



ASSESSMENT OF MERCURY TOXICITY ON SEED GERMINATION, SHOOT AND ROOT GROWTH OF *Cajanus cajan* (L.) MILLSP.

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Abstract:

The present study was carried out to test the phytotoxic effect of mercury on germination of seed and seedling growth of pigeon pea. The seeds of pigeon pea were treated with different concentrations (10, 50, 100, 150, 200, 250 and 300 ppm) of mercuric nitrate ($\text{Hg}(\text{NO}_3)_2$) solution. The germination percentages were calculated after two days (48 hrs.) and growth parameters like root length, shoot length, fresh weight and dry weight were calculated after seven days. In the present study it was observed that mercury at low concentrations (< 100 ppm) was found to show no significant effect on seed germination. However increasing concentrations of mercury (>100) decreases germination percentages significantly. It was observed that seed germination was completely inhibited at Hg concentrations above 250 ppm. Root length, shoot length and dry weight were found to be decreased with increasing concentrations of $\text{Hg}(\text{NO}_3)_2$. The inhibitory effect of mercury on shoot and root of seedling was more pronounced at 200 ppm.

Index terms:

Cajanus cajan, Mercuric nitrate $\text{Hg}(\text{NO}_3)_2$.

Introduction:

Heavy metal contamination caused by either natural processes or by human activities is one of the most serious eco-toxicological problems. Since, photosynthetic plants function as the primary and principal entry point of heavy metals into the food chain leading to animals and ultimately to man. These heavy metals enrich, bio accumulate, and bio magnifies in the food chain and ultimately a significant amount of these heavy metals are found in the animal body. Heavy metal toxicity in plants has been established by various authors exhibited from morphological to molecular levels of organization [1 and 2]. Most studied aspect of heavy metal toxicity is the damaging effect on seed germination and seedling growth of different plant species. Several authors reported in different plant species that plants showed reduction in growth and recorded decrease in rates of seed germination when they are exposed to the heavy metals [3, 4, 5, 6, 7 and 8].

Mercury

Of all the heavy metals, mercury is found to have significant environmental concern. Once mercury is introduced to the soil, it lasts for a long time because of its indestructible and non-degradable nature and therefore causes potential risk for ecosystems [9 and 10]. Mercury is not essential for any of the biological functions; rather it is toxic to both plants and animals. In living organisms, mercury is thought to interfere with the mode of enzyme action and protein synthesis by binding with the sulfhydryl groups due to its strong affinity for sulphur [11, 12 and 13]. Mercury is known to be the most hazardous heavy metal. Global release of mercury in the atmosphere has been raising three to four folds. However, the anthropogenic origin of mercury at local level is much more. Several researchers have studied the effect of mercury on growth, physiology and metabolism of several plant species including *Vigna ambacensis* [14], *Vigna radiata* [15], *Solanum melongena* [16], *Arabidopsis thaliana* [17], *Sesbania drummondii* [18], *Medicago sativa* [19], *Arachis hypogaea* L. [20], *Vigna unguiculata* [21], *Zea mays* [22], *Vigna radiata* (L.) [23]. Hg inhibits seed germination not only due to unavailability of sugars to embryo by impairing the solubilization of starch but may also be due to damage caused to embryos produced by Hg treatment [24].

MATERIALS AND METHODS

A pulse crop, *Cajanus Cajan*, (L) Millsp. (Pigeon pea) was selected for the present study. Pure line uncontaminated seeds of Pigeon pea were obtained from the seed market of Visakhapatnam. Seed treatment was not done by any seed dressing chemical and by any antifungal chemicals to avoid any interference during the experiments. Healthy seeds were hand sorted and selected for the experiments.

