RESPONSE OF MERCURY ON GROWTH AND NADH-GLUTAMATE DEHYDROGENASE ACTIVITY IN *PHASEOLUS VULGARIS*

Dr. (Mrs) Jot Sharma
Vice Principal/ Asso.Prof.
Department of Biotechnology,
Vijayaraje Institute of Science and Management,
Turari,NH-75, Jhansi Road .Gwalior-475001,
Madhya Pradesh,India.

Abstract – The present study was designed to investigate the effects of mercury on morphological and NADH-GDH activity at five different concentrations (C, 0.001, 0.01, 0.1, 1mM) of mercury. In morphological study, there was decrease in seed germination, shoot and root length and decline in germination period with the increasing concentration of mercury both treatment schedule ie when seeds were raised on Sand treated with different concentrations of mercury and when seeds were treated with different concentration of mercury for 2 and 4hrs.

NA DH- GDH enzyme of nitrate assimilation was subjected to inhibition by heavy metal treatment .In general, the inhibitory effect of heavy metals may be attributed to either blocking the supply of reducing equivalents, formation of mercurial derivatives of thiol(–SH) groups .The effect of mercury(Hg) on nitrate assimilation has rarely been reported. Hence, the present study may help to explore the possible mechanism(s) of toxicity on nitrate assimilation in Phaseolus vulgaris, an important legume crop and a great source of nutrition to millions of people. In the present study,application of mercury showed concentration dependent response on in vivo NADH- GDH activity in all three treatment schedules.

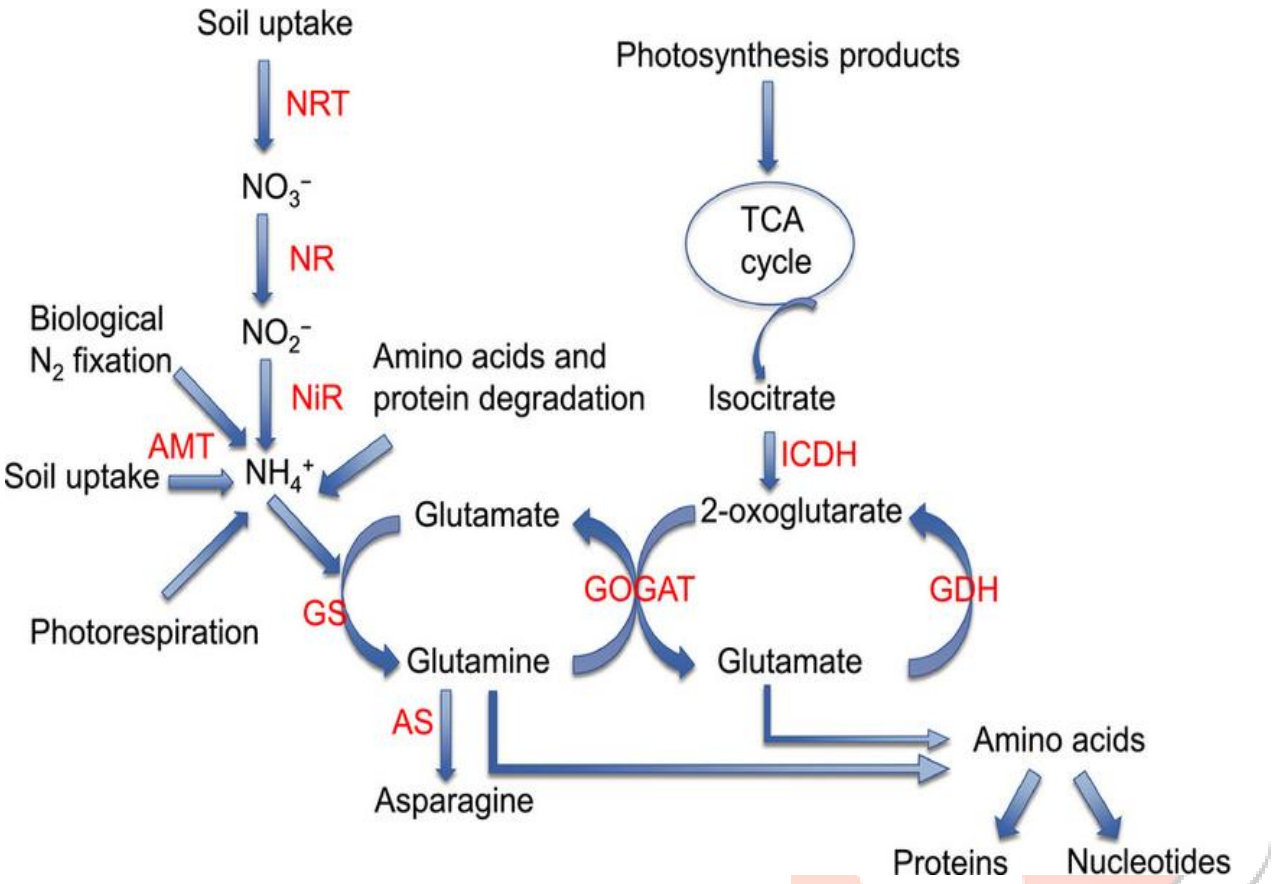
Index terms: Mercury; Phaseolus vulgaris; Morphological parameters; Glutamated dehydrogenase.

