PHYTOREMEDIATION OF INDUCED LEAD TOXICITY IN *VIGNA MUNGO* (L) HEPPER BY VETIVER GRASS

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ABSTRACT

Two inbreds of Black gram (*Vigna mungo*) were sown in the field to study the phytotoxicity of lead on growth parameters. In a separate experiment Black gram is intercropped with vetiver grass to study the phytoremediation through vetiver. In both the cases plants were treated with 9.0, 10.0 & 11.0 mg lit^{-1} concentration of $Pb(NO_3)_2$ & a control set of plants (without any treatment) was also sown in the field. In the first set of experiment, the growth and metabolism of Black gram were adversely affected with the increase in lead concentration and a concentration dependent decrease was noticed in all the growth parameters studied. The maximum reduction (% of control) was obtained with the higher dose of lead i.e. 11 mg lit^{-1} in both the varieties of Black gram. Variety T-9 was found to be more susceptible to lead as compared to PU-35.

In the second set of experiment (intercropping with vetiver) a significant recovery of this phytotoxicity was noticed in both the varieties of Black gram. Lead accumulation in roots of vetiver grown in lead treated soil is higher than growing in non contaminated soil. No stress symptoms were observed in vetiver plants. Results of the present study reveals that vetiver can be regarded as a potential phytoremediator plant that can be grown in a site contaminated with lead.

Keywords : Vigna mungo*;* phytotoxicity; Phytoremediation; Lead contamination.

1. INTRODUCTION

A rapid industrialization and its use in agriculture had led to regional and global redistribution of metals with consequent environmental pollution. The role of environmental pollution to produce various types of deleterious effects on diverse living system has been well established. Heavy metals are the most hazardous pollutants as they are non-degradable & get accumulated and become toxic both to plants & animals.

Among heavy metals, lead is one of the major contaminants found in soil, sediments, air and water. Total annual emission of lead by motor vehicles & industrial plants alone throughout the world amounts more than half a million ton. Lead can persist in the environment for 150-5000 years (Friedland, 1990). Once in water it enters the food chain & adversely affects the flora & fauna. In plants, enhanced level of lead in soil caused significant reduction in plant height, root-shoot ratio, dry weight, nodule per plant, chlorophyll content in *Vigna radiata* (Tomar et al*,* 2000) & in *Mentha spicata* (Bekiaroglou and Karatagli, 2002).

Phytoremediation is a green technology for the sustainable remediation of surface soils contaminated with heavy metals. It is an innovative, novel & potentially inexpensive technology using metal polluted soils, sludges & sediments. (Baker *et al,* 1994; Salt *et al*, 1998). It is a broad term used to classify six different remediation methods: Phytostabilization, Phytoextraction, Phytodegradation, Phytovolatilization, Rhizofilteration & Enhanced Rhizosphere Biodegradation.

Of these techniques, phytoextraction & phytostabilization are used to remediate inorganic contaminated soil especially by heavy metals.

According to Freidland (1990) an ideal plant species for phytoremediation should have either one of the following characteristics: (a) a low biomass plant with a very high metal accumulation capacity or (b) a high biomass plant with enhanced metal uptake potential. Metal tolerant plants with lower metal accumulation are preferred for phytostabilization & heavy metal hyperaccumulators are the best choice for phytoextraction as they tolerate high metal ions through various detoxification mechanisms which may include selective metal uptake, excretion, complexing by specific ligands & compartmentation of metal ligand complexes (Cobbet 2000; Clemens 2001).

The use of vetiver grass (*Chrysopogon zizanioides* (L) Roberty, family Poaceae, 2n =20) in soil conservation has been suggested throughout 20th Century. It is a versatile, fast growing hardy plant having wide climatic tolerance to extreme environments including soil pH ranging from 3.0-10.5 and temperature from 14-55^{0}C. It accumulated Pb $^{++}$, Cd^{++}, Hg^{++}, Ag^{++}, Zn^{++}, Ni^{++} & Cu^{++}, and tolerates many organic poisons making it valuable for decontaminated around mines & for lowering water table in saline soils. (Truong, P.N., 1999 a and b; Lavania *et al,* 2004; Anzani et al., 1999; Roongtanakiat N. *et al.,* 2001). As it is slow growing during initial 70-90 days, it allows the vacant interrow spaces to be used for growing an intercrop which gives cash revenue 3-4 months after planting.

