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RESEARCH ARTICLE

Effects of heavy metals on seed germination and protein content of *Vigna radiata* (l.) Wilczek

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Abstract

Rapid industrialization and urbanization processes has led to the incorporation of pollutants such as pesticides, petroleum products, acids and heavy metals in the natural resources like soil, water and air, thus degrading not only the quality of the environment, but also affecting both plants and animals. In this context, the study was carried out to assess and determine the cumulative effect of some selected heavy metals on seed germination of mung bean. Among the five selected heavy metals, mercury and copper treated seeds were found to be affected more seriously compared to other selected heavy metals. The physical parameters including shoot length and root length were recorded periodically in each sample group to know the response of germinating seeds against the selected heavy metals. Morphological changes in germinating seeds were noticeable in which colour faded from green to pale yellow and those treated with salts of Cu, Co and Mn turned brownish yellow. The selected heavy metals also showed marked effect on protein content and mitotic activity. Although reports exist on mechanisms by which the heavy metals act as stress and how plants have learnt to overcome, the future scope of this remains in excavating the signaling mechanisms in germinating seeds in response to heavy metal stress.

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INTRODUCTION

Soil is a valuable and non-renewable resource essential for germination of seeds, survival and growth of plants thus supporting every life form on earth. However in the modern world, numerous soil pollutants restrict the growth of plants. Abiotic stress factors including salinity, drought, extreme temperatures, chemical toxicity and oxidative stress from the environment are the major causes of worldwide crop loss that pose serious threats to agricultural produce. With the ongoing technological advancements in industrialization and urbanization process, the release of toxic contaminants like heavy metals in the natural resources has become a serious problem worldwide. Metal toxicity affects crop yields, soil biomass and fertility. Presence of heavy metals like lead, manganese, copper, cobalt and mercury in air, soil and water can cause bioaccumulation affecting the entire ecosystem and pose harmful health consequences in all life forms. The major sources of pollution in the state of Odisha in India are overburdens of mine, industrial effluent, fertilizers and pesticides, extra salts and elements that degrade the soil quality (Sahu S K et al., 2014). Metals and chemicals in higher concentration hamper the plant germination, growth and production mainly associated with the physiological, biochemical and genetic elements of the plant system.

Naturally plants are exposed with many adverse environmental conditions like biotic and abiotic stress. Despite all other stresses, heavy metal stress is one of great importance which has notable adverse effects on crop productivity and growth. Heavy metal stress triggers different responses in plants, ranging from biochemical responses to crop yield. The term ‘‘heavy metals’’ refers to any metallic element that has a relatively high density and is toxic or poisonous even at low concentration (Lenntech Water Treatment and Air Purification, 2004). ‘‘Heavy metals’’ in a general collective term, applies to the group of metals and metalloids with atomic density greater than 4 g/cm3, or 5 times or more, greater than water (Hawkes, 1997). However, chemical properties of the heavy metals are the most influencing factors compared to their density.

Heavy metal toxicity in plants varies with plant species, specific metal, concentration, chemical form and soil composition and pH, as many heavy metals are considered to be essential for plant growth. Some of these heavy metals like Cu and Zn either serve as cofactor and activators of enzyme reactions e.g., in forming enzymes/substrate metal complex (Mildvan, 1970) or exert a catalytic property such as prosthetic group in metalloproteins. There are different sources of heavy metals in the environment such as: natural, agricultural, industrial, domestic effluent, atmospheric sources and other sources. Activities such as mining and smelting operations and agriculture have contaminated extensive areas of world such as Japan, Indonesia and China mostly by heavy metals such as Cd, Cu and Zn (Herawati et al., 2000).

The inorganic and organic fertilizers are the most important sources of heavy metals to agricultural soil. Liming, sewage sludge, irrigation waters and pesticides, are the main sources of heavy metals in the agricultural soils. Others, particularly fungicides, inorganic fertilizers and phosphate fertilizers have variable levels of Cd, Cr, Ni, Pb and Zn depending on their sources. Cadmium is of particular concern in plants since it accumulates in leaves at very high levels, which may be consumed by animals or human beings. Cadmium enrichment also occurs due to the application of sewage sludge, manure and limes (Yanqun et al., 2005). Although the levels of heavy metals in agricultural soil are very small, the repeated use of phosphate fertilizer and its long persistence time for metals, there may be dangerously high accumulation of some metals.

