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# INFLUENCE OF CADMIUM AND LEAD ON PHYSIOLOGICAL AND BIOCHEMICAL RESPONSES OF *SESAMUM INDICUM* (L) SEEDLINGS. I. GERMINATION BEHAVIOR, TOTAL PROTEIN AND PROLINE CONTENT AND PROTEASE ACTIVITY

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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 germination behaviour, total protein and praline content and protease activity. The $CdCl_2$ at a concentration of 1000 μM was found to be completely lethal but $PbCl^2$ was not. Increasing concentration of both the metals decreased germination rate and the inhibition of root growth was more. With increasing concentration of Cd and Pb total protein increased upto 120 h and a decrease was prominent at 168 h. Activity of protease also decrease Proline accumulation was also more with increasing concentration of the heavy metals. No doubt, Cd was proved to be more toxic than Pb and the effect was maximum in roots and minimum in leaf.

## INTRODUCTION

Heavy metals play a vital role in the growth and development of plants. These may act as cofactors of some enzymes of help in the formation of intermediate metabolities. When excess amount of metals are absorbed by plants toxic effects are produced resulting in the impairment of growth, inhibition of respiration and abnormalities in cell division (Stiles, 1980) and the extent of injury being dependent on the concentration of the metal present.

It has been demonstrated that the toxic metals are capable of causing a reduction in the activity of hydrolases, viz., α-amylase, phosphates, RNase and protease in germinating seedlings; whereas the activities of catalase, peroxidase, IAA oxidase and ascorbic acid oxidase undergo considerable stimulation. Work on germinating rice seeds revealed that activities of some enzymes like catalase, peroxidase and IAA oxidase increased at the toxic concentration of $HgCl_2$ and $PbNO_3$ which synchronized with the parallel rise in the level of soluble protein (Nag *et al*., 1980). Excess copper in the germinating medium was shown to have similar effects on lettuce seedlings.

Root growth was inhibited in the presence of Lead in culture solutions (Maitra and Mukherji, 1979) and both intact plants and detached leaves supplied with lead exhibited reduced rates of photosynthesis and respiration. Cadmium, a non-essential toxic element, enters the environment through various industrial processes (Ernst, 1980 and Somashekaraiah *et al*., 1992) and to lesser extent from natural weathering (Denise *et al*., 1985). The presence

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of $Cd^{2+}$ in the environment has increased in some areas to levels which threatens the health of aquatic and terrestrial organisms. $Cd^{2+}$ was chosen as a probe metal ion because it is a wide-spread trace pollutant of high toxicity with a long biological half-life (Hilmy *et al*., 1985).

The aim of the present investigation was to check the influence of salts of Cd and Pb on germination behaviour and growth rate as well as on some biochemical responses of germinating *Sesamum Indicum* (L) were obtained from Sutton Seed Company, Calcutta. Seeds were surface sterilized with 0.1% $HgCl_2$ for 5 minutes and washed with distilled water (15' × 3 times), and finally imbibed in distilled water for 12 h. Water imbibed seeds were allowed to germinate in petri plates on filter papers soaked with different concentration of $CdCl_2$ and $PbCl_2$ (1 μM, 10 μM, 100 μM, 1000 μM) and this was considered as zero hour of the experiment.

Germination was allowed in darkness and at a temp. of 25°C ± 2°C and relative atmospheric humidity of 78% ± 2%. Germination was allowed to continue for 7 days (168 h) as was necessary for the present investigation. Percent germination, root/shoot length and total protein measurement were done in an interval of 48 hours; whereas, praline measurement and assay of protease activity were done at the end of the experiment (at 168 h) with 7 days old seedlings.

Proteins were extracted from the whole plant tissue as per the procedure described by Jayaraman, 1985 and estimated by Bradford's dye-binding method (Bradford, 1976). Quantitative estimate of proline was done following method of Sadasivan and Manikam, 1992 and assay of protease activity was according to method of Snell and Snell, 1971. The enzyme activity was determined according to Fick and Qualset, 1975.

*Seasum Indicum* (L) is a tropical pulse crop of immense importance for their higher protein contents and for the last two decades it is facing a problem of soil and water pollution by heavy metals. As this is a very common and useful legume, it was selected as our experimental material.

**Table 1a. Influence of $CdCl_2$ on seedling growth of *Sesamum Indicum* (L)**

| Concentration (M) | % decrease of root/shoot length over control | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 24 h | | 72 h | | 120 h | | 168h | |
| | Root | Shoot | Root | Shoot | Root | Shoot | Root | Shoot |
| 1 μM | 84.64 | 56.93 | 25.08 | 12.68 | 11.78 | 25.08 | 27.26 | 20.08 |
| 10 μM | 90.39 | 80.09 | 80.09 | 68.26 | 82.36 | 20.09 | 81.82 | 70.08 |
| 100 μM | 96.16 | 89.28 | 93.38 | 79.38 | 90.56 | 81.26 | 80.08 | 79.09 |

**Table 1b. Influence of $PbCl_2$ on seedling growth of *Sesamum Indicum* (L)**

| Concentration (M) | % decrease of root/shoot length over control | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 24 h | | 72 h | | 120 h | | 168h | |
| | Root | Shoot | Root | Shoot | Root | Shoot | Root | Shoot |
| 1 μM | 23.17 | 30.78 | 30.08 | 29.90 | 5.80 | 6.20 | 9.08 | 10.09 |
| 10 μM | 57.68 | 56.93 | 46.60 | 33.38 | 58.83 | 43.76 | 59.08 | 48.54 |
| 100 μM | 71.25 | 72.39 | 70.09 | 68.26 | 70.56 | 71.29 | 68.28 | 55.03 |
| 1000 μM | 100 | 100 | 93.38 | 84.13 | 88.25 | 75.09 | 90.95 | 75.09 |