Vigna mungo (L) Hepper (Black Gram or Urd Bean) is one of the most widely used pulse crop in India. It is a highly prized pulse, very rich in phosphoric acid. This pulse crop is being grown in the area adjoining the industries where industrial effluents contaminated with heavy metals is coming to the field so that the present investigation is undertaken to study the phytotoxic effects of lead on growth parameters in blackgram and its phytoremediation through vetiver grass.

2. MATERIALS AND METHODS

Present investigation is carried out in the Genetics and Plant breeding laboratory, Dept. of Plant Science, M.J.P. Rohilkhand University, Bareilly, India.

2.1. Materials

Seeds of two inbreds of *Vigna mungo* i.e PU-35 and T-9 were obtained from seed centre, Haldi, Pantnagar. Both are early maturing (70-80 days) and widely adapted for spring, rainal & rabi seasons. They were also well adapted to the surrounding regions of Bareilly with yield of about 670-900 kg ha^{-1}.Vetiver tillers (Clonal progeny of local population) were obtained from Central Institute of Medicinal and Aromatic Plants, Lucknow. Three concentrations of $Pb(NO_3)_2$: 9,10 & 11 mg L^{-1} were used for lead treatments. These doses were decided on the basis of LD-50.

2.2. Field Preparation

A field plot trial was designed in Botanical Garden of Dept. of Plant Science. The well levelled clods & weed free field was prepared to ensure adequate plant stand & early vigour. For opening up of soil, two ploughing were done. It is not only helpful in minimizing the weed but also ensure adequate trapping & conservation of moisture.

Prior to conducting the experiments a surface soil sample was collected from the plot and used for basic characteristic analysis.

2.3. Experimental Design

The whole field is divided into two parts. One is for phytotoxic experiments & other is for phytoremediation experiments. First part of the field in which phytotoxic experiments were carried out consists of 8 small plots for each having an area of 9 m^2 (3m x 3m), four plots for each inbred. Plots are arranged as follows : Control (having Black gram seeds irrigated with distilled water), Treatment A, B and C (having Black gram seeds treated with 9,10 & 11 mgL^{-1} concentration of lead respectively).Second part of the field in which phytoremediation experiments were carried out, consists of 6 small plots each having an area of 9 m^2, 3 plots for each inbreds named as D, E & F (having V*igna mungo* + vetiver treated with 9,10 & 11 mgL^{-1} concentration of lead respectively). Vetiver is planted in IInd weak of June as 4 tillers per clump at a 60 x 25 cm spacing and was cut at 30 cm high before planting. In vacant interrow spaces, Black gram seeds were sown in the field. Prior to sowing seeds were presoaked in distilled water for 8 hours to help in their easy germination. Plants were irrigated with different doses of lead at 10, 15, 30 and 35 days of sowing. The whole field is biweekly irrigated with tap water for maintaining water content to enable the heavy metal salt to reach a steady state. All the growth parameters were recorded in 25 & 45 days old plants.

2.4. Laboratory Analysis of Plant Samples

Five plants were selected randomly from each plot. Plant height was measured in 25 & 45 days old plants by laying the plants horizontally & measuring from end to end with a tape measure. Fresh weight was observed using a top loading balance. For dry weight, plants were dried in oven at 60^0C for 48 hours and dry weight was measured in grams. Chlorophyll & carotenoid contents were determined by extracting one gm. fresh leaves in 10 ml of 80% acetone. After centrifugation 1 ml of supernatent and 9 ml of 80% acetone was taken and O.D. was measured at 440 nm, 645 nm and 663 nm wavelenghts in UV-visible spectrophotometer. Total chlorophyll & total carotenoid contents were estimated as per formula given by Arnon (1949) and Ikan (1961) respectively. Nitrogen content was determined by Micro-Kjeldhal method of Lang (1958). The amount of Nitrogen was calculated by standard curve, prepared from $(NH_4)_2SO_4$. Protein content was measured by folin phenol method (Lowry *et al,* 1951). Its amount was calculated by standard curve prepared by using Bovine Serum Albumin.

