SUMMARY AND CONCLUSIONS
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Heavy metals are integrated components of the biosphere occurring naturally in the soil. Both natural and human activities introduce metals into the environment. Industrialization results into higher concentration of heavy metals into the environment. High heavy metal availability i.e. heavy metal pollution is one of the most troublesome environmental problems faced by mankind now-a-days.

The presence of heavy metals in the growing media is considered as adverse i.e. stress condition for plants. Generally plants are studied in relation to water stress, salt stress, temperature stress, but few reports are available on plant study in relation to heavy metal stress. Copper, cadmium and mercury are some of the heavy metals. Copper is essential, Cd is non-essential and mercury is phytotoxic to plants. The concentrations of all these three metals are increasing in the environment by industrialization, agriculture and many human activities. Excess amount of these metals inhibit the growth, physiological and biochemical processes in plants though the inhibitory effects depend upon the type, concentration and the availability of the metal. Moreover the sensitivity towards heavy metals depends upon the species.

Maize and wheat are important cereals and cultivated through seeds. Thus seed germination and seedling growth are
essential and important events in the life cycle of such plants. The productivity depends upon early seedling growth. Maize var Ganga safed-2 and wheat var. sonalika are extensively cultivated. As these two species are widely used, it was of interest to find out the sensitivity of the species to heavy metals. The sensitivity of the species to heavy metals was studied through the seedling growth and various metabolic studies. For this purpose, the following experiments were performed.

EXPT. 1: EFFECTS OF HEAVY METALS ON GROWTH AND METABOLISM OF MAIZE AND WHEAT SEEDLINGS (PETRIPLATE EXPT.):

Seeds of maize (*Zea mays* L. var Ganga safed-2) and wheat (*Triticum aestivum* L. var sonalika) were germinated in petriplates lined with Whatmann no.1 filter paper. The media were DW (Control), 50, 100, 150, 200, 250 and 300 μg of CuCl₂, CdCl₂, HgCl₂ per ml separately. The experiment was carried out at room temperature (28±2°C) under laboratory conditions. The percent germination was recorded and seedling growth was studied at the intervals of 24 h (48 h to 120 h for maize, 24 h to 96 h for wheat). Effects of heavy metals on seedling growth was evaluated by calculating Relative Root Growth (RRG) and Relative Growth Rate (RGR) of the seedlings.

The embryo and endosperm of 48, 72, 96 and 120 h old maize seedlings as well as 24, 48, 72 and 96 h old wheat seedlings were analysed for the following enzymic activities

The data on biochemical estimations were statistically analysed (Analysis of variance and CD).

EXPT. 2: EFFECTS OF HEAVY METALS ON GROWTH AND METABOLISM OF MAIZE AND WHEAT SEEDLINGS (POT EXPERIMENT):

The seeds of maize var Ganga safed-2 and wheat var Sonalika were raised in pots having sterilized sand. Before sowing, the heavy metals i.e. CuCl₂, CdCl₂ and HgCl₂ (50, 100, 150, 200, 250 and 300 mg/kg sand) were thoroughly mixed with sand. The necessary irrigation was done. The 6, 8 and 10 days old control (without heavy metals) and treated seedlings were studied for their growth. RRG and RGR were calculated from the seedling data. The leaf of these seedlings was also analysed for α-amylase, β-amylase, invertase, reducing and non-reducing sugars, protease, protein, total amino acid, proline, RNAse, RNA, acid phosphatase, peroxidase, polyphenol oxidase and total phenol content.
The photosynthetic pigments i.e. chlorophyll 'a', chlorophyll 'b', total chlorophyll and carotenoids were estimated from the leaf of 10 days old control and heavy metal treated maize and wheat seedlings. Data on various enzymic activities and metabolite contents were statistically analysed (ANOVA) for finding out the significance of the treatments. Heavy metal stress was evaluated through the ratio of chlorophyll 'a' to chlorophyll 'b' and total chlorophyll to carotenoids.

