J. Phytol. Res. **22**(1) : 19-22, 2009

EFFECT OF LEAD AND CADMIUM IONS ON THE LIPID AND PHOSPHOLIPID COMPOSITION IN PIGEONPEA SEEDLINGS

B. SUJATHA and B. PRIYADARSHINI
Department of Botany, Andhra University, Visakhapatnam-530003, A.P., India.

The effect of lead and cadmium were investigated on lipids and phospholipids content in pigeonpea seedlings, grown in 0.5, 1.0 and 1.5mM concentrations of Pb and Cd, in two cultivars of pigeonpea namely LRG30 and T21. The lipid and phospholipid content decreased with increasing concentrations of Pb and Cd in both the cultivars.

Keywords: Cadmium; Cultivars; Lead; Lipids; Phospholipids.

Introduction

Lead (Pb) and cadmium (Cd) is major heavy metals of the antiquity and has gained considerable importance as a potent environmental pollutants. These heavy metals are not essential and are also highly toxic[1]. Lead and cadmium have received widespread attention because of their accelerated release into the environment as the result of industrial pollution[2,3]. Recent progress in the study of toxic metals and their interactions at the biochemical level has greatly increased our understanding of the mechanism of toxicity[4]. Lipids are the important constituents of biological membranes which are involved in structural and vital biochemical functions. They also perform several other cellular functions[5]. Changes in lipid composition in response to heavy metal stress have been demonstrated in many plants[6-7]. In addition to lipid composition, heavy metals also affect the levels of fatty acids[8] and various phospholipid constituents of membranes[9]. The present study was designed to assess the lead and cadmium effects on lipid and phospholipid composition, in response to their cultivar differences.

Material and Methods

Seeds of pigeonpea [*Cajanus cajan*(L.)Millspaugh] cv.T21 (medium duration) and cv.LRG30 (long duration) supplied by ICRISAT, Patancheru, India were used in the present study. The seeds of uniform size and free from infection, were selected for the experiments. The seeds were surface sterilized by using 0.01M sodium hypochlorite for 2 min, washed thoroughly with distilled water and were placed separately in trays lined with Whatman No.1 filter papers containing 0.5, 1.0 and 1.5mM concentrations of lead (lead acetate: $(CH_3COO)_2Pb.3H_20$) and cadmium (cadmium chloride: $CdCl_2\ 2.5H_20$), respectively. Twenty-five seeds were taken in each tray. Seeds germinated and seedlings grown in distilled water (zero concentration) served as controls. The seeds were allowed to germinate at 30±2°C for 8-days under a photoperiod of 12h and at 195μ mol m^{-2} s^{-1} PPFD. Seedlings were collected for biochemical analysis. Data represent means of three separate experiments. For each treatment, three replicates (each consisting of 20 seeds) were placed in petri dishes.

Total lipids: Total lipid extraction was carried out according to the method of Bligh and Dyer[10]. One g of seedling axes or cotyledons of control and treated pigeonpea seedlings were washed and macerated with 14.4 ml boiling mixture of chloroform : methanol : water (1:2:0.6 V/V/V). The contents were filtered using muslin cloth. The filtrates were transferred into stoppered test tubes and the residues were reextracted with 8.0 ml of hot methanol and again transferred to stoppered tubes. To each of these tubes 12ml of chloroform was added. After keeping the samples over night at 0°C in the deep freeze, 11.2 ml of water was added to each tube, shaken well and centrifuged for phase separation. The final ratio of chloroform : methanol : water in each tube was 2:2:1.8 V/V/V. The aqueous phases were removed with suction. The lipid phases were washed thrice with methanol:water (2:1.8 V/V) mixture. The contents were centrifuged in a refrigerated centrifuge at 3000rpm for 10min in order to remove the water soluble compounds. The lipid extracts were evaporated to dryness.

The dried lipid residues were taken into chloroform and transferred to pre-weighed bottle and evaporated to dryness under nitrogen. The bottles were kept in a vacuum dessicator over potassium hydroxide under reduced pressure for several hours and weighed again. The difference between the weights was taken as the weight of the total lipid present in each sample. The total lipid of each sample was dissolved in chloroform,

