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Studies on effect of mercury in two verities of rice (*Oryza sativa* L.)

Poonam Misra

Department of Botany, University of Lucknow, Lucknow-226 007, India

***e-mail**: dr.poonammisra@yahoo.com

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Abstract: Mercury in excess doses caused Necrosis, distorted leaves, brown and damaged root system in both rice (*Oryza sativa* L.) varieties *i.e* Ratna and Sarju-52 seedlings. Shoot system was found to be less cylindrical than control and show some abnormality at highest dose of mercury (4.0 mM). At lowest dose of mercury, the root length was significantly decreases about 17.78% over control. The shoot seems to be more sensitive to mercury than the root in var. Ratna. 1.0 mM dose of mercury significantly activated the activity of catalase enzyme in seedlings. Various doses of mercury significantly reduced the level of total chlorophyll concentration in both the varieties of rice seedlings. The sugar content was reduced at excess dosed of Hg. At lowest concentration of mercury Sarju-52, showed stimulation in the concentration of sugar, while at highest concentration of mercury the experimental seedlings exhibited inhibition in sugar content. Mercury drastically affected the concentration of protein in these two varieties of significant decrease in protein content was found with all treatments of mercury except 1.0 mm dose of this metal in rice var. Rattan. Mercury also inhibited the activity of other important iron enzyme peroxidase in var. Ratna. However, other rice var. Sarju-52 showed enhancement in the activity of this enzyme with excess doses of the same metal.

Key words: *Vigna unguiculaia*, *Oryza sativa,* Mercury, Catalase, Sugar, Chlorophyll, Protein, Peroxidase

Introduction

Inorganic form of Hg vapour is an important hazardous substance because it enters into blood through diffusion by lungs and then goes to brain and where it causes serious damage. Alkyl group of mercury are the most toxic compounds because of their long retention time in the tissues. Therefore, they readily accumulate at high concentration in tissues of different body organs. Hg causes abnormalities in cell division and it also increase the frequency of chromosomal breakages. Some of these abnormalities may because due to the combination of mercurial with – SH groups and its resulted the inhibition of enzyme activity. Mercury toxicity in plants could be related to the pollution level of this metal in the environment. Organic content of soil, carbon exchange capacity, oxide and carbonate content, redox potential factors affecting plant uptake capacity of metal and uptake of this metal has been found to be plant specific in lichens, bryophytes, wetland plants, woody plants and various crop plants stems and leaves, while in fresh water aquatic plants uptake rate of metal concentration dependent on the species of plant, the seasonal growth rate changes and the absorbance of metal ion by plants. Atmospheric mercury is absorbed by the plant leaves and migrates to soil humus through fallen leaves. C_3 plant species uptake mercury vapour by leaves five times more than that by leaves of the C_4 plant species. Such differential uptake was largely attributed to internal resistance to mercury vapour binding. Toxicity distribution and accumulation of mercury were different between plants exposed through shoot or root, where internal mercury concentrations in the treated plants were similar. There are four important factors responsible for the Hg toxicity in plants - 1) Change in permeability of cell membrane, 2) Reaction of – SH groups with cation, 3) Replacement of essential ions, 4) Affinity for reacting with phosphate group and active group of ADP or ATP (Patra and Sharma, 2000). Mercury to plants supplied by two methods -1) direct administration, 2) Accidental exposures. Hg supplied to plants as an antifungal agent through direct administration process by used treatment of foliar spray. The resultants are better seed germination, seeding growth, and relative growth, shoot and root, studies of leaf area index, internodes development of their anatomical characters. Second accident exposure of mercury to plants occurs through air, water and soil pollution. The level of mercury toxicity in plants depends on wide range of concentrations and genetically studies. Absorption of these organic and inorganic forms of mercury from soil by plants is low. At low concentrations. Mercury has toxic effect on the decreasing capabilities of micro organism. Sensitivity to the metal was enhanced by Ca, reduction in pH and tolerance to mercury by micro-organism (Patra and Sharma, 2000).

The most important sources of contaminating agricultural soil have been the use of organic mercurials as a seed coat dressing to prevent fungal diseases of seeds (Patra and Sharma, 2000). The availability of soil Hg to plants is low, and there is a tendency for Hg accumulation in the roots, indicating that the roots serve as a barrier to Hg uptake (Tripathi and Tripathi, 1999; Munzuroglu and Geekil, 2002). Industrial effluents also contain large number of heavy metals in higher concentration. These effluents are commonly used for the irrigation in adjoining areas of drains of the industries in most of the developing countries like India, because of availability at free of cost. Some workers reported the adverse effect of these effluents on crop plants (Goel and Kulkarni, 1994; Khan and Jain, 1995; Sharma and Singh, 1999; Pandey *et al*., 2002, Pandey and Srivastava, 2002). Heavy metal toxicity to plants vary 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 (Wintz *et al*., 2002). Some of these heavy metals such as Cd, Hg, As, *etc* are strongly poisonous to metal sensitive enzymes, resulting in growth inhibition and death of organisms (Nieboer and Richardson, 1980). Such metals accumulate in ecological food chain through uptake at primary producer level and then through consumption at consumer levels.

