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Germination, Growth, Physiological and Biochemical Response of Pigeon Pea (*Cajanus cajan*) Under Varying Concentrations of Copper (Cu), Lead (Pb), Manganese (Mn) and Barium (Ba)

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ABSTRACT

With the extension of the total populace, the ecological contamination and poisonous quality by chemicals raises concern. A fast industrialization and urbanization procedure has prompted the consolidation of pollutants. For example pesticides, oil/petroleum based commodities, acids and heavy metals in the common assets like soil, water and air are corrupting the nature of the earth, and also influencing plants and creatures. Heavy metals including lead, manganese, barium and copper are also significant ecological poisons in this scenario leading to lethal impacts of plants. Hence, diminishing profitability and alarming hazardous danger to the agro-biological system. These changes act as stress to plants influencing the plant physiology. Considering the worries in the eco system, we have conducted a series of experiments to optimize the impacts of Heavy metals on seeds of Pigeon pea (*Cajanus cajan*) under varying concentrations of copper (Cu), lead (Pb), manganese (Mn) and barium (Ba) which influence the germination and other physiological procedures. The impact of selected heavy metals on seeds of Pigeon pea (*Cajanus cajan*) were observed by measuring percentage of germination, root length, shoot length, fresh weight, dry weight, number of leaves and branches respectively for various concentrations at different time intervals viz., 0,5,10,15,20,25 and 30th days. In spite of the fact that reports exists on components by which the heavy metals go about as pressure and how plants have figured out how to survive. The results of present investigations can be concluded that copper (Cu) & lead (Pb) are the most effective pollutants and shown adverse effects on physiological and biochemical properties of Pigeon pea (*Cajanus cajan*) when compared with other heavy metals. The results also indicated that as the number of days increases the yellowing of leaves and fall down of leaves were observed in all treatments. The extent of this exploration in future stays in revealing the flagging systems of germinating seeds in response to the heavy metal dosage.

Key words: Heavy metal stress, Physiology, Biochemical response, Pigeon pea (*Cajanus cajan*), Growth analysis.

INTRODUCTION

Soil is a profitable and non-inexhaustible asset basic for germination of seeds, survival and development of plants therefore supporting structure to survive on earth. In the advanced world, a wide range of soil contaminations are limiting the development and growth of plants. Wastes are the predominant source of soil pollution which originates from mining, chemical, steel processing industries and different allied industries [1]. The above mentioned wastes consists the sorts of chemical substances like heavy metals, phenolic, organic and non-metals etc. Heavy metals are the intrinsic components of the environment matrices with integral and non-imperative factors. Contaminants polluted in soil with heavy metals have turned up to be frequent due to expansion in geogenic and anthropogenic activities [2]. The unplanned dumping of municipal wastes, mining, use of high quantity of pesticides, insecticides, fungicides, and other agrochemicals leads to problems making extensive motives of environment pollution. Heavy metals, such as copper, lead, manganese and barium are fore most environmental soil pollutants, especially in areas with excessive anthropogenic pressure. Heavy metals which accumulates in soils causes damage to the food safety as the polluted soil being used in agricultural manufacturing, and also impacts on marketable and crop boom due to phytotoxicity and environmental fitness of soil organisms. The effect of these heavy metals on plant life and their metabolic activities do prompt with the aid of the geological and biological redistribution of heavy metals through pollution of the soil will be glimpsed in this article by conducting series of experiments with synthetic heavy metal polluted soils.

MATERIALS & METHOD

Preparation of Experimental Setup

Experiments were conducted for 30 days (i.e., 1/12/2018 to 31/12/2018) at Green Fields Institute of Agriculture Research & training, Ibrahimpatnam, Rangareddy, Telangana, India, to evaluate the impact of heavy metals on seeds of Pigeon pea (*Cajanus cajan*) under varying concentrations of copper (Cu), lead (Pb), manganese (Mn) and barium (Ba). For this analysis the soil samples were collected from open fields located at research institute during the season of November-2018. The collected soil samples then dried under sunlight for four days and cleaned by removing all vegetation and solid unwanted materials. The samples were transferred into plastic tubs for further experiments.

Into a series of plastic tubs equal quantity of soil (≈ 2 kg/tub) was transferred and tubs were marked as C (control), T1, T2, T3, T4, T5, T6, T7, T8 and T9. The T1 to T9 are the tubs for treating soil artificially after contaminating with different heavy metals which ranged from 100 to 900 mg. All the experiments were carried out in triplets and mean values were obtained for analysing the results.

Seed Selection & Seed Treatment

The seeds Pigeon pea (*Cajanus cajan*) used in current investigation were purchased from local market located at Ibrahimpatnam and the seeds were certified and pretreated. Hence, there is no further pretreatment performed before sowing the seeds into the experimental tubs.

The set of 30 seeds were sown into each treatment. A set of 30 seeds were sown into normal/ untreated soil and taken as control. Pigeon pea (*Cajanus cajan*) were sown into respective tubs randomly and irrigated with bore well water immediately.

Irrigation

From the time of seed sowing the experimental tubs were irrigated regularly once in a day to maintain soil moisture at saturated level. The experimental setup kept in open for better sunlight and air.

Growth Analysis

Growth analysis is a mathematical expression of environmental effects on growth and development of crop plants. This is a useful tool in studying the complex interactions between the plant and the environment. According to the proposed plan shoot measurements, root measurements and leaf measurements and dry weights of components of plant parts need to be collected at particular intervals. This data is used to calculate various indices and characteristics that describe the growth of plants and their parts which are grown in different environments and the relationship between assimilatory apparatus and dry matter production. These indices and characteristics are together called as growth parameters. Accuracy in calculation of these parameters and their correct interpretation are essential aspects in growth analysis.