GERMINATION STUDIES:

During this study, 5 test solutions with different concentrations of Hg (NO₃)₂ (10, 50, 100, 150, 200, 250 and 300 ppm) were used to investigate the effect of mercury on germination of seed and seedling growth. Deionized water was served as control. The healthy and uniform seeds of Pigeon pea were collected and thoroughly washed with the same test solution. Germination experiments were carried out in sterilized petri dishes lined with a single layer of sterilized filter paper. Always 10 no of seeds and 10 ml treatment test solutions were utilized for single treatment. The seeds were arranged on the petri plates and the toxicant was poured into the petri plates. The seeds were set under different $32 \pm 2 / 25 \pm 2$ °C day / night temperatures for 7 days. Each test was carried out in three replicates. Seedlings were removed from filter paper with the help of forceps on the 7th day of treatment. The length root and shoot of seedling was measured on the 7th day of seedling with the help of a scale. The germination percentages were recorded after 48 hours and root and shoot length of seedlings were measured after 168 hours (7 days). Plant tissues were oven dried at 80 °C for 24 hours to determine the dry weight. In all sets, the seeds were allowed to germinate in normal photo-inductive cycles and the illumination was maintained at 2400 ± 200 Lux, normal humid atmosphere and at a temperature of 28 ± 2 °C for 48 hours. After 48 hours of normal soaking and incubation, all seedlings were transferred to the growth chamber (Culture rack). To calculate the percentage of germination, periodical observations were made at an interval of 24 hours up to 168 hours. First emergence of coleorhizae (Referred as Root) about 2 mm. in length was considered as germination. Visual screening was carried out based on the growth and the percentage of seed germination and percentage of seedling establishment was also calculated.

STUDY OF SEEDLING GROWTH (PETRI PLATE CULTURE):

In petri plate culture, first the petri plates are cleaned with tap water then with chromic acid and then tap water and finally with double distilled water, and dried in an oven. Ten soaked seeds were sown in petri plates at uniform distance in all the sets. All petri plates were kept at room temperature and normal photo-inductive cycle was maintained by providing light from fluorescent tubes in daytime. For all other studies, seeds were grown up to the seedling establishment stage (up to 7th day of exposure to the toxicant), inside petri plates in different concentrations of the mercury toxicant including control set, as it was done in germination studies. On each 7th day of exposure, seedlings were harvested for respective experimental analysis.

RESULTS AND DISCUSSION

Table-1. Changes in seed germination, seedling establishment, Shoot & Root length, ratio value and percent change value, when compared to control, of 168 h old pigeon pea seedlings, grown in different concentrations of the mercury in petri plate culture. Data calculated from the mean of the samples \pm standard deviation.

Concentration of mercury	Percentage of seed germination	Percentage of seedling establishment	Shoot length in (cm)	Root length in (cm)	Shoot Fresh wt. (gm)	Root Fresh wt. (gm)	Shoot Dry wt. (gm)	Root Dry wt. (gm)
Control (0 ppm)	100	100	7.92 \pm 0.218	3.38 \pm 0.29	1.05 \pm 0.0086	0.2148 \pm 0.0182	0.171 \pm 0.005	0.0372 \pm 0.0013
10 ppm	100	100	7.08 \pm 0.140	2.86 \pm 0.35	0.906 \pm 0.008	0.1715 \pm 0.0128	0.149 \pm 0.005	0.0267 \pm 0.0027
50 ppm	100	100	6.28 \pm 0.038	2.28 \pm 0.26	0.878 \pm 0.0056	0.1204 \pm 0.0202	0.132 \pm 0.005	0.0205 \pm 0.0025
100 ppm	100	100	5.27 \pm 0.031	1.57 \pm 0.23	0.778 \pm 0.009	0.0969 \pm 0.0204	0.103 \pm 0.004	0.0144 \pm 0.0028
150 ppm	96	80	3.26 \pm 0.052	1.05 \pm 0.15	0.403 \pm 0.503	0.0405 \pm 0.0151	0.065 \pm 0.01	0.0104 \pm 0.0036
200 ppm	76	60	1.01 \pm 0.31	0.54 \pm 0.24	0.129 \pm 0.18779	0.0161 \pm 0.0086	0.025 \pm 0.02	0.0023 \pm 0.0029
250 ppm	20	0	0	0	0	0	0	0
300 ppm	0	0	0	0	0	0	0	0

