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The effects of heavy metals, Zn and Hg, on the growth and biochemical constituents of mungbean (*Vigna radiata*) seedlings

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Abstract. The effects of deleterious concentrations of $ZnSO_4$ and $HgCl_2$ were observed on the growth and biochemical constituents of germinating mungbean (*Vigna radiata*) seedlings. There was inhibition of seedling growth at all the concentrations of the chemicals used here. Gibberellic acid (GA_3), cyclic-3,5-adenosine monophosphate (cAMP) and kinetin were able to overcome partially the toxic effects of the pollutants. With increasing concentrations of $ZnSO_4$ and $HgCl_2$, there was progressive decline in the respiration rates of seedlings. Likewise, the levels of total nitrogen, total sugar, DNA and RNA declined in embryo with concomitant accumulation in the cotyledons. Gel electrophoretic studies of buffer soluble proteins isolated from polluted seedlings revealed a largescale disruption of protein bands by metal action resulting in an appreciable increase in the number of bands over those of the control. In contrast to control, the number of bands of basic proteins also increased in treatments. Rates of incorporation of ^{14}C-uracil, ^{14}C-thymine and ^{14}C-amino acid mixture into RNA, DNA and protein respectively were also lowered.

Key words: Growth reversal; Hg^{2+}; Nucleic acid and protein synthesis; Toxicity; *Vigna radiata*; Zn^{2+}.

Introduction

In a previous work, toxic action of zinc on rice seedlings was studied by Nag *et al*. (1984) who reviewed the subject and observed that deleterious concentrations of zinc brought about a significant retardation in the growth pattern of this material. They also reported that there was an increase in the levels of a few oxidizing enzymes like peroxidase, IAA oxidase and ascorbic acid oxidase and a distinct inhibition in the activities of a number of hydrolyzing enzymes in rice seedlings exposed to toxic doses of zinc. Although Zn is considered as an essential micronutrient required by plants in trace amounts for their growth and metabolic activities, mercury, on the contrary, does not have any nutritional value and it is extremely toxic even in very dilute concentrations.

Among all the heavy metals, mercurial compounds act as the effective pollutants. Photosynthetic activity is largely affected by mercury. Harris *et al*. (1970) observed a reduction in photosynthetic rate in marine diatoms and in several other phytoplanktons even by the addition of 0.1 ppb of organo-mercurial fungicides. Cells of *Chlorella pyrenoidosa* become pale-yellow by Hg treatment and there is a depression in photosynthetic rate (Kamp-Nielsen, 1971). Inhibition of photophosphorylation associated with non-cyclic electron transport has been noticed in spinach chloroplasts by the addition of phenyl mercuric acetate (Honeycutt and Krogmann, 1972). Chlorophyll development and Hill reaction activity in the leaves of rice seedlings were depressed by mercury treatments with

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concomitant increase in the activities of chlorophyllase and a few oxidizing enzymes (Mukherji and Nag, 1977; Nag *et al.*, 1981). Mitotic behaviour of onion root tip cells, water uptake and leakage of metabolites from potato tuber tissues and α-amylase formation in deembryonated rice endosperms were adversely affected by Hg toxicity (Nag *et al.*, 1980). The aim of the present work is to investigate the effects of toxic concentrations of $ZnSO_4$ and $HgCl_2$ on the growth and metabolism of mungbean which ranks in importance as a pulse food crop next to rice in tropical countries.

Materials and Methods

Mungbean (*Vigna radiata* L. Wilczek) seeds were spread over petri dishes lined with filter papers containing distilled water. After germination for 48 h in the dark at 26°C, these were transferred to different petri dishes containing $ZnSO_4$ 7 H_2O, $HgCl_2$, gibberellic acid (GA_3), cyclic-3,5-adenosine monophosphate (cAMP) and kinetin solutions and kept under the same conditions for another 72 h. Water controls were run parallel to each set. The respiration rates of seedlings were measured directly by oxygen consumption method at 30°C following Warburg's manometric method as described by Umbreit *et al.* (1957). For the estimation of total nitrogen, the micro-Kjeldahl method was adopted. Sugar estimation was done according to Somogyi (1945). The estimation of RNA and DNA was done according to the method of Smillie and Krotkov (1960). Radioprecursor incorporation was estimated according to the method described by Bray (1960). Gel electrophoresis was done according to the method described previously (Davis, 1964; Nag *et al.*, 1981).