2.5. Heavy Metal Analysis in Vetiver

Lead accumulation (mg g^{-1} DW) in different parts of vetiver (roots, shoots & leaves) were determined by using Atomic Absorption Spectrophotometer (GBC Avanta , AAS, Australia) after samples were digested with concentrated $HNO_3 + HClO_4$

2.6. Statistical Analysis

One way ANOVA was carried out to compare the means of different treatments at 5% level of significance.

3. RESULTS

3.1. Soil characteristics

Table 1 shows the physical and chemical characteristics of studied soil. Studied soil texture was sandy loamy with a low cation exchange capacity (CEC) of 10.28 $cmol_{(+)}$/Kg. The soil organic matter content was not low. Contents of N, P & K nutrients were distinctly scarce. Original lead concentration in soil digested by aqua regia was 20.27 mg/kg.

Table 1: Physical and chemical properties of studied soil

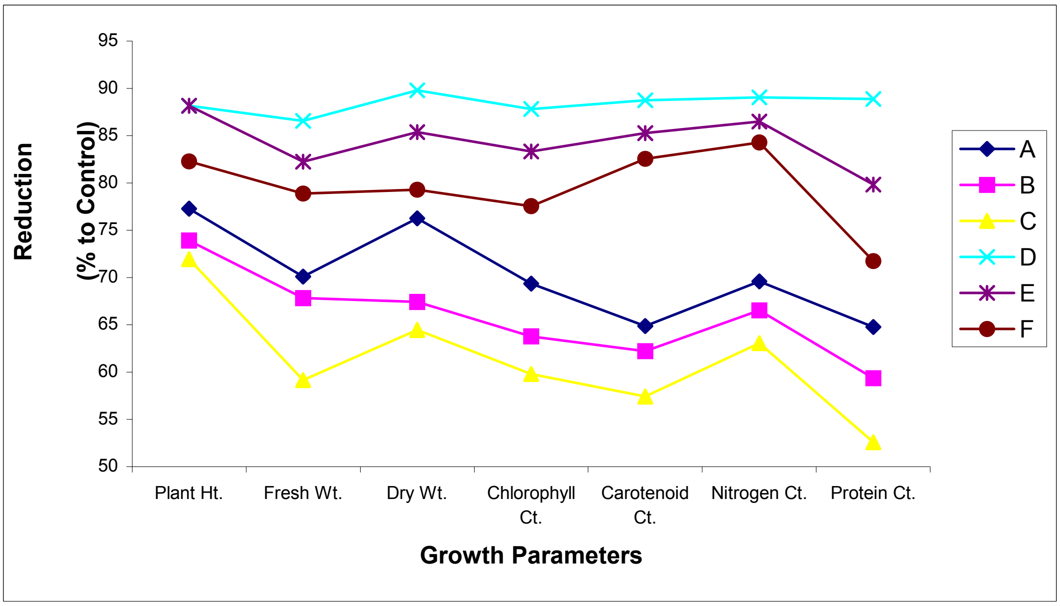
Soil Texture	Sandy loam
Soil pH	5.23
Organic Carbon (gm/kg)	27.23
CEC ($cmol_{(+)}$/kg)	10.28
Available N (gm/kg)	1.0
Available P (gm/kg)	0.43
Available K (gm/kg)	0.68
Total Pb (mg/kg)	20.27

