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Lead and Cadmium Toxicity on Seedling Growth and Metabolism of *Trigonella foenum-graecum* L.

Mallika Mukherjee

Department of Botany, UGC Centre of Advanced Study, The University of Burdwan, Golapbag, Burdwan-713104, West Bengal, India

Abstract: ***The present experiment was carried out to investigate the toxic effect of lead and cadmium on seedling growth and metabolism of medicinally important plant Trigonella foenum-graecum L. Physio-morphological changes was studied at early seedling growth under different concentrations (5, 10, 15 mg/l) of lead and cadmium. This metal showed toxic effect on seedling length, root-shoot ratio, dry weight accumulation, seedling vigour index. On the other hand, content of photosynthetic pigments viz. chlorophyll a, chlorophyll b and carotenoid were decrease in a dose dependent manner in both heavy metal solution. The content of total free amino acids, soluble proteins and soluble and insoluble carbohydrate were initially increased as compared to control but at higher concentration in both the metal treatment showed decreased in content. Oxidative stress induced by heavy metals in plant causing membrane injury as observed by enhanced level of malondialdehyde (MDA) and in all experiments, higher concentration of cadmium showed the maximum toxicity as compared to lead.***

Keywords: Lead, cadmium, seedling vigour index, *Trigonella foenum-graecum* L., malondialdehyde (MDA)

1. Introduction

Heavy metals are defined as metals with a density higher than 5 gcm^{-3}. Because they cannot be degraded or destroyed, heavy metals are persistent in all parts of the environment. Many heavy metals are naturally present in the environment. Various industrial activities which was widespread nowadays, arises environmental pollution by heavy metals (Arvind and Prasad, 2005).

Presence of heavy metals like Cd, Cu, Pb, Cr, Hg, Ni & Co in air, soil and water affect the entire ecosystem through bioaccumulation resulting hazardous health consequences in all life forms (Sethy and Ghosh, 2013). Plants growing on metal polluted sites exhibit altered metabolism, growth reduction, lower biomass production and metal accumulation. Lead and cadmium are more hazardous, nonessential and important pollutant among various heavy metals. Interaction of lead and cadmium ions with the functional groups of proteins, nucleic acids and polysaccharides and substitution of other metal ions already bound to these functional groups by these two ions can lead to metabolic disorders and reduction in growth (Costa and Spitz, 1997; Seregin and Ivanov, 2001).

Trigonella foenum - graecum L. (fenugreek) or commonly as 'methi' is an important spice as well as medicinal plant and cultivated as annual, winter legume in India, can be grown easily in low-input, marginal environment, and are promising sources of calories, proteins, B-vitamins and minerals. It is cultivated mainly in India, Pakistan, Bangladesh, Argentina, Egypt, France, Yemen, Spain, Turkey, Morocco and China and India is the largest producer in the world (Basu, 2006). The plant is aromatic, herbaceous, annual herbs and a self pollinated. Every part of this plant is utilized as leafy vegetables, fodder and condiments (Nadkarni, 1976). The seeds contain major alkaloid trigonelline which produced hypoglycaemic activity (Shani et al., 1974) and diosgenin. Fenugreek seeds can be considered as good supplement to sereals because of its high protein, lysine, insoluble and soluble dietary fiber and also rich in calcium, iron and beta-carotene (NIN report, 1987). Hence in the present investigation, the effect of lead and cadmium onmethihas been monitored in terms of morphological and physio-biochemical responses at early seedling growth.

2. Material and Methods

The seeds of *Trigonella foenum-graecum* L. cv. Pusa Early Branching were collected from the Maharashtra State Seeds Corporation Ltd., Mahabeej Bhavan, Krisna Nagar, Akola.

Different concentrations (5, 10 and 15 mg/L) of $Pb(NO_3)_2$ and $CdCl_2$ solutions were prepared with sterile double distilled water. For the control set (0) only pure double distilled water was used.

Healthy dry seeds → 0.1% $HgCl_2$ for 90 seconds.(surface sterilization) → soaked in different test solutions and control with 3 replications for 11hrs. → 20 seeds kept in each Petri dish lined with double layer of filter paper (Whatman No. 1) at 23 ± 1^0C.

2.1 Estimation of plant growth and biochemical parameters:

Germinated seeds were transferred to the plastic pots containing sterilized sand - saw dust (6:4) mixture. Data on growth parameters such as seedling length, root-shoot ratio, dry weight accumulation, seedling vigour index were recorded in 15 days old seedlings.

