Phytoremediation of Induced Lead Toxicity in *Vigna mungo* (L) Hepper by Vetiver Grass

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Problems identified in the adjoining area for cultivation of crops

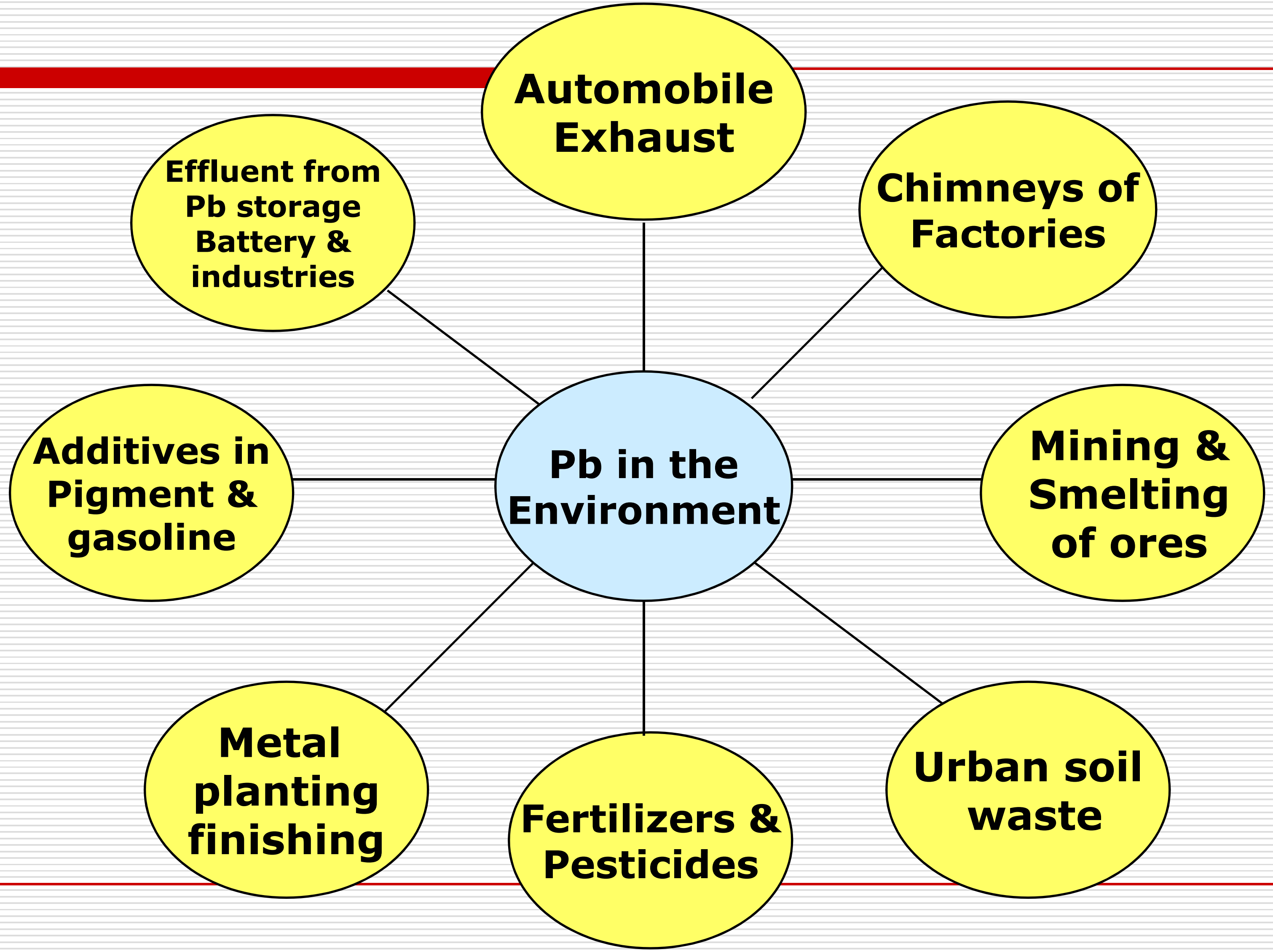
Domestic city waste water, industrial waste water, etc. that contain heavy metals (HMs) in toxic amounts are being used for irrigation purposes. The vegetables and other crops (pulse crops) grown in such areas, contain these HMs and it reaches human body through food chain and are very harmful.

Vigna mungo (L) Hepper is one of the major pulse crops grown in our area.

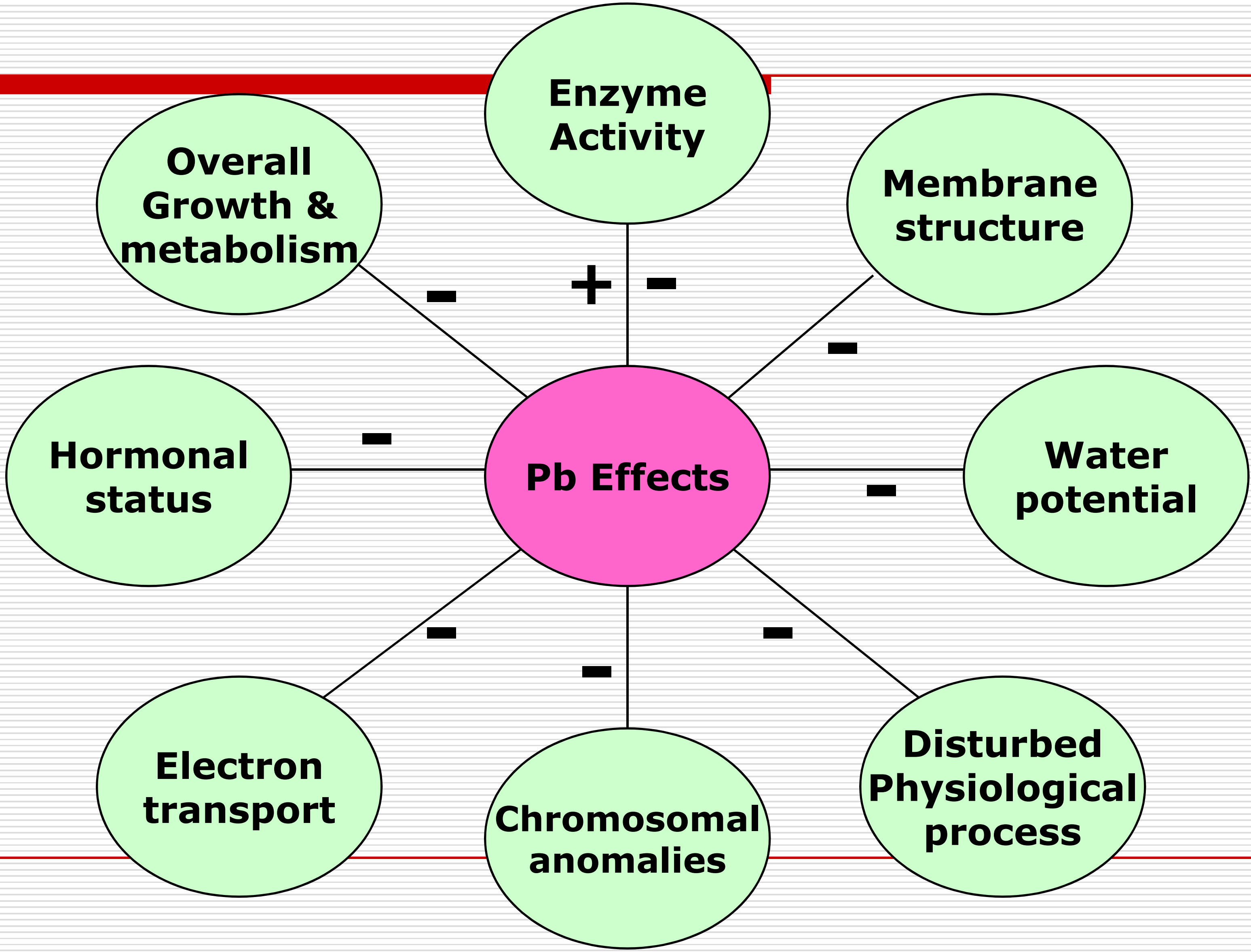
City waste water analysis showing presence of Lead (Pb)

