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Effects of Chicken Manure and Mineral Fertilizer on Some Nutritive Parameters and Lead Accumulation in Two Vigna Species Grown in Lead Contaminated Soil

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ABSTRACT
Effect of chicken manure and mineral fertilizer on *Vigna radiata* and *Vigna unguiculata* plants grown in lead contaminated soil were examined. Plants were grown in lead polluted soil, which is amended with different concentrations of chicken manure and mineral fertilizer (NPK). Accumulation of lead in the leaves of both *Vigna* species was reduced when chicken manure was added. Application of mineral fertilizer increased the accumulation of lead in *Vigna radiata*, but caused a decrease in *Vigna unguiculata*. Application of both fertilizers improved the chlorophyll, protein and carbohydrate contents of both species. Results are discussed in relation to the significance of organic and inorganic manure with accumulation of heavy metals such as lead particularly in contaminated soils and their consequences on protein and chlorophyll synthesis.

1. INTRODUCTION
Soil pollution as a result of toxic metals is one of the severe harms of the environment (Mathe-Gaspar and Anton, 2002). These toxic metals are non-biodegradable (Chaturvedi, 2004) and toxic to plants at high levels (Nedelkoska and Doran, 2000). Among the existing heavy metal pollutants, lead (Pb) is the major contaminant of the soil (Romerio *et al.,* 2006). Significant increase in the Pb content of cultivated observed near industrial areas soils has been in particular (Sharma and Dubey, 2005). The main sources of Pb pollution are exhaust fumes from automobiles, chimneys of factories, effluents from the storage battery, industry, mining and smelting of Pb ores, metal plating and finishing operations, fertilizers, pesticides and additives in pigments and gasoline (Sharma and Dubey, 2005). The soil with heavy metal contamination, Pb in particular, enhances the Pb uptake and causes their accumulation in different plants parts (Chaturvedi, 2004).

Soil improvement is a major requirement for the successful establishment of vegetation especially in metal-contaminated soils (Rotkittikhun *et al.,* 2007). The application of cow manure, poultry manure, pig manure and sewage sludge are known to support plant growth, reduce metal uptake and accumulation in plant tissue (Chiu *et al.,* 2006). It has been suggested that organic manure are improve the soil condition especially of contaminated soil through the increase in nitrogen (N), phosphorous (P) and potassium (K) contents in soil (Chiu *et al.,* 2006). Malak Ramadan *et al.,* 2007 have reported that poultry manure is a complete fertilizer due to it being rich in organic matter and organic carbon which positively influences the crop production. Awodun, 2007 indicated that poultry manure facilitates the growth of soil organisms thus promoting the soil's free mineral supply & for the reason of being evenly distributed in soil poultry manure tends to stay longer and not leaches abruptly. Blay *et al.,* 2002 have reported the beneficial effects of poultry manure on the soil and the crops grown therein. Besides being free from pathogens (infectious bacteria), poultry manure increases the fertility of aged soils, improves the soil structure by loosening of the compact soil to facilitate the ventilation.

The main objective of the present study was not only to evaluate the effect of different concentrations of chicken manure and mineral fertilizer (NPK) on Pb uptake and nutritive values such as total chlorophyll, carbohydrate and protein content of *Vigna radiata* and *Vigna unguiculata* grown in heavy metal contaminated soil but also for establishing suitable application dosage of these fertilizers for development of the lead contaminated soil. Therefore, it is presumed that compared to the inorganic manure, organic manure especially chicken manure can more efficiently improve the chemical and physical conditions of metal contaminated soil.

2. MATERIAL AND METHODS
Pot experiments were conducted on two different edible beans *Vigna unguiculata* and *Vigna radiata*. Healthy seeds of each species were selected and sterilized with 0.1% Mercuric Chloride solution for 5 minutes followed by washing with tap and distilled water. Seeds were sown in plastic bag containing 300gm sterilized soil. Two-week old seedlings were transplanted into plastic pots (12cm diameter and 15cm height) containing Pb contaminated soil which was collected from Karachi industrial region which was mixed with three levels of chicken manure and mineral fertilizer. Chicken manure was mixed with soil at different concentrations i.e. 50, 75 and 100gm chicken manure. Kg^{-1}. In addition, soil was also assorted with three different levels of mineral fertilizer addition (N:P:K= 15:15:15): 50, 75 and 100 mg fertilizer.Kg^{-1} of dry soil. Soil without mineral fertilizer and chicken manure served as the control. Plants were watered on alternate days throughout the experimental periods till maturity

3. SOIL ANALYSIS
Soil samples were collected before treatment and analyzed for following chemical parameters:

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3.1 *Soil Ph and Ec*
Soils pH and EC were measured before the addition of NPK and chicken manure. 10gm dried sieved soil mixed and homogenized in 100ml-distilled water. The mixture was then allowed to settle. Soil suspension was filtered, pH and EC were recorded using a glass-electrode pH meter (Mettler Toledo, Serial MP-220 P.H 200223M) and conductivity meter (Mettler Toledo, Serial 76785 C.M.D. 500 conductivity meter) respectively.