Results and Discussion

Seedling Growth

Inhibition of growth of mungbean seedlings was noticed at all the concentrations of $ZnSO_4$ used here (Table 1). Inhibition of hypocotyl elongation measuring about 90% was observed at the highest dose, i.e., 40 mM. Root growth was almost nil at this concentration. Growth inhibition was found to be associated with swelling of the cotyledons. Fresh weight decrease of treated seedlings was more than 90% from the control seedlings at this dosage. Growth inhibition by Zn excess has been noticed by many workers, viz., Polson and Adams (1970) in *Phaseolus vulgaris* and Milbocker (1974) in corn plant.

Seedling growth inhibition caused by $ZnSO_4$ treatment was relieved to different degrees when applied jointly with GA_3, cAMP and kinetin. Most significant effect was produced by GA_3 treatment. When applied in combination with 10 mM $ZnSO_4$, GA_3 lowered the inhibition from 48% to 5% level. Such reversal was, however, less pronounced with cAMP which could bring down the inhibition from 48% to 20% under the same concentration of $ZnSO_4$. Under higher doses of $ZnSO_4$, however, the effects of GA_3 and cAMP were relatively less. Kinetin was not only ineffective in reversing Zn-induced inhibition, the seedlings were found to be further shortened by Zn-kinetin interaction. Responsiveness of hypocoyl to GA_3 and cAMP reversal was better marked than root. Similar growth pattern of plants grown in metalliferous medium has been reported by previous workers (Dasgupta, 1975; Mukherji and Maitra, 1977; Nag *et al.*, 1984).

With $HgCl_2$, inhibition of seedling growth started at 0.4 mM concentration and gradually increased at higher doses and root growth inhibition was stronger than hypocotyl inhibition (Table 2). At 0.8 mM $HgCl_2$, hypocotyl and root lengths suffered more than 90% inhibition from control. Data further indicate that GA_3 and cAMP applied simultaneously with different concentrations of $HgCl_2$ were partially effective in relieving Hg-induced inhibition. When applied with 0.8 mM $HgCl_2$, GA_3 showed an almost complete relief of elongation inhibition. Similarly at the highest dose of $HgCl_2$, the joint application of cAMP resulted in the reduction of hypocotyl inhibition from 94 to 88% and of root from 98 to 90%. On the other hand, the effect of joint application of $HgCl_2$ with kinetin was relatively less than either with GA_3 or cAMP.

Respiration Rates

The same decreasing trend in respiration rates was maintained in $HgCl_2$ and $ZnSO_4$ treated seedlings (Fig. 1 A, 1 B). At 0.4 mM $HgCl_2$, the seedlings respired at about 40% lower rate on per seedling basis. A gradually decreasing trend was observed at the increasing concentrations and maximum reduction of about 85% was noticed at 0.8 mM $HgCl_2$. Seedlings treated with $ZnSO_4$ showed a sudden fall in respiration rate at 10 mM which was 83% less than control. At the next

Table 1. *Effect of various concentrations of $ZnSO_4$ applied alone or in combination with GA_3, kinetin and cAMP on the elongation of mungbean seedlings*

Treatment	Hypocotyl		Root		Fresh wt	
	Length (cm)	Inhibition (−) or Promotion(+),%	Length (cm)	Inhibition (−) or Promotion(+),%	Wt (g)	Inhibition (−) or promotion(+),%
Water control	6.3	—	4.40	—	1.64	—
$ZnSO_4$, mM						
10 (A)	3.3	−48	0.70	−85	0.21	−87
20 (B)	2.1	−66	0.30	−94	0.23	−86
40 (C)	0.5	−92	0.17	−97	0.12	−93
GA_3, 10^{-4}M	11.2	+77	6.00	+36	2.05	+25
$ZnSO_4$ + GA_3 (10^{-4}M)						
A + GA_3	6.0	− 5	0.90	−77	0.82	−50
B + GA_3	3.0	−52	0.40	−91	0.62	−62
C + GA_3	0.9	−85	0.31	−93	0.21	−87
cAMP, 10^{-4}M	9.3	+50	6.00	+36	1.83	+12
$ZnSO_4$+cAMP (10^{-4}M)						
A+cAMP	5.0	−20	1.10	−75	0.41	−75
B+cAMP	2.5	−63	0.50	−88	0.41	−75
C+cAMP	1.0	−84	0.50	−89	0.11	−93
Kinetin, 10 ppm	3.1	−50	4.86	+10	0.60	−63
$ZnSO_4$+kinetin (10 ppm)						
A+kinetin	2.1	−66	1.54	−65	0.20	−88
B+kinetin	1.0	−70	0.80	−82	0.12	−93
C+kinetin	0.9	−85	0.80	−82	0.12	−93

Germination time 5 days.

higher concentrations, the rates were further lowered by about 90% from control.

