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Phyto-extraction of heavy metals and biochemical changes with *Brassica nigra L.* grown in rayon grade paper mill effluent irrigated soil

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Abstract:
In this study, distribution of metal accumulation and their biological changes of Indian mustard plants (*Brassica nigra* L.) grown in soil irrigated with different concentration of rayon grade paper effluent (RGPE, 25%, 50%, 75%, 100%, v/v) were studied. A pronounced effect was recorded at 50% (v/v) RGPE on germination of seeds, amylase activity and other growth parameters in Indian mustard plants. An increase in the chlorophyll and protein contents was also recorded at <50% (v/v) RGPE followed by a decrease at higher concentrations of RGPE (>75%). A significant increase lipid peroxidation was recorded, which was evidenced by the increased malondialdehyde (MDA) content in shoot, leaves and seeds in tested plant at all the concentrations of RGPE. This Indian mustard plants (*Brassica nigra* L.) are well adapted for tolerance of significant amount of heavy metals due to increased level of antioxidants (cysteine and ascorbic acid) in root shoot and leaves of treated plants at all concentration of RGPE. Moreover, it is also important that RGPE should be treated to bring down the metal concentration well within the prescribed limit prior to use in agricultural soil for ferti-irrigation.

Keywords: Rayon grade paper mill effluent; Indian mustard plant; Phytoremediation; Heavy metals; Lipid peroxidation; biochemical parameters

Background:
Today, the heavy metal contamination is a global concern, caused by natural occurring process (volcanism and erosion) and several anthropogenic activities. Metals tend to bio-accumulate in food chain as large areas of agricultural land have been contaminated with heavy metals. Rayon grade pulp-paper effluent (RGPE), which represents a foul smelling, dark brown and complex waste effluent discharged by pulp paper industries is also a major source of soil and aquatic pollution. It is estimated that about 270-455 M^3 (60,000-1, 00,000 gallons) of water is required per tonne of paper production and discharge more than 47,000-80,000 gallons of wastewater containing lignin and Chlorophenols. Along with high pollution parameters a significant quantity of heavy metals (Cd, Cu, Fe, Mn, Ni and Zn) is also contributed from pulp originated from plant extract and during the different industrial process **[1]**

thereby turning pulp and paper mill effluents into 'a Pandora's box of waste chemicals'. This waste effluent when released as such into water bodies, it adversely affected the aquatic ecosystem by reducing the penetration power of sunlight and ultimately reduces the photosynthetic activities and dissolved oxygen content **[2]** whereas in agricultural soil, it causes inhibition of seed germination and depletion of vegetation by reducing the manganese availability and amylase activity **[3].**

Provided conventional approaches (Physical and chemical) to the remediation of contaminated wastewater are inefficient and costly and can also lead to the formation of toxic intermediate **[2].** Particularly, phytoremediation technique may be a better approach for industrial waste minimization because these are cost-effective and environment-friendly technology. Ideal plants for phytoremediation should have a high biomass production, deep penetration of roots, fast growing and able to tolerate and accumulate high amount of potentially toxic heavy metals in their aerial and harvestable parts. Recently, the Indian mustard plants (*Brassica* sp.) have been reported to have high potential to tolerate and accumulate high quantities of potentially toxic heavy metals and can be used for phytoremediation of metal-polluted soils. Indian mustard plant (*Brassica* sp.), the major oil seed crop and used as food, is reported as a most promising and interesting model plant for phytoremediation **[4].**

The effect of different industrial effluents as a source of irrigation and their effect on growth and productivity of various crop plants have been studied by various workers **[5].** Instead of various pollutants, the effluent from paper mill is rich source of organic and inorganic matter such as nitrogen, phosphorus, calcium, magnesium and the trace elements. This showed a significant scope for pulp-paper mill effluent recycling for land application. Moreover, the direct use of RGPE in agricultural field for irrigational purposes is generally not recommended safe for environmental health and plant growth due to high pollution parameters of RGPE. On the other hand, there is no data or study to support the irrigation and its toxic effect on long-term application on environment health problems. In addition, many farmers in developing countries including India are cultivating mustard plants in soil contaminated with potentially toxic heavy metals. The status of metals in different parts of mustard plants grown on contaminated soil is still ambiguous. Hence, the experiments were planned and conducted to study the accumulation of various heavy metals (Cd, Mn, Ni, Cu, Fe and Zn) into its different parts and their effect on the biochemical parameters on the potential of Indian mustard plant (*Brassica nigra* L.) cultivated in agricultural soil irrigated with RGPE.