This paper presents an overview of the mercury toxicity on early stage in seedling of two verities (Ratna and Sarju – 52) of rice (*Oryza sativa* L.) plant.

Materials and Methods

Short term experiment was carried out by using petridish culture. Two verities (Ratna and Sarju- 52) of rice (*Oryza sativa* L.) seeds were soaked in the controlled nutrient solution and thirty-five germinated seeds were transferred in each petridish with varying concentration of mercury (1.0, 2.0 and 4.0 mM) on the filter paper. The nutrient solution had the following composition: as M eq./1- $Ca(NO_3)_2$-8; KNO_3-4; $MgSO_4$-4, NaH_2PO_4-4; as ppm – Fe-5.6; Mn-0.55; Cu-0.046; Zn-0.065; B-0.37; Mo-0.05; Co and Ni-0.006 each (Hewitt, 1966; Agarwala and Sharma, 1976). Mercurous chloride was used to produce Hg concentration of 1.0, 2.0 and 4.0 mM. Growth in terms of root and shoot lengths were measured at regular intervals. Chlorophyll, sugar and protein concentration were estimated respectively by the method of Petering *et al.* (1940), Dubais *et al*. (1956) and Lowry *et al*. (1951) respectively. Catalase (CAT) and peroxidase (POD) activities were assayed by the method of Euller and Josephson (1927) and Luck (1963).

Results

Mercury in excess doses caused distorted leaves, brown and damaged root system in both the varieties i.e ratna and sarju-52 of rice seedlings. Necrosis was observed at leaf tip of Hg treated seedlings. Seedlings get shortened and change into yellow brown color. Shoot system was found to be less cylindrical than control and show some abnormality at highest dose of mercury (4.0 mM). Basal part of shoot system in Sarju-52 was week and yellow in color and after some time changes into brown color. In general, results demonstrate a dose dependent inhibition in root and shoot growth of most of the given seedling species with mercury toxicity. Concentration in rice var. ratna. At 4.0 mM of Hg, the shoot length was found to be as low as 27% of the control. At lowest dose of mercury, the root length was significantly decreases about 17.78% over control. The shoot seems to be more sensitive to mercury than the root in var. ratna. it is interesting to note that the other rice var. Sarju-52 showed more sensitivity at 4.0 mM dose of mercury so far as shoot length of seedlings was concerned. Root length of this variety showed an increasing tendency with excess doses of mercury. Maximum enhancement in root length was observed dose of Hg (Table 1). The catalase activity in var. Sarju-52 was stimulated or inhibited, depending on the mercury concentration in the nutrient solution. 1.0 mM dose of mercury significantly activated the activity of catalase enzyme in seedlings. In contrast mercury concentration higher than 1.0 mM inhibited the specific activity of this enzyme. The same trend was observed in rice var. Ratna at 2.0 and 4.0 mM of mercury (Table 1).

Various doses of mercury significantly reduced the level of total chlorophyll concentration in both the varieties of rice seedlings. In fact the presence of mercury caused a greater reduction in chlorophyll content in var. Ratna than Sarju-52. Chlorophyll concentration in var. Sarju-52 treated with mercury in the concentration of 4.0 mM decreased by 7.25 fold as compared to control seedlings, However, seedlings treated with 1.0 mM dose decreased chlorophyll content up to five folds were observed (Table 2). The sugar content was reduced at excess dosed of Hg. Seedlings subjected to 1.0 mM dose of mercury had a 29% reduction of this parameter in var. Ratna. At lowest concentration of mercury Sarju-52, showed stimulation in the concentration of sugar, while at highest concentration of mercury the experimental seedlings exhibited inhibition in sugar content. However, no change in sugar content was observed at 2.0 mM Hg (Table 2). The presence of mercury in the solution culture drastically affected the concentration of protein in these two varieties of significant decrease in protein content was found with all treatments of mercury except 1.0 mm dose of this metal in rice var. Ratna (Table 2). Mercury inhibited the activity of other important iron enzyme peroxidase in var. Ratna. However, other rice var. Sarju-52 showed enhancement in the activity of this enzyme with excess doses of the same metal (Table 2).

Discussion

Heavy metals in excess amounts normally show deleterious effects in plants. In the present study the effects of three commonly occurring heavy metals i.e. nickel, lead and mercury were investigated. It was found that these three heavy metals in excess amounts cause adverse effects in the growth and metabolism of the plants. Sugarcane, maize barley, pea, rice, mustard, cowpea and wheat were taken for the present study.