2.2 Analysis of chlorophyll a, chlorophyll b and carotenoid:

Chlorophyll pigments were extracted and estimated according to the formulas of Lichtentaler and Wellburn (1985) slightly modified by Dere et al. (1998). 100mg of fresh leaves were homogenized using 5 ml of 95% (v/v) methanol. The green slurry was centrifuged at 4000rpm for

10 minutes. The supernatant was taken in the test tubes. The residual pigments were re-extracted using 5 ml of same methanol and centrifuged as same as earlier. This supernatant was added to the previously collected supernatant. The optical density was recorded at 666 nm, 653 nm and 470 nm against 95% (v/v) methanol as blank in UV – Visible Spectrophotometer. Chlorophyll and carotenoid contents were expressed in mg / g of fresh weight. The amount of pigments present in the pigment extract was determined employing the following formulas:

$C_a = 15.65 \times A_{666} - 7.340 \times A_{653}$

$C_b = 27.05 \times A_{653} - 11.21 \times A_{666}$

$C_{x+c} = 1000 \times A_{470} - 2.860 \times C_a - 129.2 \times C_b/245$

[C_a = chlorophyll a, C_b = chlorophyll b, C_{x+c}= total carotenoid.

2.3 Estimation of total free amino acid and total soluble protein:

Freshly collected 100 mg leaf samples were homogenized with 80% boiling ethanol and then centrifuged at 5000 rpm for 10 minutes.

Quantitative analysis of total free amino acids was done by the following method of Moore and stein (1948), modified by Bhattacharjee (1984) using ether and 0.1% % ninhydrin solution. The absorbance of the solution was measured with a UV-Visible spectrophotometer at 580 nm. The quantitative estimation was made by using the OD values from a standard curve prepared from glycine. Total free amino acids contents were expressed in mg/g fresh weight.

Quantitative analysis of total protein was done by the following method of Kar and Mishra (1976) using 10% (w/v) cold trichloroacetic acid (twice), ethanol (once), ethyl alcohol:chloroform (3:1, v/v,), ether, NaOH and finally the Folinciocalteau reagent and measuring the OD values at 650nm according to the method of Lowry et al. (1951). Quantitative determination was made by comparing the OD values with a standard curve previously prepared using Bovine serum albumin (BSA). Content of protein was expressed as mg/g of fresh weight.

2.4 Estimation of soluble and insoluble carbohydrate:

Quantitative analysis of soluble and insoluble carbohydrate was determined by the method of Mc Cready *et al.* (1950), after modifications. 100 mg of fresh leaf tissues were thoroughly homogenized using 5 ml boiling 80% ethanol and centrifuged at 5000 rpm for 10 minutes. After dilution of the supernatant, for soluble carbohydrate analysis, 1 ml sample and 3 ml freshly prepared, precooled, 0.2% anthrone reagent was taken in a test tube. For insoluble sugar the residue was digested with 5 ml 25% H_2SO_4 at 80^0C in a water bath for 30 minutes. The extracted material was taken and diluted 10 times with double distilled water and used as a source of insoluble carbohydrate. After that 1 ml of diluted sample was taken in test tube and insoluble carbohydrate level was determined with 0.2% anthrone reagent were added. In both cases after 30 minutes the intensity of green colour (OD) was measured in a UV-Visible spectrophotometer at 620 nm. The quantitative estimation was made by using the OD values from a standard curve prepared from glucose and the sugar contents were expressed in mg/ g fresh tissue.

2.5 Estimation of MDA content

The MDA content was estimated using the procedure of Heath and Packer (1968). Freshly collected 100 mg of leaf tissues were homogenized in a 5 ml solution of 1% Trichloroacetic acid (TCA), followed by centrifugation at 10000 rpm for 5 min. The test solution content1 ml of enzyme extract and 3 ml of 5% TCA containing 1% thiobarbituric acid (TBA) were mixed and the mixture was heated in a hot water bath at 95^0 C for 30 min. The reaction mixture was again centrifuged at 5000 rpm for 5 min. and the absorbance was measure at 532 nm and 600 nm in a spectrophotometer against a blank. The non-specific turbidity if any was corrected by subtracting A_{600} from A_{532} values. The concentration was calculated from its extension coefficient of 155 μM^{-1} cm^{-1} by the formula employed as;

$$\text{MDA content} = \frac{\text{Absorbance X Total volume (ml) x100}}{\text{Extinction coefficient x Vol. of Sample (ml) x weight of Plant tissue}}$$

The MDA concentration was finally expressed in nmol per g fresh tissue.

3. Statistical Analysis

Data were analyzed statistically for determining mean and standard errors and statistical significance using analyses of variance (ANOVA) following Panse and Sukhatme (1978).

4. Results and Discussions

Table 1: Root length (cm), shoot length (cm), root-shoot ratio, seedling vigour index and dry weight accumulation/g of fresh weight (g) of seedlings raised from the seeds pretreated with different concentrations of lead (Pb) and cadmium (Cd). [Each value is mean (n=3) ±S.E.]

