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**RESEARCH ARTICLE**

# Influence of lead on biochemicals and proline contents of *Vigna unguiculata* (L.) Walp

■ **M. KRISHNAVENI, J. SURESH KUMAR AND P.S. SHARVANAN**

**SUMMARY**

Among heavy metals, lead is an element that is easily accumulated in soil and sediments. The level of lead found in plants is often correlated with the level present in the environment. Cowpea [*Vigna unguiculata* (L.) Walp.], a member of Fabaceae, it is an annual multi-purpose grain legume plants suitable in a variety of cropping systems. The present study on ecophysiological effect of lead is undertaken to analyse the influence of ead on biochemicals and proline contents of cowpea [*Vigna unguiculata* (L.) Walp.]. In this study, Co -1 was considered to be more tolerant than other varieties tested. The lead treatment up to 10mg kg $^{-1}$ soil concentration was, however, beneficial for the overall growth parameters**.** From those treatments under lead values were increased at 10mg kg$^{-1}$ lead treatment. The uptake and accumulation of lead in cowpea plants increased with increased of lead level in the soil under field / pot culture experiments. From 25 -200mg kg$^{-1}$ biochemical contents were decreased (except proline).

**Key Words :** Influence, Biochemicals, Proline, *Vigna unguiculata*

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A pollutant is any substance in the environment which causes objectionable effects, impairing the welfare of the environment, reducing the quality of life and may eventually cause death. If present such a substance in the environment beyond a set or tolerance limit, the environment is contaminated. Our environment is affected by a great variety of pollutants. Road laying activity gave different concentrations of the metals *viz.*, lead, copper, zinc, cadmium and nickel increased rapidly within 10 to 50 meters from the roadsides. The level of lead in the environment is currently a great concern. Their high concentrations in soils could be very toxic for plants resulting in different effects on plant physiology (Sandalio *et al.*, 2001). Lead was shown to induce changes in the composition of protein and lipids of red blood cell's membrane (Fukumoto *et al*., 1983) and to inhibit haemoglobin synthesis (Monteiro *et al.*, 1989). Lead occurs naturally in the earth's crust and its natural levels remain below

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50mg $kg^{-1}$ (Arias *et al*., 2010).

Lead occurs naturally in the environment (Motto *et al*., 1970). However, most lead concentrations that are found in the environment are a result of human activities (Davis and Holmes, 1972). Neumann *et al.* (1990) have extended the sources of lead pollution by paints, lead wastes, cell batteries, lead solders and forms. Due to the application of lead in gasoline an unnatural lead cycle has consisted. In car engines lead is burned so, that lead salts (chlorines, bromines and oxides) will originate (Creasen *et al*., 1971) sources. Soil contamination by lead reduces the quality of both soil and cultivated plants which often limits the production of some food products and animal feed.

Lead administrated to potted sugar beet plants at rates of 100 - 200ppm caused chlorosis and growth reduction (Hewilt, 1953). Low amounts of lead (0.005ppm) caused significant reduction in growth of lettuce and carrot roots was reported by Baker (1972). Effect of heavy metal on growth and biochemical characteristic of photosynthesis of barley and maize seedlings were observed by Stiborova *et al.* (1987). The primary cause of cell growth inhibition arises from a lead-induced simulation of indol - 3 acetic acid (IAA) oxidation. Lead is also known to affect photosynthesis by inhibiting activity of carboxylating enzymes. High level of lead also causes inhibition of some enzyme activities were studied by Sinha *et al.* (1988).

The effect of lead and cadmium on the bio-chemical changes in the leaves of *Vigna* and *Hydrilla* was reported by Battacharyya and Choudhuri (1994). Bhattacharjee and Mukherjee (1994) analyzed the influence of cadmium and lead on germination behaviour, protein and proline content, protease activity, cell injury, pigment, sugar, nucleic acid content and peroxidase activity of *Vigna unguiculata*.