3.2 *Soil Organic Matter*
Loss on Ignition (LOI) method was used for measuring the soil organic matter, which involves burning off organic matter in soil samples (Konen *et al.*, 2002). Air-drying of the soil sample was followed by passing of the air-dried soil through a 2 mm mesh sieve. 5g of air-dried soil was placed in a crucible and weighed (pre-weight). The crucible with soil was placed in a furnace and heated to about 360^{o}C for 3 hours. Later, the sample was weighed (post-weight). Percent organic matter was calculated using the following formula

$$\% \text{ Organic Matter} = \frac{(\text{pre-weight} - \text{post-weight})}{\text{pre-weight}} \times 100$$

3.3 *Soil Heavy Metal Analysis*
Soil samples, NPK and chicken manure were dried in oven at 40°C for 48 h and then passed through a 2 mm sieve to discard non soil particles. After that 5 gm of both fertilizer and soil sample were digested in 50 ml of 2 M Nitric acid for 2 h at 100°C. The extracts were analyzed for total lead by atomic absorption spectrophotometer (Nowack *et al.*, 2001).

3.4 *Biochemical Analysis of Plants*
The leaf samples from both control and treated plants were collected randomly in early hours of the morning and were kept in labeled sample bags. Later total chlorophyll (Maclachlam and Zalik, 1963) total protein (Lowry *et al.*, 1951) and carbohydrate (Yemm and Willis, 1959) were examined.

3.5 *Plants Heavy Metal Analysis*
Plants leave samples were dried for 42 h at 40°C. 20ml of 70% Nitric acid was added in 1 g plant leave material, covered with glass Petri dish and incubated for 30 minutes. The sample was heated until the entire solid particle disappeared. Later 10 ml of 70 % perchloric acid was added to the sample. The heating was continued until the volume was reduced upto to 3ml, then solution was cooled and volume was made upto 100 ml with de-ionized water followed by filtering through Whatman Number 1 filter paper and then sample used to study the amount of lead present. Lead was analyzed by atomic absorption spectrophotometer (Richards, 1954).

3.6 *Statistical Analysis*
The data for chlorophyll, protein, carbohydrate and heavy metal concentrations of plant under different treatments were analyzed using the "COSTAT" statistical program by two-way analysis of variance (ANOVA) to compare the means of different treatment and "SIGMA PLOT" program was used for graphic presentation of the data.

4. RESULTS
4.1 *Soil characteristics*
Table-1 reveal that amount of extractable lead was higher in contaminated soil 345.260 µg.mg^{-1} as compare to chicken manure 53.760 µg.mg^{-1} and mineral fertilizer 32.910 µg.mg^{-1}. The contaminated soil contained low level of organic matter (0.323%), while nitrogen, phosphorus and potassium content of polluted soil was low as compare to chicken manure and mineral fertilizer.

Table-1: Chemical properties of experimental soil, chicken manure and NPK.

Parameters	Soil	Chicken manure	Mineral Fertilizer
pH	7.04	6.23	6.91
EC (µs/m)	1226	4220	-
Organic matter (%)	0.323	59.10	-
Nitrogen (%)	0.050	2.260	33.50
Phosphorus (%)	0.030	2.170	15.50
Potassium (%)	0.040	1.480	48.00
Extractable Pb (µg.mg^{-1})	345.26	53.76	32.91

The data in table 2 illustrate the EC of contaminated soil was lower as compare to other treatments. The pH of the polluted soil was neutral, whereas pH of soil with chicken manure was nearly neutral and soil with mineral fertilizer was basic. Table 2 also shows that amount of extractable lead was decrease after chicken manure addition, whereas in mineral fertilizer treatment was increased as compare to non amended soil sample.

Table-2: pH, EC and extractable Pb in the different treatments before plant growth experiment

Treatments	pH	EC (μs/m)	Extractable Pb (μg.mg^{-1})
Soil	7.04	1226	345.260±10.844
Soil+50g chicken manure	7.16	2814	218.327±12.374
Soil+75g chicken manure	7.15	3786	187.462±10.666
Soil+ 100g chicken manure	7.18	4223	156.753±10.431
Soil+50mg mineral fertilizer	7.83	2347	261.616±15.363
Soil+75mg mineral fertilizer	7.85	2528	283.167±11.561
Soil+ 100mg mineral fertilizer	7.96	2639	313.714±14.536

4.2 *Accumulation of Lead*

Significant (P<0.001) accumulation of lead in leaves of *Vigna unguiculata* and *Vigna radiata* was observed by mineral fertilizer (NPK) application. In *Vigna radiata* (Fig. 1a) accumulation of lead increased with increasing the concentration of applied NPK, whereas in Vigna *unguiculata* (Fig. 1b) variable trend of lead accumulation was observed. In both species maximum lead accumulation was found in 100gm/Kg NPK treatment compared to control. This increase sustained till the end of experiment. Hence the inorganic fertilizer addition was not very effective in reducing the lead uptake in plant tissue.

