus uptake of metals into roots of plants was changed when metals were applied in combinations. Clarkson and Lüttge (1989) reported that Cu and Zn, Ni and Cd compete for the same membrane carriers for uptake of heavy metals from soil solution into the root. An increased uptake of both Cd and Ni was observed after sUV-B exposure in the test plant. Agrawal and Mishra (2009) also reported increased uptake of Cd after UV-B exposure to pea plants. The accelerated uptake of metals not only suggested an increase in cell permeability but also support the earlier reports on the damage of biomembranes due to UV-B and heavy metals.

Translocation factor (TF) represents the ability of plants to pump heavy metals from roots to shoots. Heavy metals are transported from roots to shoots in terrestrial plants to different extents. In present study, Ni showed more mobility than Cd. Ni might be translocated extensively as it has been classified among essential micronutrients and remains associated with some metallo-enzymes; however it is toxic at supraoptimal level to plants. The TF values obtained in the present study suggests that the tested spinach plants tended to accumulate more Ni and Cd in underground parts than above-ground parts. Singh et al. (2008) also reported that TF values were less than a unit for Cd and Ni in *Beta vulgaris* L. plants grown on sewage sludge and fly ash-amended soils, respectively. The higher accumulation of both Cd and Ni in roots may be complexation of heavy metals with the sulfhydryl groups, resulting in less translocation of heavy metals to shoots (Singh *et al.,* 2004). Plants may have an adaptation mechanism for regulating heavy metal allocation in roots rather than in aerial parts (shoots), thereby reducing the incidence of heavy metal-induced oxidative stress in photosynthetic organs. The result related to the uptake of metals in this study suggests that roots of *Spinach* are efficient barriers to Cd and Ni translocation to above ground plant parts. These strategies employed by plants, at least in part, counteract the prevailing stress conditions.

The present study clearly showed that the combined application of sUV-B radiation and Cd and Ni caused more adverse effect on the growth and development of plants than their individual treatment, and not only confirmed the hypothesis of synergistic inhibition of different metabolic processes jointly by sUV-B and heavy metals. It also suggests that the environmental hazards of UV-B radiation as expected in future would be more intensified than expected in the natural systems already contaminated with heavy metals.

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