Advantages of Growth Analysis

- One can study the growth of the population or plant community in precise way with the availability of raw data on different growth parameters.
- These studies involve an assessment of the primary production of vegetation in the field i.e. at the ecosystem level of organization.
- The primary production plays an important role in the energetic of the whole ecosystem
- The studies also provide precise information on the nature of the plant and environment interaction in a particular habitat.
- It provides accurate measurements of whole plant growth performance in an integrated manner at different intervals of time.

Disadvantages of Growth Analysis

In classical growth analysis sampling for primary values which consists of harvesting (destructively) represents set of plants or plots and is impossible to follow the same plants or plots throughout the experiment.

Growth Characteristics – Definition and Mathematical Formulae

The following data are required to calculate different growth parameters in order to express the instantaneous values and mean values over time interval. In the following discussion W, W_L, W_S and W_R are used to represent the dry weights of total plant, dry leaves, stem and roots respectively. Whereas A is the leaf area.

Relative Growth Rate (RGR)

The term RGR was coined by Blackman. It is defined as the rate of increase in dry matter per unit of dry matter already present. This is also referred as Efficiency index, since the rate of growth is expressed as the rate of interest on the capital. It provides a valuable overall index of plant growth. RGR can be calculating by following formulae.

$$Relative\ Growth\ Rate = \frac{loge^{W_2} - loge^{W_1}}{T_2 - T_1}$$

Net Assimilation Rate (NAR)

The NAR is a measure of the amount of photosynthetic product going into plant material i.e. it is estimate of net photosynthetic carbon assimilated by photosynthesis minus the carbon lost by respiration. The NAR can be determined by measuring plant dry weight and leaf area periodically during growth and is commonly reported as grams of dry weight increase per square centimeter of leaf surface per a particular time period. This is also called as unit leaf rate because the assimilatory area includes only the active leaf area in measuring the rate of dry matter production. The mean NAR over a time interval from T1 to T2 is given by

$$NAR = \frac{W_2 - W_1}{T_2 - T_1} X \frac{loge^{A_2} - loge^{A_1}}{A_2 - A_1}$$

Leaf Area Ratio (LAR)

The LAR is a measure of the proportion of the plant which is engaged in photosynthetic process. It gives the relative size of the assimilatory apparatus. It is also called as capacity factor. It is defined as the ratio between leaf area in square centimeters and total plant dry weight. It represents leafiness character of crop plants on area basis.

$$Leaf\ Area\ Ratio = \frac{A}{W}$$

Leaf Weight Ratio (LWR)

It is one of the components of LAR and is defined as the ratio between grams of dry matter in leaves and total dry matter in plants. Since the numerator and denominator are on dry weight basis LWR is dimensionless. It is the index of leafiness of the plant on weight basis.

$$Leaf\ Weight\ Ratio\ (LWR) = \frac{W_L}{W}$$

Specific Leaf Area (SLA)

It is another component of LAR and defined as the ratio between leaf area in cm^2 and total leaf dry weight in grams. This is used as a measure of leaf density. The mean SLA can be calculated as follows.

$$Specific\ Leaf\ Area\ (SLA) = \frac{A}{W_L}$$

Specific Leaf Weight (SLW)

The reciprocal of SLA is called SLW. It is defined as the ratio between total dry weight and leaf area. It indicates the relative thickness of the leaf of different genotypes.

$$SpecificLeafWeight(SLW) = \frac{W_L}{A}$$

Leaf Area Duration (LAD)

It is usually expressed as a measure of leaf area integrated over a time period. Some takes into account both the magnitude of leaf area and its persistence in time. It also represents the leafiness of the crop growing period. Thus the unit of measurement of LAD may be in day or weeks or months [3].

$$LeafAreaDuration(LAD) = \frac{LA_1 + LA_2\ (T_2 - T_1)}{2}$$

Plant Sampling and Analysis

A seed was considered as germinated when root had emerged more than 2 mm. The number of germinated seeds per time is termed as seed germination rate. Germination percentage and tolerance indices determined by the following formula [4].

$$\%\ of Germination = \frac{NumberofSeedsGerminated}{TotalNumberofSeedsPlanted} X\ 100$$

$$Toleranceindices = \frac{Meanrootlengthoftreatedseed}{Meanrootlengthofcontrol}$$

The inhibition of seedling growth was expressed according to the formula [5].

$$percentageofinhibition = \frac{Lengthofcontrol - Lengthoftreatedseed}{Lenghtofcontrol} X\ 100$$

Seedling Vigor Index

Seedling vigor index are those properties of the seed which determine the levels of activity and performance of the seed during germination and seedling emergence. It is a single measurable property like germination describing several characteristics associated with various aspects of the performance of seed. Seedling vigor index is calculated by using formula [6-7].

SVI = Germination percentage × Seedling length

Percentage Phyto-toxicity

Percentage phytotoxicity of heavy metals on root and shoot growth of pigeon pea (*Cajanus cajan*) were calculated at regular time interval (5 to 30 days of seedling growth). The following formula was used for calculating the percentage phytotoxicity [8].

$$PercentageofPhytotoxicity = \frac{\frac{S}{R} lengthofcontrol - \frac{S}{R} lengthoftreatedseed}{\frac{S}{R} lengthofcontrol} X\ 100$$

ESTIMATION OF BIOCHEMICAL ATTRIBUTES

Biochemical attributes were studied in term of photosynthetic pigments. The chlorophyll-a, chlorophyll-b and total chlorophyll (a + b) were determined spectrophotometrically. Leaves were cut into small pieces, mixed thoroughly and 0.25 g of leaves were taken into a mortar to grind them finely by using pestle with 25 ml of 80% acetone for 5 minutes. The homogenate was filtered through filter paper (Whatman No.42) and was made a volume of 25 ml with 80% acetone. The total Carbohydrates were determined by Anthrone method, total proteins by Biuret method and peroxidase activity by O-diansidine method enzymatically [9-11].