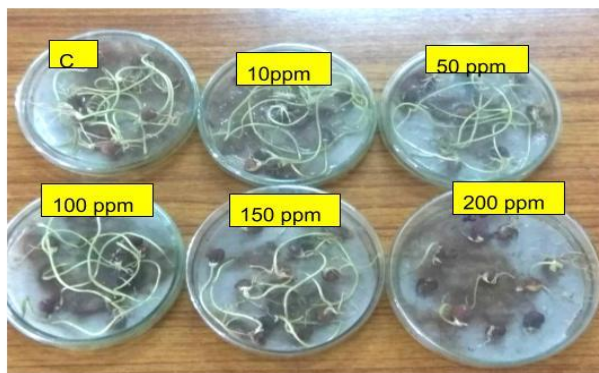


Figure-1: Growth of pigeon pea seedlings treated with mercury.

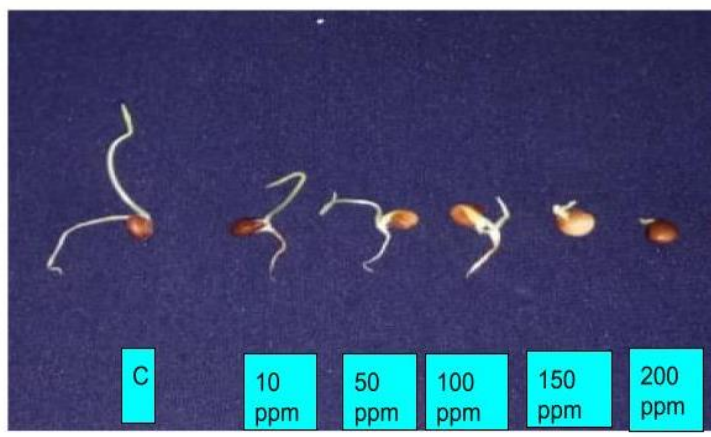


Figure-2: Growth of pigeon pea seedlings treated with mercury after 7 days.

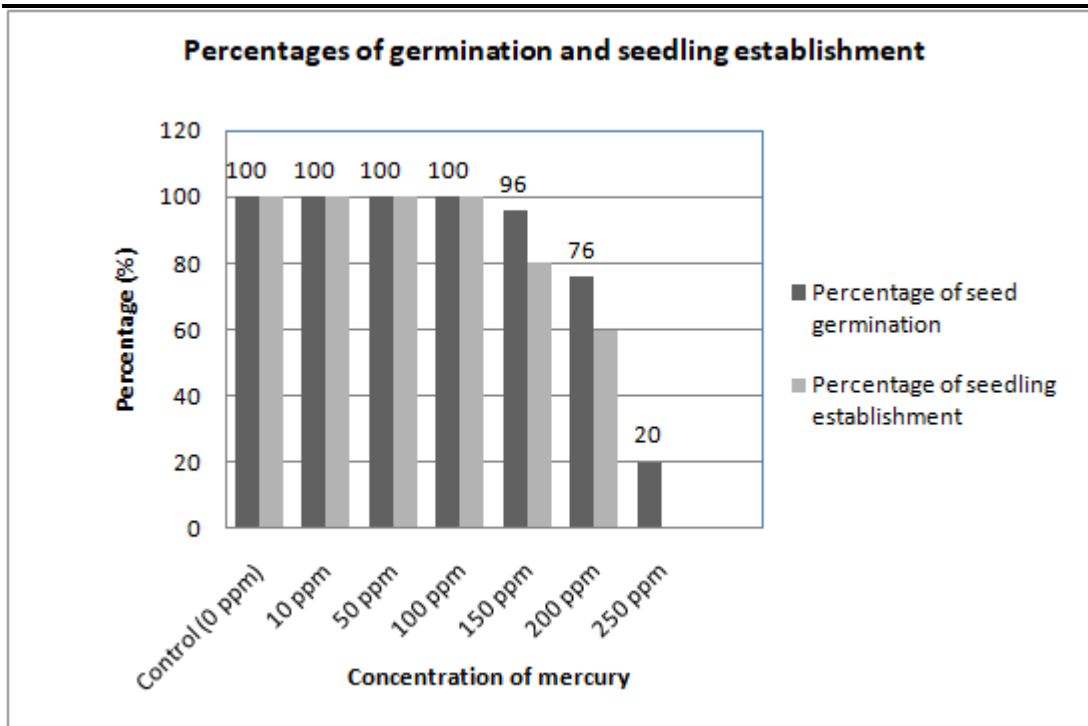


Figure 3: Showing percentage of seed germination and seedling establishment of pigeon pea in control and selected concentrations of mercury.