There are many reports which show the adverse effects of these heavy metals in plants (Tandon and Gupta, 2002). Xiong (1998) was of the view that roots are more responsive to toxic metals in environment because to accumulation of more heavy metals than any were affected earlier and subjected to accumulation of more heavy metals than any of the organ. The lead also reduces root growth and causes mitotic irregularities *etc*. Reduced root and shoot length due to excess amounts of lead might be due to abnormal transport of some essential nutrients including zinc which is responsible for auxin synthesis in plant. Lane *et al*. (1978) indicated that inhibiting effect of lead on growth might be due to the interference of lead with auxin regulated cell elongation. Hg is reported to be one of the most toxic heavy metals present in the environment. It is used in industries which produce electrical equipments, pesticides, batteries, domestic thermometers etc. increasing dose of Hg in all plants investigated in this study caused severe reduction in the growth of plants. Although not much work has been done to study the effect of this most toxic element *i.e*. mercury on plants, but whatever studies has been done they indicate the adverse effects of mercury on the growth and metabolism of plants.

Munzuroglu and Geckil (2002) studied the effect of Hg, Cd, Co, Cu ,Pb and Zn on wheat and cucumber plants, they found a lot of variation in the degree of inhibition caused by these heavy metals. Both plants had a reduction in seed germination, rate of root and hypocotyls or coleoptile length was increasing concentration of heavy metals. Among these metals Hg was found to be most inhibitory effect on this parameter in wheat and cucumber seed at certain concentration *i.e.* more than 1.5 mM in cucumber and at 1.7 mM in wheat. No other metal cause this kind of inhibition even at the highest concentration. Earlier studied have showed that at certain

Table - 1: Effect on root and shoot length and enzymes (catalase and peroxidase) of rice (*Oryza sativa* L.) seedlings ver. Ratna and Sarju - 52 treated with different doses of mercury

Treatments	Ratna				Sarju - 52			
	Shoot length (cm)	Root length (cm)	Catalase	Peroxidase	Shoot length (cm)	Root length (cm)	Catalase	Peroxidase
Control	2.30±0.28	8.65±0.02	1.28±0..31	0.038±0.00	18.83±0.70	5.70±0.00	0.84±0.02	0.05±0.00
1.0 mM Hg	10.75±0.35	7.60±0.05	0.86±0.02	0.030±0.002	13.75±0.02	7.06±0.00	1.35±0.00	0.17±0.01
2.0 mM Hg	10.20±0.00	7.37±0.10	0.42±0.11	0.032±0.007	13.63±0.04	6.16±0.00	0.54±0.14	0.08±0.01
4.0 mM Hg	9.10±0.14	7.11±0.07	0.14±0.00	0.029±0.00	12.86±0.02	5.95±0.02	0.24±0.01	0.11±0.01
CD at 5% P	**1.032**	**0.278**	**0.242**	**0.00109**	**1.556**	**0.046**	**0.3039**	**0.01151**

Table - 2: The concentration of chlorophyll, sugar and protein in younger leaves of rice seedling ver. Ratna and Sarju - 52 treated with different doses of mercury

Treatments	Ratna			Sarju-52		
	Chlorophyll (mg g^{-1} F.W.)	Sugar (mg g^{-1} F.W.)	Protein (mg g^{-1} F.W.)	Chlorophyll (mg g^{-1}F.W)	Sugar (mg g^{-1}F.W.)	Protein (mg g^{-1} F.W.)
Control	1.65±0.00	1.11±0.014	23.36±0.56	1.18±0.004	0.21±0.01	29.70±57
1.0 mM Hg	1.58±0.004	0.32±0.035	23.36±0.56	0.774±0.02	0.296±0.00	11.09±0.00
2.0 mM Hg	1.53±0.002	0.55±0.00	29.33±1.08	0.62±0.002	0.206±0.00	23.36±0.55
4.0 mM Hg	1.49±0.004	0.67±0.003	35.00±0.00	0.16±0.002	0.062±0.01	20.99±0.55
CD at 5% P	**0.1544**	**0.1130**	**2.905**	**0.0492**	**0.0534**	**2.1132**

heavy metals including mercury, reduced germination in lentil, radish, mustard and rice plants (Ayaz and Kadioglu, 1997; Mishra and Choudhury, 1998). Results of Mozungrum and Geekil (2002) regardinig inhibition of germination and root growth was in agreement with some earlier studies showing that in most cases Hg with no known beneficial function had the highest toxicity potential in plants (Mishra and Choudhury, 1998) and micro organism (Nies, 1999). Ti appears that Hg had a severe toxic effect on the growth hormone especially IAA (indole acetic acid) which might be a reason of reduced overall growth of all the plants taken in this study. The TEM analyis of chloroplast ultra structure marked increase in level of distortion of thylakoids and plasto-globule was found at higher lead concentration in moss plant. The damage in thylakoids structure related with the important disturbance in the metabolic function of organelles effecting chlorophyll biosynthesis. Photosynthesis and the activities of redox enzymes justifying a decrease in growth (Baryla *et al.*, 2001) Reduction in chlorophyll content resulting in inhibition of photosynthesis with different concentration of heavy metals, because of that a decrease in dry biomass of plants was also seen. Total chlorophyll content was found to be decreased at higher concentration of three heavy metals *i.e.* nickel, lead and mercury on the plants taken in this study except pea.

