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INFLUENCE OF CADMIUM AND LEAD ON PHYSIOLOGICAL AND BIOCHEMICAL RESPONSES OF *SESAMUM INDICUM* (L) SEEDLINGS. II. *CELL INJURY*, PIGMENT, SUGAR, NUCLEIC ACID CONTENT AND PEROXIDASE ACTIVITY

P.K. MISRA

Ex-Department of Chemistry, Ravenshaw College, Cuttack-753003 (Odisha)

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Physiological and biochemical responses of Sesamum Indicum (L) seedlings under the influence of Cadmium and Lead were investigated with reference to the cell injury, photosynthesis pigment contents, variation in soluble and insoluble sugar contents, changes in nucleic acid contents and peroxidase activity. With the increasing concentration of both the metals cellular damage was proment. Pigments like chlorophyll a and b and Carotenoids also decreased. The total soluble sugar increased with the increasing concentration of heavy metals but the insoluble sugar decreased. In case of both Cd and Pb treatment the activity of peroxidase clearly decreased in root, stem and leaf. RNA increased remarkably after 24h of treatment and later started decreasing, but a decreasing trend in DNA content was recorded from the beginning of the treatment. Cadmium was more toxic than Lead and the root was the most sensitive part when compared with stem and leaf.

INTRODUCTION

Seed germination is largely affected by toxic concentration of heavy metals (Nag *et al*., 1980). It has been found that toxicity imposed by the heavy metals involves an overall disruption in the synchronization of different metabolic processes occurring in the cells, the resultant effect being manifest in the inhibition of cell division and consequent retardation of growth (Nag *et al*., 1981).

Excess of copper and chromium in the growing medium caused a drastic reduction in the growth of diatom *Nitzschia palea* and green alga *Chlorella pyrenoidosa* and this phenomenon was attributed to a permanent injury to the photosynthetic mechanism (Nielsen and Kamp-Nielson, 1970; Nielsen and Wium-Anderson, 1971; Nielsen *et al*, 1969 and Wium-Andersen, 1974). Mercury also behaved similarly to copper as poison to biological material (Kamp-Nielsen, 1971). RNA, DNA and protein contents greatly reduced both in embryo and endosperm with increasing concentrations of lead and with concomitant increase in amino acid content in rice embryo (Maitra and Mukherjee, 1979).

In the present investigation an attempt has been made to study the toxic effect of two heavy metals, cadmium and lead on the cell membrane injury and changes of some biochemical parameters in a tropical legume crop *Sesamum Indicum* (L) which is considered as one of the important pulses in our country and reputed for its higher protein content.

Materials and methods

Seeds of *Sesamum Indicum* (L) Walp. Were obtained from Sutton Seed Company of Calcutta. Seeds were always surface sterilized with 0.1% $HgCl_2$ for 5 minutes and washed with distilled water for 15' × 3 times and ultimately allowed to imbibe in distilled water for 12 h. Imbibed seeds were allowed to germinate in Petri plates on filter papers soaked with varied concentrations of $CdCl_2$ and $PbCl_2$ (1 μM, 10 μM, 100 μM and 1000 μM). Germination was allowed in darkness and at a temp. of 25°C $\pm$ 2°C and relative humidity of 78% $\pm$ 2%. This was considered as initiation of the experiment.

The injury index of leaf and root was one of the subjects of present investigation. For the injury index of leaf, 30 leaf were taken in a vial containing 20 ml deionized water and were incubated at 25°C $\pm$ 2°C for 24 h. The electrical conductivity of the leachet was measured in a direct reading conductivity meter. For the root, 3 pieces weighing 0.25 g were treated in the same way.

The tissue along with the leachatl was then autoclaved and the total electrolytes were measured in terms of conductivity. The injury index was calculated according to Asare-Boamah and Fletcher, 1986 as

$$\% \text{ injury} = \frac{1 - T_1 / T_2}{1 - C_1 / C_2} \times 100$$

When,

C_1 and C_2 = Conductivity of the control (untreated) sample before and after autoclaving.

T_1 and T_2 = Conductivity of the chemical-treated sample before and after autoclaving.

Photosynthetic pigments like chlorophyll a and b and carotenoids were extracted and estimated following the method of Sadasivam and Manikam, 1992 in 7 days old seedlings.

Extraction and estimation of soluble and insoluble sugar and done as per procedure of Jayaraman, 1985. For determination of peroxidase activity the method of Kar and Mishra, 1976 was followed here. Nucleic acids were extracted and estimated following the method of Jayaraman, 1985.

Throughout the investigation, the 7 days old (168 h) seedlings were used except in case of nucleic acid estimation where the study was initiated at 24 h and continued at each 48 h interval upto 168 h.

