

EFFECT OF LEAD NITRATE-PB (NO₃)₂ ON PLANT NUTRITION, AS WELL AS PHYSICAL AND CHEMICAL PARAMETERS ON LOBIA (VIGNA UNGUICULATA LINN. WALP.)

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Abstract: Lead is a major pollutant in both terrestrial and aquatic ecosystem wherein it may adversely affect the faunal and floral health. When accumulated in plants, it is necessary to know the tolerance to lead by plants/parts and Pb-detoxification, phytoremediation and rhizofiltration in polluted soils is the remedy. In the present communication, the effect of lead nitrate on the growth, biomass and plant nutrition of lobiya has been reported and tolerance dose of plants have been found.

INTRODUCTION

Lead is used in various industries and in high concentrations; it is one of the top three heavy metal pollutants of soil, water, air and biosystems with tremendous health consequences (ATSDR, 1993). Lead Nitrate, a colourless oxidizing agent alone finds application in (1) Lead paints (2) Nylon and polystyres as heat stabilizer (3) electrolysis (4) Coating for photographic paper (5) Rodenticides (6) leaching process in gold cyanidation (7) tanning material for leather making (8) floatation for ore (9) making fireworks and matches and (10) as medical astringent (Pracheta, 2008).

Human exposure to lead occurs through drinking water, airborne lead particulates from the exhaust of automobiles and lead-based industrial products. In animal systems, lead pollution can cause irreversible encephalopathy, seizure, coma and even death. Lead fatigue, irritability, memory loss (Ehle et al, 1990), Anaemia (Goyez, 1988), high blood pressure, myocardial infarction (USEPA, 1990), nephropathy (Goyer *et al.*, 1988), gastrointestinal disturbances, weight loss, liver impairment and immunosuppression are other common toxic effects of lead exposure in animals apart from prenatal exposures that may cause birth defects, miscarriage and underdeveloped babies. Biotoxic effects of organic and inorganic forms of lead pollution include CNS, PNS, GIT and other biosystems including cardiovascular system (Duribe *et al.*, 2007).

In plants especially vegetable excess lead accumulation may result in stunted growth, chlorosis, and blackleg of root system etc. (Liu, 1990).

In view of toxic effects of lead on animal and plant systems (Buck, 1970; Miller and Koeppel, 1971; Coper, Wong and Kheifets, 1985; Ehle and McKee, 1990; Liu *et al.*, 1990) and in triggering allergies (Singh and Vats, 2006), it was considered only appropriate to investigate the effects of lead nitrate on nutrition and growth of Lobiya in Meerut. The plant was chosen because (1) cow

pea has desirable agronomic and nutrient traits as food & feed stuffs (2) it has amino acid profile similar to soyabean (Defang, 2008).

MATERIALS AND METHODS

1- Plant material and metal ions used:

Were dry, healthy seeds of *Vigna unguiculata* procured locally and lead nitrate in 5, 10 and 15 ppm concentrations.

2- Root and shoot length analysis:

Thoroughly washed viable seeds were placed on cotton padded filter paper disc in 10 petriplates each with 20 seeds on it. One plate was control while remaining 9 having different concentration of lead nitrate served as treatments. Root and shoot length were measured after 7 days. After recording fresh weight they were kept at 60°C for 2 days, for the measurements of their dry weights. Data were statistically analyzed with repeat to means standard errors range and Response coefficient RC value.

RC estimated toxicity as per following formula:

$$RC = \frac{VT-VC}{VC}$$

Where VT= value of treated set; VC= value of control set.

The negative values for RC's indicated inhibition while positive values indicated stimulation.

RC's values obtained against different concentration were classed as

A = >-0.20 (Tolerant)

B = -0.20 to 0.39

C = -0.40 to -0.59

D = -0.60 to -0.79 (B – D = Partially tolerant).

E = <-0.80 (Non-tolerant)

3- Physical as well as chemical parameters and nutrition:

After washing seeds thoroughly they were soaked in water for 24 hrs before sowing in pots containing soil which was pre-analyzed for soil characterization especially macro nutrients.

Soil samples were huge soil mass and were composite taken up to a depth of 0-15 cm of earth. 500g soil per sample was taken in steel augur samples were labeled. For further processing soil samples were air dried in shed and then was passed via 2mm stainless steel sieve.

