Morphological Response of *Phaseolus vulgaris* in presence of Mercury in Laboratory Conditions

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Abstract
Heavy metals are major environmental pollutant when they are present in high concentration in soil. They have toxic effects on growth, nodulation and nitrogen fixation of legumes and development of plants. Mercury stress triggers disturbances in cellular structure and metabolism which is poorly understood. Therefore, responses on seedling growth and other physical and chemical parameters of Phaseolus vulgaris to different concentrations (0.001, 0.01, 0.1, 1 mM) and control of mercury (Hg) salt solutions were studied.

Morphological parameters like root and shoot length, dry weight, total leaf area and biochemical constituents of plants were recorded. The successive growth deformities in seedlings were recorded at lower concentration, but marginal and higher level of Hg salt solution showed significant suppression. Most morphological and biochemical characteristics showed concentration-dependent alterations, which may help to identify appropriate bio-indicators of heavy metal contamination.

Keywords: Heavy metals, Mercury, *Phaseolus vulgaris*, Toxicity.

Introduction
The various agro ecosystems are severely threatened by heavy metals that are released into the environment as a result of industrial operations and their buildup in various ecological systems[14]. Accumulation of heavy metals in soil significantly impacts local eco-safety and constitutes a hazard to relevant animals and plants because of their cumulative effects and long-term interactions[5].

According to the Agency for Toxic Compounds and Disease Registry, mercury is one of the harmful substances that should be avoided at all costs due to its toxicity, mobility and prolonged atmospheric presence. It is also regarded as one of the planet's most deadly natural substances[20].

Higher metal concentrations have demonstrated toxicity to a number of physiological processes including the growth, synthesis of pigments and the synthesis of chlorophyll. Nodulation for legume crops has recently attracted a lot of interest[10]. Heavy metal causes several toxic effects on plants such as down regulation of seed germination[3,15], reduction in plant growth and yield[4,13]. The tendency of heavy metals to bind to enzymes, which results in changes to their catalytic activities and inhibition, is primarily responsible for their toxicity[24].

As a heavy metal, mercury has hazardous consequences when it builds up in living things like plants, animals and people. One of the biggest issues in biological study is the interactions between such heavy metal elements and higher plants, especially those with high nutritional value like pulses and legumes. Recently, the use of enrichment factors has been broadened to include studies on soil, water systems and sediment as well as assessments of heavy metal contamination in environmental geochemistry. These factors include transfer factor, bio concentration factor (BCF) and plant uptake factor[16,20]. The ratio of an element's content in a plant to that in soil is known as BCF in soil science[5].

Additionally, BCF is a crucial quantitative indication of crop contamination and is frequently used to calculate the amount of metal that is transferred from the soil to plants[11,18,20]. According to BCF-based research, leaf vegetables have the highest levels of metal enrichment in vegetables followed by tubers and fruit vegetables[6,12]. There is a large body of literature on the toxicity of lead, cadmium, arsenic etc. but relatively little on the toxicity of mercury on plants. Understanding the mechanism of how plants struggle heavy metal adverse effects, is hence of great importance.

The present investigation relates the effect of mercury concentration on level of growth and biochemical parameters in *Phaseolus vulgaris* in order to contribute to an understanding of effect of environmental stress on *Phaseolus vulgaris*.

Material and Methods
River sand was used for growing of plants. Collected sand was washed thoroughly followed by 24 hrs sterilization with acid and finally washed with water thrice. 1 kg per pot dry sand was used for experiments. Chitra seed variety of *P. vulgaris* was used for experiments. Chitra was taken from local market of Gwalior (MP). Healthy 10 seeds (for each treatment plan) were treated with different concentration of mercury (0.001 to 1 mM $HgCl_2$) for 2 hours followed by washing with autoclaved distilled water three times and then potted.

After suitable growth following morphological parameters were noted: germination rate, root and shoot length, leaf area, root and shoot wet weight and dry weight. Leaf area was observed by computer software Easy Leaf Area with the help of phone camera[9]. The various morphological parameters such as root length, shoot length (cm $plant^{-1}$),

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total leaf area and dry weight of root and shoot per plant were determined for every sample. To obtain fresh weight, excess water from root washing was removed with a paper towel. To obtain dry weight, the plants were left at 65 °C to a constant temperature. Chlorophyll estimation was done using method of Strain and Svec[23].

Results

Root and shoot lengths of bean plants at different stages of growth under Hg stress are represented in tables. Thus, higher concentration of Hg salt solution inhibited the growth of plants. The degree of inhibition varied significantly depending on the concentration of the mercury salt solution. Maximum root length was observed at 0.01 mM Hg with respect to control. The shoot length of *Phaseolus vulgaris* seedlings showed a gradual increasing nature with concentration of mercury salt solutions, except at 1mM concentration. From the research, it was observed that there is gradual decrease of leaf area with subsequent increase in the mercury salt solution. The maximum leaf area was observed in case of treatment0.01 mM concentration with respect to control and minimum Leaf area was in 1 mM concentration.

