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Effects of industrial waste water on heavy metal accumulation, growth and biochemical responses of lettuce (*Lactuca sativa* L.)

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Abstract: *The waste water showed high values of total solid (TS), hardness and chloride with slightly alkaline pH along with high concentrations of Cr (2.03 mg l^{-1}), Ni (1.59 mg l^{-1}) and Zn (0.46 mg l^{-1}). The concentration of Cu (0.21 mg l^{-1}) and Zn in industrial waste water was low than Ni and Cr. The diluted (25 and 50%), undiluted (100%) waste water was used to irrigate the lettuce plants grown in alluvial soils. Plants accumulated heavy metals in their shoot (Ni, 13.65; Cr, 19.73; Zn, 21.6 and Cu 14.76 µg g^{-1} dry weight) and root (Ni, 41.4; Cr, 31.6; Zn, 30.2 and Cu 15.85 µg g^{-1} dry weight) in high concentrations after irrigation with undiluted industrial waste water. Maximum accumulation of heavy metals was found in the root than the shoot (13.65-21.60 µg g^{-1} dry weight). Dry matter yield and biomolecules (Chlorophyll a, b and sugar contents) was found to increase with increase in concentration of waste water upto 50 %, which declined at the exposure of undiluted waste water. Catalase activity was found to increase with increase in waste water concentrations upto 100%, while carotenoids content increased in plants only upto the 50% waste water irrigation. Use of industrial waste water, in such form, on agricultural lands is not found suitable without proper treatment. It could be injurious to plants growth and may be a potential threat to food web.*

Key words: *Heavy metals, Waste water, Biochemical responses, Lettuce*
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Introduction

Heavy metal pollution in the environment is quite relevant in the present scenario due to its deleterious effect on human health via food chain. Biosphere pollution by heavy metals has accelerated dramatically during the last few decades as a result of discharge of waste waters from various industries and urban population. The consumption of plants grown in heavy metals rich soil, results in introduction of substantial amount of potentially toxic metals into the food chain (Epniewska and Bucior, 2001; Pandey and Nautiyal, 2008; Perez and Sharma, 2008; Agoramoorthy, 2009; Handique and Handique, 2009).

Although, some heavy metals, such as Cu and Zn, have known functions as micronutrients in plants (Sharma, 2006), they become toxic at high levels (Pandey, 2008). High accumulation of metals affects both growth and metabolism of plants (Baccouch *et al*.,1998). These phytotoxic effects of heavy metals depends on metal concentration, plant species, pH and other factors in soil (Barman *et al.,* 2000; Chandra *et al*., 2004). Due to heavy metal stress the production of reactive oxygen species (ROS) causes damage to the plant cells (Gajewska *et al*., 2006). The antioxidants produced in cells defend the biochemical activities and provide tolerance in plants under stressed conditions (Asada, 1992). The accumulation of heavy metals in plants exposed to industrial waste water, and their effects on growth and metabolism of plants need extensive studies for various research purposes. Common effluent treatment plant located in Unnao district , U. P. state, India discharges waste water from a large number of industries into a drain after treatment. This waste water is used for irrigational purposes in agricultural fields on both sides of the drain. Therefore, the present study was aimed to explore the uptake of some heavy metals present in industrial waste water by lettuce (*Lactuca sativa* L.) plants after exposure. Also, the irrigational impact of the industrial waste water was observed to find tolerance limit to metal concentration, growth and biochemical changes in lettuce plants.