Methodology:

Sample collection and investigational setup

Rayon grade paper mill effluent (RGPE) was collected from wash machine section of *M/s.* Century Pulp Paper Mill, Lalkuan, Nainital, Uttrakhand, India located (79 °10'E longitude and 29 °3'N latitude) at foot hills of Himalayas. The samples were taken into pre-sterilized container (20 liter capacity), immediately preserved at 4 °C. Further, the physico-chemical parameters were accomplished as described in standard methods for the examination of water and wastewaters (**6**). Metals and different salts (chloride, sodium and nitrate) were analyzed by atomic absorption spectrophotometer and ion meter (Orion Model 960) using selective ion electrode.

For the construction of pot experiment uncontaminated garden soil were collected from the Scientific and Applied Research Center (SARC), Meerut, India. The uncontaminated garden soil was completely air-dried, finely powdered and sieved (2.0 mm mesh) and equal amount (20 kg dw) was filled in pots having dimensions 50 X 35 cm and were irrigated with equal volume (4 liter) of 25%, 50%, 75% and 100% (v/v) RGPE and control with tap water. The seeds of *Brassica nigra* L. were surface sterilized in 75% (v/v) ethanol for 2 min, followed by 10% (v/v) sodium hypo chloride for 10 min to avoid any fungal contamination, washed thrice with double distilled water. The sterilized 10 seeds of tested plant were equally sown in each pot to a depth of 2.0-2.5 cm. The experiment pots were kept in outdoor natural conditions with temperature (20-30 ^{0}C) and moisture (50-55%) variation. This pot experiment was conducted in triplicate with an analogous set of controls.

Physico-chemical analysis of garden soil

pH (Orion ion meter Model-960) and electrical conductivity (EC) (Thermo Orion Model-162A, USA) were determined after mixing a soil and water (1:5 w/v slurry) for 1 h, total nitrogen (TN) by TOC-V_{csn} analyzer (Shimadzu, JAPAN). Water content was determined by drying the soil samples at 80 ^{o}C for 24 h. Phosphate and sulfate was measured by vanadomolybdo-phosphoric acid colourimetric and $BaCl_2$ precipitation methods, respectively (6). Prior to total heavy metal analysis, soil samples were air dried (25 ^{o}C, 14 days). The concentration of different heavy metals (Cu, Cr, Zn, Fe, Ni, Mn and Pb) was measured using inductively coupled plasma spectrophotometer (Thermo Electron; Model IRIS Intrepid II XDL, USA).

Seed germination test & amylase activity

Four dilutions of RGPE (25%, 50%, 75% and 100%, v/v) were made with double distilled water. A (10 ml) aliquot of these dilutions was transferred separately to petri dishes. Ten seeds of *Brassica nigra* L. were placed in each dish and left for 10 days at 25^{0}C. The germinated seeds were counted at the initial appearance of the radical by continuous visual observation for 10 days. The percent germination and amylase activity was calculated according to the method of Ana *et al.* **[7].**

Assessment of growth and biochemical parameters of tested plant

The tested plants with their respective controls were harvested manually after 30, 60 and 90 days of sowing and washed with distilled water to remove any contiguous particle. The chlorophyll content in fresh leave samples of mustard plants were estimated by the method of Arnon's **[8].** Protein content in root, shoot and leaves of treated plants including control was estimated by using BSA as standard protein Lowry *et al.* **[9]**. Lipid peroxidation in plant tissue was determined indirectly in terms of malondialdehyde (MDA) content. The MDA content was measured by thiobarbituric acid (TBA) reaction Heath and Packer, **[10].** The ascorbic acid and cysteine content in freshly harvested plant samples (root, shoot and leaves) of treated and untreated (control) mustard plants was estimated according to the method given by Gaitonde, **[11]** respectively.

Heavy metal analysis in different parts of plants

The different plant parts (root, shoot and leaves) were washed separated chopped into small pieces and dried in oven (60-65 ^{0}C). The dried plant parts were used for the estimation of various heavy metals (Cd, Cu, Fe, Mn, Ni and Zn).

Statistical analysis

To confirm the variability of data obtained and validity of results, all the data were subjected for the statistical significance using one-way Analysis of Variance (ANOVA) to make comparison between more than two means followed by Tukey's test (Ott, 1984) for comparison of individual means using the Graph Pad software (Graph Pad Software, San Diego, Calif.). Analytical precision of the method was improved by including the triplicate samples.