3.2. Phytotoxic effects on growth parameters in *Vigna mungo* (Fig. 1 & 2)

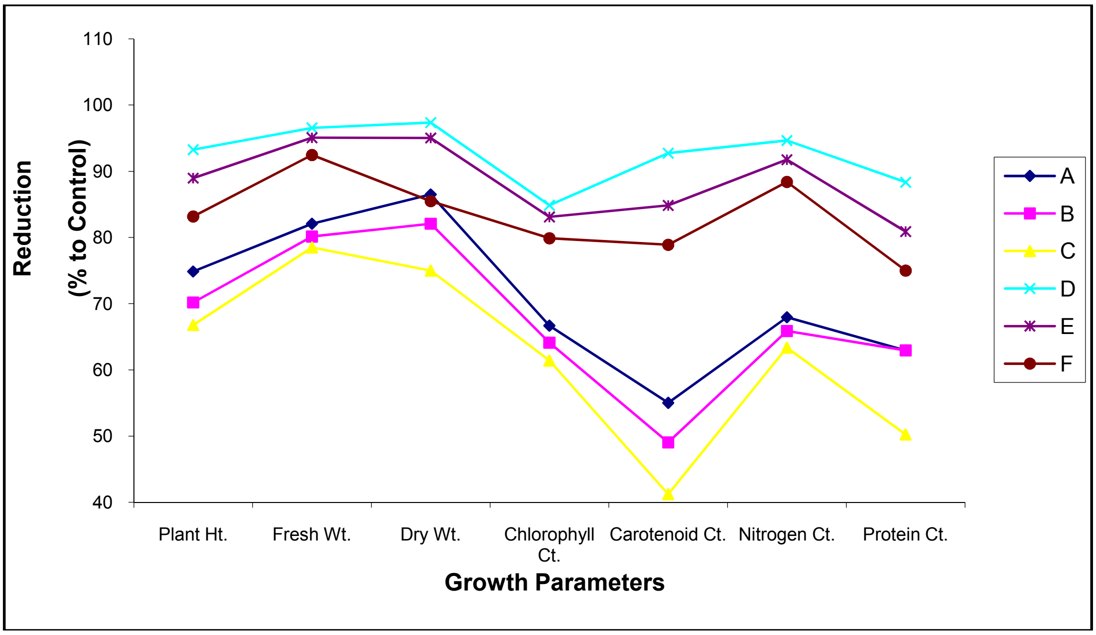
Plant height measured at 25 & 45 days of sowing showed a significant reduction as the lead concentration increases & it was limited upto about 33% as compared to control in 45 days old plants of both the varieties.

Fresh weight & dry weight of plants also showed a gradual reduction with increase in concentrations of lead. Maximum reduction was noticed in 45 days old plants of variety T-9 where it is about 21% in case of fresh weight & about 31% in case of dry weight.

Fig. 1: Effects of Lead on growth parameters in *Vigna mungo* (Variety PU-35) and its phytoremediation through *C. zizanioides*.