Animal manure enriches the soil by the addition of Mn, Zn, Cu and Co and sewage sludge by Zn, Cr, Pb, Ni, Cd and Cu (Verkleji, 1993). The increase in heavy metal contamination of agricultural soil depends on the rate of application of the contributors with its elemental concentration and soil characteristics to which it is applied. Heavy metal accumulation in soil is also due to application of soil amendments such as compost refusing and nitrate fertilizers (Ross, 1994). Liming increases the heavy metal levels in the soil more than the nitrate fertilizers and compost refuse. Sewage sludge is one of the most important sources of heavy metal contamination to the soil (Ross, 1994). Several heavy metal based pesticides are used to control the diseases of grain and fruit crops and vegetables and are sources of heavy metal pollution to the soil (Ross, 1994).

Other sources of heavy metals include refuse incineration, landfills and transportation (automobiles, diesel-powered vehicles and aircraft). Two main anthropogenic sources that contaminate the soil are fly ash produced due to coal burning and the corrosion of commercial waste products, which add Cr, Cu, Pb and galvanized metals (primarily Zn) into the environment. Coal burning adds heavy metals such as Cd, Hg, Mn, Ni, Al, Fe and Ti into the soils (Verkleji, 1993). Although reports exist on mechanisms by which the heavy metals act as stress and how plants have learnt to overcome, the future scope of this review remains in excavating the signaling mechanisms in germinating seeds in response to heavy metal stress.

Materials and Methods

Plant material used for the present study was the seeds of *Vigna radiata* (L.) R. Wilczek commonly known as mung bean, belonging to the family leguminosae. It was purchased from the local market and was identified. For the present study, five heavy metal salts were selected in five different concentrations. They included $Pb(NO_3)_2$ (Lead nitrate), $HgCl_2$ (Mercuric chloride), $CuSO_4.5H_2O$ (Copper sulphate), $CoCl_2.6H_2O$ (Cobalt chloride) and MnO(Manganese(ll) oxide).The seeds of the mung bean were thoroughly washed with distilled water three times and a group of viable 20 seeds were transferred into a sterile conical flask containing 20ml different molar concentrations of 5 heavy metal salts viz. 0.5mM, 1mM, 1.5mM, 2mM and 2.5mM. After a period of 4 hours at dark chamber incubation, the swollen seeds were transferred into petriplates having double layered sterile whatman number 1 filter paper. After that 9 ml of the corresponding heavy metal solutions were added to petriplates and the petriplates were transferred to incubation chamber for a period of 72 hrs.

The physical parameters like shoot length and root length were analyzed systematically.

Analysis of shoot length -The length of the shoot of the germinating seeds was recorded in every 24 hr using the standard methods.

Analysis of root length- Root initiation and length of root were noted and were subjected to comparative studies.

Quantitative Analysis: Quantitative analysis was carried out in each group of the germinating seeds to know the physiological changes which were affected by the heavy metal stress.

Total protein estimation: The total protein was estimated as per Lowry et al (1951).

Determination of mitotic activity: Roots from the germinated seeds were used for the mitotic study (Badria et al., 2001)

Results and Discussion

Analysis of Physical parameters

In the preliminary study the physical parameters including shoot length and root length were recorded periodically in each sample group to know the response of germinating seeds against the selected heavy metals.

The shoot length was recorded systematically in a time interval of 24 hrs. Maximum growth of 6.3cm was noticed against cobalt chloride at 0.5mM after 72 hrs of incubation and the least was about 0.3cm for 2.5mM copper sulphate at 24 hrs. The shoot showed stunted growth and chlorosis when treated with heavy metals compared with that of positive control. All the seedlings failed to develop healthy shoot (Table 1).

The root analysis also supports the shoot development. When compared with shoot, the heavy metals severely affected the root initiation as well as the development of root system. There were no roots produced in the case of mercuric chloride and copper sulphate even after 72 hrs of treatment and similarly no roots were developed in case of rest of the three metal salts at the initial 24 hrs treatment. But a root length of maximum 7.6cm was recorded against 0.5 mM manganese oxide treated seeds after 72 hrs incubation (Table 2).

