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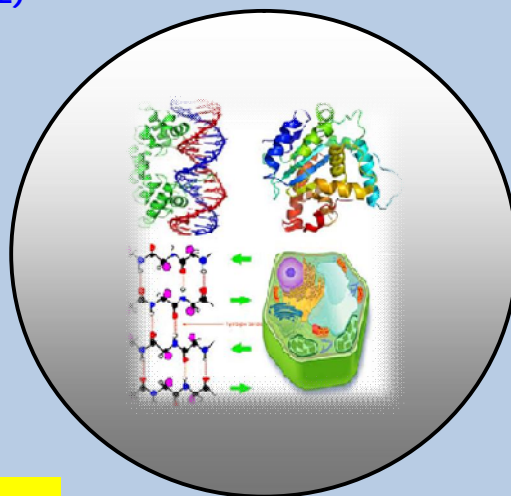
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Bio-accumulation of Heavy Metals (Zn, Cu, Fe, Cd, Ni and Cr) and Biochemical Responses of Wild Plants near Express Highway (NH 25) in Unnao District, Uttar Pradesh State (India)

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ABSTRACT

The study was carried out to evaluate the tissue accumulation of heavy metals (Zn, Cu, Fe, Cd, Ni and Cr) and some biochemical constituents (pigments and protein contents) in wild plants exposed to environmental conditions near express highway (NH 25) in Unnao district (U.P. state, India). Wild plants accumulated high content of iron (9.8 to 112.8 $\mu\text{g Fe g}^{-1}$ dr. wt.), Cd (8.2 to 17.5 $\mu\text{g Cd g}^{-1}$ dr. wt.), Ni (6.3 to 17.2 $\mu\text{g Ni g}^{-1}$ dr. wt.) and Cr (12.4 to 16.6 $\mu\text{g Cr g}^{-1}$ dr. wt.) near NH 25, the accumulation of these metals was lower in wild plants away from the NH 25. The biochemical responses with respect to chlorophylls (a, b and total), carotenoids, and protein contents in wild plants showed low value near NH 25 as compared to wild plants away from the NH 25. Therefore, transport activities contribute heavy metals accumulation and decreased pigments and protein content in wild plants near NH 25.

Key words: Heavy Metals, Pigments, Protein and Express Highway.

INTRODUCTION

The development of a wide range of transportation system in all over the world and safety of environment from its various activities is a global challenge. The issue of transport activities and the environment is paradoxical in nature since transportation conveys substantial socio-economic benefits, but at the same time transportation is also contribute pollutants including heavy metals in to the environmental systems.

The metals are classified as "heavy metals" if, in their standard state, they have a specific gravity of more than 5 g/cm³.

There are sixty known heavy metals (Du Laing *et al.*, 2009). Heavy metals can accumulate over time in soils and plants and their excess levels could have a negative influence on physiological activities of plants such as photosynthesis, gaseous exchange, nutrient absorption and causing reductions in plant growth and dry matter yield (Devkota and Schmidt, 2000).

Heavy metals in the atmosphere, soil and water, some are even in traces (Cd, Cr, Hg, Ni, As etc.) can cause serious problems to all living organisms, and their bioaccumulation in the food chain especially can be highly dangerous to human health (Afyoni *et al.*, 1998; Pilon-Smits, 2005). The entry of pollutants in the environment and their adverse effects on living beings increased greatly in few decades due to transport activities (Kumar and Pandey, 2010). Elevated levels of heavy metals in plants growing near highways may tolerate their adverse effects upto a critical limits of toxicity, thereafter pose negative effects on metabolic activities ultimately may cause vegetation loss (Pandey, 2014).

Heavy metals accumulate in living organisms, circulate in the trophic chain and moreover their dangerous concentrations persist in ecosystems for a long time (Tiller, 1989; Pandey and Sharma, 2002; Tlustoš *et al.*, 2006). Plant roots participate primarily in the heavy metal ions uptake (Lasat, 2002). The elevated concentration of heavy metals cause disturbances in cell membrane functioning in the photosynthetic and mitochondrial electron transport and in the inactivation of many enzymes activity in the basic cell metabolism regulation, which as the result leads to diminishing energy balance and disturbances in cell mineral nutrition (Kosobrukhov *et al.*, 2004). The accumulation of heavy metals in vascular plants provokes significant biochemical and physiological responses (Meenakshi and Pandey, 2009) and modify several metabolic processes (Macfarlane *et al.*, 2003). Wild plants grow near express highway, face various adverse environmental conditions. The pollutants including heavy metals emitted from transport activities enter into plant system either through root from the soil or leaves through foliar dust. Least informations are available about the extent of heavy metals accumulation and biochemical constituents level in wild plants grow near highways transport system. Therefore, study was carried out on these aspects may be helpful for eco-friendly management of transport system to protect the vegetation.