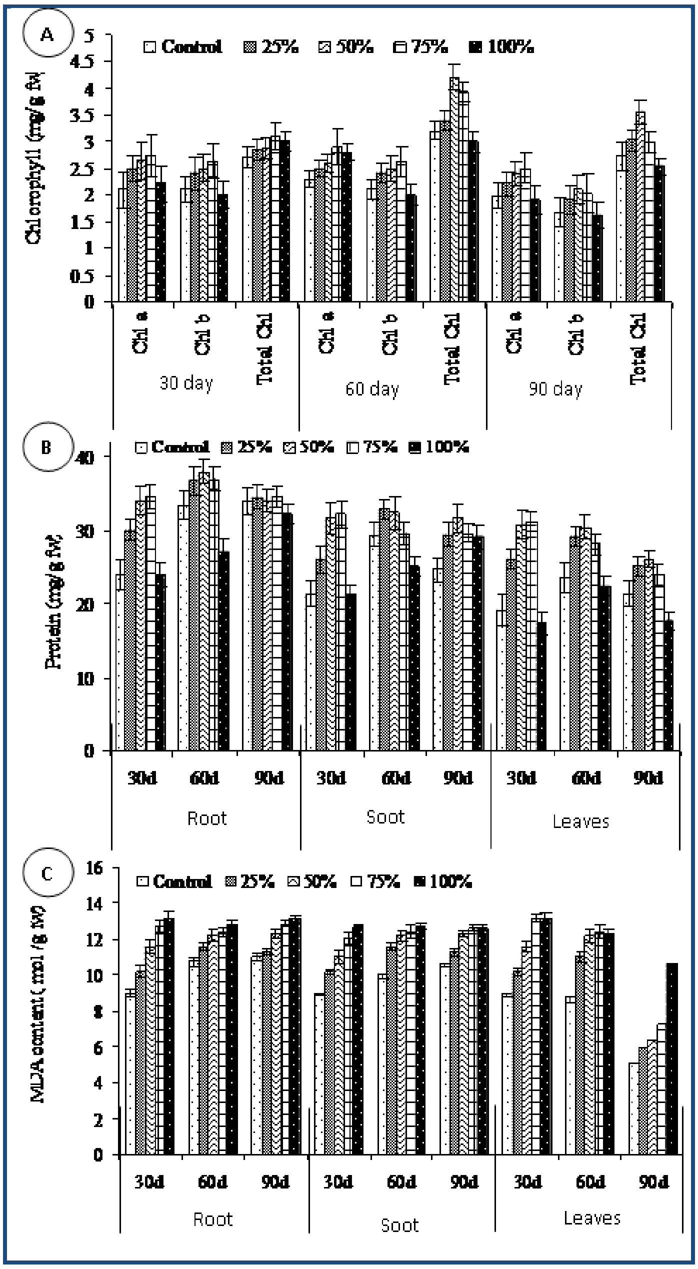

Figure 1: Chlorophyll-a, b, total chlorophyll and carotenoid content (mg g^{-1}) in leaves (A), Protein content (mg g^{-1}) (B), MDA content (µmol g^{-1}) (C), of Indian mustard plants irrigated with RGPE and tap water (control).

Results:

Physico-chemical characteristics of RGPE and garden soil

The chemical characteristics of RGPE and garden soil are showed in **Table 1 (see supplementary material).** Moreover, in present study, the germination of seed was recorded and it was observed that 50% (v/v) RGPE has pronounced effect on seed germination and early seedling growth. But, higher concentration (i.e. 75% and 100% (v/v) of RGPE has shown 30 and 50% inhibitory effect on seed germination and amylase activity, respectively as shown in **Table 2 (see supplementary material)**.

Evaluation of Physiological and biochemical effects

The increases chlorophyll contents (chlorophyll a, b and total chlorophyll) in leaves of tested plant were recorded at all concentrations of RGPE (25, 50, 75 and 100% v/v) at 30 and 60 days of growth period. But, these pigments decreased significantly beyond 50% RGPE at 60 and 90 days of growth period compared to their respective controls (**Figure 1 A)**. The maximum increase of 44.7%, 40.6% and 47.0% were recorded in chlo-a, b and total chlorophyll content respectively in mustard plants treated with 50% RGPE at 60 days of growth period as compared to their respective controls. The protein content in root, shoot and leaves also increased compare to respective controls in mustard plants irrigated with 50% (v/v) RGPE at 60 days of growth period **(Figure 1 B).** Nevertheless at higher concentrations (>50%, v/v) of RGPE, there was a decrease in protein content of root, shoot and leaves at 60 and 90 days of growth period. Moreover, the MDA content in root and leaves of mustard plants treated with RGPE increased with rise in RGPE content versus to control at all the exposure periods indicating the enhanced lipid peroxidation in mustard plants grown in RGPE irrigated soil **Figure 1C.**

Antioxidants are considered to play an important role in the detoxification of toxic oxygen species generated in presence of metal ions. However, to protect from oxidative stress conditions induced by free radicals, plants have adopted several cellular entities consisting non-enzymatic cellular entities as ascorbic acid, cysteine and non-protein thiol etc. The maximum induction in ascorbic acid content of root, shoot and leaves of the treated plants were recorded as 68.8%, 42.2%, and 38.1% at 90, 60 and 60 days of growth period respectively as compared to their respective controls **(Figure 2A).** Moreover, cysteine, a –SH containing amino acid is a key constituent of phytochelatins and plays an important role in metal detoxification. The cysteine content in root, shoot and leaves of tested plants treated with various concentrations of RGPE has increased at all the RGPE concentrations and growth periods versus to their respective controls **(Figure 2B).** The maximum increase in cysteine content of root was recorded as 77.8% at 50% RGPE concentration after 90 days of growth period compared to control plants. Further, the cysteine content in leaves of treated plant increases up to 50% RGPE concentration at all the tested growth periods except at 90 days, while higher RGPE concentration showed inhibitory effects compared to control plants **(Figure 2B).**