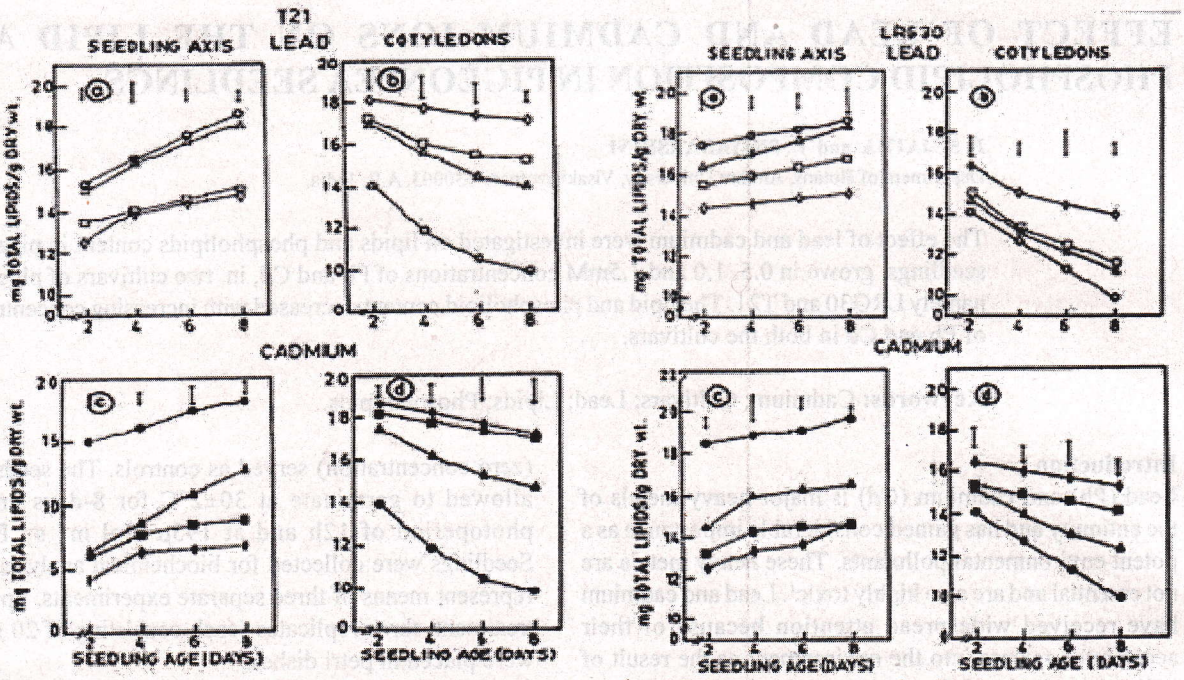

Fig.1. A. Total lipid content of seedlings of pigeonpea, cv.T21 and cv.LRG30 in response to lead and cadmium stresses (Vertical lines represent S.E.).; Seedling axis : a and c ; Cotyledons : b and d ; Lead : a and b; Control : O - O; 0.5: mM Δ-Δ ; 1.0: mM □-□; 1.5: mM ◇- ◇; Cadmium : c and d; Control : •-• ; 0.5: mM ▲- ▲; 1.0: mM ■ - ■; 1.5: mM ◆-◆.