Treatments	Concentrations (mg/l)	Root length (cm)	Shoot length (cm)	Root-Shoot ratio	Seedling vigour index	Dry weight accumulation/g of fresh weight (g)
Control	0	8.46±0.02	10.34±0.02	0.81±0.004	987.93±8.41	0.34±0.004
Lead	5	7.85±0.04	9.29±0.04	0.84±0.008	846.59±11.81	0.32±0.02*
	10	6.55±0.03	9.03±0.02	0.72±0.001	782.74±10.76	0.31±0.01
	15	5.82±0.02	8.53±0.03	0.68±0.005	688.15±7.33	0.29±0.01
Cadmium	5	5.83±0.04	8.63±0.04	0.67±0.001	696.85±7.47	0.32±0.01*
	10	5.63±0.03	8.05±0.03	0.69±0.001	584.85±14.32	0.30±0.01
	15	5.43±0.06	7.92±0.04	0.68±0.004	539.81±7.27	0.27±0.01
CD at 5% level		0.1236	0.1373	0.2962	38.1720	0.0420

* Significant at 5% level

Table 2: Chlorophyll a and b, carotenoid, free amino acid, protein, soluble and insoluble sugar content of seedlings raised from the seeds pretreated with different concentrations of lead (Pb) and cadmium (Cd). [Each value is mean (n=3) ±S.E.]

Insoluble carbohydrate content (mg/g of fresh tissue)	9.50±0.01	13.0±0.02	18.75±0.06	11.00±0.06	9.75±0.03	18.50±0.23	8.50±0.05	0.3869
Soluble carbohydrate content (mg/g of fresh tissue)	6.50±0.06	7.25±0.06	6.50±0.05	6.0±0.02	16.0±0.14	11.0±0.05	10.50±0.05*	0.2718
Protein content (mg/g of fresh tissue	2.15±0.001	3.15±0.01	2.55±0.01	2.50±0.02	2.95±0.01	2.70±0.01	2.30±0.01	0.0391
Free amino acid content l(mg/g of fresh tissue)	1.75±0.01	1.80±0.01	2.75±0.004	1.90±0.01	2.05±0.01*	2.65±0.01	1.75±0.01	0.0882
Carotenoid content (mg/g of fresh tissue)	2.14±0.07	2.09±0.09	2.0±0.08	1.97±0.009	2.11±0.03	1.95±0.04	1.89±0.02	0.3435
Chlorophyll b content (mg/g of fresh tissue)	9.24±0.10	9.0±0.10	8.93±0.03	8.67±0.03	8.54±0.05	8.54±0.05	7.66±0.06	0.2746
Chlorophyll a content (mg/g of fresh tissue	8.17±0.09*	7.66±0.14	7.33±0.12	7.11±0.12*	6.94±0.21**	6.94±0.21**	6.25±0.14	0.5414
Concentration(mg/l)	0	5	10	15	5	10	15	
Treatments	Control	Lead			Cadmium			CD at 5%level

The results revealed that a gradual decline took place with rise of doses of both the metals in the plant growth at seedling stage. Both lead and cadmium showed negative effects on lengths of root and shoot, seedling vigour index, root-shoot ratio and seedling vigour index. Shoot length and root length are considered as very useful indicator of metal toxicity in plants. Perveen et al. (2011) pointed out that Cd reduced plant growth as well as plant length, dry weight, possibly by changes in water and nutritional status of plants. The inhibitory effect of Cd ions on root elongation is mediated through the altered cell growth. After being attached with cell walls and middle lamella, Cd enhances the cross-linking among the wall components inhibiting cell growth as well as root elongation. Prasad (1995) pointed that Cd affected directly or indirectly on auxin metabolism or auxin carriers resulting inhibition of growth by disturbing cell division and cell expansion.

Result exhibited that there was a decline in chlorophyll and carotenoid content in heavy metals treated plants and cadmium shows the highest effect than the lead in comparison to control. John et al. (2008) pointed out that reasons for declining chlorophyll biosynthesis in plants under Pb and Cd stress were (i) inhibition of important enzymes for chlorophyll biosynthesis, such as delta-aminolevulinic acid dehydratase (ALA-dehydratase) and protochlorophyllide reductase. (Van Assche and Clijsters 1990 and Kupper et al., 1996) (ii) adversed effect on the supply of Mg2and Fe2+ required for chlorophyll biosynthesis (iii) deficiency of Zn2+ resulting in the inhibition of carbonic anhydrase (Van Assche and Clijsters 1990) (iv) Mg2+ ions, which is associated with tetrapyrrole ring of chlorophyll, is replaced by heavy metals. Interference of heavy metals with enzyme protochlorophyllide reductase, inhibit chlorophyll biosynthesis at protochlorophyllide stage (Bhattacharyya and Choudhari, 1994).

