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ORIGINAL PAPER

Comparative growth response of two varieties of *Vigna radiata* L. (var. PDM 54 and var. NM 1) grown on different tannery sludge applications: effects of treated wastewater and ground water used for irrigation

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Abstract This study evaluates the possibility of using contaminated soil by treated tannery wastewater and the use of tannery sludge in agriculture. The plants of *Vigna radiata* var. PDM 54 grown on contaminated soil and irrigated with ground water have not shown the translocation of toxic metal (Cr) in the upper part. The biomass of the plant increased when irrigated with treated tannery wastewater compared to ground water, whereas no significant change was observed in chlorophyll and protein contents. In both the varieties (var. PDM 54 and var. NM 1) of *V. radiata* grown on tannery sludge amendments, the growth parameters exhibited a pronounced positive growth response up to 35% tannery sludge amendments compared with the plants grown on garden soil. Despite the Cr accumulation at lower amendments, no toxicity symptoms were observed in both the varieties of the plants. Higher amendments affected various growth parameters, NR activity, and carbohydrate content of the plants. The results suggest that the plants of *V. radiata* (var. PDM 54) may be grown on contaminated soil or lower sludge amendments and irrigated with ground water. No translocation of toxic metal Cr was found in the seeds of the plants grown in up to 25% tannery sludge. However, periodical monitoring is required before the consumption of seeds. Overall, the results showed that plant growth patterns were influenced to some extent by the level of soil contamination and the water used for irrigation.

Keywords *Vigna radiata* · Tannery sludge · Chromium · Growth · Scanning electron microscopy · Accumulation

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Introduction

Unmindful disposal of industrial waste has created problems twofold: it has degraded the soil fertility and contaminated the food-chain. As a cheap management practice, lower amounts of sludge are amended with agricultural soil to enhance the productivity, and treated wastewaters are also used for irrigation purpose. The beneficial effects of using sewage sludge in agriculture have been proven. It improves the physical, chemical, and biological properties of the soil and increases crop production through the addition of nutrients and organic matter (Mantovia et al. 2005; Cassado-Vela et al. 2006; Dolgen et al. 2007), at the same time preventing it from entering other ecosystems and further polluting.

India is one of the largest producers of leather in the world and there are at present more than 3,000 tanneries, with annual processing capacity of 0.7 million tonnes of hides and skins. In India, Jajmau, Kanpur (about 400 tanneries) is a major industrial town which specializes in processing hides into heavy leather. The wastewaters discharged from leather tanning industries also contain significantly high amounts of heavy metals, particularly chromium. It has been common practice to use the treated wastewater for irrigation of crops, vegetables, etc. The prevalent practice has contaminated the top soil of the adjoining agricultural fields. Thus, edible plants growing on such contaminated soil represent an important pathway for the movement of potentially toxic elements from soil to human beings (Singh et al. 2004a). Today, however, the decline of arable land and an increasing food demand, has left no choice but to continue growing crops on such contaminated lands (Barman et al. 2000; Fytianos et al. 2001; Sinha et al. 2005; Gupta and Sinha 2006a; Sinha et al. 2007; Sinha and Gupta 2007). Recently, Sinha et al. (2006a) have demonstrated healthy growth of vegetables and crops grown on tannery wastewater contaminated soil. The accumulation and translocation of metal varies from plant to plant. It was observed that leafy vegetables have shown more accumulation and translocation of toxic metal chromium than in the edible parts of other vegetables and crops. Metals such as Fe, Zn, and Mn are essential as micronutrients for many cellular processes, however, at higher concentrations their ions act as efficient generators of reactive oxygen species. Moreover, metals at excessive concentrations affect plant growth, development, and yield by suppressing metabolism and translocation.

Vigna radiata (mung bean) is widely grown as mixed, intercrop, or in rotation to improve nitrogen status of soil or to break the disease pest cycles in most parts of the World. Mung is a very tolerant crop, therefore, it is expected that the plant may grow healthy in contaminated soil, and may accumulate toxic metal ion in edible parts which can enter the food chain through dietary intake. Therefore, the growth alone should not be criteria for choosing crops to be grown in contaminated areas. Singh et al. (2003) reported inhibition of seedling (*Vigna radiata*) growth and nitrate reductase activity in response to Pb^{2+} and salt stress; they also reported the synergestic effect due to these stresses. Sinha et al. (2006b) reported that Mn stress alters phytotoxic effects of Cr in *Vigna radiata* grown in solution culture. Similarly, Shankar et al. (2004) studied antioxidant response of enzymes and metabolites to chromium speciation in *V. radiata* roots grown in solution culture. These studies were carried in solution culture and the extrapolation of these studies to field conditions is not feasible due to many environmental factors which may interfere synergistically or antagonistically. Besides, there are several reports (Singh et al. 2004b; Sinha and Gupta 2007; Sinha et al. 2007) demonstrating better growth of the plants when grown on tannery sludge amended soils. Thus, it is necessary to experimentally validate the suitable dose to be amended with soil to grow *V. radiata*.

This study was planned to take place in the contaminated soil of Jajmau, Kanpur where up-flow anaerobic sludge blanket (UASB) treated tannery wastewater has been used for irrigation for many decades. Experiments were planned using *Vigna radiata* var. PDM 54 on naturally contaminated soil (field) with two different types of waters for irrigation: UASB treated wastewater and ground water, in order to check the use of contaminated land for cultivation. In another experiment (pots), two varieties of *Vigna radiata* (var. PDM 54 and var. NM 1) were grown in different amendments of tannery sludge in order to simulate various level of contamination and its effect on morpho-anotomical parameters, chlorophyll, and protein contents. The plants were irrigated with ground water.

Materials and methods

Study location

In India, Jajmau (Kanpur) is a major industrial town and lies in the Indo-Gangetic plains between the parallels of 26°28′ N and 80°24′ E. The discharge from these industries is treated in an up-flow anaerobic sludge blanket (UASB) treatment plant before release in an agricultural field (~2,100 acre). The present study was conducted in an experimental plot in the same area.

Seed procurement

The seeds of *Vigna radiata* (var. PDM 54 and var. NM 1) were obtained from the Indian Institute of Pulse Research, Kanpur (Uttar Pradesh, India). All the seeds were sterilized in 3% formalin for 5 min to avoid fungal contamination, then washed with distilled water for three changes and soaked in water overnight.

Experimental set-up

In the field, seeds of *Vigna radiata* (var. PDM 54) were cultivated on contaminated soil of Jajmau, Kanpur in plots measuring 2 m × 2 m, in five replicates from March until June 2005. The plants were irrigated by two different types of water: UASB treated wastewater and ground water, then harvested after 90 days.

In the laboratory, the experiment was designed to simulate different levels of contamination prevailing in the field by amendments made out of tannery sludge. Dried tannery sludge cakes were collected from the sludge beds of the Wastewater Treatment Plant in Jajmau, Kanpur (Uttar Pradesh, India) in large plastic bags and brought to the field laboratory. Various amendments (10, 25, 35, 50, 75, and 100%) of tannery sludge (TS) were prepared after pulverizing and sieving (2 mm) using garden soil (GS), where garden soil served as control. For the purpose of convenience, the tannery sludge amendments have been denoted as 10% TS, 25% TS, 35% TS, 50% TS, 75% TS, and 100% TS. Soaked seeds of both varieties of *V. radiata* (var. PDM 54 and NM 1) were evenly sown in pots (14′′ in diameter) filled with different amendments (10, 25, 35, 50, 75 and 100%) of tannery sludge (10 kg dw), along with one set of control in soil, and each in three replicates. Ten seeds were sown in each pot to a depth of 5 cm. A total of 21 pots were randomly divided into 7 groups (amendments), each group with 3 pots (replicates). The pots were watered daily until seed germination. When the seedlings had developed five or six leaves, they were thinned out to retain three uniform ones per pot and allowed to grow. Pots were placed in a greenhouse in a randomized block design at an average diurnal temperature of 25–45°C. Pots were irrigated using tap water when required. The experiments were repeated twice, each time with three replicates. The plants were harvested monthly for three consecutive months (90 days).

Metal accumulation

Oven dried contaminated soil (CS), tannery sludge, and soil samples (0.5 g) were digested in HNO_3 (70%) and HF (3:1) using the Microwave Digestion System MDS 2000 (CEM Corp., USA) for the estimation of metal contents.

