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ScienceDirect
Journal of Hazardous Materials 152 (2008) 431–439
Journal of Hazardous Materials
www.elsevier.com/locate/jhazmat

Effect of distillery sludge on seed germination and growth parameters of green gram (*Phaseolus mungo* L.)

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Received 24 May 2007; received in revised form 29 June 2007; accepted 29 June 2007
Available online 7 July 2007

Abstract

Experiments were carried out to study the effect of distillery sludge amendments with garden soil (10, 20, 40, 60, 80 and 100%) on seed germination and growth parameters of *Phaseolus mungo* L. Germination percentage and index values decreased with rise in sludge concentration. Soil amended with 10% (w/w) sludge showed favorable growth while >10% was inhibitory for plant growth. Soil amended with 10% (w/w) distillery sludge induced the growth in root length, shoot length, number of leaves, biomass, photosynthetic pigment, protein and starch while 20% (w/w) sludge amended soil had variable effects on the root, shoot, leaves and nodules of *P. mungo* L. At concentrations (>40%) reduced all the growth parameters, viz., root length, shoot length, number of leaves, biomass, photosynthetic pigment, protein and starch of *P. mungo*. Malondialdehyde (MDA) product of lipid peroxidation was also enhanced in both root and leaves of sludge amended soil grown *P. mungo* at all the sludge amendments and exposure periods. A coordinated increase in cysteine, non-protein thiol and ascorbic acid antioxidants was up to 40 days of growth. After this period a decrease was observed. The N, P, K and Mg accumulation followed the order shoot > leaf > root. Calcium accumulation was highest in the upper part of the plants (including shoot and leaves). Furthermore, heavy metals content were also increased in different parts of *P. mungo* grown on increasing concentration of sludge amended garden soil with time. Zinc and copper accumulation was maximum versus other heavy metals. Based on these studies, sludge having concentrations ≤10% (w/w) can be applied as a fertilizer.

Keywords: Distillery sludge; Antioxidants; Growth parameters; *Phaseolus mungo* L.; Heavy metals; Soil amendments

1. Introduction

The disposal of industrial wastes is a worldwide problem. Among various industries, sugarcane molasses based (distilleries) industries occupy a prominent place in Indian economy. There are approximately 300 distilleries in India, releasing 3.5×10^{15} L effluent annually [1]. The distillery effluent is characterized by high biochemical oxygen demand (BOD) (40,000–50,000 mg/L), chemical oxygen demand (COD) (90,000–100,000 mg/L) [2–5], phenolic compounds, sulphate and heavy metal load [3,5]. When discharged into water bodies, it damages the aquatic ecosystem by reducing photosynthetic activities and dissolved oxygen [6]. In addition, distilleries produce approximately 1500 tonnes of sludge per day during anaerobic digestion of spent wash [7] which is characterized by high organic matter (OM), total dissolved solid (TDS), phenol, sulphate and heavy metals concentrations [2].

There is no set methodology for sludge disposal. The common disposal process for sewage includes land filling, land application and incineration. Among these, land filling is the first choice for sewage disposal. However, the future of sludge disposal through land filling is not very bright due to the fact that a large volume of soil is required to cover the waste in order to prevent the leaching of potentially toxic compounds including metals and phenols. Landfill sites are not easy to locate and strictly regulated to prevent odor emission. Leaching of various toxic compounds also contaminated the ground and surface waters [8]. Metals at supraoptimal concentration in the growth media can function as stressors causing physiological constraints that decrease plant vigour and affect plant growth, development and yields [9]. This will cause negative impact on root weight, number of leaves and shoots weight of plants [10]. It has also

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doi:10.1016/j.jhazmat.2007.06.124

been reported that at high concentrations, nodule plant^{-1} and nodule g^{-1} root reduced due to the higher concentrations of heavy metals leached from sewage sludge [11]. The generation of reactive oxygen species is also stimulated in the presence of metals, which can seriously disturb normal metabolism through oxidative damage of cellular compartments. To counteract this damage, highly efficient antioxidant defense mechanism in plant cell can deactivate metals stress generated by reactive oxygen radical [12–14]. Sludge generated from various sector have also been utilized in various agricultural uses due to the fact that significant amount of nitrogen, phosphorus and other organic matters are available in the industrial and domestic sludge [15–18]. In view of this, distillery sludge having high organic content may be a good alternative to be used as a fertilizer for soil amendments. Therefore, the optimization of distillery sludge concentrations with garden soil for different commonly growing crops is required. This may affect the physico-chemical properties and microbiological processes. In addition, the presence of heavy metals, phenolic and sulfur compounds in distillery sludge are ambiguous for plant growth and their effect on environment is not very well known. A very few studies are reported on environmental fate of distillery waste using very dilute effluents [19,20]. To the best knowledge of the authors, no report is available where the effects of distillery sludge under soil-amended conditions with legume crops are studied.

In view of above, pot scale studies were conducted to assess the effects of different concentrations of distillery sludge (0–100% w/w) on seed germination, different physiological parameters of *Phaseolus mungo* L. Translocation of metals and their effects on various antioxidants involvement were also studied. Pot scale studies were chosen due to the fact that the plant production under controlled conditions can easily be evaluated.

2. Materials and methods

2.1. Sludge collection and experimental design

Dried distillery sludge cakes were collected in clean sterile polythene bags from sludge bed of wastewater treatment plant of M/s Unnao Distillery located at Unnao in Uttar Pradesh, India. The plant has an installed capacity of 9000 kL for alcohol production and generates ~800 tonnes of sludge annually [1].

