

Proline accumulation in lemongrass (*Cymbopogon flexuosus* Stapf.) due to heavy metal stress

G.K. Handique¹ and A.K. Handique^{*2}

¹Department of Botany, Nalbari College, Nalbari-781 335, India

²Department of Biotechnology, Gauhati University, Guwahati - 781 014, India

(Received: March 20, 2007; Revised received: August 03, 2007; Accepted: September 20, 2007)

Abstract: Toxic heavy metals viz. lead, mercury and cadmium induced differential accumulation of proline in lemongrass (*Cymbopogon flexuosus* Stapf.) grown in soil amended with 50, 100, 200, 350 and 500 mg kg⁻¹ of the metals have been studied. Proline accumulation was found to be metal specific, organ specific and linear dose dependant. Further, proline accumulation following short term exposure (two months after transplantation) was higher than long term exposure (nine months after transplantation). Proline accumulation following short term exposure was 2.032 to 3.839 μ moles g⁻¹ for cadmium (50 -200 mg kg⁻¹); the corresponding range for mercury was 1.968 to 5.670 μ moles g⁻¹ and 0.830 to 4.567 μ moles g⁻¹ for lead (50 -500 mg kg⁻¹ for mercury and lead). Proline accumulation was consistently higher in young tender leaf than old leaf, irrespective of the metal or duration of exposure. For cadmium treatment proline level was 2.032 to 3.839 μ moles g⁻¹ for young leaves while the corresponding value for old leaf was 1.728 to 2.396 μ moles g⁻¹ following short term exposure. The same trend was observed for the other two metals and duration of exposure. For control set proline accumulation in root was 0.425 μ moles g⁻¹ as against 0.805 and 0.533 μ moles g⁻¹ in young and old leaves respectively indicating that proline accumulation in root are lower than leaves, under both normal and stressed condition.

Key words: Proline, Heavy metal, Lemongrass, *Cymbopogon flexuosus*
PDF of full length paper is available with author (*akhandique@gmail.com)

Introduction

Pollution due to heavy metal is a matter of growing concern because of their toxicity to all forms of life. Heavy metals mostly accumulate in soil and water and so they are a particular threat to plants since they are rooted to soil and hence get maximum exposure. Not all heavy metals are toxic; on the contrary certain metals like iron, zinc, copper, magnesium, manganese, molybdenum etc. are required by plants in trace amount and have well defined biological function. But some metals like lead, mercury, cadmium, nickel, arsenic, chromium etc., have no known biological functions and are toxic to life even at very low concentration (Salt *et al.*, 1995). Toxic heavy metals like lead, mercury and cadmium emanate mostly from various industrial effluents, mining and smelting of metalliferous ores, sewage sludge etc. Cadmium pollution mostly emanate from excessive use of phosphate fertilizers and compared to other heavy metals cadmium is readily absorbed in higher amount by various plants, which is a matter of serious concern (Mejare and Bulow, 2001). High levels of heavy metals in industrial, agricultural and residential areas have been well documented by various workers for a long time (Klein, 1972; Saikia *et al.*, 1988; Singh *et al.*, 1992; Zengin and Kirbag, 2007). Heavy metal pollution is of anthropogenic origin and so in future their intensity is likely to increase. Since heavy metals are not biodegradable they keep on accumulating in soil and water and hence they are a major and far reaching threat. Therefore, study of plant's exposure to heavy metals particularly at biochemical level deserve priority. There are evidences that plants like tomato (De and Mukherjee, 1998), *Vigna unguiculata* (L) Walp (Bhattacharjee

and Mukherjee, 1994) respond to heavy metal stress through accumulation of proline. There are evidences that nickel hyperaccumulator *Alyssum* exhibit accumulation of another free amino acid histidine under condition of nickel stress (Kramer *et al.*, 1996).