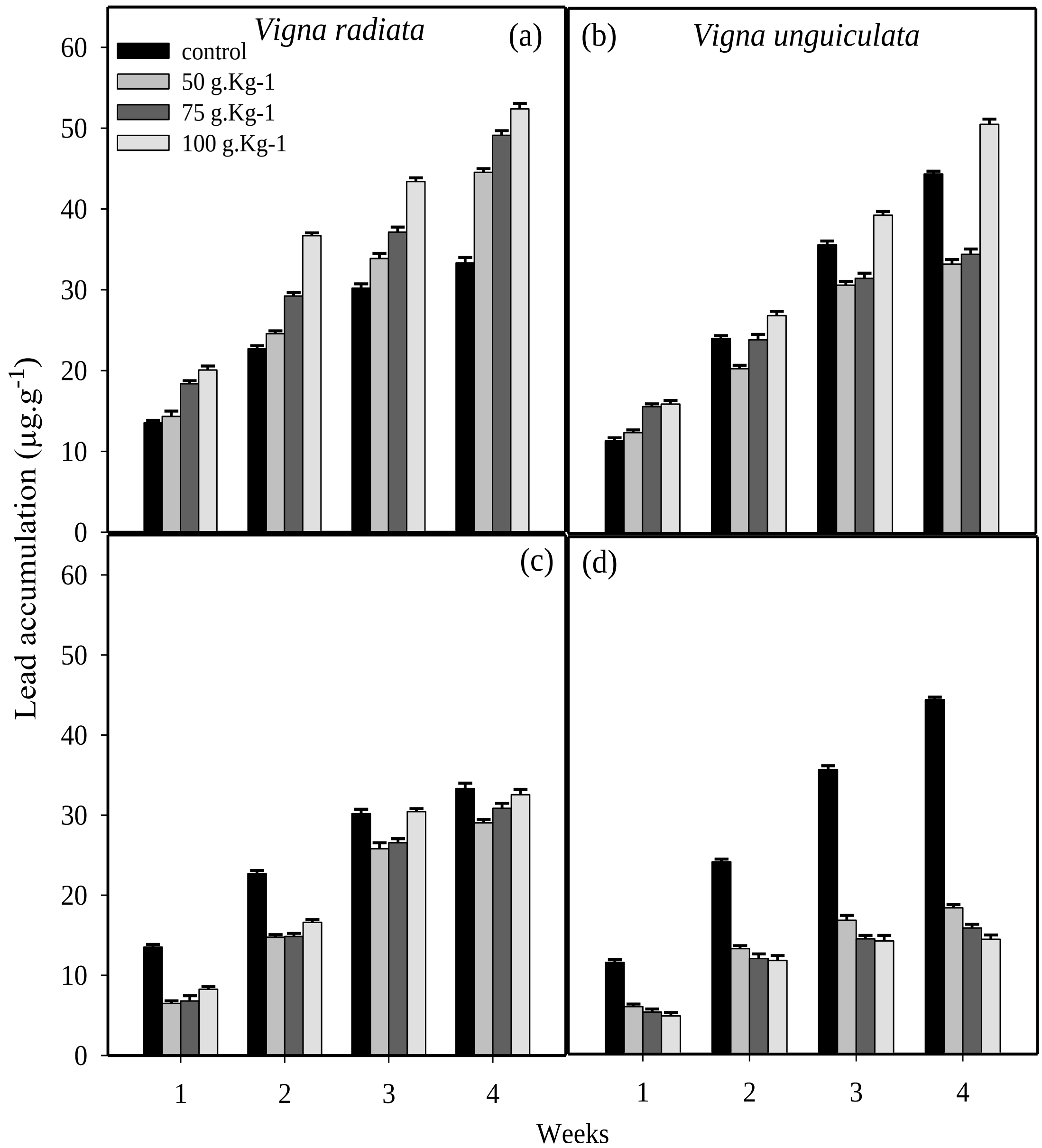

Fig-1: Effect of different concentration of mineral fertilizer (a, b) and chicken manure (c, d) on lead accumulation in leave of *Vigna radiata* and *Vigna unguiculata* grown on lead contaminated soil.

From Fig. 1d and 1c, it is obvious that increasing addition of chicken manure reduces the uptake of lead by *Vigna radiata* and *Vigna unguiculata*. Significant impacts of chicken manure were observed in reducing accumulation of lead in leaves of *Vigna unguiculata* ($P<0.001$) and *Vigna radiata* ($P<0.01$) throughout the experimental period, however highest accumulation of lead was observed in non amended control of *Vigna radiata* and *Vigna unguiculata*. Result suggested that chicken manure addition was very effective in reducing the lead uptake by plants. In *Vigna unguiculata* (Fig. 1d) lead accumulation was reduces with increasing addition of chicken manure however uneven trend of heavy metal concentration in leaves of *Vigna radiata* was observed (Fig. 1c).