Extract Monitoring by Spectrophotometer

After the extraction, chlorophyll contents were monitored by UV-Vis spectrophotometer [12]. The optical density/absorbance of each solution were measured at 663 and 645 nm against 80% acetone blank in 1 cm quartz cuvette at room temperature. The Arnon's equation was used to calculate the amount of chlorophyll-a, chlorophyll-b and total chlorophyll (a + b) [13-14].

Chl a (mg·g−1) = [(12.7 × A663) − (2.69 × A645)] × ml acetone/mg leaf tissue

Chl b (mg·g−1) = [(22.9 × A645) − (4.68 × A663)] × ml acetone/mg leaf tissue

Total Chl = Chl a + Chl b

STATISTICAL ANALYSIS

Data was statistically analyzed using one-way ANOVA on Graphpad Prism 6.01 software [14]. The results were presented as mean ± S.D. (standard deviation) and data from different treatments and control were compared by Duncan's multiple-range test at $p < 0.05$.

RESULTS & DISCUSSION

Germination

The effect of various concentrations of Barium (Ba), Lead (Pb), Manganese (Mn) and Copper (Cu) on seed germination of *Cajanus cajan* (Pigeon Pea), presented in figure-1-4. There was a reduction in seed germination as metal concentration increases in all the experiments in general, when compared to control treatment except seeds treated with various concentrations of Mn. The experiments conducted with barium treated soils, the germination % at 5th day decreased with increase in concentration up to (T1 –T4) 400 mg/2 kg of soil. The moderate and higher concentrations of T5 – T9 shows increased germination %. These results indicating the higher concentration of barium metal ions favors the germination process. From 10th day there is no significant germination observed in all the treatments including control. The maximum germination rate observed between the treatments T6 to T8 (600 – 800 mg/2kg of soil) with the % of 80 – 83% respectively (Fig-1). The lowest germination rate was observed for T4 and T9 (400 & 900 mg/2kg of soil) with germination percentage of 58 and 67. The result also indicates that pigeon pea (*Cajanus cajan*) is responding mixed type of behavior with barium. All the treatments of barium metal did not shown any visible toxicity symptoms up to 17th day of growth period. As duration of growth period increases the chlorosis, yellowing of leaves and drying edges in seedlings were observed.

The impact of lead (Pb), on seed germination of pigeon pea (*Cajanus cajan*) was presented in figure-2. The results indicate that the % of germination is decreased with increase in lead (Pb) concentrations. On 5th day the % of germination is 79% in control and the % germination decreased from 77 to 30% with respect to T1 – T9. The maximum % germination after 10 days, it was observed that T1 with 85% and minimum % germination was observed for T9 with 47%. The result also concludes that higher concentrations of Pb, does not favor the germination process. Similar types of results were observed with Heidari and Sarani 2011 [15], where they studied the effect of lead and cadmium on seed germination seedling growth and antioxidant enzymes activity of mustard. The same type of results were observed by Shafiq et.al., 2008 [16], when they studied the effect of lead and cadmium on germination and seedling growth of *Leucaena leucocephala*. The current investigation also concludes the rate of germination in lead treatments was high when compared to the treatments of barium, which indicates that the accumulation efficiency of lead is more when compared to barium for pigeon pea (*Cajanus cajan*) seedlings.

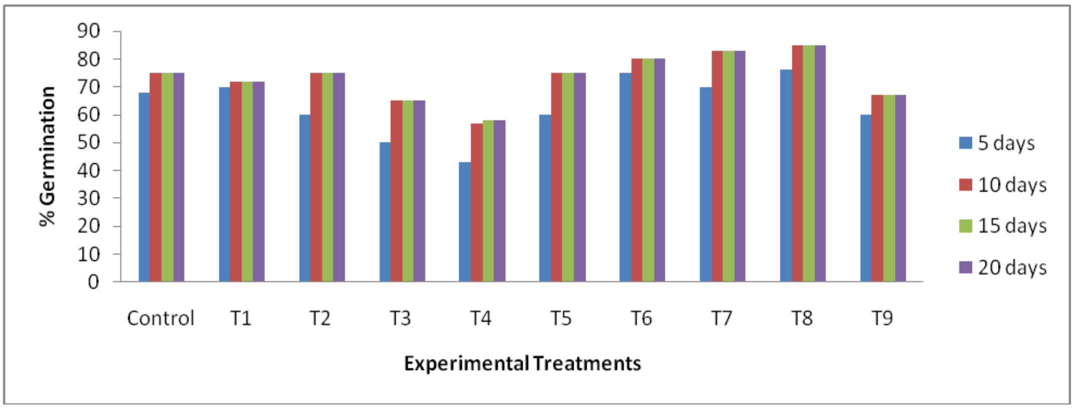

Figure-1: Effect of Barium (Ba) concentrations on germination of pigeon pea (*Cajanus cajan*) seeds.