Different parts of the treated and control plants were separated manually, washed thoroughly with running deionized water, blotted dry, cut in small pieces and oven dried (70°C for 1 week). After dry weight determination, the oven-dried samples were ground and digested in HNO_3 (70%) using the Microwave Digestion System MDS 2000 and metal contents were estimated using the Atomic Absorption Spectrophotometer (GBC Avanta $\sum$, GBC Scientific Equipment, USA). The concentration of metals in the control plant had already been subtracted from the data of metal accumulation in the treated plants.

Growth parameters

The plants (one plant from each pot) were harvested after 30, 60, and 90 days after sowing and repeatedly washed with double distilled water. At each harvest, the length of roots and shoots of all the plants was measured by Vernier calipers, photosynthetic area was determined by a delta T device, and the number of leaves was recorded manually. Chlorophyll content in the fresh leaves of the plant (100 mg) was estimated by the method of Arnon (1949). Protein content in the roots and leaves of the control and treated plants was estimated using BSA as a standard protein (Lowry et al. 1951). Total carbohydrate content was determined according to Dubois et al. (1956). The nitrate reductase activity was estimated in the leaves using the method of Srivastava (1974).

Scanning electron microscopic studies

The leaves of the plant (pots) *V. radiata* (var. PDM 54 and var. NM 1) grown on 50 and 100% TS along with control were harvested after 90 days of growth and examined by scanning electron microscope. The leaf samples were kept in 2.5% gluteraldehyde for fixation and left overnight. The material was passed through alcohol series (30, 50, 70, 90, and 100%) for dehydration, and drying was done in BAL-TEC CPD-030 critical point drier using liquid CO_2 as the carrier gas. The mounted specimens (six samples from each category) were coated with 15 nm thin gold and studied with the Philips XL-20 scanning electron microscope (USA).

Data analysis and interpretation

The experiment was performed in a completely randomized block design involving seven amendments of tannery sludge and at three growth periods. To confirm the variability of data and validity of results, all the data were subjected to an analysis of variance (ANOVA), and to determine the significant difference between treatments, least significant difference (LSD) analysis was performed (Gomez and Gomez 1984).

Quality control and quality assurance

The standard reference material of metals (E-Merck, Germany) was used for calibration and quality assurance for each analytical batch. For sludge and soil analysis, analytical data quality of metals was ensured through repeated analysis of sewage sludge (catalogue no. CRM029-050; lot no. JC029a) and soil (catalogue no. CRM 028-050; lot no. IH020) samples of the Resource Technology Corporation (EPA Certified Reference Material), and results were found to be within predicted levels. The reference solution (BND 1101.02) for multi-elements (Zn, Fe, Cu) and (BND 402.02) for Cr was also used for calibration of the atomic absorption spectrophotometer and validation of the test methods provided by the National Physical Laboratory (NPL), New Delhi (India), and the results were found to be within ±1.50% of certified values. The recoveries of metals from the plant tissues were found to be more than 98.5% as determined by digesting three samples each from spiked plant samples.

Results and discussion

Physico–chemical properties

The analysis of physico-chemical properties of UASB treated tannery wastewater (Table 1) used for the irrigation of agricultural soil showed high salinity, EC, TDS, BOD, and COD due to processing of raw hides where a large number of chemicals are used which find their way into wastewater. The physico–chemical properties of the soil irrigated with UASB treated wastewater (T1) and ground water (T2) have shown that the level of EC, CEC, OC, and OM were found higher in T1 than T2 (Table 2). Similarly, the results of physico-chemical analysis of CS, GS, and its different amendments (Table 2) showed higher levels in CS and TS than the level of respective parameters in GS except Fe. The level of Fe was higher in GS as compared to the CS and TS, which is due to the presence of Fe as one of the major constituents in the earth's crust. There are some recent reports showing high level of EC, salinity, CEC, OM, and OC in tannery waste contaminated soil (Sinha and Gupta 2007) due to long-term inputs of treated tannery wastewater in agricultural fields.

Table 1 Physico–chemical parameters of treated tannery wastewater[a]

Parameters	UASB treated tannery wastewater
pH	7.26 ± 0.01
Salinity (g kg^{-1})	8.50 ± 0.01
Electrical conductivity (μSm^{-1})	81.1 ± 0.07
Total dissolved solids (mg l^{-1})	7,990 ± 102
Biological oxygen demand (mg l^{-1})	273 ± 28
Chemical oxygen demand (mg l^{-1})	1,360 ± 126
Metals (mg l^{-1})	
Fe	3.93 ± 0.58
Cr	3.20 ± 0.52
Zn	0.39 ± 0.03
Mn	0.12 ± 0.007
Ni	Not detected

[a] All values are means of five replicates ±SD

Metal accumulation

In the field experiment, the plants of *V. radiata* (var. PDM 54) were grown on contaminated soil and irrigated with treated tannery wastewater (routine practice) and ground water to critically evaluate the use of contaminated soil for cultivation in different irrigation practices. The analysis of results (Table 3) showed significantly higher levels of Cr in the plant parts when irrigated with UASB treated tannery wastewater after 90 days of growth than in the plants irrigated with ground water. This is due to the presence of high levels of Cr in the treated wastewater used for irrigation. The order of accumulation of all the metals was found as roots > leaves > shoots.

Table 2 Physico–chemical properties of different substrates[a]

Parameters	Substrates				
	T1	T2	GS[b]	CS[b]	TS[b]
pH (1:2 ratio)	7.90 ± 0.24	7.18 ± 0.007	6.63 ± 0.04	7.86 ± 0.005	7.84 ± 0.005
Salinity (g kg^{-1})	7.10	0.80	0.2	1.3	7.5
EC (μS m^{-1})	2188 ± 10.9	1,660 ± 13.8	708 ± 1.6	2,506 ± 5.47	3,044 ± 11.4
CEC (cmol kg^{-1})	91.52 ± 1.84	60.04 ± 0.06	55.69 ± 0.83	83.8 ± 1.2	146.25 ± 1.87
OC (%)	0.80 ± 0.007	0.23 ± 0.007	0.48 ± 0.04	1.27 ± 0.07	5.47 ± 0.27
OM (%)	0.60 ± 0.02	0.35 ± 0.01	0.793 ± 0.121	2.18 ± 0.01	9.39 ± 0.49
Total concentration of heavy metals (mg kg^{-1} dw)					
Fe	14,285 ± 1244	13,442 ± 127	38,862 ± 3684	22,898 ± 1740	19,401.44 ± 61
Cr	197.76 ± 12.83	48.50 ± 3.69	5.1 ± 0.34	145.87 ± 21.51	7,489.76 ± 203
Zn	104.91 ± 0.97	147.18 ± 14.8	45.15 ± 0.86	217.03 ± 22.81	340.69 ± 11.10
Mn	360.78 ± 31.12	356.27 ± 31.02	238.93 ± 15.3	310.76 ± 68.8	294.19 ± 15.50
Cu	43.79 ± 1.68	53.03 ± 5.82	18.67 ± 0.85	18.67 ± 0.85	188.09 ± 5.81

[a] All values are means of three replicates ±SD

[b] Sinha et al. (2007)

Table 3 Accumulation of metals in various plant parts (mg kg^{-1} dw) irrigated with UASB treated wastewater (T1) and ground water (T2) after 90 days[a]

Treatments	Plant parts	Metals			
		Fe	Cr	Zn	Mn
T1	R	825.14 ± 76.70	147.96 ± 9.30	42.43 ± 4.44	25.48 ± 1.62
	S	831.89 ± 79.80	19.18 ± 3.10	104.35 ± 12.13	39.59 ± 2.96
	L	1,244.41 ± 47.52	21.01 ± 2.79	45.89 ± 1.35	78.34 ± 1.56
T2	R	335.63 ± 32.13	10.35 ± 1.01	31.43 ± 1.93	17.53 ± 1.79
	S	386.72 ± 32.79	9.74 ± 0.73	51.04 ± 4.86	21.24 ± 0.24
	L	1,107.56 ± 8.06	bdl[b]	65.67 ± 0.67	94.41 ± 1.00

[a] All values are means of three replicates ±SD

[b] Ni was found bdl

Both varieties of *V. radiata* grown in pots showed accumulation of metals in different part of the plants and increased progressively with increase in amendments (Tables 4 and 5). In var. PDM 54, the maximum increase of Cr, i.e. 517.99, 399.8, and 276.5% in its roots, shoots and leaves, respectively, was recorded in 100% TS as compared to 10% TS (Table 4). In var. NM 1, the maximum increase in accumulation of Cr, i.e. 443.43, 333.10, and 319.32%, was recorded in 100% TS in roots shoots and leaves, respectively as compared to 10% TS (Table 5). Overall, the variety var. PDM 54 showed more accumulation of metals than var. NM 1 in all parts of the plants. Interestingly, no accumulation of Cr in the seeds of the plants was recorded in lower amendments of tannery sludge (10 and 25% TS).