Parameters	Quality/Quantity
Physical	
Color	**Blackish**
Odour	**Pungent**
Chemical	
pH	**9.4**
T.D.S	**3390**
T.S.S.	**790**
C.O.D.	**1520**
B.O.D.	**785**
Heavy Metals	
Cd	**0.204**
Cu	**7.500**
Pb	**3.374**
Zn	**7.270**

Source of Pb pollution in the environment (Gupta, 2008).



A generalized view of Pb toxicity in plants. '+' and '-' signs indicate positive and negative effects respectively (Gupta, 2008).



Strategies to overcome this problem

- **Mutations:**

  In pulses, especially in *V. mungo*, we have induced mutants through chemical mutagenesis. The mutants induced showed no harmful effects even when they are grown in HM contaminated soils. These mutants showed resistance against the hazardous effects of HMs.

  Physiol. Mol. Biol. Plants. 6: 157-161 (2000).

- **Phytoremediation:**

  Intercropping of pulses along with a phytoremediator plant also gives a positive response against this problem.

❖ *Cajanus cajan* with *Brassica juncea*	Mercury
❖ *Cajanus cajan* with *Helianthus annuus*	Zinc
❖ *Vigna mungo* with *Vetiveria zizanioides*	Lead

Phytoremediation

- A novel, potentially inexpensive green technology for sustainable remediation of surface soils contaminated with HMs.

- Considered to be a “Green Technology” in the field of innovative clean up strategies. It is on average tenfold cheaper than other physical, chemical or thermal remediation methods (Hooda, 2007).

- Includes a range of plant mediated processes that are useful in treating environmental problems. These are:

  Phytoextraction/Phytoaccumulation
  Phytostabilization/Phytorestoration
  Phytovolatilization
  Rhizofilteration
  Phytodegradation
  Phytostimulation
  Phytotransformation

Advantages and limitations of some of the sub-processes of the Phytoremediation (Prasad, 2004).

Advantages	Limitations
Phytoextraction	
The plant must be to produce abundant biomass in short time, e.g. in green house experiments, gold was harvested from plants.	**Metal hyperaccumulators are generally slow-growing and bioproductivity is rather small and shallow root systems. Phytomass after process must be disposed off properly**
Phytostabilization	
It circumvents the removal of soil, low cost and is less disruptive and enhances ecosystem restoration revegetation.	**Often requires extensive fertilization or soil modification using amendments, long term maintenance is needed to prevent leaching**

Phytovolatilization

Contaminant / pollutant will be transformed in to less-toxic forms, e.g. elemental mercury and dimethyl selenite gas. Atmospheric processes such as photochemical degradation for rapid decontamination / transformation.

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Rhizofilteration

It can be either *in situ* (floating rafts on ponds) or *ex situ* (an engineered tank system); terrestrial or aquatic.

pH of the medium to be monitored continually for optimizing uptake of metals; chemical speciation and interactions of all species in the influent need be understood; functions like a bioreactor and intensive maintenance is needed.

Intercropping

Intercropping of grasses and legumes are recommended for vegetation of wasteland in order to ensure a long-term stability of vegetation due to contribution of nitrogen by legume species.

Vigna mungo (L) Hepper/ Black gram; Family Fabaceae

- One of the most widely used pulse crop in India.
- Highly prized pulse, very rich in phosphoric acid.
- Some important nutritive values (per 100 g) of black gram are:

Vitamins:	Carotene	0.038
(mg)	Thiamine	0.420
	Riboflavin	0.020
	Niacin	2.0
	Choline	0.206
	Folic acid	0.132
Protein (mg):		24.0
Fat (mg):		1.4
Fibres (mg):		0.9
Carbohydrates (mg):		59.6
Minerals (mg):		3.2
Calorific Values (k cal):		347

Vetiver Grass (Vetiveria zizanioides (L) Nash)

- Agriculture:

  - **Used to trap sediments, increase soil moisture recharge and stabilize soils during intense rainfall and flood.**

  - **Protect rural structure viz. roads, ponds, drains, canals and building sites.**

  - **As it is slow growing initial 70-90 days, it allows the vacant inter row spaces to be used for growing an intercrop that gives cash revenue 3-4 months after planting.**

Bioremediation:

- Vetiver has already been established as tolerance to extreme environments including soil pH ranging from 3.0-10.5 and temp. from 14-55^{0}C.
- Also accumulates extremely high levels of heavy metals (Pb^{++} Cd^{++} Hg^{++} Ag^{++} Zn^{++} Ni^{++} Cu^{++}) and tolerate many organic poisons.
- Used to reclaim soil and increases site productivity of totally unprotected sites.
- It has been proved to rehabilitate:
  - Cu mines in China
  - Coal mines in Indonesia
  - Diamond mine spoils in South Africa
  - Control erosion & leachate from municipal landfills in China.

Materials

- ***Vigna mungo* (L) Hepper**
  - **Variety PU-35**
  - **Variety T-9**

- ***Vetiveria zizanioides* (L) Hepper**
  (*Chrysopogon zizanioides* (L) Hepper)
  - **Clonal progeny of local population**

- **Lead as Lead nitrate**
  Doses: 9.0 mg L^{-1}
  10.0 mg L^{-1}
  11.0 mg L^{-1}

Experimental Design

- The whole field was divided into two parts:

- First for phytotoxic experiments :consisted of 8 small plots for each having an area of 9 m^2 (3m x 3m), four plots for each inbred.
  - Control (having *Vigna* seeds irrigated with distilled water),
  - Treatment A, B and C (having *Vigna* seeds treated with 9.0, 10.0 and 11.0 mg L^{-1} concentration of lead respectively).

- Second part for phytoremediatiion experiments: consisted of 6 small plots each having an area of 9 m^2, 3 plots for each inbred.
  - Treatment D, E and F (having *Vigna mungo* + Vetiver treated with 9.0, 10.0 and 11.0 mg L^{-1} concentration of lead respectively).