I. INTRODUCTION

Mercury (Hg)is a strong phytotoxic as well as genotoxic metal (Fridovich, 1986; Suszcynsky and Shann, 1995). Its distribution in the environment has been a focus of scientific attention because of the potential health risks posed by Hg exposure (Sahni, 2011). Anaerobic organism's bio-transform the inorganic form to methyl mercury which gets bio-accumulated in food chain. (Azevedo and Rodriguez, 2012). The influence of metals on development and reproduction of plants can be firstly quantified by determining the germination traits of seed and growth performance of seedling (Patra and Sharma, 2000).

Toxic effects of mercury in plants include abscission of older leaves, growth reduction, decreased vigour inhibition of root and leaf development, decreased chlorophyll content and nitrate reductase activity (Vyas J and Puranik RM, 1993) Other adverse effects caused by excessive mercury include membrane structure integrity disruption (Ma C, 1998), mineral nutrient uptake reduction(Cho U, Park J, 2000..Patra M, Sharma A, 2000) and photosynthesis and transpiration reduction(Krupa .Z and Baszynski .T, 1995) Higher concentrations (> 1-2 mg/l) of mercury decreased the growth of pea (Beaford W .etal 1977), tobacco(Suszeynsky E.M and Shann J.R, 1995], tomato (Cho U and Park J, 2000)and alfalfa (Beaford W, et al, 1977).Inhibition of enzymes of different metabolic pathways has also been reported by mercury toxicity.(Morch, V. M. ,et al 2002;Lenti, K., et al,2002 ; Basak. M,etal,2001; Shaw, B.P and Rout N.P, 2002)

Considerable amounts of mercury may be added to agricultural land with fertilizers, lime and manures. The most important sources of contaminating the agricultural soils have been the use of organic mercurials as a seed- coat dressing to prevent fungal diseases in seeds. The absorption of organic and inorganic mercury from soil by plants is low, and there is a barrier to mercury translocation from plant roots to tops. Thus, large increase in mercury levels in soil produce only modest increase in mercury levels in plants by direct uptake from soil (Patra M. and Sharma A., 2000). Higher plants growing in the soil receive inorganic nitrogen mostly in the form of nitrate. The nitrate in the plant tissue is reduced to ammonium via nitrite by the sequential action of enzymes as depicted the following scheme.



Glutamate dehydrogenase (NADH-GDH,Ec 1.4.1.3) is of central importance in nitrogen metabolism,as it forms a link between nitrogen and carbohydrate metabolism and possess regulatory properties.The enzyme is shown to be regulated by various metabolic and environmental factors and seems to be important in the assimilation of ammonia under stress (Srivastava H.S., and Singh R.P, 1987)

Phaseolus vulgaris, an important legume crop and a great source of nutrition to millions of people. The effect of Hg on morphological and physiological characteristics of P. vulgaris has not been studied yet.

The objectives of the present study were: (1) to investigate the effects of Hg on the growth parameters like shoot, root length, and seed germination (2) to evaluate the effects of Hg on glutamate dehydrogenase activity with a view to gain some insight into the mechanism on The results should be helpful to correlate the nitrogen static of the plant with growth and yield of crop.