Accumulation and distribution of heavy metals

The distribution pattern of different heavy metals (Cd, Cu, Fe, Mn, Ni and Zn) in different parts of mustard plant was apparently related to the RGPE concentration and growth period as shown in **Table 3, 4 & 5 (see supplementary material)**. The accumulation of all the tested heavy metals was found maximum in root followed by shoot and/or leaves, which increases with increase in RGPE concentration and growth period. The RGPE concentration dependent maximum accumulation of Fe was observed in root and leaves of mustard plant at initial days while at later days of plant growth (i.e. 90 days) shoot accumulated the higher Fe content. The accumulation of all tested heavy metals was found minimum in the edible parts (seeds) of treated mustard plants as than the other plant parts **Table 5 (see supplementary material)**. The order of cumulative heavy metals accumulation in irrigated mustard plants was root > shoot >leaves at 30 days and roots > leaves > shoots at 60 and 90 days of growth period as shown in **Table 3, 4 & 5 (see supplementary material).**

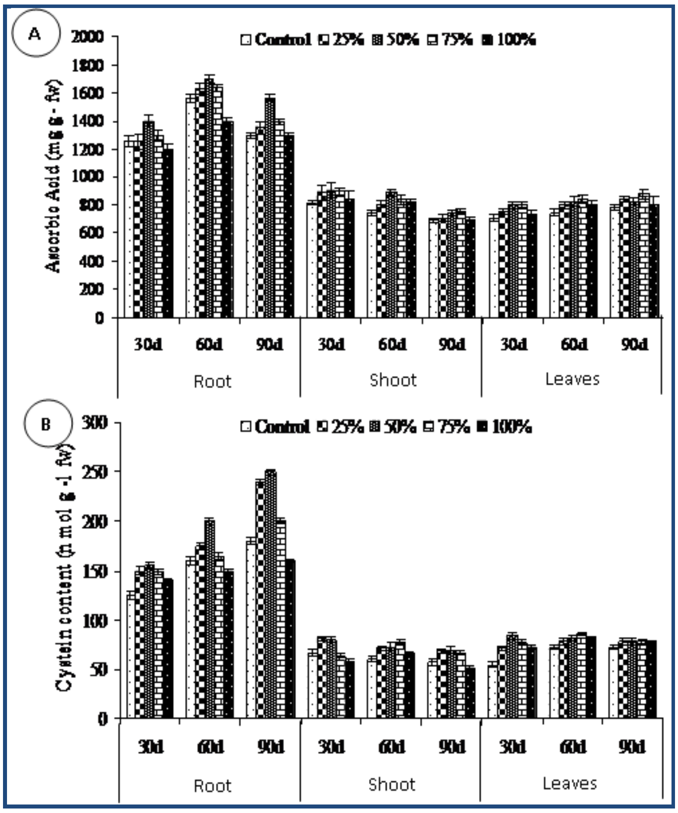

Figure 2: Ascorbic acid content (µg g^{-1}) (A), and Cysteine content (nmol g^{-1}) (B) in root, shoot and leaves of Indian mustard plants irrigated with RGPE and tap water (control).

Discussion:

RGPE is a complex aqueous system containing mainly lignin and its chloro-derivatives and many other organic pollutants. The physic-chemical characteristics of RGPE and garden soil have revealed that pH values and EC of RGPE were higher than of garden soil. The Century industry uses kraft pulping process, where wood pieces are heated with sodium hydroxide and sodium sulphite. Use of sodium hydroxide makes the effluent alkaline in nature. The high EC value of RGPE may b due to presence of different salts in RGPE as TDS (8,780± 26.7), TOC (1,200± 25.8), organic matter (3.48± 0.53), nitrate (36.73±1.04), sulphate (986 ±25.1), chloride (600± 26.9) etc. Moreover, high COD/BOD ratio (approximately 2.8) in RGPE is due to presence of high molecular weight compounds, i.e. chloro-phenols, lignin and its derivatives which contribute high COD and color, respectively instead of BOD **[1].** The source of sulfate ions in effluent might be sodium sulfite, which is used in pulping process and the nitrates detected in RGPE indicated the presence of nitrogen in lignin **[2].** The high concentrations of heavy metals in RGPE are mainly due to the corrosion of digestion vessels and possibly due to bioaccumulation of these metals by plants which are used as raw material.