The metal accumulation ability of different plants varies with species. Furthermore, the irrigation practice greatly influence the rate of heavy metal accumulation in the plants grown on contaminated soils (Barman et al. 2000; Fytianos et al. 2001). Similar to the present findings, more accumulation of Cr in the roots was recorded than shoots, which may be due to reduction of Cr (VI) to Cr (III), which reduces its mobility from roots to shoot. A similar study reported by Singh et al. (2003) on *V. radiata* seedlings grown in the presence of Pb and NaCl showed higher accumulation of Pb^{+2} and Na^{+} in their roots than in their leaves. Shankar

Table 4 Accumulation of metals (μg g^{-1} dw) in different parts of *V. radiata* var. PDM 54 grown (pots) on different amendments of tannery sludge after 90 days[a,b]

Substrates	Plant parts	Metals			
		Cr[c]	Fe	Zn	Mn
GS	Roots	bdl	68.41 ± 6.68	16.24 ± 3.15	17.53 ± 1.79
	Shoots	bdl	105.21 ± 10.51	13.25 ± 1.05	21.24 ± 0.24
	Leaves	bdl	101.21 ± 9.98	25.24 ± 4.05	24.41 ± 1.00
10% TS	Roots	70.06 ± 5.67	80.74 ± 4.79	16.24 ± 3.15	32.29 ± 2.83
	Shoots	12.36 ± 0.93	79.46 ± 2.26***	12.01 ± 1.17	20.53 ± 1.82
	Leaves	16.72 ± 1.19	153.04 ± 13.8**	11.06 ± 0.89	27.87 ± 1.07
25% TS	Roots	138.16 ± 13.67**	153.04 ± 13.8**	35.13 ± 1.49**	57.83 ± 5.02**
	Shoots	17.98 ± 2.27*	68.41 ± 6.68**	24.73 ± 0.81***	41.30 ± 2.61***
	Leaves	20.83 ± 1.55	79.46 ± 2.26***	22.19 ± 1.71***	45.99 ± 0.83***
35% TS	Roots	165.27 ± 14.49***	173.30 ± 13.35***	45.79 ± 2.97***	82.43 ± 2.29***
	Shoots	19.36 ± 1.53*	77.19 ± 5.88***	37.62 ± 1.46***	64.20 ± 2.52***
	Leaves	26.71 ± 0.77***	105.05 ± 10.81***	31.65 ± 1.31***	72.77 ± 3.34***
50% TS	Roots	250.05 ± 13.56***	295.95 ± 15.59***	61.77 ± 6.61***	105.22 ± 10.30***
	Shoots	27.46 ± 1.31***	103.33 ± 10.19***	52.64 ± 8.38**	77.11 ± 7.07***
	Leaves	28.67 ± 0.94***	120.95 ± 3.47†	45.42 ± 2.74***	82.63 ± 2.20†
75% TS	Roots	312.45 ± 30.16***	378.91 ± 15.42†	76.08 ± 1.78***	145.60 ± 13.14***
	Shoots	36.38 ± 3.43***	125.80 ± 10.63***	59.73 ± 5.04***	103.57 ± 7.03***
	Leaves	43.40 ± 2.78***	148.13 ± 8.75***	52.19 ± 5.19***	118.21 ± 10.01***
100% TS	Roots	432.97 ± 20.45***	493.26 ± 15.60†	97.38 ± 5.75***	196.83 ± 11.69***
	Shoots	54.37 ± 4.56***	152.97 ± 5.49†	81.24 ± 7.24***	157.08 ± 8.64***
	Leaves	62.95 ± 5.66***	209.97 ± 19.51***	70.73 ± 3.25***	166.11 ± 5.90†

[a] All values are means of three replicates ±SD

[b] Student *t*-test (two tailed as compared to 10% TS)

[c] Cr bdl in seeds at 10 and 25% TS

*$p < 0.05$, **$p < 0.02$, ***$p < 0.01$, †$p < 0.001$

et al. (2004) observed that roots and shoots of *Vigna radiata* varied highly in their ability to accumulate Cr regardless of the speciation in the nutrient medium. These studies were conducted in solution culture, however, there are many reports (Sinha et al. 2007; Sinha and Gupta 2007) on the plants grown in naturally contaminated soil or simulated field conditions showing variable level of translocation of metals. Similar to the present findings, the sunflower seeds grown on lower amendments have not shown the accumulation of Cr in the seeds (Singh et al. 2004b). Besides high levels of Cr in the tannery sludge and contaminated soil, the transfer of metal from substrate to plant was not found to be high, which is due to binding of metal to different fractions of the soil. There are many reports on tannery waste contaminated soil where most of the Cr was bound to the Fe–Mn oxide fraction of the sludge and not available to plants for uptake (Gupta and Sinha 2006b, 2007a). Thus, the addition of a proper amendment contributes towards the beneficial effects on the plant. A comparative study using two types of irrigation water was adopted, and the effect on plant productivity can help to understand the potential harmful effect of treated wastewater irrigation in agricultural soil. These studies showed that the application of sludge is dose and crop dependent.

Morphological parameters

The plants of *V. radiata* (var. PDM 54) grown in field and irrigated with UASB treated tannery wastewater (T1) have shown no significant difference in root and shoot lengths, whereas, number of leaves ($p < 0.02$) and fresh weight ($p < 0.01$) increased significantly as

Table 5 Accumulation of metals (μg g^{-1} dw) in different parts of *V. radiata* var. NM-1 grown (pots) on different amendments of tannery sludge after 90 days[a,b]

Substrates	Plant parts	Metals			
		Cr[c]	Fe	Zn	Mn
GS	Roots	bdl	59.31 ± 7.68	18.25 ± 4.91	20.33 ± 3.79
	Shoot	bdl	115.31 ± 14.11	17.29 ± 3.09	29.22 ± 2.14
	Leaves	bdl	142.21 ± 10.28	35.44 ± 3.07	14.21 ± 3.05
10% TS	Roots	63.04 ± 11.03	64.35 ± 6.58	14.73 ± 1.18	23.78 ± 2.79
	Shoots	10.42 ± 1.42	24.44 ± 1.99	9.02 ± 1.31	18.38 ± 1.43
	Leaves	12.21 ± 0.33	29.88 ± 3.42	10.40 ± 0.79	22.06 ± 1.66
25% TS	Roots	136.93 ± 12.81**	123.97 ± 7.7***	31.47 ± 2.02***	36.91 ± 1.48***
	Shoots	15.23 ± 1.04*	57.69 ± 9.59*	19.36 ± 1.09***	29.22 ± 1.59***
	Leaves	18.05 ± 1.15**	65.88 ± 5.05***	19.95 ± 0.73***	31.69 ± 1.27***
35% TS	Roots	153.08 ± 4.57***	155.53 ± 7.4***	39.96 ± 3.50***	40.81 ± 2.15***
	Shoots	16.87 ± 2.08*	64.42 ± 2.60***	28.85 ± 0.94***	31.02 ± 0.81***
	Leaves	19.88 ± 0.83***	74.36 ± 2.45***	29.81 ± 2.89***	35.88 ± 0.88†
50% TS	Roots	214.25 ± 7.02***	202.6 ± 6.35***	54.91 ± 5.40***	75.95 ± 4.74***
	Shoots	21.09 ± 1.10***	84.31 ± 7.69***	42.74 ± 2.10***	55.13 ± 6.01***
	Leaves	25.54 ± 1.36***	92.96 ± 2.75***	46.95 ± 1.59†	66.48 ± 3.52***
75% TS	Roots	266.72 ± 15.90***	255.28 ± 12.37***	72.07 ± 2.77†	98.13 ± 9.13***
	Shoots	29.24 ± 1.19***	102.51 ± 5.97***	54.80 ± 6.12***	69.82 ± 7.25***
	Leaves	38.89 ± 1.23†	121.86 ± 5.51***	58.14 ± 5.32***	74.43 ± 3.67***
100% TS	Roots	342.58 ± 22.47***	354.52 ± 11.40†	88.12 ± 4.37***	152.66 ± 3.27†
	Shoots	45.13 ± 1.92***	130.66 ± 9.40***	69.73 ± 4.89***	102.69 ± 5.93***
	Leaves	51.20 ± 6.48***	183.83 ± 7.44†	76.81 ± 8.05***	110.75 ± 8.03***

[a] All values are means of three replicates ±SD

[b] Student *t*-test (two tailed as compared to 10% TS)

[c] Cr bdl in seeds at 10 and 25% TS

*$p < 0.05$, **$p < 0.02$, ***$p < 0.01$, †$p < 0.001$

compared to ground water irrigated plants (T2; Table 6). Thus, the plants irrigated with treated tannery wastewater have shown better growth than irrigated with ground water which may be due to the presence of nutrients and richness of organic matter.