Sharavanan *et al.* (2007) studied the effect of cadmium on germination, seedling growth and biochemical changes of cowpea. Influence of heavy metals like cadmium and lead on growth, biochemical parameters and uptake in *Lemna polyrrhiza* were studied by John *et al.* (2008). Effects of lead on the structure and function of photosystem II of *Spirodela polyrrhiza.* Heavy metal toxicity and its effect on plant growth, biochemical parameters and metal accumulation by *Brassica juncea* were examined John *et al.* (2009).

Proteins play a major role in the tolerance of the plant to lead. In contrast, low concentrations of lead increased total protein content. However, certain amino acids, like proline increase under lead stress was observed by Qureshi *et al.* (2007). Effects of heavy metal on chlorophyll, proline, protein and abscisic acid level of sunflower (*Helianthus annus* L.). This protein accumulation may defind the plant against lead stress (Gupta *et al.*, 2010), particularly for proteins involved in cell redox maintenance. Mundra and Bhati (1994) reported the effect of iron, manganese and *Rhizobium*, inoculation on growth, nodulation, iron, manganese ratio and protein contents of cowpea. The effect of sulphur and zinc on protein and methionine contents of chick pea (Tripathi *et al.*, 1999); rice (Hsu and Kao, 2003) were reported the changes in protein and amino acids contents in two cultivars of rice seedlings with different level of cadmium. Effect of high concentrations of lead may decrease the total of protein content was observed by Chatterjee *et al.* (2004).

Melnichuk *et al.* (1982) observed effect of cadmium on free amino acid content in germs of pea seeds at early germination stages. The effects of copper and cadmium on the uptake and leakage of potassium ion in *Betula pendula* roots were investigated by Gussarsson and Jenson (1992). Costa and Morel (1994) were analysed the effect of cadmium on gas exchange and amino acid contents of lettuce. Mundra and Bhati (1994) reported the effect of iron, manganese and *Rhizobium* inoculation on growth, nodulation, iron manganese ratio and protein content of cowpea. The water relations, gas exchange and amino acid contents in cadmium treated lettuce were registered by Costa and Morel (1994).

Bassi and Sharma (1993) observed proline accumulation in wheat seedlings exposed to heavy metals (zinc and copper). Proline is known to play a role in the detoxification of active oxygen in plants under heavy metal stress. Accumulation of proline may be due to increased synthesis from glutamate, lower rate of protein oxidation and slowed incorporation of proline in to protein stated by Bohnert *et al.* (1995). Accumulation of proline has been observed in various plant species subjected to heavy metal stress (Shah and Dubey, 1998). Chen *et al.* (2001) analysed regulation of proline accumulation in detached rice leaves exposed to excess copper. Effects of copper on chlorophyll, proline, protein and abscisic acid level of sunflower (*Helianthus annuus* L.) seedlings were discussed by Zengin and Kirbag (2007). Schat *et al.* (1997) investigated heavy metal-induced accumulation of free proline in a metal tolerant and a non-tolerant ecotype of *Silene vulgaris* plants. Shafi Tantrey and Agnihotri (2010) analysed chlorophyll and

proline content of gram (*Cicer arietinum* L.) under cadmium and mercury treatments. Lead tolerance of certain legume species grown on lead or tailing studied by Sudhakar *et al.* (1992).

## MATERIAL AND METHODS

### Material :

The phytotoxicity of lead acetate was examined through our selected legume cowpea [*Vigna unguiculata* (L.) Walp.] (Family : Fabaceae). In our study we chosen CO -1 variety and the seeds were procured from National Pulses Research Centre, Vamban, Pudukottai district, Tamil Nadu, India. The duration of the crop was 120 days. Seeds of uniform size, colour and weight were chosen for the experiments.

### Methods :

*Pot culture experiment* :

Plants were grown in pots in untreated soil (control) and in soil to which lead acetate had been applied (0, 10, 25, 50, 75, 100 and 200mg $kg^{-1}$ soil). The inner surface of pots were lined with a polythene sheet. Each pot contained 3kg of air dried soil. Lead acetate ($PbCH_3COO$) was finely powdered, applied to the soil and mixed thoroughly. Twenty five seeds were sown in each pot. All pots were watered to filled capacity twice a day. Plants were thinned to a maximum of five per pot after a week of germination. The treatments were replicated five times.