These were brought to the Industrial Toxicology Research Centre, Lucknow for further studies. The unexposed and non-contaminated garden subsoil collected from the Gheru campus of Industrial Toxicology Research Centre (ITRC), Lucknow, India was used for various amendments. Sludge and soil were completely air-dried, powdered and sieved (2.0 mm mesh) before mixing together. The amendments of distillery sludge with garden soil were carried out at different concentrations (0, 10, 20, 40, 80 and 100%). A control of garden soil was also used. Different concentrations of sludge-amended soils (10 kg dw) were filled in different earthen pots having dimensions 20 in. × 14 in. The soaked and sterile ten seeds were evenly sown in each pot to a depth of 2 in. Prior to sowing, the *P. mungo* seeds were sterilized in 3% formalin for 5 min to avoid any fungal contamination, washed thrice with double distilled water and soaked overnight in water. Experiments were conducted in triplicate with a parallel set of control. All the pots were watered daily till seed germination. The natural moisture condition of loamy soil required for plant growth was maintained using tap water. All the pots were kept in a natural condition with an average durable temperature (25–35 °C) and low humidity (50–55%). The humidity was measured by auto weather station (WM-200, Envirotech, India). The photoperiod was 10 h having light inten-

Table 1
Physico-chemical characteristics of distillery sludge and garden soil used in the experiment

S. no.	Physico-chemical parameters	Distillery sludge	Garden soil
1	pH	8.5 ± 0.30	7.4 ± 0.39
2	Cation exchange capacity	36.96 ± 2.93	12.00 ± 1.03
3	Moisture (%)	80 ± 2.0	70 ± 1.8
4	Organic matter (%)	21.64 ± 0.83	0.460 ± 0.51
5	Electrical conductivity	3.70 ± 0.21	2.03 ± 0.01
6	Phosphate	22670 ± 45.43	8701 ± 15.23
7	Sulphate	144.06 ± 9.61	155.24 ± 7.58
8	Phenol	500.3 ± 26.46	26.56 ± 1.34
9	Total nitrogen	644 ± 34.49	103.56 ± 9.45
10	Ammonical nitrogen	11.90 ± 1.17	21.30 ± 2.51
11	Sodium	80.52 ± 2.464	31.3 ± 4.23
12	Chloride	355 ± 59.25	107 ± 7.35
13	Metals		
	Cd	2.05 ± 0.53	BDL
	Cr	8.12 ± 0.18	12.76 ± 0.21
	Cu	118.96 ± 2.54	26.04 ± 0.84
	Fe	85.23 ± 2.016	33.54 ± 1.75
	Mn	88.24 ± 0. 44	16.43 ± 1.32
	Ni	27.20 ± 2.64	14.40 ± 1.63
	Pb	19.76 ± 0.65	15.32 ± 0.35
	Zn	130.26 ± 8.43	91.32 ± 4.65

All values are mean ($n = 3$) ± S.D. and presented in mg kg^{-1} except electrical conductivity (mS cm^{-1}), CEC (Mequiv./10 g). BDL, not detected (minimum detection limit of Cd, 0.02 μg g^{-1}).

Table 2
Chemical properties of garden soil after mixing with 10, 20, 40, 60 and 80% (w/w) distillery sludge

Physico-chemical parameters	Sludge				
	10	20	40	60	80
pH	8.26 ± 0.12 d	8.40 ± 0.13 d	8.40 ± 0.12 d	8.14 ± 0.12 d	8.01 ± 0.13 d
EC	0.94 ± 0.02 a	1.17 ± 0.03 a	1.37 ± 0.03 a	1.55 ± 0.03 a	3.42 ± 0.07 a
CEC	18.35 ± 2.53 d	24.69 ± 3.17 d	27.73 ± 3.0 d	30.53 ± 3.14 d	33.34 ± 4.12 d
Moisture (%)	66 ± 1.3 c	68 ± 0.4 c	69 ± 0.32 d	70 ± 0.24 d	68 ± 0.15 c
Organic matter (%)	10 ± 0.09 a	15 ± 0.96 a	17.52 ± 1.27 c	18.58 ± 0.72 d	20.60 ± 0.81 d
Phosphate	10097 ± 1.52 a	11494 ± 3.05 a	14288 ± 3.55 a	17082 ± 4.75 a	24569 ± 6.85 a
Sulphate	107 ± 9.51 d	121 ± 7.80 d	130 ± 3.64 d	134 ± 3.33 d	138 ± 5.52 d
Phenol	70.25 ± 2.68 b	116.8 ± 9.00 b	218 ± 8.39 a	321 ± 12.53 a	432 ± 12.54 a
Total nitrogen	154.54 ± 4.89 b	198.78 ± 7.95 b	235 ± 10.25 c	354 ± 9.84 a	420 ± 19.3 a
Ammonical nitrogen	16.34 ± 0.58[a]	22.54 ± 0.73[a]	26.58 ± 0.32 d	31.54 ± 2.65 d	33.21 ± 3.32 d
Sodium	35.84 ± 1.72 d	44.52 ± 2.56 d	53.42 ± 4.95 d	62.32 ± 4.56 d	72.12 ± 5.64 d
Chloride	102.69 ± 5.13 a	134.85 ± 3.30 a	235.45 ± 3.85 a	254.98 ± 6.35 b	312.42 ± 5.32 a
Cd	0.20 ± 0.01 a	0.62 ± 0.01 a	0.82 ± 0.04 a	0.98 ± 0.05 a	1.69 ± 0.02 a
Cr	7.25 ± 0.28 d	7.98 ± 0.16 d	8.12 ± 0.32 d	10.52 ± 0.36 b	11.32 ± 0.57 d
Cu	32.88 ± 0.99 d	36.00 ± 1.12 d	60.64 ± 1.92 a	100.92 ± 3.65 a	110.80 ± 4.05 b
Fe	35.84 ± 1.32 d	39.12 ± 1.79 d	54.35 ± 1.85 a	70.54 ± 2.91 a	79.54 ± 3.58 b
Mn	20.35 ± 0.83 a	32.15 ± 1.29 a	40.89 ± 1.39 b	66.54 ± 3.89 a	70.54 ± 1.27 d
Ni	14.52 ± 0.43 d	15.68 ± 0.55 d	18.78 ± 0.71 a	19.85 ± 0.54 d	20.12 ± 0.70 d
Pb	15.42 ± 0.49 d	16.54 ± 0.53 d	18.21 ± 0.72 d	18.98 ± 0.57 d	19.45 ± 1.09 d
Zn	134.08 ± 4.05 d	164.45 ± 6.25 d	251.74 ± 9.02 a	257.76 ± 10.1 d	281.59 ± 10.3 c