The following conclusions were drawn from the above experiments:

1. Heavy metals i.e. CuCl₂, CdCl₂ and HgCl₂ having 100 μg/ml and above concentration inhibited percent germination of maize var Ganga safed-2 and wheat var Sonalika. Inhibitory effect was positively related with concentration. The adverse effect caused by heavy metals was in the order of Hg > Cd > Cu. Inhibitory effect was more on maize than on wheat.

2. 50 μg/ml and above concentrations of all the metals effectively inhibited the root elongation in maize seedlings. Shoot elongation was inhibited, when concentration was more than 100 μg/ml. Hg caused most adverse effect. The inhibitory effects of all the heavy metals on elongation was positively related with concentration.

3. The root shoot elongation in wheat was also adversely
affected but effective concentration differed with metals i.e. 200 μg/ml Cu, 150 μg/ml Cd and 50 μg/ml Hg significantly inhibited the elongation. Adverse effect was metal concentration and duration dependent.

4. In both the crops root was more affected than shoot by heavy metals suggesting that root is the target organ for heavy metal. Inhibitory effect of maize root was more in comparison to wheat indicating that maize is more sensitive to heavy metals.

5. Root shoot ratio in maize seedlings was lowered by Cu, Cd and Hg. Lowering was related with concentration of heavy metals. In wheat, root shoot ratio was significantly lowered only in mercury treated seedlings, confirming that wheat seedling is comparatively more tolerant to heavy metals in comparison to maize. Root shoot ratio in maize seedlings followed decreasing trend with advancing germination. In wheat, root shoot ratio was more or less similar in copper and cadmium treated seedlings during germination. Data confirms sensitive nature of maize seedlings.

6. Relative Root Growth (RRG) in maize and wheat seedlings was decreased by heavy metals, decrease was significant in maize.

7. Copper, cadmium and mercury lowered the fresh and dry weight of maize embryo, mercury was most effective. Reverse effect was found for endosperm. The effect of
heavy metals was concentration dependent.

8. Copper and cadmium did not affect the fresh weight and dry weight of young embryo axis of wheat, in older seedlings adverse effect was noted but mercury decreased the fresh weight of embryo (24 to 96 h old). Higher fresh weight of endosperm of treated seedlings indicates less utilization of reserve nutrients.

9. Heavy metals particularly cadmium and mercury caused more adverse effect on fresh weight of maize embryo in comparison to that in wheat indicating that maize is more sensitive to heavy metal. Toxic effect varies with metal and species.

10. Relative growth rate (RGR) of growing axis of maize and wheat was lowered when seeds were germinated with higher concentration of heavy metals for longer period. RGR was higher in seedlings treated with heavy metals for shorter duration, indicating physiological adaptation against strainful condition. The toxic effects of individual heavy metal can not be differentiated with the help of RGR. RGR was lower in metal treated seedlings of maize, when compared with RGR of metal stressed wheat seedlings suggesting that maize may be considered as more sensitive to heavy metals than wheat.

11. Percent moisture was higher in embryo axis of older treated maize seedlings. Water uptake in endosperm was reduced by higher concentration of heavy metals during
first few hours of germination. In case of wheat embryo, percent moisture was lowered when exposure time was longer. Percent moisture in endosperm of heavy metal treated seedlings was reduced at the time of radicle emergence. The toxicity may be assessed by studying percent moisture in the endosperm at the time of radicle emergence.

12. The heavy metals irrespective of their nature, i.e. copper, cadmium and mercury lowered the α-amylase activity in embryo, but stimulated in endosperm suggesting revival strategy by producing higher amount of sugars in the endosperm of heavy metal treated maize seedlings.

13. Generally α-amylase activity was lowered by copper, cadmium and mercury in the embryo of 24h old wheat seedlings. During that period activity was stimulated in endosperm then it was lowered. The higher concentration significantly lowered the activity in the embryo. Mode of action of copper, cadmium and mercury was more or less similar on enzymic activity. Different concentrations gave more or less similar effects on enzymic activity.

14. Copper stimulated the β-amylase activity in maize embryo. Cadmium and mercury lowered it in older seedlings. Generally copper and cadmium stimulated the activity in endosperm of 48h old seedlings. Stimulation in β-amylase activity may provide more soluble
nutrients, thus, seedlings may remain alive under stress condition. There was no consistency between different concentrations and alterations in enzymic activity. The toxic nature of heavy metals can be evaluated through determination of enzymic activity in the embryo while tolerance can be correlated by determining the enzymic activity in endosperm.