The plant material used for the present study is mung bean because they are used as a source of food and have the capacity to absorb heavy metals from polluted soil (Bishnoi et al., 1993). Excess concentrations of some heavy metals in soils such as Cd^{+2}, Cr^{+6}, Cu^{+2}, Ni^{+2} and Zn^{+2} have caused the disruption of natural aquatic and terrestrial ecosystems (Meagher, 2000) .Irrespective of the source, heavy metals have toxic effects on plants and heavy metals are known to interfere with the environment. The treatment with heavy metals inhibits the germination percentage of seed, though the extent of inhibition is more at higher concentration. The decrease in seed germination may be due to the breakdown of stored food material in seed (Kalimuthy and Siva, 1990).

In the preliminary phytochemical and physical analysis of the germinating seed to different heavy metal stress, it was found that the seeds showed negative response to all metals salts irrespective of time and concentration in terms of stunted growth, growth inhibition, chlorosis , poor metabolic activity, lowest respiratory efficiency and water mineral absorption efficiency. Heavy metals are day to day deposited to the earth crust and are absorbed by plants and in turn they enter into different food chain and nutrient cycling.

In the case of seeds treated with lead nitrate, the shoot length decreased with increasing concentration. Highest shoot length was noted for 0.5mM concentration at 72 hours, whereas lowest was for 2.5mM in 24 hours incubation time. No roots were formed after 24 hours incubation time. Root growth was seen only after 48 hours. Highest root length was observed after 72 hours for seeds treated with 0.5mM concentration and lowest were observed for seeds in 2.5mM concentration.

The shoot length of germinated seeds treated with mercuric chloride was found to decrease with increase in concentration till 1.5mM. But after that, the shoot length increased for 2mM and 2.5mM concentrations. Among the five different concentrations of mercuric chloride, the highest shoot length was observed for seeds in 2.5mM solution after 72hours whereas lowest was for 1.5mM seeds in 24hours incubation time. No root developed in any of the concentrations even after 72 hours treatment.

The seeds treated in different concentrations of copper sulphate showed stunted growth pattern. This was clearly observed in shoot and root of germinated seeds. The shoot lengths of seeds treated with copper sulphate were minimum when compared with the other heavy metals of concern in this study. The highest shoot length recorded here was 1.5cm (in 0.5mM) and lowest was 0.3 cm (in 2.5mM), which is the least value in this shoot length analysis. It also showed no root development in any of the 5 concentrations. The same findings were reported earlier due to effluent treatment by Rajesh Kumar and Bhargava (1998), Hariom et al. (1994). The reduction in shoot and root growth at higher concentration of effluent may be due to the fact that germinating seeds under higher concentrations would get less amount of oxygen which might have restricted the energy supply and retarded the growth and development(Kumar A, 2000).

When the seeds treated with cobalt chloride were observed under different concentrations, there was a decline in shoot and root length with increase in concentrations. That is after 24 hours incubation time, the shoot length reduced from 0.8cm (0.5mM) to 0.4cm (2.5mM). Similar reduction pattern in shoot length were observed in 48 hours and 72 hours. Root was not developed in 24 hours of treatment. Very minute sized roots aroused after an incubation period of 48 hours. The root length in different molar concentrations varied from 0.1 cm to 0.8cm. The least value was observed for 1mM and 1.5mM concentrations after 48 hours of treatment. The highest value recorded was for 0.5mM concentration after 72 hours of treatment.

The shoot length of germinated seeds treated with Manganese (ll) oxide decreased with increasing concentrations. This pattern was observed even after 72 hours of treatment. The shoot length slowly decreased from the lowest concentration to the highest. The lowest value observed was 1.4cms and the highest was 6.3cms in 2.5mM and 0.5mM respectively. The root developed only after 48 hours of treatment. The root length was seen reduced with increase in concentration. Of all the five selected heavy metals, the root length was observed to be maximum for MnO that is 7.6 cms after a period of 72 hours in 0.5mM concentrations. The least value in this set was 2.5cms (for 2mM and 2.5mM after 48 hours).

Tables and Figures

Table 1. Physical parameter of shoot length of heavy metal treated seeds of *Vigna radiata* (in cm).