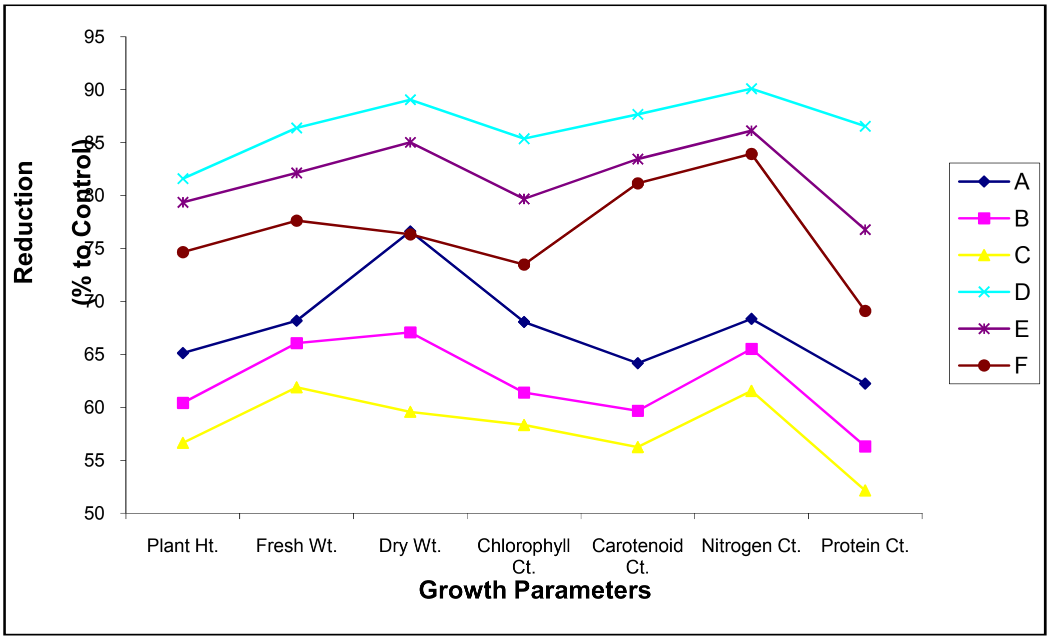
AT 45 DAYS



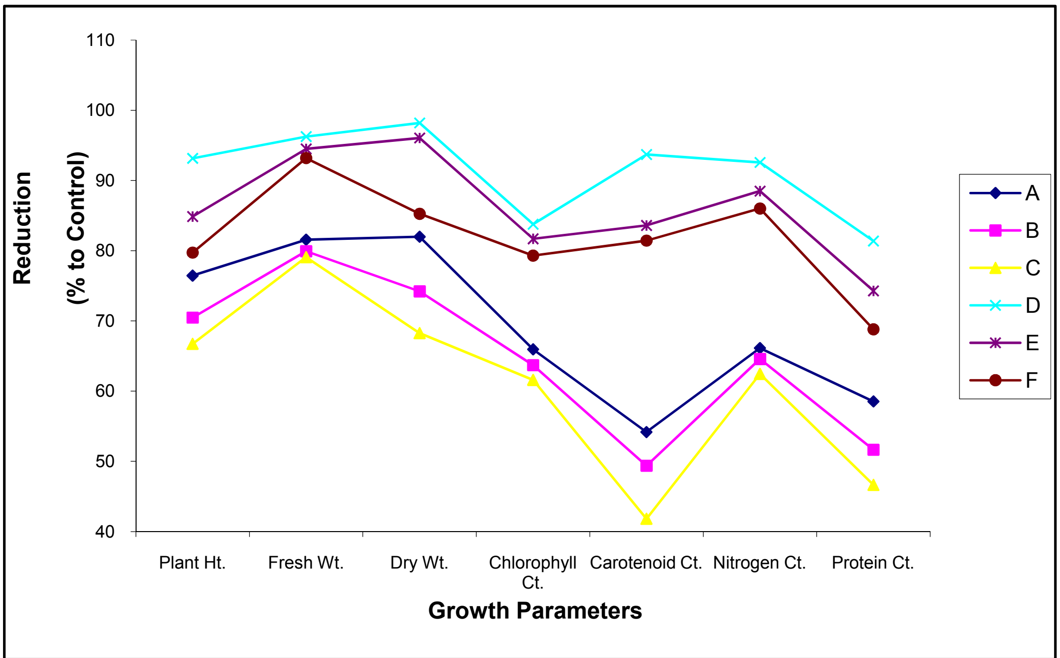
A=*Vigna mungo* treated 9 with mgL^{-1} Lead Nitrate, **B**= *Vigna mungo* treated with 10 mgL^{-1} Lead Nitrate, **C**= *Vigna mungo* treated with 11 mgL^{-1} Lead Nitrate, **D**= *Vigna mungo* + Vetiver treated with 9 mgL^{-1} Lead Nitrate, **E=** *Vigna mungo* + Vetiver treated with 10 mgL^{-1} Lead Nitrate, **F=** *Vigna mungo* + Vetiver treated with 11 mgL^{-1} Lead Nitrate

Fig. 2: Effects of Lead on growth parameters in *Vigna mungo* (Variety T-9) and its phytoremediation through *C. zizanioides*.