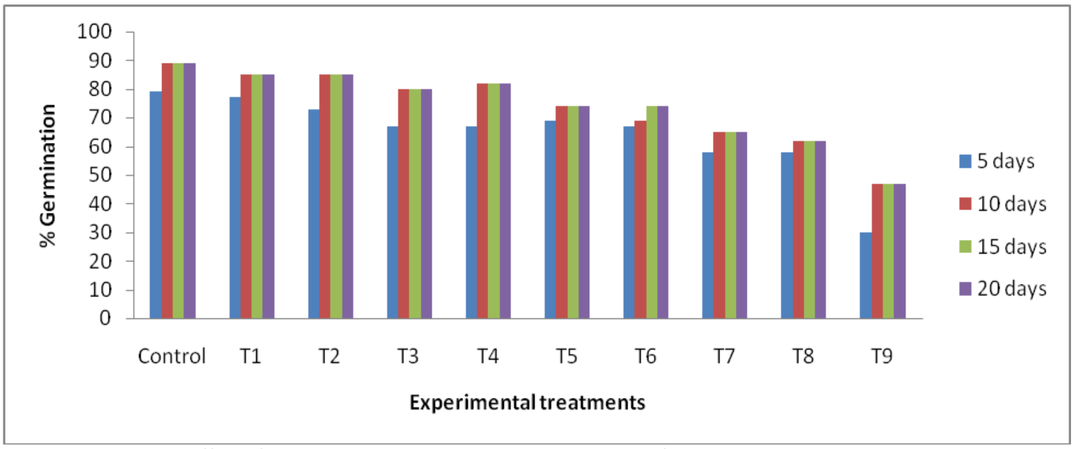

Figure-2: Effect of Lead (Pb) concentrations on germination of pigeon pea (*Cajanus cajan*) seeds.

The effect of manganese concentrations on germination of pigeon pea (*Cajanus cajan*) were presented in figure-3. The results indicating pigeon pea (*Cajanus cajan*) showed mixed type of behavior with manganese. The % of germination increased with increase in metal ion concentration when compared to control treatment. The reason behind this is the ionic (Mn^{+2}) metal may actively involve as a cofactor in various biochemical pathways during seedling growth, which favors the healthy growth of pigeon pea (*Cajanus cajan*). The similar kind of results presented by Keerthi kumara et. al., 2016 [17], they had investigated the impact of cadmium and manganese on *in-vivo* seed germination and seedling growth of horse gram. The same type of results were also described by Roy & Bera 2000 [18], they observed 100 ppm manganese solution favoring 100% seed germination in mung bean. As, it is essential heavy metal for plant growth. In the present study maximum % of germination was observed for T7 with 90%, after 15 days of seed sowing. This result indicates the approach of manganese and the extraction rate is very slow in pigeon pea (*Cajanus cajan*) seeds. The % of germination significantly higher than the % germination of seedlings treatments of barium and lead. This statement concludes that soils and water which are polluted with 'Mn' may favor healthy growth of pigeon pea (*Cajanus cajan*). There is no any other morphological effects (chlorosis, yellowing of leaves and dry the edges of seedling) observed in the treatments even after 25 days of crop period.

The effect of copper on seed germination of pigeon pea (*Cajanus cajan*) was shown in figure-4. The result clearly indicates that, % of germination was promoted with increasing concentration. But, the % germination is decreased when compared to control. After 5 days of seed sowing the % of germination in control treatment was 75% and T1, T2, T3, T4, T5, T6, T7, T8 and T9 were with 69%, 56%, 73%, 65%, 58%, 78%, 78% and 78% respectively. The lower copper concentrations shown mixed type of behavior. Whereas, the higher concentrations shows stable % of germination rate (from T7 to T9 with 78% of germination). The mixed type of response maybe due to improper solubility of $CuSO_4.5H_2O$ in the soil, which was failed during accumulation and uptake process. The results given by melania & micle, 2015 [19], indicated that 10 ppm showed greater % of germination compare to 1 ppm and 50 ppm. No growth of seedlings was observed at 100 ppm, in their experiments. These studies indicate that copper may enhance the germination process up to optimum. However, most of the studies indicated that 'Cu' significantly decreases the seed germination. With 10 µM Cu treatment wheat and rice seed germination was reduced by more than 35% and 60% respectively [20]. In Alfa alfa, 40 mg/L Cu inhibited significantly seed germination by 39% [21]. In Crambe, higher concentrations of 'Cu' decreased the seed germination significantly [22].

The % of germination and growth rate is almost similar as studied in seeds treated with barium and lead. Overall results of % germination of pigeon pea (*Cajanus cajan*) seeds with various concentrations of Ba, Pb, Mn and Cu can be concluded that all the concentrations of 'Mn' favor the germination and growth of pigeon pea (*Cajanus cajan*) seedlings when compared to other metals studied in present investigation.