Heavy metal	Time interval (in hrs)	0.5mM	1mM	1.5mM	2mM	2.5mM
$Pb(NO_3)_2$	24 48 72	1.5 3.1 5.2	1.1 2 2.9	0.6 1.2 1.6	0.6 1.1 1.5	0.5 0.9 1.3
$HgCl_2$	24 48 72	0.8 0.8 0.9	0.7 0.8 0.8	0.6 0.7 1.4	1.5 1.9 2.4	1.6 2.1 2.5
$CuSO_4.5H_2O$	24 48 72	1.5 1.5 1.6	0.5 0.5 0.7	0.4 0.7 0.9	0.4 0.5 0.8	0.3 0.3 0.4
$CoCl_2.6H_2O$	24 48 72	0.8 1.2 1.9	0.7 0.9 1.1	0.7 0.7 0.8	0.6 0.7 0.7	0.4 0.5 0.7
MnO	24 48 72	1.8 3.4 6.3	1.6 3.2 5.9	1.5 2.4 3.1	1.5 2.4 2.9	1.4 2.3 2.6

Table 2. Physical parameter of root length of heavy metal treated seeds of Vigna radiata (in cm).

Heavy metal	Time interval (in hrs)	0.5mM	1mM	1.5mM	2mM	2.5mM
$Pb(NO_3)_2$	24	No root	No root	No root	No root	No root
	48	0.6	0.3	0.3	0.1	0.1
	72	1.3	0.9	0.7	0.2	0.2
$HgCl_2$	24	No root	No root	No root	No root	No root
	48	No root	No root	No root	No root	No root
	72	No root	No root	No root	No root	No root
$CuSO_4.5H_2O$	24	No root	No root	No root	No root	No root
	48	No root	No root	No root	No root	No root
	72	No root	No root	No root	No root	No root
$CoCl_2.6H_2O$	24	No root	No root	No root	No root	No root
	48	0.2	0.1	0.1	0.2	0.2
	72	0.8	0.3	0.2	0.2	0.2
MnO	24	No root	No root	No root	No root	No root
	48	3.0	2.9	2.6	2.5	2.5
	72	7.6	6.4	6.0	5.8	5.7

Table 3. Quantitative analysis of total protein in the heavy metal treated seeds of *Vigna radiata* (in mg/g FW).

Heavy Metals	Protein content(mg/g)					Control
	0.5mM	1mM	1.5mM	2mM	2.5mM	1511.622
$Pb(NO_3)_2$	1375.6	1700.908	1740.898	1702.366	1654.178	
$HgCl_2$	1479.6	2276.76	2290.094	1428.976	1420.97	
$CuSO_4.5H_2O$	2351.41	2729.984	2772.658	2708.64	1597.184	
$CoCl_2.6H_2O$	1431.64	1571.34	1675.32	1479.397	1181.312	
MnO	1647.58	2300.7	2199.45	1730.2	1706.24	

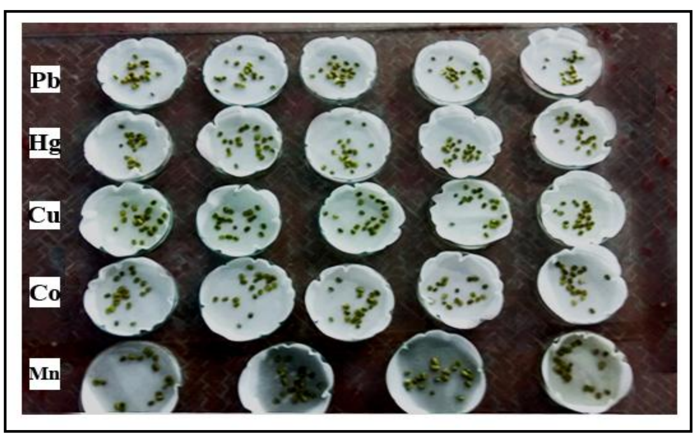

Figure 1.Germination of seeds of *Vigna radiata* L.(R) Wilczek after 24 hrs of treatment with heavy metals

