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EFFECT OF MERCURY AND EFFLUENTS ON SEED GERMINATION, ROOT-SHOOT LENGTH, AMYLASE ACTIVITY AND PHENOLIC COMPOUNDS IN *VIGNA UNGUICULATA*

P. Uma Devi, S. Murugan, S. Akilapriyadharasini*, S. Suja* and P. Chinnaswamy**
Department of Microbiology, Dr. N.G.P. Arts and Science College, Coimbatore-641 014, T. N.
*Department of Biochemistry, Dr. N.G.P. Arts and Science College, Coimbatore-641 014, T. N.
**Institute of Laboratory Medicines, Kovai Medical Center and Hospitals, Coimbatore-641 014

ABSTRACT

The lethal toxicity tests for mercury and effluents were performed in *Vigna unguiculata* (cow pea). There was a significant alteration in the growth of plants. Effects of mercury and effluents reduce the germination of seeds. An abnormality in root-shoot length as well as variation in amylase activity was observed. The amount of phenolic compounds increase with increase in mercury content. Since *Vigna unguiculata* is an annual plant, results can be seen quickly.

INTRODUCTION

Mercury is one of the most toxic metals. It causes neurological disorder in humans. Mercury is widely distributed in the environment in a lower concentration of about (0.08ppm) and up to 1.7 ppm in soils.

Major source through which mercury enters the environment is natural weathering. Other sources are industrial effluents of electrical equipment, paints, pesticides, pulp, paper, thermometers, medicines, batteries and dental preparations. In addition, burning of fossil fuels, smelting, cement manufacturing, gold mine activities and wastes also contribute some amount of mercury.

Mercury in humans comes through ingestion of food and drinking water. The safe level of mercury in blood and hair is considered to be 0.02 ppm as per WHO. In man, it is mainly concentrated in liver, kidney and brain. The toxicity of mercury is due to its high lipid solubility, and high penetrating action and absorption in cells.

The standard of mercury in drinking water as recommended by WHO is 0.001ppm. The Bureau of Indian Standards (BIS) has recommended 0.01 ppm as maximum allowable level of mercury in water discharged into inland water bodies.

Raw sewage contains significant concentration of mercury and other heavy metals. Long term and indiscriminate application of raw sewage effluent directly to agricultural fields may cause accumulation of mercury in soils.

Contamination of soils by mercury affects germination of seeds, early growth of crops and yield. Exposure of plants to 5.6-6.8 mg Hg/kg decreases metabolite level, amylase activity, and germination process in seeds (Greenway & Munn 1996). The soils polluted with heavy metals may have ecological and biological problems.

The present study was carried out with the main objective to test the effect of mercury and effluents on seed germination, root-shoot length, amylase activity and phenolic compounds in *Vigna unguiculata*.

MATERIALS AND METHODS

The seeds of *Vigna unguiculata* were obtained from Seed Technology Division, Tamilnadu Agricultural University, Coimbatore, Tamilnadu, India.

The selected concentration of effluent was prepared as 1.50 mM, and mercury (as $HgCl_2$) between 0.05-2.0mM. Because of its highly inhibitory effect preparations were made in double distilled water and some comparisons were made using distilled water as control.

Germination of Seeds and Root Shoot Length

The seeds were washed in distilled water and sterilized by immersing in 0.1% mercuric chloride for 2 minutes followed by 5 times washing in double distilled water, and incubated at 28°C for 24hr. The seeds were distributed in Petri-dishes over the surface of Whatman No. 1 filter paper with 10 mL of heavy metal solution of mercury. Petri dishes were closed and kept at room temperature in illuminated cabinet for 15 days. Germination percentage and root-shoot length were determined at 5, 10, 15 and 20 days. The number of germinated seeds was counted every 24 hr for 3 days and the data were expressed as percentage.

Amylase Activity

Amylase extract was obtained from mercury treated seeds. One mL extract was taken and to this 2 mL of dinitro salicylic acid was added and boiled. To this potassium sodium tartaraic was added and cooked. Reduced brown coloured product amino salicylic acid formed was read at 560nm.