II. Material and Methods

2.1. **Morphological assay**: For this study, acid washed sand was used. The pH of sand was 6.2. Then in each pot 1kg of sand was treated with different concentration of Hg for 24h. Followed by the transfer of 20 properly sterilized and washed rajma seeds into each pot. For regular watering Hoagland solution was used. After a week seedlings were harvested then root and shoot were separated for measurements. Seed germination was also counted. All values counted in three replicates of experiment (Ling et al., 2010; Sharma et al., 2009).

2. 2. **Enzyme Assay**: Phaseolus vulgaris(Rajmah) Seeds of Phaseolus vulgaris purchased from local dealer were surface sterilized with HgCl2 (0.1%) for 1-2 minutes and then washed thoroughly with distilled water. Twenty seedlings were raised in plastic pots (10" diameter, 3.5" depth) containing approximately 1.5 Kg acid washed sand for 7-8 days in continuous light of 30 wm-2 intensity supplied by fluorescent tubes at 26±2 º C. They were watered with ½ strength Hoagland's solution (pH 6.0) containing no nitrogen (Hoagland DR, Arnon DI, 1938) Primary leaves from healthy seedlings were cut into about 0.5x0.5 cm segments and floated on 1/4th strength Hoagland's solution containing desired compounds for 24 hrs at continuous light intensity of 40wm -2 at a temperature of 26±2º C inside BOD (Model No 6783, INDOSAW).

2.2.1 Metal Treatment

Seeds were treated with metal at three levels in order to correlate uptake, accumulation

A) Excised primary leaves were cut into small segments and treated with metals of desired concentrations in continuous light.

B) Seeds were treated with metals for 4 hrs followed by thorough wash and subsequently planted on acid washed sand contained in plastic pots. Primary leaves of the seedling were kept aside for various analytical determinations.

C) Seeds were planted on metal treated acid washed sand contained in plastic pots. Primary leaves of the seedlings were subjected to various analytical determinations.

2.2 2 Enzymatic analyses- Enzyme glutamate dehydrogenase was isolated as described in (Puranik and Shrivastava). NADH-GDH activity was assayed by monitoring the decrease in absorbance at 340nM according to method of (Singh and Srivastava). The unit of enzyme activity has been expressed as n mole of NADH oxidized per min.

Data presented in the paper are average of at least three independent experiments with ± S.E..Significance of difference obtained for various treatments was tested by student's t test .

III.RESULT

3.1.Effect on Germination, Shoot, and Root Length

In sand treatment the highest percentage of seed germination was observed in 0.01with 65% in comparison to control then continuously decreased. In 1 mM there was only 36% germination (Table 1). In seed treated for two hour 48% germination was observed in 0.1 where as in four hour treated seed in 0.01 with 43% highest germination. In seed treated for two hour 1 mM have only 23%, where as in four hour treated seed 28 % in 0.001 least germination followed by 1 mM with no germination was found.

The growth of phaseolus vulgaris assessed in terms of shoot and root length sand treated, shoot length was linearly decreased from control to 1 mM concentrations of Hg or significantly ($P < 0.01$) inhibited at higher concentrations. A 58 % reduction in shoot length was observed at a concentration of 0.001mM, with respect to the control. Root length was continually decreased from control to 1 mM concentration.In seed treated with two hour, shoot length was more inhibited at 0.1 mM with 78.23% reduction where as in four hour treatment more inhibition in 0.001 mM concentration. Root length was decreased form control to 1mM in two hour seed treated where as in four hour 0.01mM shows more inhibition followed by 0.001 mM. (Table 2 and 3)

Period of seedling germination was found 130% times at 1mM concentration more inhibited than control during sand treatment. In seed treatment with two hour at higher concentration decline in period of seedling germination than control where as in four hour seed treatment there was significantly inhibition ($P < 0.01$) shown in 0.1 mM concentration. (Table 4)

3.2 Effect of Mercury on NADH-GDH activity

Supply of HgCl2 (0.001 to 1.0mM) to excised bean leaf segments caused significant decrease in NADH-GDH activity (Table5). However, the increase in NADH-GDH was observed due to supply of 0.001 mM and 0.01 mM mercury. The activity increased in leaves raised in soil treated with HgCl2 (0.001 to 0.1 mM) .The activity however, decreased at 1.0mM of mercury (Table6). Similarly, the increase in NADH-GDH activity was monitored in leaves raised from Hg treated seeds (0.001 to 1.0mm) and the increase was more pronounced at 0.01mM of Hg (Table7).