In pot experiments, in general, comparison of all the growth parameters has shown greater increase in var. PDM 54 than NM 1 in all the amendments in all growth periods (Fig. 1).

The analysis of the data showed significant ($p < 0.01$) increase in shoot length with increase in amendment ratio (Fig. 1a) in both the varieties except at 10% TS in var. PDM 54. The maximum increase ($p < 0.01$) in shoot length of var. PDM 54 (43.79%) and NM 1 (59.60%) was found in 75% TS after 90 days against their respective GS. Root length

Table 6 Morphological parameters, plant pigments, and protein contents of *V. radiata* (PDM 54) grown on soil irrigated with UASB treated wastewater (T1) and ground water (T2)[a,b]

Parameters	T1	T2
Root length (cm)	19.30 ± 3.54	16.70 ± 0.10
Shoot length (cm)	45.40 ± 1.56	48.70 ± 2.40
Number of leaves	53.50 ± 4.95*	27.00 ± 1.41
FW (g)	83.00 ± 1.41**	65.00 ± 1.41
Total Chl (mg g^{-1} fw)	2.51 ± 0.12	2.60 ± 0.33
Chl a/b	2.17	1.76
Carotenoid (mg g^{-1} fw)	0.63 ± 0.04	0.73 ± 0.13
Leaf protein (mg g^{-1} fw)	14.91 ± 0.52	14.59 ± 2.52

[a] All values are means of three replicates ±SD

[b] Student *t*-test (two tailed as compared to T2)

*$p < 0.02$, **$p < 0.01$

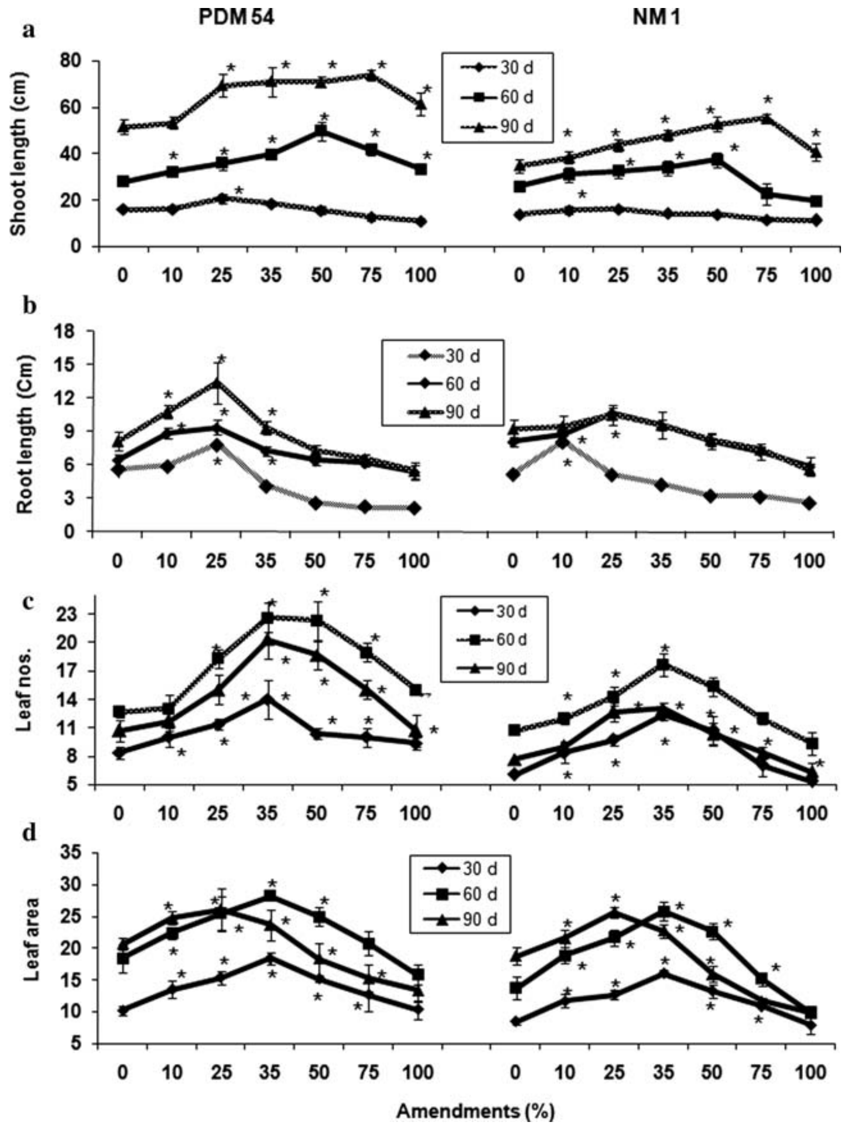


Fig. 1 Effect of tannery sludge amendments on root length (cm) (**a**), shoot length (cm) (**b**), number of leaves (**c**), and leaf area (cm^2) (**d**) of *V. radiata* (var. PDM 54 and NM 1). All values are means of three replicates ± SD. *$p < 0.01$ as compared to GS

increased significantly ($p < 0.01$) in var. PDM 54, until 35% TS with maximum increase (64.38%) in 25% TS, and in var. NM 1 a maximum increase of 13.59% was recorded in 25% TS at 90 days, against their respective GS (Fig. 1b), followed by a decrease. Both varieties exhibited increases in root length at lower amendments; however, the increase was more prominent in var. PDM 54 than var. NM 1.

In var. PDM 54, the number of leaves (Fig. 1c) of the plant increased in all the amendments and in all the growth periods except at 100% TS at 90 days; however, in NM 1, the increase was recorded up to 75% TS. In both varieties, the maximum increase ($p < 0.01$) in number of leaves was recorded up to 35% TS at all the growth periods as compared to their respective GS. Maximum increases of 90.53% (var. PDM 54) and 69.49% (var. NM 1) were observed in the plants grown on 35% TS at 90 days over their GS values. Both varieties exhibited an increase in growth parameters at lower amendments; however, the increase was more prominent in var. PDM 54 than var. NM 1. In both varieties, the leaf area (Fig. 1d) increased up to 75% TS at 30 and 60 days and up to 35% TS at 90 days over their respective GS.

Similar to earlier reports (Sinha et al. 2007; Sinha and Gupta 2007), the plants grown on lower amendments facilitate the growth parameters in both varieties. This phenomenon could be due to the availability of essential metals in excess at lower amendments; however, at higher TS amendments, the same had become toxic, and hence this manifestation could be seen in the form of reduction in growth parameters. Shankar et al. (2004) reported that Cr (VI) treatments exhibited significant reduction in all the growth parameters (root length, root dry weight, and total leaf area).

There are several reports of metal tolerance and healthy growth of different edible crops grown on contaminated soil and plants tend to adapt themselves to cope with stress conditions which vary from one plant to another. The leafy vegetables have shown higher levels of toxicity (Sinha et al. 2007; Sinha and Gupta 2007; Gupta and Sinha 2007b) compared to other oil bearing plants when grown on contaminated soil (Singh et al. 2004b). Sinha et al. (2006b) recently reported that the effects of low Mn were more pronounced on Cr toxicity than adequate and excess manganese in the plants of *Vigna radiata*, cv. PU 19.