All values are mean ($n = 3$) ± S.D. and presented in mg kg^{-1} except electrical conductivity (mS cm^{-1}), CEC (Mequiv./10 g). Means of individual parameters are compared at different concentrations of sludge within row: (a) highly significant, ANOVA $p < 0.001$; (b) significant, ANOVA $p < 0.01$; (c) less significant, ANOVA $p < 0.05$; (d) non-significant.

sity of 1600 lx. The light intensity was measured with a light meter (light meter-1131, Korea). All the metal analyses were carried out on inductively coupled plasma spectrophotometer (Thermo Electron; Model IRIS Interepid II XDL, USA). pH measurements were made on the Orion ion meter model 960. Conductivity was carried out using Orion conductivity meter (Model 150).

2.2. Physico-chemical analysis of sludge and garden soil

The physico-chemical parameters of soils and sludge-amended soils were analyzed in triplicate. The physico-chemical parameters of sludge, i.e. organic matter, cation exchange capacity (CEC) were estimated using standard methods [21]. pH of the garden soil and sludge amended soils (1:2.5 sludge water suspension) was determined using Orion ion meter (Model 960). The electrical conductivity of the sludge (1:2.5 sludge water suspensions) samples (EC) was measured using Orion conductivity meters (Model 150).

2.3. Biochemical and plant growth parameters analysis

Sludge extractions were carried out by mixing 3.0 g of sludge with 30 mL distilled water. The resultant suspension was centrifuged and filtered using Whatman no. 1 filter paper. Filtered (0.5 mL) aliquot was transferred into a Petri dish. Ten seeds were

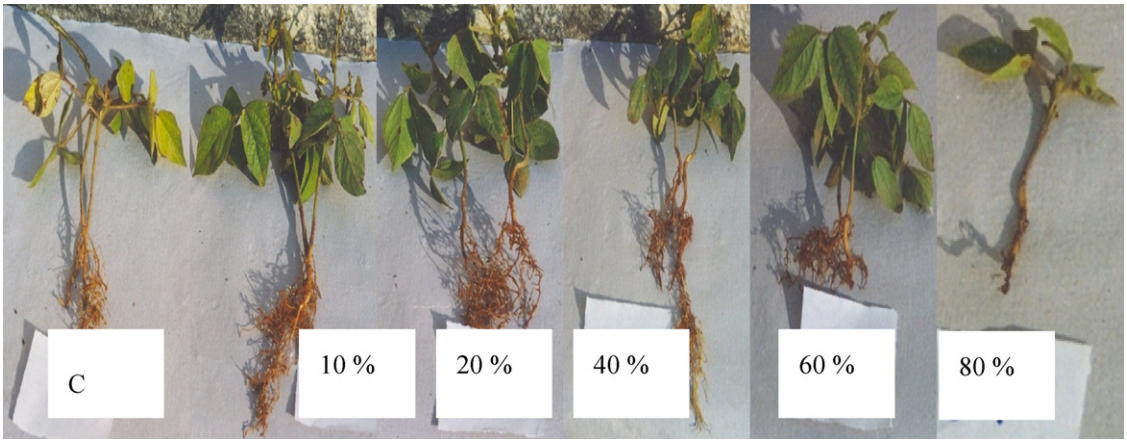

Fig. 1. Morphological effect of distillery sludge amendment soils at different concentrations (10–80%) on the growth of root, shoot and leaves of *P.mungo* after 60 days vs. the control.

Table 3
Effect of distillery sludge amended soil on seed germination of *P. mungo*

Sludge concentration (%)	Germination (%)	Speed of germination index	No. of plant(s) after 1 month	No. of plant(s) after 2 months
Control	100	230 ± 6.32	10 ± 0.69	10 ± 0.51
10	100	238 ± 5.34	8 ± 0.59	8 ± 0.34
20	80	236 ± 4.21	8 ± 0.35	8 ± 0.27
40	70	154 ± 3.12	6 ± 0.35	6 ± 0.19
60	70	130 ± 2.84	5 ± 0.19	3 ± 0.13
80	60	105 ± 4.87	4 ± 0.23	3 ± 0.10
100	50	60 ± 1.85	–[a]	–[a]

All values are mean ($n = 3$) ± S.D.
[a] No growth or germination was observed.

Table 4
Effect of distillery sludge on root length, shoot length, leaves number and their respective biomass of *P. mungo*

Sludge concentration (%)	Root length (cm)	Biomass of root (g/plant)	Shoot length (cm)	Biomass of shoot (g/plant)	No. of leaves plant^{-1}	Biomass of leaves (g/plant)
Control	12.73 ± 0.48	0.221	19.67 ± 0.75	0.525	12.00 ± 0.45	0.354
10	13.17 ± 0.53	0.268	21.17 ± 0.7	0.569	14.00 ± 0.55	0.390
20	9.17 ± 0.32	0.198	9.00 ± 0.65	0.612	9.67 ± 0.42	0.234
40	8.17 ± 0.35	0.122	8.57 ± 0.31	0.312	9.40 ± 0.40	0.186
60	7.83 ± 0.26	0.098	8.50 ± 0.31	0.102	8.33 ± 0.35	0.102
80	6.50 ± 0.22	0.018	2.50 ± 0.08	0.069	8.00 ± 0.33	0.026

All values are mean ($n = 3$) ± S.D.

placed in each dish. The Petri dishes were incubated at 25 °C. The germinated seeds were counted to the initial appearance of the radical by continuous visual observation for 10 days. The germination index (GI) was calculated by the method reported elsewhere [22].