It may be suggested that the respiration rate is directly proportional to seedling elongation whereby seedlings dwarfed by metal treatments are characterized by reduced rates of respiration. The existence of a direct relationship between respiration rate and seedling vigour has been reported by several workers (Woodstock and Pollock, 1965; Paul and Mukherji, 1972; Mukherjee *et al.*, 1973). In the present work, the seedling growth under metal treatments has always been found to be stunted which is naturally coupled with less demand for energy obtainable from the breakdown of ATP.

Nitrogen, Sugar and Nucleic Acid Contents

The salient features regarding the influence of Hg and Zn on nitrogen contents of both embryo and cotyledons are more or less similar in that the toxic concentrations of both the metals resulted in an appreciable reduction in total nitrogen content of embryo coupled with an increment in cotyledons which can be easily correlated with growth inhibition (Fig. 1C, 1D). About 74% diminished value of embryo nitrogen was recorded even at 0.4 mM $HgCl_2$. The reduction became gradually magnified at 0.6 mM and total nitrogen was found to be most adversely affected at 0.8 mM $HgCl_2$ where 90% reduction was recorded. Similar to total nitrogen, soluble nitrogen of embryo suffered a drastic fall of about 80% at 0.6 mM $HgCl_2$ beyond which no further significant reduction was observed (Fig. 1E, 1F). $ZnSO_4$ treatment showed about 96%

Table 2. *Effect of various concentrations of $HgCl_2$ applied alone or in combination with GA_3, kinetin and cAMP on the elongation of mungbean seedlings*

Treatment	Hypocotyl		Root		Fresh wt	
	Length (cm)	Inhibition (−) or promotion (+), %	Length (cm)	Inhibition (−) or promotion (+), %	Wt (g)	Inhibition (−) or promotion (+), %
Water control	6.5	—	4.30	—	1.80	—
$HgCl_2$, mM						
0.4 (A)	3.2	−50	0.85	−80	0.21	−88
0.6 (B)	1.9	−70	0.20	−95	0.27	−85
0.8 (C)	0.4	−94	0.11	−98	0.09	−95
GA_3, 10^{-4}M	11.5	+77	5.85	+36	2.25	+25
$HgCl_2+GA_3$ (10^{-4}M)						
A+GA_3	6.3	− 3	1.10	−74	0.76	−58
B+GA_3	3.6	−45	0.40	−90	0.63	−65
C+GA_3	0.8	−88	0.30	−93	0.18	−90
cAMP, 10^{-4}M	9.7	+50	5.90	+36	2.01	+12
$HgCl_2$+cAMP (10^{-4}M)						
A+cAMP	7.6	+17	1.20	−72	0.42	−77
B+cAMP	2.6	−60	0.50	−88	0.47	−74
C+cAMP	0.8	−88	0.40	−90	0.09	−95
Kinetin, 10 ppm	3.3	−50	4.75	+10	0.67	−63
$HgCl_2$+kinetin (10 ppm)						
A+kinetin	2.1	−68	1.60	−62	0.24	−87
B+kinetin	1.7	−75	0.64	−85	0.15	−92
C+kinetin	0.7	−90	0.55	−67	0.09	−95

Germination time 5 days.

reduction at the maximum concentration, i.e., 40 mM. As opposed to the effect on embryo, soluble nitrogen of cotyledons was always maintained at much higher level than control. In contrast to water control, cotyledons showed a 3-fold rise in soluble nitrogen at 0.8 mM $HgCl_2$ whereas its accumulation was more than 6-fold with 40 mM $ZnSO_4$.