MATERIAL AND METHODS

The study areas were selected at express highway (NH 25) in Unnao district (26° 28' N Latitude and 80° 43' E longitude). The express highway (NH 25) also links Lucknow to Kanpur district in Uttar Pradesh state (India). A composite soil sample collected from site I and II just near (0-10 m) to NH 25 and analyzed for some physico-chemical properties (Table 1). To evaluate the accumulation level of heavy metals (Zn, Cu, Fe, Cd, Cr and Ni) in wild plants near express highway (NH 25), and their biochemical responses against heavy metals, the experiment was carried out. Wild plant species were collected from the Unnao district near the express highway (NH 25). These collected wild plants were analyzed for tissue accumulation of heavy metals after digestion with nitric and perchloric acid (Piper, 1969). Plants were also analyzed for some biochemical constituents.

The harvested wild plants collected from different study sites I and II (0-10 m near NH 25) and III and IV (about 5 km away from NH 25).

These collected wild plants were crushed finely in pestle and mortar in 10 ml cold 80% acetone centrifuged at 1000 rpm of 20 minutes in centrifuged tubes for pigments content. Later supernatant was transferred to glass tubes and volume was made 10 ml by adding 80% cold acetone. Absorbance of the supernatant for chlorophyll a, chlorophyll b and carotenoids content was taken at wavelengths 663.6, 646.6, 510.0 and 480.0 nm, respectively in spectrophotometer (Perkin Elmer Lambda 40, USA). Chlorophyll a and b and carotenoids content were calculated on leaf fresh weight basis, as method given by Porra *et al.* (1989). Protein content was estimated by the method of Lowry *et al.* (1951).

The concentration of heavy metals in shoots was determined in oven dried plants by Atomic Absorption Spectrophotometer. Wet digestion of plant samples was carried out in nitric acid: perchloric acid (HNO₃: HClO₄) (3:1 v/v) by heating till insepoint dryness. These evaporated samples were diluted, filtered with Whatman No. 42 and finally volume was made upto 25 ml with distilled water and analyzed for heavy metals (Zn, Cu, Ni and Cr) respectively in Atomic Absorption Spectrophotometer (A Anylist Perkin Elmer 700, USA). Concentration of heavy metals in plant tissue is expressed as $\mu\text{g g}^{-1}$ dry weight.

The heavy metals were estimated in soil by the method of Lindsay and Norvwell (1978). The physico-chemical properties of the soil were determined by the methods described by Piper (1969). Data presented in the table are mean \pm S.E. value (n=3) and statistically analyzed using student 't' test for significance.

RESULTS

Assessment of heavy metals (Zn, Cu, Fe, Cd, Cr and Ni) accumulation and some biochemical constituents such as pigments (chlorophyll a, b, total chlorophyll and carotenoids) and protein contents were determined in some wild plants (*Nerium*, *Bougainvillea* and *Croton*) and compared. Plants were collected from nearby areas of express highway – NH 25 (0 to 10 m) at sites I and II exposed continuously to the vehicular environment; and same plant species were also collected from about 5 km away (site III and site IV) from NH 25. These wild plant species analyzed for metals accumulation in their tissues and for some biochemical constituents.

Soil properties

Composite soil samples collected from different study sites at NH 25 were analyzed for some important physico-chemical properties, data are presented the Table 1. The pH of the soil was slightly alkaline, sandy loam in texture, poor in organic matter (< 0.4%) with moderate calcium carbonate content (0.7 to 3.6%). Soil contained carbonate and bicarbonate ions. The heavy metal Fe content was high (40-60 ppm), whereas other studied heavy metals content was low (< 0.5 ppm) in soil.

Tissue concentration of heavy metals

Wild plants estimated for heavy metals accumulation in pre-monsoon period (2011) are presented in table 2A and 2B. These plants showed elevated levels of metal accumulation. The range of heavy metals accumulation was determined 2.5 to 35.6 $\mu\text{g g}^{-1}$ dry weight. These plants also accumulated some toxic metals (such as Cd and Cr) which are not essential to the plant growth.

The wild plants showed accumulated heavy metals at site I and II ranged from 1.2 to 119.8 $\mu\text{g g}^{-1}$ dry weight as compared to plants away from the NH 25 was 0.8 to 22.8 $\mu\text{g g}^{-1}$ dr. wt. Maximum accumulation of Fe in wild plants was observed (1.2 to 119.8 $\mu\text{g g}^{-1}$ dry weight). Thus, the tissue accumulation of heavy metals was higher at site I and II just near to NH 25 (0-10 m) than the sites III and IV away from the NH 25.