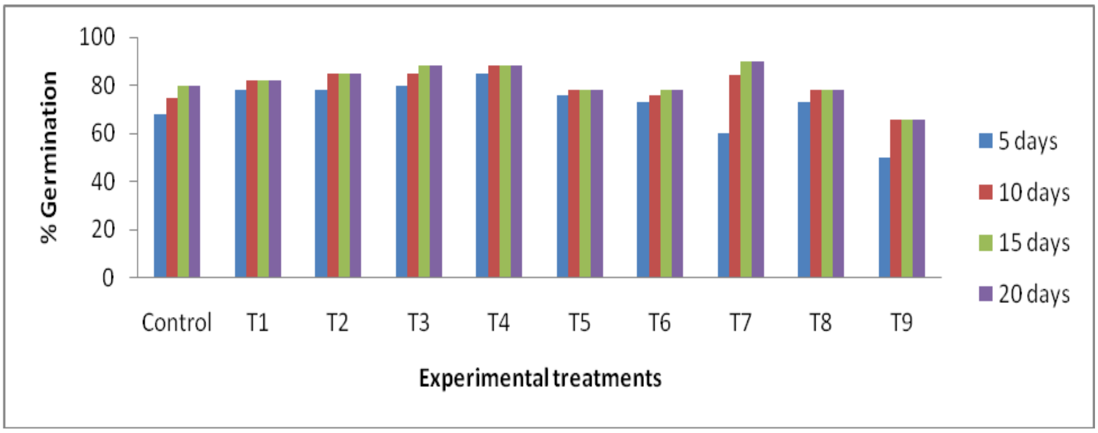

Figure-3: Effect of Manganese (Mn) concentrations on germination of pigeon pea (*Cajanus cajan*) seeds.

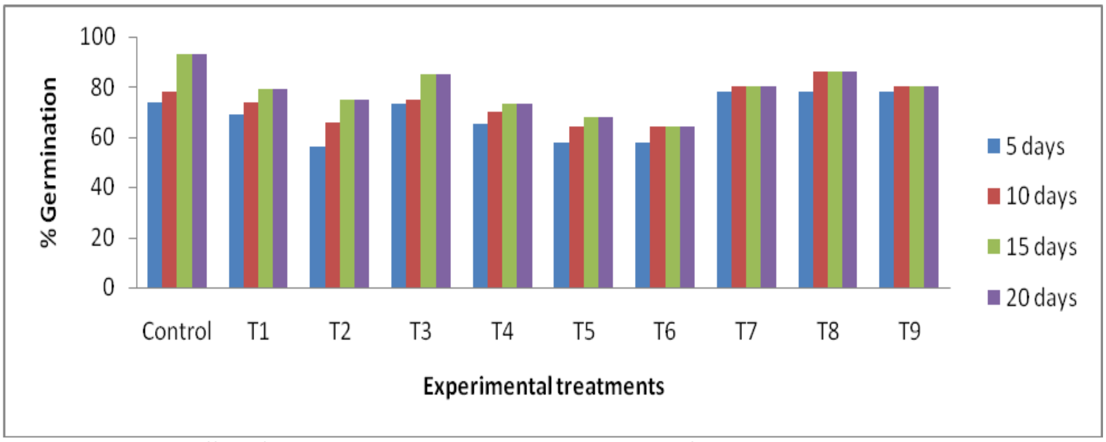

Figure-4: Effect of Copper (Cu) concentrations on germination of pigeon pea (*Cajanus cajan*) seeds.

Shoot length

The data representing the shoot growth of pigeon pea (*Cajanus cajan*) at various concentrations of barium heavy metal reported in this investigation shown in figure-5. From the results, it is observed that reduction in shoot growth in all treatments when compared to control treatment during all the experimental durations. The day 5th control seedling length was 5.8 cm. The lower concentrations of barium (T1 to T3) showed shoot length of 5 cm and T4 to T5 showed 4cm. A gradual increase in shoot growth was observed for T6 (4.8 cm) to T9 (5.2 cm), indicating higher concentration of 'Ba' which promotes the growth or plant developing a strategy to overcome higher metal concentration stress. A few studies reported the genetic and biochemical elements in plants helping them to overcome heavy metal stress [23]. The seedling growth was increased with increase of growth period in all treatments including control. The mean shoot length of control treatment was 7.9 cm on 20th day and maximum shoot growth observed for T2 treatment (6.2 cm) on the same day.

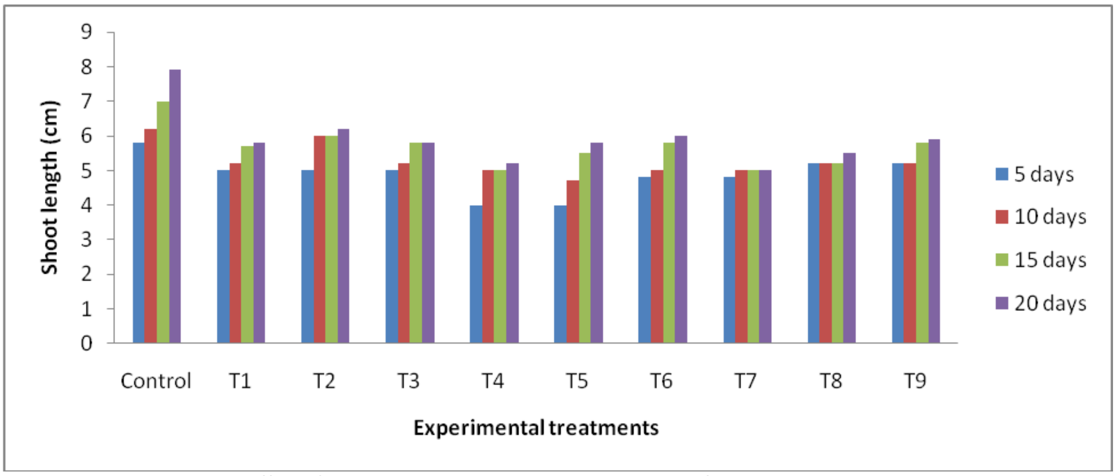

Figure-5: Effect of barium concentrations on shoot growth of pigeon pea (*Cajanus cajan*)

The data representing the shoot growth of pigeon pea (*Cajanus cajan*) at various concentrations of lead (Pb) were shown in figure-6. From the results it was observed that lead promoted the shoot growth at lower concentrations (T1 – T5) compared to control treatment. The higher concentrations of lead in present investigation showed almost similar shoot growth and no significant change was observed during all the days of crop period. A few studies have reported the 'Pb' showing decreased growth in shoot length and promotes fresh weight increase [24]. The shoot growth rate was significantly decreased when compared to shoot growth rate of barium treatments.

