



Impact of mercuric chloride on the seedlings of *Macrotyloma uniflorum* growth profile

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ABSTRACT

Various toxic substances are regularly released into the environment as effluents. Among these, mercury is highly toxic. It affects the growth of plants, seed germination, etc. This study aims to understand the effects of mercury on seed germination and seedling growth of fifteen seeds of *Macrotyloma uniflorum* (Horse gram) at different concentrations. Mercuric chloride is used as the mercury source at concentrations of 0.2, 0.4, 0.6, 0.8, and 1.0 M and compared against a control. Horse gram seeds were soaked in a mercuric chloride solution in a petri dish at ambient temperature and germination and seedling growth were monitored after 10 days; the average was reported. Concentrations of 0.2 M and 0.4 M didn't exhibit any significant reduction, and concentrations of 0.6 M, 0.8 M, and 1.0 M had a remarkable reduction in the germination of horse gram seeds. Furthermore, 0.8 M and 1.0 M concentrations significantly affected root length. The 1.0 M concentration of mercury was found to be sufficient to cause significant reductions in seedling dry weight as compared to the control. High concentrations of Mercury decreased seed germination, shoot, and root length, and seedling dry weight. An increase in mercury concentration up to 1.0 M showed the highest percentage decrease in seed germination (52%), with no significant effect on shoot length and root length (2.4%) of horse gram relative to the control. *Macrotyloma uniflorum* was more sensitive to mercury in seedling growth and root elongation than in seed germination. Minimal use of mercury compounds in fungicides and nematicides is recommended. Special care should be taken to monitor toxic pollutants, as larger concentrations can produce harmful effects to crops and the ecosystem as well.

Keywords: Mercury chloride, horse gram (*Macrotyloma uniflorum*), soil contamination, plant growth

1. INTRODUCTION

Soil contamination by heavy metals poses a significant threat to agriculture and the ecosystem. This pollution arises from natural geologic processes and anthropogenic activities, including rapid industrialisation, urbanisation, mining, and the extensive use of chemical fertilisers and pesticides (Chand *et al.*, 2015; Adrees *et al.*, 2015).

The introduction of untreated industrial effluents and wastewater contaminated with toxic metals leads to soil and water resource degradation that causes various environmental and health hazards (Simon, 2014).

Heavy metals can be characterized by their high density and atomic weight; some elements (Cu, Fe, Mn, Mo, Ni and Zn) are essential micronutrients for organisms, and are profoundly toxic at higher concentrations (Chibuike, 2014). Mercury (Hg) and arsenic (As) are recognized as highly dangerous due to their toxicity, persistence in the environment, and the bioaccumulation and biomagnification within food chains (Bini & Bech, 2014; Comino *et al.*, 2015; Zhang & Wong, 2007). Human exposure to heavy metals is linked to severe health concerns such as renal and hepatic damage, neurotoxicity, and carcinogenic effects (Iqbal *et al.*, 2014).

Mercury in the form of mercuric chloride is an extremely toxic pollutant that raises worldwide concerns. Mercuric chloride is used as a catalyst and reagent in organic synthesis, as a wood preservative, pesticide and in battery manufacturing. As a result of industrial applications, coal combustion, gold mining, smelting, and the disposal of electronics, mercury is released into the atmosphere, where it can travel long distances before returning to land or water (Baya & Van Heyst, 2010; Wang *et al.*, 2012; Deng *et al.*, 2011) and has been added to the soil and plants over time through the use of mercury in historical and current fungicides, pesticides, and nematicides in agriculture (Simon, 2014). Mercury acts as a strong phytotoxin to plants, as it disrupts important physiological and biochemical functions necessary for seed germination and seedling development, and also reduces the amount of photosynthetic pigments and disrupts chloroplast function, as well generating reactive oxygen species (ROS), leading to oxidative damage of plant cells, protein, and fatty acid membranes (Lux *et al.*, 2015; Ilektra Sperdouli, 2022; Dunergan *et al.*, 2007). One of the most obvious signs of mercury toxicity is the very strong inhibition of root elongation and subsequent inability to take up water and nutrients (Wang *et al.*, 2013).