### Sampling :

Plant samples were collected at random, at regular intervals (20, 40, 60, 80, 100 and 120$^{th}$ day) and used for biochemical, and proline contents of cowpea. Five plants from each replicate of a pot were analyzed for its various parameters and the average was calculated. These mean values of the replicates were used for statistical analysis.

### Biochemical analysis :

Total sugars (Nelson, 1944) 2g of fresh leaf materials were plunged in boiling ethanol and allowed to boil for 5 to 10 minutes. Five ml of alcohol was used for every gram of leaf tissue. The extract was cooled and then the tissue was crushed thoroughly in a mortar with pestle. The residue was again reextracted with 80 per cent alcohol, filtered, combined and the volume was adjusted (Loomis and Shull, 1937).

### Hydrolysis :

1ml of ethanol extract was taken in a test tube and evaporated to dryness. To the residue, 1ml of distilled water and 1ml of 1N sulphuric acid was added and incubated at 49°C for 30 min. The solution was neutralized with 1N NaOH using methyl red indicator.

### Estimation :

1ml of Nelson's reagent was added to each tube prepared by mixing reagent A and B in the ratio 25 : 1 (Reagent A : 25g sodium carbonate, 25g sodium potassium tartarate, 20g sodium bicarbonate and 200g an hydrous sodium sulphate dissolved in 1000ml ; reagent B: 15g of cupric sulphate in 100ml of distilled water with 2 drops of concentrated sulphuric acid). The test tubes were heated for 20 min in a boiling water bath, cooled and 1ml of arseno - molybdate reagent (25g ammonium molybdate, 21ml concentrated sulphuric acid, 5g of sodium arsenate dissolved in 475ml of distilled water and incubated at 37°C in a water bath for 48 hr) was added. The solution was thoroughly mixed and diluted to 25ml and read at 495nm in a spectrophotometer. The sugar content of unknown samples were calculated from glucose standard.

### Starch (Summer and Somers, 1949) :

The ethanol insoluble residues taken from ethanol extraction were dried at 60°C for 48 hour in an oven. 3ml of 6N HCl was added to 200mg of the powdered residue and autoclaved at 100°C for an hour. The flask was cooled and the volume was raised to 25ml with distilled water. 1ml of aliquot was withdrawn, neutralized with 1N NaOH and sugar was estimated by Nelson (1944) method. The amount of starch was arrived by multiplying the sugar by the factor 0.9.

### Amino acids (Moore and Stein,1948) :

Ethanol extract of leaf tissues was made as mentioned in the case of sugars. One ml of ethanol extract was taken in 25ml test tube and neutralized with 0.1N NaOH using methyl red indicator. 1ml of ninhydrin reagent was added (800mg stannous chloride in 500ml citrate buffer, pH - 5.0; 20g ninhydrin in 500ml methyl cellosolve, both solutions were mixed). The contents were boiled in a water bath for 20 min, 5ml of diluent solution (distilled water and n - propanol mixed in equal volume) was added, cooled and made upto 25ml with distilled water. The absorbance was measured at 570nm

in a spectrophotometer. The standard graph was prepared using leucine.

**Proline (Bates *et al.*, 1973) :**

500mg of plant tissue was homogenized in 10.0ml of 3 per cent aqueous sulphosalicylic acid. The homogenate was filtered through Whatman number 42 filter paper. 3ml of acid ninhydrin (1.25g ninhydrin in 30ml of glacial acetic acid and 20ml of 6M phosphoric acid) and 2ml of glacial acetic acid in a test tube was heated for an hour at 100 $^0$C. The reaction mixture was extracted with 4ml of toluene and mixed vigorously by using a vortex mixture for 15 - 20 sec. The chromophore containing toluene was aspirated from the aqueous phase. The absorbance of the toluene layer was measured in a spectrophotometer at 520nm using toluene as blank.

**Protein (Lowry *et al.*, 1951) :**

Fresh tissue weighing 0.5g was macerated in 20 per cent trichloroacetic acid using mortar and pestle. The homogenate was then centrifuged at 600rpm for 30 min and the supernatant was discarded. 5ml of 0.1N NaOH was added to the pellet and it was centrifuged for 30 min. The supernatant was saved for the estimation of protein.