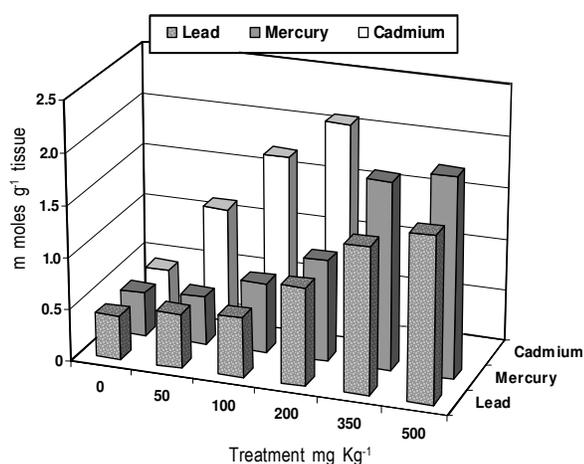
The present study deals with the study of proline accumulation in lemongrass (*Cymbopogon flexuosus* Stapf.) which is a hardy perennial industrial cash crop as a source of lemongrass oil, extensively used in perfumery, toiletry and pharmaceutical industries. *Java citronella* which is related to lemongrass has been found to be significantly tolerant to lead and mercury (Deka Boruah, 1999). Lemongrass has wide adaptability and ability to withstand adverse climatic condition for which it was selected as test plant to study heavy metal response. In recent years phytoremediation is gaining importance as an eco-friendly technique for decontamination or clean up of metal polluted soil which is user friendly and very cost effective (McGrath, 1998). The present study was aimed at studying the variability in proline accumulation due to lead, mercury and cadmium and to find out which metal is the strongest inducer. It was also intended to see whether there is differential accumulation in different organs viz young and old leaves and root. Variability in accumulation over short and long term exposure to heavy metals was also another objective of the present study.

Materials and Methods

Planting materials for lemongrass (*Cymbopogon flexuosus* Stapf.) were collected from the experimental garden of Department of Biotechnology, Gauhati University. Lemongrass is traditionally

Table - 1: Proline accumulation (values are mean \pm SD) in leaves due to short term and long term treatment with heavy metals

| Treatment mg kg ⁻¹ | μ moles g ⁻¹ tissue | | | | | | | | | | | |
|----------------------------------|------------------------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|
| | Short term exposure | | | | | | Long term exposure | | | | | |
| | Young leaves | | | Old leaves | | | Young leaves | | | Old leaves | | |
| | Pb | Hg | Cd | Pb | Hg | Cd | Pb | Hg | Cd | Pb | Hg | Cd |
| 0 | 0.805 \pm 0.06 | 0.805 \pm 0.06 | 0.805 \pm 0.06 | 0.533 \pm 0.03 | 0.533 \pm 0.03 | 0.533 \pm 0.03 | 0.705 \pm 0.03 | 0.705 \pm 0.03 | 0.705 \pm 0.03 | 0.480 \pm 0.02 | 0.480 \pm 0.02 | 0.480 \pm 0.02 |
| 50 | 0.830 \pm 0.04 | 1.968 \pm 0.08 | 2.032 \pm 0.03 | 0.580 \pm 0.00 | 1.505 \pm 0.05 | 1.728 \pm 0.02 | 0.749 \pm 0.06 | 1.796 \pm 0.02 | 2.305 \pm 0.00 | 0.502 \pm 0.02 | 1.054 \pm 0.00 | 1.687 \pm 0.00 |
| 100 | 0.797 \pm 0.04 | 2.265 \pm 0.06 | 2.665 \pm 0.05 | 0.640 \pm 0.03 | 1.907 \pm 0.05 | 1.880 \pm 0.05 | 1.287 \pm 0.06 | 1.691 \pm 0.00 | 3.450 \pm 0.03 | 0.870 \pm 0.00 | 1.409 \pm 0.03 | 2.116 \pm 0.07 |
| 200 | 1.814 \pm 0.10 | 3.512 \pm 0.08 | 3.839 \pm 0.12 | 0.756 \pm 0.04 | 2.037 \pm 0.08 | 2.396 \pm 0.07 | 1.695 \pm 0.07 | 2.287 \pm 0.05 | 3.984 \pm 0.05 | 1.293 \pm 0.04 | 1.507 \pm 0.05 | 3.866 \pm 0.11 |
| 350 | 3.794 \pm 0.09 | 4.119 \pm 0.11 | - | 2.090 \pm 0.09 | 3.494 \pm 0.07 | - | 3.450 \pm 0.11 | 3.734 \pm 0.08 | - | 2.525 \pm 0.06 | 2.712 \pm 0.08 | - |
| 500 | 4.567 \pm 0.15 | 5.670 \pm 0.11 | - | 2.190 \pm 0.09 | 3.756 \pm 0.13 | - | 3.984 \pm 0.10 | 4.080 \pm 0.08 | - | 2.718 \pm 0.05 | 2.734 \pm 0.07 | - |
| C.D at 5% | 0.029 | 0.019 | 0.025 | 0.013 | 0.007 | 0.009 | 0.067 | 0.018 | 0.020 | 0.010 | 0.014 | 0.018 |
| C.D at 1% | 0.039 | 0.027 | 0.037 | 0.018 | 0.010 | 0.013 | 0.091 | 0.025 | 0.029 | 0.014 | 0.019 | 0.025 |