3.1- Physical Parameters:

After determining soil texture, electrical conductivity was determined Elico made conductivity meter and pH was determined by the pH meter of same manufacturer company.

3.2- Determination of macronutrients:

Standard procedure were employed for the determination of available nitrogen, soil organic Carbon, available Phosphorus, Potassium and Sulphur as earlier described by Maheshwari, Datta *et al.*, 1962; Walkey and Black, 1934; Olsen *et al.*, 1954; Hanway and Heidel, 1952; Palaskar *et al.*, 1981.

Estimations of these were made using following calculations:

Nitrogen:

1 ml of 0.02N H₂SO₄ = 0.00028g N.

$$\text{N released (mg/kg)} = \frac{(A-B) \times 0.00028}{\text{Wt. of soil sample (g)}} \times 10^6 \times \frac{\text{Vol. of KCl or NaCl (ml)}}{\text{Vol. of leachate/filtrate (ml)}}$$

A and B stands for the net titre values for incubated and un-incubated samples (in aerobic and anaerobic conditions).

Carbon:

$$\text{Organic carbon (\% in soil)} = \frac{10 (B-S)}{B} \times 0.003 \times \frac{100}{\text{Wt. of sample (g)}}$$

B and S Stands for the net titre values (ml) of blank and sample, respectively

Phosphorous:

$$\text{K (kg ha}^{-1}\text{)} = \frac{Q \times V \times 2.24 \times 10^6}{A \times S \times 10^6} = \frac{Q \times V \times 2.24}{A \times S}$$

Q = quantity of P in ug on X-axis

V = volume of extracting reagent used (ml)

S = weight of soil sample (g)

A = volume of aliquot used for colour development (ml).

Potassium:

$$\text{K (kg ha}^{-1}\text{)} = \frac{C \times 25}{5} \times \frac{10^6}{10^6} \times 2.24 = C \times 11.2$$

C stands for the concentration of potassium in the sample obtained on X-axis, against the reading.

Sulphur:

$$\text{S in soil (mg/kg)} = R \times 100 / 10 \times 20 = R/2$$

Where, R stands for the quantity of s in mg as obtained on X-axis against a reading.

Micronutrient (Zn, Cu, Fe, and Mn): Were determined using Atomic absorption spectrophotometer using DTPA extractant. Calculations were made as under:

$$\text{Zn in soil (mg kg}^{-1}\text{)} = \frac{A \times 20}{A \times 2} = 10$$

A stands for the Zn concentration in aliquot as read from X-axis of standard curve against the sample reading.

Atomic Absorption Spectrophotometer is the meathod for other micronutrients using working standards as described by Pracheta (2008).

Layout of Treatment (Lead Nitrate):

Control	Plant		
	T ₁ R ₁	T ₂ R ₁	T ₃ R ₁
	T ₁ R ₂	T ₂ R ₂	T ₃ R ₂
	T ₁ R ₃	T ₂ R ₃	T ₃ R ₃
	5 ppm	10 ppm	15 ppm

- *First treatment:* After 30 DAS.
T₁, T₂, T₃= 2 ppm, 3ppm, 5ppm
- *Second treatment:* after 40 DAS.
T₁, T₂, T₃= 2 ppm, 4 ppm, 6ppm
- *Third treatment:* after 50 DAS.
T₁, T₂, T₃= 1 ppm, 3 ppm, 4ppm

Plant analysis was done by two methods i.e. first for nitrogen analysis (Kieldall method) and second for all macro and micro nutrient analysis.

For nitrogen analysis 0.5g samples and add 10 ml sulphuric acid and 10g mixture of C_uSO₄ and K₂SO₄ taken in (1:10), For P, K and micronutrients analysis, samples after crushing then digest with the help of HClO₄: HNO₃ (9:4).

The other analysis was done as aliquot of soil analysis described earlier.

RESULTS AND DISCUSSION

To distinguish tolerant (T), partially tolerant (PT), and non tolerant (NT) plants against Lead nitrate solution, three

concentrations i.e. 5 ppm, 10 ppm, 15 ppm were used in 1-7 old seedlings and RC's for root, shoot and whole seedlings as well as for fresh and dry weight of root, shoot and whole seedlings were worked out.