Materials and Methods

The experiment was designed to study the irrigational effect of industrial waste water (CETP, Unnao) on heavy metal accumulation, growth and biochemical responses of *Lactuca sativa* L. grown in alluvial soil of Lucknow (26°52' N latitude and 80°56' E longitude). The composite surface soil sample (0-20 cm) was collected from the Badshahbagh area of Lucknow, Uttar Pradesh. It was ensured that soil had not received any fertilizers or contaminants before sampling. The soil was air dried, sieved and analyzed for its physico-chemical properties. The above soil was analysed for DTPA (Diethyl triamine pentaacetic acid), extractable metals by the method of Lindsay and Norwell (1978). A basal application of NPK (60:30:30 ppm) *i.e.*, N (urea), P_2O_5 (sodium dihydrogen phosphate) and K_2SO_4 (potassium sulphate), was added in soil before sowing the seeds. Ten Kg of basal soil was filled in polythene container (20 cm diameter x 6 cm depth). Industrial waste water samples collected from the drain of common effluent treatment plant (CETP) located in Unnao district, Uttar Pradesh, India were pooled to make a composite sample and its physico-chemical characteristics and heavy metal contents (Cu, Cr, Ni and Zn) were analysed following the standard

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methods of APHA *et al.* (2005). Four irrigational treatments of composite sample (0, 25, 50 and 100%) were made in five replicates. Ten seeds of *Lactuca sativa* var. iceberg were grown in above collected soil up to 40 days and later thinned to two plants per pot, and irrigated only with distilled water. After that, graded supply of industrial waste water was given thrice a week and the visible symptoms on plants were observed regularly. The plants were harvested for dry matter yield at 90 days of treatment. The root and shoot samples were collected for heavy metal (Zn, Cu, Cr and Ni) accumulation in *L. sativa*. Biochemical responses were observed at 60 days of treatment of industrial waste water. Pigment (chlorophyll a and b and total chlorophyll) content was estimated by the method of Lichtenthaler and Wellburn (1983), sugar by Dubois *et al.* (1956) carotenoids by Duxbury and Yentsch, (1956) and catalase by Euler and Josephson (1948). Data were statistically analysed by ANOVA for least significant difference (p=0.05).

Results and Discussion

A large number of industries discharge their waste water in a common effluent treatment plant (CETP) located in Unnao district (U.P. state, India). The industrial waste water showed higher values of pollutants than those of ISI (1974) standards discharge limits (Table 1) even after treatment. High level of total solids (7970 mg l^{-1}) was determined, which is the cause of salinity in water and soil (Pandey and Srivastava, 2002). The levels of heavy metals were in the order of Cr>Ni>Zn>Cu in industrial waste water. These heavy metals when discharged into the aquatic system or agricultural fields pose risk of transfer into the food web (Kisku *et al.*, 2000; Pandey, 2008) thereby adversely affecting human health (Desi *et al.*, 1998).

When *Lactuca sativa* L. plants growth in composite soil (Table 2) were irrigated with undiluted industrial waste water, they accumulated high content of heavy metals in root (Ni, 41.3 and Cr, 31.8 μg g^{-1} dry weight) and shoot (Ni, 12.8 and Cr, 19.5 μg g^{-1} dry weight). These values indicated that the accumulation

Table - 1A: Some physico-chemical properties of industrial waste water samples from CETP, Unnao

Parameters	Value	ISI standards
pH	7.24	5.5 – 9.0
Total solids (mg l^{-1})	7970	-
Total dissolved solids (mg l^{-1})	7840	2100
Total suspended solids (mg l^{-1})	130	100
Hardness (mg l^{-1})	1430	600
Chloride (mg l^{-1})	2550	1000

Table - 1B: Heavy metals concentration (mg l^{-1}) in industrial waste water samples from CETP, Unnao

Parameters	Value	ISI standards
Copper	0.21	0.05-1.5
Chromium	2.03	0.05
Nickel	1.59	<0.01
Zinc	0.46	5.0 - 15.0

*ISI standards No. 2490 (1974)