Morpho–anatomical

The effect on the epidermal structures of leaves of *V. radiata* var. PDM54 (Fig. 2a–f) and var. NM 1 (Fig. 3a–f) grown on higher sludge amendments (50 and 100% TS) after 90 days was recorded. The analysis of figures showed that most of the stomata were found partially or totally closed in the leaves of the plants growing in the tannery sludge amended soil. The number of stomata per unit area was increased with increase in sludge amendment ratio as compared to GS. The observations showed slightly higher elevation of stomata than that of GS. The stomata was more highly affected in var. NM 1 plants grown on 100% TS after 90 days (Fig. 3) than var. PDM 54. The epidermal layer of the plants after 90 days was also observed to be highly convoluted (Figs. 2 and 3), as compared to GS. This could be due to the decrease in turgor pressure in the epidermal layer. The drop in turgor pressure in the epidermal cytoplasm could be due to inhibition of the cell wall to uptake requisite

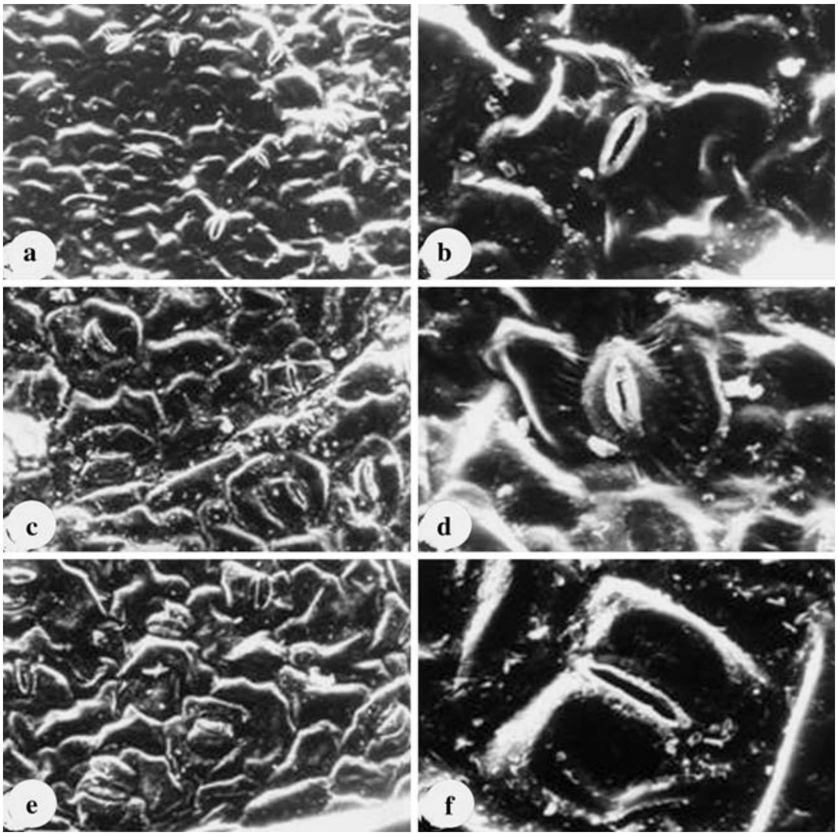


Fig. 2 SEM micrographs of leaf surface of *V. radiata* var. PDM 54. (**a**) Leaf epidermis of control plant (400×). (**b**) Magnified view of plate A (1,550×). (**c**) Leaf epidermis of plants growing in 50% tannery sludge (400×). (**d**) Magnified view of plate C (1,550×). (**e**) Leaf epidermis on plants growing in 100% tannery sludge. (**f**) Magnified view of plate E (1,550×)

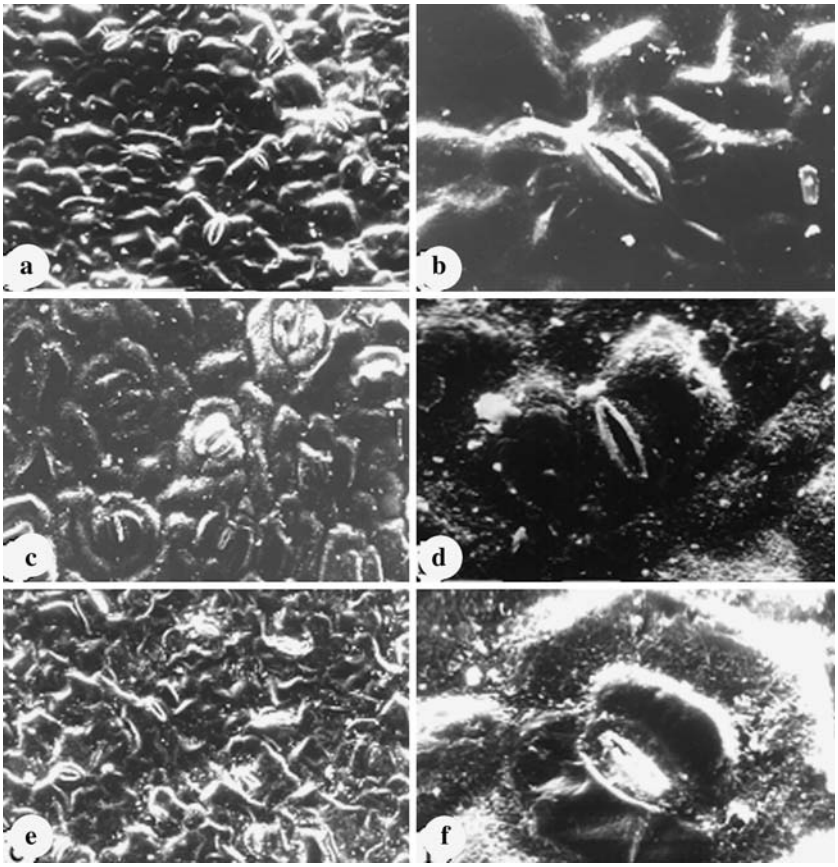


Fig. 3 SEM micrographs of leaf surface of *V. radiata* var. NM 1. (**a**) Leaf epidermis of control plant (400×). (**b**) Magnified view of plate A (1,550×). (**c**) Leaf epidermis of plants growing in 50% tannery sludge (400×). (**d**) Magnified view of plate C (1,550×). (**e**) Leaf epidermis on plants growing in 100% tannery sludge. (**f**) Magnified view of plate E (1,550×)

amount of water from the vascular bundle. The sap of the plants growing on 100% TS is likely to contain high salts and metals; therefore, reverse osmosis cannot be ruled out to be occurring on the cell walls, forcing water from the cytoplasm to the intercellular spaces. As micrographs suggest (Figs. 2 and 3), the plants of *V. radiata* var NM 1 were more sensitive than var. PDM 54. The elongation in stomatal length with increase in TS amendments has been reported earlier in plants grown under stress conditions (Sinha et al. 2007; Singh and Sinha 2004).

Photosynthetic pigments

UASB treated tannery wastewater irrigated (T1) plants of *V. radiata* var. PDM 54 have shown no significant difference in total chlorophyll and carotenoid contents (Table 6) in comparison with ground water irrigated plants (T2).

Overall, the photosynthetic pigments in the plant of *V. radiata* var. PDM 54 (Table 7) were higher than in var. NM 1 (Table 8) growing in all the amendments at all growth periods. Total chlorophyll content increased with increase in TS amendments until 35% TS at 90 days in both varieties and then decreased. A maximum increase of 20.3% in var. PDM 54 and 7.8% in var. NM 1 at 10% TS in total chlorophyll content was recorded after 90 days over their respective GS. The total chlorophyll content results were compared for both the varieties and the increase (%) was more pronounced in var. PDM 54 than var. NM 1. At 90 days, the chl a/b ratio decreased in both varieties with increase in amendment ratio greater in PDM than in var. NM 1.