Subsequently, the plants were harvested after 60 days of sowing and repeatedly washed with tap water to remove any attached particles. Furthermore, these were rinsed with 10 mmol $CaCl_2$ solution along with washing with deionised water. Height of the plants, root length, shoots length, number of leaves and leaf area were measured manually using an inch-scale immediately after harvesting. The number of nodules/plant was also enumerated by counting. For dry matter estimation, the roots, shoots and leaves were separated and dried at 72 °C in an oven for 72 h [23]. The chlorophyll content was estimated by spectrophotometer following Arnon's method [24]. Protein content in the root, shoot and leaves was determined according to the method of Lowry et al. [25]. Starch content was estimated by the method of Dubois et al. [26]. Lipid peroxidation in the plant tissue was determined indirectly in terms of malondialdehyde (MDA) content. The malondialdehyde (MDA) content was measured by thiobarbituric acid (TBA) reaction [27]. Cysteine content was estimated by the method of Gaitonde [28]. Ascorbic acid content was estimated according to the method of Keller and Schwager [29]. Non-protein thiol (acid soluble thiol) content was measured using Ellman's reagent (5, 5′-dithio bis 2-nitrobenzoic acid) [30]. All experiments were performed in triplicates.

2.4. Metal analysis

The uprooted *P. mungo* plants were washed thoroughly first by distilled water (to remove sand clinging) to the roots followed by a 10 mmol L^{-1} solution of $CaCl_2$. The roots, stem and leaves were separated, chopped into small pieces and oven-dried at 70 °C for 7 days. The dried plants were ashed in a muffle furnace at 460 °C for 6 h. The weighed ash were digested in 2% HNO_3 and filtered through 0.45 μm glass fiber filter

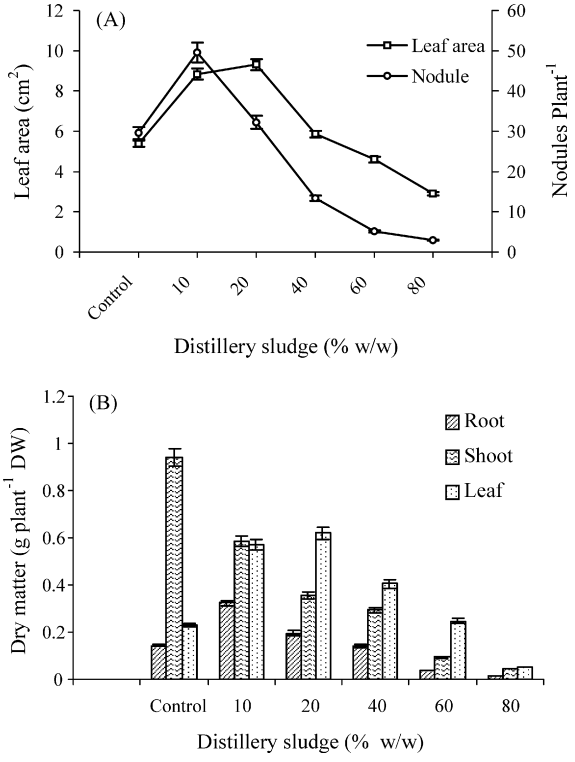

Fig. 2. Effect of distillery sludge on (A) leaf area and nodule formation and (B) dry matter in leaf, shoot and root of *P. mungo*.

Table 5
Effect of different concentration of sludge-amended soil on chlorophyll of *P. mungo*

Sludge concentration (%)	Chlorophyll *a*	Chlorophyll *b*	Chl *a*/Chl *b*	Total chlorophyll
Control	0.332 ± 0.003	0.120 ± 0.005	2.77	0.432 ± 0.017
10	0.570 ± 0.020	0.176 ± 0.004	3.24	0.750 ± 0.015
20	0.558 ± 0.021	0.162 ± 0.003	3.44	0.723 ± 0.019
40	0.536 ± 0.019	0.158 ± 0.012	3.39	0.688 ± 0.024
60	0.506 ± 0.017	0.140 ± 0.007	3.61	0.642 ± 0.018
80	0.383 ± 0.015	0.122 ± 0.001	3.14	0.466 ± 0.008

All values are mean ($n = 3$) ± S.D. and in mg g^{-1}.

Table 6
Effect of different concentration of distillery sludge on protein and starch of *P. mungo*

Sludge concentration (%)	Leaf protein (mg g^{-1} FW)	Shoot protein (mg g^{-1} FW)	Root protein (mg g^{-1} FW)	Leaf starch (mg g^{-1} FW)	Shoot starch (mg g^{-1} FW)	Root starch (mg g^{-1} FW)
0 (Control)	4.79 ± 0.13	10.79 ± 0.44	15.12 ± 0.68	17.79 ± 0.68	10.10 ± 0.35	10.64 ± 0.42
10	8.64 ± 0.39	17.36 ± 0.68	18.21 ± 0.79	16.10 ± 0.71	9.40 ± 0.29	10.32 ± 0.34
20	7.13 ± 0.30	14.67 ± 0.52	15.94 ± 0.71	13.70 ± 0.60	7.98 ± 0.26	8.36 ± 0.19
40	6.43 ± 0.29	14.00 ± 0.49	14.94 ± 0.50	5.94 ± 0.22	3.57 ± 0.09	4.11 ± 0.07
60	5.81 ± 0.22	12.25 ± 0.45	12.87 ± 0.57	5.37 ± 0.19	2.71 ± 0.05	2.32 ± 0.03
80	5.05 ± 0.19	10.83 ± 0.38	9.13 ± 0.35	3.53 ± 0.09	1.83 ± 0.04	1.74 ± 0.02

All values are mean ($n = 3$) ± S.D.; FW, Fresh weight.