Phenolic Compounds

Phenol reacts with phosphomolybdic acid in folin ciocalteau reagent (0.5mL). After 3 minutes, 2mL of 20% sodium carbonate was added. The tubes were boiled and colour developed was read at 660nm.

RESULTS AND DISCUSSION

Mercury is highly toxic in nature and causes neurological disorders in human beings. Mercury displayed relatively high toxicity to *Vigna unguiculata* at 24 h incubation, compared to the effluents at higher concentration for the same period. Treatment reduces germination of seeds at high concentrations (Table 1, Fig. 1).

Table 1: Effect of mercury and effluent on the germination percentage of cowpea seeds after 5 days incubation.

S.No	$HgCl_2$		Effluent	
	Conc. (mM)	Germination	Conc. (mM)	Germination
1	0.00	96.0	0.00	96.0
2	0.05	66.0	0.10	1.0
3	0.10	61.0	0.50	77.0
4	0.50	16.0	1.00	69.0
5	1.00	14.0	5.00	49.0
6	1.50	6.0	10.00	24.0
7	2.00	2.0	50.00	3.0

Table 2: Effect of mercury and effluent on the root and shoot length.

S.No	Conc.	Length (mm)					
		Root			Shoot		
		5th day	10th day	15th day	5th day	10th day	15th day
Mercury							
1	0.00	15.00	20.00	25.00	20.00	49.0	56.00
2	0.05	4.70	9.80	14.50	14.50	30.0	41.00
3	0.10	2.50	3.70	6.80	7.80	18.0	23.00
4	0.50	0.40	0.90	4.30	5.20	11.0	15.00
5	1.00	0.30	0.60	1.00	2.00	6.0	9.00
6	1.50	0.20	0.35	0.50	0.90	2.0	5.00
7	2.00	0.05	0.06	0.10	0.40	0.5	1.00
Effluent							
1	0.00	15.00	20.00	25.00	20.00	49.0	56.00
2	0.05	4.70	9.80	14.50	14.50	30.0	41.00
3	0.10	2.50	3.70	6.80	7.80	18.0	23.00
4	0.50	0.40	0.90	4.30	5.20	11.0	15.00
5	1.00	0.30	0.60	1.00	2.00	6.0	9.00
6	1.50	0.20	0.35	0.50	0.90	2.0	5.00
7	2.00	0.05	0.06	0.10	0.40	0.5	1.00

Table3: Effect of mercury and effluent on the amylase activity.

S.No	Actiity (Units/g of fresh weight)		
	Conc.(mM)	Amylase	Peroxidase
Mercury			
1	0.00	241.3	266.2
2	0.05	243.6	272.5
3	0.10	235.1	278.4
4	0.50	212.4	269.6
5	1.00	169.7	289.7
6	1.50	142.5	318.4
7	2.00	141.7	296.2
Effluent			
1	0.0	236.0	253.9
2	0.1	241.5	249.2
3	0.5	238.6	254.5
4	1.0	240.8	261.6
5	5.0	245.8	266.4
6	10.0	221.6	279.3
7	50.0	230.8	274.1

Treatment at low concentrations of mercury (0.05, 0.1mM) affected root length adversely compared to shoot length. The rapid absorption and faster rate of detoxification in the shoot leads to more accumulation in the root. (Table 2, Figs. 2 and 3).

Amylase activity showed lower levels with increase in concentration of mercury than with effluents. Amylase activity was higher in control than the treated seeds at 1mM and 2mM of mercury (169 units and 141 units) (Table 3, Figs. 4 and 5).

Table 4: Effect of mercury and effluent on the percentage of phenols.

S.No	Conc. (mM)	$HgCl_2$ Phenols (g/100g)			Conc. (mM)	Efflunt Phenols (g/100g)		
		1D	7D	15D		1D	7D	15D
1	0.00	2.6	3.7	4.1	0.00	2.4	5.7	4.11
2	0.05	3.5	3.8	5.2	0.10	2.7	5.9	6.20
3	0.10	3.4	5.6	6.0	0.50	2.4	5.6	6.00
4	0.50	4.6	5.4	6.1	1.00	3.8	5.9	5.9
5	1.00	4.5	5.2	8.4	5.00	4.1	5.6	6.0
6	1.50	6.3	6.3	8.6	10.00	4.2	5.7	6.1
7	2.00	5.9	7.0	8.9	50.00	4.4	5.6	6.2