In both varieties, the carotenoid content increased in all the amendments at 30 days as compared to GS. The comparison of carotenoid content in both varieties (var. PDM 54 and var. NM 1) has shown a greater increase (%) in var. PDM 54 than var. NM 1 in all growth periods (Tables 7 and 8). A maximum increase of 39.67% at 10% TS (var. PDM 54) and 24.48% at 25% TS (var. NM 1) was recorded at

Table 7 Effect of tannery sludge amendments on total chlorophyll and carotenoid contents (mg g^{-1} fw) and chl a/b ratio in the leaves of *V. radiata* var. PDM 54[a]

Substrates	Durations			Photosynthetic pigments
	30 days	60 days	90 days	
GS	1.96 ± 0.45	2.14 ± 0.15	2.00 ± 0.21	Total chl
	1.36	2.70	2.58	Chl a/b
	0.51 ± 0.04	0.56 ± 0.02	0.50 ± 0.03	Carotenoid
10% TS	2.18 ± 0.27	2.42 ± 0.15*	2.41 ± 0.12*	Total chl
	1.63	2.49	2.48	Chl a/b
	0.54 ± 0.08	0.66 ± 0.07*	0.70 ± 0.03*	Carotenoid
25% TS	2.60 ± 0.19*	2.61 ± 0.22*	2.24 ± 0.17	Total chl
	2.03	2.42	1.81	Chl a/b
	0.71 ± 0.04*	0.68 ± 0.06*	0.58 ± 0.06*	Carotenoid
35% TS	2.71 ± 0.21*	3.01 ± 0.27*	2.17 ± 0.13	Total chl
	2.14	1.81	2.00	Chl a/b
	0.73 ± 0.13*	0.78 ± 0.02*	0.55 ± 0.03	Carotenoid
50% TS	2.40 ± 0.33*	2.71 ± 0.38*	1.99 ± 0.18	Total chl
	1.67	1.94	1.63	Chl a/b
	0.59 ± 0.07*	0.75 ± 0.05*	0.49 ± 0.07	Carotenoid
75% TS	2.30 ± 0.07*	2.44 ± 0.13*	1.89 ± 0.1	Total chl
	1.85	2.46	1.43	Chl a/b
	0.55 ± 0.04	0.66 ± 0.03*	0.49 ± 0.03	Carotenoid
100% TS	2.20 ± 0.29	2.293 ± 0.11	1.62 ± 0.05	Total chl
	0.68	0.70	0.56	Chl a/b
	0.52 ± 0.09	0.61 ± 0.02	0.36 ± 0.03	Carotenoid

[a] All values are means of three replicates ±SD

*$p < 0.01$ as compared to GS

90 days, as compared to their respective GS. The maximum carotenoid content in var. PDM 54 at a lower amendment (10% TS) than NM 1 (25% TS) indicated that var. PDM 54 has developed better tolerance mechanisms towards metal induced stress than NM 1.

In both varieties, the increase in photosynthetic pigments in the lower amendments (up to 35% TS) in the early stages of growth could be attributed to the presence of essential metal ions in tannery sludge required for chlorophyll biosynthesis (Singh et al. 2004b; Sinha et al. 2007; Sinha and Gupta 2007). However, reduction in chlorophyll content at higher sludge amendments may be attributed to the interference of heavy metals in the formation of chlorophyll through direct inhibition of an enzymatic step. Dhir et al. (2004) also reported a decline in chlorophyll (Chl *a* and Chl *b*) levels in Cd treated plants of *Vigna radiata* grown in solution culture.

Carotenoid, which is considered as non-enzymatic antioxidant, plays an important role in protection of chlorophyll pigment under stress conditions by quenching the photodynamic reactions and replacing peroxidation. In conformity with earlier findings (Sinha et al. 2007; Sinha and Gupta 2007), an increase in the carotenoid content with increase in amendments and growth has been observed. However, at higher amendments and longer growth period, there was an overall decrease in chlorophyll pigments in both the varieties. However, more decrease in chlorophyll content of var. NM 1 than var. PDM 54 was recorded which may be due to varietal difference. Sinha et al. (2006b) observed the combined effect of Cr on *Vigna radiata* cv. PU 19 in Mn deficiency as well as excess conditions. Visible symptoms such as depression in biomass, chlorophyll a and b, activity of ribonuclease, and increasing peroxidase activity were observed in the plant.

Table 8 Effect of tannery sludge amendments on total chlorophyll and carotenoid contents (mg g^{-1} fw) and Chl a/b ratio in the leaves of *V. radiata* var. NM 1[a]

Substrates	Durations			Photosynthetic pigments
	30 days	60 days	90 days	
GS	1.69 ± 0.19	2.05 ± 0.07	1.99 ± 0.32	Total chl
	3.58	2.77	2.20	Chl a/b
	0.421 ± 0.04	0.47 ± 0.06	0.44 ± 0.05	Carotenoid
10% TS	2.02 ± 0.24*	2.42 ± 0.12*	2.14 ± 0.23	Total chl
	2.73	1.88	2.03	Chl a/b
	0.465 ± 0.08	0.52 ± 0.03	0.52 ± 0.07*	Carotenoid
25% TS	2.34 ± 0.12*	2.56 ± 0.45*	2.12 ± 0.13	Total chl
	2.84	2.11	1.75	Chl a/b
	0.53 ± 0.08*	0.64 ± 0.06*	0.55 ± 0.06*	Carotenoid
35% TS	2.28 ± 0.05*	2.28 ± 0.24	2.08 ± 0.15	Total chl
	2.68	2.15	2.16	Chl a/b
	0.51 ± 0.07*	0.57 ± 0.05*	0.53 ± 0.06*	Carotenoid
50% TS	2.04 ± 0.16*	2.19 ± 0.22	1.56 ± 0.19	Total chl
	2.68	2.31	2.01	Chl a/b
	0.49 ± 0.08	0.49 ± 0.06	0.39 ± 0.05	Carotenoid
75% TS	2.14 ± 0.05*	2.02 ± 0.21	1.47 ± 0.28	Total chl
	2.73	2.25	1.98	Chl a/b
	0.49 ± 0.03	0.46 ± 0.05	0.36 ± 0.09	Carotenoid
100% TS	2.14 ± 0.20*	1.69 ± 0.11	1.17 ± 0.34	Total chl
	2.80	2.21	1.98	Chl a/b
	0.46 ± 0.04	0.36 ± 0.05	0.32 ± 0.09	Carotenoid

