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EFFECTS OF PHOTOSYNTHETIC PIGMENTS OF *VIGNA RADIATA* L. & *VIGNA UNGUICULATA* L. CULTIVATED IN LEAD CONTAMINATED SOIL AMENDED WITH CHICKEN MANURE AND MINERAL FERTILIZER

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ABSTRACT

Vigna radiata and *Vigna unguiculata* plants were grown in lead (Pb) contaminated soil which is amended with different concentrations of organic fertilizer (chicken manure) and mineral fertilizer (NPK). In present investigation polluted soil was from industrial area of Karachi with elevated Pb content. The concentration of photosynthetic pigments that is chlorophyll “a”, “b” and carotenoides in leaf of developing plants were monitored every one week interval up to the period of 4 weeks. Application of both fertilizer improve the concentration of chlorophyll “a”, “b” and carotenoids, with time. In control of both *Vigna radiata* and *Vigna unguiculata,* chlorophyll “a”, “b” and carotenoids concentration was reduces, such redution in the concentration of leaf pigment due to the toxic effect of metals (Pb).

Key words: Carotenoides, Chlorophyll “a”, Chlorophyll “b”, Chicken manure, Mineral fertilizer, Metal polluted soil, *Vigna radiata*, *Vigna unguiculata*.

INTRODUCTION

Heavy metal contamination of soil, water and air has caused severe environmental hazard in the biosphere due to rapid industrialization and urbanization (Xiong, 1998). Heavy metals are a dangerous group of soil pollutants, the contamination by heavy metals causes a serious problem because they cannot be naturally degraded like organic pollutants and they accumulate in food chain (Smejkalova *et al*., 2003). Among the existing heavy metal pollutants, lead (Pb) is the major contaminant of the soil (Gratao *et al*., 2005; Romerio *et al*., 2006). Significant increases in the Pb content of cultivated soils have been observed near industrial areas (De Abreu *et al*., 1998; Mishra and Choudhuri, 1999; Sharma and Dubey, 2005).

Contamination of soil by lead enhances plant uptake and causing their accumulation in different plants organ (Ernst, 1996; Zayed *et al*., 1998; Gimmler *et al*., 2002; Chaturvedi, 2004; Gaspar *et al*., 2005). Lead and other heavy metal have no known biological functions (Antosiewicz, 1992; Xiong, 1998 Nicholls and Mal, 2003). In plants, the exposure to toxic concentration of lead causes oxidative stress, growth inhibition, chlorosis, browning of root tips, disturbance of photosynthesis, reduction of water and nutrient uptake and inhibition of enzymatic activities of various enzyme (Sharma and Dubey, 2005).

Soil improvement is the most important step for successful establishment of plants in heavy metal polluted soil. Addition of organic manures (Cow manure, Poultry manure and Pig manure) are well established to be involved in fertilization of plants due to its beneficial effect on physical, chemical and biological characteristics of the soil, this in turns, influences growth and increase production of plant (Scialdone *et al*., 1980; Wong and Lau, 1985; Ye *et al*., 1999).

The main objective of this investigation was to study the effect of heavy metal polluted soil which is amended with different concentration of chicken manure and mineral fertilizer (NPK) by moitoring the concentration of photosynthetic pigment Chlorophyll “a”, “b” and carotenoides in leaves of Vigna *radiata* and *Vigna unguiculata*.

MATERIAL AND METHODS

A pot Experiment was conducted with 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 rinsing with tap and distilled water. Seeds of *Vigna unguiculata* and *Vigna radiata* were sown in plastic begs each containing 300gm sterilized soil. After germination 12 days old seedlings were transplanted in plastic pots (12cm diameter and 15cm height) contains lead polluted soil (polluted soil collected from industrial area of Karachi), Soil was mixed with three levels of chicken manure and mineral fertilizer (50, 75 and 100 g/Kg). Soil without NPK and chicken manure served as the control. Regular watering was carried, plants watered according to requirement throughout experimental period.

Samples of soil, mineral fertilizer and chicken manure were analyzed for total lead by atomic absorption spectrophotometer following the procedure use by the Swiss national soil monitoring network (Nowack *et al*. 2001).

The leaf samples from both control and treated plants were collected in early hours of the morning and were kept in labeled sample bags. The plants samples were analyzed for following leaf pigments.

Chlorophyll ("a" and "b") and carotenoids were extracted from the leaves and estimated by the method of Maclachlam and Zalik (1963). Data were statistically analyzed by "SPSS" program and "SIGMA PLOT" program was used for graphic presentation of the data.

RESULTS

The polluted soil contains higher lead (Pb) content (245.260µg/mg) as compare to chicken manure (53.760µg/mg) and mineral fertilizer (32.910µg/mg).