Mercury was found to be most toxic element as it caused reduction in this parameter even at its lower dose in all the plant studied. Adverse effect of the three heavy metals even at its lower dose in all the plant studied. Adverse of three heavy metals *i.e*. nickel, lead and mercury on the iron metabolism of studied plants. Soikheli (1981) reported change in the structure of chloroplast under the effect of metal and other toxic substance resulting in to swelling of thylakoids accompanied by an increase of volume of thylakoid loculi. Parekh and Puranik (1992) reported inhibitory effect of lead acetate on the total chlorophyll content in maize plants. Induction of chlorosis or decrease in chlorophyll content of plant subjective to heavy metal toxicity is often associated with impairment of iron utilization in plants. Khan and Khan (1983) reported importance of iron and magnesium in photosynthesis of plant. Magnesium is necessary for chlorophyll formation while iron is needed in feerodoxins in photosynthesis redox system. Chlorophyll sysnthesizing system and chlorophyllase activity were affected by heavy metals. Quzounidou *et al*. (1987) found that plant growth inhibited with increased heavy metal concentration was attributed to indirect effect of the toxic heavy metals on the concentration of essential plant nutrient or structural damage of chloroplast in wheat plants.

Patra and Sharma (2000) reported mercury affects both light and dark reaction of photosynthesis. They were to the view that substitution of the central atom of chlorophyll, magnesium by mercury. In vivo prevents photosynthesis reaction varies with light intensity. Mercury induced reduction in both chlorophyll a and b and consequently the total chlorophyll content also. The reduction may be due to sensitivity of enzymes involved in chlorophyll biosynthesis and degradation towards heavy metal ions. Earlier also Tandon *et al*. (2000) found increased in total sugar content at increasing doses of nickel in moong plants. Such increase in total sugar content at lower doses of some heavy metals might have resulted due to the improper translocation of sugars from leaf to other parts of the plants. Tripathi and Tripathi (1999) reported significant reduction in some metabolic parameters including carbohydrates and sugars in leaves of *Albezia lebbek* at excess amounts of nickel and mercury. All metals decrease the contents with increasing the concentration in agricultural crops. The lower

sugar level may be due to lowered synthesis or diversion of the metabolites to other synthesis processes (Tripathi and Tripathi, (1999).

The increase or decrease in total protein was related to the concentration of different metals supplied to the studied plants. Changes in protein composition *i.e.* protein metabolic perturbation during mercury stress reflect the de-arrangement in protein degradation. Aminotransferases served as a strategic link between carbohydrates and protein metabolism under environment stress condition. In present study total protein content was found to be increased in most of the cases. However, Agrawala *et al*. (1977) have reported decreased soluble protein content at excess amounts of some heavy metals in barley plants. This is inconformity with some of the observations of this study. In *Albizia lebbek* also a significant reduction was found in protein contents in excess amounts of Ni and Hg by Tripathi and Tripathi (1999). Possibly the decrease amount of total protein at excess doses of heavy metals might have resulted from toxic effects of these metals on nitrogen metabolism of the plant. On the other hand in the majority of studied plant the total protein content was found to be increased at excess doses of these of these three heavy metal i.e. nickel lead and mercury. Activities of both iron enzymes viz. catalase and peroxide was found to be either decreased or increased as the case may be in different plants. Panda and Patra (1997) reported oxidative damage and biochemical lesions in plant cells due to heavy metals. Heavy metal toxicity results in praline accumulation and alteration of various enzyme activities changes in growth and metabolism of plant in vitro uptake of heavy metals resulted secondary responses such as oxidation damage by producing highly reactive oxygen species (ROS). Activity of antioxidative enzyme directly related with the steady state level of ROS in the cell and augmentation of the antioxidative defense plays a pivotal role in regulating oxidation stress. Decrease in CAT activity due to metal toxicity in plants could result from the attack caused by metal ion induced ROS, Which may possible cause a non redox metal causing elevated lipid peroxidation indirectly resulting in free radical production. A greater activity of the enzymatic components of the antioxidative system indicates the high metal stress condition in plants and changes in the enzymes depend on plants species and heavy metal type.

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