In embryo, total sugar content was greatly reduced by $HgCl_2$ and $ZnSO_4$ applications (Fig. 2 A, 2 B). At the highest concentration of $HgCl_2$, there was 80% decrease of total sugar content of embryo. At the highest concentration of Zn, however, the reduction was only 11%. The situation was reverse in case of cotyledons in which total sugar progressively increased under increasing Hg and Zn treatments. Reducing sugar contents of Hg and Zn treated embryo were always maintained at lower levels than control, and the effect increased with increasing concentrations (Fig. 2 C, 2 D). At 0.8 mM $HgCl_2$, there was about 20% reduction in reducing sugar which was further diminished by about 50% at the maximum concentration of Zn. In cotyledon, there was an accumulation of reducing sugar at higher levels of Hg and Zn treatments.

DNA content of embryo suffered the maximum loss of about 40% at 0.8 mM $HgCl_2$ (Fig. 2 E). A further diminished value amounting to more than 80% from control was noticed at 40 mM $ZnSO_4$ (Fig. 2 F). DNA content of cotyledons showed an increasing trend following metal treatments. Cotyledons of Hg-treated seedlings exhibited more than 2-fold rise in DNA content while $ZnSO_4$ showed only a little increase from

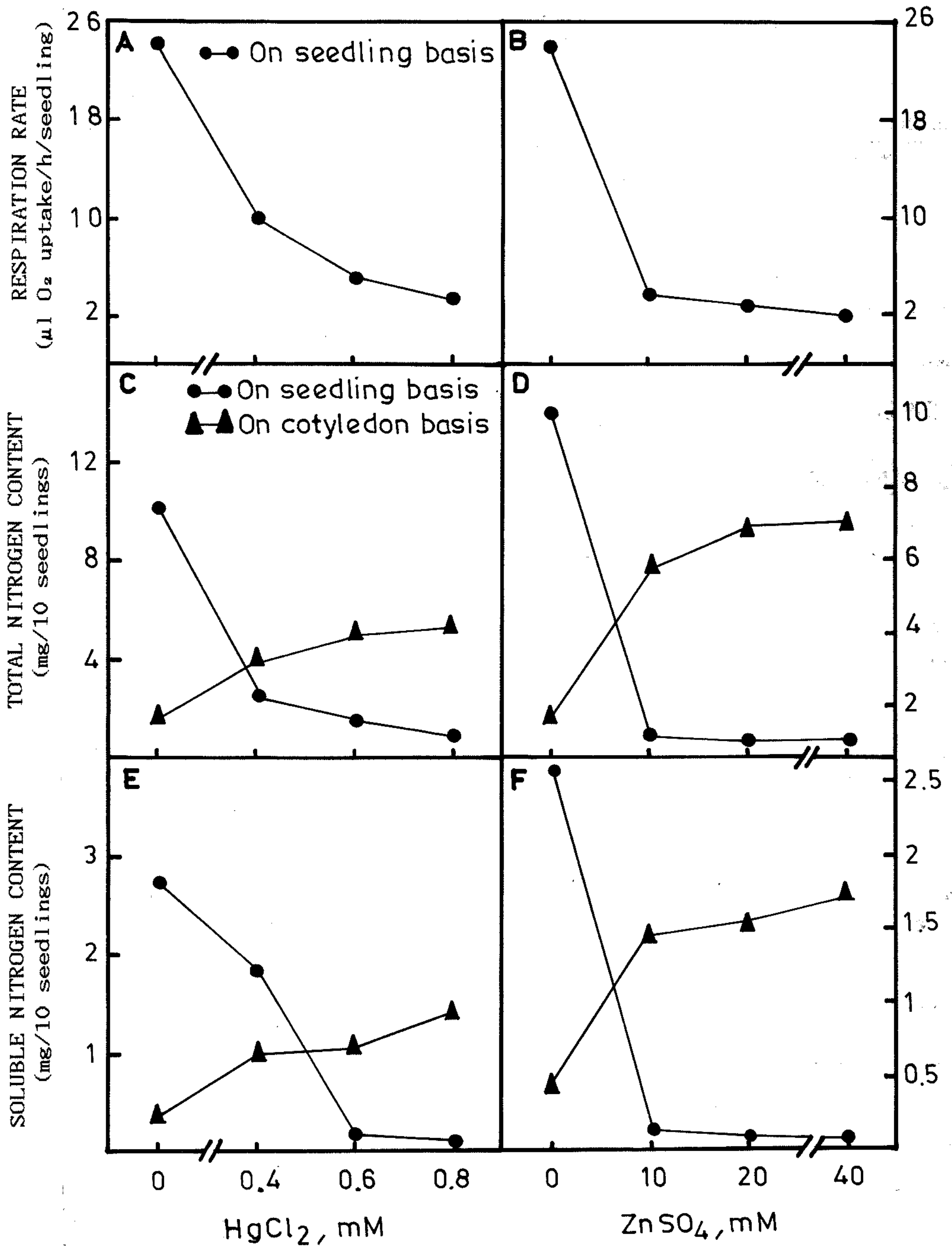

Fig. 1. Effects of various concentrations of $HgCl_2$ and $ZnSO_4$ solutions on (A, B) respiration rates (μl O_2 uptake/h/seedling), (C, D) total nitrogen contents (mg N/10 seedlings) and (E, F) soluble nitrogen contents (mg N/10 seedlings) of germinating mungbean seedlings.

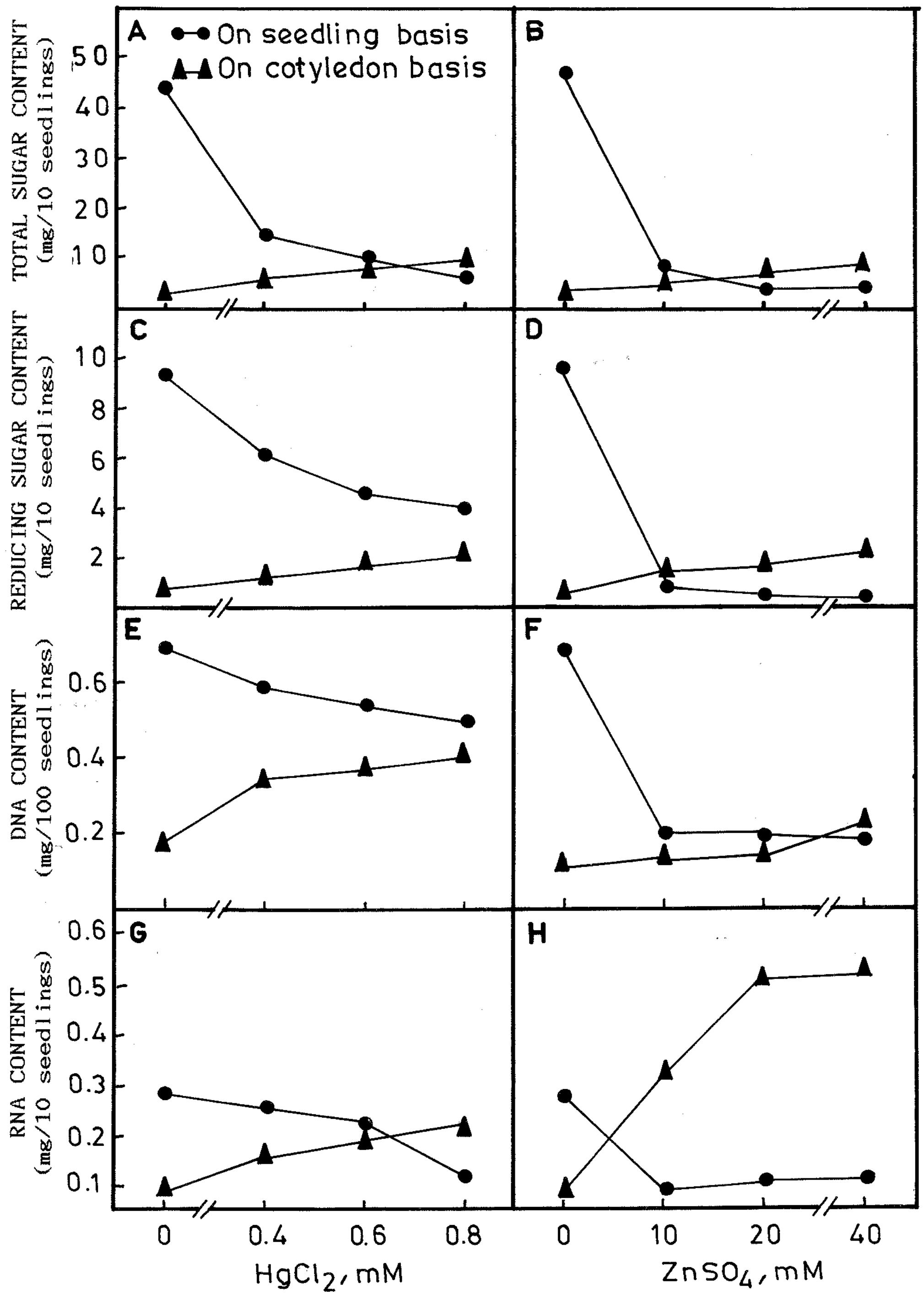

Fig. 2. Effects of various concentrations of $HgCl_2$ and $ZnSO_4$ solutions on (A, B) total sugar contents (mg sugar/10 seedlings), (C, D) reducing sugar contents (mg sugar/10 seedlings), (E, F) DNA contents (mg DNA/100 seedlings) and (G, H) RNA contents (mg RNA/10 seedlings) of germinating mungbean seedlings.