**Fig. 1:** Proline accumulation in root due to heavy metal treatment

propagated through stem portion referred to as 'slips'. Such healthy, uniform slips were used as planting material for the present study. Cadmium nitrate [Cd(NO₃)₂ · 4H₂O], mercuric nitrate Hg(NO₃)₂ · H₂O], and lead nitrate [Pb(NO₃)₂], were used for the present study. Nitrate salts were preferred for their high solubility and hence easy availability for plants. Garden soil was collected by digging to a depth of about 20 inches and taking representative soil of the depth profile. The soil were sun dried, grounded and mixed with FYM in 6:1 ratio. The soil FYM mixture was amended by adding calculated amount of respective heavy metal salt and thoroughly mixing it. Soil were amended with each of the three heavy metals to have soil with concentration of 50, 100, 200, 350 and 500 mg kg⁻¹ (w/w). Earthen tubs were filled with amended soil, (8 kg per tub) allowed to stand for 15 days and then 3 slips were planted per tub. During rainy season transparent polythene sheets were erected over the plantation so that the rainwater could not cause flooding. Watering was done as

and when necessary with precaution so that the soil remains moist without flooding. For leaf, proline estimation was done at two growth stage viz. short term exposure (two month after transplantation) and long term exposure (nine month after transplantation). At both stage proline estimation was done separately for young leaf and old leaf. Newly emerged and the first leaf together were considered as young leaf while the 4th leaf and subsequent leaves were considered as old leaf. For root, proline estimation was made after long term exposure since the plants had to be uprooted for the purpose. *In vivo* proline estimation was made as per the method described by Sadasivam and Manikam (1992). The control set comprised of unamended soil and three replications were made for each treatment. The data were subjected to one way analysis of variance and CD values at 5 and 1% level were computed.

Results and Discussion

The results show that all the three heavy metal induced proline accumulation. Irrespective of the type of metal proline accumulation has been found to be linear dose dependent. It is noteworthy that cadmium treatment of 350 mg kg⁻¹ and above was lethal and so data were generated for cadmium treatment up to 200 mg kg⁻¹. It has been observed that under normal circumstances proline level in young leaves were higher than older leaves. This is evident from the fact that in control, for young leaf proline level was 0.805 μ moles g⁻¹ while the corresponding value for old leaves was 0.533 μ moles g⁻¹. Generally young leaf is metabolically more active where proline is actively synthesised and this appears to be the reason for this difference. It has been observed that irrespective of short term or long term exposure and for both young and old leaves proline accumulation following cadmium treatment were highest. Accordingly cadmium was the strongest inducer while lead was the least (Table 1). The general trend was that following short term exposure proline accumulation was little higher than that of long term exposure. However the impact of the three metals differed to some

extent. For instance for lead treatment exposure to low dose of 50 mg kg⁻¹ induced proline accumulation little higher than that of control; whereas at highest dose of 500 mg kg⁻¹ there were 5.6 and 4.1 times increase over control for young and old leaves respectively. Unlike lead, mercury even at the lowest dose induced 2.44 and 2.82 times more proline accumulation than that of control for young and old leaves respectively following short term exposure. Similar was the impact for cadmium also (Table 1).

Proline accumulation in root under normal condition was found to be little lower than that of leaves. Although there was linear dose dependent increase in proline accumulation in root for all the three metals, yet their magnitude was lower than the corresponding values for leaves. For instance, following treatment with lead, proline accumulation were 1.24 to 3.78 times higher than that of control for lowest and highest dose respectively. These values are much lower than the corresponding values for leaves. The situation was similar for mercury and cadmium (Fig. 1).