4.3 *Total Chlorophyll*

The results obtained are illustrated in Fig 2; which indicate that chlorophyll content of *Vigna unguiculata* ($P<0.001$) and *Vigna radiata* ($P<0.05$) were significantly affected by mineral fertilizer application. Increasing concentration of applied NPK markedly increased the chlorophyll contents. The stimulating influence of mineral fertilizer amendments on *Vigna radiata* grown in lead polluted soil was uneven; 50g/Kg NPK treatment gave highest chlorophyll content as compared to 75 and 100g/Kg NPK treatments (Fig. 2a). However in *Vigna unguiculata* chlorophyll content increased with increasing addition of mineral fertilizer. In *Vigna unguiculata* increasing trend of chlorophyll content was observed till the end of experimental period. The chlorophyll stimulating influence of mineral fertilizer on *Vigna radiata* and *Vigna unguiculata* grown in lead polluted soil may be due to improvement in availability of the nutrients of polluted soil.

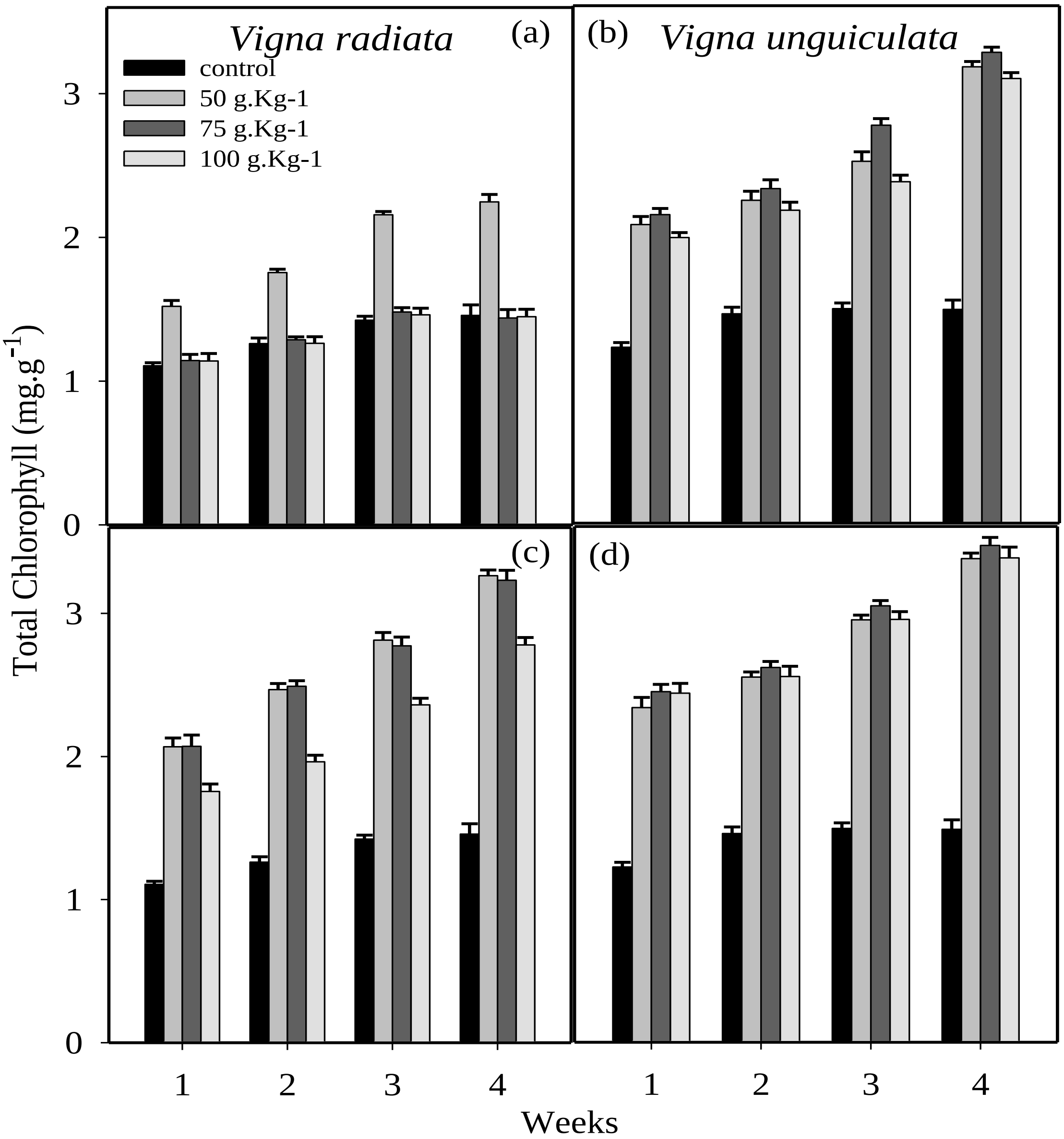

Fig-2: Effect of different concentration of mineral fertilizer (a, b) and chicken manure (c, d) on total carbohydrate content of *Vigna radiata* and *Vigna unguiculata* on grown lead contaminated soil.