- **Vetiver was planted in second weak of June as 4 tillers per clump at 60 x 25 cm spacing and was cut at 30 cm high before planting. In vacant interrow spaces, *Vigna* seeds were sown in the field.**

- **Plants were irrigated with different doses of lead at 10, 15, 30 and 35 days of sowing.**

- **All the growth parameters were recorded in 25 and 45 days old plants.**

Methodology

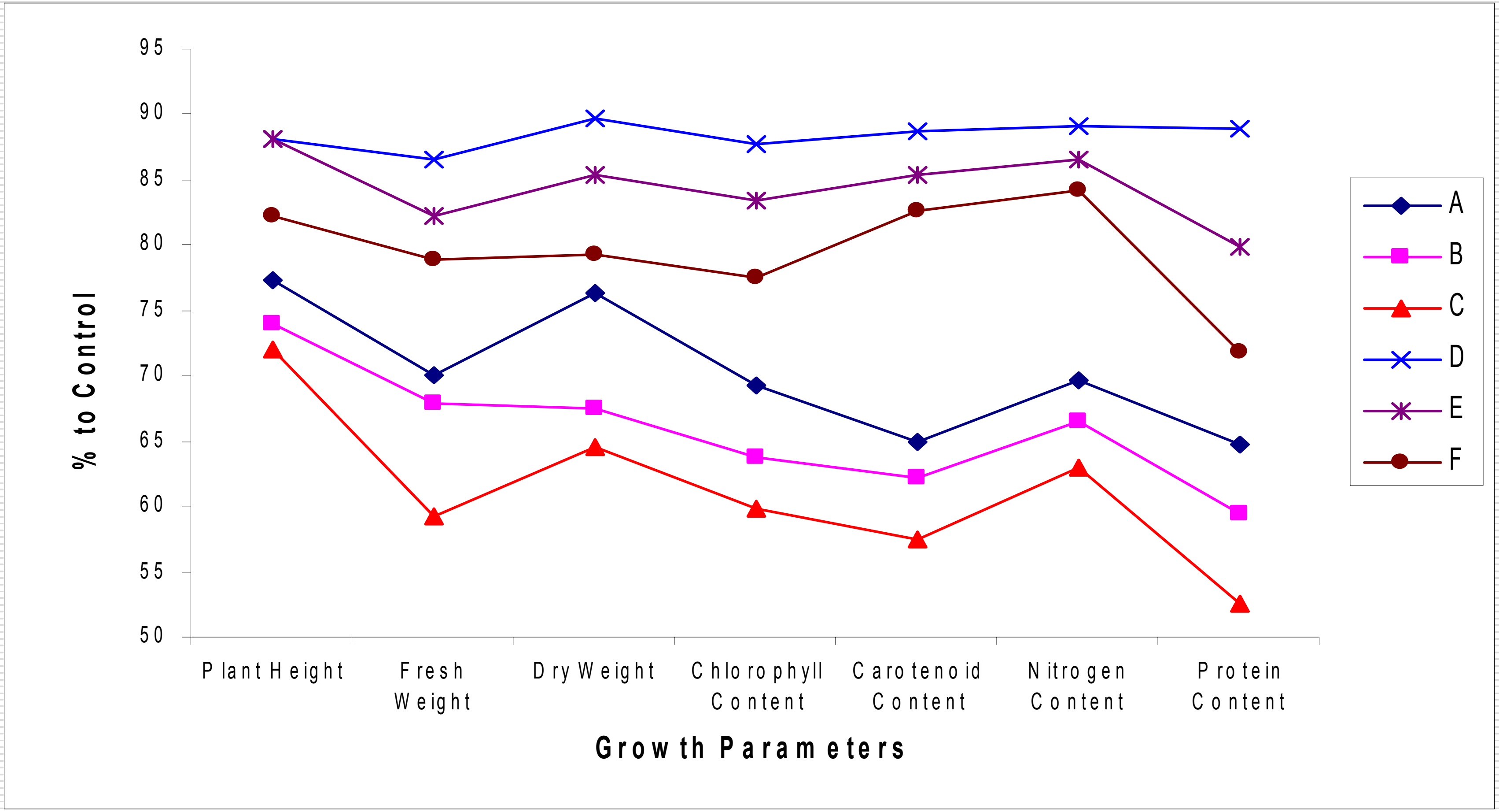
- Plant height, Fresh weight and Dry weight of plants
- Chlorophyll content by Arnon (1949) method.
- Carotenoid content by Ikan (1961) method.
- Nitrogen content by Lang (1958) Micro-kjeldhal method.
- Protein content by Lowry et al. (1951) method.
- Bioaccumulation of Pb in different parts of vetiver by AAS (GBC, Avanta, AAS, Australia).

RESULTS AND DISCUSSION

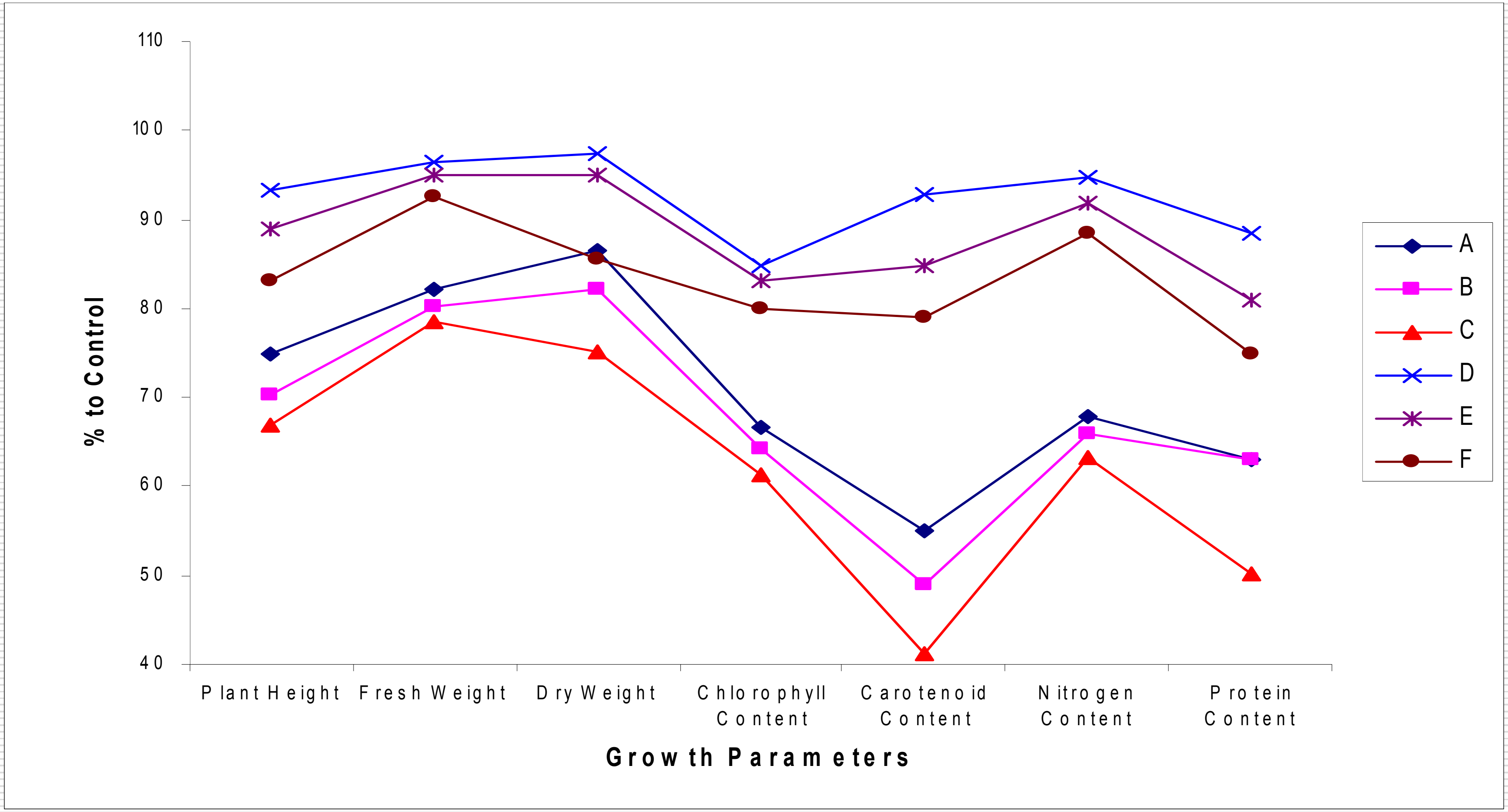
Table 1: Physical and chemical properties of soil

