**International Research Journal of Pharmaceutical and Applied Sciences (IRJPAS)**
Available online at **www.irjpas.com**
Int. Res J Pharm. App Sci., 2014; 4(4):1-3

**Research Article**

# IMPACT OF LEAD ON GROWTH, BIO CHEMICAL AND ENZYMATIC CHANGES IN BLACK GRAM.

**S. Ravikumar* , P. Thamizhiniyzn**
Department of Botany Annamalai University, Annamalai nagar-609 002, Tamilnadu, India

*Corresponding Author: S. Ravikumar; Email: drsrk74@gmail.com

**Abstract:** Lead (Pb) is one of the non essential and toxic heavy metals which can cause oxidative stress in plants. The effects of lead toxicity on growth and some biochemical parameters of black gram were studied under laboratory condition. Different treatments of Pb (0, 5, 10, 25, 50, 100 and 200 mg/l) were used in order to consider changes in germination, seedling growth, dry weight, proline content, Total chlorophyll and enzyme activity (catalase and peroxidase). Compared with the control, Pb treatment caused a significant decrease in root and shoot growth and dry weight and chlorophyll content. In contrast, a significant increase in proline, Catalase and peroxidase activity of leaves was observed in Pb treatments.
**Key words:** Lead, Black gram, proline,enzymes, Growth, Dry weight

## INTRODUCTION

Lead (Pb) is one of the potentially toxic heavy metal pollutants of the environment with no known biological function and its concentrations are rapidly increased in agricultural soil[1]. The most significant factors which can distribute lead as a pollutant in the environment are burning of fossil fuels, agricultural manufacturing, mining, pesticides and fertilizers[2]. According to Oliver and Naidu plants show different reactions against Pb toxicity[3]. Some of them are sensitive and the others have more tolerance. In plants, it has been widely reported that accumulation of Pb may cause many physiological, biochemical and structural changes like decline in photosynthetic rate and essential elements absorption[4] , the roots and shoots growth inhibition, chlorosis and decrease in water potential and plant hormones[5]. Plant cells have a protective system against oxidative stress including enzyme antioxidant system like catalase and guaiacol peroxidase and non-enzyme antioxidant system.[6] Nowadays, the usage of fertilizers including micronutrients and macronutrients has increased. One of the most toxic heavy metals which may be found in some kind of fertilizers is lead that can be absorbed and accumulated in plants. Then they enter the food chain to form hazard for human health.[7] Therefore, it is essential to investigate the effects of lead toxicity on some biochemical characteristics of the seedlings in blackgram (*Vigna mungo* (L)Hepper).

## MATERIALS AND METHODS

Black gram (*Vigna mungo* (L) Hepper) seeds were obtained from the Regional Rice Research station, Aaduthurai, Tamil Nadu, India. Uniform seeds were selected and surface sterilized with 0.01% $Hgcl_2$ solution for 2 minutes. Petriplates lined with filter paper and 20 black gram seeds were placed in each petriplate. They were treated with different concentrations (control, 5, 10,25,50,75,100 and 200 mg /l) of Lead. Three replicates were maintained. Plant Growth were measured by using centimeter scale and recorded. The same plant samples were taken for morphological studies were also used for the determination of dry weight by using electrical single pan balance. Their dry weights were determined by keeping the plant materials in a hot air oven at 80 °C for 24 hrs and recorded. Similarly the Proline[8], Total chlorophyll [9], Catalase and Peroxidase[10] were also measured.

## RESULTS AND DISCUSSION

The common symptoms caused by lead toxicity were yellowing of the leaves and appearance of dark brown pigments, especially in 200 Mg/l, which might be attributed to nutrient imbalance induced by excess lead. Pb treatment dramatically inhibited the germination, seedling growth and accumulation of both shoot and root biomass and consequently, decreased their dry weight. Lead toxicity can induce complex changes in plants at biochemical and physiological aspects. The most obvious symptoms include leaf chlorosis and reduction of plant growth[11], Decrease in DNA, RNA and protein synthesis[12], imbalance of chlorophyll metabolism[13].