Results indicated that chicken manure had stimulating effect on total chlorophyll content of *Vigna radiata* and *Vigna unguiculata* as statistically significant (*Vigna radiata* $P<0.01$ *Vigna unguiculata* $P<0.001$) results were attained. The chlorophyll enhancement in *Vigna radiata* and *Vigna unguiculata* by the application of chicken manure may be due to increased nutrient contents of the lead polluted soil. Data presented in Fig 2 indicated that the chlorophyll content of *Vigna radiata* and *Vigna unguiculata* were markedly increased with increasing concentration of application of chicken manure.

4.4 *Total Protein*

Fig 3 indicates that applied mineral fertilizer levels caused increase in protein content of *Vigna radiata* and *Vigna unguiculata* grown in lead polluted soil. These increases might be due to improvement in nutrient content of the polluted soil after NPK addition. The result obtained for the affect of mineral fertilizer on protein content of *Vigna radiata* and *Vigna unguiculata* were statistically significant (P<0.01).

Results in Fig 3 illustrated that chicken manure had a significant (P<0.01) effects on protein contents of *Vigna radiata* and *Vigna unguiculata*. Increasing concentration of applied chicken manure from 50 to 100g/Kg markedly increased the chlorophyll contents. The stimulating influence of chicken manure amendments on *Vigna radiata* and *Vigna unguiculata* continued till the end of experiment.