Sand	47.4%
Silt	30.2%
Clay	22.1%
Soil Texture	Sandy Loam
Soil pH (H_2O)	5.23
Organic Carbon (gm kg^{-1})	27.23
CEC (cmol(+) kg^{-1})	10.28
Total N (gm kg^{-1})	1.0
Available P (gm kg^{-1})	0.43
Available K (gm kg^{-1})	0.68
Total Pb (mg kg^{-1})	13.27±0.18
Total Zn (mg kg^{-1})	30.23±2.18
Total Cd (mg kg^{-1})	00.58±0.02
Total Cu (mg kg^{-1})	10.41±1.23

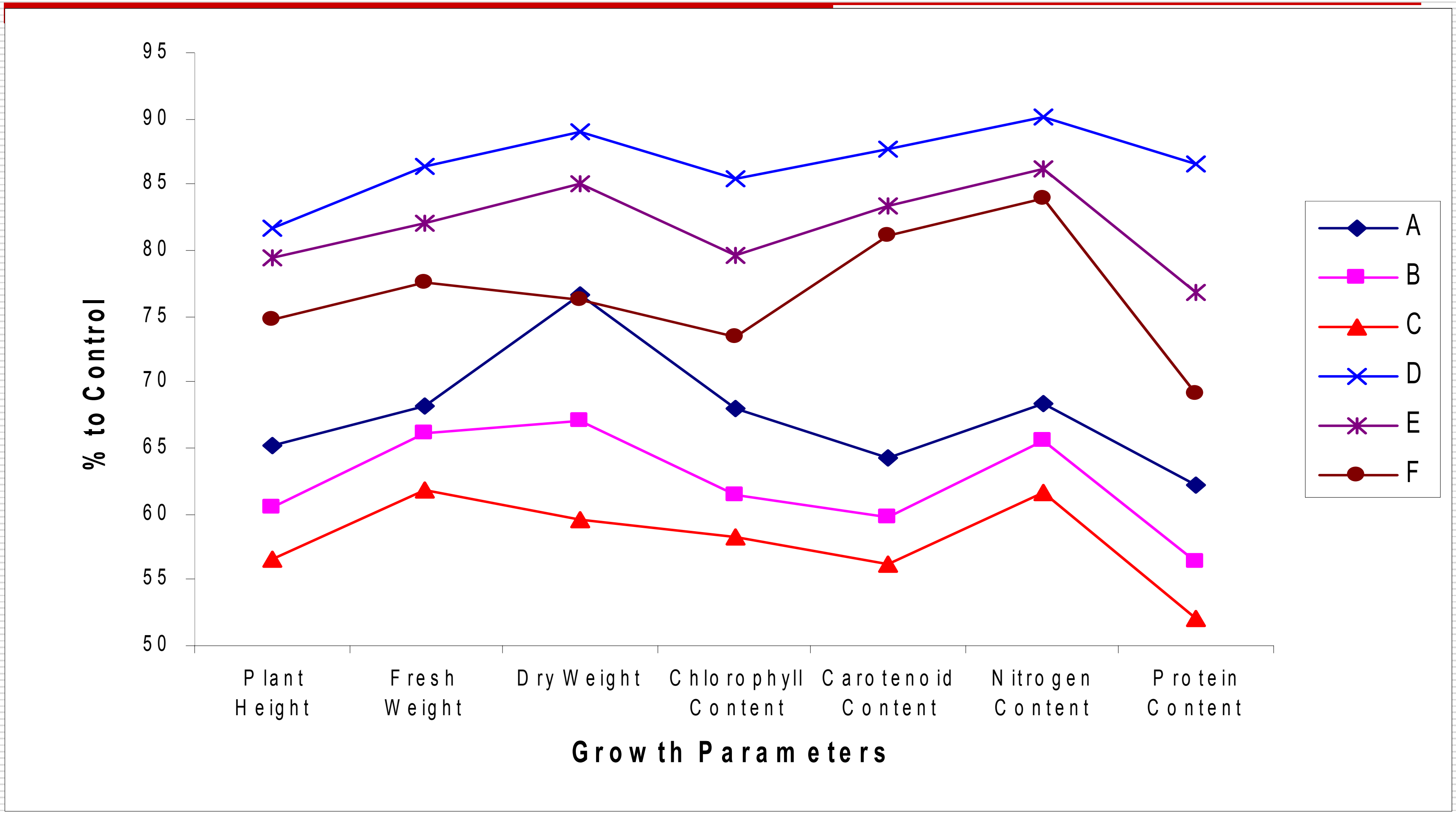
Effects of Pb on growth parameters in *V. mungo* (var. PU-35) at 25 days and its phytoremediation through vetiver grass.