The initial phases of plant development (germination of seeds and establishment of seedlings) are extremely sensitive to environmental conditions (abiotic stresses) such as contamination with heavy metals.

These two phases determine the density of your plant's agricultural stand and contribute significantly to final yield. For this reason, root elongation bioassays are one of many empirical approaches that can quantify the effects of various environmental pollutants (phytotoxicants) on plants (Source: US EPA, 1982). Although many researchers have published studies on how other heavy metal phytotoxicants (Cd, Pb, and Cu, for example) affect agriculture, few published scientific studies address the specific detrimental effects of mercury on leguminous plants during germination and early-seedling development stages (Source: Nijara *et al.*, 2019).

Macrotyloma uniflorum (Lam.) Verdc. (horse gram) is a drought-resistant legume with high nutrient value, grows naturally in the warm tropics of southern Asia and has become an important dietary staple for many people living in the region, especially those living in India, where it accounts for a large part of the diet of many people (Purseglove 1974). Horse gram has a high concentration of protein, fibre and minerals, and some studies have shown that it can have medicinal properties (anti-urolithic and hypocholesterolemic effects) (Chaitanya *et al.* 2010; Kashid 2021). Despite its nutritional importance and agronomic significance, studies on the physiological response of horse gram to heavy metal stress have been largely limited. Horse gram germination is more sensitive to cadmium than other legumes; however, manganese can stimulate germination when grown under in vitro conditions (Kumari *et al.* 2016). Therefore, there is a need to conduct a comprehensive study on the concentration-dependent phytotoxic effects of mercury on horse gram from germination through the early stages of seedling development.

To achieve this objective, a series of experiments was designed to determine the effects of mercury in the form of mercuric chloride (HgCl₂) on seed germination and growth of *Macrotyloma uniflorum*. Our approach is focused on three aspects: the percentage of seeds germinated with increasing mercury concentration (i.e., 0.2 M – 1.0 M); the effect of mercury on the length of the roots and shoots and the total fresh and dry weight of the seedlings. The results of this study will enable the identification of a mercury toxicity threshold in horse gram and provide additional insight into the ecological risks associated with mercury exposure in the agricultural environment.

MATERIALS AND METHODS

2.1 Plant Material and Sterilisation

Viable horse gram was chosen and was imbibed in distilled water for about ten hours and surface sterilised with 70% ethanol for one minute, followed by 0.1% sodium hypochloride for five minutes. This was rinsed thrice with sterile distilled water. Surface sterilisation was followed by blotting of the seeds on sterile filter paper sheets. Petri dishes containing media were autoclaved for 15 min at 121 °C at 15 lb pressure in an autoclave. This was followed by wet sterilisation in a laminar air flow for inoculation; all the requirements for inoculation were transferred inside the laminar air flow chamber for seed inoculation under in vivo conditions.

2.2 Mercury Treatment

The sterilised seeds are placed in petri dishes containing sterile absorbent cotton and filter paper immersed in water. Further, mercuric chloride (HgCl_2) was used to treat the seeds in different concentrations for 30 seconds. This was transferred to petri dishes containing sterile absorbent cotton and filter paper. Triplicates were maintained along with the control plate, with different concentrations (i.e., 0.2, 0.4, 0.6, 0.8, and 1.0 M). Seed germination was initiated by the addition of water. Distilled water is used as the medium for the control plate.

2.3 Experimental Setup

Seeds were placed on sterile absorbent cotton and filter paper in Petri dishes, with triplicates per treatment. Dishes were maintained under controlled conditions. Seed germination was observed from day 3, which shows the initial growth of the seedlings. After a fifteen-day time period, various parameters such as Germination percentage, shoot and root length and their fresh and dry weight. The dry weight was determined by drying the plant material in a hot air oven at about 160°C for 30 minutes.

2.4 Data Collection

After fifteen days, the seed germination percentage, shoot and root length of the seedlings and their fresh and dry weight were noted.