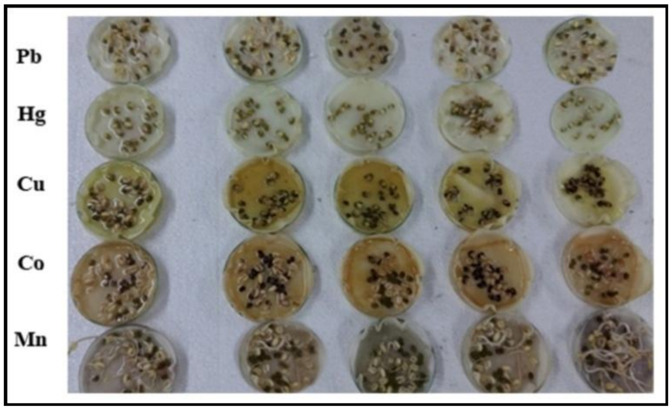

Figure 2.Germination of seeds of *Vigna radiata* L.(R) Wilczek after 72 hrs of treatment with heavy metals

Figure 3. Measurement of shoot length of *Vigna radiata* L.(R) Wilczek

Figure 4. Mitotic activity of the root cells of *Vigna radiata* L.(R) Wilczek (control)

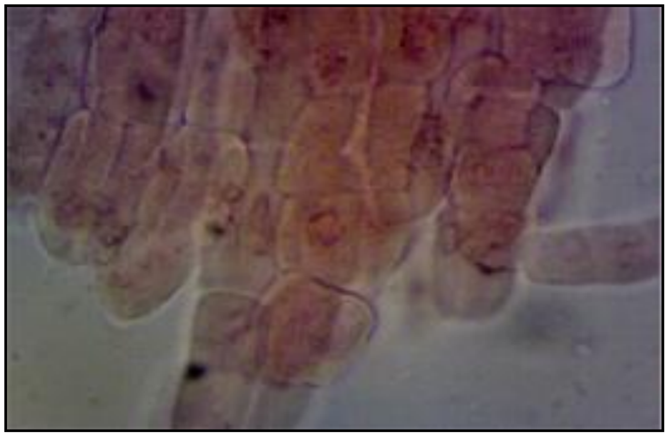

Figure 5. Mitotic activity of the root cells of *Vigna radiata* L.(R) Wilczek (Treated)

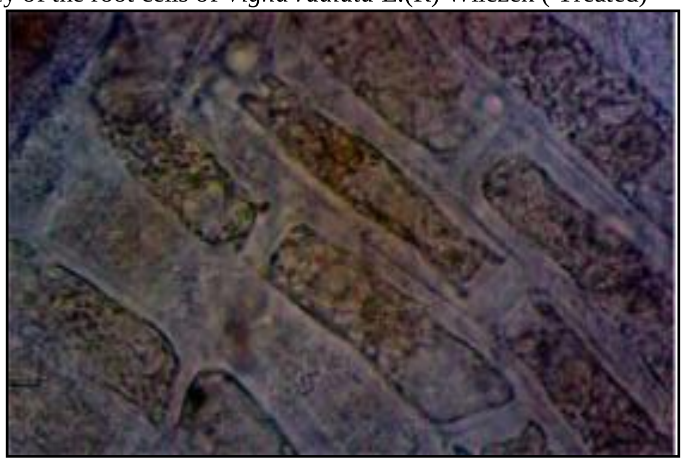

Figure 6. Mitotic activity of the root cells of *Vigna radiata* L.(R) Wilczek (Treated

Figure 7. Mitotic activity of the root cells of *Vigna radiata* L.(R) Wilczek (Treated)

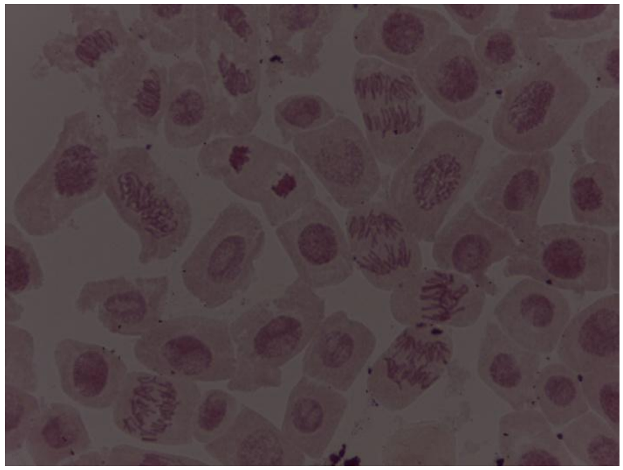

Figure 8. Mitotic activity of the root cells of *Vigna radiata* L.(R) Wilczek (Treated)