[a] All values are means of three replicates ±SD

*$p < 0.01$ as compared to GS

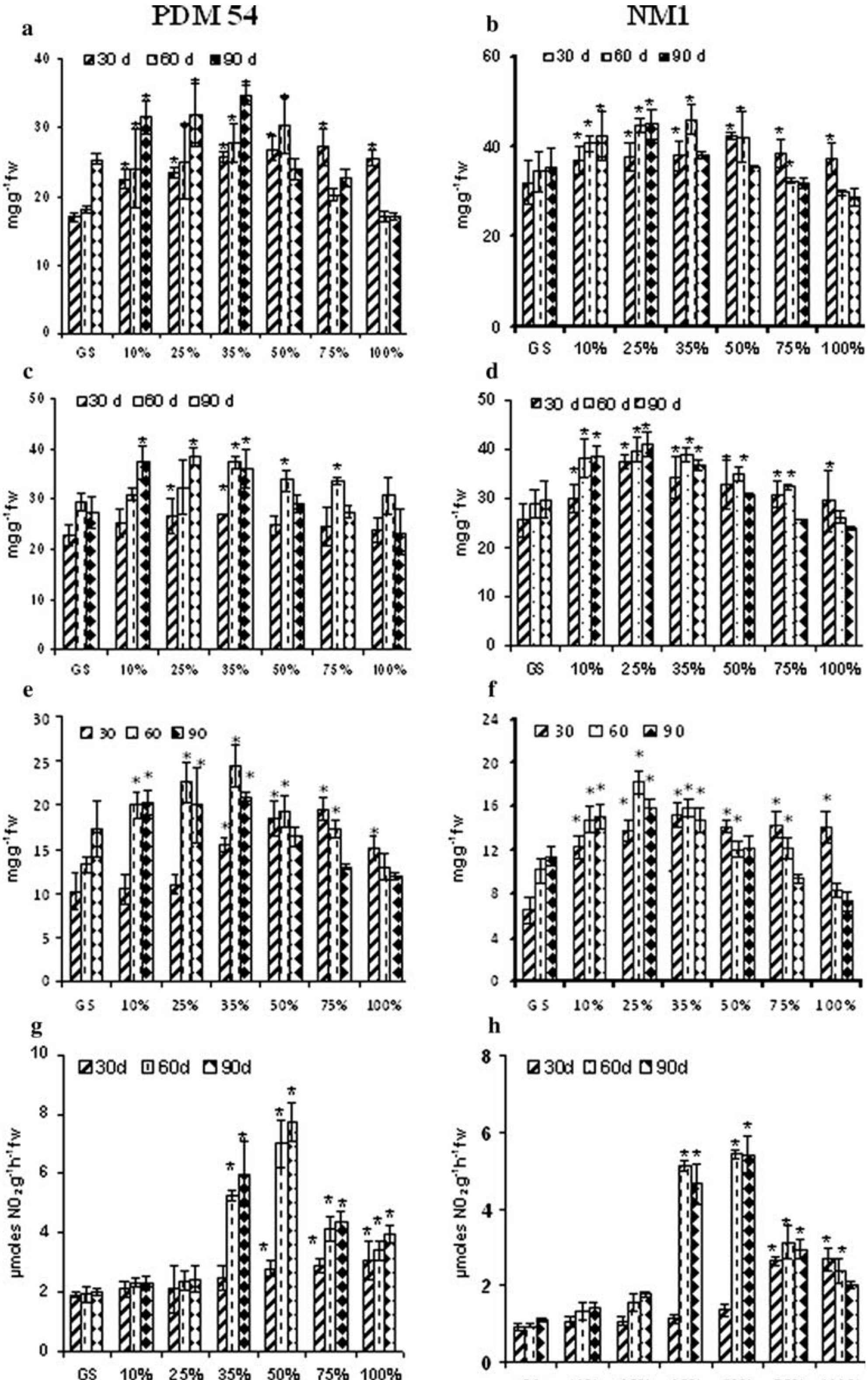
Protein and carbohydrate contents

In leaves of var. PDM 54, the protein content increased significantly in all amendments (30 days), up to 50% TS (60 days), and 35% TS (90 days) as compared to their respective GS, with maximum increase of 37.2% at 35% TS (90 days) (Fig. 4a). In roots, the protein content increased at lower amendment of TS with maximum increase of 39.68% at 25% TS after 90 days of growth. In the leaves of var. NM 1 (Fig. 4b), the protein content increased up to 35% TS after 90 days with maximum increase of 26.6% at 25% TS after 90 days as compared to GS. In roots, a significant increase of 37.11% was recorded in the plants grown in 25% TS (90 days), as compared to GS. Overall comparison showed that the var. PDM 54 variety showed better response than var. NM 1.

In both varieties, the carbohydrate content increased significantly up to 75% TS at 60 days and 35% at 90 days as compared to their respective GS samples. The maximum increases of 21.49% and 29.09% were observed in var. PDM 54 and var. NM 1, respectively, in the leaves of the plants grown on 35% TS at 90 days of growth (Fig. 4e, f).

Both varieties showed similar trends, wherein, the maximum protein content was obtained at later stage of growth (90 days), at lower amendments, which may be due to the availability of essential elements for healthy growth. Hsu and Kao (2003) reported that Cd induced a notable decrease in protein content in the leaves of Cd-sensitive rice genotype, but not for

Fig. 4 Effect of different amendments of tannery sludge on protein content of *V. radiata* leaves. (**a**) Var. PDM 54. (**b**) Var. NM1. (**c**) On protein content in roots, var. PDM 54. (**d**) Var. NM1. (**e**) Total carbohydrate content in leaves, var. PDM 54. (**f**) Var. NM1. (**g**) Nitrate reductase activity in leaves var. PDM 54. (**h**) Var. NM1. All values are mean of three replicates. *$p < 0.05$ as compared to GS


PDM 54
a
30 d
60 d
90 d
mgg⁻¹fw
GS
10%
25%
35%
50%
75%
100%
NM1
b
c
d
e
30
60
90
f
g
30d
60d
90d
µmoles NO₂g⁻¹h⁻¹fw
h

tolerant type. Demirevska-Kepova et al. (2004) reported that high Cu level induced the reduction in leaf total soluble protein in barley plants. The higher concentrations of carbohydrate detected in the plants in both the varieties at lower amendments was in good coincidence with the report by Verma and Dubey (2001), who suggested that higher concentration of soluble sugar could possibly provide an adaptive mechanism in maintaining favorable osmotic potential under Cd toxicity. Guo et al. (2007) reported poor increase in soluble sugar and decrease in soluble protein contents in the barley plants treated with multimetals.

In vivo NR activity

The overall NR activity was found higher in var. PDM 54 than in var. NM 1 (Fig. 4g, h). After 30 days of growth, the activity was found to increase significantly ($p < 0.01$) with increase in amendments in both varieties. After 60 and 90 days of growth, the maximum increase was observed in 50% TS amendment in both varieties. Maximum increase of 288.36% in NR activity was observed after 90 days of growth in var. PDM 54, whereas, 395.23% increase was observed in var. NM 1 in 50% TS, as compared to their respective GS samples. Nitrate reductase activity was also found to increase significantly ($p < 0.01$) and consistently with increase in duration of growth at higher amendments (35% TS onwards) in both varieties except 100% TS in var. NM 1, as compared to initial duration (30 days).

In vivo NR activity catalyzes the nitrate assimilation, which appears to be a rate limiting process in acquisition of nitrogen in most cases (Campbell 1999). Inhibition in NR activity has been observed in several plants (Sinha et al. 1994; Singh et al. 1998). In plants, nitrate (NO_3^-) is the most important source of N, an essential mineral element (Crawford and Glass 1998; Hirsch and Sussman 1999) and the nitrate must be taken up across the plasma membrane for nitrogen metabolism. Once inside the symplast of a plant, NO_3^- reduced to NO_2^- by nitrate reductase (NR), and NO_2^- is converted to NH_4–N by nitrite reductase. The resulting NH_4–N is then assimilated into amino acids, nucleic acids, proteins, chlorophylls, and other metabolites (Marschner 1995; Stitt et al. 2002). Therefore, nitrogen influences all levels of plant function, from metabolism to resource allocation, growth, and development (Stitt and Krapp 1999; Scheible et al. 2004). Recently, Xiong et al. (2006) suggested that Cu toxicity in the plants of *Brassica pekinensis* growth decreased nitrate reductase activity and total chlorophyll content. Adverse effects due to increasing Cu concentration in plant tissues included shortened root length, fewer leaves, and decline in plant biomass.

Conclusion

The results of the present study demonstrated that, among both varieties, the plant *V. radiata* (var. PDM 54) shows more accumulation of Cr but also exhibits a significant increase in growth parameters when grown on lower amendments of sludge or contaminated soil and irrigated with ground water. Interestingly, no translocation of toxic metal Cr was found in the seeds of the plants grown in up to 25% tannery sludge and/or the plants grown on contaminated soil irrigated with ground water. Therefore, an optimum level of tannery sludge amendment (25%) with the agricultural soil can act as a partial nutrient supplement and, at the same time, manage the waste without posing a potential hazard to the plant. However, care must be taken while growing edible plants, and metal levels may be monitored at regular intervals. Higher concentrations of metals in the plants grown on higher amendment of tannery sludge (>25%) affected various growth parameters and also affected NR activity and carbohydrate content of the plants. Based on these findings, it is recommended that the plants may be grown on 25% tannery sludge amendment which has shown no accumulation of toxic metal Cr in the seed part.

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References

Arnon, I. (1949). Copper enzymes in isolated chloroplast. Polyphenol oxidase in *Beta vulgaris*. *Plant Physiology, 24*, 1–15.

Barman, S. C., Sahu, R. K., Bhargava, S. K., & Chaterjee, C. (2000). Distribution of heavy metals in wheat, mustard and weed grown on field irrigated with industrial effluents. *Bulletin of Environmental Contamination and Toxicology, 64*, 489–496.

Campbell, W. H. (1999). Nitrate reductase structure, function and regulation: bridging the gap between biochemistry and physiology. *Annual Review of Plant Physiology. Plant Molecular Biolology, 50*, 277–303.

Casado-Vela, J., Selleś, S., Dıáz-Crespo, C., Navarro-Pedrenõ, J., Mataix-Beneyto, J., & Gómez, I. (2006). Effect of composted sewage sludge application to soil on sweet pepper crop (*Capsicum annuum* var. annuum) grown under two exploitation regimes. *Waste Management*, *27*, 1509–1518. doi: 10.1016/j.wasman.2006.07.016.