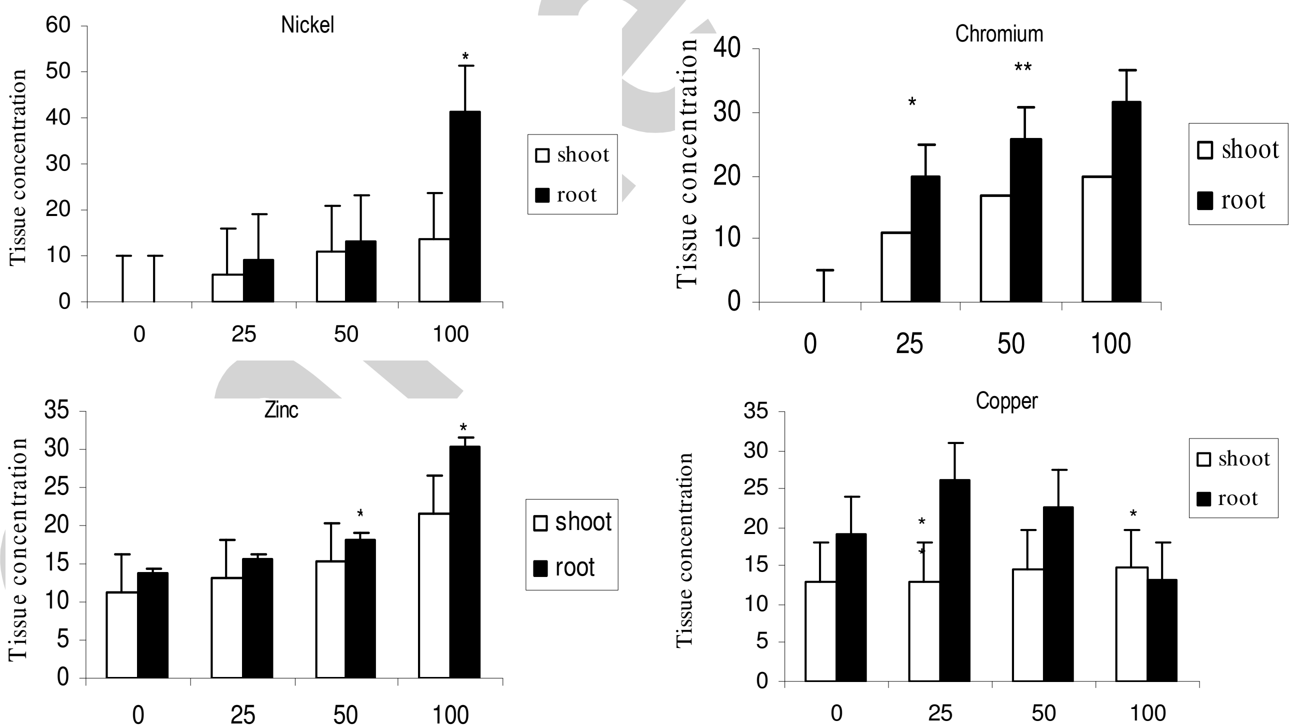

Fig. 1: Tissue concentration (μg g^{-1} dry weight) of Ni, Cr, Zn and Cu of root and shoot *Lactuca sativa* plants ± SE value (n=5); * - significant at p<0.05 level, ** - significant at p<0.01 level

Table - 2: Physico-chemical properties of alluvial soil (Lucknow) before sowing the plant (*Lactuca sativa* L.)

Texture	Bulk density	pH	Organic matter	$CaCO_3$	E.C.	Heavy metals (ppm)			
	($g\ cc^{-1}$)		(%)	(%)	($mS\ cm^{-1}$)	Zn	Cu	Ni	Cr
Loamy sandy	1.40	6.5	0.21	0.45	1.16	0.26	0.72	0.18	0.12

E.C. = Electrical conductance

Table - 3: Irrigational impact of industrial waste water on dry matter production and biochemical responses of *Lactuca sativa* plants (at 60 days after treatment)

Parameters	Waste water (%)				L.S.D.
	0	25	50	100	$p<0.05$
Dry weight ($g\ plant^{-1}$)	0.94	1.10	1.63	0.71	0.62
Chlorophyll a ($mg\ g^{-1}$ FW)	1.05	1.31	2.02	1.59	0.65
Chlorophyll b ($mg\ g^{-1}$ FW)	0.29	0.37	0.68	0.43	0.26
Carotenoids ($mg\ g^{-1}$ FW)	0.42	0.48	0.70	0.56	0.18
Sugar ($\mu g\ g^{-1}$ FW)	24.63	27.23	33.19	21.05	5.70
Catalase ($mg\ g^{-1}$ FW)	140.6	148.6	168.3	172.6	24.4