[31]. According to EPA method (3050B) [32], the dried and sieved soil and sludge were also digested using a mixture of HNO_3: H_2O_2. The concentrations of Cd, Cu, Cr, Fe, Mn, Ni, Pb, and Zn were measured using inductively coupled plasma spectrophotometer (Thermo Electron; Model IRIS Interepid II XDL, USA).

2.5. Statistical analysis

One-way analysis of variance (ANOVA) and Turkey's test [33] were employed to test the data variability and validity of the results using the Graph Pad software (Graph Pad Software, San Diego, CA).

3. Results and discussion

High concentrations of nitrogen, phenol, chloride and heavy metals were detected in distillery sludge versus the garden soil (Table 1). The values of pH (8.5) and EC (3.70 mS cm^{-1}) were also higher in sludge than the garden soil where the pH and EC were 7.4 and 2.03 mS cm^{-1}, respectively. High values of pH and EC in the sludge may be due to the presence of high concentrations of soluble salt. High concentrations of heavy metals and salts in sludge are due to the condensation process, which takes place during sugar manufacturing and alcohol production. Also, CEC and OM of distillery sludge were significantly higher than garden soil.

Table 7
Level of MDA content (μmol g^{-1} FW) in the root and leaves of *P. mungo* grown on different sludge amendment soil

Samples	Plant parts	Exposure period (days)		
		20	40	60
Control	Root	2.05 ± 0.09	3.30 ± 0.23	4.43 ± 0.15
	Leaf	1.32 ± 0.07	2.72 ± 0.10	3.51 ± 0.08
10	Root	2.32 ± 0.05 b	4.53 ± 0.23 b	5.38 ± 0.11 b
	Leaf	1.72 ± 0.03 b	3.58 ± 0.06 a	3.86 ± 0.19 d
20	Root	2.67 ± 0.02 b	4.77 ± 0.32 d	5.55 ± 0.42 d
	Leaf	1.93 ± 0.01 d	3.49 ± 0.04 d	4.05 ± 0.05 d
40	Root	2.89 ± 0.03 c	4.89 ± 0.03 d	6.59 ± 0.24 b
	Leaf	2.23 ± 0.02 c	3.65 ± 0.02 d	5.39 ± 0.31 a
60	Root	3.13 ± 0.0.15 c	5.00 ± 0.42 d	6.91 ± 0.20 d
	Leaf	3.05 ± 0.09 a	3.65 ± 0.19 d	4.37 ± 0.19 a
80	Root	3.53 ± 0.02 a	5.42 ± 0.30 d	7.50 ± 0.20 d
	Leaf	3.13 ± 0.23 d	4.69 ± 0.07 a	6.32 ± 0.29 a

All values are mean ($n = 3$) ± S.D.; means of individual MDA content of control plant root to the treated plant root and MDA content of control plant leaves to the treated plant leaves are compared at different concentrations of sludge 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.

The soil's characteristics amended with different sludge concentrations are presented in Table 2. The pH of the soil increased from 7.4 to 8.0 with rise in sludge amendment rate indicated that sludge could act as a buffer medium for garden soil. Additions of distillery sludge also increased the EC significantly of sludge amendment soil, which adversely affected the growth of *P. mungo* L. (Fig. 1). These observations were in agreement with the earlier reported results [34,35], where increased EC have been found inhibitory for the plant growth. Simultaneously, phosphate and total nitrogen content in garden soil increased with increase in the amendment rate of distillery sludge.

In general, percentage seed germination decreased (except at 10%) with increase in sludge concentrations (Table 3). After 10 days of sowing, soil amended with 20% (w/w) sludge caused 20% inhibition in seed germination followed by 30, 40 and 50% inhibition at 60, 80 and 100% (w/w), respectively versus the control. However, the seedling died within 15 days of germination at 100% (w/w) sludge. The inhibition of seed germination at higher concentrations of sludge-amended soils may be due to the high salt concentrations in distillery sludge creating high osmotic pressure. The high osmotic pressure caused inhibition of seed germination. Similar observations have been reported earlier [20,36].

Furthermore, the vegetative growth parameters of *P. mungo* (shoot length and root length) at all tested concentrations showed inhibitory effect (except 10% sludge amended soil) versus the control (Table 4). The root length and number of leaves of *P. mungo* at 10% (w/w) sludge concentration increased by 3.45 and 16.66%, respectively compared to control (Table 4). At 80% (w/w) sludge-amended soil (after 60 days of study), 48.94 and 21.66% reduction in shoot length and root length was observed.

In general 10% (w/w) sludge-amended soil exhibited vigorous growth along with an increase in the number of nodules plant^{-1} but delayed flowering was observed as compared to control (Fig. 2A). However, at higher concentration of the