RESULTS AND DISCUSSION

The rate of seed germination and its growth profile were tested by exposing the seeds to different concentrations of mercuric chloride (i.e., 0.2 M, 0.4 M, 0.6 M, 0.8 M, 1.0 M), which was compared against a control plate. The seeds treated with 0.2 M mercuric chloride did not show any significant reduction in horse gram seed germination. In contrast, an increase in mercuric chloride concentration from 0.2 M to 0.4 M significantly reduced seed germination. This was reduced along with the increase in the concentrations of mercuric chloride (0.6 M, 0.8 M, 1.0 M). Root and shoot development of the seedlings was also affected by the treatment of mercury, which is noteworthy. Remarkable reduction was found in the concentrations 0.8 M and 1.0 M when compared with lower concentrations, which also exhibited a reduction in growth of the root and shoot.

Cellular damage is caused in plants due to the oxidative stress inflicted by heavy metals. These metal ions are accumulated in plants that in turn disturbs cellular ionic homeostasis. The detrimental effects of heavy metal exposure and accumulation are controlled by detoxification mechanisms in plants. The decrease in horse gram seedling and root growth offers more proof that too much mercury may impede plant growth and development. Each of the components has a distinct role that is known to be related to the activity of enzymes. However, large amounts of trace elements are more likely to have a negative impact on plants that are under stress. (2015, Muhammad). By reducing photosynthesis and impairing chlorophyll synthesis, mercury ingestion may cause major harm to plants (Lavado *et al.*, 2007).

Table 1: Impact of mercuric chloride on seed germination of horse gram (*Macrotyloma uniflorum*)

Sr. NO	Concentration of HgCl_2 (M)	No. Of seeds Sown	No. Of seeds germinated			Total no. Of seeds	Mean value	Percentage of germination
			1	2	3			
1.	0.2	15	5	5	5	15	5	100
2.	0.4	15	4	5	5	14	4.6	92
3.	0.6	15	5	3	5	13	4.3	86
4.	0.8	15	3	4	4	11	3.6	72
5.	1.0	15	3	3	2	8	2.6	52
6.	CONTROL	15	5	5	5	15	5	100

Table 2: Impact of mercuric chloride on shoot length and root length of horse gram (*Macrotyloma uniflorum*)

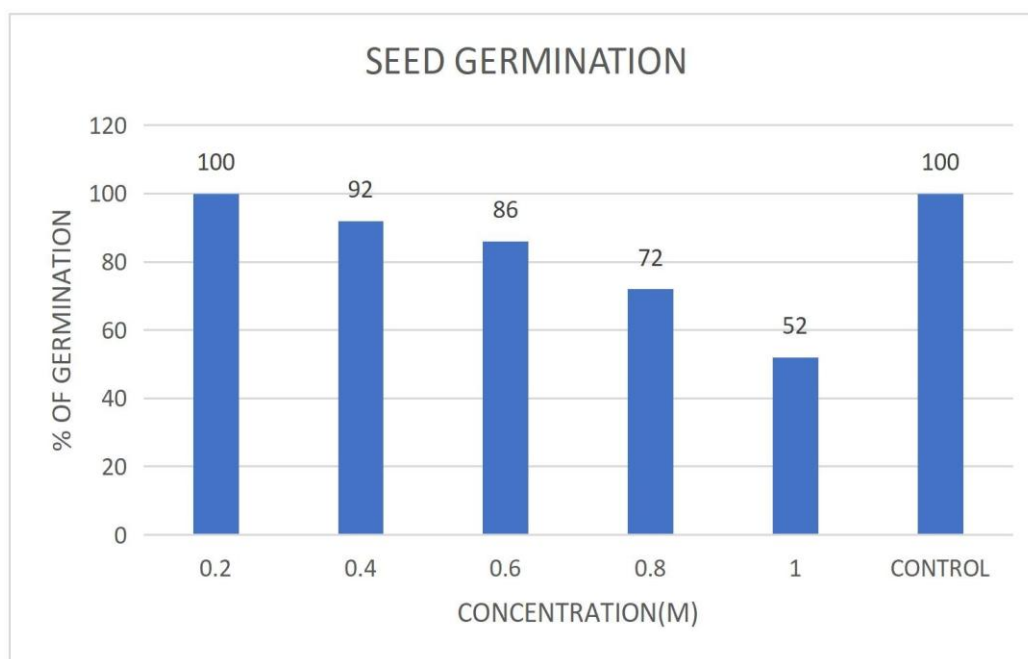
Sr. NO	Concentration of HgCl ₂ (M)	No. of seedlings taken	Total root length (cm)	Total shoot length (cm)	Root mean value	Shoot mean value
1.	0.2	15	9.2	25.8	8.6	3.06
2.	0.4	15	4.6	14.7	4.9	1.53
3.	0.6	15	4.1	13	4.3	1.36
4.	0.8	15	3.6	9.9	3.3	1.2
5.	1.0	15	0.1	7.2	2.4	0.03
6.	CONTROL	15	99.1	111.7	37.23	33.0