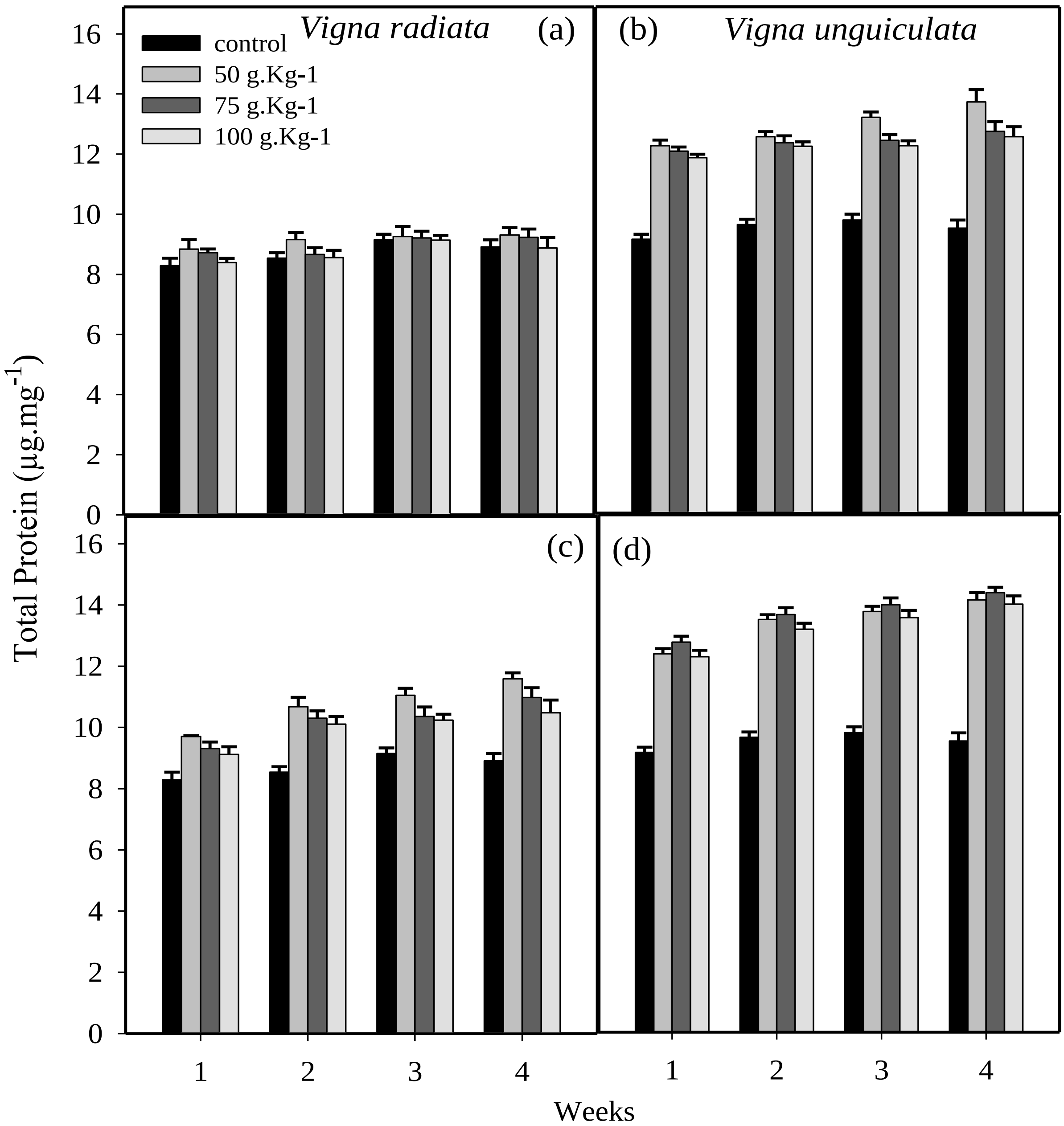

Fig-3: Effect of different concentration of mineral fertilizer (a, b) and chicken manure (c, d) on total protein content of *Vigna radiata* and *Vigna unguiculata* frown on lead contaminated soil.

4.5 *Total Carbohydrates*

Result indicated increase in total carbohydrate content of *Vigna radiata* and *Vigna unguiculata* in all mineral fertilizer treatments as compare to control (Fig. 4), and the results obtained were statistically significant (P<0.01). Mineral fertilizer amendment had stimulating influence on carbohydrate content of both species. Maximum increase in total carbohydrate content of *Vigna radiata* and *Vigna unguiculata* was observed in 50 and 75 g/Kg mineral fertilizer treatment respectively.

Different level of applied chicken manure showed significant (P<0.01) changes in carbohydrate content of *Vigna radiata* and *Vigna unguiculata* which grown in lead contaminated soil. Data presented in Fig. 4 indicated increase in all chicken manure treatment of both species as compare to control. In all chicken manure treatment increased in carbohydrate content continued till the end of experiment and maximum increased was observed in 75g/Kg chicken manure treatment of both species.