Zinc

Wild plants accumulated high content of Zn at sites I and II exposed to environmental conditions near (0-10 m) express highway NH 25 ranged from 7.8 to 35.5 $\mu\text{g g}^{-1}$ dry weight. Maximum accumulation of Zn was found in *Bougainvillea* (35.5 $\mu\text{g g}^{-1}$ dr. wt.). The order of accumulation of Zn in wild plants was *Bougainvillea* > *Calotropis* > *Croton* > *Parthenium* > *Ageratum* > *Euphorbia* > *Nerium*. The accumulation of Zn did not followed any regular pattern in between plants, and showed variable concentrations at different sites.

Copper

The accumulation of Cu in wild plants ranged from 3.8 to 25 $\mu\text{g g}^{-1}$ dr. wt. Maximum accumulation of Cu was found in *Calotropis*. The order of Cu accumulation was *Calotropis* > *Parthenium* > *Bougainvillea* > *Nerium* > *Ageratum* > *Croton* > *Euphorbia* was observed. The least accumulation of Cu was determined in *Euphorbia* species grown near express highway (NH 25).

Iron

Iron accumulation in wild plants showed maximum value as compared to other metals studied at site I and II just near to (0 – 10 m) express highway (NH 25). Maximum tissue concentration of iron (Fe) was determined in *Calotropis*. The accumulation of iron was determined in order *Calotropis* > *Nerium* > *Croton* > *Euphorbia* > *Parthenium* > *Ageratum*. Least accumulation of iron was determined in *Ageratum* species (9.8 $\mu\text{g g}^{-1}$ dr. wt.) at site II. The tissue concentration of iron was ranged from 9.8 to 119.8 $\mu\text{g g}^{-1}$ dr. wt. in wild plants grown near express highway (NH 25) was low than site III and IV.

Cadmium

Cadmium (Cd) concentrations in wild plants determined in the range of 6.5 to 22.5 $\mu\text{g g}^{-1}$ dr. wt. *Croton*, *Parthenium* and *Bougainvillea* showed higher accumulation of Cd (8.2 to 17.5 $\mu\text{g g}^{-1}$ dr. wt.). Comparatively, low accumulation of Cd was determined in *Nerium*, *Euphorbia*, *Croton* and *Calotropis* sps. than other wild plants studied.

Chromium

Chromium accumulation in wild plants near (0-10 m) the express highway (NH 25) was ranged from 2.5 to 16.6 $\mu\text{g g}^{-1}$ dr. wt. The wild plants *Bougainvillea*, *croton* and *Parthenium* showed higher accumulation of Cr (12.4 to 16.6 $\mu\text{g Cr g}^{-1}$ dr. wt.) as compared to other plants studied. The order of accumulation of chromium in wild plants near expressway was *Bougainvillea* > *Croton* > *Nerium* > *Parthenium* > *Ageratum* and *Euphorbia*.

Nickel

A high accumulation of nickel (Ni) was quantified in wild plants at site I and II near express highway (NH 25). The nickel concentration of wild plants ranged from 6.3 to 27.6 $\mu\text{g g}^{-1}$ dr. wt. The plants showed higher accumulations of Ni were *Ageratum*, *Bougainvillea* and *Nerium* (6.3 to 17.2 $\mu\text{g Ni g}^{-1}$ dr. wt.). Whereas, *Euphorbia*, *Croton*, *Parthenium* and *Calotropis* showed low Ni content (5.8 to 11.6 $\mu\text{g g}^{-1}$ dr. wt.) in their tissues.

The accumulation of heavy metals (Zn, Cu, Fe, Cd, Ni and Cr) in wild plants showed higher values at sites I and II (just near to express highway as compared to sites III and IV (about 5 km away from the express highway (NH 25).

Biochemical responses

Pigments

Wild plants collected from adjacent areas to the NH 25 showed chlorophyll 'a' content ranged from 0.18 to 0.51 mg g⁻¹ fr. wt. as compared to the wild plants away from the NH 25 (ranged 0.6 to 0.92 mg g⁻¹ fr. wt.). The *Croton* sps. showed maximum value of chlorophyll 'a' content than other species studied. The chlorophyll 'b' content at sites I and II (ranged 0.35 to 0.72 mg g⁻¹ fr. wt.) in leaves determined was lower than in leaves of wild plants at sites III and IV (ranged 1.0 to 1.45 mg g⁻¹ fr. wt.). Similarly, total chlorophyll content in wild plants leaf at site I and II (ranged 1.0 to 1.6 mg g⁻¹ fr. wt.) was lower than at sites III and IV (ranged 1.42 to 2.53 mg g⁻¹ fr. wt.) about 5 km away from the NH 25. The carotenoids content in wild plants also followed similar trend like chlorophyll 'a' and 'b' at sites I and II (ranged 0.65 to 1.12 mg g⁻¹ fr. wt.) and sites III and IV (ranged 1.1 to 1.65 mg g⁻¹ fr. wt.). Comparatively, *Croton* species showed higher values of all the pigments content estimated than the *Nerium* and *Bougainvillea* at all the study sites at NH 25 in Unnao district.