Results revealed that content of total free amino acids significantly increased initially and after that decreased at the maximum concentration of both Pb and Cd. Protein content was also found to increase at the initial concentration of both the Pb and Cd after that it was decreased. Protein degradation occurs as a result of increased protease activity (Palma *et al.*, 2002) which was found under stressed conditions. Increase in proteolytic activity in response to heavy metal stress was found by Lee *et al.* (1976).Some toxic effect of reactive oxygen species due to heavy metal stress, may be the cause of fragmentation of proteins which reduced the protein content. Reasons for reduced levels of protein in the plant raised from seeds pretreated with by heavy metal stress a) reduction in soluble protein level due to extensive hydrolysis of protein b) catalytic activity of different metals c) any stressed conditions d) enhanced protein degradation process e) decreased availability of amino acids and denaturation of enzymes involved in protein synthesis.

Results shows the content of soluble carbohydrate was found to increase initially over the control in Pb and Cd treated plants and then decline except Cd treatment at 15 mg/l concentration showed the higher content than the control.On the other hand, insoluble carbohydrate increased over the control in Pb and Cd treated seedlings with maximum at 10 mg/l concentration but it decreased below the content found in control at higher Cd concentration (15 mg/l). John *et al.* (2008) opined that the photosynthetic inhibition or stimulation of rate of respiration probably caused the decrease in total sugar content in plants under Pb and Cd stress. Heavy metals react with ribulose bisphosphate carboxylase resulting reduction of carbon metabolism (Stiborova *et al.*, 1987).Reduction in total sugar content of the stressed leaves is due to the inhibition of photosynthesis or stimulation of respiration rate.

Tested heavy metals induced significantly enhancement of lipid peroxidation in a dose dependent manner and cadmium showed the maximum effect. MDA is a low molecular weight end product formed by the decomposition of certain primary and secondary lipid peroxidation, is an oxidative stress indicator (Saffer *et al.*, 2009).Increased Cd concentration is correlated with increased lipid peroxidation. MDA content was markedly affected by the highest concentration of cadmium in *Trigonella foenum-graecum* L. In lower concentrations *Trigonella* seedlings showed capability to adapt and produce lower amount of MDA but in higher concentrations MDA concentrations increases in Cd exposure. The results suggested that heavy metals create excessive generation of superoxide radicals by deficient

antioxidant defenses which result increase in lipid peroxidation and oxidative stress in *Trigonella* (Bhat *et al.*, 2012).

5. Conclusion

The study shows that cadmium had more toxic effect than lead on various growth factors of *Trigonella foenum-graecum* L. Further research is needed at molecular level to understand theadaptive mechanism of this important medicinal plant under heavy metal stress.

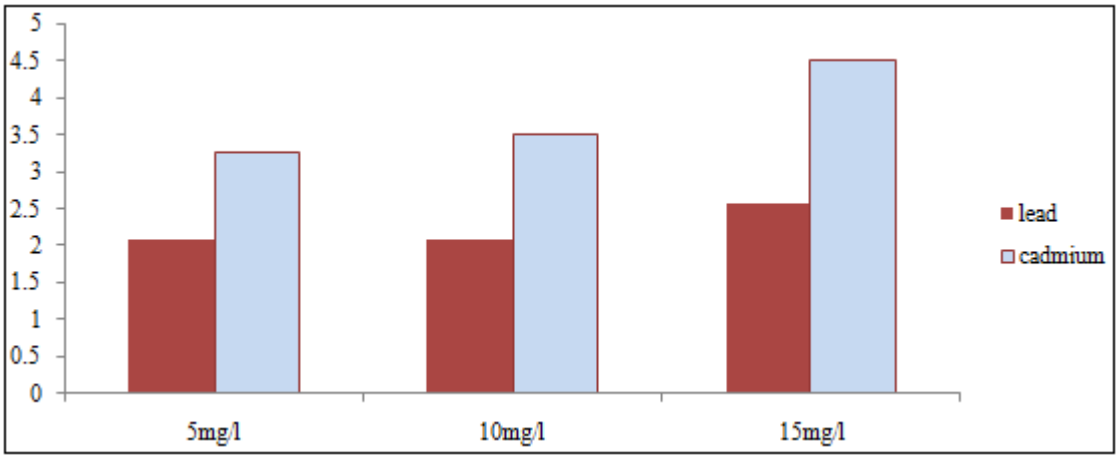

Figure 1: Effect of lead and cadmium on MDA content of the 15 days old leaves.

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Author Profile

Mallika Mukherjee was the former research scholar in the Dept. of Botany, The University of Burdwan.