Compared to the control, total chlorophyll slightly declined by increase in lead concentrations. Table 2 shows the changes of chlorophyll in different Pb concentrations. The highest level refers to control condition and the lowest level refers to the highest concentration of Pb. In the present study, leaf total chlorophyll significantly decreased by increase in lead amount which coincides with Hamid *et al.*[1] . It could be attributed to oxidative stresses and inhibition in chlorophyll synthesis. Lead may prevent enzyme activity like aminolevulinic acid dehydratase[14] or decrease essential elements absorption such as $Mg^{2+}$ and $Fe^{2+}$ to inhibit chlorophyll synthesis. [15] Also, some previous investigations have indicated that chlorophyll decrease can be due to its decomposition by increase in chlorophyllase activity.[16-17]

Table 2 illustrates proline changes in blackgram seedlings by increase in Pb. As can be seen, increase in lead results in a rise in proline. High level of proline, especially in roots, can eliminate hydroxyl radicals, maintain osmoregulation, prevent enzyme destruction[18] and decrease toxicity of heavy metals[19].

There was a dramatic rise in catalase and peroxidase activity with the increase in different Pb treatments (Table 2). Increase in catalase and peroxidase activity was significant in all treatments except 200 Mg/l. It has been shown lead can cause oxidative stress in plants.[12] Plant cells are equipped with a protective system including antioxidant enzymes like catalase and peroxidase which can bridle free radicals.[20] Decrease in enzyme synthesis or change in subunits arrangement may be a reason for decline in enzyme activity[17] and increase in peroxidase activity can be due to releasing from cell wall [21]. Most of the previous studies indicated that heavy metals like lead are responsible for catalase activity decrease in rice[12] and peroxidase activity increase in rice and soybean [12] which coincide our results.

**Table 1.Effect of Lead on growth parameters of Black gram**

| | Seed germination | Root length | Shoot length | Dry weight |
|---|---|---|---|---|
| Control | 95 | 6.56 | 9.28 | 0.778 |
| 5 | 89<br>(-6.31) | 5.54<br>(-15.54) | 7.92<br>(-14.65) | 0.722<br>(-7.19) |
| 10 | 76<br>(-20.0) | 4.91<br>(-25.15) | 6.84<br>(-26.29) | 0.647<br>(-16.83) |
| 25 | 65<br>(-31.57) | 4.16<br>(-36.58) | 5.61<br>(-39.54) | 0.612<br>(-21.33) |
| 50 | 54<br>(-43.15) | 3.77<br>(-42.53) | 5.00<br>(-46.12) | 0.493<br>(-36.63) |
| 75 | 48<br>(-70.52) | 3.20<br>(-51.21) | 4.31<br>(-53.55) | 0.447<br>(-42.54) |
| 100 | 39<br>(80.0) | 2.37<br>(63.87) | 3.60<br>(61.20) | 0.391<br>(-49.74) |
| 200 | 28<br>(84.0) | 2.00<br>(66.50) | 3.00<br>(63.42) | 0.312<br>(-52.54) |

Percentage over control is expressed in parentheses
F values are significant at 1 % level.

**Table 2.Effect of Lead on Total chlorophyll, Proline content, Catalase and Peroxidase activity of Black gram**

| | Total chlorophyll | Proline | Catalase | Peroxidase |
|---|---|---|---|---|
| Control | 0.692 | 0.567 | 8.38 | 0.285 |
| 5 | 0.647<br>(-6.50) | 0.614<br>(+ 8.28) | 9.03<br>(+ 7.75) | 0.312<br>(+ 9.47) |
| 10 | 0.612<br>(-11.56) | 0.689<br>( 21.51) | 9.89<br>(+ 18.81) | 0.339<br>(+ 18.94) |
| 25 | 0.563<br>(-18.64) | 0.712<br>(+25.57) | 11.26<br>(+ 34.36) | 0.375<br>(+ 31.57) |
| 50 | 0.544<br>(-21.38) | 0.779<br>(+37.38) | 11.76<br>(+40.33) | 0.395<br>(+38.59) |
| 75 | 0.502<br>(-27.45) | 0.825<br>(+45.50) | 12.25<br>(+46.18) | 0.414<br>(+45.26) |
| 100 | 0.476<br>(-31.21) | 0.860<br>(+51.67) | 12.93<br>(+54.29) | 0.437<br>(+53.33) |
| 200 | 0.352<br>(-37.50) | 0.976<br>(+55.50) | 13.38<br>(+56.18) | 0.585<br>(+55.26) |

Percentage over control is expressed in parentheses
F values are significant at 1 % level.

**ACKNOWLEDGMENT**

The authors are proudly thanked to the Professor and Head, Department of Botany, Annamalai University to carry out the study.