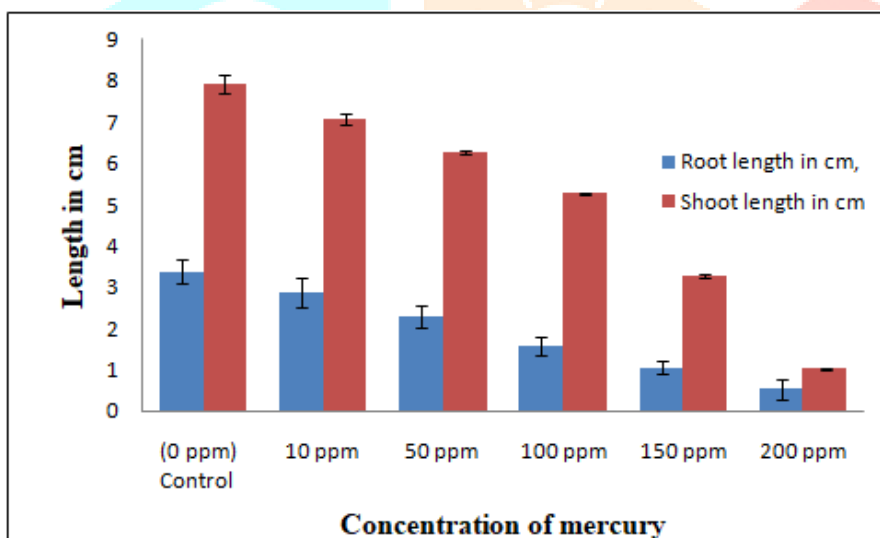


Figure 4: Showing changes in shoot length and root length of pigeon pea seedlings after 168 hrs of exposure in control and mercury exposed seedlings