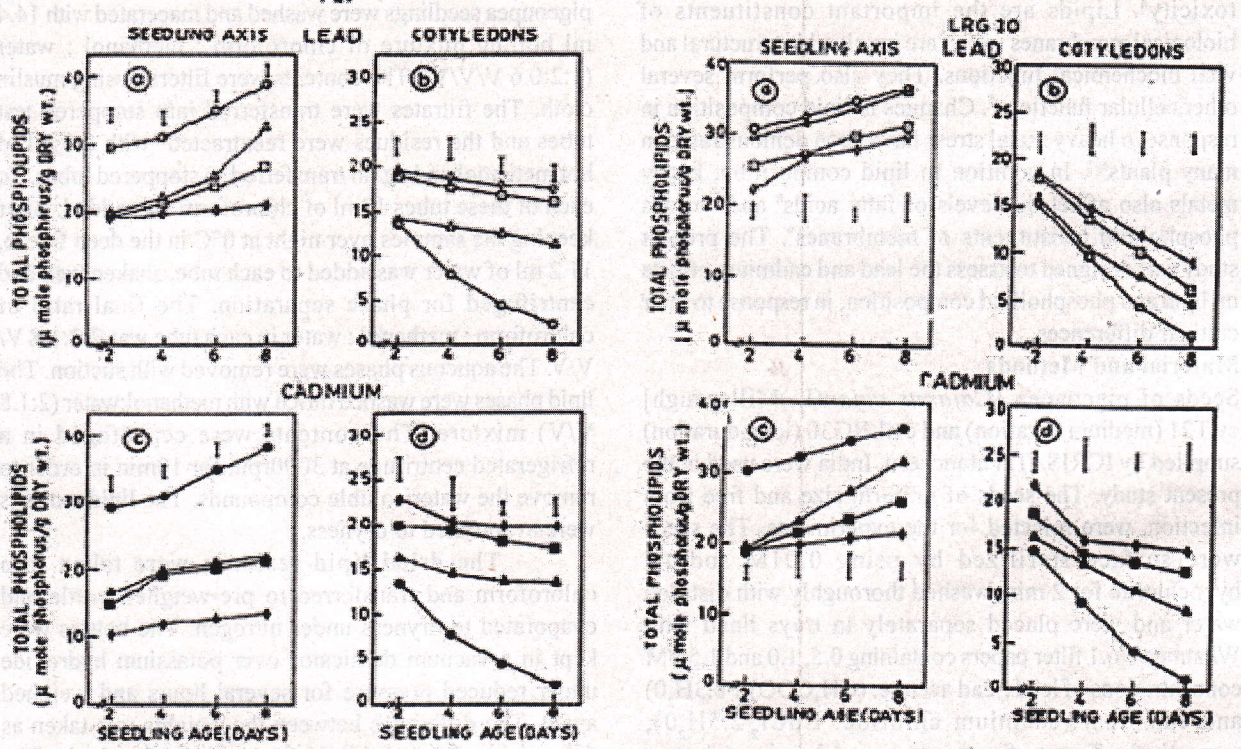

Fig.1. B. Total phospholipid content of seedlings of pigeonpea, cv.T21 and cv.LRG30 in response to lead and cadmium stresses (Vertical lines represent S.E.).
Seedling axis : a and c ; Cotyledons : b and d ; Lead : a and b; Control: O - O; 0.5: mM Δ- Δ ; 1.0: mM □-□ ; 1.5: mM ◇- ◇ ; Cadmium : c and d; Control •-• ; 0.5: mM ▲- ▲; 1.0: mM ■ - ■; 1.5: mM ◆-◆.

made upto 5ml and was preserved at 0°C in tightly stoppered standard flask until further analysis. The total lipid content was expressed as mg per gram dry weight.

Total Phospholipids: The total phospholipids content was determined as the phosphorus content of the phospholipids by the procedure outlined by Bartlett[11]. The phospho lipid samples were taken into test tubes marked with 10ml. The organic solvent was removed by passing compressed air into the test tubes. One ml of 60% perchloric acid was added to each tube and digested at 170-180°C on a heating mantle. Digestion was continued until the samples were clear. After digestion, to each tube 4.5ml of 0.44% ammonium molybdate reagent was added, followed by 0.2ml of 1-amino-2-napthol-4-sulphonic acid reagent. The contents of the tubes were shaken well and heated over a boiling water bath for 10min. After cooling the volume was adjusted to 10ml with distilled water. The colour intensity of each sample was measured at 660 nm using schimadzu (UV-240) spectrophotometer. Aliquots of KH_2PO_4 solution containing 2mg phosphorus/ml were used as standards. Blanks were run simultaneously. For computing the total phospholipid content, the lipid phosphorus was multiplied by factor 2.5. The results were expressed as μ mole phosphorus per organ and μ mole phosphorus per g dry-weight.

Results and Discussion

The lipid content of the seedlings axes of lead and cadmium treated seedlings registered lower values when compared to their respective controls. The cotyledons of control seedlings of the two cultivars of pigeonpea showed a continuous decline in the lipid content, during the period of study. The cotyledons of the lead and cadmium treated seedlings retained considerable quantities of lipid when compared to their respective controls (Fig.1A).

Lipids form an important component of cell membranes. Changes in membrane lipid composition under heavy metal stress conditions can alter the fluidity of membranes integrity[12]. Changes in lipid composition by heavy metal treatment were studied by several workers[13]. Under a variety of stress conditions, the lipid composition of root cells is known to alter markedly, which includes changes in the level of unsaturation of the fatty acids[8] and changes in the total and relative abundance of the various phospholipid classes of cells and plasma membranes[9]. Such changes inevitably affect the phase transition temperature of the membranes which in turn, could influence the activity of a number of membrane bound enzymes[14]. Cadmium may induce premature senescence of leaves and it results in the progressive degradation of thylakoid membrane lipids[15]. Further, the plasma membrane, the most exposed cell organelle, is thought to be the major target for heavy metal stress which was clearly noticed in our previous ultrastructural studies[16] due to changes in composition and structure of water and lipid bodies[17]. The disorder and disassembly within a lipid bilayer could contribute to the fluidity of the membrane and membrane leakage under abiotic stress[18-19].

The total phospholipids content of the seedling axes of the two pigeonpea cultivars decreased with increasing concentrations of lead and cadmium treatments and registered lower values at all the stages of seedling growth when compared to their respective controls. The cotyledons of the controls showed a continuous decline in the phospholipids with increasing age of the seedlings. The cotyledons of the lead and cadmium treated seedlings retained more levels of phospholipids when compared to their respective controls (Fig.1B). In addition to the lipids, phospholipids also play an important role in the structure and functions of plant cells. It is presumed that the reduction in the content of lipids and phospholipids in response to lead and cadmium exposure may be a consequence of their reduced synthesis or increased lipase activity or lipid peroxidation, either individually or in different combinations[20].