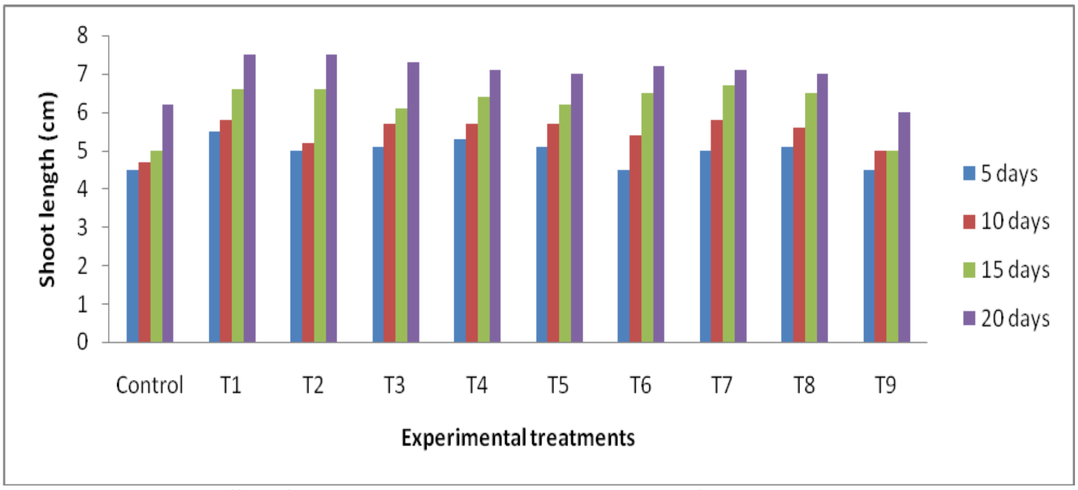

Figure-6: Effect of Lead (Pb) concentrations on shoot growth of pigeon pea (*Cajanus cajan*)

The impact of manganese (Mn) on the shoot growth of pigeon pea (*Cajanus cajan*) at different dosage was given in figure-7. From the figure it can be observed that, initially there is no significant growth of shoot length at all concentrations of 'Mn' including control treatment. As the duration of crop period increases, the growth rate of shoot increased at lower concentrations. But, no significant growth was observed at higher concentrations (T8 & T9). The minimum growth of shoot length at higher concentrations was 5 cm and is continued stable from 5^{th} day to 20^{th} day.

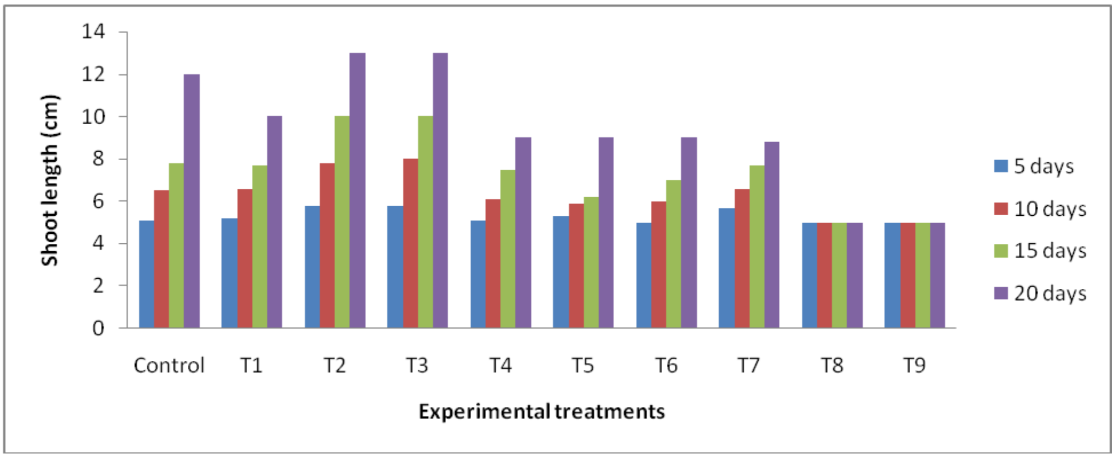

Figure-7: Effect of Manganese (Mn) concentrations on shoot growth of pigeon pea (*Cajanus cajan*)

The stable length of shoot at higher concentration indicates that pigeon pea (*Cajanus cajan*) may utilize energy to overcome heavy metal stress than other physiological functions [24]. The maximum shoot length of control treatment at 20th day was 12 cm and maximum shoot length observed at the seed treated with 200 mg & 300 mg 'Mn' for 2kg of soil. The shoot growth rate significantly increased when compared to shoot growth rate of barium and lead treatments, indicating that pigeon pea (*Cajanus cajan*) having more tolerance towards manganese.

The impact of copper on shoot growth rate of pigeon pea (*Cajanus cajan*) depicted in figure-8. From the figure it can be observed that there is no significant change in shoot growth at lower concentrations of copper treatment. The higher concentrations showed adverse effects and decreased shoot growth. At the 20th day maximum growth was observed for T1, T2 and T3 with 11 cm of shoot length. The control treatment showed 12 cm shoot length and lower concentration showed decreased shoots growth of 8.4% when compared with control treatment. The similar kind of results were reported by Aydinalp 2009 [22], in their studies where the shoot elongation of Alfa alfa reduced by 69% at the dose of 40 ppm.