Table 1 indicates that the RC values of R_1 shows higher tolerance to LN as compared to R_2 and R_3 . It follows the order of sequence: $R_1 > R_2 > R_3$ in case of root length at 5 ppm conc. or 100 ppm conc. the effect of LN on length R_3 shows higher tolerance. The order being: $R_3 > R_2 > R_1$, while for 15 ppm conc. R_2 shows higher tolerance with the order: $R_2 > R_3 > R_1$. All the treatment have 60% tolerance against LN conc.

In case of shoot length the R_3 shows higher tolerance to R_1 and R_2 with the sequence: $R_3 > R_2 = R_1$. R_1 and R_2 show equal tolerance. For 10 ppm shoot length showed that R_3 shows higher tolerance to LN than R_1 and R_2 with the sequence: $R_3 > R_2 = R_1$. Shoot length against 15 ppm conc. of LN indicates that the R_3 shows higher tolerance to LN with the sequence: $R_3 = R_2 > R_1$. Overall effects of all treatment on shoot show tolerance towards LN.

In case of 7 days old, total of shoot and root length of Lobiya against 5 ppm conc. indicates that R_3 and R_1 shows equal tolerance. While in case of 100 ppm conc. R_3 shows higher tolerance than R_1 and R_2 for 15 ppm conc. R_3 and R_1 shows equally tolerance.

Fresh and dry weight:

Table 2 and 3 show the RC values, respectively show higher tolerance towards different concentrations.

Nutrients before experiments:

In the initial soil samples before experiment the conc. of N, P, K and S was poor whereas the conc. of micronutrient was medium in range. 37.530, 8.080, 34.635 and 5.705 ppm for macronutrients and 2.790 ppm for Mn, 1.495 for Fe, 0.711 for Cu and 0.373 for Zn.

Nutrients analysis of treatments:

The positive RC value of most of the samples Root, shoot and leaves, after the crop maturity indicates tolerance towards lead (Table 4-5)

Table 6, 7 and 8 show that the treatment increasing concentration of the $Pb(NO_3)_2$ simultaneously enhanced the plant growth as well as nutritional composition. Tolerance capacity of lead for the plant and its parts showed no toxicity at crop maturity.

Generated by humans, agricultural, industrial and aerosol sources, lead is well known pollutant in both terrestrial and aquatic ecosystems (Laxen et al, 1977). Upon inhalation or ingestion its exposure may target bones, brain, blood, kidneys and thyroid glands of human being adversely (ATSDR, 1993). Depending upon the level and duration of exposure this heavy metal may contaminate soils and when accumulated may show wide range of biological effects in plants. e.g:(1) it may inhibit photosynthesis, (2) upset mineral nutrition and water balance, (3) Change hormonal status and (4) affect membrane structure and permeability (Liu, 1999) or may accumulate in leaves of *zea mays* or *Brassica juncea* (Martin, 1977).

As far as nutrients are concerned, their balance may be disturbed by Pb accumulation. Needless to say that nitrogen is important constituent of Protein, Phosphorous supplies energy for growth, Potassium activates certain enzymes and regulates stomatal opening, Sulphur is required in all protein, Likewise Molybdenum is required for nodulation, Iron for photosynthesis, Manganese is a cofactor and Cobalt is needed in photosynthesis, NPK are plant mobile nutrients while Co, Zn, Mg, Fe are intermediate in plant mobility. Thus, the data of the present study are significant because (1) Pb-detoxification of heavy metal containing soils, (2) tolerance to Pb by plants and (3) Remediation of Pb containing soils by phytoremediation and rhizofiltration technologies are the remedial measures.

Table – 1

[illegible]