The data reported in the present study suggest that increasing concentrations of Mercury had an increased inhibitory effect on seed germination percentage, root length, shoot length, as well as NADH-GDH activity of ***PHASEOLUS VULGARIS*** However, at low levels of mercury showed a significant increase in morphological growth parameters, but growth process on beyond these levels are impacted adversely. The highest concentrations of heavy metal solution had the most negative influence on all the parameters which are considered for examination.

IV.DISCUSSION

The seed germination of phaseolus vulgaris was decreased with increased in mercury concentration which was significantly ($P < 0.01$) affected at higher concentrations of Hg (Table 1 and 2). Reduction in biomass at high levels of Hg may be correlated to high Hg accumulation by seedlings. In that case, cells might have to produce extra energy to cope with the high Hg concentration in the tissues. (Gregger, M., 1999). The growth pattern of phaseolus seedlings in the presence of Hg was different from pea and spear mint(Beauford, W., et al,1977), ryegrass(Attar, A.F etal,1988)tomato (Cho U,and Park J,2000)grandiflora (S. Malar, et al,2014)Mentha arvensis (R. Manikandan, S. V. et al,2015).

In present study, as the mercury concentration increased in sand and soil there was observed decreased in growth parameter . Furthermore no growth in 1 mM concentration was found in four hour treated seed.

Phytotoxic effects of heavy metal ions have been widely reported. The morphological and physiological aspects of metal toxicity, however, have been explored only in a few processes. The relatively strong affinities of heavy metal ions for side chain ligands of proteins indicate that enzyme activities and other functional proteins are one of the primary targets of metal toxicity (Vallee and Ulmer 1972; Hampp et al., 1976).The visible toxicity symptoms of HgCl2 treatment was stunting and chlorosis, browning of leaf tip, reduction in growth, and stunting of seedlings and root were the morphological symptoms of Hg toxicity. The inhibition of root growth and development of lateral roots are symptoms of toxicity due to Hg which can be attributed in the inhibition of mitosis, reduced synthesis of cell wall components and changes in photosynthetic activity (Patnaik and Mohanty, 2013)

The results demonstrated inhibition of in vivo NADH-GDH activity by mercuric chloride. The effect of Hg on enzyme inhibition seems to involve decreased mobilization of storage pool of NO3- to metabolic pool. It may be noted that dealleviated protein, chlorophyll content, endogenous nitrate pool has been previously reported (Vyas J and Puranik R.M, 1993]. Our present results also substantiate that inhibition of NADH-GDH activity by mercury, might be caused either by suppressing the synthesis of enzyme molecule or by inhibiting the activity of existing molecule (Table5).

Metals are known to induce biphasic dose response curves which allow a gross division into two general regions (Valle B.L and Ulmer D.D, 1972.) The stimulatory effect of low concentration against a background of a deficiency state that constitutes phase one of the dose response curves. For some metals, the requirement may be at such low concentration that experimental production of a deficiency state may be technically difficult. As a result, information on the possible stimulatory effect of metals at low concentrations is inadequate. On the other hand, the inhibitory or toxic effect of high concentration that constitute phase two of the dose response curve has been reported for most of the metals about which sufficient information exists (Davis R.D et al ,1978;Dash S.,et al 1988), including mercury (Subhadra A.V., et al,1991) (Table5). In case of leaf segments raised from seeds as well as soil treatment schedules the peak concentration where the maximum activity was observed was 0.01 mM Hg (Table 6 and Table7).

Increase in NADH- GDH activity has been reported in soybean roots and nodules under heavy metal stress. Involvement of the enzyme s in ammonia assimilation during Hg supply has been indicated in the present study. The enzyme activity was studied in presence of NH_4Cl ,ammonium ,the substrate of the enzyme seem to have counteracting effects and Hg stress which may be relived by NH_4Cl..