The application of higher concentration of RGPE in agricultural soils may result in an increase the pollution parameters, which adversely affected the growth of mustard plants. Similar findings also have been reported by different authors **[5, 12].** Indeed, a variety of crop plant species differed widely in response to different concentration of industrial effluent with respect to the germination and other biochemical parameters **[13]**. The inhibition in seed germination at higher concentrations of RGPE might be attributed to the high pollution parameters load in RGPE, which induces high osmotic pressure and anaerobic conditions **[2]**. This high osmotic pressure and anaerobic condition render various biochemical processes such as movement of solute, respiration process of seeds and enzymatic steps of seed germination. Further, it has been reported that high RGPE content act as inhibitor for plant growth hormone (s) (auxin and gibberline), which are mainly required for the growth and development of plants. At the same time, higher biomass was noted up to 50% (v/v) RGPE concentration versus the control and thereafter, it decreased drastically in all tested samples of above 50% RGPE. The higher biomass production has given strong evidence for the inducible effect of RGPE on plant growth parameters. This finding was also corroborated well with Chandra *et al.* **[1].**

The increase in photosynthetic pigments was observed in *Brassica nigra* plants due to higher accumulation of Fe in leaves. These findings of our results were also strengthened by Chandra *et al.* **[14]**, who reported an increase in total chlorophyll and protein contents of *Phaseolus aureus* L. treated with industrial effluent. On the other hand, the reduction in chlorophyll contents observed in present study may be attributed to the interaction of potentially toxic heavy metals present in effluent with the functional –SH group of chlorophyll-synthesizing enzyme **[15]**.

In the same way, the raise in total protein content might be due to the induction of several stress proteins in treated plants at higher concentration of industrial effluent **[16]**. The decrease in chlorophyll and protein content in plants at any given concentration might be attributed to the overall decrease in plant growth parameters and photosynthetic activity **[14]**. Furthermore, formation of melondialdehyde (MDA), a major cytotoxic product of lipid peroxidation acts as an indicator of peroxidation of membrane lipids in plants **[17]**. The level of MDA formation can be considered as the level of lipid peroxidation. Ascorbic acid, which is a powerful secondary antioxidant, plays a prominent role in scavenging free oxy-radicals **[17]**.

The increased level of cysteine content corresponds to the level of tolerance exhibited by metal-treated plants. The decrease in cysteine content in the plants treated with higher RGPE concentrations (>50%) and at higher exposure periods was probably due to the decreased activities of sulfate reduction enzymes, ATP sulfurylase and adenosine-5-phosphosulfate sulfo transferase under metal stress **[18]**.

The higher accumulation of heavy metals in different parts of *Brassica nigra* L. has also shown by different authors during the comparison of heavy metals accumulation in other plant species. Studies carried out with different varieties of *Brassica* have shown that these plants were able to take up and accumulate the high concentration of heavy metals in intercellular spaces **[19]**. Interestingly, the edible parts (seeds) of treated mustard plants have shown the least accumulation of all the tested heavy metals **(Table 6)** than the other plant parts. It may be attributed to the slow translocation of metals from root to the aerial parts. The high accumulation of Fe, Zn and Cu in various parts of *Brassica nigra* L. indicated the fast mobility of these metals in the plants.

Conclusion:

In present study, a significant higher accumulation of heavy metals was observed in the various parts of the Indian mustard plant treated with different concentrations of RGPE. Moreover, the 50% level of RGPE application has been just enough for phytoremediation as the seed germination, amylase activity and other biochemical parameters had no adverse effect. Thus, the study concluded that the indiscriminate application of RGPE is health hazards during the irrigation in Indian agricultural practices.

Conflict of Interest:

Authors declare that there is no conflict of interest in preparation of the manuscript.

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References:

[1] Chandra R *et al. Bioresour Technol.* 2011 **102:** 6429 [PMID: 21482463]
[2] Pokhrel D & Viraraghavan T, *Sci Total Environ.* 2004 **333:** 37 [PMID: 15364518]
[3] Kumar P & Chandra R, *Bull Environ Contam Toxicol.* 2004 **73:** 903 [PMID: 15669736]
[4] Minglin L *et al. Gene* 2005 **363:** 151 [PMID: 16226851]
[5] Jain N *et al. Env Monit Assess.* 2005 **110:** 243 [PMID: 16308790]
[6] Chandra R & Abhishek A, *Biodegrad.* 2010 **22:** 603 [PMID: 21061144]
[7] Ramana S *et al. Bioresour Technol.* 2002 **81:** 117 [PMID: 11764762]
[8] APHA. 2005 Standard methods for the examination of water and wastewater, 21sted. American Public Health Association, Washington, DC, New York.
[9] Ana F *et al. J Hazard Mat.* 2004 **108:** 161 [PMID: 15120869]
[10] Arnon DI, *Plant Physiol.* 1949 **24**: 1 [PMID: 16654194]
[11] Lowry OH *et al. J Biol Chem.* 1951 **193:** 265 [PMID: 14907713]
[12] Heath RL & Packer L, *Arch Biochem Biophys.* 1968 **125:** 189 [PMID: 5655425]
[13] Gaitonde MK, *Biochem. J.* 1967 **104:** 627 [PMID: 6048802]
[14] Chandra R *et al. J Environ Biol.* 2004 **25:** 381 [PMID: 15907064]
[15] Singh S & Sinha S, *Ecotoxicol Environ Safe.* 2005 **62:** 118 [PMID: 15978297]
[16] Zhu YL *et al. Plant Physiol.* 1999 **121:** 1169 [PMID: 10594104]
[17] Singh S *et al. Chemosph.* 2004 **57:** 1663 [PMID: 15519412]
[18] Nussbaum S *et al. Plant Physiol.* 1988 **88:** 1407 [PMID: 16666474]
[19] Lee JH *et al. Environ Poll.* 2002 **117**: 61 [PMID:11843538]

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Supplementary material:

Table 1: Physico-chemical characteristics and Heavy metals in RGPE and garden soil.

Parameters	RGPE	Parameters	Garden soil
pH	8.0± 0.31	pH	7.0 ±0.39
Lignin	22,000±24.2	Sand (%)	48.0± 10.2
BOD (mg l^{-1})	9,000± 245	Silt (%)	33.0± 8.0
COD(mg l^{-1})	23,800± 297	Clay (%)	14.0± 1.0
TOC(mg l^{-1})	1,200± 25.8	Moisture (%)	63 ±1.8
TDS(mg l^{-1})	8,780± 26.7	OM (%)	1.88± 0.51
Color	6,300± 33	TN (mg kg^{-1})	113.50± 9.5
TN(mg l^{-1})	337± 23	EC (ms cm^{-1})	2.63± 0.01
OM(mg l^{-1})	3.48± 0.53	CEC (meq/10g)	11.0± 1.0
CEC(meq/10ml)	11.01± 1.0	Sulphate (mg kg^{-1})	143.24± 7.58
Nitrate	36.73±1.04	Phosphate (mg kg^{-1})	681.0± 15.2
EC(mS cm^{-1})	2.93± 0.03	Chloride (mg kg^{-1})	131± 7.3
Sulphate (mg l^{-1})	986 ±25.1	Phenolics (mg kg^{-1})	28.3± 4.2
Phosphate (mg l^{-1})	31.0± 6.2		
Chloride (mg l^{-1})	600± 26.9		
Phenolics (mg l^{-1})	430± 31.0		
Na	293±1.34		
Heavy metals (mg l^{-1})		Heavy metals (mg kg^{-1})	
Cd *(0.01)	2.28± 0.06	Cd	BDL
Cu *(0.20)	8.12± 0.84	Cu	0.83± 0.03
Fe *(5.0)	74.01± 1.98	Fe	12.76± 1.2
Mn *(0.20)	6.03± 0.92	Mn	1.91± 0.04
Ni *(0.20	5.60± 0.31	Ni	1.34± 0.03
Zn *(2.0)	18.11± 1.16	Zn	2.66± 0.10

All the values are means of triplicate (N=3) ± SD. BOD-Biological oxygen demand; COD-Chemical oxygen demand; TOC-Total organic carbon; TDS-Total dissolved solids; OM-Organic matter. TN-Total nitrogen; EC-Electrical conductivity; CEC-Cation exchange capacity; BDL-Bellow detection limit. *Permissible limit of trace elements in wastewaters (USEPA, 2000).

Table 2: Effect of RGPE on seed germination and amylase activity on Indian mustard plants.

Conc. of RGPE (%)	No. of seeds sown	No. of seeds germinated	% Germination	Amylase Activity (U/gm)
Control	10	10	100	1.30±0.14
25%	10	10	100	1.60±0.20
50%	10	10	100	1.25±0.17
75%	10	7	70	0.97±0.10
100%	10	5	50	0.76±0.08

All the values are Means of triplicate (N=3) ± SD.