AT 25 DAYS



AT 45 DAYS



A=*Vigna mungo* treated 9 with mgL^{-1} Lead Nitrate, **B**= *Vigna mungo* treated with 10 mgL^{-1} Lead Nitrate, **C**= *Vigna mungo* treated with 11 mgL^{-1} Lead Nitrate, **D**= *Vigna mungo* + Vetiver treated with 9 mgL^{-1} Lead Nitrate, **E**= *Vigna mungo* + Vetiver treated with 10 mgL^{-1} Lead Nitrate, **F**= *Vigna mungo* + Vetiver treated with 11 mgL^{-1} Lead Nitrate

Lead also induced reduction in chlorophyll & carotenoid contents significantly. It induced an appreciable reduction in chlorophyll pigment upto 30% in 45 days of old plants in both the varieties. The percentage of carotenoid content is drastically decreased in both the varieties. In this case plants showed a significant reduction upto 58% in 45 days old plants of both the varieties.

Nitrogen contents expressed on the basis of mg g^{-1} dry weight reduced progressively with increase in lead concentrations. Nitrogen contents when analysed after 45 days of sowing showed an appreciable decline upto 36% in varieties PU-35 & up to 38% in variety T-9.

Protein contents were determined as mg g^{-1} dry weight. As compared to control a maximum significant reduction was also recorded in both the varieties among all the lead concentrations. Decline in protein content in 45 days old plants was comparatively more pronounced having considerable reduction up to 49% in variety PU-35 & up to 53% in variety T-9.

3.3. Recovery through phytoremediation

The phytoremediation with vetiver has achieved which is evident from recovery of phytotoxicity induced by lead in growth parameters studied to a significant level in both the varieties of Black gram (Fig. 3). The maximum recovery (% to reduction) was upto 73% in plant height, 80% in fresh weight of plants, 80% in dry weight of plants, 54% in chlorophyll content, 83% in carotenoid content, 83% in nitrogen content & 68% in protein content in case of variety PU-35 whereas, in case of variety T-9, a maximum recovery was recorded in plant height (70%), in fresh weight of plants (79%), in dry weight of plants (90%) , in chlorophyll content (52%), in carotenoid content (86%), in nitrogen content (78%) & in protein content (55%).

3.4. Lead concentration in vetiver

Significant difference was found between concentrations of lead in different parts of vetiver grown in contaminated soil in comparison to its concentration in plants grown in non-contaminated soil (Table 2). Lead concentration in roots of vetiver grown in contaminated soil treated with the highest dose i.e. 11 mg L^{-1} (374.90 mg kg^{-1} DW) was about 2.3 times higher than in shoots (166.3 mg kg^{-1}DW). Pb concentration in roots of vetiver grown in contaminated soil was about 69 times higher than grown in non contaminated soil (Fig. 4).

4. DISCUSSION

The growth and metabolism of Black gram was adversely affected when the plants were exposed to different concentrations of lead. Lead stress causes multiple direct and indirect effects on plant growth and metabolism and also alters some physiological processes (Diaz *et al.* 2001).

Plant height is decreased with increase in lead concentration. This is due to decrease in mitotic frequency and lead accumulation in cell wall components especially pectic substances and hemicelluloses (Tomer *et al*. 2000). Lead was also reported to retard cell division and differentiation and also reduce their elongation thus affect the plant growth & development (Kastori *et al,* 1993). Decrease in fresh weight of plants was might be due to heavy loss of moisture. Decrease in dry weight might be due to accumulation of certain nutrients, reduction in photosynthesis and chlorophyll 'a' synthesis as suggested for cowpea by Joshi *et al* (1999). Chlorophyll content, in general, gradually decreased with increase in lead concentration as compared to its control.