Table 1. Physico-chemical properties of soils collected from area just near to express highway (NH-25) in Unnao district in the year, 2011.

Parameters	Minimum	Maximum	Average
pH (1: 2.5, soil: water ratio)	7.2	7.9	7.5±
E.C. (mS/cm)	0.73	1.6	1.28±
Texture	Sandy loam	-do-	-do-
Bulk density (g/m ³)	0.98	1.2	1.0±
Organic matter (%)	0.21	0.34	0.25±
Calcium carbonate (%)	0.72	3.6	1.46±
Calcium (meq./100 g soil)	2.5	4.8	3.2±
Magnesium (meq./100 g soil)	7.8	10.5	8.5±
Carbonate (meq./l)	0.3	1.5	0.5±
Bicarbonate (meq./l)	0.2	0.6	0.36±
Phosphorus (µg/g)	6.0	20.0	18.0±
Iron (ppm)	40	60	55±
Available zinc (ppm)	0.58	1.4	0.8±
Available copper (ppm)	0.28	0.8	0.52±
Nickel (ppm)	0.09	0.12	0.09±0.01
Chromium (ppm)	0.046	0.08	0.05±0.01
Cadmium (ppm)	0.001	ND	ND

ND - not detectable, ± S.E. value (n=3).

Protein

Total protein content estimated in wild plant leaves at NH 25 and data presented in the table 3. The protein content in leaves was estimated higher at sites III and IV as compared to sites I and II (just near to NH 25). At sites I and II, total protein content in leaves of *Croton*,

Bougainvillea and *Nerium* ranged from 325 to 480 $\mu\text{g g}^{-1}$ fr. wt. as compared to sites III and IV ranged from 510 to 780 $\mu\text{g g}^{-1}$ fr. wt. The lowest value of protein content was observed in *Bougainvillea* species (325.5 to 398 $\mu\text{g g}^{-1}$ fr. wt.) as compared to *Croton* (480 $\mu\text{g g}^{-1}$ fr. wt.) and *Nerium* (428 $\mu\text{g g}^{-1}$ fr. wt.) at sites just near (0-10 m) to NH 25.

Table 2 A. Heavy metals accumulation in wild plant species at various locations near (0-10 m) express highway (NH 25) in Unnao district at Pre-monsoon period (2011).

Sites	Plants	Heavy metals ($\mu\text{g g}^{-1}$ dry weight)					
		Zn	Cu	Fe	Cd	Cr	Ni
I	<i>Euphorbia</i> sp.	20.4 \pm 1.0*	4.7 \pm 0.5*	25.8 \pm 1.5*	7.1 \pm 0.1	1.2 \pm 0.1	5.8 \pm 0.1*
	<i>Croton</i> sp.	26.5 \pm 0.5*	3.8 \pm 0.2	75.0 \pm 0.5	8.2 \pm 0.1	12.4 \pm 0.1	11.6 \pm 0.2*
	<i>Nerium</i> sp.	15.8 \pm 0.5	8.7 \pm 0.1*	98.6 \pm 0.5**	10.8 \pm 0.1	9.5 \pm 0.1	16.2 \pm 0.1
	<i>Bougainvillea</i> sp.	35.5 \pm 0.6	9.4 \pm 0.5	119.8 \pm 5.5	15.5 \pm 0.1	16.6 \pm 0.1	17.2 \pm 0.1*
	<i>Ageratum</i> sp.	20.5 \pm 0.2	7.2 \pm 0.2	25.6 \pm 1.5	16.0 \pm 0.1*	14.8 \pm 0.2	6.3 \pm 0.5
	<i>Parthenium</i> sp.	21.5 \pm 0.5*	14.8 \pm 0.1**	26.5 \pm 1.5	17.5 \pm 0.5	15.2 \pm 0.1	8.3 \pm 0.2
	<i>Calotropis</i> sp.	30.0 \pm 0.5	21.5 \pm 1.0*	14.8 \pm 0.5**	7.0 \pm 0.1	7.3 \pm 0.1*	13.7 \pm 0.2
II	<i>Euphorbia</i> sp.	12.6 \pm 0.5	6.5 \pm 0.1	35.6 \pm 0.5	9.6 \pm 0.2	2.5 \pm 0.3*	7.6 \pm 0.1*
	<i>Croton</i> sp.	10.5 \pm 1.0*	5.2 \pm 1.0	46.7 \pm 1.5	22.5 \pm 0.5	15.6 \pm 0.1	9.8 \pm 0.1
	<i>Nerium</i> sp.	8.0 \pm 1.0	10.5 \pm 0.5	60.8 \pm 1.2	6.9 \pm 0.5	15.7 \pm 0.1	18.8 \pm 0.1*
	<i>Bougainvillea</i> sp.	25.5 \pm 0.5*	12.4 \pm 0.5	25.0 \pm 1.0*	8.6 \pm 1.0	10.2 \pm 0.5*	20.5 \pm 0.5
	<i>Ageratum</i> sp.	12.0 \pm 1.0**	8.5 \pm 0.5*	9.8 \pm 0.1	6.5 \pm 0.1	5.5 \pm 0.1	27.6 \pm 0.2*
	<i>Parthenium</i> sp.	7.50	18.5 \pm 0.5**	12.2 \pm 0.1	10.4 \pm 0.1	7.2 \pm 0.5*	6.8 \pm 0.2
	<i>Calotropis</i> sp.	18.6 \pm 1.5	25.0 \pm 1.0	112.6 \pm 0.5**	7.5 \pm 0.1	12.5 \pm 0.1	8.6 \pm 0.1