Results

While analyzing the effect of various concentrations of $CdCl_2$ and $PbCl_2$, it was seen that the injury was more in case of increasing concentration of both the heavy metals. The results also reflected that the per cent injury was more in case of root than in leaf and the effect of Cd was also greater in contrast to Pb.

Photosynthetic pigments like chlorophyll *a* and *b* and carotenoids showed a decreasing trend with the increasing concentration of Cd and Pb. No remarkable difference between the results of Cd and Pb treatment was noticed (Table 1).

Table 1. Influence of $CdCl_2$ and $PbCl_2$ on photosynthetic pigment contents of *Sesamum Indicum* (L) seedlings (7 days old)

Concentration (M)	Percent increase or decrease (-) over control					
	Chlorophyll a		Chlorophyll b		Carotenoids	
	Cd	Pb	Cd	Pb	Cd	Pb
0 μM	1.48	1.42	0.64	0.64	0.28	0.28
1 μM	1.15	1.35	0.38	0.58	0.25	0.24
10 μM	0.84	0.68	0.26	0.26	0.18	0.18
100 μM	0.54	0.64	0.18	0.18	0.16	0.14
1000 μM	Lethal	0.51	Lethal	Lethal	Lethal	0.08

The soluble sugar increased with the increasing concentration of Cd and Pb, while the insoluble sugar steadily decreased. When the increase/decease in root, stem and leaf was compared, root was seen as the most responsive tissue in contrast to leaf and stem. Also, Cd effect in the changing pattern of both soluble and insoluble sugar was more than the influence of Pb (Table 2 and 3).

Table 2. Influence of $CdCl_2$ on sugar content (mg/g fresh wt.) of *Sesamum Indicum* (L) seedlings (7 days old)

Concentration (M)	Amount of pigment (mg/g fresh wt.)					
	Soluble Sugar			Insoluble Sugar		
	Root	Stem	Leaf	Root	Stem	Leaf
1 μM	25.74	12.34	13.44	-3.94	-2.23	-2.99
10 μM	64.16	40.28	38.33	-9.44	-6.94	-7.48
100 μM	86.74	56.33	57.95	-14.35	-10.33	-9.83
1000 μM	Lethal	Lethal	Lethal	Lethal	Lethal	Lethal

Table 3. Influence of $PbCl_2$ on sugar content (mg/g fresh wt.) of *Sesamum Indicum* (L) seedlings (7 days old)

Concentration (M)	Amount of pigment (mg/g fresh wt.)					
	Soluble Sugar			Insoluble Sugar		
	Root	Stem	Leaf	Root	Stem	Leaf
1 μM	24.35	18.39	19.20	-4.64	-2.40	-3.04
10 μM	51.00	29.29	27.18	-10.38	-10.80	-11.18
100 μM	68.38	42.63	44.38	-13.75	-12.23	-13.20
1000 μM	81.29	59.08	60.04	-16.85	-14.00	-13.93

Peroxidase activity decreased in case of both the heavy metal (Cd & Pb) treatments and the results were more or less comparable except at the concentration of 100 μM when the effect of Cd was more pronounced. No remarkable difference in case of root, stem and leaf was recorded (Table 4).

When the seedlings were treated with both $CdCl_2$ and $PbCl_2$, initially after 24h the amount of RNA increased (Table 5 & 6). But, no further remarkable change compared with the value at 23 h was noticed and instead, a decreasing pattern was evident with the prolonged duration of treatment. The increased concentration of the heavy metals, here, could not produce steady increase of RNA quantity always. The maximum quantitative increase of RNA was at 10 μM concentration of both Cd and Pb treatment. But, on the other hand, DNA decreased steadily with the enhanced concentration of both Cd and Pb as well as with the longer duration of treatment (upto 168 h).

Table 4. Influence of $CdCl_2$ on peroxidase activity/g fresh wt./h in *Sesamum Indicum* (L) seedlings (7 days old)

Concentration (M)	Percent increase or decrease (-) over control					
	Root		Stem		Leaf	
	Cd	Pb	Cd	Pb	Cd	Pb
1 μM	12.35	10.40	12.44	10.05	10.04	9.70
10 μM	18.45	25.36	17.33	23.96	14.39	20.60
100 μM	54.93	26.75	48.18	25.33	40.33	25.33
1000 μM	Lethal	36.64	Lethal	32.34	Lethal	30.34

Table 5. Influence of $CdCl_2$ on nucleic acid content (mg/g fresh wt.) of *Sesamum Indicum* (L) seedlings (7 days old)