FW = Fresh weight

of heavy metals was higher in root than the shoot possibly due to its slow translocation to upper parts (Barman *et al*., 2000). The tissue concentration of Zn and Cu in *L. sativa* root (Zn 15.5 and Cu 19.0 $\mu g\ g^{-1}$ dry weight) and shoot (Zn 11.7 and Cu 13.0 $\mu g\ g^{-1}$ dry weight) was found below the critical limit of toxicity.

The visible symptoms of toxicity that appeared in treated plants, were stunted growth and chlorosis which started from upper margins of leaves towards the base then turned necrotic at severe stage. Leaves were found to wilt and dry after irrigation with undiluted industrial waste water. However, these symptoms were not visible in plants irrigated with diluted waste waters (25 and 50%). High content of heavy metals, particularly Ni and Cr, in industrial waste water decreased dry matter yield of test plants by 35.4%. The retardation in growth and development of toxicity symptoms in plants could be attributed due to high uptake of heavy metals and their accumulation in plant parts (Pandey, 2006). The uptake of heavy metals in plants was found to increase with increase in waste water concentrations.

The pigment (chlorophyll a, b and carotenoids) and sugar contents were increased in plants exposed at 25 and 50% concentration of waste water, whereas they were less when plants were treated with undiluted waste water (100%) irrigation. Heavy metals at higher concentrations inhibit uptake and transportation of other metal elements such as Fe, Zn and Mn, by antagonistic effects (Parida *et al*., 2003). These metals play an important role in the synthesis of biomolecules as well as enzymatic activities in plant cells (Gautam and Pandey, 2008). Lettuce plants, irrigated with 50 % diluted waste water showed maximum growth and biochemical responses (Table 3) at the tissue concentration - Ni 13.3, Cr 25.7, Cu 19 and Zn 18.2 $\mu g\ g^{-1}$ dry weight in root and Ni 11, Cr 16.7, Cu 13 and Zn 15.2 $\mu g\ g^{-1}$ dry weight in shoot were quantified.

The increase in sugar content and some antioxidative responses (activity of catalase and carotenoid content) of lettuce was found to be maximum at the exposure of 50% waste water and may be correlated with maximum increased growth at this level. Heavy metals like Ni and Cr cause oxidative stress and produce reactive oxygen species (ROS), which damage cells in plants (Khan, 2007). Catalase and carotenoids behave as antioxidants and play an important role in the defence mechanism of plants (Khan and Patra, 2007). Reduction in catalase activity and carotenoids content following treatment with undiluted waste water might be due to the damage by high levels of Ni and Cr to the defence system of lettuce plants (Noctor,1998). The results of the present study indicated that industrial waste water contained high levels of heavy metals (Ni>Cr>Zn>Cu), and through its exposure, *L. sativa* plants were accumulated high level of heavy metals in the order: Ni>Cr>Zn>Cu in root and Zn>Cr>Ni>Cu in shoot. The translocation of Ni and Cr in shoot was found to be less than the Zn even after high accumulation in the root. The concentration of Cr in waste water was higher than Ni and Zn but its uptake in root and translocation to shoot portion was highly affected due to synergistic and antagonistic effects (Barman *et al*., 2000; Pandey, 2006). The study concluded that industrial waste water (CETP, Unnao) is not suitable for irrigational purposes, in its present form. *Lactuca sativa* L. plants produced visible symptoms of toxicity and growth retardation, due to high accumulation of heavy metals (Ni and Cr), when exposed to undiluted industrial waste water. These responses of lettuce plants indicated its behaviour as a bioindicator of heavy metal pollution in waste water and may be helpful in research studies and phytoremedial approaches.

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