The data in table 1 illustrate that chicken manure and mineral fertilizer treatment has positive effect on the amount of chlorophyll “a” of both *Vigna radiata* and *Vigna unguiculata* as compared to control. In all chicken manure treatment of *Vigna radiata* and *Vigna unguiculata* significant ($P<0.01$) increase in the amount of chlorophyll “a” was observed through out the experiment (Fig. 1a & 1b). In *Vigna radiata* amount of chlorophyll “a” increases with the increased intensity of applied chicken manure, however, uneven increasing trend was observed in *Vigna unguiculata*. In 1st week of investigation decrease in the amount of chlorophyll “a” of *Vigna radiata* was observed after treatment with different concentration of mineral fertilizer as compare to control (Fig. 1c), afterward significant ($P<0.05$) increase was observed till the end of investigation period, on the other hand mineral fertilizer treatment of *Vigna unguiculata* showed significant ($P<0.01$) increase in the amount of chlorophyll “a” (Fig. 1d), which is continued till the end of experiment.

From Table 2 it is obvious that Significant increase in amount of chlorophyll “b” of *Vigna radiata* and *Vigna unguiculata* ($P<0.01$) was observed throughout the investigational period. The amount of chlorophyll “b” of *Vigna unguiculata* and *Vigna radiata* showed a sharp increase with the increase in the concentration of chicken manure supplied. In both species the high increase in chlorophyll “b” content was observed in chicken manure treatments as compare to control (Fig. 2a & 2b). The chlorophyll “b” content of *Vigna unguiculata* treated with mineral fertilizer elucidates increase which was higher than control (Fig. 2c) and *Vigna radiata* demonstrate higher increase in control as compare to treatment (Fig. 2d).

The result obtained for the effect of different concentration of chicken manure treatment on carotenoid amount of *Vigna radiata* and *Vigna unguiculata* were statistically significant ($P<0.01$). The carotenoid content of *Vigna radiata* treated with different concentration of chicken manure and mineral fertilizer showed a maximum increase in 50 g/Kg fertilizer treatment (Fig. 3a & 3c) and all fertilizer (chicken manure and mineral fertilizer) treatment of *Vigna unguiculata* display increase in all treatment as compare to control, the result obtained were uneven (Fig. 3b & 3d).

Table 1. Weekly changes in the amount of Chlorophyll “a” (mg/g) of *Vigna unguiculata* and *Vigna radiata* grown on lead contaminated soil under different fertilizer treatments.

species	Treatment	Fertilizer applied (mg/Kg)	Weeks			
			1st	2nd	3rd	**4**th
Vigna radiata	Control	-	1.233±0.025	1.057±0.063	1.033±0.033	0.964±0.061
	Chicken manure**	50	1.259±0.057	1.535±0.033	1.980±0.081	2.105±0.017
		75	1.345±0.061	1.629±0.038	2.016±0.023	2.332±0.067
		100	1.389±0.033	1.826±0.086	2.236±0.063	2.554±0.025
	Mineral fertilizer*	50	1.133±0.051	1.232±0.086	1.328±0.022	1.447±0.089
		75	1.065±0.076	1.156±0.089	1.196±0.061	1.249±0.033
		100	0.941±0.061	0.978±0.036	1.013±0.058	0.939±0.081
Vigna unguiculata	Control	-	1.351±0.061	1.232±0.066	1.061±0.041	1.057±0.026
	Chicken manure**	50	1.488±0.028	1.735±0.091	2.343±0.021	2.736±0.041
		75	1.561±0.032	1.721±0.026	2.372±0.051	2.730±0.066
		100	1.483±0.023	1.622±0.061	2.242±0.091	2.526±0.033
	Mineral fertilizer**	50	1.434±0.081	1.613±0.099	2.169±0.061	2.133±0.057
		75	1.342±0.046	1.611±0.044	2.128±0.066	2.032±0.066
		100	1.300±0.021	1.572±0.063	1.776±0.021	1.842±0.059

Significance level = *$P<0.05$ and **$P<0.01$

Table 2. Weekly changes in the amount of Chlorophyll "b" (mg/g) of *Vigna unguiculata* and *Vigna radiata* grown on lead contaminated soil under different fertilizer treatments.

species	**Treatment**	**Fertilizer applied (mg/Kg)**	**Weeks**			
			1st	2nd	3rd	**4**th
Vigna radiata	Control	-	0.428±0.061	0.449±0.041	0.666±0.022	0.693±0.036
	Chicken manure**	50	0.762±0.073	0.833±0.029	0.809±0.029	0.959±0.055
		75	0.832±0.061	0.868±0.055	0.930±0.023	0.986±0.023
		100	0.916±0.061	0.936±0.026	0.976±0.026	1.059±0.036
	Mineral fertilizer**	50	0.435±0.033	0.458±0.077	0.481±0.026	0.495±0.033
		75	0.414±0.033	0.422±0.066	0.431±0.081	0.476±0.026
		100	0.229±0.054	0.263±0.025	0.341±0.025	0.388±0.041
Vigna unguiculata	Control	-	0.705±0.026	0.762±0.022	0.438±0.038	0.336±0.038
	Chicken manure**	50	0.939±0.022	0.978±0.026	1.145±0.056	1.166±0.028
		75	0.932±0.042	0.993±0.078	1.240±0.086	1.246±0.033
		100	0.922±0.066	0.976±0.047	1.045±0.054	1.133±0.086
	Mineral fertilizer**	50	0.867±0.081	0.932±0.091	0.954±0.026	0.966±0.041
		75	0.852±0.072	0.933±0.043	0.973±0.026	0.976±0.036
		100	0.872±0.088	0.941±0.033	0.977±0.033	0.989±0.089