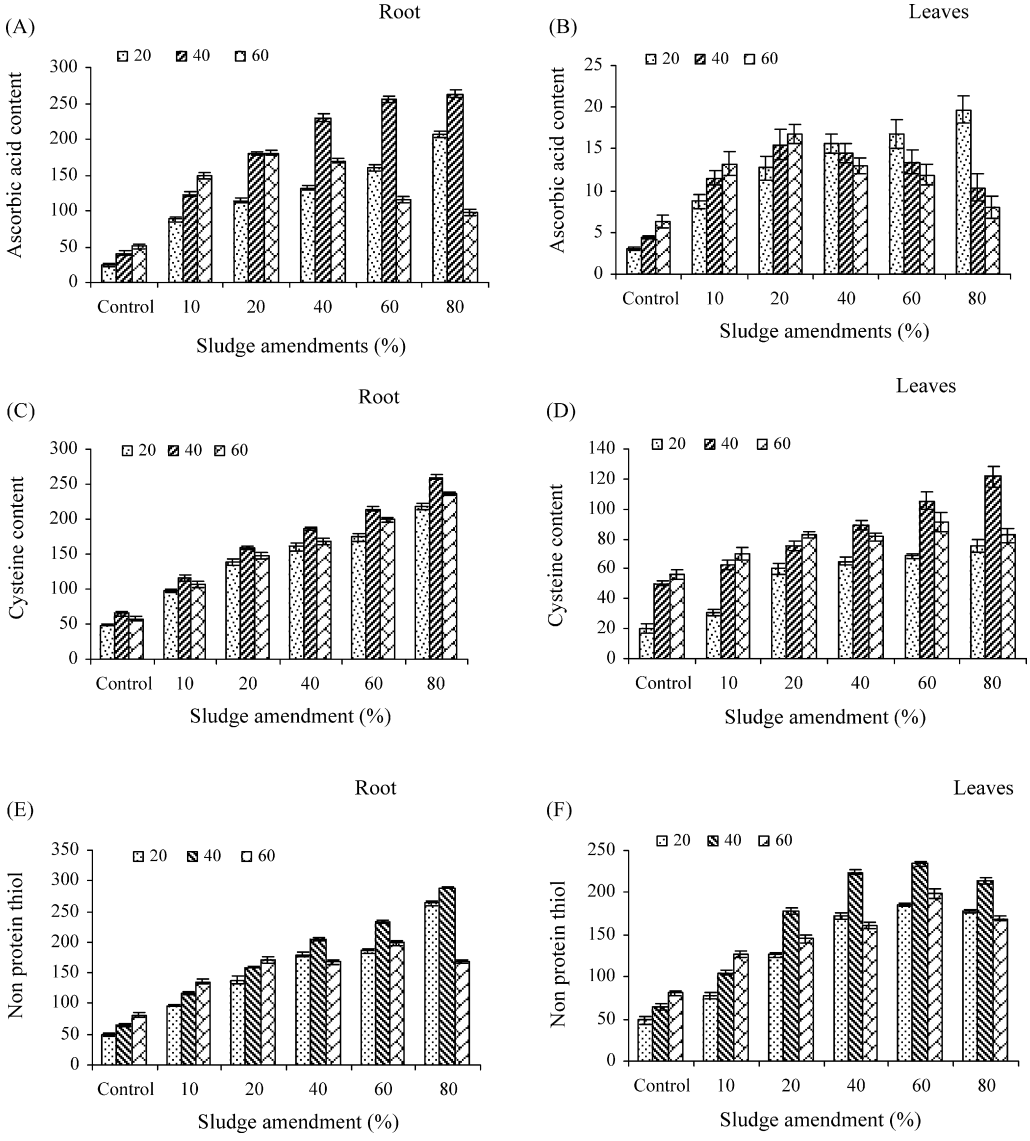

Fig. 3. The effect on levels of ascorbic acid (μg g^{-1}): (A and B) cystein (nmol g^{-1}); (C and D), non-protein thiol (μmol g^{-1}); (E and F) contents in the root and leaves of *P. mungo* grown on different concentrations of sludge.

sludge-amended garden soil resulted in marked reduction in nodule plant^{-1}. The reduction of nodule plant^{-1} was 89.65% at 80% (w/w) sludge-amended soil versus the control along with drastic decrease in leaves area and nodule plant^{-1} (Fig. 2A, 1) and numbers (Table 4). This decrease in *P. mungo* L. nodulation may be due to the presence of soluble salts and heavy metals [18,37,38], which are toxic to *Rhizobia*.

These results supported the induction of plant growth at concentrations ≤10%. This might be due to the induction of plant growth hormone while at higher sludge concentrations reversal in plant growth was observed. It has been reported that high sludge content was suppressive for plant growth hormone(s) (auxin and gibberline) which are responsible for the growth and development of plants [39]. The reduction in plant growth at high concentrations of distillery sludge might be due to the entrance of the metal into the protoplasm resulting in the loss of intermediatory metabolites which are essential for further growth and development of plants [40]. These findings are very much in accordance with the earlier reported results [40].

Dry matter of shoot, roots and leaves were inducible up to 20% (w/w) sludge concentration versus the control. However, it decreased drastically at 80% (w/w) sludge amended soil. The maximum biomass (0.94 g) was in shoot of the control sets (Fig. 2B). Dry matter follows the order leaves > shoot > root at 10% (w/w) sludge amended soil. The chlorophyll content was more in 10% (w/w) sludge amended soil which was gradually decreased with increase in concentration (Table 5). The control showed low chlorophyll content than amended soil (up to 80% w/w sludge concentration).

The photosynthetic surface area and leaf chlorophyll content were the key factors for determining dry matter production of *P. mungo* L. These findings are in full agreement with the earlier reported results [41,42]. The increase in the chlorophyll content at low sludge concentration was due to the presence of essential nutrients and metal ions [19,43]. However, these metals, which were acting as nutrient crossed the threshold limit at high sludge concentrations and work as toxic agent through direct inhibition of photosynthesis [43,44].

The protein content was increased with 20% (w/w) sludge-amended soil. However, >20% sludge there was a slight decrease in root, shoots and leaves protein (Table 6). The higher content of protein may be due to the induction of other stress protein in plant at higher sludge concentrations reflecting the manurial properties of distillery sludge at lower concentrations. The exposure of *P. mungo* L. to sludge amended soil resulted in a decrease of starch content of root, shoots and leaves (Table 6). The decrease in starch content of plants at any given concentration can be attributed to overall decrease in plant growth parameters and photosynthetic activity [45].