Table 3: Concentration of Heavy metals (mg kg^{-1}) in roots of control and treated Indian mustard plants

Metals (mg kg^{-1})	Days	Concentration of RGPE (in %)				
		Control	25%	50%	75%	100%
	30	BDL	1.96±0.12^{a}	4.19±0.13^{a}	5.01±0.08^{b}	5.07±0.08^{a}
Cd	60	BDL	2.90±0.33^{b}	6.10±0.28^{a}	6.11±1.1^{a}	6.97 ±0.05^{a}
*(0.21)	90	1.00±0.04	4.32±0.22^{b}	7.03±0.14^{a}	7.11±1.1^{a}	8.06±0.09^{a}
	30	3.04±0.21	3.46±0.18^{c}	4.66±0.36^{a}	4.94±0.20^{a}	5.0±0.52^{a}
Cu	60	4.21±0.38	6.18±0.24^{a}	7.06±1.19^{a}	7.46±1.6^{a}	8.59±1.96^{b}
*(3.00)	90	5.47±0.67	6.39±0.1^{b}	8.09±1.26^{a}	8.87±1.3^{a}	9.86±1.97^{b}
	30	268.27±3.1	337.56±3.3^{b}	399.14±4.17^{b}	476.20±2.2^{c}	491.20±8.19^{d}
Fe	60	393.73±6.6	497.70±7.2^{d}	526.05±8.16^{d}	613.97±3.16^{d}	713.10±6.19^{d}
*(20.0)	90	690.43±1.2	743.60±5.0^{d}	893.09±7.0^{d}	928.88±7.6^{d}	964.60±3.96^{d}
	30	13.16±1.2	25.06±1.7^{a}	30.12±2.1^{c}	39.18±1.57^{b}	42.05±3.26^{b}
Mn	60	20.73±1.8	35.85±1.0^{a}	39.20±1.1^{b}	50.47±2.0^{b}	53.95±3.6^{c}
*(2.00)	90	34.95±1.87	38.58±1.0^{a}	40.87±1.4^{b}	53.11±2.7^{c}	56.33±1.5^{d}
	30	BDL	BDL	BDL	BDL	3.42±0.05^{a}
Ni	60	BDL	BDL	BDL	3.05±1.2^{a}	5.39±0.45^{a}
*(1.63)	90	1.09±0.31	2.25±1.16^{a}	3.29±1.96^{a}	4.17±2.1^{a}	6.63±2.1^{a}
	30	9.50±1.2	22.30±1.8^{b}	27.46±3.7^{b}	35.46±1.8^{a}	36.57±1.46^{b}
Zn	60	18.08±3.5	36.37±3.6^{b}	39.71±3.26^{c}	44.54±2.9^{b}	51.43±2.1^{b}
*(27.4)	90	25.59±2.3	48.97±2.56^{c}	54.74±2.4^{d}	60.46±2.1^{b}	66.38±1.7^{c}

All the values are means of triplicate (N=3) ± SD. BDL- Bellow detection limit. Means of different heavy metals of control to the treated plant root are compared at different concentrations of RGPE within column. ^{a}highly significant; ANOVA $p<0.001$,^{b}significant; ANOVA $p<0.01$,^{c}less significant; ANOVA $p<0.05$, ^{d}non significant. *Permissible limit of trace elements.

Table 4: Concentrations of Heavy metals (mg kg-1) in shoot of control and treated Indian mustard plants.

Metals (mg kg-1)	Days	Concentration of RGPE (in %)				
		Control	25%	50%	75%	100%
	30	BDL	0.23±0.03[a]	1.57±0.03[a]	1.86±0.06[a]	2.32±0.03[a]
Cd	60	BDL	1.65±0.05[a]	2.19±0.24[a]	2.64±0.10[a]	3.35±0.05[a]
*(0.21)	90	0.19±0.07	2.14±0.14[a]	3.98±0.14[a]	4.21±0.14[a]	5.33±0.03[a]
	30	2.03±0.06	3.48±0.19[a]	3.05±0.68[a]	4.32±0.15[a]	4.30±0.12[a]
Cu	60	2.61±0.15	4.58±1.59[b]	5.05±1.1[a]	6.61±1.95[b]	7.42±1.9[b]
*(3.00)	90	2.91±0.11	4.91±1.59[a]	6.87±1.1[a]	8.18±2.1[b]	10.38±2.2[a]
	30	30.60±2.59	67.79±3.57[c]	138.26±4.1[a]	177.55±2.1[c]	216.34±3.58[d]
Fe	60	96.87±3.56	144.57±4.0[d]	186.88±3.56[a]	268.40±3.1[a]	269.60±4.6[b]
*(20.0)	90	101.70±4.0	219.86±6.1[a]	285.22±4.58[d]	323.80±4.1[d]	377.40±5.0[d]
	30	1.41±0.74	4.33±1.7[a]	7.16±1.0[a]	11.58±2.7[b]	14.69±2.57[a]
Mn	60	3.35±1.0	8.03±2.56[a]	13.30±2.0[a]	17.60±3.0[a]	22.54±2.58[a]
*(2.00)	90	4.25±2.0	11.02±2.36[c]	16.99±2.58[b]	27.04±3.0[d]	23.55±3.0[a]
	30	BDL	BDL	BDL	BDL	BDL
Ni	60	BDL	BDL	BDL	BDL	5.11±1.1[a]
*(1.63)	90	BDL	BDL	7.61±1.6[a]	8.68±1.1[a]	9.55±1.0[a]
	30	3.82±0.06	6.95±2.16[a]	15.76±2.56[a]	22.08±2.1[b]	32.18±3.1[b]
Zn	60	5.75±1.0	17.35±2.1[c]	26.53±3.6[a]	26.05±4.0[c]	39.41±2.2[a]
*(27.4)	90	9.77±1.5	20.89±2.2[b]	29.78±3.2[b]	31.40±3.59[a]	43.25±3.1[d]