The loss in membranes integrity is often caused by an increase in saturation of membrane phospholipids and a decrease in lipid and phospholipids contents. The pigeonpea cv.LRG30, which recorded lower phospholipids breakdown, showed lower leaching of solutes when compared to cv.T21 as a function of lead and cadmium toxicity. The studies on the cultivars of pigeonpea clearly suggests that membrane deterioration as indicated by a decline in total lipids and phospholipids and consequent loss of membrane integrity represents an important physiological event associated with lead and cadmium. The rapid decline in the lipid components in the cv.T21 may be due to greater destabilisation of membranes under lead and cadmium toxicity when compared to cv.LRG30.

References

1. Kupper H, Lombi E, Zhao F-J and McGrath SP 2000, Cellular compartmentation of cadmium and zinc in relation to other elements in the hyperaccumulator *Arabidopsis halleri. Planta* **212** 75-84.
2. Surosz W and Palinska KA 2004, Effects of heavy metals stress on cyanobacterium *Anabaena flosaquae. Archives of Environmental Contamination and Toxicology* **48** 40-48.
3. Sharma Pallavi and Dubey Rama Shanker 2005, Lead toxicity in plants. *Braz.J. Plant Physiol.* **17 (1)** 35-52.
4. Abdulla M, Nair BM and Chandra RK 1985, Health

effects and interactions of essential and toxic elements. *Nutr. Res.***1** 1-751.

5. Kates M and Marshall MO 1975, In: *Recent Advances in the Chemistry and Biochemistry of plant lipids* (Eds.T.Gaillard and E.I. Mercer). Academic Press, New York. pp.115.
6. Krupa Z, Siedlecka A, Maksymiec W and Baszynski T 1993, *In vivo* response of photosynthetic apparatus of *Phaseolus vulgaris* L. to nickel toxicity. *J. Plant Physiol.* **142** 664-668.
7. Krupa Z 1988, Cadmium induced changes in the composition and structure of the light-harvesting chlorophyll a/b protein II in radish cotyledons. *Physiol.Plant.* **73** 518-524.
8. Svennignsson M and Lilijenberg C 1986, Changes in cuticular transpiration rate and cuticular lipids of oat *(Avena sativa)* seedlings induced by water stress. *Physiol.Plant.* **66** 9-14.
9. Norberg P and Lilijenberg C 1991, Lipids of plasma membranes prepared from root cells. *Plant Physiol.* **96** 1136-41.
10. Bligh EG and Dyer WJ 1959, A rapid method of total lipid extraction and purification. *Can.J. Biochem. Physiol.* **59** 388-393.
11. Bartlett GR 1959, Phosphorus assay in column chromatography. *J. Biol. Chem.* **234** 466-468.
12 Brown MC, Dudeja PK and Brasitus TA 1998, S-Adenosyl-L-Methionine modulates Na^++K^+-ATPase activity in root colonic basolateral membranes. *Biochemistry Journal* **251** 215-22.
13. Maksymiec W and Baszynski T 1988, The effect of Cd^{2+} on the release of proteins from thylakoid membranes of tomato leaves. *Acta Soc. Bot. Pol.* **57** 465-474.
14. Cook DT, Burden RS, Clarkson DT and James CS 1989, Xenobiotic –induced changes in membrane lipid composition ; effects on plasma-membrane ATPase. In:Atkin R. Clifford, D.R. (eds.) *Mechanism and regulation of transport processes.*
15. Fong F and Heath RL 1977, Age dependent changes in phosphor-lipid and galactolipids in primary bean leaves (*Phaseolus vulgaris*).*Phytochem.* **16** 215-217.
16. Sujatha B and Priyadarshini B 2008, Ultrastructural changes in the root cells of pigeonpea (*Cajanus cajan* (L.)) Millspaugh) induced by low levels of cadmium in the medium. *Indian J. Plant Physiol.* **13 (2)** 107-110.
17. Ravi Valluru and Wim Van den Ende 2008, Plants fructans in stress environments: emerging concepts and future prospects. *J.Exp.Bot.* **59** 11, 2905-2916.
18. Verslues PE, Agarwal M, Katiyae-Agarwal S, Zhu J and Zhu JK 2006, Methods and concepts in quantifying resistance to drought, salt and freezing, abiotic stresses that affect plant water status. *The Plant Journal* **45** 523-539.
19. Beck EH, Fettig S, Knake C, Hartig K and Bhattarai T 2007, Specific and unspecific responses of plants to cold and drought stress. *J. Bioscience* **32** 501-298.
20. Summerfield FW and Tappel AL 1984, Effects of dietary polyunsaturated fats and vitamin E on ageing and peroxidative damage to DNA. *Arch. Biochem. Biophys.* **282** 408-416.