DISCUSSION

The composite soil samples collected from nearby areas of NH 25 (0-10 m) analyzed for their physico-chemical properties (Table 1). The value of pH (>7.0), electrical conductance (>1.0), CaCO_3 (more than 1.5%) and organic matter content (0.21 to 0.34%) showed poor fertility of the soil (Brady and Weil, 1996). Soil contained some essential elements such as Ca, Mg and phosphorus content supported plant growth has been reported earlier (Sharma, 2006, Marschner, 2003).

Some micronutrients Zn and Cu showed normal critical range of sufficiency for plant growth as DTPA extractable 0.6 to 1.4 ppm Zn and 0.3 to 0.8 ppm Cu in soil (Sharma, 2006). The iron content in soil determined to be very high (40 to 60 ppm) may adversely effects plants growth (Rodriguez *et al.*, 2007). In soil, concentration of Ni and Cr was low, even not detectable at the some places.

Table 2 B. Heavy metals accumulation in wild plant species at various locations about 5 km away from the express highway (NH 25) (Unnao district, U.P.) at Pre-monsoon period (2011).

Sites	Plants	Heavy metals ($\mu\text{g g}^{-1}$ dry weight)					
		Zn	Cu	Fe	Cd	Cr	Ni
III	<i>Euphorbia</i> sp.	12.5 \pm 2.0	14.8 \pm 1.0	16.5 \pm 1.5**	0.8 \pm 0.1	ND	2.1 \pm 0.1*
	<i>Croton</i> sp.	10.5 \pm 1.0	4.5 \pm 1.0	14.2 \pm 1.5	4.2 \pm 0.5*	ND	0.8 \pm 0.1
	<i>Nerium</i> sp.	6.0 \pm 0.1*	2.5 \pm 0.1*	10.5 \pm 1.0	2.9 \pm 0.2	0.6 \pm 0.1	2.5 \pm 0.1
	<i>Bougenvellia</i> sp.	9.5 \pm 0.5	2.0 \pm 0.5	22.8 \pm 1.0	6.0 \pm 0.5	ND	3.5 \pm 0.1
	<i>Ageratum</i> sp.	7.8 \pm 0.1	6.5 \pm 0.1	20.5 \pm 1.0	ND	1.2 \pm 0.1	2.0 \pm 0.1
	<i>Parthenium</i> sp.	5.4 \pm 0.1	2.3 \pm 0.1	15.6 \pm 0.5	ND	ND	ND
	<i>Calotropis</i> sp.	6.5 \pm 0.5	1.5 \pm 0.1	10.2 \pm 0.1	ND	2.4 \pm 0.1	3.5 \pm 0.1
IV	<i>Euphorbia</i> sp.	5.8 \pm 0.2	2.8 \pm 0.1**	8.6 \pm 0.2	ND	ND	1.8 \pm 0.1
	<i>Croton</i> sp.	8.6 \pm 0.5	7.6 \pm 0.2*	6.5 \pm 0.1	1.5 \pm 0.1	0.8 \pm 0.1	2.0 \pm 0.1
	<i>Nerium</i> sp.	6.7 \pm 0.1	5.2 \pm 0.2	5.6 \pm 0.2	2.1 \pm 0.1	ND	ND
	<i>Bougenvellia</i> sp.	7.2 \pm 0.2*	3.8 \pm 0.1*	5.2 \pm 0.1	ND	ND	ND
	<i>Ageratum</i> sp.	5.5 \pm 0.2	4.5 \pm 0.1	4.2 \pm 0.1	ND	ND	0.8 \pm 0.1
	<i>Parthenium</i> sp.	5.2 \pm 0.2	10.5 \pm 0.5*	10.2 \pm 0.5	ND	ND	1.5 \pm 0.1
	<i>Calotropis</i> sp.	2.5 \pm 0.1	4.6 \pm 0.2	8.6 \pm 0.1**	1.0 \pm 0.1	1.5 \pm 0.1	ND

ND- not detectable; \pm - S.E. (n=3); * - value significant at 0.05 level and ** - value significant at 0.01 level.