The present study showed that variation in proline accumulation due to heavy metal stress is metal specific. Irrespective of root or leaf cadmium induced maximum proline accumulation, followed by mercury and lead in lower order. Proline accumulation in plants following water stress is a well established fact (Yamada *et al.*, 2005) but there are evidences that abiotic stresses like toxic heavy metal stress also induce proline accumulation. Heavy metal induced proline accumulation has been reported in wheat (Lalk and Dorfling, 1985), *Vigna unguiculata* (Bhattacharjee and Mukherjee, 1994), tomato (De and Mukherjee, 1998) *etc.* Roy and Bera (2003) reported that, in mungbean heavy metal stress induced not only proline accumulation but phenol also and the same were found to be organ specific. In pea genotypic variation in proline accumulation was reported (Metwally *et al.*, 2008) indicating that it has a genetic basis. Tissue specific variation in proline accumulation under normal condition may be due to differences in metabolic activities in different tissues. There are reports that proline content in young leaf was higher than old leaf in *Amaranthus lividus* (Bhattacharjee and Mukherjee, 1995). Similarly in tomato, compared to cultured cell in seedling, proline level was higher under normal condition (De and Mukherjee, 1998). In the present study, compared to old leaf in young leaf proline level was higher and compared to roots leaf proline level was higher under normal condition. The tissue specific variation observed in the present study is in agreement with the earlier reported findings. Due to heavy metal stress proline accumulation profile may change depending upon the duration of exposure. Study with *Vigna* sp showed that following long term exposure proline level declined compared to that of short term exposure due to lead and cadmium stress (Bhattacharjee and Mukherjee, 1994). In the present study also such variation due to short and long term exposure was observed but the variation pattern was different for the three metals. For instance for lead, in case of young leaf proline accumulation profile remained more or less same irrespective of short and long term exposure while for old leaf proline accumulation increased following long term exposure. Saradhi and

Saradhi (1991) working with *Cajanus cajan* showed that of the four heavy metals *viz* cadmium, cobalt, zinc and lead; cadmium was the strongest inducer of proline accumulation while zinc was the weakest inducer. The fact that compared to other metals cadmium is a strong inducer for proline was observed in *Vigna radiata* (Arora and Saradhi, 1995), *Vigna unguiculata* (Bhattacharjee and Mukherjee, 1994). In the present study also cadmium induced maximum proline accumulation in both young and old leaves followed by mercury and lead in lower order. Since roots are in direct and constant contact with metal amended soil it was expected that proline accumulation in root would be very high. However, in the present study proline accumulation in root was found to be lower compared to that of leaf (Fig. 1). On the other hand in *Vigna unguiculata* proline accumulation in root was found to be higher than that of leaf following exposure to lead and cadmium (Bhattacharjee and Mukherjee, 1994). One reason may be that in leaves, particularly young leaves proline level was higher because it is actively synthesized there. Another reason may be photoactivation of key enzymes involved in proline synthesis in leaves (Arora and Saradhi, 1995). The present findings are in corroboration with the report that heavy metal induced proline accumulation can be used as biochemical indicator of heavy metal pollution (Saradhi and Saradhi, 1991).

The exact mechanism of how proline accumulation helps the plant to cope up with heavy metal stress is difficult to elucidate. However, available evidences suggest that proline acts by protecting the key enzymes from being inactivated by toxic heavy metal ions. *In vitro* experiment involving cadmium and zinc showed that proline appear to protect enzymes like glucose-6-phosphate dehydrogenase and nitrate reductase against toxic effect of zinc and to a lesser extent by cadmium through a reduction of the free metal ions due to formation of metal proline complex. On the basis of this it is opined that the main function of metal induced proline accumulation may be associated with osmoregulation and enzyme protection rather than metal sequestration (Sharma *et al.*, 1998). Proline is not the only amino acid to be accumulated to a conspicuously high level due to metal stress. In *Alyssum* enhanced production of histidine has been found to be associated with nickel hyperaccumulation (Kramer *et al.*, 1996). On the other hand cadmium stress is known to induce accumulation of cysteine in *Arabidopsis thaliana* (Dominguez Solis *et al.*, 2004). However, proline accumulation is not the only mechanism to cope up with heavy metal stress, because it is a well established fact that many plant respond to heavy metal stress by synthesizing a class of metal binding proteins called phytochelatin (Woolhouse, 1983; Steffens, 1990). Apart from these, plants try to cope up with metal stress by synthesizing some other amino acids, oligopeptides, betaine, nicotianamine, polyamines *etc.* and out of these polyamines function as signalling and antioxidant agent (Sharma and Dietz, 2006). The present study showed that proline accumulation can be used as a biochemical indicator for heavy metal stress in lemongrass and that cadmium is the strongest inducer of proline accumulation. From the present study it appears that young leaf is the ideal organ to assess proline accumulation. The notable aspect of the present study is that over long term exposure proline accumulation decreases indicating

that over long term, the plants can possibly cope up with metal stress, which deserve further study.