control. RNA content of embryo was depleted by about 57% from control at 0.8 mM $HgCl_2$ treated seedlings (Fig. 2 G). Maximum reduction of 75% in RNA content occurred at 10 and 20 mM $ZnSO_4$, while the highest dose, i.e., 40 mM was not able to cause any further reduction (Fig. 2 H). Like DNA, RNA content of Hg and Zn treated cotyledons also showed an increasing trend. The highest concentration of Hg showed more than 2-fold increment while in case of Zn this rate was gradually increased and finally magnified more than 5-fold at 20 and 40 mM $ZnSO_4$.

Gel Electrophoretic Studies

The electrophoretic patterns of buffer soluble proteins isolated from intact mungbean seedlings show that the number of bands corresponding to Hg and Zn treatments was in excess of those of water control (Fig. 3). Starting from the origin, 4 consecutive bands

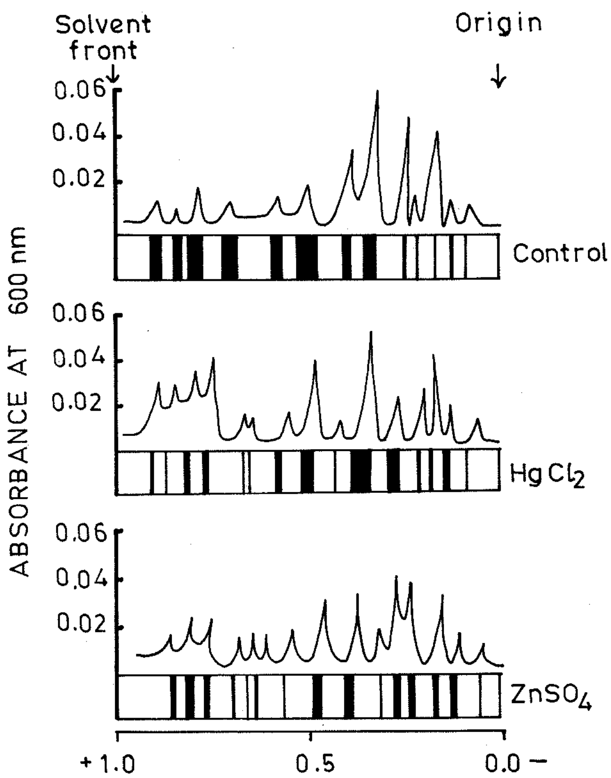

Fig. 3. Gel electrophoretic analysis of buffer soluble proteins of mungbean seedlings grown in presence of $HgCl_2$ and $ZnSO_4$ solutions. Gel slices homogenized with 7.5% acetic acid, diluted to 5 ml, optical density measured at 600 nm.

with Rf 0.08, 0.16, 0.21, 0.58 were present both in control and Hg treated sets. In addition, 11 new protein bands with Rf 0.13, 0.27, 0.38, 0.42, 0.51, 0.65, 0.66, 0.76, 0.81, 0.87 and 0.90 appeared in Hg treatment which could not be detected in control. The colour of the band with Rf 0.21 appeared almost 2-fold intense in Hg treatment, whereas the other bands showed no appreciable change in colour intensity. In $ZnSO_4$ treatment, 3 bands with Rf 0.12, 0.23 and 0.40 were found to be identical to control, whereas 12 fresh bands corresponding to Rf 0.05, 0.17, 0.27, 0.31, 0.48, 0.56, 0.63, 0.65, 0.68, 0.77, 0.81 and 0.86 appeared in Zn treatment only. It was also noticed that the band with Rf 0.12 appeared more intense in Zn treatment as compared to control.