Mercury concentration in aboveground parts of the plants appears to depend on soli or sediment organic content,carbon exchange capacity, oxide and carbonate content, redox potential, formulation used and total metal content. In general ,mercury uptake in plants could be related to pollution level. (Patra and Sharma, 2000)

Table 1. Effect of HgCl2 on germination of P. vulgaris seed

Concentration of mercury (mM)	Sand treated with mercury concentrations Percentage %	Sand treated with mercury concentrations Percentage %	
	24 hour sand treated	2 hrs	4hrs
Control (without treatment	76	53	53
0.001	56	40	28
0.01	65	41	43
0.1	53	48	33
1	36	23	No Growth

Table 2. Effect of HgCl2 on shoot lengths

Concentration of Hg mM	Sand treated with Hg 24 Hrs	Seeds treated with Mercury 2 Hrs	4Hrs
Control	*211.5 ± 59.94*	129.33 ± 13.28	*109 ± 45.03*
0.001	*155 ± 34.87*	*98 ± 20.78*	*56 ± 1.5*
0.01	154 ± 13.08	122 ± 45.03	102 ± 14.43
0.1	97.67 ± 4.93	99 ± 10.39	66 ± 17.9

Table 3. Effect of HgCl2 on root lengths

Concentration of Hg mM	Sand treated with Hg 24 Hrs	Seeds treated with Mercury 2 Hrs	4Hrs
Control	81.67±2.52	69.33±32.33	45.33±5.77
0.001	61±27.62	57±24.25	20.33±4.04
0.01	83 ±27.84	59.33±25.4	39.67±0.59
0.1	45±20.81	51±13.86	232.67±20.21
1.0	20.67±3.21	15.67±4.62	No growth

Table 4. Decline in periods of seed germination

Concentration of Hg mM	Sand treated with Hg 24 Hrs	Seeds treated with Mercury 2 Hrs	4Hrs
Control	9±2.65	12±2	9.67±2.52
0.001	61±27.62	57±24.25	11.33± 1.15
0.01	8.33±1.53	9.67±2.52	13.67 ± 1.53
0.1	10.67±2.52	10.33±1.53	19.33 ± 1.15
1.0	10±3.46	10.67±2.31	No Growth

Table 5. Effect of mercury on NADH-GDH activity in excised bean leaf segments

HgCl2 Conc,mM	NADH-GDH activity (min/gm fresh weight) Mean value ± SEM
0.000	84.00± 1.47
0.001	111.00 ± 2.7
0.01	94.8 ± 2.15
0.1	65.60 ±2.71**
1.0	52.80 ± 1.49 **

(** P< 0.001, *P< 0.01)

Leaf segments from 7d old bean seedlings raised in continuous light were floated on 1/4th strength Hoagland solution containing 10mM NH_4 Cl and desired concentration of mercury chloride in continuous light in BOD incubator for 24h at 25± 1° C. The values are average of five consecutive experiments

Table6. Effect of mercury on NADH-GDH activity in seedlings raised from mercury treated soil.

HgCl2,Conc. mM	NADH-GDH activity (min/gm fresh weight) Mean value ± SEM
0.000	85.60±2.04
0.001	-
0.01	124.00 ± 1.78**
0.1	112.00 ± 2.8**
1.0	Sample lost

(** P< 0.001, *P< 0.01)

Leaf segments from 7d old bean seedlings (raised from mercury treated soil for 24 hrs) were floated on 1/4th strength Hoagland solution containing 10mM NH_4 Cl in continuous light in BOD incubator for 24h at 25± 1° C. The values are average of five consecutive experiments.

Table7. NADH-GDH activity in seedlings raised from seeds treated with mercury

HgCl2 Conc. mM	NADH-GDH activity (Min/gm. Fresh Weight) Mean value± SEM
0.000	88.00± 1.78
0.001	-
0.01	103.20 ± 1.49**
0.1	72.00 ± 1.78 **
1.0	60.80 ± 1.49 **

(** P< 0.001, *P< 0.01)

Leaf segments from 7d old bean seedlings raised (from seeds treated with Hg for 4 hrs) were floated on 1/4th strength Hoagland solution containing 10mM NH_4Cl in continuous light in BOD incubator for for 24h at 25± 1° C. The values are average of five consecutive experiments.

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