Morphological changes in germinating seeds

On the first day that is after 24 hours of incubation period, all the seeds germinated and the seed size enlarged in all the 5 concentrations. The seed colour faded from green to pale yellow. After 72 hours of treatment, seeds treated with salts of Cu, Co and Mn turned brownish yellow. Similar results were reported by Nilima D Gajbhiye (2013) when 0.1 to 0.9% concentrations of sodium nitrate were used for the study of toxic effects on germinating seeds of mung bean. The treatment may have affected water and osmotic potential thus preventing the development of turgor pressure in the seed, which has been considered as one of the key factors for the initiation of radicle growth during seed germination (Maia et al., 2001).

Total protein estimation

The protein profile of the germinating seeds are strongly corroborating with the physical parameters. A drastic reduction in the protein content was noticed in the germinating seeds with respect to time and molarity of the heavy metals. For 0.5mM concentration, Cu & Mn are higher than the control and remaining showed lower protein content than the control. For 1mM, all the seeds treated with 1mM concentrations of heavy metals showed higher protein content than that of control and also the values increased when compared with the control. For 1.5mM, the seeds treated with Pb, Hg, Cu, and Co showed a slight increase in the protein content when compared with that of 1mM concentration but those treated with Mn showed a decrease in protein content for the above said condition. For 2mM and 2.5mM concentrations, all the seeds showed decreased protein content with all the metals when compared with that of 1.5mM concentration. The reduction of protein may be due to inhibition of biosynthesis and/or increased degradation of protein (Batish et al., 2006).

Oxidative stress is one of the main disturbances of metabolic pathways and damage to macromolecules (He-gedus et al., 2001). High levels of heavy metals interrupt the mitochondrial activity and induce oxidative stress by triggering the generation of ROS. This leads to the disruption of biomembrane lipids and cellular metabolites in plants (Messer et al., 2005; Cargnelutti et al., 2006). Thus the ROS generated during the heavy metal absorption leads to oxidative burst at the cellular level and interferes with the metabolic pathways and lower protein profile in the germinating seeds of *Vigna radiata* substantiating the above facts.

During the last few years, a significant increase in the amount of metal has appeared in the environment with the constant increase in the industries raising a serious question. We have investigated how heavy metal treatment affected the seed germination of *Vigna radiata.* As metal processing industries are increasing, it is necessary to have a good knowledge of plants and their responses to heavy metal stress (Bewely and Black, 1982). The highest risk for human health is when plants develop tolerance mechanisms against metals and when those plants are incorporated into the food chain. Growth changes are the first most obvious reactions of plants under stress (Breckle et al., 1991). Thus preliminary investigation revealed the impact of common heavy metals stress to seed germination. Further studies are warranted to know the molecular mechanism and genetic aberrations due to heavy metal absorption.

Determination of mitotic activity

The control and the treated seeds were used for the mitotic study. The control seeds showed normal mitotic activity (Figure 4). But in case of the treated seeds, they behaved differently in terms of mitosis. All most all the root tips were elongated and damaged when compared with the control (Figure 5). In case of seeds treated with Co, Cu and Mn, there observed no typical mitotic activity (Figure 6). Similar results were reported by Nilima D Gajbhiye (2013) for *Vigna radiata* seeds grown in 0.1%NaNO3. The cells showed mitotic cells but in 0.9% NaNO3concentration, cells were elongated and damaged when compare with control. The similar results were reported by other workers that the methanol extract of dried latex, the crude dried latex of *C. procera* has earlier been shown to exhibit anti-mitotic activity in the *Allium cepa* model (Sehgal R et al., 2006). An association between nitrate exposure and incidence of childhood leukemias was found in one study (Moller H et al., 1997). Living in areas with high nitrate levels in drinking water during childhood was associated with a higher incidence of testicular cancer (Moller H, 1997 and Thorpe N, 2005). Significant reduction in plant growth induces disorientation of roots and shoots, plant tissue, and finally the cell wall destruction occurs (Sharma et al., 2000; Patra and Sharma, 2000).