To 0.5ml of the extract, 5ml of copper reagent 'C' was added (Reagent C : mixture of reagents 'A' and 'B' in the 50 : 1 ratio. Reagent A : 2 % $Na_2CO_3$ in 0.1N NaOH; Reagent B: equal volume of 1 % $CuSO_4$ and 2 % sodium potassium tartarate). The tubes were shaken well and allowed to stand in dark for 10 min at room temperature, 0.5ml of properly diluted Folin Ciocalteau reagent was added to the solution and mixed thoroughly. The absorbance was read at 550nm in a spectrophotometer against an appropriate blank. Bovin serum albumin was used as the standard.

**Statistical analysis :**

The statistical analysis of experimental results were carried out by standard deviation. In order to analyze the data statistical tool such as ANOVA (Analysis of Variance) was used. Standard deviation calculated by following methods of O'Brien (1981) and Neter *et al.* (1990).

## RESULTS AND DISCUSSION

Cowpea [*Vigna unguiculata* (L.) Walp.] belongs to Fabaceae family is an annual legume. The phytotoxicity of lead acetate was examined in present investigation. The contamination of soil and water with lead mostly results from human activities. Various industries, such as mining and smelting, use of lead in paints, gasoline and linings have resulted in its spreading all over the world. Lead is extremely toxic to all intermediates of food chains (Piechalak *et al.*, 2002). In this study, Co -1 was considered to be more tolerant than other varieties tested. The lead treatment up to 10mg $kg^{-1}$ soil concentration was, however, beneficial for the overall growth parameters**.**

Impact of different treatment of lead on total sugar content (mg/$g^{-1}$/fresh weight) of CO -1 on different days (20, 40, 60, 80, 100 and 120) are presented in Table 1. The highest values of total sugar content of CO -1 (5.427, 9.512, 11.848, 12.187, 9.007 and 6.698, respectively)

**Table 1 : Impact of lead on total sugar content (mg/$g^{-1}$/fresh weight) of cowpea variety CO -1**

| Lead treatments (mg $kg^{-1}$ soil) | Sampling days | | | | | |
|---|---|---|---|---|---|---|
| | 20 | 40 | 60 | 80 | 100 | 120 |
| Control | 5.109 | 9.035 | 11.058 | 11.024 | 8.551 | 6.285 |
| 10 | 5.427 (+5.89) | 9.512 (+5.06) | 11.848 (+6.66) | 12.187 (+9.56) | 9.007 (+5.05) | 6.698 (+6.24) |
| 25 | 4.817 (-6.02) | 7.431 (-17.71) | 9.260 (-16.25) | 10.813 (-1.91) | 8.035 (-6.04) | 5.994 (4.55) |
| 50 | 4.566 (-10.59) | 6.923 (-23.33) | 8.425 (-23.81) | 10.529 (-4.49) | 7.519 (-12.07) | 5.674 (-9.64) |
| 75 | 4.182 (-18.08) | 6.276 (-30.48) | 7.644 (-30.88) | 10.182 (-7.63) | 6.694 (-21.72) | 5.169 (-17.69) |
| 100 | 3.775 (-26.08) | 5.613 (-37.84) | 7.031 (-36.41) | 9.514 (-13.69) | 6.262 (-26.77) | 4.757 (-24.25) |
| 200 | 3.212 (-37.08) | 4.816 (-46.67) | 6.969 (-39.43) | 8.095 (-26.56) | 5.457 (-36.19) | 3.895 (-37.99) |

* Per cent over control values are given in the parentheses

ANOVA

| Source of Variation | SS | df | MS | F | P-value | F crit |
|---|---|---|---|---|---|---|
| Rows | 61.06826 | 6 | 10.17804 | 37.474 | 1.2412 | 2.420523 |
| Columns | 162.5005 | 5 | 32.50009 | 119.6604 | 7.0319 | 2.533555 |
| Error | 8.148084 | 30 | 0.271603 | | | |
| Total | 231.7168 | 41 | | | | |

were observed at 10mg $kg^{-1}$ lead treatment in above said days. The lowest values of total sugar content of cowpea was observed (3.212, 4.816, 6.969, 8.095, 5.457 and 3.895, respectively) at 200mg $kg^{-1}$ lead treatment.