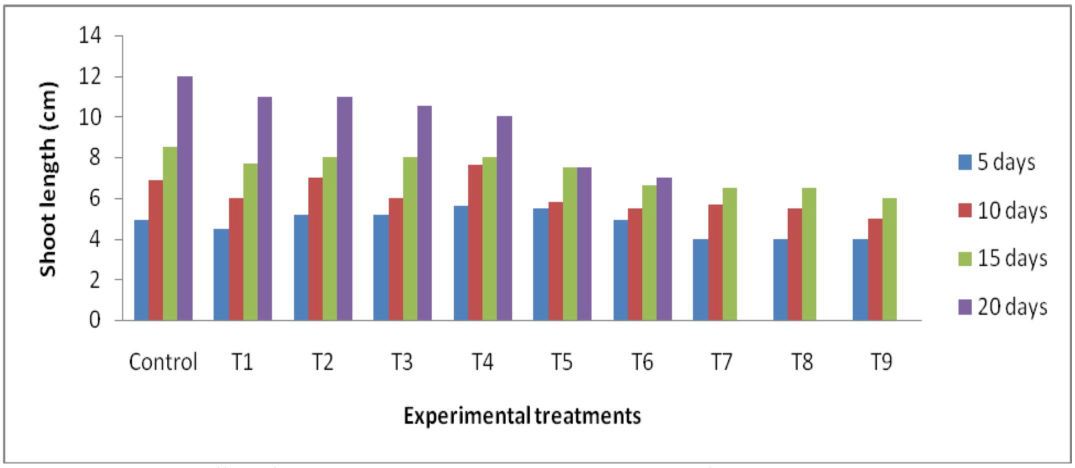

Figure-8: Effect of Copper (Cu) concentrations on shoot growth of pigeon pea (*Cajanus cajan*)

Root length

The effect of various concentrations of barium on root growth of pigeon pea (*Cajanus cajan*) showed in figure-9. From the figure, it is observed that the growth rate of root got decreased as increase in barium concentration throughout the experimental periods. The T1 showed significant greater root growth (12 cm), when compared to control treatment at 20th day of seedling growth. The root growth rate decreased by 47% compared to control treatment at higher concentrations. The literature concludes that higher concentrations of barium enhanced root elongation, showed positive significant growth rate of root with Tanzania guinea grass which were grown in nutrient solution. The total root length and root surface were not changed by barium concentrations [25]. According to Fitter 1996 [26], the greater root length and specific root length indicates relative greater proportion of thin roots, which was grown as a strategy in search of nutrients in media with low nutrient availability. The current investigations and root growth behavior of pigeon pea (*Cajanus cajan*) suggests that it should be grown away from higher barium concentrations.

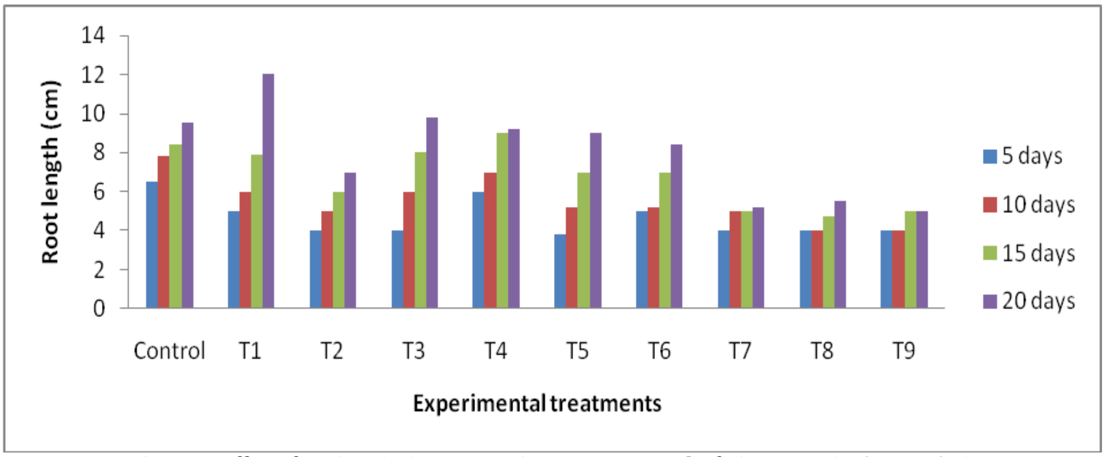

Figure-9: Effect of Barium (Ba) concentrations on root growth of pigeon pea (*Cajanus cajan*)