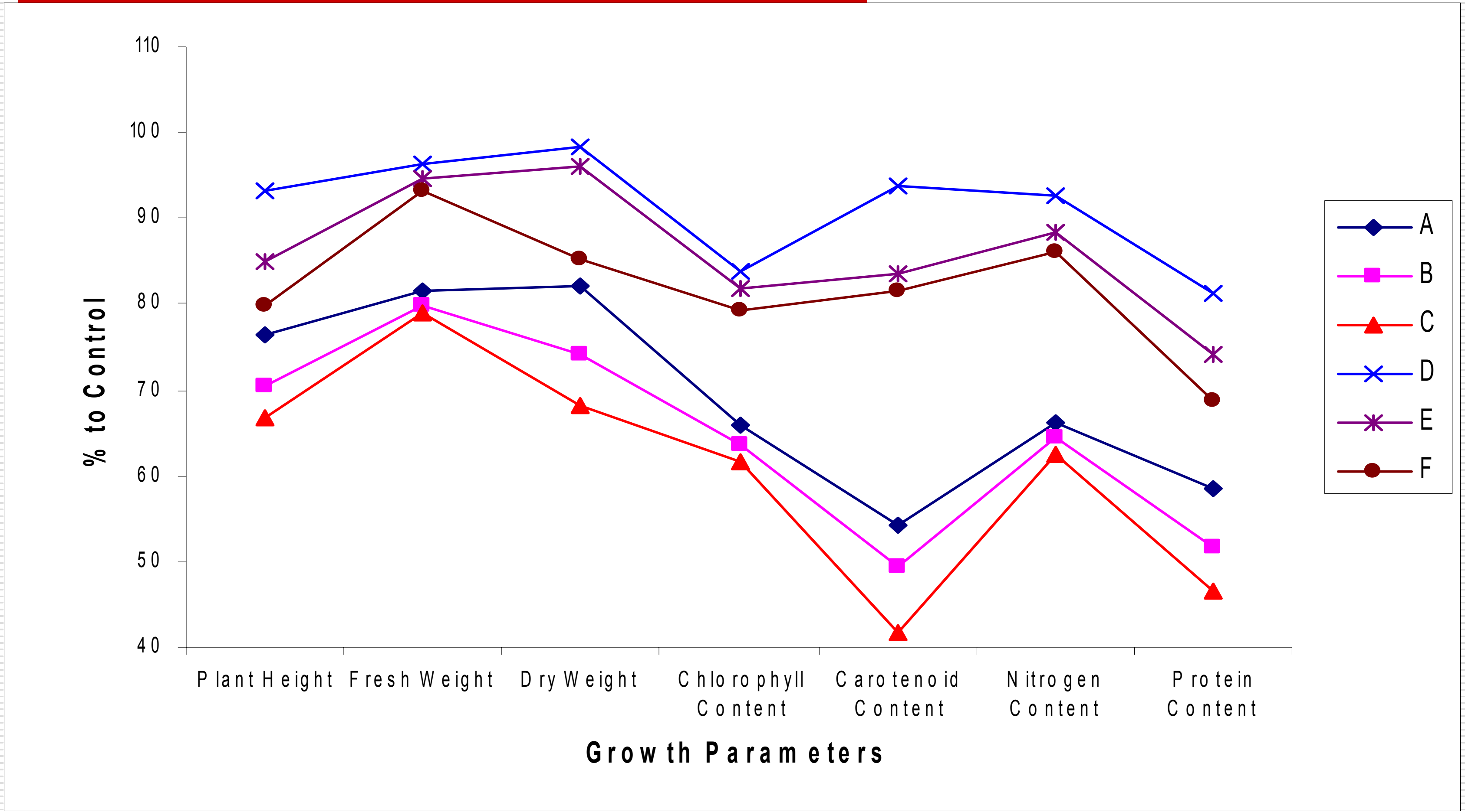
Effects of Pb on growth parameters in *V. mungo* (var. PU-35) at 45 days and its phytoremediation through vetiver grass.



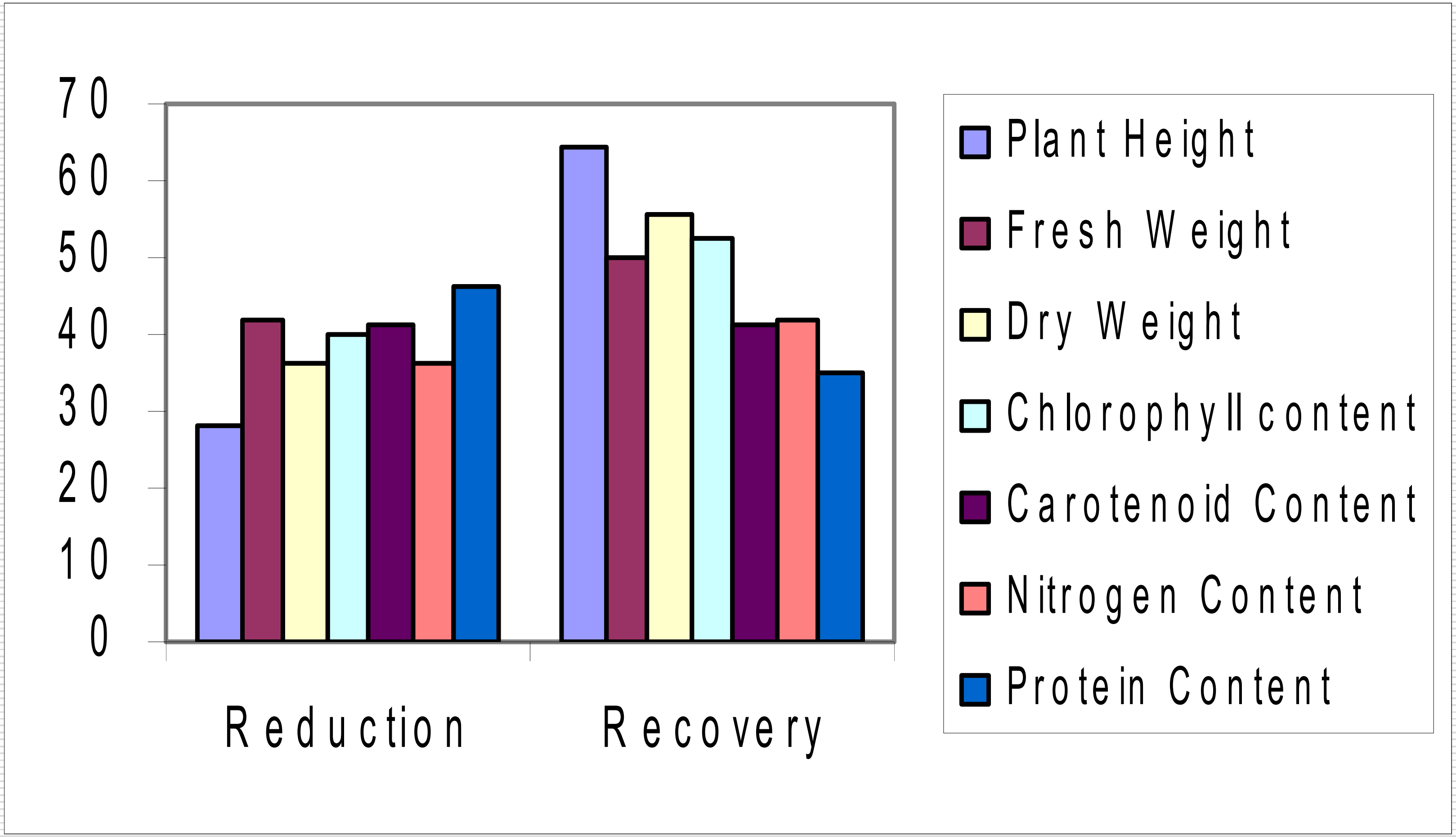
Effects of Pb on growth parameters in *V. mungo* (var. T-9) at 25 days and its phytoremediation through vetiver grass.