Chlorophyll biosynthesis is inhibited by heavy metals, particularly by inhibiting □□-amino levulinic acid dehydrogenase & protochlorophyllide reductase (Ouzounidou, 1995). Sulphydryl interaction of these enzymes was proposed as mechanism of this inhibition. Carotenoid content of 25 and 45 days old seedlings progressively declined with increase in lead level. This reduction might be due to interference of lead in pigment metabolism as reported by Arvind (2004). Nitrogen & protein contents gradually decrease as the amount of lead increases. When heavy metal toxicity cross threshold, protein level decreases & this might be due to breakdown of protein synthesis mechanism at toxic concentration of heavy metals or due to reduced incorporation of free amino acids in to protein. (Cheetri *et al,* 2004) Protein content under heavy metal influence may be affected due to enhanced protein hydrolysis resulting in decreased concentration of soluble proteins. Binding of heavy metals to sulphydryl (-SH) group of proteins also disturb the organisation of protein molecules. (Tomer *et al*, 2000).

Table 2: Concentration of Lead (mg kg^{-1} DW) in different parts of vetiver after harvesting *Vigna mungo* (75^{th} day after vetiver transplantation).

Concentration Of Pb (mg lit^{-1})	**Roots**	**Shoots**	**Leaves**
Control (vetiver without any treatment)	4.3*±1.6	3.1*±1.2	2.3*±1.6
Vigna mungo +vetiver treated with 9 mg lit^{-1}	299.63*±14.5	133.00*±4.5	29.96*±3.1
Vigna mungo +vetiver treated with 10 mg lit^{-1}	340.00*±12.7	151.53*±9.5	37.5*±4.5
Vigna mungo +vetiver treated with 11 mg lit^{-1}	374.90*±19.2	166.33*±6.4	45.1*±4.0

* Significant at 5% level of significance

Results of phytoremediation experiments (where vetiver is intercropped along with Black gram) indicate significant recovery of phytotoxicity induced by lead in all the parameters studied to a significant level. Growth of Black gram plants in artificially contaminated soil was significantly retarded in comparison to plants grown as intercrop with vetiver. AAS studies also confirm that lead has been accumulated by vetiver where the accumulation is primarily in roots as compared to shoots & leaves and this makes vetiver useful for phytostabilization indicating that lead is accumulated more in below ground parts (roots) and is weakly translocated through vascular system. Thus vetiver acts as a powerful phytoremediator & hyperaccumulator for lead & makes the soil less toxic. As a result, phytotoxicity of lead on growth parameters has been drastically reduced. The mechanisms behind this hyperaccumulation & detoxification include chelation to organic acids (Oven *et al* 2002) or proteins (Martens et al. 1996) or it may be due to its larger biomass apart from the stronger metal uptake ability. Furthermore, it could yield better covering and revegitating benefits. (Xia, 2004). The fact that vetiver can live some 50 yrs. (National Resource Council, 1995) make this species an efficient, enduring, low cost & long term remedial option for phytoremediation. Thus, the present study demonstrates that vetiver grass may be used as potential phytoremediator plant at industrial sites contaminated with lead.

5. CONCLUSION

Based on the results of the present work, following is concluded:

1. Lead causes adverse effects on growth and metabolism of Black gram. This is evident from the reduction in growth parameters studied.
2. Variety T-9 is more susceptible to lead toxicity as compared to variety PU-35.
3. A significant recovery of this induced toxicity is noticed, when grown along with vetiver as an intercrop. This was found to be due to lead accumulation in vetiver (mostly in its roots) confirming its potentiality as a phytoremediator.
4. The dual cash revenue can be procured by intercroping Black gram with vetiver along with phytoremediation of heavy metals contaminated soil.

6. ACKNOWLEDGEMENT

We are thankful to University Grants Commission, New Delhi (UGC Project No. F-30-147/2004 (SR)) for providing financial support.

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