Presence of chromosomal bridges was observed in few anaphases. Some adjacent cells were also of unequal sizes, some diagonal and transverse metaphases and anaphases were also observed. These indicated abnormal orientation of mitotic apparatus or distorted mitotic spindles causing dis-oriented mitosis (DOM). Occurrence of disoriented chromosomes might be due to action of heavy metals on the microtubules (MT) of the spindle fibers. Sticky chromosome is a result of the improper folding of chromosome fibers into chromatids and thus there is an intermingling of the fibers and chromosomes become attached to each other by means of subchromatid bridge. Disturbed metaphase indicated by chromosome spreading irregularly all over the spindle apparatus and bridges recorded in the present studies are the result of chromosome breakage and reunion (Badr, 1983).

Summary and Conclusion

Naturally plants are exposed with many adverse environmental conditions like biotic and abiotic stress. Despite all other stresses, heavy metal stress is one of great importance which has notable adverse effects on crop productivity and growth. Heavy metal stress triggers different responses in plants, ranging from biochemical responses to crop yield. There are different sources of heavy metals in the environment such as: natural, agricultural, industrial, domestic effluent, atmospheric sources and other sources. Plant material used for the present study was the seeds of *Vigna radiata* (L.) R. Wilczek commonly known as mung bean belonging to the family leguminosae.

In the preliminary study the physical parameters including shoot length and root length were recorded periodically in each sample group to know the response of germinating seeds against the selected heavy metals. When compared with shoot, the heavy metals severely affected the root initiation as well as the development of root system. There were no roots produced in the case of mercuric chloride and copper sulphate even after 72 hrs of treatment and similarly no roots were developed in case of rest of the three metal salts at the initial 24 hrs treatment. The seed colour faded from green to pale yellow. After 72 hours of treatment, seeds treated with salts of Cu, Co and Mn turned brownish yellow. The protein profile of the germinating seeds was strongly corroborating with the physical parameters. Mitotic observation also explained the effects of heavy metals. The cells were elongated and damaged in all most all the cases and for the seeds treated with metal salts of Co, Cu and Mn, no typical mitotic stages were observed.

Increasing environmental pollution caused by heavy metals, released by industrial and agricultural activities, is a major problem in the world. Plants grown on soils with parent material rich in heavy metals or polluted by industrial effluents are known to absorb heavy metals in quantities that may be toxic to plant growth and metabolism. Excess of heavy metals cause phytotoxic effects in several ways, one of these being the excessive production of reactive oxygen species (ROS) which disturb the cellular redox environment causing oxidative stress. The highest risk for human health is when plants develop tolerance mechanisms against metals and when those plants are incorporated into the food chain. Growth changes are the first most obvious reactions of plants under stress (Breckle et al., 1991). Thus preliminary investigation revealed the impact of common heavy metals stress to seed germination. Further studies are warranted to know the molecular mechanism and genetic aberrations due to heavy metal absorption.

References

Badria, F.A., Houssein,W.E., Zaghloul, M.G., and Halim, A.F. (2001). Antimitotic activity of gossypol and gossypolone. Pharmaceutical Biology, Vol 39: 120-126.

Badr, A. **(1983**). Mitodepressive and chromotoxic activities of two herbicides in Allium cepa. Cytol, 48: 451-457.

Batish, D.R., Gupta, P., Singh, H.P., Kohli, R.K. **(2006).** L DOPA (L-3,4-dihydroxyphenylalanine) affects rooting potential and associated biochemical changes in hypocotyls of mungbean and inhibits mitotic activity in onion root tips. Plant Growth Regul, 49: 229–235.

Bewley, J.D., and Black, M. (1982). The Physiology and Biochemistry of Seeds, Berlim, Springer-Verlag, Vol 2: 375.

Bishnoi, U.R., Oka, G.M., Fearon, A.L. (1993). Quantity and quality of forage and silage of pearl millet in comparison to sudax, grain, and forage sorghum harvested at different growth stages, J. Trop. Agric. (Trinidad), 70: 98-102.

Breckle, S.W., Waisel, Y., Eshel, A., Kafkafi, U. and Marcel Dekker. **(1991).** Growth under Stress: Heavy Metal., Plant Roots, The Hidden Half. Edition, New York, 351-373.

Cargnelutti, D., Tabaldi, L.A., Spanevello, R.M., Jucoski, G.O., Battisti, V., Redin, M., Linares, C.E.B., Dressler, V.L., Flores, M.M., Nicoloso, F.T., Morsch, V.M. and Schetinger, M.R.C. (2006). Mercury toxicity induces oxidative stress in growing cucumber seedlings, Chemosph, 65: 999–1106.