Table 3: Impact of mercuric chloride on fresh weight of horse gram (*Macrotyloma uniflorum*)

Sr. No.	Concentration (m)	No. of Seedlings taken	Shoot weight (g)	Root weight (g)
1.	0.2	15	1.5	0.9
2.	0.4	15	0.9	0.7
3.	0.6	15	0.7	0.5
4.	0.8	15	0.6	0.3
5.	1.0	15	0.2	0.1
6.	CONTROL	15	1.8	1.1

Table 4: Impact of mercuric chloride on dry weight of horse gram (*Macrotyloma uniflorum*)

S. No	Concentration (m)	No. of seedlings Taken	Shoot weight (g)	Root weight (g)
1.	0.2	15	0.07	0.07
2.	0.4	15	0.05	0.06
3.	0.6	15	0.04	0.05
4.	0.8	15	0.03	0.03
5.	1.0	15	0.01	0.01
6.	CONTROL	15	0.08	0.08

**Figure 1: Impact of mercuric chloride on seed germination of horse gram (*Macrotyloma uniflorum*)**

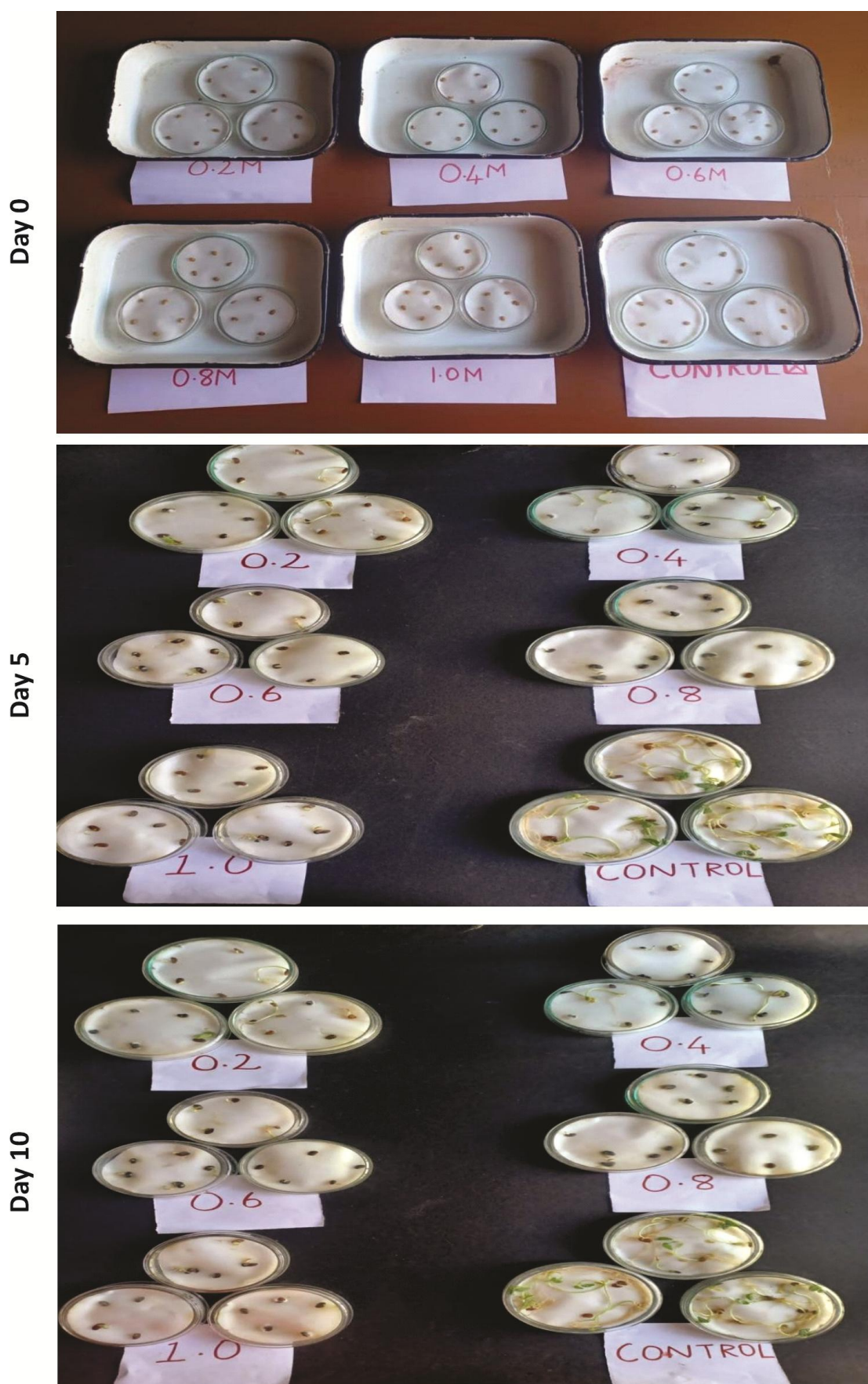


Plate 1: impact of mercuric chloride on seed germination of horse gram (*Macrotyloma uniflorum*)



Plate 2 : Impact of mercuric chloride on shoot length and root Length of horse gram (*Macrotyloma uniflorum*)

The response of horse gram seed germination to mercuric chloride treatment differed from that of the control. A high concentration of heavy metals reduces horse gram seed germination and suggests that excessive mercury treatment may limit plant growth and development. According to reports, inorganic mercury can have negative effects on aquatic plant culture media (Boeing 2000). Root elongation and germination rate provide a number of benefits, including sensitivity, ease of use, affordability, and compatibility with unstable substances or samples. The ecological risk assessment of harmful contaminants requires toxicity information (Wang *et al.*, 2001). As a quick phytotoxicity test technique, germination rate and root elongation have several benefits, including sensitivity, ease of use, affordability, and compatibility with unstable substances or materials. The ecological risk assessment of harmful contaminants requires toxicity information (Wang *et al.*, 2001).