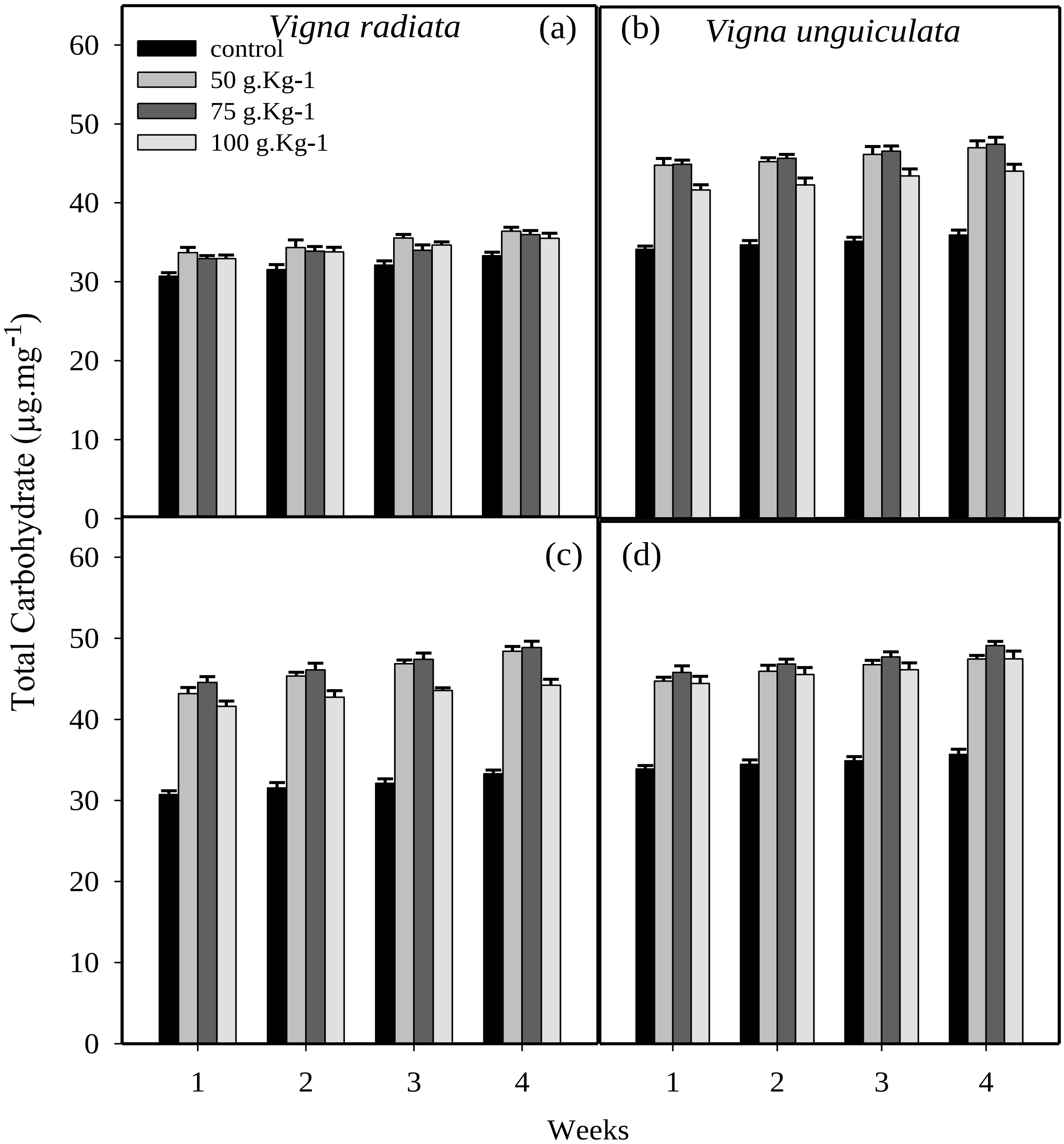

Fig-4: Effect of different concentration of mineral fertilizer (a, b) and chicken manure (c, d) on total carbohydrate content of Vigna radiata and Vigna unguiculata grown on lead contaminated soil.

5. DISCUSSION

The present study demonstrated that the application of chicken manure was very effective in reducing the uptake of lead by plants as compare to inorganic fertilizer. Lead polluted soil amended with fertilizer enhances the plant growth as compare to non-amended Pb polluted soil. Similar results were reported by Rotkittikhun *et al.,* 2007 by the addition to the pig manure in polluted soil thus reducing the uptake of lead by plants while the inorganic fertilizer addition enhanced the uptake of lead by plants. Rahman *et al.,* 2007 also suggested that heavy metal uptake was comparatively higher in inorganic fertilizer treated plants than plants treated with organic fertilizer. Further, many factors such as poor physical structure, low water and nutrient holding capacity, deficiency of major nutrients (N, P, K), acidity and alkaline, water supply, toxic materials, salinity, stability, surface temperature are known to affect plant establishment on polluted soil (Bradshaw and Chadwick, 1980). The uptake of heavy metals by plants from soils is affected by management factors like application of organic fertilizers, despite the fact that organic fertilizers themselves can be a source for heavy metals, nevertheless organic matter added with manures, compost, and sludge's greatly increases soil physical features like colloidal stability and cation exchange capacity and chemical features like plant available nutrients and chelating compounds which have a beneficial effect on soil biology (Gaj *et al.,* 2006). Decomposing poultry manure slowly releases the nutrients into the soil thus reducing the nutrient loss (Ramadan *et al.,* 2007). Organic matter improves soil structure, reduces erosion, increases infiltration, in addition organic amendments also help to immediately decrease metal bioavailability and provide a slow-release fertilizer (Mendez and Maier, 2008). NPK and organic material of domestic refuse could improve the poor physical properties and microbial activities of the polluted soil although reduced heavy metal toxicity to plants by complexing (Wong and Lau, 1985). Organic matter amendment reduced the heavy metal in polluted soil, this may be due to chelation, complexation, adsorption between metals in soils and the organic matter that is contained in organic amendments and also the dilution effect when they are mixed with soils (Chiu *et al.,* 2006). It was suggested that soil had a comparatively large capability for the immobilization of ionic lead and that the precipitation of lead was primarily through the fixation by organic matter (Alloway, 1995). Organic fertilization may affect the transfer of heavy metals to plants by several mechanisms like enhanced metal adsorption through increased surface charge, increased formation of organic and inorganic metal complexes, precipitation of metals, and reduction of metals from higher valency mobile form to lower valency immobile form (Bolan and Duraisamy, 2003). Various reasons could be attributed to the increase in the reduction of heavy metal in the presence of the organic amendments. These include the supply of carbon and protons, and stimulation of microorganisms that are considered to be the major factors for enhancing the reduction of heavy metal (Losi *et al.,* 1994). The oxidation of organic matter releases the proton and lowers the pH of soil, which results in lowering the mobility of heavy metal (Khoshgoftarmanesh and Kalbasi, 2002). Awodun (2007) proposed that in presence of poultry manure the high pH of the soil could be brought closer to neutral. The organic amendments were rich in Nitrogen (part of which was in Ammoniacal form). Oxidation of Ammoniacal N to Nitrate N (nitrification) resulted in the release of protons, which may be one of the reasons for the decrease in soil pH with the addition of the organic amendment. (Bolan *et al.,* 2003) suggested that the organic fertilization improve soils storage capacity of heavy metals by increasing cation exchange capacity and increased concentration of chelating agents, in addition this function acts as a buffer system against heavy metal transfers in ecosystems either by preventing them from leaching or by counteracting their accumulation in plants (Rechcigl, 1995).