Root wet weight showed decrease with mercury treatment maximum at 0.001 mM $Hgcl_2$ concentration. Dry weight of root was comparable to control except 0.1 mM concentration which showed decrease in dry weight with comparison to control. Wet weight of shoot showed decrease with increase in concentration of $HgCl_2$. Dry weight of shoot results showed decrease except at 0.001 mM concentration. Leaf wet weight showed maximum increase at 0.001and minimum at 0.1 mM concentration of $HgCl_2$. Dry weight of leaf showed almost same increase in all concentrations of mercury.

Chlorophyll: The effect of different concentrations of Hg treatment (0.001 mM to 1 mM $HgCl_2$ concentration on photosynthetic pigments is depicted in table 3. Photosynthetic pigment contents of leaves decreased by increasing the Hg concentration.

Discussion

The findings of the study showed that as mercury levels increased, root, shoot and leaf structure gradually changed. This is most likely caused by the breakdown of plant vascular system and cortical cells. The biomass of many plant species has decreased as the supply of Hg has increased[17,21]. *Phaseolus vulgaris* growth has changed, as observed. When mercury concentrations were low, similar outcomes were seen with tomato seedlings[7]. Present study clearly shows that with increase in Hg concentration, there is a decrease the leaf area and it shows concentration dependent results.

Table 1
Effect of Mercury on Germination, Length and Area

Concentration of Hg (mM)	Germination %	Root Length (cm)	Shoot Length (cm)	Leaf Surface Area (cm^2)
Control	100%	7.3±0.14	11.9±0.16	3.460 ±0.47
0.001	250%	9.0±0.63	15.7±0.09	3.577 ±0.63
0.01	200%	10.6±0.16	15.4 ±0.12	4.306 ±0.04
0.1	125%	8.2±0.04	15.5± 0.12	3.831 ±0.09
1	75%	9.1±0.04	11.8± 0.09	2.145 ±0.06