T1R2	R	0.275	+	0.13	0.000	-	2.500	-0.848	T2R2	0.0345	+	0.103	0.000	-	1.200	-0.889	T3R2	0.335	+	0.084	0.000	-	1.000	-0.814
	S	0.050	+	0.034	0.000	-	0.500	-0.868		0.050	+	0.034	0.000	-	0.500	-0.868		0.165	+	0.072	0.000	-	1.000	-0.566
	T	0.325	+	0.163	0.000	-	3.000	-0.851		0.395	+	0.123	0.000	-	1.500	-0.819		0.500	+	0.148	0.000	-	2.000	-0.771
	R	1.025	+	0.303	0.000	-	4.000	-0.688		1.315	+	0.352	0.000	-	5.000	-0.600		1.375	+	0.302	0.000	-	5.000	-0.581
2 day	S	0.285	+	0.124	0.000	-	2.000	-0.657		0.300	+	0.088	0.000	-	1.000	-0.639		0.385	+	0.090	0.000	-	1.000	-0.536
	T	1.310	+	0.420	0.000	-	6.000	-0.682		1.615	+	0.433	0.000	-	6.000	-0.608		1.760	+	0.352	0.000	-	5.000	-0.572
	R	2.615	+	0.566	0.500	-	9.000	-0.399		2.875	+	0.495	0.000	-	6.000	-0.339		4.775	+	0.513	1.000	-	8.000	0.098
3 day	S	0.565	+	0.170	0.000	-	3.000	-0.474		0.800	+	0.143	0.000	-	2.000	-0.256		1.018	+	0.231	0.000	-	3.000	-0.053
	T	3.180	+	0.684	0.500	-	12.000	-0.414		3.675	+	0.611	0.000	-	8.000	-0.323		5.793	+	0.685	1.000	-	11.000	0.068
	R	3.770	+	0.463	1.000	-	9.200	-0.302		4.800	+	0.372	2.000	-	8.000	-0.111		5.740	+	0.498	1.800	-	8.500	0.063
4 day	S	1.040	+	0.189	0.000	-	3.000	-0.235		1.370	+	0.180	0.200	-	3.000	0.007		1.770	+	0.334	0.000	-	4.000	0.301
	T	4.810	+	0.593	1.000	-	11.700	-0.288		6.170	+	0.463	2.500	-	9.000	-0.087		7.510	+	0.775	2.000	-	12.000	0.111
	R	4.975	+	0.406	2.000	-	9.000	-0.095		7.550	+	0.580	3.000	-	13.000	0.374		6.625	+	0.427	2.500	-	10.000	0.206
5 day	S	1.550	+	0.221	0.500	-	4.000	-0.199		2.335	+	0.263	0.200	-	5.000	0.207		3.275	+	0.303	1.000	-	5.500	0.693
	T	6.525	+	0.537	2.500	-	13.000	-0.122		9.885	+	0.685	5.500	-	18.000	0.330		9.900	+	0.488	6.000	-	15.000	0.332
	R	5.925	+	0.564	2.500	-	12.000	-0.033		8.790	+	0.636	3.500	-	13.500	0.434		8050	+	0.461	3.000	-	11.000	0.313
6 day	S	2.575	+	0.206	1.200	-	4.000	-0.300		3.010	+	0.265	0.500	-	5.400	0.134		4.280	+	0.303	2.500	-	7.000	0.612
	T	8.500	+	0.678	3.700	-	16.000	-0.032		11.800	+	0.629	7.500	-	16.500	0.343		12.330	+	0.627	7.000	-	17.000	0.404
	R	6.440	+	0.543	2.800	-	12.200	-0.015		9.255	+	0.574	4.000	-	13.500	0.415		8.755	+	0.432	5.000	-	12.000	0.339
7 day	S	3.675	+	0.253	1.600	-	6.000	-0.032		3.755	+	0.308	1.500	-	5.500	-0.011		5.465	+	0.314	2.800	-	8.000	0.440
	T	10.115	+	0.578	6.500	-	18.000	-0.021		13.010	+	0.693	8.000	-	17.500	0.259		14.220	+	0.587	9.500	-	19.500	0.376
	R	10.115	+	0.578	6.500	-	18.000	-0.021		13.010	+	0.693	8.000	-	17.500	0.259		14.220	+	0.587	9.500	-	19.500	0.376