The number of basic protein bands also increased in Hg and Zn treated seedlings. In control, 8 bands were found, while in Hg treatment, 10 new bands with Rf 0.06, 0.13, 0.25, 0.31, 0.46, 0.54, 0.57, 0.61, 0.65 and 0.76 appeared and thus the total number was 12 (Fig. 4). Total number of basic protein bands in Zn treated seedlings was 10 and here 6 new bands appeared with Rf 0.29, 0.36, 0.44, 0.51, 0.55 and 0.61 which were not detected in control. There was no significant change in colour intensity of the bands caused by either treatments.

The influence of metal treatments was greater in buffer soluble protein in general, whereas the basic protein profile was less affected. It is known that the basic proteins are responsible for their regulatory action on DNA and reports are there to indicate that DNA synthesis is inhibited by metals (Sigel, 1974). An interesting fact emerging from this investigation is that the heavy metals which are known to cause depression in enzyme activity by forming metal-protein complexes particularly in *in vitro* systems actually induces or activates some new enzymes in this system *in vivo* (Mahler and Cordes, 1974). In this connection, it is noteworthy that cycloheximide, a potent inhibitor of protein synthesis and actinomycin D, the inhibitor of DNA dependent RNA synthesis have been shown to exhibit paradoxical effects by inducing new enzymes in different systems (Klis and Hak, 1972; Nanda *et al.*, 1973). In a more recent work, another protein synthesis inhibitor, streptomycin has been shown to be capable of inducing α-amylase formation in embryoless rice half-seeds both in the presence and absence of GA_3 and cyclic AMP (Bag and Mukherji, 1980). It has also

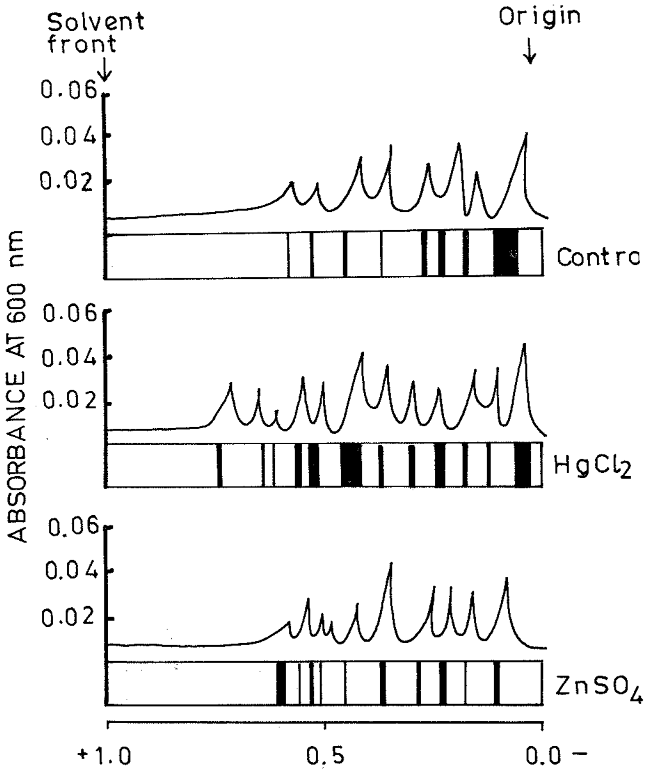

Fig. 4. Gel electrophoretic analysis of basic proteins of mungbean seedlings grown in presence of $HgCl_2$ and $ZnSO_4$ solutions. Gel slices homogenized with 7.5% acetic acid, diluted to 5 ml, optical density measured at 600 nm.

been demonstrated that the number of protein bands isolated from streptomycin-treated rice seedlings is in excess of water control. The control of protein synthesis may be brought about by an unstable repressor protein operating at the level of transcription and translation (Tomkins *et al.*, 1969). Heavy metal treatment may be assumed to reduce the concentration of the repressor, thus leading to a partial or complete derepression of the synthesis of certain other proteins. In view of this postulate, one may expect that the resultant rate of enzyme synthesis in a treatment should become higher than the untreated control.

It is evident from Fig. 5 that the electrophoretic pattern of peroxidase isoenzymes are distinctly influenced by heavy metals. In the control set, 6 isoenzyme bands were detected but some of these bands originally present in control disappeared in the treatment sets. It is noteworthy that although the number of isoperoxidase bands was reduced in treatments as compared to control, the peroxidase properties of some of the protein bands measured individually far exceeded those of the control.