When exposed to heavy metals, plants undergo oxidative stress, which damages their cells (Kunjam *et al.*, 2015; Nagati *et al.*, 2015). Furthermore, metal ions that disrupt cellular ionic homeostasis are accumulated by plants (Acharya and Sharma, 2014; Siddiqui *et al.*, 2014). Plants have developed detoxifying systems to reduce the harmful effects of

exposure to the buildup of heavy metals (Yadav, 2010).

CONCLUSIONS

The seeds of horse gram (*Macrotyloma uniflorum*) had a significant impact when exposed to various concentrations of mercury. Increased concentrations had a toxic effect on the germination and growth of the seedlings. 0.2 M concentration promoted tolerance towards mercury while 1.0 M exhibited the lowest tolerance. Anthropogenic activities, including industrialisation, have caused the concentration of mercury to increase rapidly in the environment. Higher levels of heavy metals in the soil are a cause for concern as they have severe impacts on plant growth and cause lower yields. Studies on seedling growth by treating them with mercury help shed some light on the issue. To mitigate the harmful impacts caused by mercury, reducing the use of fungicides, pesticides, and nematicides that contain traces of mercury is essential. It is also important to monitor pollutants in the environment, as their accumulation harms plants and ecosystems by travelling within food chains. More efforts are necessary to identify sources causing mercury toxicity and control their usage. More studies are vital to carry on the ongoing efforts to curb their usage and to develop tolerance indices.

Conflict of interest

The authors declare no competing interests.

Data Availability Statement: Not applicable.

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