Impact of different treatment of lead on starch content (mg/$g^{-1}$/fresh weight) of CO -1 on different days (20, 40, 60, 80, 100 and 120) is presented in Table 2. The highest values of total starch content of CO -1 (5.244, 6.119, 7.548, 9.233, 7.244 and 5.224, respectively) were observed at 10mg $kg^{-1}$ lead treatment in above said days. The lowest values of total starch content of cowpea was observed (2.217, 2.872, 4.632, 6.647, 2.327 and 2.341, respectively) at 200mg $kg^{-1}$ lead treatment.

Changes of different treatment of lead on amino acid content (mg/$g^{-1}$/fresh weight) of CO -1 on different days (20, 40, 60, 80, 100 and 120) is presented in Table 3. The highest values of amino acid content of CO -1 (4.523, 5.576, 7.565, 8.674, 6.677 and 5.725, respectively) was observed at 10mg $kg^{-1}$ lead treatment in above said days. The lowest values of amino acid content of cowpea was observed (1.185, 1.287, 3.722, 5.059, 3.300 and 3.155, respectively) at 200mg $kg^{-1}$ lead treatment. The higher amount of amino acid contents of cowpea seedlings under various lead treatment in all sampling days was observed in 10mg $kg^{-1}$soil. The amino acid content decreased gradually with progressive increase of lead treatment (from 25mg $kg^{-1}$ soil). The

**Table 2 : Effect of lead on starch content (mg/$g^{-1}$/fresh weight) of cowpea variety CO -1**

| Lead treatments (mg $kg^{-1}$ soil) | Sampling days | | | | | |
|---|---|---|---|---|---|---|
| | 20 | 40 | 60 | 80 | 100 | 120 |
| Control | 4.734 | 5.528 | 6.924 | 8.895 | 6.869 | 4.798 |
| 10 | 5.244 (+9.78) | 6.119 (+9.67) | 7.548 (+8.25) | 9.233 (+3.66) | 7.244 (+5.17) | 5.224 (+8.12) |
| 25 | 4.565 (-3.50) | 5.248 (-5.02) | 6.798 (-1.83) | 8.645 (-2.81) | 6.786 (-1.22) | 4.428 (-7.70) |
| 50 | 4.224 (-10.73) | 5.102 (-7.68) | 6.432 (-7.11) | 8.243 (-7.32) | 6.392 (-6.94) | 4.234 (-11.77) |
| 75 | 3.538 (-25.21) | 4.429 (-19.86) | 5.824 (-15.91) | 7.792 (-12.41) | 5.679 (-17.32) | 3.863 (-19.48) |
| 100 | 2.825 (-40.30) | 3.569 (-35.42) | 5.123 (-26.02) | 7.122 (-19.93) | 3.912 (-43.04) | 3.123 (-34.92) |
| 200 | 2.217 (-53.13) | 2.872 (-48.01) | 4.632 (-33.11) | 6.647 (-25.26) | 2.327 (-66.13) | 2.341 (-51.19) |

* Per cent over control values are given in the parentheses

ANOVA

| Source of variation | SS | df | MS | F | P-value | F crit |
|---|---|---|---|---|---|---|
| Rows | 48.90785 | 6 | 8.151308 | 63.78842 | 9.8616 | 2.420523 |
| Columns | 87.69622 | 5 | 17.53924 | 137.2541 | 9.9320 | 2.533555 |
| Error | 3.833599 | 30 | 0.127787 | | | |
| Total | 140.4377 | 41 | | | | |