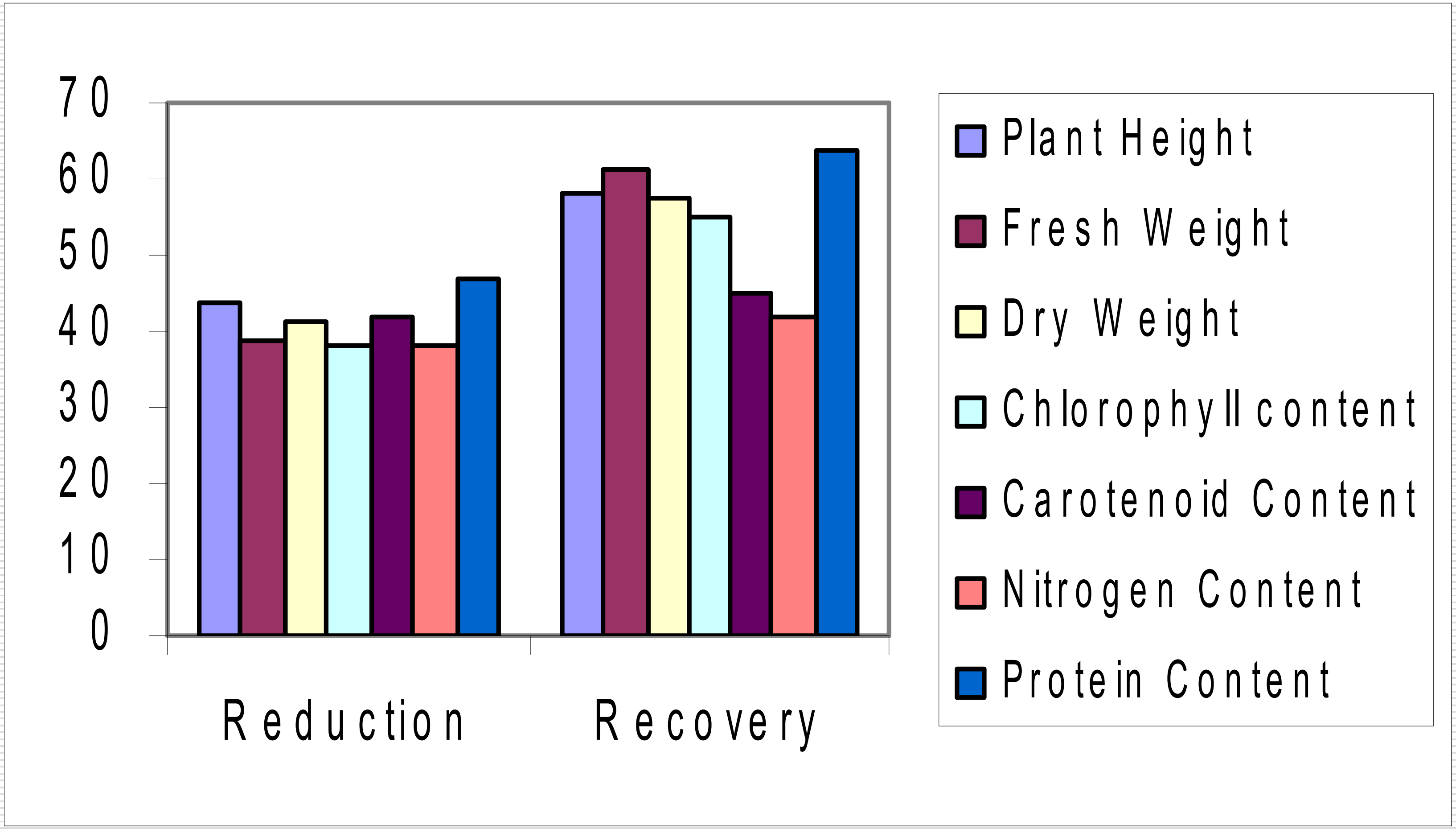
Effects of Pb on growth parameters in *V. mungo* (var. T-9) at 45 days and its phytoremediation through vetiver grass.



Effects of Lead on growth parameters in *Vigna mungo* (var. PU-35) showing reduction (% to control) and maximum recovery (% to reduction).



Effects of Lead on growth parameters in *Vigna mungo* (var. T-9) showing reduction (% to control) and maximum recovery (% to reduction).

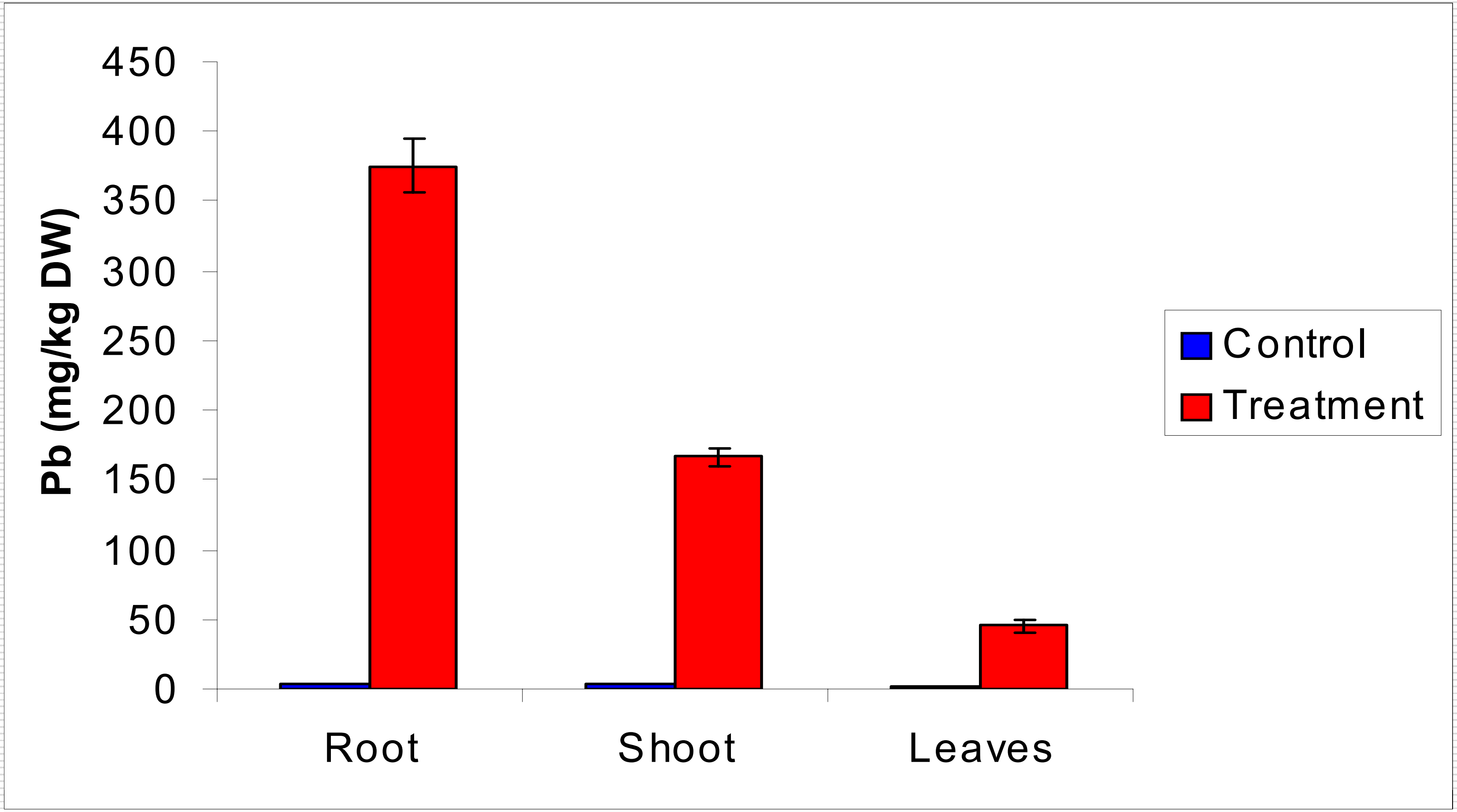


Concentration of Lead (mg kg^{-1} DW) in different parts of Vetiver after harvesting *Vigna mungo* var. PU-35 (75th day after vetiver transplantation).