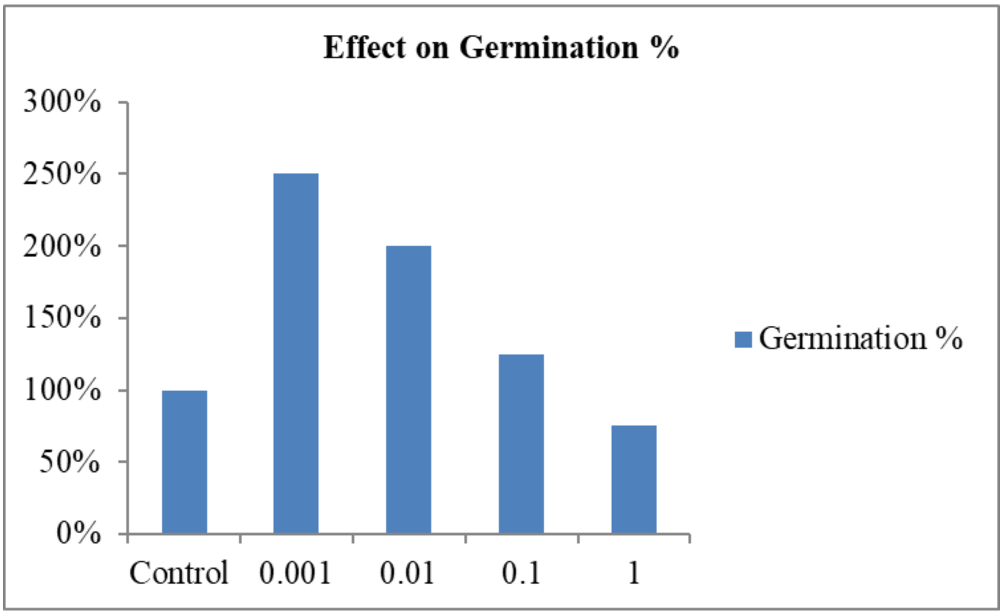

Figure 1: Effect of Mercury on Germination

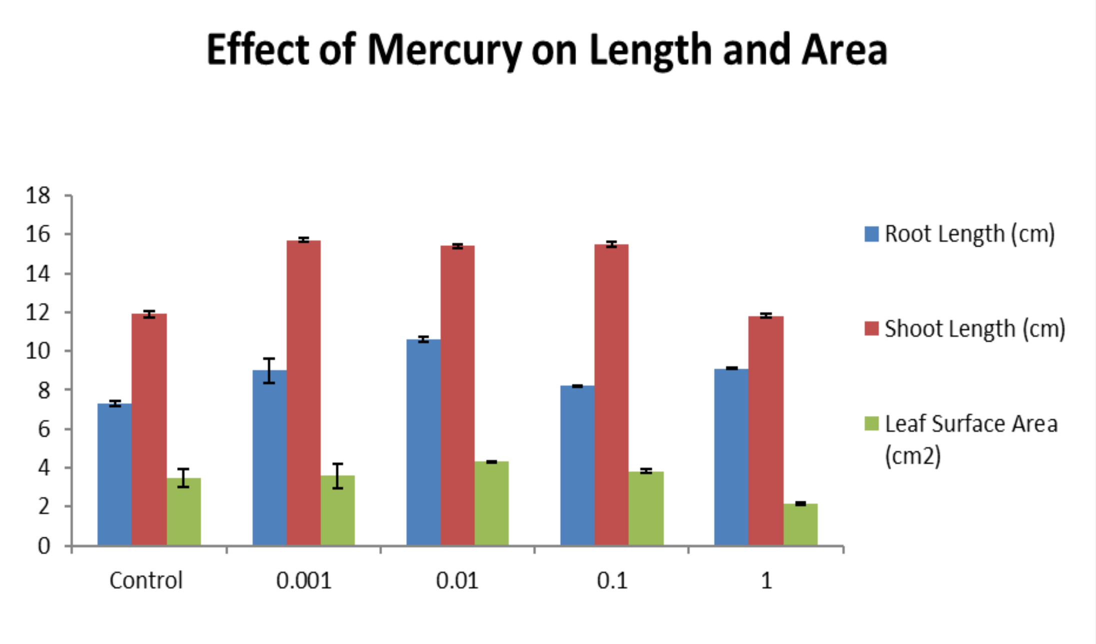

Figure 2: Effect of Mercury on Length and Area

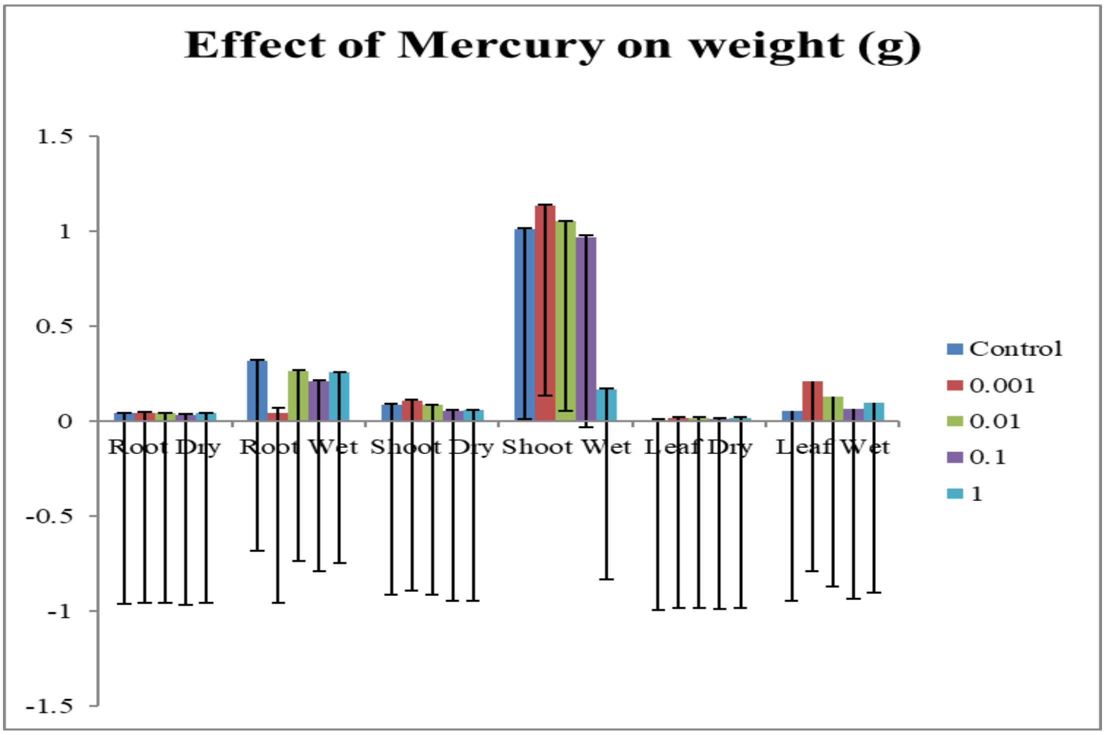

Figure 3: Effect of Mercury on weight

Table 2
Effect of Mercury on weight

Concentration of Hg (mM)	Weight of Root (gm)		Weight of Shoot (gm)		Weight of Leaf (gm)	
	Dry	Wet	Dry	Wet	Dry	Wet
Control	0.042±0.003	0.319±0.007	0.088±0.006	1.012±0.007	0.008±0.001	0.056±0.002
0.001	0.043±0.004	0.043±0.004	0.110±0.027	1.136±0.008	0.019±0.002	0.209±0.004
0.01	0.043± 0.004	0.266±0.004	0.087±0.006	1.055±0.005	0.019±0.002	0.130±0.002
0.1	0.033± 0.004	0.210±0.035	0.055±0.003	0.969±0.007	0.013±0.001	0.065±0.003
1	0.043±0.004	0.257±0.004	0.058±0.003	0.169±0.004	0.019±0.003	0.100±0.004