**Table 3 : Changes of amino acid contents (mg/$g^{-1}$/fresh weight) of cowpea variety CO -1as affected by lead treatment**

| Lead treatments (mg $kg^{-1}$ soil) | Sampling days | | | | | |
|---|---|---|---|---|---|---|
| | 20 | 40 | 60 | 80 | 100 | 120 |
| Control | 3.292 | 4.312 | 6.283 | 7.463 | 5.352 | 4.594 |
| 10 | 4.523 (+27.19) | 5.576 (+22.66) | 7.565 (+16.95) | 8.674 (+13.96) | 6.677 (+19.85) | 5.725 (+19.75) |
| 25 | 2.168 (-34.16) | 3.456 (-19.82) | 5.329 (-15.17) | 6.435 (-13.78) | 5.124 (-4.24) | 4.378 (-4.68) |
| 50 | 2.037 (-38.11) | 3.225 (-25.20) | 5.062 (-19.44) | 6.321 (-15.30) | 4.674 (-12.62) | 4.043 (-11.99) |
| 75 | 1.786 (-45.76) | 2.867 (-33.51) | 4.675 (-25.58) | 5.785 (-22.48) | 4.372 (-18.29) | 3.867 (-15.82) |
| 100 | 1.443 (56.21) | 2.547 (-40.95) | 4.249 (-32.36) | 5.473 (-26.67) | 3.764 (-29.65) | 3.425 (-25.45) |
| 200 | 1.185 (-64.01) | 1.287 (-70.15) | 3.722 (-40.75) | 5.059 (-32.21) | 3.300 (-38.32) | 3.155 (-31.32) |

* Per cent over control values are given in the parentheses

ANOVA

| Source of variation | SS | df | MS | F | P-value | F crit |
|---|---|---|---|---|---|---|
| Rows | 48.77255 | 6 | 8.128759 | 140.5474 | 1.420 | 2.420523 |
| Columns | 73.7503 | 5 | 14.75006 | 255.0306 | 1.2623 | 2.533555 |
| Error | 1.735093 | 30 | 0.057836 | | | |
| Total | 124.2579 | 41 | | | | |

same trend was reported by Shah and Dubey (1997) due to cadmium on rice ; Lee *et al*. (2001) lead on soybean ; Chatterjee *et al*. (2004) lead on rice ; Zare *et al*. (2007) lead and John *et al*. (2008) cadmium and lead on *Lemna polyrrhiza*.

Lead level above 10mg kg-1 significantly reduced amino acid contents in cowpea plants. Nitrogen is a precursor for the synthesis of amino acids (Devlin, 1975). Since the nitrogen content of the metal treated plants was found reduced, ultimately amino acids content of plant was also reduced (Mayz and Cartwright, 1984) because there was only limited availability of nitrogen for the synthesis of amino acids.

Efficacy of different treatment of lead on protein content (mg/g$^{-1}$/fresh weight) of CO -1 on different days (20, 40, 60, 80, 100 and 120) was presented in Table 4 The highest values of protein content of CO -1 (37.274, 51.395, 65.369, 71.304, 57.779 and 50.929, respectively) were observed at 10mg kg$^{-1}$ lead treatment in above said days. The lowest values of protein content of cowpea was observed (20.185, 30.308, 38.396, 44.478, 35.219 and 32.279, respectively) at 200mg kg$^{-1}$ lead treatment. The protein content of cowpea variety CO-1 leaves increased with increase of lead level (10mg kg$^{-1}$) in the soil and then it decreased gradually, in all sampling days. Increased and decreased protein content due to some heavy metals on plants were observed by Kim *et al.* (1978) copper, nickel, chromium, cobalt, manganese; Nag *et al*. (1981) copper, zinc, mercury, lead and cadmium in rice; Mesmar and Jaber (1991) on wheat and lensculinaris due to lead; Stiborova *et al.* (1986) copper and cadmium on *Hordeum vulgare*; Shah and Dubey (1997) on rice due to cadmium; Kastori *et al.* (1992) lead, cadmium, copper and zinc on sunflower; Bhattacharjee and Mukherjee (1994) cadmium and lead on *Vigna unguiculata* and Pinero *et al*. (2002) on bean, alfalfa, oat and ryegrass due to lead.