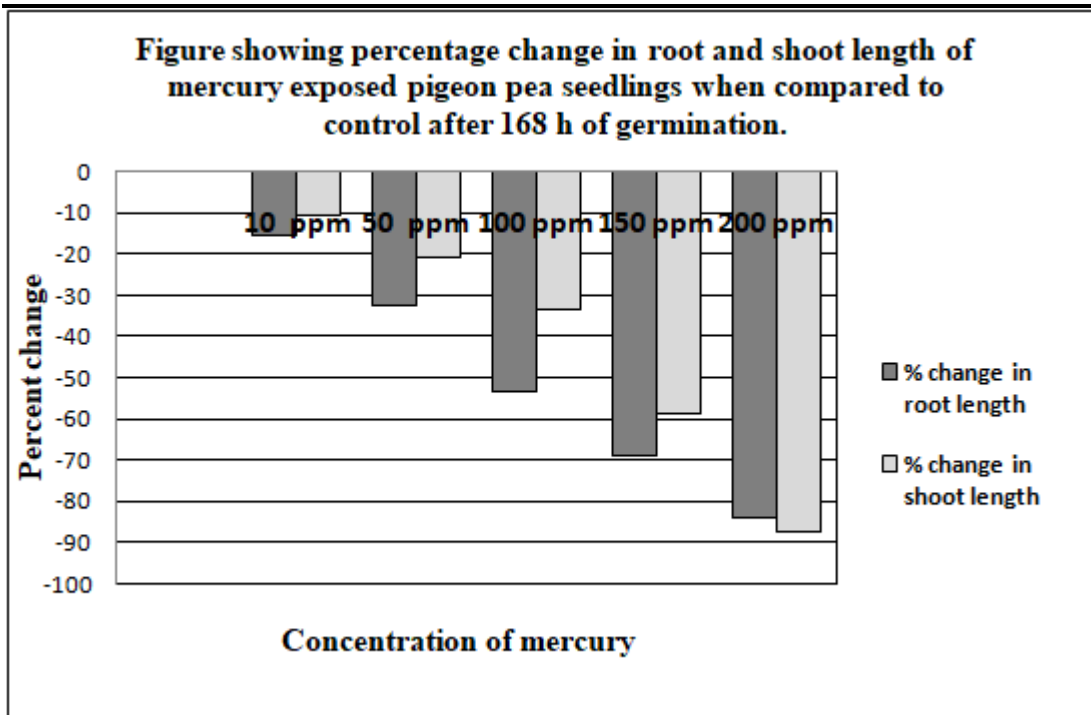


Figure-5: Percent change in length of root and shoot of pigeon pea seedlings treated with mercury after 7 days.

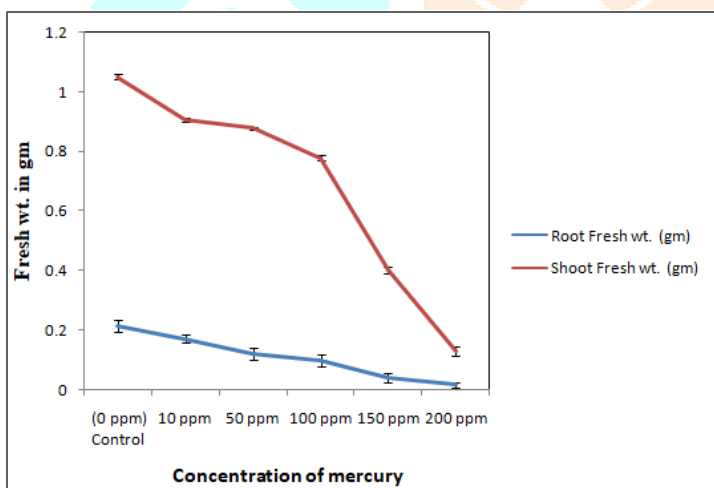


Fig. 6: Showing changes in shoot and root fresh weights of pigeon pea seedlings in different concentrations of mercury after 168 hrs, of exposure.

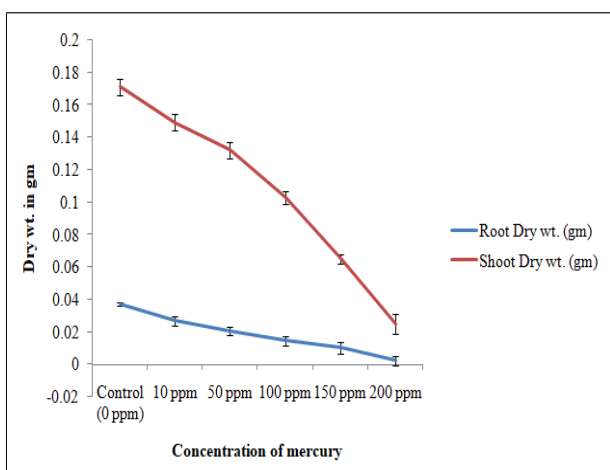


Fig. 7: Showing changes in shoot and root dry weights of pigeon pea seedlings in different concentrations of mercury after 168 hrs, of exposure.