The accumulation of heavy metal was determined and presented in table 2 (A, B). In wild plants studied, accumulation of heavy metals (Zn, Cu, Fe, Cd, Ni and Cr) was observed in variable range. Zn and copper content was found in normal range of plant growth (Pandey, 2006, Gerndas *et al.*, 1999), whereas Fe, Cd, Ni and Cr accumulation indicated toxic range to plant growth (Baccouch *et al.*, 1998; Sahu *et al.*, 2007). The tissue concentration of Fe (ranged from 14.8 to 112.6 $\mu\text{g g}^{-1}$ dr. wt.) showed from sufficient to toxic in range in wild plants (Guo and Marschner, 1995). The accumulation of Zn, Cu, Fe and Ni may support plant growth upto a critical limit (Marschner, 2003), but at higher concentrations pose toxic effects to the plants (Khan, 2007). Tissue accumulation of Cd, Cr and Ni may pose adverse effects on growth and metabolism of plants (Chatterjee and Chatterjee, 2000), even in traces.

The wild plants *Nerium* and *Bougainvillea* accumulated high content of Fe (60.8 to 119.8 $\mu\text{g g}^{-1}$ dr. wt.) at sites I and II may be due to exposed to vehicular exhaust and dust of transport activities (Kumar and Pandey, 2010) continuously as compared to sites III and IV (4.2 to 22.8 $\mu\text{g g}^{-1}$ dr. wt.). The accumulation of estimated heavy metals in wild plants near NH 25 was higher than the plants away from the express highway (NH 25) it could be attributed due to high concentration of these heavy metals in soil and air near NH 25 (Mckenzie et al., 2005).

Table 3. Some biochemical constituents [Pigments (mg g^{-1} fr. wt.) and protein content ($\mu\text{g g}^{-1}$ fr. wt.)] in wild plant species at study sites I and II (0-10 m near NH 25) and III and IV (about 5 km away from NH 25) in Unnao district.

Study sites	Plants	Pigments			Protein
		Chl. a	Chl. b	Carotenoids	
I	<i>Nerium</i> sp.	0.39 \pm 0.10*	0.72 \pm 0.1*	0.84 \pm 0.1*	415.0 \pm 2.5
	<i>Bougainvillea</i> sp.	0.22 \pm 0.10	0.42 \pm 0.1	0.85 \pm 0.1	325.5 \pm 1.5
	<i>Croton</i> sp.	0.51 \pm 0.05	0.56 \pm 0.1	1.12 \pm 0.2	480.0 \pm 5.5
II	<i>Nerium</i> sp.	0.46 \pm 0.1	0.57 \pm 0.1*	0.85 \pm 0.1	428.0 \pm 10.0
	<i>Bougainvillea</i> sp.	0.18 \pm 0.1	0.35 \pm 0.1	0.65 \pm 0.1	398.0 \pm 5.5
	<i>Croton</i> sp.	0.31 \pm 0.1	0.40 \pm 0.1	0.89 \pm 0.2	475.0 \pm 10.5
III	<i>Nerium</i> sp.	0.62 \pm 0.2	1.45 \pm 0.2	1.12 \pm 0.2**	665.0 \pm 15.5
	<i>Bougainvillea</i> sp.	0.75 \pm 0.1	1.0 \pm 0.1	1.10 \pm 0.2	576.0 \pm 15.0
	<i>Croton</i> sp.	0.60 \pm 0.1*	1.35 \pm 0.2	1.50 \pm 0.5	510.0 \pm 10.0
IV	<i>Nerium</i> sp.	0.71 \pm 0.2	1.10 \pm 0.1	1.54 \pm 0.5	780.0 \pm 20.0
	<i>Bougainvillea</i> sp.	0.68 \pm 0.2	0.98 \pm 0.2	1.12 \pm 0.1	610.0 \pm 25.0
	<i>Croton</i> sp.	0.92 \pm 0.1**	1.12 \pm 0.1*	1.65 \pm 0.5	650.0 \pm 30.5

\pm - S.E. value; * - value significant at <0.05 level and ** - value significant at <0.01 levels. Site I and II – near (0-10 m) express highway and Site III and IV – away (about 5 km) from the express highway NH 25.