T1R3	R	0.342	+	0.131	0.000	-	1.500	-0.810	T2R3	0.440	+	0.131	0.000	-	1.500	-0.756	T3R3	0.145	+	0.071	0.000	-	1.000	-0.920
1 day	S	0.083	+	0.042	0.000	-	0.500	-0.781		0.075	+	0.036	0.000	-	0.500	-0.803		0.060	+	0.038	0.000	-	0.700	-0.842
	T	0.421	+	0.159	0.000	-	2.000	-0.807		0.515	+	0.159	0.000	-	2.000	-0.764		0.205	+	0.102	0.000	-	1.700	-0.906
	R	0.974	+	0.225	0.000	-	3.000	-0.704		1.185	+	0.268	0.000	-	3.000	-0.639		1.200	+	0.253	0.000	-	3.000	-0.635
2 day	S	0.221	+	0.067	0.000	-	0.800	-0.734		0.270	+	0.084	0.000	-	1.000	-0.675		0.305	+	0.088	0.000	-	1.000	-0.633
	T	1.195	+	0.286	0.000	-	3.700	-0.710		1.455	+	0.343	0.000	-	4.000	-0.646		1.505	+	0.320	0.000	-	4.000	-0.634
	R	3.368	+	0.379	0.500	-	7.000	-0.226		4.125	+	0.783	0.500	-	10.000	-0.052		3.100	+	0.438	0.500	-	6.500	-0.287
3 day	S	1.121	+	0.181	0.000	-	2.500	0.043		1.820	+	0.253	0.500	-	4.000	0.693		1.975	+	0.265	0.200	-	4.000	0.837
	T	4.489	+	0.466	0.500	-	9.500	-0.172		5.945	+	1.016	1.000	-	14.000	0.096		5.075	+	0.635	1.500	-	10.000	-0.065
	R	5.3.42	+	0.449	1.000	-	9.000	-0.011		6.175	+	0.774	1.000	-	11.500	0.144		5.200	+	0.327	2.000	-	7.500	-0.037
4 day	S	2.132	+	0.292	0.500	-	4.500	0.567		2.425	+	0.210	0.800	-	4.000	0.783		1.805	+	0.258	0.300	-	4.000	0.327
	T	7.474	+	0.627	2.500	-	13.500	0.106		8.600	+	0.937	2.000	-	14.500	0.272		7.005	+	0.468	4.000	-	10.500	0.036
	R	7.868	+	0.353	5.000	-	10.500	0.432		9.725	+	0.642	3.000	-	13.000	0.770		6.770	+	0.449	3.000	-	10.000	0.232
5 day	S	3.000	+	0.274	1.000	-	5.000	0.550		3.275	+	0.273	1.000	-	5.000	0.693		3.580	+	0.342	1.000	-	6.500	0.850
	T	10.868	+	0.569	6.000	-	15.500	0.463		13.000	+	0.769	5.000	-	17.500	0.750		10.350	+	0.673	4.000	-	15.000	0.393
	R	8.616	+	0.313	5.200	-	11.000	0.406		6.675	+	0.713	2.000	-	12.000	0.089		7.585	+	0.424	3.500	-	11.000	0.237
6 day	S	4.084	+	0.241	2.000	-	6.000	0.538		2.535	+	0.218	1.000	-	4.800	-0.045		3.845	+	0.367	1.500	-	7.000	0.448
	T	12.700	+	0.453	9.000	-	17.000	0.446		9.210	+	0.798	3.000	-	16.800	0.048		11.430	+	0.622	6.500	-	17.200	0.301
	R	8.747	+	0.436	5.000	-	12.000	0.338		6.150	+	0.587	1.500	-	9.500	-0.060		8.695	+	0.395	5.000	-	12.000	0.330
7 day	S	4.521	+	0.321	2.200	-	7.000	0.191		3.230	+	0.211	2.000	-	4.500	-0.149		4.545	+	0.378	1.800	-	7.500	0.198
	T	13.268	+	0.549	8.800	-	17.500	0.284		9.380	+	0.761	3.500	-	13.700	-0.092		13.240	+	0.645	8.700	-	18.400	0.281