15. Copper generally stimulated the β-amylase activity in the embryo axis of wheat while cadmium and mercury decreased it. Generally, higher β-amylase activity was found in the endosperm of 24h old heavy metal treated seedlings, the activity was lowered in the endosperm of older seedlings. The mode of action of these metals was different in growing axis but it was similar in endosperm.

16. Starch content was higher in the endosperm of heavy metal treated seedlings. Though copper being the essential, while cadmium and mercury being phytotoxic elements all gave similar effects on starch content, indicating that excess amount of heavy metals cause similar effects on starch.

17. Lower concentration of copper stimulated the invertase activity in embryo axis of younger maize seedlings, while cadmium and mercury decreased it. The significant effect was found with mercury. During 48h, all the heavy metals lowered the activity in endosperm. Copper stimulated it in older seedlings while cadmium and
mercury decreased it. The inhibitory action of heavy metal was in the order of Hg > Cd > Cu.

18. Copper, cadmium and mercury lowered the invertase activity in embryo axis of wheat seedlings. In younger seedlings, activity was correlated with concentration. Higher concentration of copper, cadmium and mercury stimulated the activity in endosperm, the lowering was found in the endosperm of 48h old seedlings.

19. Heavy metals i.e., copper, cadmium and mercury accumulated non-reducing sugar in embryo. The accumulation was increasing with increase in concentration. In endosperm, higher concentration of each metal gave similar effects.

20. The non-reducing sugar was accumulated in embryo and endosperm of wheat seedlings germinated with higher concentration of copper, cadmium and mercury. The intensity of the effect was not differentiated with the nature of the metal.

21. Copper, cadmium and mercury increased the level of reducing sugar in embryo of maize seedlings. The accumulation was progressively increased with increase in concentration. The accumulation was more or less similar in copper, cadmium and mercury treated seedlings. In endosperm, similar effects were found only with higher concentration of metal. The determination of reducing and non-reducing sugar may be selected as one of the good parameter for evaluating
toxic effects of heavy metals in embryo.

22. Reducing sugars were accumulated in the embryo of wheat seedlings germinated with copper, cadmium and mercury. Higher amount of reducing sugar was also found in the endosperm of metal treated seedlings during early hours of germination. The different metals gave similar effects.

23. Copper stimulated the protease activity. Lower concentration was effective in embryo, while higher concentration gave highly significant effect in the endosperm. Cadmium and mercury lowered the activity in maize embryo and endosperm. The adverse effect was correlated with concentration. The decrease in activity was more with mercury than with cadmium.

24. Copper stimulated the protease activity in embryo and endosperm of wheat seedlings during first 24h of germination. Cadmium did not alter the protease activity significantly in embryo, but in endosperm activity was promoted. Mercury increased the activity in embryo as well as in endosperm during first 24h of germination.

25. The protein content in the embryo and endosperm of maize was not generally altered by copper. Cadmium did not affect the levels of protein significantly in the embryo. Mercury decreased the protein content in the embryo during initial period, then such effect was not reported. Similarly, mercury treatment did not alter
the protein content in the endosperm.

26. Copper, cadmium and mercury did not affect the protein content in the embryo but lowered in endosperm of wheat seedlings.

27. Copper, cadmium and mercury caused accumulation of amino acids in embryo of maize and more specifically in endosperm. The accumulation was maximum in copper treated embryo axis and minimum in mercury treated seedlings. Thus tolerance of the seedlings may be correlated with accumulation of amino acids.

28. Amino acids were accumulated in growing axis of copper, cadmium and mercury treated wheat seedlings. Copper was the most effective. Amino acids were also accumulated in endosperm.

29. Copper, cadmium and mercury accumulated proline in embryo and endosperm of maize seedlings. The accumulation was progressively increasing with increase in concentration. The higher accumulation was found in mercury treated seedlings. Accumulation of proline indicates heavy metal toxicity.