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

- Data are expressed as mean values of triplicates (n=3) and are significant at *P=0.05.

Comparison of amounts of Pb accumulated by different parts of vetiver after 75 days transplanting.

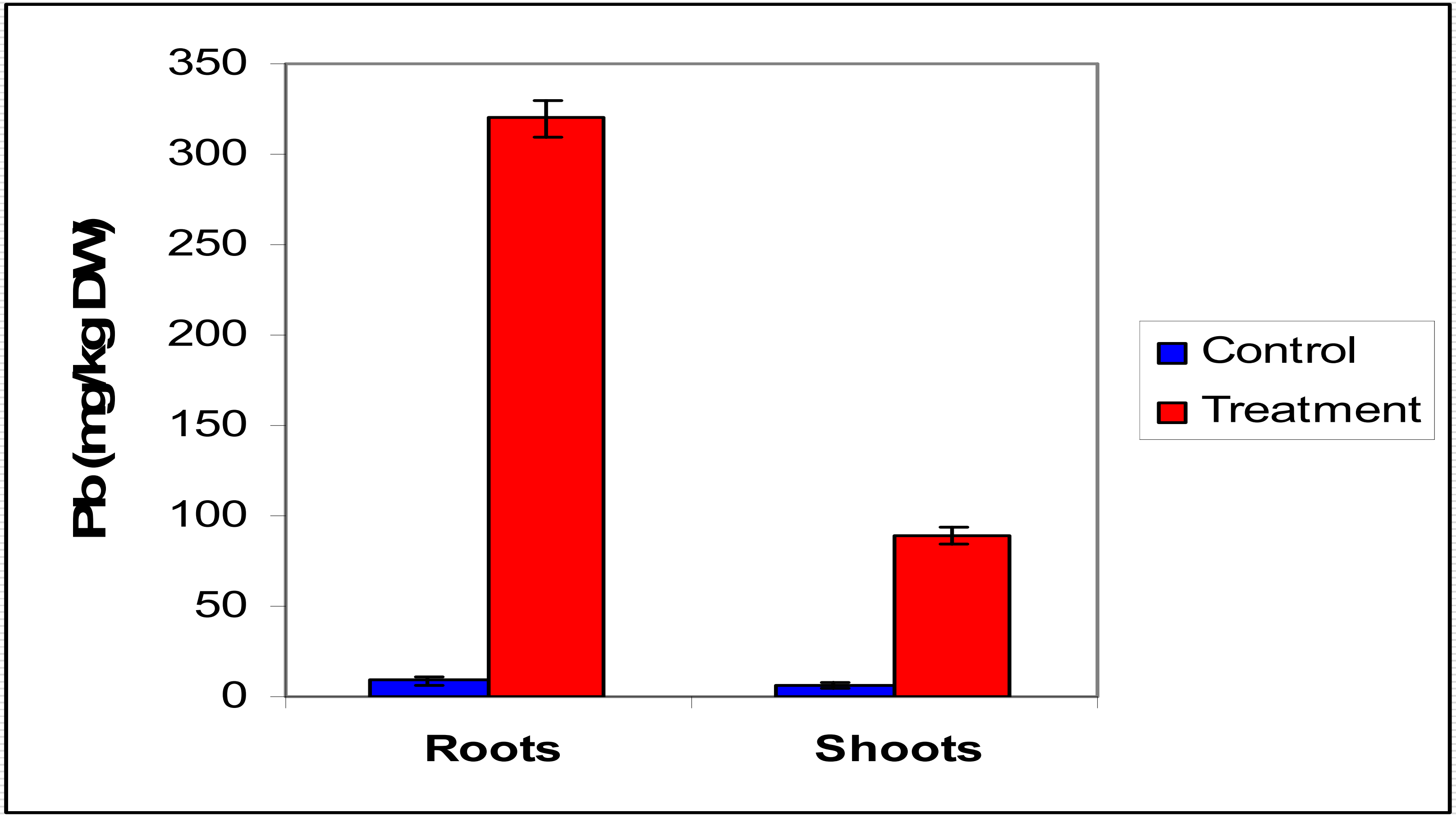


Pb Content (mg kg^{-1} DW) in different parts of *Vigna mungo* at 45 days.