Isoperoxidases represent an enzyme complex involved in auxin catabolism. Increase in the activity of some of the isoperoxidase bands observed here is in agreement with our previous findings on growth inhibitory properties of heavy metals. The present finding on peroxidases thus confirm our general hypothesis that seedlings with reduced growth have higher peroxidase activity as compared to their normal counterparts and the treated seedlings are thus associated with diminished level of endogenous auxin.

Radioprecursor Incorporation Studies

In metal treated samples, the rate of incorporation of a mixture of radioactive amino acids into proteins was largely depressed (Table 3). It was noticed that the rate of incorporation was reduced, on the average,

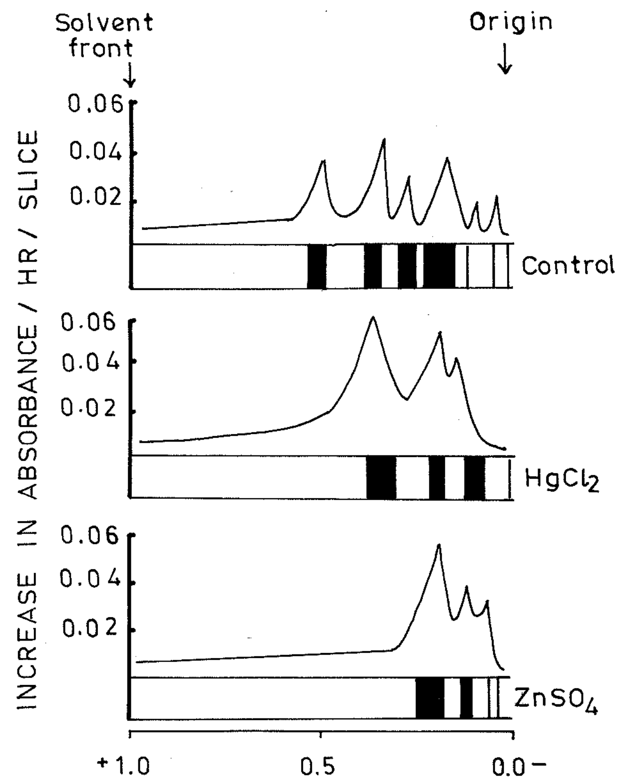

Fig. 5. Gel electrophoretic analysis of peroxidase isoenzymes of mungbean seedlings grown in presence of $HgCl_2$ and $ZnSO_4$ solutions. Gel slices homogenized with buffer solution and tested for peroxidase activities. Reaction mixture: entire homogenate, 5 ml phosphate buffer, 1 ml H_2O_2 (0.5 vol) and 1 ml catechol (0.5%).

Table 3. *Effect of $ZnSO_4$ and $HgCl_2$ on the incorporation of ^{14}C uracil, ^{14}C thymine and ^{14}C amino acid mixture (glycine + valine) into RNA, DNA and protein respectively by germinating mungbean seedlings*

Treatment	RNA		DNA		Protein	
	CPM/g f. wt.	% decrease	CPM/g f. wt.	% decrease	CPM/g f. wt.	% decrease
Water control	454	—	590	—	8356	—
$ZnSO_4$, 20 mM	367	−19	472	−20	3510	−58
$HgCl_2$, 0.6 mM	340	−25	360	−39	2757	−67

by about 60% under toxic levels of Hg and Zn. Incorporation of radioactive uracil and thymine into RNA and DNA of mungbean seedlings was also inhibited by the presence of metals in the medium (Table 3). The rate of incorporation was reduced by about 25 and 20% in case of RNA and about 40 and 20% in case of DNA at toxic concentrations of Hg and Zn respectively.

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重金屬鋅與汞對綠豆幼苗之生長與生化成份之影響

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本文就鋅與汞，在達致毒濃度時，對綠豆幼苗之生長或生化成份的影響加以觀察。硫酸鋅在 10 mM，氯化汞在 0.4 mM 以上均會抑制幼苗之生長，GA_3、cAMP 與細胞分裂素可以使毒害減輕。隨着鋅與汞濃度之提高，幼苗之呼吸率逐漸下降，同時，胚之總氮量、總糖量、DNA 和 RNA 下降，而子葉內所含者則增加。以膠體電泳分析抽取自幼苗之水溶性蛋白或鹼性蛋白，發現重金屬處理者，其蛋白質帶增加，推測重金屬可能引起蛋白質之破壞，又處理組之 RNA、DNA 和蛋白質的合成速率也下降。