Concentration (M)	Per cent increase or decrease (-) over control after							
	24 h		72 h		120 h		168 h	
	DNA	RNA	DNA	RNA	DNA	RNA	DNA	RNA
1 μM	-2.33	24.25	-8.54	25.29	-20.54	19.39	-18.59	10.08
10 μM	-11.63	42.43	-17.03	39.48	-25.65	37.29	-31.26	30.38
100 μM	-25.59	24.26	-24.28	26.34	-25.63	32.56	-37.59	24.25
1000 μM	Lethal	Lethal	Lethal	Lethal	Lethal	Lethal	Lethal	Lethal

Table 6. Influence of $PbCl_2$ on nucleic acid content (mg/g fresh wt.) of *Sesamum Indicum* (L) seedlings (7 days old)

Concentration (M)	Per cent increase or decrease (-) over control after							
	24 h		72 h		120 h		168 h	
	DNA	RNA	DNA	RNA	DNA	RNA	DNA	RNA
1 μM	-2.04	20.24	-8.09	19.74	-12.69	20.48	-15.39	16.79
10 μM	-10.04	39.94	-16.83	40.28	-21.33	35.98	-26.78	22.38
100 μM	-20.43	21.33	-19.28	19.33	-23.45	17.33	-28.90	14.38
1000 μM	-25.08	19.48	-22.38	19.90	-24.34	17.06	-29.34	14.09

Discussion

Quantitative changes in pigments and determination of injury index are considered as the most important parameters among others to assess the disorganization and metabolic injury to the tissues. In case of treatment with Cd and Pb, the injury was more with greater concentrations, which can be attributed to change in membrane permeability (Nag *et al*., 1980). The more injury in root tissue than in stem and leaf might be due to the heavy metals reaching the shoot is usually lower than the amount left in the roots because of formation of Cd binding proteins and binding of Cd to cell walls in the roots (Brinkhuis *et al*., 1980; Gregar and Lindberg, 1986; Rohinson and Jackson, 1986; Greger and Johansson, 1992). Fadmium depresses plant growth, especially that of roots, so that the root absorption area decreases (Greger and Johansson, 1992).

Impaired chlorophyll development by heavy metal may be due to the interference with the synthesis of proteins, the structural components of chloroplasts. Treatment with $CdCl_2$ and $PbCl_2$ presumably block the synthesis of activities of enzyme proteins responsible for chlorophyll biogenesis. Detached corn and sunflower leaves exposed to various concentration of Cadmium exhibited reduced photosynthesis and transpiration (Bazzaz *et al*., 1974). Impaired chlorophyll development by heavy metals may be due to interference with the synthesis of proteins, the structural component of chloroplasts (Nag *et al*., 1981). Experimental data on the reduction in chlorophyll content with concurrent inhibition of photosynthesis by treatment with Hg, Cu and Zn are available with unicellular alga *Chlorella* (Nag *et al*., 1981). Apart from inhibition of Biosynthetic enzymes of chlorophyll formation, the increased activity of lipoxygenase may also contribute to the decreased level of chlorophyll with Cd^{2+} treatment (Somashekaraiah *et al*., 1992).

The accumulation of total soluble sugar could be the result of a greater degree of conversion of starch into soluble sugars and/or to low sugar utilization. Such a high soluble sugar content might lower the solute potential of heavy metal-stressed cells which could help them maintain turgidity in an adverse environment.

The accumulation of more peroxide means the increasing toxicity in the concerned tissues and normally the peroxidase activity remains high under such condition to combat the adverse situation. In our results, the fall in the activity of scavenging enzyme like peroxidase almost in all the cases correlates well with the mounting injury indeed and not in agreement with the earlier findings of Nag *et al*., 1980 where work on germinating rice seeds reveals that activity of enzyme like peroxidase increased at the toxic concentration of $HgCl_2$ and $PbNO_3$.

Growth rate of the germinating seeds is known to be controlled by RNA content (Maitra and Mukherjee, 1979) and the influence in RNA metabolism should be reflected in the growth rate. Conversely, the effects which are correlated with the growth rate are known to affect RNA metabolism. In our results, the quantity of RNA increased in maximum initially after 24 h and no further marked increment was noticed afterwards. Instead, the amount decreased with the prolonged duration of treatment. This might be due to initial shock the tissue immediately tried to fight against the adverse situation by synthesizing some new RNAs and proteins. When a steady state was attained, perhaps, there was no more any need of RNA level could be linked to decreasing synthetic capacity of the tissue. At the later period of treatment, some RNA might have been utilized and degraded. But under the influence of toxic effect of heavy metals like Cd and Pb fresh synthesis of DNA was inhibited and might have been degraded with the increasing concentration of Cd and Pb as well as with longer duration of treatment.

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