Malondialdehyde (MDA), a major cytotoxic product of lipid peroxidation acts as an indicator for free radical production. Therefore, MDA formation can be considered as a measure of lipid peroxidation [12]. The effect of distillery sludge amend-

Table 8
Accumulation of heavy metals (mg kg^{-1} DW) in different part of *P. mungo* grown in different concentration of distillery sludge-amended soil

Sludge concentration (%)	Plant part	Cd	Cr	Cu	Ni	Zn	Fe	Mn
Control	Root	BDL	7.87 ± 0.22	31.56 ± 0.95	1.69 ± 0.05	62.47 ± 2.65	17.64 ± 0.60	5.18 ± 0.17
	Shoot	0.04 ± 0.001	3.16 ± 0.12	7.76 ± 0.3	BDL	18.3 ± 0.81	14.31 ± 0.44	3.48 ± 0.09
	Leave	0.04 ± 0.001	3.16 ± 0.11	7.79 ± 0.24	BDL	18.66 ± 0.73	11.22 ± 0.44	2.01 ± 0.06
	Seeds	0.04 ± 0.001	3.21 ± 0.11	7.81 ± 0.24	BDL	24.76 ± 1.06	13.90 ± 0.61	2.58 ± 0.05
10	Root	0.39 ± 0.02 a	11.02 ± 0.51 a	65.74 ± 2.41 a	7.97 ± 0.33 a	138.0 ± 4.29 a	23.77 ± 0.85 b	9.26 ± 0.37 b
	Shoot	0.25 ± 0.12 b	8.86 ± 0.37 a	10.64 ± 0.42 a	5.97 ± 0.22 a	37.12 ± 1.42 a	27.62 ± 1.07 a	14.16 ± 0.44 a
	Leaves	0.32 ± 0.015 d	9.69 ± 0.36 a	15.95 ± 0.62 a	7.48 ± 0.31 a	62.29 ± 2.97 a	25.61 ± 0.80 a	8.18 ± 0.36 a
	Seeds	0.35 ± 0.012 a	2.71 ± 0.08 a	7.21 ± 0.29 a	4.81 ± 0.16 a	16.82 ± 0.61 a	25.61 ± 0.80 a	15.18 ± 0.47 a
20	Roots	0.52 ± 0.022 a	1.38 ± 0.04 a	74.7 ± 3.23 c	2.38 ± 0.07 a	13.95 ± 0.44 a	31.74 ± 0.92 a	12.18 ± 0.37 c
	Shoots	0.32 ± 0.012 d	2.50 ± 0.08 a	6.52 ± 0.23 a	4.62 ± 0.19 a	12.93 ± 0.40 a	36.47 ± 1.62 a	15.28 ± 1.55 d
	Leaves	0.78 ± 0.033 c	2.89 ± 0.12 a	13.28 ± 0.61 a	BDL	61.88 ± 2.66 d	26.19 ± 1.11 d	11.20 ± 0.35 a
	Seeds	0.62 ± 0.024 a	9.79 ± 0.42 a	14.15 ± 0.46 a	7.00 ± 0.22 a	64.19 ± 2.05 a	33.11 ± 0.99 a	16.16 ± 0.50 d
40	Roots	1.37 ± 0.04 a	0.97 ± 0.04 d	78.57 ± 3.67 d	BDL	78.73 ± 3.8 a	42.12 ± 2.0 a	23.10 ± 1.01 a
	Shoots	0.31 ± 0.01 d	2.35 ± 0.09 a	2.89 ± 0.1 a	BDL	7.03 ± 0.20 d	46.05 ± 1.90 a	26.20 ± 0.79 a
	Leaves	3.58 ± 0.15 a	3.22 ± 0.13 c	11.50 ± 0.41 c	BDL	53.05 ± 2.01 c	37.49 ± 1.62 a	22.22 ± 0.68 a
	Seeds	0.52 ± 0.02 a	3.52 ± 0.11 a	5.50 ± 0.22 a	BDL	9.11 ± 3.65 c	47.17 ± 2.01 a	8.00 ± 1.1 a
60 e	Roots	0.77 ± 0.03 a	8.58 ± 0.30 d	79.47 ± 3.10 d	10.56 ± 0.37 a	147.8 ± 6.3 a	54.47 ± 1.70 a	37.20 ± 1.29 a
	Shoots	0.59 ± 0.02 a	4.31 ± 0.14 a	16.37 ± 0.64 a	2.58 ± 0.08 a	148.9 ± 6.27 a	60.17 ± 1.84 a	41.16 ± 1.54 a
	Leaves	0.50 ± 0.016 a	1.29 ± 0.04 d	12.05 ± 0.44 d	BDL	62.21 ± 2.98 c	48.92 ± 1.95 a	32.81 ± 0.97 a
80 e	Roots	1.52 ± 0.04 a	10.12 ± 0.42 d	82.50 ± 4.02 d	10.80 ± 0.37 d	160.75 ± 7.5 d	70.04 ± 2.21 a	44.37 ± 1.61 a
	Shoots	0.62 ± 0.027 d	7.35 ± 0.28 a	22.32 ± 0.88 a	3.52 ± 0.12 a	148.98 ± 5.7 d	78.2 ± 3.21 a	52.40 ± 2.44 a
	Leaves	0.55 ± 0.02 d	1.80 ± 0.07 a	16.80 ± 0.71 a	BDL	82.50 ± 4.01 a	66.44 ± 3.1 a	40.91 ± 1.75 a

All the values are mean ($n = 3$) ± S.D.; means of different metals content of control to the treated plant root, different metals content of control to the treated plant leaves and different metals content of control to the treated plant leaves are compared at different concentrations of sludge 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; (e) bioaccumulation in seeds not seen due to very rudimentary growth; BDL, not detected.