**REFERENCES**

1. Hamid, N., N. Bukhari and F. Jawaid, Physiological responses of *Phaseolus vulgaris* to different lead concentrations. *Pak. J. Bot.*, **2010**; 42(1): 239-246.
2. Eick, M.J., J.D. Peak, P.V. Brady and J.D. Pesek. Kinetics of lead absorption and desorption on goethite: Residence time effect. *Soil Sci*., **1999**; 164: 28-39.
3. Oliver, D. and R. Naidu,. Uptake of Cu, Pb, Cd, As and DDT by vegetables grown in urban environments. Proceedings of the 5th National workshop on the assessment of site contamination.

Natio. Environ. Prot. Coun. Ser. Corp., **2003**; 151-161.
4. Larbi, A., F. Morales, A. Abadia, Y. Goforcena, J.J. Lucena and J. Abadia,. Effect of Cd and Pb in sugar beet Plants grown in nutrient solution: Induced Fe deficiency and growth inhibition. *Funct. Plant Biol*., **2002**; 29: 1473-1481
5. Sharma, P.R. and S. Dubey,. Lead toxicity in plants. *Braz. J. Plant Physiol*., **2005**; 17(1): 35-52.
6. Van Assche, F. and H. Clijster,. Effects of metal on enzyme activity in plants. *Plant Cell Environ*., **1990**; 13: 195-206.
7. Hendrickson, L.L. and R.B. Corey,. Effect of equilibrium metal concentration on apparent selectivity coefficients of soils complexes. Soil Sci., **1981** 131: 136-171.
8. Bates, L.S., S.P. Waldren and I.D. Teare,. Rapid determination of free proline for water- stress studies. *Plant Soil*., **1973**; 39: 205-208.
9. Arnon, D.I.,. Copper enzymes in isolated chloroplasts polyphenol oxidase in Beta vulgaris. *Plant Physiol*., **1949**; 24: 1-15.
10. Maehly, A.C. and B. Chance, Methods of biochemical analysis. *In*: Glick, D. (ed.), Vol. 1, Inter Science Publications, Inc., New York. **1967**.
11. Burton, K.W., E. Morgan and A. Roig,. The influence of heavy metal on the growth of Sitka-spruce in South Wales forests. II greenhouse experiment. *Plant Soil*., **1984**; 36: 301-313.
12. Verma, S. and R.S. Dubey,. Lead toxicity induces lipid peroxidation and alters the activities of antioxidant enzymes in growing rice plants. *Plant Sci.*, **2003**; 164: 645-655.
13. Dalla-Vecchia, F., N. La Rocca, I. Moro, S. De-Faveri, C. Andreoli and N. Rascio,. Morphogenetic, ultrastructural and physiological damages suffered by submerged leaves of elodea canadensis exposed to cadmium. *Plant Sci.*, **2005**; 168(2): 329-338.
14. Prasad, D.D.K. and A.R.K. Prasad,. Altered aminoluvelinic acid metabolism by Pb and Hg in germinating seedling of Bajra (*Pennisetum typhoidenum*). *J. Plant Physiol*., **1987**; 127: 241-249.
15. Burzynski, M.. The influence of lead and cadmium on absorption and distribution of $K^+$ , $Ca^{2+}$ , $Mg^{2+}$ and $Fe^{2+}$ in cucumber seedling. *Acta. Physiol. Plant.*, **1987**; 9: 229-238.
16. Drazkiewicz, M.,. Chlorophyll-occurrence, functions, mechanism of action effects of internal and external factors. *Photosynthetica*, **1994**; 30: 321-331.
17. Hertwig, B., P. Streb and J. Feierabend,. Light dependence of catalase synthesis and degradation in leaves and the influence of interfering stress conditions. *Plant Physiol*., **1992**; 100: 1547-1553.
18. Kuznetsov, W. and N.L. Shevyakova,. Stress responses two tobacco cells to high temperature and salinity, proline accumulation and phosphorylation of polypeptids. *Physiol. Plantarum*., **1997**; 101: 477-482.
19. Alia, P. and P. Saradhi,. Proline accumulation under heavy metal stress. *J. Plant Physiol*., **1991**; 138: 554-558.
20. Cho, V.H. and J.O. Park,. Mercury-induced oxidative stress in tomato seedlings. *Plant Sci.*, **2000** 156: 1-9.
21. Gaspar, T., C. Penel, T. Throp and H. Greppin,. Peroxidases (1970-1980). A Survey of their Biochemical and Physiological Roles in Higher Plants. Geneva University Press, Switzerland. **1982**
22. Bradford, M.M., A rapid sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.*, **1976.** 72: 248-254.