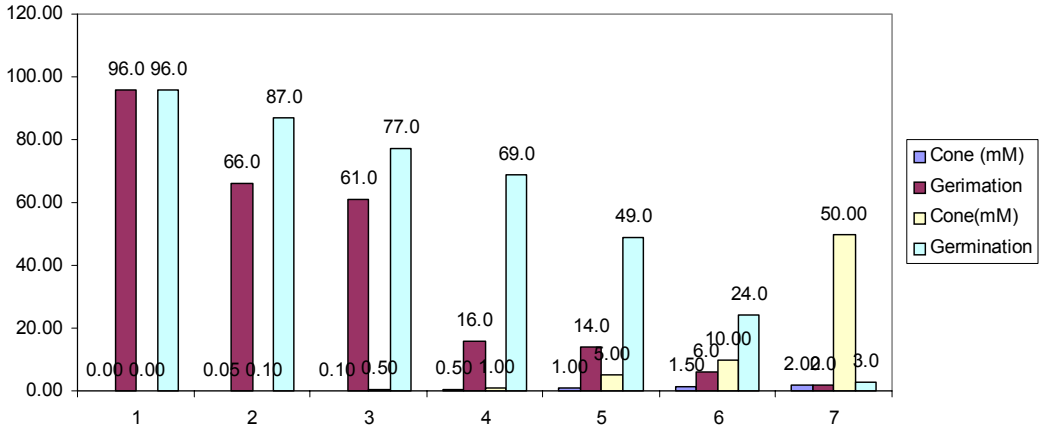

Fig. 1: Effect of $HgCl_2$ and effluent on the germination percentage of cowpea seeds after (5 days) incubation.

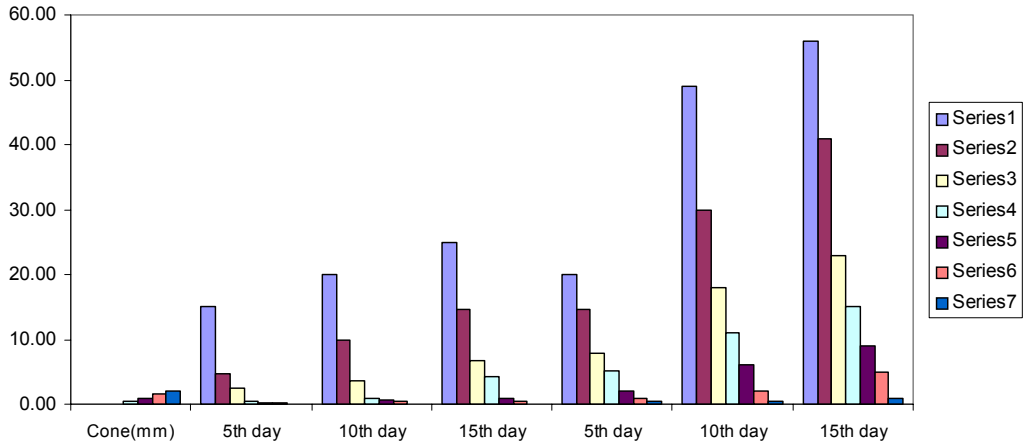

Fig. 2: Effect of mercury on the root and shoot length.

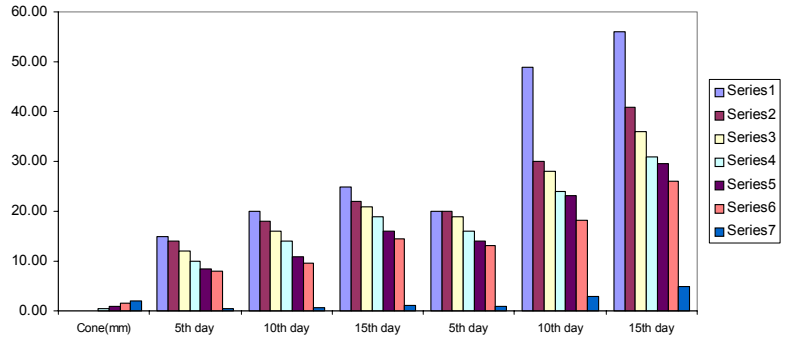

Fig. 3: Effect of effluent on the root and shoot length.