The high accumulation of some heavy metals in wild plants near NH 25 could be due to settled dust particles along with heavy metals on the leaves as also observed earlier (Rogge et al., 1993). The wild plants near NH 25 were poor in growth, leaves were small in size, showed chlorotic and necrotic symptoms might be due to the high accumulation of Fe, Ni, Cd and Cr and their adverse effects on biochemical activities (Pandey and Sharma, 2002, Pandey, 2004). Due to the accumulation of heavy metals like Cr, Cd and Ni may pose risk of loss of biodiversity (Sharma, 2012). The phytotoxic effects resulted due to interaction between heavy metals and plants (Barman et al., 2001). Wild plants contained low contents of pigments chlorophylls and carotenoids content at sites I and II (Table 3) as compared to sites III and IV could be attributed due to high concentration of toxic metals along with other pollutants near NH 25 (Deng et al., 2004).

The decrease in chlorophyll 'a' and 'b' content may affect photosynthetic activity of plants (Kosobrukhov *et al.*, 2004). The decrease in pigments content lead to limited growth of plants has been reported (Gautam and Pandey, 2008). Total protein content was found in the range of 325 to 480 $\mu\text{g g}^{-1}$ fr. wt. in wild plants at sites I and II, it was lower than the plants grown at sites III and IV away from the NH 25, increased protein content in wild plants could be attributed due to the availability of essential elements to the plants (Sammantarary *et al.*, 1998) and also could be due to protein synthesized in response to stress conditions (Schutzendubel and Polle, 2002).

CONCLUSION

Study concluded that, wild plants near the express highway (NH 25) in Unnao district accumulated high content of heavy metals such as Ni, Cr, Cd and Fe. Some essential elements such as Zn and Cu also accumulated in wild plants in sufficient range which can promote the wild plants growth. A higher accumulation of above heavy metals possibly decreased pigments and protein content in wild plants studied near NH 25. These adverse effects may be controlled by eco-friendly management system of transport activities.

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REFERENCES

- Afyoni, M., Rezainejad and Khayyambashi, B. 1998. Effect of effluent on function and absorption of heavy metals by spinach and lettuce. *Journal of Agriculture and Natural Resources*, 2 (1): 19-30.
- Baccouch, S., Chaoui, A. and Feriani, E.E 1998b. Nickel toxicity effect on growth and metabolism of maize. *Journal of Plant Nutrition*, 21(3): 577-588.
- Barman, S.C., Kisku, G.C., Salve, P.R., Mishra, D., Sahu, R.K., Ramteke, P.W. and Bhargava, S.K. 2001. Assessment of industrial effluent and its impact on soil and plants. *Journal of Environmental Biology*, 22(4): 251-256.
- Brady, N.C. and Weil, R.R. 1996. The nature and properties of soils (11th ed.). Prentice Hall, New York.
- Chatterjee, J. and Chatterjee, C. 2000. Phytotoxicity of chromium, cobalt and copper in cauliflower. *Environmental Pollution*, 109 (1): 69-74.
- Deng, H., Ye, Z.H. and Wong, M.H. 2004. Accumulation of lead, zinc, copper and cadmium by 12 wetland plant species thriving in metal contaminated sites in China. *Environmental Pollution*, 132 (1): 29-40.
- Devkota, B. and Schmidt, G.H. 2000. Accumulation of heavy metals in food plants and grasshoppers from the Taigetos Mountains, Greece. *Agricultural Ecosystem Environment*, 78 (1): 85-91.
- Dowling, D.N. and Doty, S.L. 2009. Improving phytoremediation through biotechnology. *Current Opinion in Biotechnology*, 20: 204-206.