Crawford, N. M., & Glass, D. M. A. (1998). Molecular and physiological aspect of nitrate uptake in plants. *Trends in Plant Science, 3*, 389–395.

Demirevska-Kepova, K., Simova-Stoilova, L., Stoyanova, Z., Hölzer, R., & Feller, U. (2004). Biochemical changes in barley plants after excessive supply of copper and manganese. *Environmental Experimental Botany, 52*, 253–266.

Dhir, B., Sharmila, P., & Saradhi, P. P. (2004). Hydrophytes lack potential to exhibit cadmium stress induced enhancement in lipid peroxidation and accumulation of proline. *Aquatic Toxicology, 66*, 141–147.

Dolgen, D., Alpaslan, M. N., & Delen, N. (2007). Agricultural recycling of treatment-plant sludge: a case study for a vegetable-processing factory. *Journal of Environmental Management, 84*, 274–281.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric method for determination of sugar and related substance. *Analytical Chemistry, 28*, 350–356.

Fytianos, K., Katsianis, G., Triantafyllou, P., & Zachariadis, G. (2001). Accumulation of heavy metals in vegetables grown in an industrial area in relation to soil. *Bulletin of Environmental Contamination and Toxicology, 67*, 423–430.

Gomez, K. A., & Gomez, A. A. (1984). *Statistical procedure for agricultural research*. New York: Wiley.

Guo, T. R., Zhang, G. P., & Zhang, Y. H. (2007). Physiological changes in barley plants under combined toxicity of aluminum, copper and cadmium. *Colloids and Surfaces B: Biointerfaces, 57*, 182–188.

Gupta, A. K., & Sinha, S. (2006a). Chromium levels in vegetables and grains grown on tannery effluent irrigated area of Jajmau, Kanpur (India): influence on dietary intake. *Bulletin of Environmental Contamination and Toxicology, 77*, 658–664.

Gupta, A. K., & Sinha, S. (2006b). Chemical fractionation and heavy metal accumulation in the plant of *Sesamum indicum* (L.) var. T55 grown on soil amended with tannery sludge: selection of single extractants. *Chemosphere, 64*, 161–173.

Gupta, A. K., & Sinha, S. (2007a). Phytoextraction capacity of the plants growing on tannery sludge dumping sites. *Bioresource Technology, 98*, 1788–1794.

Gupta, A. K., & Sinha, S. (2007b). Phytoextraction capacity of the *Chenopodium album* L. grown on soil amended with tannery sludge. *Bioresource Technology, 98*, 442–446.

Hirsch, R. E., & Sussman, M. R. (1999). Improving nutrient capture from soil by the genetic manipulation of crop plants. *Trends in Biotechnology, 17*, 356–361.

Hsu, Y. T., & Kao, C. H. (2003). Changes in protein and amino acid contents in two cultivars of rice seedlings with different apparent tolerance to cadmium. *Plant Growth Regulators, 40*, 147–155.

Lowry, O. H., Rosebrought, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with folin-phenol reagent. *Journal of Biological Chemistry, 193*, 265–275.

Mantovia, P., Baldonib, G., & Toderib, G. (2005). Reuse of liquid, dewatered, and composted sewage sludge on agricultural land: effects of long-term application on soil and crop. *Water Research, 39*, 289–296.

Marschner, H. (1995). *Mineral nutrition in higher plants* (2nd ed.). London, UK: Academic.

Scheible, W.-R., Morcuende, R., Czechowski, T., Fritz, C., Osuna, D., Palacios-Rojas, N., Schindelasch, D., Thimm, O., Udvardi, M. K., & Stitt, M. (2004). Genome-wide reprogramming of primary and secondary metabolism, protein synthesis, cellular growth processes, and the regulatory infrastructure of *Arabidopsis* in response to nitrogen. *Plant Physiology, 136*, 2483–2499.

Shankar, A. K., Djanaguiraman, M., Sudhagar, R., Chandrashekar, C. N., & Pathmanabhan, G. (2004). Differential antioxidative response of ascorbate glutathione pathway enzymes and metabolites to chromium speciation stress in green gram (*Vigna radiata* (L.) R. Wilczek. Cv. CO4) roots. *Plant Science, 166*, 1035–1043.

Singh, R. P., Dabas, S., Choudhary, A., & Maheshwari, R. (1998). Effect of lead on nitrate reductase activity and alleviation of lead toxicity by inorganic salts and 6-benzylaminopurine. *Biology Plant, 40*, 399–404.

Singh, K. P., Mohan, D., Sinha, S., & Dalwani, R. (2004a). Impact assessment of treated/untreated wastewater toxicants discharged by sewage treatment plants on health agricultural and environmental quality in the wastewater disposal area. *Chemosphere, 55*, 227–255.

Singh, S., Saxena, R., Pandey, K., Bhatt, K., & Sinha S. (2004b). Response of antioxidants in sunflower (*Helianthus annuus* L.) grown on different amendments of tannery sludge: its metal accumulation potential. *Chemosphere, 57*, 1663–1673.

Singh, S., & Sinha, S. (2004). Morphoanatomical response of two cultivars of *Brassica juncea* (L.) Czern grown on tannery waste amended soil. *Bulletin Environmental Contamination and Toxicology, 72*, 1017-1024

Singh, R. P., Tripathi, R. D., Dabas, S., Rizvi, S. M. H., Ali, M. B., Sinha, S. K., Gupta, D. K., Mishra, S., & Rai, U. N. (2003). Effect of lead on growth and nitrate assimilation of *Vigna radiata* (L.) Wilczek seedlings in a salt affected environment. *Chemosphere, 52*, 1245–1250.

Sinha, P., Dube, B. K., & Chatterjee, C. (2006b). Manganese stress alters phytotoxic effects of chromium in green gram physiology (*Vigna radiata* L.) Cv. Pu 19. *Environmental and Experimental Botany, 57*, 131–138.

Sinha, S., Gupta, A. K., & Bhatt, K. (2007). Uptake and translocation of metals in fenugreek grown on soil amended with tannery sludge: involvement of antioxidants. *Ecotoxicology Environmental Safety, 67*, 267–277.

Sinha, S., Gupta, A. K., Bhatt, K., Pandey, K., Rai, U. N., & Singh, K. P. (2006a). Distribution of metals in the edible plants grown at Jajmau, Kanpur (India) receiving treated tannery wastewater: relation with physico-chemical properties of the soil. *Environmental Monitoring and Assessment, 115*, 1–22.

Sinha, S., Mallick, S., Misra, R. K., Singh, S., Basant, A., & Gupta, A. K. (2007). Uptake and translocation of metals in *Spinacia oleracea* L. grown on tannery sludge-amended and contaminated soils: effect on lipid peroxidation, morpho-anatomical changes and antioxidants. *Chemosphere, 67*, 176–187.

Sinha, S., Pandey, K., Gupta A. K., & Bhatt, K. (2005). Accumulation of metals in vegetables and crops grown in the area irrigated with river water. *Bulletin of Environmental Contamination and Toxicology, 74*, 210–218.

Sinha, S. K., Srivastava, H. S., & Tripathi, R. D. (1994). Inhibition of nitrate reductase activity in presence of divalent cations and growth hormones in maize leaves. *Chemosphere, 29*, 1775–1782.

Srivastava, H. S. (1974). *In vivo* activity of the nitrate reductase in maize seedlings. *Indian Journal of Biochemistry and Biophysics, 11*, 230–232.

Stitt, M., & Krapp, A. (1999). The molecular physiological basis for the interaction between elevated carbon dioxide and nutrients. *Plant Cell Environment, 22*, 583–622.

Stitt, M., Müller, C., Matt, P., Gibon, Y., Carillo, P., Morcuende, R., Scheible, W.-R., & Krapp, A. (2002). Steps towards an integrated view of nitrogen metabolism. *Journal of Experimental Botany, 53*, 959–970.

Verma, S., & Dubey, R. S. (2001). Effect of cadmium on soluble sugars and enzymes of their metabolism in rice. *Biology Plant, 44*, 117–123.

Xiong, Z.-T., Liu, C., & Geng, B. (2006). Phytotoxic effects of copper on nitrogen metabolism and plant growth in *Brassica pekinensis* Rupr. *Ecotoxicology and Environmental Safety, 64*, 273–280.