Table 3
Effect of Mercury on Chlorophyll content

Concentration of Hg (mM)	Average Chlorophyll (mg/g fresh weight)
Control	144.6±3.26
0.001	166.6±2.37
0.01	100.2±0.09
0.1	157.6±2.46
1	136.7±1.65

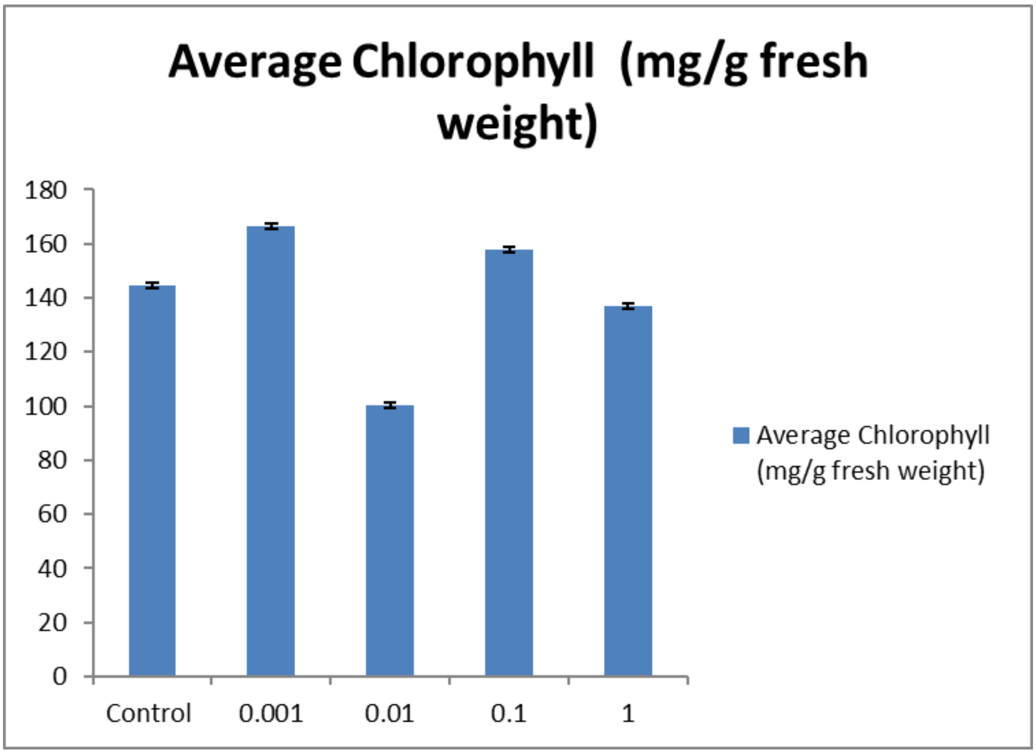

Figure 4: Effect of Mercury on Chlorophyll

Our results are in conformity with the results of various researchers[19,20]. In tomato plant, the leaf area had linear negative relationship with the dose of cadmium[22]. This perhaps may be due to decrease of the activities of many enzymes involved in the fixation of CO_2, alteration of thylakoid organization, reduction of chlorophyll content, inhibition of photosynthetic activity, disruption of interactions of chlorophyll molecules into stable complexes[25].

However, findings from plant biomass suggested that these changes were likely brought on by Hg interfering with the abnormal growth of root cells. Arduini et al[2] reported an observation that was remarkably similar influenced by cadmium toxicity.

Biochemical constituents: With the accumulation of Hg ions in water *Phaseolus vulgaris* plant tissues, we observed effects on the photosynthetic pigments and decreased levels of chlorophyll content in all treatments with respect to control in this study., Di Sanità and Gabbrielli[8] pointed out that all heavy metals, even at low doses, cause perturbations in various plant processes such as development of chloroplasts, photosynthesis, biological nitrogen fixation, sulphate assimilation and respiration.

Conclusion

This report reveals that *Phaseolus vulgaris* was affected by mercury and most prominent effect was recorded on ultra structure of root followed by shoot and leaf. Furthermore, since the effluents of many industries are contaminating the agronomic soils and making them unsuitable for sustainable agriculture, therefore, we suggest the growers to avoid the use of sewage water for irrigation.

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