Table-2: Effect of Lead nitrate on the Fresh weight of Root, Shoot and Res.S. of Lobiya.

	ROOT	RC	SHOOT	RC	Res.s	RC
CONTROL	0.250		1.950		2.570	
T₁R₁	0.560	-0.156	3.730	0.000	2.950	-0.014
T₁R₂	0.380	0.178	2.150	0.372	2.730	0.076
T₁R₃	0.530	0.244	2.950	0.735	2.980	0.065
T₂R₁	0.750	-0.222	3.640	0.316	3.680	0.523
T₂R₂	0.420	-0.067	3.070	0.428	3.650	0.318
T₂R₃	0.350	0.667	2.830	0.693	4.220	0.329
T₃R₁	0.650	-0.267	3.000	0.447	3.380	-0.018
T₃R₂	0.640	0.422	4.250	0.977	3.380	0.220
T₃R₃	0.330	0.444	3.110	0.395	2.720	0.220

Table-3: Effect of Lead nitrate on the Dry weight of Root, Shoot and Res.S. of Lobiya.

	ROOT	RC	SHOOT	RC	Res.s	RC
CONTROL	0.040		0.270		0.660	
T₁R₁	0.230	0.750	0.490	-0.111	0.910	0.439
T₁R₂	0.070	1.000	0.240	0.222	0.950	0.409
T₁R₃	0.080	4.750	0.330	0.815	0.930	0.379
T₂R₁	0.100	1.000	0.440	0.296	1.140	0.803
T₂R₂	0.080	1.250	0.350	0.333	1.190	1.152
T₂R₃	0.090	1.500	0.360	0.630	1.420	0.727
T₃R₁	0.090	1.250	0.450	0.667	1.030	0.561
T₃R₂	0.090	1.250	0.410	0.519	0.740	0.121
T₃R₃	0.190	3.750	0.350	0.296	0.880	0.333

Table-4: Effect of Lead nitrate on the Fresh weight of Root, Shoot and Leaves of Lobiya.

	ROOT	RC	LEAVES	RC	SHOOT	RC
CONTROL	2.470		31.730		10.530	
T₁R₁	3.060	0.239	28.280	-0.109	8.750	-0.169
T₁R₂	6.270	1.538	46.640	0.470	16.250	0.543
T₁R₃	3.980	0.611	26.100	-0.177	10.400	-0.012
T₂R₁	4.320	0.749	21.060	-0.336	8.700	-0.174
T₂R₂	7.450	2.016	50.480	0.591	25.880	1.458
T₂R₃	4.890	0.980	38.230	0.205	13.370	0.270
T₃R₁	5.720	1.316	40.670	0.282	18.710	0.777
T₃R₂	5.610	1.271	34.950	0.101	12.650	0.201
T₃R₃	4.770	0.931	48.400	0.525	15.790	0.500

Table-5: Effect of Lead nitrate on the Dry weight of Root, Shoot and Leaves of Lobiya.

	ROOT	RC	LEAVES	RC	SHOOT	RC
CONTROL	0.550		4.100		1.210	
T₁R₁	1.000	0.818	3.800	-0.073	1.290	0.066
T₁R₂	1.590	1.891	6.540	0.595	2.310	0.909
T₁R₃	1.05	0.909	3.810	-0.071	1.420	0.174
T₂R₁	1.040	0.891	3.140	-0.234	1.220	0.008
T₂R₂	1.840	2.345	7.130	0.739	3.740	2.091
T₂R₃	1.370	1.491	5.200	0.268	1.820	0.504
T₃R₁	1.510	1.745	6.410	0.563	3.310	1.736
T₃R₂	1.450	1.636	4.850	0.183	1.970	0.628
T₃R₃	1.280	1.327	6.780	0.654	2.700	1.231

Table-6: Effect of Lead nitrate on the different nutrient components of Root of Lobiya.

Nutrients	Control	T ₁	T ₂	T ₃
N%	0.110	0.140	0.166	0.187
P%	0.090	0.090	0.120	0.160
K%	0.940	1.200	1.320	1.460
S ppm	0.096	0.720	0.784	0.920
Mn ppm	6.390	9.560	11.640	12.860
Fe ppm	2.860	3.360	9.960	10.240
Cu ppm	3.140	8.000	10.100	12.890
Zn ppm	1.840	1.140	3.460	4.860

Table-7: Effect of Lead nitrate on the different nutrient components of Shoot of Lobiya.

Nutrients	Control	T ₁	T ₂	T ₃
N%	0.220	0.220	0.236	0.277
P%	0.110	0.132	0.144	0.162
K%	0.870	1.140	1.670	1.760
S ppm	0.106	0.220	0.266	0.272
Mn ppm	8.140	24.840	26.320	28.320
Fe ppm	7.120	18.120	22.860	24.000
Cu ppm	6.280	9.100	11.100	10.160
Zn ppm	2.360	8.210	9.240	8.780

Table-8: Effect of Lead nitrate on the different nutrient components of Leaves of Lobiya.