Chlorophyll is the central part of the energy manifestation of every green plant system and therefore, any significance alteration in their levels is likely to cause marked effects on the entire metabolism of plants (Knudson *et al.,* 1977). In the present investigation, total chlorophyll content of control plants was more affected as compare to treatments. Present work is supported by the work of Sinha and Gupta, 2005 who suggested that chlorophyll contents of *Sesbania cannabina* increased with an increase in the fly ash amendment, but fly ash amendment enhanced the accumulation of metals (Fe, Mn, Zn, Cu, Pb and Ni). Lead inhibits the chlorophyll synthesis by impairing uptake of essential elements such as Mg and Fe by plants (Burzynski, 1987). Negative effect on chlorophyll development by heavy metals may be due to the interference to proteins (enzymatic proteins) responsible for chlorophyll biosynthesis (Jaleel *et al.,* 2009). It has also been proposed that plants exposed to lead ions showed a decline in the photosynthetic rate as a result of distorted chloroplast, restrained synthesis of chlorophyll, obstructed electron transport (Sharma and Dubey, 2005). Tawfik, (2008) suggested that heavy metal could accumulate to higher levels in the aerial part preferentially in the chloroplast and disturb the enzyme of chlorophyll biosynthesis by interacting with SH group of chloroplast synthesizing enzyme. The key enzyme of chlorophyll biosynthesis, d-amino laevulinate dehydrogenase, is strongly inhibited by lead ions (Prassad and Prassad, 1987). An enhancement of chlorophyll degradation occurs in lead treated plants due to increased chlorophylase activity (Drazkiewicz, 1994). The results indicate that in *Vigna unguiculata* and *Vigna radiata* chlorophyll content increase with fertilizer application. The significant impact of nutrient sources (organic and inorganic fertilizers) was observed on chlorophyll content of plant (Amujoyegbe *et al.,* 2007).

In the present work, protein content of *Vigna radiata* and *Vigna unguiculata* was increased by increasing the levels of chicken manure and mineral fertilizer amendments compared to non amended control. Increase in protein content of *Sesbania cannabina* was observed with increasing levels of the fly ash amendment (Sinha and Gupta, 2005). The decrease in protein content in control samples may be due to enhanced protein degradation process as a result of increased protease activity that is found increased under stress conditions (Palma *et al.,* 2002). Increased proteolytic activities in response to heavy metals were found by Lee *et al.,* (1976) who observed increased activities of hydrolytic enzyme of soybean leaves in response to heavy metal stress. It is also possible that these heavy metals may

have induced lipid per oxidation in fragmentation of proteins due to toxic effects of reactive oxygen species leading to reduce protein content (Davies *et al.,* 1987). The application of both organic and inorganic fertilizer stimulated the protein content of plants by improving nutrient status of the soil terms of both physical and biological characteristic (Zeidan, 2007).

In present study, carbohydrate content of control showed decrease as compare to fertilizer treatment. Sugars considered key metabolites in plant metabolism, not only because the first complex organic compounds formed in plants as the result of photosynthesis, but also provides a major source of respiratory energy (Deef, 2007). Sugars also play a number of ecological roles in plant protection against wounds and infection as well as in the detoxification of foreign substances (Sativir *et al.*, 2000). Carbohydrates perform important hormone-like functions as primary messengers due to their essential role in plant growth, development and metabolic links with the initial physiological processes (Rolland *et al.*, 2002). Warrier and Saroja (2008), found reduction in carbohydrate content as the result of heavy metal pollution and suggested this decline might be the result of decreased photosynthesis. Our result corroborated with finding of Ahmed (1978), who found that treatment of plant with lead increased respiration rates of its organ and reduced the photosynthetic rates. The negative effect of heavy metals on carbon metabolism is a result of their possible interaction with the reactive centre of ribulosebisphosphate carboxylase (Stiborova *et al.*, 1987). The results indicate increase in carbohydrate content of *Vigna unguiculata* and *Vigna radiata* which grown in fertilizer amended soil. Fertilization of soil affects the photosynthesis and controls plant development, possibly through carbohydrate metabolism which is involved in other important plant signaling and regulatory pathways (Mcintyre, 2001).

6. CONCLUSION

The study showed that applications of both chicken manure and mineral fertilizer to the lead contaminated soil improve the growth, protein, chlorophyll and carbohydrate contents of leaves of both *Vigna* species. Application of chicken manure and mineral fertilizer decreases the lead uptake by *Vigna unguiculata* and *Vigna radiata* indicated that application of chicken manure and mineral fertilizer used for successful establishment of plants in heavy metal polluted soil. Knowledge obtained in present investigation showed that chicken manure seem to be well suited for *Vigna unguiculata* and *Vigna radiata* cultivation in lead polluted soil since it reduces the lead uptake and improves the nutritive values as compare to mineral fertilizer.

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