Hariom, N. O. Singh. and S.A. Mayak. **(1994).** J. Environ. Biol, 15-171.

Hawkes, J.S. (1997). Heavy metals, J. Chem. Edu, 74: 1369–1374.

Hegedus, A., Erdei, S. and Horvath, G. **(2001)**. Comparative studies of H_2O_2 detoxifying enzymes in green and greening barley seedings under cadmium stress. Plant. Sci, 160: 1085–1093.

Herawati, N., Suzuki, S., Hayashi, K., Rivai, I.F. and Koyoma, H. **(2000).** Cadmium, copper and zinc levels in rice and soil of Japan, Indonesia and China by soil type. Bull. Environ. Contam. Toxicol, 64: 33–39.

Kalimuthu, K. and Siva, S.R. **(1990).** Physiological effects of heavy metals on zea maize seedlings. Ind.j.of plant physiology, 33: 242-244.

Kumar, A.(2000). Adv. Plant Sci, 13-427.

Kumar, R. and Bhargava, A.K. **(1998).** Adv. Plant Sci. 11-221.

Lenntech,Water Treatment and Air Purification. **(2004).** Water Treatment, Published by Lenntech, Rotterdamseweg, Netherlands.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., and. Randall, R.J. (1951). Protein Measurement with the Folin Phenol Reagent, J. Biol. Chem. 193: 265-275.

Maia, A., and Rainer, L.(2001). Changes in water relations, solute leakage and growth characters during seed germination and seedling development in Trigonella coerulea (Fabaceae), Jounal of Applied Botany, 75: 144-151.

.

Meagher, R. B. **(2000).** Phytoremediation of Toxic Elemental and Organic Pollutants, Current Opinion in Plant Biology, 3: 153-162.

Messer, R.L., Lockwood, P.E., Tseng, W.Y., Edwards, K., Shaw, M., Caughman, G.B., Lewis, J.B. and Wataha, J.C. (2005). Mercury (II) alters mitochondrial activity of monocytes at sublethal doses via oxidative stress mechanisms, J. Biomed. Mat. Res, 75: 257–263.

Mildvan, A.S. (1970). Metal in enzymes catalysis. In: Boyer DD (ed) The enzymes, vol 11. Academic Press, London. 445–536.

Moller, H. (1997). Work in agriculture, childhood residence, nitrate exposure, and testicular cancer risk: a case-control study in Denmark. Cancer Epidemiol. Biomarkers Prev. 6(2): 141-144.

Nilima .D. Gajbhiye. **(2013).** Toxic Effect of Sodium Nitrate on Germinating Seeds of Vigna radiata . World Academy of Science, Engineering and Technology International Journal of Biological, Food, Veterinary and Agricultural Engineering Vol 7: No.10.

Patra, M., and Sharma, A. **(2000).** Mercury toxicity in plants, Bot. Rev., 66: 379–422.

Ross, S.M. **(1994)**. Toxic metals in soil–plant systems, Wiley, Chichester. 469.

Sahu, S.K., and Pradhan, K.C. **(2004**). Sarangi Soil Pollution in Orissa. Orissa Review.

Sehgal, R., Roy, S. and Kumar, V.L. **(2006).** Evaluation of cytotoxic potential of latex of Calotropis procera and podophyllotoxin in Allium cepa root model. Biocell 30: 9-13.

Thorpe, N. and Shirmohammadi, A. (2005). Herbicides and nitrates in groundwater of Maryland and childhood cancers: a geographic information systems approach. J Environ Sci Health C.Environ Carcinog. Ecotoxicol. Rev. 23(2): 261-278.

Verkleji, J.A.S. (1993). The effects of heavy metals stress on higher plants and their use as bio monitors. In: Markert B (ed) Plant as bioindicators: indicators of heavy metals in the terrestrial environment. VCH, New York.415–424.

Yanqun, Z., Yuan, L., Jianjun, C., Haiyan, C., Li, Q. and Schratz, C. **(2005).** Hyper accumulation of Pb, Zn and Cd in herbaceous grown on lead-zinc mining area in Yunnan, China, Environ. Int. 31: 755–762.