The dissolubility of proteins increased under metal which might be a detoxifying mechanism (e.g. protein produced to reduce the toxicity of cadmium). Increment of soluble protein amount can be a consequence of *de novo* synthesis of some stress proteins as result of exposure to exogenous factor. Similar findings were observed by Li and Yu (1990); Hong *et al*. (1991) on wheat due to cadmium; Li *et al*. (1992) on tobacco due to cadmium Gonçalves *et al*. (2007) on cucumber due to cadmium; Azmat and Haider (2007) *Phaseolus mungo* and *Lens culinaris* due to lead.

Decreased protein content was observed at higher concentrations of lead on protein degradation process as a result of increased protease activity which was found to increase under stress conditions. It was also likely that these heavy metals induced lipid peroxidation and fragmentation of proteins due to toxic effects of reactive oxygen species which lead to reduced protein content Palma *et al.* (2002).

In the present investigation, proline content was observed gradual increase, it could be noted with increasing lead treatment. Thus, proline accumulation under such condition may also be operative genotypes tolerant to stress. Influence of different treatment of lead on proline content (mg/g$^{-1}$/fresh weight) of CO -1 on different days (20, 40, 60, 80, 100 and 120) is presented in Table 5. The highest values of proline CO-1 (0.619,

**Table 4 : Efficacy of lead on protein content (mg/g$^{-1}$/fresh weight) of cowpea variety CO -1**

| Lead treatments (mg kg$^{-1}$ soil) | Sampling days | | | | | |
|---|---|---|---|---|---|---|
| | 20 | 40 | 60 | 80 | 100 | 120 |
| Control | 34.452 | 47.642 | 62.714 | 67.424 | 53.202 | 47.506 |
| 10 | 37.274 (+7.57) | 51.395 (+7.30) | 65.369 (+4.06) | 71.304 (+5.44) | 57.779 (+7.92) | 50.929 (+6.72) |
| 25 | 32.643 (-5.25) | 44.395 (-6.81) | 57.368 (-8.52) | 62.285 (-7.62) | 48.734 (-8.39) | 43.287 (-8.88) |
| 50 | 29.813 (-13.46) | 39.106 (-17.91) | 53.426 (-14.82) | 58.144 (-13.76) | 43.230 (18.75) | 39.775 (-16.27) |
| 75 | 27.132 (-21.24) | 36.856 (-22.61) | 48.201 (-23.14) | 53.923 (-20.01) | 40.485 (-23.90) | 37.235 (-21.61) |
| 100 | 24.351 (-29.31) | 33.922 (-28.79) | 42.478 (-32.25) | 47.624 (-29.36) | 38.173 (-28.25) | 35.509 (-25.25) |
| 200 | 20.185 (-41.40) | 30.308 (-36.38) | 38.396 (-38.77) | 44.478 (-34.03) | 35.219 (-33.80) | 32.279 (-32.05) |

* Per cent over control values are given in the parentheses

ANOVA

| Source of variation | SS | df | MS | F | P-value | F crit |
|---|---|---|---|---|---|---|
| Rows | 2336.453 | 6 | 389.4089 | 110.349 | 4.4919 | 2.420523 |
| Columns | 3507.541 | 5 | 701.5081 | 198.7904 | 4.7522 | 2.533555 |
| Error | 105.8665 | 30 | 3.528883 | | | |
| Total | 5949.86 | 41 | | | | |

0.895, 1.275, 1.482, 1.026 and 0.982, respectively) were observed at 200 mg $kg^{-1}$ lead treatment in above said days. The lowest values of proline (0.334, 0.537, 0.739, 1.216, 0.667 and 0.619, respectively) at control.

The proline content of cowpea showed a marked increase in cowpea plants treated with lead. Increased of proline content due to different stress, particularly drought, and salinity is a common observation. But it is the fact that metal stress also increases the proline content which has been meagerly reported in the literature. This would be evident from the study of Hong *et al.* (1991) cadmium on wheat; Qin *et al.* (1994) lead on *Beassica chinensis.*

Proline is such amino acid that plays a therapeutic role in plants (Singh *et al*., 1973; Flowers *et al*., 1977). Accumulation of proline may be due to increased synthesis from glutamate, lower rate of protein oxidation and slowed incorporation of proline in to protein. Proline, sugar, glycine, betaine and other organic solutes are believed to improve metal tolerance by contributing to osmotic and preventing enzyme activity in the presence of toxic ions (Greenway and Munns, 1980 and Bremberger and Luttge, 1992). Proline increases the stress tolerance of the plants through such functions as osmoregulation, the protection of enzymes against denaturation, and the stabilization of protein synthesis (Kuznetsov and Shevyakova, 1997). Increase in proline content may be either due to *de novo* synthesis or decreased degradation or both (Kasai *et al.,* 1998).