Nutrients	Control	T ₁	T ₂	T ₃
N%	0.360	0.620	0.660	0.700
P%	0.160	0.120	0.130	0.140
K%	0.750	1.090	1.120	0.160
S ppm	0.087	0.220	0.266	0.287
Mn ppm	3.260	19.120	22.070	26.120
Fe ppm	6.540	14.120	16.180	18.710
Cu ppm	4.120	4.000	6.120	8.150
Zn ppm	3.890	5.180	5.190	4.860

REFERENCE

- ATSDR** (Agency for Toxic Substances and Disease Registry, 1993). U.S.P. H.S., Atlanta, GA.
- Buck, W.B.** (1970). Lead and organic pesticide poisoning in cattle. *J. Amer. Vet. Med. Ass.* **156**: 1468.
- Cooper, W.C., Wong, O and Kheifets, L.** (1985). Mortality among employees of lead battery plants and lead-producing plants. *Scand. J. Work Environ. Health* **11**:331-345.
- Datta, N.P.; Khera, M.S. and Saini T.R.** (1962). A rapid colorimetric procedure for the determination of the organic carbon in soils. *J. Indian Soc. Soil Sci.* **10**: 67-74.
- Ehle, A.L. and McKee, D.C.** (1990). Neuropsychological effect of lead in occupationally exposed workers. *Crit. Rev. Toxicol.* **20**:237-255.
- U.S. Environmental protection agency (EPA)** (1986). An addendum to EPA Air Quality Criteria for Lead (1986). In: *Air Quality Criteria for Lead*, Vol. I. Environmental Criteria and Assessment Office, Research Triangle Park.
- Defang, H.F.; Teguio, A.; Awah-Ndukum, Kenfack, A.; Ngoula, F and Mehuge, F.** (2008). Performana and carcas characteristics of broilers fed boiled cow-pee meal diet. *African J. Biotech.* **7**(9):1351-1356.

- Duruibe, J.O., Ogwuegbu, Moc and Egwurugwu, JN** (2007). Heavy metal pollution and human biotoxic effects. *International J. Physical Sciences*, **2**(5): 112-118.
- Goyer, R.A.** (1988) Lead. In: *Handbook on Toxicity of Inorganic Compounds*. H.G. Seiler and H.Sigel, eds. Marcel Dekker, Inc.: New York, pp. 359-382.
- Hanway, J.J. and Heidel, H.** (1952) Soil analyses methods as used in Iowa State College Soil Testing Laboratory. *Iowa Agric.* **57**:1-31.
- Lane, S.D. and Martin, E.S.** (1997). A histochemical investigation of lead uptake in *Raphanus sativus*. *New Phytol.* **79**:281-286.
- Liu, Donghua, A, Wusheng Jianga; Changjun Liua; Changhong, Xina and Wenqiang Hou** (1999). Uptake and accumulation of lead by roots, hypocotyls and shoots of Indian mustard [*Brassica juncea*(L.)] Depart. of Biol., College of Chemistry and Life Sci., Tianjin Normal University, Tianjin 300074, Vol-71, ISSUE3, 2000, 273-277.
- Miller R.J. and Koeppe DE** (1971). Acculation and physiological effect of lead in corn. In: *Proceeding of University of Missouri, Columbia* pp. 186-193.
- Olsen, S.R. Cole.; Watanabe, F.S. and Dean, L.A.** (1954). Estimatio of available phosphorous in soils by extraction with sodium bicarbonate. *Circ. U.S. Dept. Agric.* 939.
- Palaskar, M.S., Babrekar, P.G. and Ghosh, A.B.** (1981). A rapid analytical technique to estimate sulphur in soil and plant extracts. *J.Indian Soc. Soil Sci.* **29**:249-256.
- Pracheta** (2008). To evaluate the effet of lead nitrate on plant nutrition as well as physical and chemical parameters on Lobiya (*V. unguiculata*). M.Sc. Dissertation in Biotechnology M.I.E.T., Meerut.
- Singh, Lokendra and Vats, Preeti** (2006). An update of Aeromycological research in India. *Plant Archives* **6**(2):399-421.
- Walkley, A.J. and Black, I.A.** (1934) Estimation of soil organic carbon by the chromic acid titration method. *Soil sci.* **37**:29-38.