References

- Arora, S. and P.P. Saradhi: Light induced enhancement in proline levels in *Vigna radiata* exposed to environmental stresses. *Australian J. Plant Physiol.*, **22**, 383-386 (1995).
- Bhattacharjee, J. and A.K. Mukherjee: Divalent calcium in heat and cold stress induced accumulation of proline in *Amaranthus lividus* Linn. *Geobios*, **22**, 203-207 (1995).
- Bhattacharjee, S. and A.K. Mukherjee: Influence of cadmium and lead on physiological and biochemical responses of *Vigna unguiculata* (L.) Walp. Seedling germination behaviour, total protein, proline content and protease activity. *Pollut. Res.*, **13**, 269-277 (1994).
- De, B. and A.K. Mukherjee: Mercury induced metabolic changes in seedlings and cultured cells of tomato. *Geobios*, **23**, 83-88 (1998).
- Deka Boruah, H.P.: Biochemical and Physiological Assessment of the Impact of Heavy Metal Pollutants on *Java citronella*. Ph.D Thesis, Gauhati University (1999).
- Dominguez Solis, J.R., M.C. Lopez Martin, M.C. Ager, M.D. Ynsa, L.C. Romero and C. Gotor: Increased cysteine availability is essential for cadmium tolerance and accumulation in *Arabidopsis thaliana*. *Plant Biotechnol. J.*, **2**, 469-476 (2004).
- Klein, D.: Mercury and other metals in urban soil. *Environ. Sci. Technol.*, **6**, 560-562 (1972).
- Kramer, U., J.D. Cotter-Howells, J.M. Charnock, A.J.M. Baker and J.A.G. Smith: Free histidine as a metal chelator in plants that accumulate nickel. *Nature*, **379**, 635-638(1996).
- Lalk, I. and K. Dorfling: Hardening, ABA, proline and freezing resistance in the winter wheat varieties. *Physiol. Plant*, **63**, 287-292 (1985).
- McGrath, S.P.: Phytoextraction for Soil Remediation. Plants That Hyperaccumulate Heavy Metals (Ed.: Robert, R. Brooks). CAB International (1998).
- Mejare, M. and L. Bulow: Metal binding proteins and peptides in bioremediation and phytoremediation of heavy metals. *Trends in Biotechnology*, **19**, 67-73 (2001).
- Metwally, A., V.I. Safronova, A.A. Belimov, K.J. Dietz: Genotypic variation of the response to cadmium toxicity in *Pisum sativum*. *J. Exp. Bot.*, **56**, 167-178 (2008).
- Roy, Samadrita Barman and A.K. Bera: Individual and combined effect of mercury and manganese on phenol and proline content in leaf and stem of mungbean seedlings. *J. Environ. Biol.*, **24**, 78-81 (2003).
- Sadasivam, S. and A. Manikam: Biochemical Methods for Agricultural Sciences, Wiley Eastern Ltd., New Delhi (1992).
- Saikia, D.K., R.P. Mathur and R.K. Srivastava: Heavy metals in water and sediments of upper Ganga. *Ind. J. Environ. Hlth.*, **31**, 11-17 (1988).
- Salt, D.E., M. Blaylock, N.P.A. Kumar, V. Dushenkov, B.D. Ensley, I. Chet, and I. Raskin: Phytoremediation – A novel strategy for removal of toxic metals from environment with plants. *Biotechnology*, **13**, 468-474 (1995).
- Saradhi, A. and P.P. Saradhi: Proline accumulation under heavy metal stress. *J. Plant Physiol.*, **138**, 554-558 (1991).
- Sharma, Shanti S. and Karl-Josef Dietz: The significance of amino acids and amino acid-derived molecules in plant responses and adaptation to heavy metal stress. *J. Exp. Bot.*, **57**, 711-726 (2006).
- Sharma, S.S., H. Schat and R. Voojjs: *In vitro* alleviation of heavy metal induced enzyme inhibition by proline. *Phytochemistry*, **49**, 1531-1535 (1998).
- Singh, H.P., L.R. Mahaver and J.P. Mishra: Heavy metals in sediment and water in the river Ganga. *J. Assam Sci. Soc.*, **34**, 52-56 (1992).
- Steffens, J.C.: Stress response in plants: Adaptation and acclimatization mechanisms. Wiley Liss Inc., New York. p. 377 (1990).
- Woolhouse, H.W.: Encyclopaedia of Plant Physiology. Vol. 12C, New Series, Springer-Verlog, New York. p. 245 (1983).
- Yamada, M., H. Morishita, K. Urano, N. Shiozaki, K. Yamaguchi, K. Shinozaki, Y. Yoshiba: Effects of free proline accumulation in petunia under drought stress. *J. Exp. Bot.*, **56**, 1975-1981 (2005).
- Zengin, F.K. and S. Kirbag: Effects of copper on chlorophyll, proline, protein and abscisic acid level of sunflower (*Helianthus annuus* L.) seedlings. *J. Environ. Biol.*, **28**, 561-566 (2007).