- Du Laing, G., Rinklebe, J., Vandecasteele, B. and Tack, F.M.G. 2009. Trace metal behaviour in estuarine and riverine floodplain soils and sediments: A review. *The Science of the Total Environment*, 407 (1): 2919-2930.
- Gautam S. and Pandey, S.N. 2008. Growth and biochemical responses of nickel toxicity on leguminous crop (*Lens esculentum*) grown in alluvial soil. *Research Environment Life Science*, 1: 25-28.
- Gerendas, J., Sattelmacher, I.B., Polaccoj. and Freyermuth, S. 1999. Significance of Ni for plant growth and metabolism. *Journal of Plant Nutrition and Soil Science*, 162: 241-256.
- Guo, Y. and Marschner, H. 1995. Uptake, distribution, and binding of cadmium and nickel in different plant species. *Journal of Plant Nutrition*, 18: 2691-2706.
- Khan, M.H. 2007. Induction of oxidative stress and antioxidant metabolism in *Calamus tenuis* leaves under chromium and zinc toxicity. *Indian Journal of Plant Physiology*, 12(4): 353-359.
- Kosobrukhov, A., Knyazeva, I. and Mudrik, V. 2004. Plantago major plants responses to increase content of lead in soil, growth and photosynthesis. *Plant Growth Regulators*, 42: 145-151.
- Kumar, R. and Pandey, S.N. 2010. Monitoring of trace metals in air at different locations of Lucknow. *Asian Journal of Microbiology Biotechnology Environment Science*, 12(1): 1-4.
- Lasat, M.M. 2002. Phytoextraction of toxic metals: a review of biological mechanisms. *Journal of Environmental Quality*, 31: 109-120.
- Lindsay, W.L. and Norwell, W.A. (1978): Equilibrium relationships of Zn²⁺, Fe³⁺, Ca²⁺ and H⁺ with EDTA and DTPA in soils. *Soil Science Society American Proceedings* 33, 62-68.
- Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. 1951. Protein determination with folin reagent. *Journal Biological Chemistry*, 193: 265-276.
- MacFarlane, G.R., Pulkownik, A. and Burchett, M.D. 2003. Accumulation and distribution of heavy metals in the grey mangrove, *Avicennia marina* (Forsk.) Vierh.: biological indication potential. *Environmental Pollution*, 123: 139-151.
- Marschner, H. 2003. Mineral nutrition of higher plants (second ed.) Academic press, London. pp. 889-899.
- Meckenzie, C.H., Lim Godwin, A.A. and Morawska, L. 2005. Characterization of element and polycyclic aromatic hydrocarbon composition of urban air in Brisbane, *Atmos. Environ.*, 39: 463-476.
- Meenakshi, A. and Pandey, S.N. 2009. Effect of chromium stress on growth and physiological responses of linseed (*Linum usitatissimum* L.) plants. *Asian Journal of Microbiology Biotechnology and Environmental Science*, 11: 123-126.
- Pandey, N. and Sharma, C.P. 2002. Effect of heavy metals Co²⁺, Ni²⁺ and Cd²⁺ on growth and metabolism of cabbage. *Plant Science*, 63: 753-758.
- Pandey, S.N. 2004. Industrial effluent and its effect on seed germination and seedling growth of *Zea mays* Linn. and *Oryza sativa* Linn. *Biological Memoirs*, 30(2): 104-107.
- Pandey, S.N. 2014. Effects of soil sodicity on growth, biochemical constituents and Zn content in wheat plants. *J. Biol. And Chem. Res.*, 31(1): 317-324.

- Pilon-Smits, E. 2005. Phytoremediation- A Review. *Annual Reviews Plant Biology*, 56: 15-39.
- Piper, C.S. 1969. Soil and Plant analysis. Hassel Press. Adelaide, Australia.
- Porra, R.J., Thompson, W.A. and Kriedemann, P.E. 1989. Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls *a* and *b* extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. *Biochimica Biophysica Acta*, 975: 384-394.
- Rodriguez, M.C., Barsanti, L., Passarelli, V., Conforti, V. and Gualtieri, P. 2007: Effects of chromium on photosynthetic and photoreceptive apparatus of the alga *Chlamydomonas reinhardtii*. *Environment research*, (2): 234-239.
- Rogge, W.F., Hildemann, L., Mazurek, M.A. 1993. Sources of fine organic aerosol, natural gas and catalyst equipped automobiles and heavy duty diesel trucks, *Environmental Science and Technology*, 27: 636-651.
- Sahu, R.K., S. Katiyar, Jaya Tiwari and Kisku, G.C. 2007. Assessment of drain water receiving effluent from tanneries and its impact on soil and plants with particular emphasis on bioaccumulation of heavy metals. *Journal of Environmental Biology*, 28: 685-690.
- Samantaray, S., Rout, G.R. and Das, P. 1998. Role of chromium on plant growth and metabolism. *Acta Physiol. Plant*, 20: 201-212.
- Schützendübel, A. and Polle, A. 2002. Plant responses to abiotic stresses: heavy metal-induced oxidative stress and protection by mycorrhization. *J. Exp. Bot.*, 53: 1351-1365.
- Sharma, C.P. 2006. Plant micronutrient. 1st Edition, Science Publisher, New Hampshire, USA. pp 5-10.
- Sharma, P.D. (2012). Environmental monitoring and impact assessment. In: Ecology and Environment (Eds. Sharma, P.D.) Rastogi Publications, Meerut: pp. 574-584.
- Tiller, K.G. 1989. Heavy metals in soil and their Environmental significance. *Soil Science*, 9: 113-142.
- Trstos, P., Szdkovd, J.L., Hrub, J.L., Hartmanj., Najmanovd, J., Nedilnik, J.L., Pavlikovd, D. and Batysta, M. 2006: Removal of As, Cd, Pb, and Zn from contaminated soil by high biomass producing plants. *Plant Soil Environment*, 52: 413-423.

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