30. Higher concentrations of copper, cadmium and mercury accumulated proline content in embryo and endosperm of wheat seedlings. Longer duration of the treatment gave significant effect. Copper, cadmium and mercury acted similarly. Wheat var Sonalika is more tolerant in comparison to maize var Ganga safed-2 to metal stress. Under metal stress condition proline accumulation was
significant in maize than in wheat. Study on proline accumulation may be one of the good parameters for knowing the level of phytotoxicity in metal sensitive variety. In tolerant variety, it is difficult to know the phytotoxicity level by determining the amount of proline in metal treated seedlings.

31. The higher concentrations of copper and all the concentrations of cadmium and mercury decreased the RNAse activity in the embryo of maize seedlings at the time of sprouting. Heavy metals suppressed the activity in the endosperm of 96h old seedlings. Cadmium and mercury enhanced the activity in embryo of older seedlings.

32. RNAse activity in embryo axis of wheat was lowered by copper, cadmium and mercury. Inhibitory effect was correlated with concentration. In endosperm, it was stimulated by heavy metals during first 24h of germination, then it was declined.

33. RNA content was higher in the embryo of older maize seedlings germinated with heavy metals. In endosperm, increased level of RNA was noted with higher concentrations of heavy metals.

34. Lower concentration of copper, cadmium and mercury decreased the RNA content in the embryo of younger wheat seedlings but higher concentrations enhanced it in older seedlings. The effects of heavy metals on RNA content in embryo differed with the age of seedlings.
Higher concentrations of heavy metals decreased RNA in endosperm of 24 and 96h old seedlings.

35. Acid phosphatase activity was suppressed by higher concentrations of heavy metals in maize embryo at the time of emergence of radicle, but with advancing growth, activity was stimulated. Copper stimulated the activity in endosperm. Cadmium and mercury stimulated upto 72h then decreased it.

36. Heavy metals decreased the APase activity in the embryo of wheat during initial hours of germination. During 72h the activity was increased by lower concentration. The activity was increased in the endosperm of the heavy metal treated seedlings in 24h old seedlings and then lowered it.

37. Heavy metals enhanced the peroxidase activity in embryo and endosperm of maize seedlings. The effect was positively correlated with concentration. The peroxidase activity in endosperm indicates the toxic nature of the heavy metals, i.e. higher peroxidase activity denotes more toxic effect.

38. Peroxidase activity in the embryo and endosperm of wheat seedlings was increased by copper, cadmium and mercury. Significant increase was noted in older seedlings. The increase in activity was correlated with concentration.

39. The higher concentration of copper, cadmium and mercury suppressed the polyphenol oxidase activity in embryo of
younger maize seedlings. The opposite effect was noted for endosperm.

40. Polyphenol oxidase activity in wheat was decreased significantly in the embryo axis during first 24h, but stimulated in endosperm. In older seedlings, activity was inhibited. Effects of all the metals were more or less similar.

41. Heavy metals accumulated phenol in embryo and endosperm of maize seedlings. The accumulation was positively correlated with concentration. The effect of copper, cadmium and mercury was similar on phenol content in embryo and endosperm. Accumulation was more in endosperm than that in embryo.

42. Total phenol was accumulated in embryo and endosperm of wheat seedlings germinated under heavy metals. The accumulation in the embryo was related with concentration. The effect was more in embryo than that in endosperm.

43. Seed germination of maize and wheat in sand culture experiment was decreased by higher concentrations (more than 100 mg/kg sand). The percent inhibition was similar in both the crops and with all the three metals.

44. The elongation of root and shoot in 6, 8 and 10 days old maize seedlings raised in pots was decreased by copper, cadmium and mercury. The inhibition was progressively increased with increase in concentration.
The inhibitory effects were in the following sequence:

\[ \text{Hg} > \text{Cd} > \text{Cu} \]

The inhibitory effects were more on root than on shoot.

45. Root elongation of 6, 8 and 10 days old wheat seedlings grown in pots, was inhibited by copper, cadmium and mercury. The inhibitory effect was more with mercury than cadmium and copper. Shoot elongation was not much affected, only higher concentrations inhibited it. Shoot elongation may be considered as indicator of heavy metal tolerance of wheat.