To conduct experiments in the seed germinator the selected mercury concentrations were: 10 ppm, 50 ppm, 100 ppm, 150 ppm, 200 ppm, 250 ppm and 300 ppm. In the control set, 100% seed germination and 100% seedling establishment was observed in petri plate culture. All the seeds were germinated and 100% seedlings established up to 100 ppm mercury concentrations. In the 150 ppm mercury treatment, 100% seeds germinated, out of which only 96% seedlings established. The rest of the germinated seedlings died because of the toxicant. In 150 ppm of mercury concentration, seeds swelled and the seeds started germinating only 96% seeds germinated, the rest could not reach to 2 mm size to be counted as germinated, and only 80% seedlings were established. At this stage, no further germination or prolongation of plumule was marked. In 200 ppm mercury treatment, 76% seeds germinated, but only 60% seedlings established with stunted growth. In the case of 250 ppm of mercury, 20% pigeon pea seeds were germinated but these germinated seeds could not establish altogether (Table.1 and figure 3). No germination was marked beyond 300 ppm of the mercury. Selected healthy seeds were exposed to graded series of concentrations of mercury in sterilized petri plates to find out the lethal concentration and percent survival values for the present set of experiments. Series of pilot tests were conducted and the experiments were repeated at least thrice to determine the lethal concentration values. From the toxicity study, five different concentrations (10, 50, 100, 150, 200 ppm) of the mercury were selected for future experiments. During concentration studies, seeds and seedlings were carefully watched to find out the abnormalities during seed germination and seedling establishment. No significant morphometric change was recorded. Browning of the shoot at the base of the exposed seedlings was marked. The entire root became brown after a few days of exposure (7th day). Control seedlings remained clinically healthy. At very high concentrations of the mercury, the exposed roots became small and less. At higher concentrations of the toxicant, germination was observed but no seedling establishment was marked. Cent percent germination after 168 h of exposure was recorded in the control and at 10, 50, and 100 ppm of mercury concentration. The percentage of seed germination decreased with the increase in mercury concentration. The values obtained in the toxicity testing were significant and indicate the toxic nature of mercury. The values obtained were statistically significant. The effect of mercury on germination was more pronounced at 200 ppm of mercury concentration as only 76% seeds germinated and 60% established. At 250 ppm of mercuric concentration the pigeon pea seeds 20% seeds germinated but these germinated seeds could not establish altogether. No germination was marked beyond 300 ppm of mercuric nitrate (Table-1 and figure 3).

STUDY OF MORPHOLOGICAL PARAMETERS:

A) Shoot length and Root length:

From three replicas 10 seedlings were selected randomly and shoot and root lengths were measured, with the help of a scale. The shoot length of the germinated seedlings showed significant changes in the mercury exposed pigeon pea seeds in petri plate culture. In the control set, the shoot length of the seedling was 7.92 ± 0.29 cm after 168 hrs of germination and the root length was 3.38 ± 0.29 cm in petri plate culture. In case of mercury at 10 ppm the shoot length of the exposed seedling decreased from 7.92 to 7.08 cm showing 10.6% decrease over the control value. In case of 50 ppm of mercury concentration, the shoot length of the exposed seedling further declined from 7.92 to 6.28 cm showing 20.7% decrease when compared to the control value (Table-1). With the increase in mercury concentration, the shoot length decreased significantly to 5.27 cm showing a decrease of -33.45% at 100 ppm mercury concentration (Table-1, Fig. 4 and Fig. 5). When the concentration of mercury increased to 150 ppm, the shoot length decreased significantly from 7.92 to 3.26 cm and recorded a decrease of 58.8%. At 200 ppm mercury concentration, the shoot growth declined significantly and showed 87.2% decrease when compared to control.

The root length of the germinated seedlings showed significant changes in the mercury exposed pigeon pea seeds. In the control set, the root length of the seedling was 3.38 after 168 h of germination. In case of 10 ppm mercury concentration, the root length of the exposed seedling decreased from 3.38 to 2.28 cm showing 15.2% decrease over the control value. In case of 50 ppm of mercury concentration, the root length of the exposed seedling decreased significantly from 3.38 to 2.28 cm showing 32.5% decrease when compared to the control value (Table-1; Fig. 4 and Fig. 5). With the increase in the mercury concentration, the root length further declined to 1.57 cm at 100 ppm concentration (Table-1.Fig. 4 and Fig. 5) showing 53.5% decrease when compared to root length value of control. When the seeds are treated with high concentration of mercury (150 ppm and 200 ppm), the root length decreased significantly from 3.38 to 1.05 and 0.54 showing 68.93% and 84.02% decrease in root length respectively.