Increased proline content occurred due to increase of lead in root and aerial part of the cowpea plant. This is common process in plant; increased proline in plants is a defensive mechanism. Proline increase tolerance of plant by several mechanisms such as elimination of hydroxyl radicals, osmosis adjustment, inhibition of destroying of enzymes and maintaining protein synthesis (Kuzentsov and Shevyakova, 1997). One of the defensive mechanisms to Cr is synthesis and accumulation of some amino acids like proline that is a osmotic alignment and reduce the toxicity of heavy metals (Alia and Saradhi, 1991). More accumulation of proline in root of plant can show the importance of osmosis alignment in absorbance places. Metal stress also increases the proline content which has been meagerly reported in the literature.

## Conclusion :

The objectives of the present investigation were the effects of different concentrations (Control, 10, 25, 50, 75, 100 and 200mg $kg^{-1}$ soil) of lead on the variety (CO -1) of cowpea [*Vigna unguiculata* (L.) Walp.] based on the bio-chemical changes like, total sugar, starch, amino acids, protein and proline activities. From those treatments under lead values were increased at 10mg $kg^{-1}$ lead treatment. The uptake and accumulation of lead in cowpea plants increased with increased of lead level in the soil under field / pot culture experiments. From 25 -200mg $kg^{-1}$ biochemical contents were decreased (except proline). It might be suggested that nutrients available in 10mg $kg^{-1}$ lead in the soil and it was suitable for crops and it provides nutrients to the field and plants. Proper care should be taken in disposal of lead contaminated effluent to avoid soil pollution.

**Table 5 : Influence of lead on proline content (mg/$g^{-1}$/fresh weight) of Cowpea variety CO -1**

| Lead treatments (mg $kg^{-1}$ soil) | Sampling days | | | | | |
|---|---|---|---|---|---|---|
| | 20 | 40 | 60 | 80 | 100 | 120 |
| Control | 0.334 | 0.537 | 0.739 | 1.216 | 0.667 | 0.619 |
| 10 | 0.349 (4.297) | 0.555 (3.24) | 0.764 (3.27) | 1.244 (2.25) | 0.686 (2.76) | 0.630 (1.74) |
| 25 | 0.366 (9.58) | 0.586 (9.12) | 0.818 (10.69) | 1.268 (4.270 | 0.726 (8.84) | 0.665 (7.43) |
| 50 | 0.408 (22.15) | 0.659 (22.71) | 1.026 (38.70) | 1.297 (6.57) | 0.815 (22.32) | 0.734 (18.57) |
| 75 | 0.537 (60.47) | 0.796 (48.23) | 1.086 (46.81) | 1.420 (16.770 | 0.962 (44.07) | 0.872 (40.87) |
| 100 | 0.558 (67.36) | 0.835 (55.49) | 1.125 (52.09) | 1.457 (19.81) | 0.995 (49.03) | 0.914 (47.65) |
| 200 | 0.619 (85.32) | 0.895 (66.85) | 1.275 (72.53) | 1.482 (21.95) | 1.026 (53.67) | 0.982 (58.64) |

*Per cent over control values are given in the parentheses

ANOVA

| Source of Variation | SS | df | MS | F | P-value | F crit |
|---|---|---|---|---|---|---|
| Rows | 0.756624 | 6 | 0.126104 | 82.26551 | 2.8717 | 2.420523 |
| Columns | 3.10793 | 5 | 0.621586 | 405.4992 | 1.3826 | 2.533555 |
| Error | 0.045987 | 30 | 0.001533 | | | |
| Total | 3.91054 | 41 | | | | |

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