46. Root shoot ratio in maize and wheat seedlings was lowered by copper, cadmium and mercury. Mercury was the most effective in both the crops. Root shoot ratio was remarkably reduced in wheat seedlings. The adverse effect was concentration dependent in both the crops.

47. Relative root growth (RRG) in maize was decreased by heavy metals. Inhibition was maximum with mercury. The inhibitory effect of all the metals was concentration dependent. RRG value for wheat seedlings was also lowered by copper, cadmium and mercury, maximum effect was found with mercury, then with copper and then with cadmium.

48. Fresh weight of root, stem and leaf of 6, 8 and 10 days old maize seedlings was significantly lowered by higher concentrations of heavy metals (150 mg and above concentration of heavy metals/kg sand). Mercury induced most toxic effects. Leaf emergence was delayed. Higher
fresh weight of metal treated endosperm suggested the less mobilization of reserve nutrients from endosperm to growing axis.

49. Root fresh weight of wheat seedlings was progressively decreased with increase in concentration. Stem fresh weight was affected by higher concentration of heavy metals. Leaf fresh weight was also decreased significantly with higher concentration, but fresh weight of endosperm was higher in the same seedlings. Mercury caused most adverse effect on fresh weight of leaf. Mercury was the most toxic for both the crops. The inhibitory effects were more in maize than in wheat.

50. Relative Growth Rate (RGR) was generally increased in maize seedlings by heavy metals, effects were increasing with increase in concentration. Significant effect was found in the seedlings treated for shorter duration. Higher RGR was also found for wheat seedlings treated with mercury for short duration. Longer treatment, especially with copper and mercury, decreased RGR in wheat seedlings.

51. The dry weight of root, stem and leaf of 6, 8 and 10 days old maize and wheat seedlings was inhibited by copper, cadmium and mercury. Inhibitory effect was related with concentration. Higher concentrations reduced the mobilization of reserve nutrients from endosperm. The inhibitory effect on dry weight of root,
stem and leaf was comparatively more significant in maize than in wheat. Root was highly affected than leaf and stem. Mercury caused most adverse effect.

52. Generally, percent moisture in root, stem and leaf of both maize and wheat seedlings were not affected by copper and cadmium. Higher concentrations of mercury decreased the percent moisture in root, stem and leaf of both the crops. Percent moisture was remarkably decreased in endosperm of both the crops. Mercury caused significant lowering. The inhibitory effect was more in maize than in wheat. Thus percent moisture in endosperm may be selected as a physiological marker for studying phytotoxic effects of heavy metals at seedling stage.

53. Copper, cadmium and mercury stimulated α- and β-amylase activities in the leaf of 6 days old maize seedlings but suppressed in 8 and 10 days old maize seedlings. Generally, effects were correlated with concentration. Copper, cadmium and mercury remarkably stimulated α-amylase activity in wheat leaf but β-amylase activity was more in 6 and 8 days old heavy metal treated wheat seedlings. In 10 days old seedlings, cadmium stimulated the activity while copper and mercury lowered it.

54. Reducing and non-reducing sugars were accumulated in the leaf of maize and wheat treated with copper, cadmium and mercury. Accumulation was increasing with increase in concentration of heavy metals. Copper,
cadmium and mercury gave similar effects on sugar accumulation in maize leaf.

55. Generally, heavy metals decreased the invertase activity in the leaf of maize. Inhibitory effects were correlated with concentration. In 6 days old wheat seedlings, copper, cadmium and mercury stimulated the activity. Stimulation in activity was also found in 10 days old seedlings treated with cadmium while copper and mercury suppressed the activity.

56. In 8 days old maize seedlings, heavy metals stimulated protease activity, but in 10 days old seedlings, it was lowered. The effects were correlated with concentration up to some extent. In wheat, copper, cadmium and mercury enhanced the protease activity in the leaf of 6 days old seedlings, but suppressed it in 10 days old seedlings.

57. Protein content was more in the leaf of maize seedlings raised with high concentrations of heavy metals. Mode of action of all the metals was similar. Accumulation was positively correlated with concentration of heavy metal. Copper, cadmium and mercury enhanced the level of protein in the leaf of 6, 8 and 10 days old wheat seedlings. Prominent effect was found with higher concentrations.