B) Shoot weight and Root weight:

Ten seedlings from three replicates were taken and the shoots were separated from the roots. These were washed thoroughly with distilled water, surface dried by means of blotting paper. Then fresh weights of roots and shoots were taken separately by a single pan electronic balance (Dhona make).

After weighing the fresh shoots and roots, they were kept in an oven for 48 hours at a temperature of 80°C and their dry weights were recorded at 24 hrs. of interval till we got a constant weight of the samples.

The shoot fresh weight of the effluent exposed pigeon pea seedlings decreased from 1.05 to 0.906, 0.878 and 0.778 mg at 10 ppm, 50 ppm, and 100 ppm of mercury concentration, showed 14%, 16% and 26% reduction over the control value. Whereas, at higher concentration of mercury at 150 ppm, the shoot weight decreased significantly from 1.05 to 0.403 mg showed a decrease by 61.6% and at 200 ppm mercury concentration, the fresh weight of shoot decreased from 1.05 to 0.129 mg that showed 87.7% decrease over the control value (Table.1 and Fig. 6).

The root fresh weight of the mercury exposed pigeon pea seedlings decreased from 0.248 to 0.1715, 0.1204, 0.0969, 0.0405 and 0.0161 mg at respective concentrations of 10 ppm, 50 ppm, 100 ppm, 150 ppm and 200 ppm of mercury, where a decrease by 20.15%, 43.9%, 54.8%, 81.1% and 92.5% were recorded over the control value (Table-1 and Fig. 6).

The shoot dry weight of the mercury exposed pigeon pea seedlings decreased from 0.171 to 0.149, 0.005, 0.132, 0.103, 0.065 and 0.025 mg that showed 12.8%, 22.8%, 39.7%, 61.9% and 85% decrease in shoot dry weight at respective concentrations ranging from 10 ppm, 50 ppm, 100 ppm, 150 ppm and 200 ppm when compared to the control value (Table.1 and Fig.7).

The root dry weight of the mercury exposed pigeon pea seedlings decreased from 0.0372 to 0.026, 0.0205, 0.0144, 0.0104, 0.0023 mg at concentrations ranging from 10 ppm, 50 ppm, 100 ppm, 150 ppm and 200 ppm of mercury showed 28.2%, 44.8%, 61.2%, 72.04% and 93.8% indicating drastic decrease when compared to the control value (Table.1 and Fig.7).

In the present investigation, the effect of mercury on seed and seed biology during germination and seedling establishment and growth parameters of seedling were studied in mercury treated seedlings. All the parameters of the exposed pigeon pea seedlings were found to be less than the control values. The effect is increasing with increasing concentrations of mercury. Similar observations by previous findings of decrease in germination percentages by higher concentrations of mercury metal reported by in mung bean [25]. The reduction in germination percentages might be attributed to the toxic effect of heavy metals on the activity of enzymes such as amylase, protease and ribonuclease [26] and mobilization of food reserves [26]. The mercury toxicity causes water deficit the seedling that leads to inhibition of root and shoot growth [27]. The decrease in root and shoot length of the treated seedlings at higher concentration was in agreement with the previous findings of [28 and 29]. It was observed from the data that the root was more affected than the shoot. The reduction of growth of shoot and root may be attributed to the change of properties of plasma membranes and cell walls by replacement of cations by mercury metal ions [11]. It has been proved time and again that heavy metals hamper normal functioning of plants and acts as a barrier to metabolic processes in several ways such as the bonding of HMs with sulfhydryl groups of proteins results in the disruption of protein structure [30] and affect the functional groups of various cellular molecules such as pigments or enzymes [31]. Metal toxicity also disrupts the integrity of membranes [32]. All such events lead to the repression of enzyme mediated vital metabolic events such as photosynthesis and respiration.

CONCLUSION:

The results obtained in this investigation show that mercury at higher concentrations show adverse effects on seed germination and seedling growth. Moreover the release of mercury into the immediate environment may enter the cereals, pulses and vegetables we eat and affect the health of human beings. Hence it is an urgent necessity to minimize the use of mercury in industries and mercury containing pesticides and fungicides.

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