Table 9
Effect of distillery sludge amendment soil on nutrient (mg kg^{-1}) content of different parts of *P. mungo*

Sludge Concentration (%)	Plant parts	N	P	K	Ca	Mg
Control	Leaves	526 ± 16.12	313 ± 12.31	843 ± 49.65	5053 ± 195	1381 ± 52.45
	Shoot	853 ± 17.06	423 ± 20.32	14011 ± 298	8532 ± 420.23	2551 ± 96.94
	Root	543 ± 20.43	337 ± 15.94	1190 ± 33.65	3321 ± 149	618 ± 25.95
10	Leaves	632 ± 18.69	374 ± 20.65	9238 ± 198.4	5382 ± 172	1418 ± 26.92
	Shoot	995 ± 25.13	469 ± 16.35	16023 ± 307	9321 ± 223.70	2998 ± 170.28
	Root	689 ± 22.32	356 ± 12.98	12315 ± 421	4232 ± 287	888 ± 40.13
20	Leaves	682 ± 13.23	409 ± 9.68	10132 ± 354	3432 ± 130	1218 ± 50.18
	Shoot	832 ± 10.20	489 ± 20.89	14321 ± 186	4753 ± 101	2481 ± 74.43
	Root	629 ± 14.53	423 ± 8.96	11532 ± 186	3278 ± 61.30	732 ± 35.14
40	Leaves	713 ± 24.32	326 ± 6.63	7453 ± 168	3923 ± 23.89	1173 ± 38.46
	Shoot	854 ± 30.54	397 ± 40.32	9132 ± 168	4796 ± 215	1018 ± 40.68
	Root	769 ± 32.11	338 ± 23.12	8318 ± 207	3289 ± 70.87	613 ± 18.64
60	Leaves	743 ± 16.23	336 ± 30.01	6819 ± 132	3589 ± 180	1069 ± 49.65
	Shoot	866 ± 11.35	411 ± 26.35	8321 ± 316	4313 ± 345	1917 ± 28.95
	Root	760 ± 26.18	432 ± 24.35	7524 ± 376	3013 ± 90.39	532 ± 20.54
80	Leaves	669 ± 38.18	354 ± 18.65	6008 ± 360	3000 ± 150	953 ± 63.54
	Shoot	713 ± 18.15	403 ± 9.68	7983 ± 275	4132 ± 214	1771 ± 51.53
	Root	632 ± 32.19	383 ± 14.45	6432 ± 192	2913 ± 133.96	497 ± 18.63

All the values are mean ($n = 3$) ± S.D.

ments on MDA content is shown in Table 7. MDA content in roots and leaves increased with rise in sludge amendment ratio versus the control at all exposure periods, indicating the enhanced lipid peroxidation in sludge grown *P. mungo*. To protect from oxidative stress conditions induced by free radicals, plants adopted cellular entities consisting of non-enzymatic cellular entities. These included ascorbic acid, cysteine, non-protein thiol, etc. Therefore, after 20, 40 and 60 days growth of *P. mungo* at all sludge concentrations, the non-enzymatic cellular entities in both root and leaves increased versus the control (Fig. 3A–F). After a period of 40 days, cysteine, non-protein thiol and ascorbic acid content in leaves of *P. mungo* increased with rise in sludge concentrations. At 80% distillery sludge, the values of cysteine (300%), non-protein thiol (541%) and ascorbic acid (229%) were maximum. The increase in cysteine, non-protein thiol and ascorbic acid contents in root and leaves were up to a period of 60 days and at 10 and 20% sludge amendment soil grown plants. At higher sludge concentrations an inhibitory trend of these parameters were observed. Maximum increase in ascorbic acid (21%) was in root followed by non-protein thiol (20%) in root and cysteine (11%) in leaves of *P. mungo* versus the 40 days content ascorbic acid, cysteine and non-protein thiol at 60 days of growth. These findings corroborated with the report of [12].

Metal accumulation in the plants grown at different sludge concentrations of amended soil showed different magnitude and relative distribution (Table 8). The trend of metal accumulation follows the order Zn > Fe > Mn > Cu > Cr > Ni > Cd. This trend varied from one part to another depending on distillery sludge amendment ratio. The accumulation of lead was below detection limit, therefore, not shown in Table 8. The results revealed that the accumulation of heavy metals at ≥40% (w/w) concentrations was highest in the roots except iron and manganese, which accumulated maximum in the shoots. Overall, Cu and Zn were accumulated maximum while Cd and Ni were accumulated least in *P. mungo* L. The accumulation of all heavy metals was minimum in fruit parts at 10% sludge-amended soil grown *P. mungo*. However, accumulation pattern of N, P, K and Mg were in the order shoot > leaf > root. Accumulation of Ca was maximum in upper part of the plants including shoot and leaves (Table 9). High accumulation of Zn, Fe and Cu, in various parts of *P. mungo* indicated the fast mobility of these metals in the plant [46] while lead absence may be due to its poor availability in soil for plant uptake.

4. Conclusion

Dried distillery sludge cakes were collected from sludge bed of a local wastewater treatment plant located at Unnao in Uttar Pradesh, India. This sludge was characterized and utilized for soil amendments. High concentrations of phenol, heavy metals and significant concentrations of N, P, and K were present in the sludge versus the garden soil. Experiments were carried out to study the effect of distillery sludge amendments with garden soil (10, 20, 40, 60, 80 and 100%) on seed germination and growth parameters of *P. mungo* L. Soil amended by 10% of distillery sludge (w/w) may be used as a fertilizer, whereas high concentration of distillery effluent is harmful for plants and soil. A risk assessment of toxic metals present in the edible parts of the plant, grown in the distillery sludge-amended soil, is required as these are being consumed by the animals and general population.

Acknowledgement

The financial assistance by Department of Biotechnology (DBT), Govt. of India to carry out this work is highly acknowledged.

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