# Results

The effect of various concentration of Cd and Pb on percent germination and seedling growth (Figs.1a, 1b and Tables 1a and 1b) showed that the Cd at a concentration of 1000 µM was absolute toxic in contrast to same concentration of both the metals deceased the germination rate. The most effective inhibitory concentration for Cd was 100 µM and for Pb, 1000 µM. At a later period (168 h), inhibitory effect was lesser in comparison with earlier periods. Comparatively, Cd was more inhibitory than Pb.

When the length of root and shoot of the seedlings was measured separately, increasing inhibition with increasing concentration of both the metals was evident. Inhibition of root growth was seen to be more than shoot growth in all the cases. The inhibitory effect of Cd was more pronounced than that of Pb.

There was a steady increase of total protein content upto 120 h and surprisingly enough, this amount decreased when seen at 168 h. When the increment of total protein in Cd-treated plant was compared to that of Pb-treated plant, Cd treatment seemed to be more effective in increasing total protein. There was also a steady increase with the increase in concentration upto 10 µM. No appreciable increase was noticed after that even though the concentration was increased upto 1000 µM. The decrease of protein at 168 h of treatment was more in Pb-treated plant than in Cd-treatment.

Activity of protease steadily decreased with the increase of concentration in case of both the metals and the rate was more in case of Cd treatment.

Proline accumulation was also more in case of Cd treatment with the increase of concentration and the response was highest in case of root and lowest in leaf (Table 2).

**Table 2. Effect of $CdCl_2$ and $PbCl_2$ on accumulation of proline (mg/g fresh wt.) in *Sesamum Indicum* (L) seedings (7 days old)**

| Concentration (M) | Percent increase over control | | | | | |
|---|---|---|---|---|---|---|
| | Root | | Stem | | Lead | |
| | Cd | Pb | Cd | Pb | Cd | Pb |
| 1 µM | 66.68 | 67.29 | 60.28 | 59.28 | 60.38 | 59.29 |
| 10 µM | 179.23 | 170.48 | 129.33 | 121.03 | 101.73 | 87.78 |
| 100 µM | 184.33 | 175.82 | 147.93 | 140.38 | 119.93 | 100.28 |
| 1000 µM | Lethal | 177.25 | Lethal | 141.93 | Lethal | 103.44 |

# Discussion

In case of germination and seedling growth, both Cd and Pb proved to be inhibitory and the effect of Cd was more pronounced. Photosynthesis and respiration in higher plants are highly sensitive to Cadmium and Lead and the $Cd^{2+}$ in particular inhibits chlorophyll biosynthesis by reacting with protochlorophyllide reductase and synthesis of 5-aminolevulinic acid (Bazzar *et al*., 1974 and Stobard *et al*., 1985). Interaction of heavy metals with functional –SH groups was generally proposed as the mechanism of inhibition for several physiological reactions (Shio *et al*., 1978 and Sandamann and Boger, 1980). In our findings it was seen that at a later period (168 h), inhibitory effect of Cd and Pb was lesser in comparison with that of the earlier period. This might be due to initial metablic impairment for the sudden shock and gradually the tissues triggered on their resistance mechanism inside and as a result, at the later period of germination the inhibitory effect as minimum. Also at a later period, the mobilization of protein from cotyledons to actively growing tissues might occur. While plant growth may be severely restricted by heavy metals, plants possess a unique ability to rapidly adapt and evolve tolerance to toxic or lethal levels of heavy metals (Steffens, 1990 and Woolhouse, 1983). Plant cells subjected to heavy metals rapidly synthesize a class of metal binding polypeptides whose function is to sequester and detoxify excess metal ions. Among the common metals, Cd is by far the strongest inducer of Physochelatins (Huang *et al*., 1987).

Study with the estimation of total protein also showed increasing pattern with prolonged duration of treatment and decreased at the later part (168 h). This might be due to the fact that with the longer duration the tissues reached in a state of extreme toxicity when the protein synthesizing system failed to function or might be some protein degrading mechanism came into work.

Proline accumulation in plant tissues can also be considered as a soluble nitrogen sink. Accumulation of praline upon dehydration due to water deficit or upon decreasing osmotic potential has been recorded in bacteria, algae and higher plants. More recently, some authors (Charest and Phan, 1990) have proposed that praline accumulation can play an important role in cellular pH control. Our results of praline accumulation in Cd-treated plants was more than that in Pd-treated plants and this might be due to some osmotic imbalance within the metal treated plants inhibiting the water transport system and leading to water deficit. As root is considered the tissue of primary response in comparison with shoot and leaf, the greater accumulation of praline in root might be due to that.

Fall in the protease activity of cotyledonary reserves as well as disruption in the rate of mobilization of precursors of protein production in the developing seeds causes an accumulation of proteins in the heavy metal treated seedlings. Our findings of decreasing protease activity with the increasing concentration of the metals and longer duration of treatment could be considered in the same perspective.

Cadmium was more toxic than lead in the growth *Sesamum Indicum* (L) seedlings and the effect was maximum in root than in shoot and leaf.

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