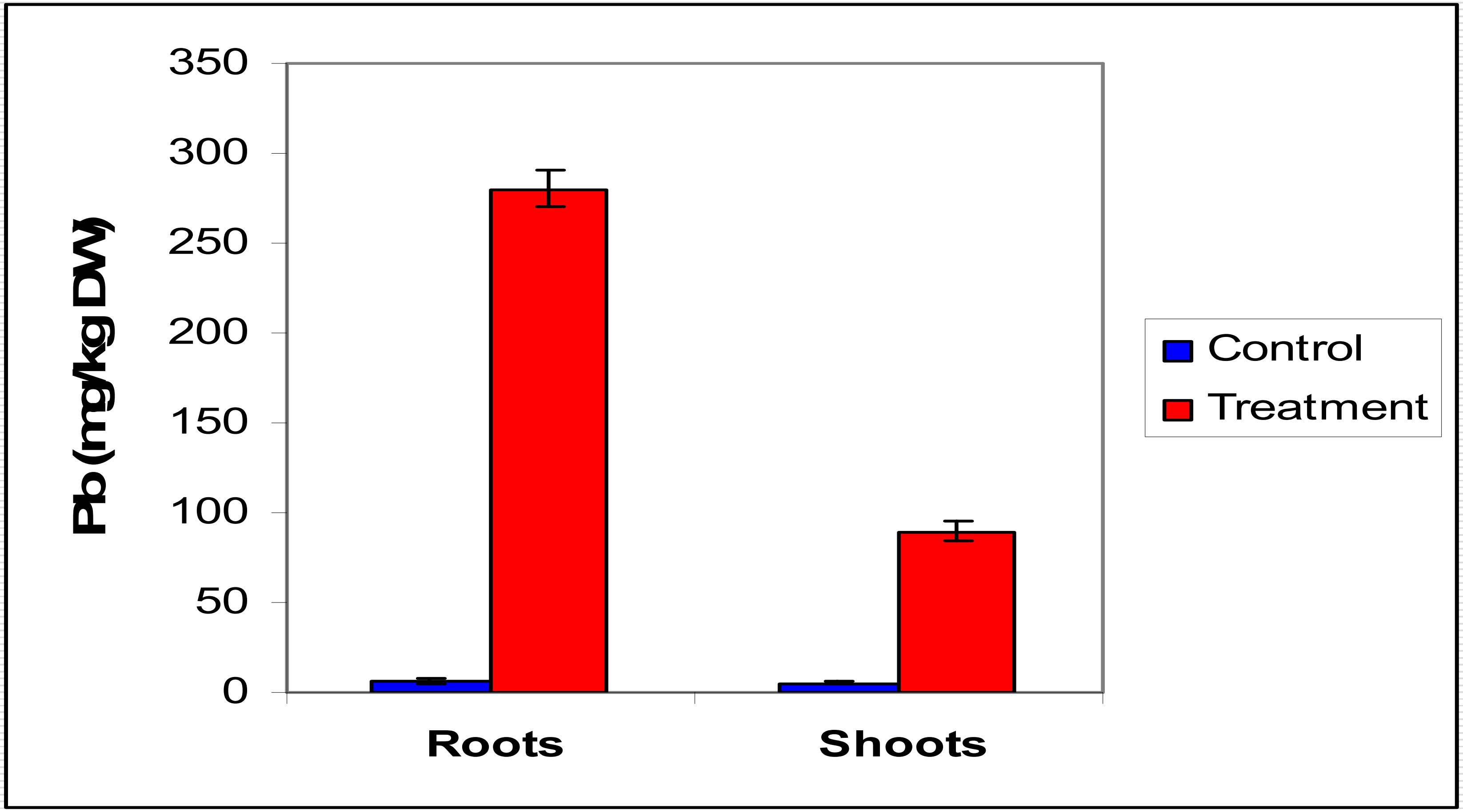
Pb Concentration (mg L^{-1})	Roots Mean ± SE	Shoots Mean ± SE
VARIETY PU-35		
Control	8.60*±1.9	6.20*±1.8
9.0	180.29*±1.9	70.21*±3.2
10.0	202.71*±7.2	78.62*±4.8
11.0	320.21*±10.2	89.21*±5.1
VARIETY T-9		
Control	6.30*±2.1	5.30*±0.9
9.0	156.21*±5.8	71.21*±3.2
10.0	193.21*±7.0	71.31*±4.6
11.0	280.24*±9.8	89.81*±5.6

- Data are expressed as mean values of triplicates (n=3) and are significant at *P=0.05.

Comparison of different amounts of Pb accumulated by different parts of *V. mungo* (PU-35) at 45 days.



Comparison of different amounts of Pb accumulated by different parts of *V. mungo* (T-9) at 45 days.



- **Decrease in plant height might be due to decrease in mitotic frequency, retardation of cell division and cell differentiation & Pb accumulation in cell wall components especially pectic substances and hemicelluloses.**

- **Decrease in fresh weight of plants might be due to heavy loss of moisture.**

- **Decrease in dry weight of plants might be due to accumulation of certain nutrients, reduction in photosynthesis and Chl a synthesis.**

- **Decrease in pigment biosynthesis (Chlorophylls and carotenoids) was due to interference of Pb in pigment metabolism, particularly with sulphydryl interaction of the key enzymes (amino levulinic acid dehydratase and protochlorophyllide reductase).**

- Reduction in nitrogen and protein contents might be due to breakdown of protein synthesis mechanism, reduced incorporation of free amino acids into protein or binding of HMs to sulphydryl group of proteins.

- The reduction was more pronounced in var. T-9 as compared to var. PU-35.

- Result of phytoremediation experiments (where vetiver is intercropped along with black gram) indicate a marked recovery of phytotoxicity induced by Pb in all the parameters studied to a significant level.

- An analysis of Pb accumulation in roots and shoots (stem and leaves) of vetiver showed that a small amount of Pb was already present in these organs which showed a ground contamination of the environment and subsequently vetiver plants with the metals.

- A concentration dependent increase in internal Pb level was well correlated with exogenous supply of Pb. The Pb accumulation was, however, considerably higher in root tissues than shoot and leaves.

- Pb retention in the roots is based on binding of Pb to ion exchangeable sites on the cell wall and extra cellular precipitation, mainly in the form of Pb carbonate deposited in the cell wall.

  Pb binds strongly to the carboxyl groups of the carbohydrates galacturonic acid and glucuronic acid in the cell wall, which restricts its transportation via apoplast (Jarvis and Leung, Environ. Exp. Bot. 48, 21-32, 2002).

- Once inside the plant, Pb moves predominantly into the root via apoplastic pathway and accumulate near the endodermis. It appears that casparian strips of the endodermis are the major limiting factor restricting Pb transport across endodermis into the central cylinder tissue (Seregin and Ivaniov, Russion J Plant Biol, 48, 606-630, 1997).

- This provides evidence that Pb moves into the symplast. Symplastic transport (mediated by membrane transport proteins) of HMs probably takes place in the xylem after they cross the casparian strips.

- Endodermis acts as a partial barrier to the movement of lead between the root and shoot. This may in part account for the reports of higher lead accumulation in roots as compared to shoots (Verma and Dubey, Plant Sci., 164, 645-655, 2003).

- Recent studies have shown that lead accumulation in plant parts is correlated with formation of Pb-EDTA complex and this complex is major form of lead absorbed and translocated by plant (Epstein et al., Plant Soil, 208, 87-94, 1999; Barona et al., Environ. Pollu., 113, 79-85, 2001).

- It has been suggested that metal chelate complex may enter the root at breaks in the root endodermis and casparian strip and be rapidly transported to shoots (Shen et al., J Environ Qual., 31, 1893-1900, 2002).

Conclusion

- Growth and metabolism of black gram was adversely affected when the plants were exposed to different concentrations of lead as evident from the reduction in growth parameters studied.

- Variety T-9 was found to be more susceptible to lead toxicity as compared to variety PU-35.

- A significant recovery of this induced toxicity in *V. mungo* was noticed, when grown along with vetiver as an intercrop. This was found to be due to significant bioaccumulation of Pb in vetiver (mostly in its roots) confirming its potential use in phytostabilization.

- **Vetiver can live up to about 50 years, therefore, it is suggested that its shoots should be cut regularly in order to stimulate its growth into thick clumps.**

- **The dual cash revenue can be procured by intercropping *Vigna* with vetiver along with phytoremediation of HMs contaminated soil.**

Publications

- Deepak Kr. Gupta (2008): “Studies on the Effects of Lead on *Vigna mungo* (L) Hepper with its Phytoremediation through *Vetiveria zizanioides* (L) Nash”. Ph.D. Thesis, M.J.P. Rohilkhand University, Bareilly, India.

- Gupta, D.K., Srivastava, A. and Singh, V.P. (2008: *In Press*): EDTA enhances lead uptake and facilitates phytoremediation by vetiver grass. *J Environ. Biol.,* 84(3/4).

Work in Progress

Use of various soil chelates viz. EDTA, HEDTA, NTA, Citric Acid, etc. in enhancing the phytoremediation potential of vetiver grass under different soil conditions.

Acknowledgement

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THANK YOU