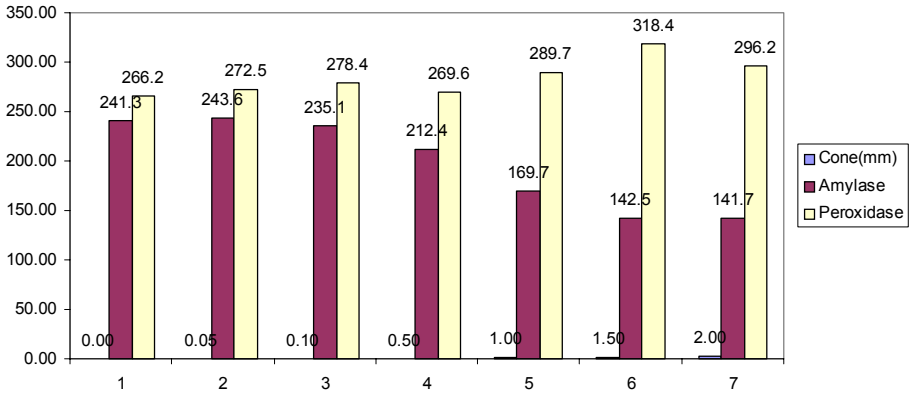

Fig. 4: Effect of mercury on the amylase activity.

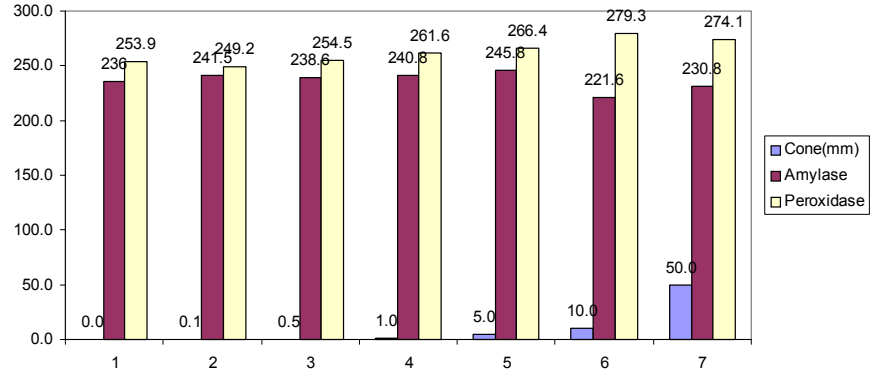

Fig. 5: Effect of effluent on the amylase activity.

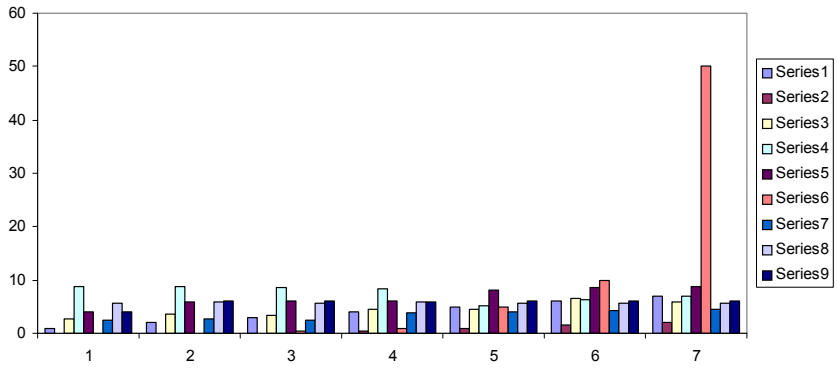

Fig. 6: Effect of mercury and effluent on the percentage of phenols.

High level of mercury in treatment was found to increase the level of phenolic compounds which may be responsible for inhibition of germination and growth. According to Colars & Dunlop (1974), phenolic acids exert a marked effect on membrane permeability and membrane electrical potential (Table 4 and Fig. 6).

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