All the values are means of triplicate (N=3) ± SD. BDL- Bellow detection limit. Means of different Heavy metals of control to the treated plant shoot are compared at different concentrations of RGPE within column. ahighly significant; ANOVA p<0.001,bsignificant; ANOVA p<0.01,cless significant; ANOVA p<0.05, dnon significant. *Permissible limit of trace elements

Table 5: Concentration of Heavy metals (mg kg-1) in leaves of control and treated Indian mustard plants.
All the values are means of triplicate (N=3) ± SD. BDL- Bellow detection limit. Means of different Heavy metals of control to the treated plant leaves

Metals (mg kg-1)	Days	Concentration of RGPE (in %)				
		Control	25%	50%	75%	100%
	30	0.15±0.06	2.77±0.09[a]	3.19±0.32[a]	4.05±0.05[a]	4.98±0.05[a]
Cd	60	0.20±0.05	5.73±1.1[a]	6.22±1.24[a]	7.73±1.2[a]	9.14±1.1[a]
*(0.24)	90	0.36±0.06	6.99±1.7[b]	8.49±1.96[a]	9.04±2.1[a]	9.98±1.1[a]
	30	3.26±0.30	6.76±1.9[a]	7.75±1.0[a]	9.74±2.0[b]	10.52±2.58[b]
Cu	60	5.17±1.0	11.29±1.8[b]	13.85±2.0[a]	15.45±2.6[a]	18.03±2.57[a]
*(4.84)	90	8.72±1.0	14.25±2.0[c]	19.67±2.1[a]	22.20±3.1[a]	25.63±2.57[a]
	30	206.17±4.6	326.83±4.3[d]	556.07±5.5[b]	737.55±5.2[b]	672.47±2.6[d]
Fe	60	376.00±6.0	566.16±5.6[d]	774.00±4.5[d]	846.67±4.5[d]	883.84±4.1[d]
*(20.0)	90	218.03±8.0	384.22±3.6[b]	464.39±4.1[d]	486.84±3.6[b]	636.15±5.6[c]
	30	22.15±2.0	49.14±3.0[c]	174.03±3.96[b]	167.86±4.56[b]	179.96±3.65[a]
Mn	60	57.26±3.0	99.48±5.1[a]	187.48±4.1[b]	264.66±2.5[c]	192.49±2.0[a]
*(2.0)	90	96.36±4.6	167.30±4.5[a]	236.30±3.6[c]	297.81±2.5[a]	268.42±2.4[b]
	30	BDL	BDL	BDL	BDL	1.83±0.06[a]
Ni	60	BDL	BDL	BDL	BDL	1.98±0.07[a]
*(2.14)	90	BDL	BDL	0.56±1.85[a]	2.04±1.0[a]	2.8040±1.0[a]
	30	23.27±3.56	23.07±2.6[a]	38.57±2.56[a]	46.53±2.1[a]	68.48±2.56[a]
Zn *(38.1)	60	29.57±3.56	47.43±2.6[b]	52.18±3.3[a]	76.17±3.9[a]	79.93±3.1[a]
	90	46722±3.58	76.16±3.0[b]	94.18±4.1[c]	97.42±3.16[b]	99.40±4.0[a]
Heavy metals in seeds of control and treated Indian mustard plants						
Cd *(0.11)		0.08±0.04	BDL	BDL	0.10± 0.03[a]	0.13± 0.04[a]
Cu *(3.52)		0.13± 0.08	1.21± 0.03[a]	1.49± 0.06[a]	1.86± 0.05[a]	1.90± 0.07[a]
Fe *(10.0)		3.56± 0.50	4.45± 0.57[b]	5.50± 1.6[c]	5.86± 1.56[c]	6.64± 1.6[c]
Mn *(2.0)		3.10± 0.15	4.22± 0.72[b]	5.16± 1.45[b]	6.89± 1.62[c]	7.06± 1.59 [c]
Ni *(0.95)		BDL	BDL	BDL	BDL	BDL
Zn *(27.7)		1.33± 0.03	1.79± 0.06[a]	2.10± 0.04[a]	2.23± 0.05[a]	2.46± 0.06[a]

are compared at different concentrations of RGPE within column. [a]highly significant; ANOVA p<0.001,[b]significant; ANOVA p<0.01,[c]less significant; ANOVA p<0.05, [d]non significant. *Permissible limit of trace elements.

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