Significance level = **P<0.01

Table 3. Weekly changes in the amount of Carotenoid (mg/g) of *Vigna unguiculata* and *Vigna radiata* grown on lead contaminated soil under different fertilizer treatments.

species	**Treatment**	**Fertilizer applied (mg/Kg)**	**Weeks**			
			1st	2nd	3rd	**4**th
Vigna radiata	Control	-	0.238±0.036	0.468±0.051	0.423±0.044	0.418±0.038
	Chicken manure**	50	0.386±0.063	0.798±0.036	0.924±0.051	0.971±0.061
		75	0.378±0.021	0.766±0.071	0.826±0.033	0.884±0.088
		100	0.344±0.026	0.742±0.074	0.800±0.023	0.816±0.058
	Mineral fertilizer**	50	0.366±0.033	0.671±0.033	0.773±0.074	0.835±0.066
		75	0.325±0.029	0.647±0.034	0.713±0.043	0.806±0.052
		100	0.317±0.026	0.536±0.055	0.583±0.033	0.681±0.061
Vigna unguiculata	Control	-	0.403±0.022	0.423±0.042	0.451±0.022	0.413±0.063
	Chicken manure**	50	0.669±0.061	1.023±0.026	1.131±0.046	1.326±0.033
		75	0.852±0.044	1.121±0.066	1.286±0.072	1.386±0.046
		100	0.650±0.033	0.819±0.022	1.026±0.036	1.079±0.066
	Mineral fertilizer**	50	0.654±0.022	0.886±0.032	1.084±0.066	1.112±0.033
		75	0.642±0.042	0.812±0.066	0.973±0.054	1.066±0.042
		100	0.526±0.046	0.732±0.081	0.788±0.063	0.986±0.033

Significance level = **P<0.01

DISCUSSION

Lead concentration is increasing rapidly in the environment due to enlarged use of its sources by human society (Haider *et al*., 2006), increasing lead concentration has become a global problem because of its deterious influence not only on plant growth (yield and quality) and environmental quality, but also on the health of human beings, therefore, much effort has been made to decontaminate the polluted soil by using chemical, physical or biological methods (Salt *et al*., 1998).

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). Plant pigments are of tremendous significance in the biosphere, for the reason that the chlorophyll is earth's most important organic molecules as they are necessary for photosynthesis (Blackburn, 2007). Carotenoids are as well essential for plant and mammal survival through their photosynthetic and nutritional functions (Blackburn, 2007).

In the present investigation, chlorophyll "a", chlorophyll "b" and carotenoid content of control samples was more affected as compared to fertilizer treatment. Our work is supported by the work of Sinha and Gupta (2005) who suggested that chlorophyll contents of *Sesbania cannabina* increased with increase in the Fly Ash amendment. Amendments other than fly ash enhance the accumulation of metals (Fe, Mn, Zn, Cu, Pb and Ni).

Lead (Pb) inhibits the chlorophyll synthesis by impairing uptake of essential elements such as Mg and Fe by plants (Burzynski, 1987). Jaleel *et al* (2009) suggested that, impaired chlorophyll development by heavy metals may be due to the interference to proteins (enzymatic proteins) responsible for chlorophyll biosynthesis. Chlorophyll "a" and Chlorophyll "b" content was decreased in two mustard cultivars under heavy metal treatment (Mobin and Khan, 2006). Heavy metal can accumulate in 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 (Tawfik, 2008). The Key enzyme of chlorophyll biosynthesis, d-amino laevulinate dehydrogenase, is strongly inhibited by Pb ions (Prassad and Prassad 1987). An enhancement of chlorophyll degradation occurs in Pb treated plants due to increased chlorophylase activity (Drazkiewicz, 1994).

Carotenoids are a non enzymatic antioxidant, playing vital role in the protection of plant from the adverse impact of reactive oxygen species (Tawfik, 2008). Likewise, Sengar *et al*. suggested that Carotenoids protect chlorophyll from photooxidative destruction and therefore, a reduction in carotenoid could have a serious consequence on chlorophyll pigments, although the total carotenoid content was also slightly reduced by Pb, there was no correlation between Pb concentration and the fall in carotenoid content (Sengar *et al*., 2008).

CONCLUSION

Thus present study clearly indicated that the application of chicken manure and mineral fertilizer was the most effective for stimulating the growth and metabolic processes of plants in lead contaminated soils.

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