58. Amino acids were accumulated in the leaves of heavy metal treated maize and wheat seedlings. Accumulation was controlled by concentration of applied metal and
59. Higher amount of proline was found in the leaves of heavy metal treated maize and wheat seedlings. The most significant effect was found in 10 days old seedlings and it was correlated with concentration of heavy metal.

60. Heavy metals particularly, mercury, stimulated RNAse activity in 6 and 8 days old maize seedlings, but decreased in 10 days old seedlings. The fate of RNAse activity depends upon duration of the treatment. Shorter duration stimulated the activity while longer exposure decreased the activity in both the crops. Longer duration of copper and mercury lowered the activity, while cadmium enhanced it vigorously.

61. RNA content was lower in 6 days old maize seedlings, but it was more in 10 days old heavy metal treated maize seedlings in comparison to that in control seedlings. The higher concentration was effective in younger seedlings while lower concentration was effective in older maize seedlings. In wheat leaf, generally, heavy metals enhanced the RNA level. Higher concentration was effective.

62. Heavy metals stimulated the acid phosphatase activity in 6 days old maize seedlings, but in 8 and 10 days old seedlings, it was decreased. In wheat, higher APase activity was found in 6 and 8 days old seedlings. Copper and mercury lowered the activity in 10 days old
63. Peroxidase activity in the leaves of maize and wheat was stimulated by copper, cadmium and mercury. The increase was positively correlated with concentration. Rise in the activity was much more sharp in maize than that in wheat.

64. Polyphenol oxidase activity was decreased in older seedlings of maize and wheat, which were treated with copper and mercury, while cadmium stimulated it. Cadmium stimulated the activity in 6 days old maize and wheat seedlings.

65. Higher amount of total phenols were found in the leaf of maize and wheat treated with copper, cadmium and mercury. The accumulation was positively correlated with concentration of the metal.

66. Photosynthetic pigments i.e. chlorophyll 'a', chlorophyll 'b', total chlorophyll and carotenoids were decreased by higher concentrations of cadmium and mercury. Mercury was more effective and it caused more adverse effect in maize than in wheat.

67. Mercury decreased the ratio of total chlorophyll to carotenoids in maize, reverse was true for wheat. Higher amount of chlorophyll pigments in comparison to carotenoids in wheat leaf may be correlated with better tolerance of wheat in comparison to maize.
Finally it is concluded that seedling growth of maize var Ganga safed-2 and wheat var sonalika was adversely affected by copper, cadmium and mercury having 50 μg/ml or above concentrations, though adverse effect was more in maize than in wheat. Moreover inhibitory effects were related with concentration as well as nature of metal. Mercury seems to be most effective.

The presence of heavy metals in media impaired the various metabolisms, though different metals did not act similarly on particular enzyme/metabolite. Moreover changes in metabolic activities also differed with age of the seedlings, duration of treatments etc. still alterations were sharp in maize than in wheat, indicating that maize var Ganga safed-2 is more sensitive to heavy metals.

When maize and wheat seedlings were raised in pots having heavy metal contaminated sand, all the heavy metals i.e. copper, cadmium and mercury decreased the seedling growth, inhibition was more in maize than in wheat. Various enzymatic activities and metabolites in leaf of both the crops were affected, the alterations were dependent upon type of metal, its concentration, exposure period etc. Significant alterations were found in maize than in wheat.

Both the experiments (without and with soil) suggests that maize var Ganga safed-2 being more sensitive to heavy metals avoid its cultivation in areas having heavy metal
contaminated soil. Certain parameters like percent moisture in endosperm, proline accumulation, peroxidase activity may be considered as markers for heavy metal toxicity in cereal seedlings. The biochemical changes in embryo at the time of sprouting predict the toxic effects of heavy metals on seedling growth while tolerance mechanism can be predicted through metabolic changes in endosperm of very young seedlings. Generally varieties of cereals are selected on the basis of their capacity to tolerate no. of stresses like water stress, salt stress etc. but now there is a time to select the variety having heavy metal tolerance also.