The effect of various concentrations of Lead (Pb) on root growth of pigeon pea (*Cajanus cajan*) is presented in figure-10. From the figure, it is observed that lower the concentration of lead promoted root elongation process. The root growth rate is highly increased at low treatments when compared to control. In most of the plants, 90% of total Pb is accumulated in roots [27]. The primary effect of Pb toxicity in plants is rapid inhibition of root growth, probably due to the inhibition of cell division in the root tip [28] when availability of lead is high (i.e. at higher concentrations of lead). In response to Pb exposure, plants have developed a variety of tolerance mechanisms. Roots are the first organs, exposed to Pb ions [29]. The first defense strategy is to stop metal entering the root tissues by excluding it [30]. Roots rapidly respond to the available Pb by forming mechanical barrier. In certain plants, there is synthesis and deposition of callose between the plasma membrane and the cell wall. This newly formed structure acts as a barrier against stress factors including metals [31, 32]. Samardakiewicz et al. 2012 [33] examined whether callose forms an efficient barrier against Pb penetration in the roots of *Lemna minor* L. exposed to 15 µM of Pb for 6 h. This treatment resulted in the synthesis and deposition of callose in the newly formed cell wall in the protoderm in the center of the root tip. After callose deposition the Pb concentration was restricted in these superficial cells. Similar observations have been made in other species exposed to Pb including *Arabidopsis thaliana* [34] and *Funaria hygrometrica* [35]. Pb-induced callose deposition has been detected in the rhizodermis and in the center of the stele of Pb-treated soybean *Glycine max* roots tips [36]. Under metal stress, the synthesized callose inhibits cell-to-cell transport. This may result in the prevention of a wide incursion of Pb ions, but it can simultaneously inhibit the transport of other molecules. However, the synthesis of callose is not a general pattern in plants in response to Pb, in *Zea mays* and *G. max*, low level Pb treatment did not result in any callose deposition in root tissue. Although, these species synthesized callose in response to cadmium or arsenic [37], it seems that the formation of callose was closely related to the amount of Pb entering the cell, and subsequently the level of stress.

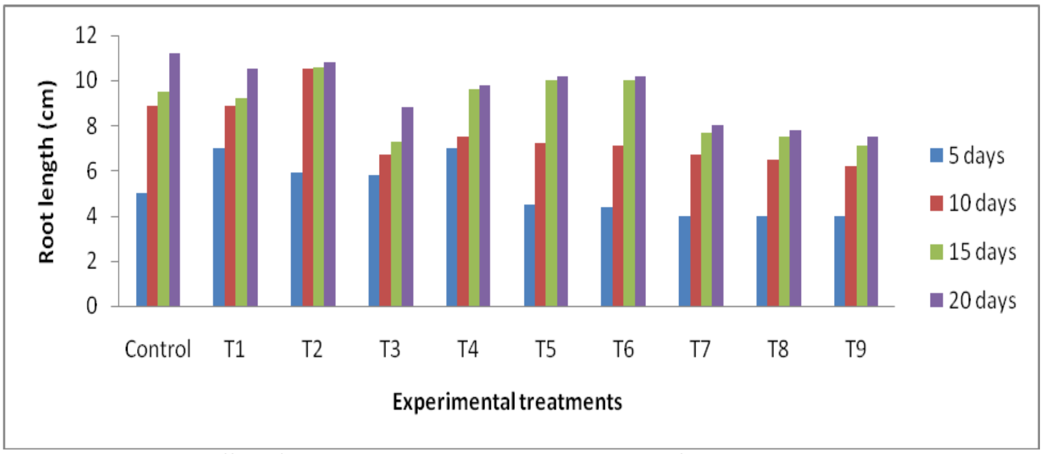

Figure-10: Effect of Lead (Pb) concentrations on root growth of pigeon pea (*Cajanus cajan*)

The effect of various concentrations of Manganese on root growth of pigeon pea (*Cajanus cajan*) is presented in figure-11. From the figure, it is observed that 'Mn' adversely effecting the root growth promotion. The root growth rate decreased, with increase in manganese concentration. Similar results were reported by Zhao et. al., 2017 [38] in their studies 'Mn' toxicity inhibited primary root growth by reducing the auxin accumulation in root tip. They also observed Mn toxicity decreased auxin accumulation in roots by reducing auxin biosynthesis and repressing auxin transport via the decrease of the expression of auxin efflux carrier's PIN4 and PIN7 proteins. Root meristematic cell division potential is an important factor that affects root growth [39, 40]. Root meristem activity is controlled by auxin and cytokinin, and their interaction [41]. Auxin controls meristem growth and cell division by mediating degradation of SHY2 protein, a repressor of auxin signaling. Mn toxicity decreased auxin accumulation in roots, and thereby reducing meristematic cell division in root tips.

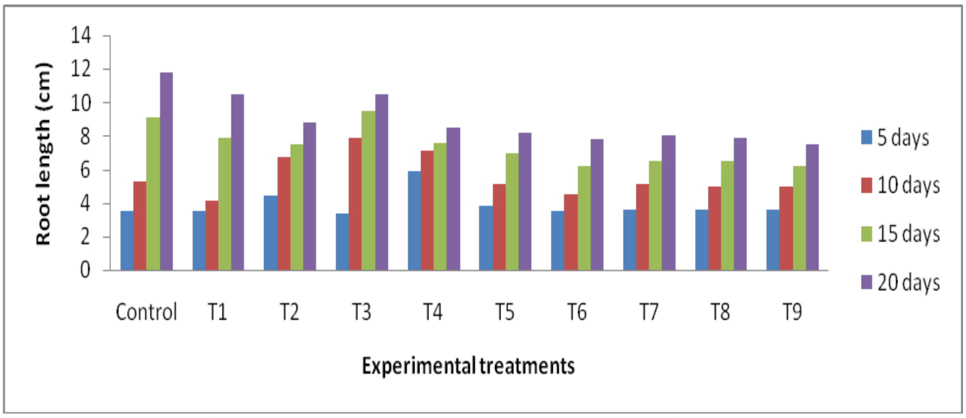

Figure-11: Effect of Manganese (Mn) concentrations on root growth of pigeon pea (*Cajanus cajan*)

The effect of various concentrations of Copper on root growth of pigeon pea (*Cajanus cajan*) is presented in figure-11. From the figure, it is observed that 'Cu' adversely effected the root growth promotion. The root growth rate decreased with increase in copper concentration. As the crop growth period increased the slow recovery in root elongation was observed at lower copper concentrations. These observations suggests that some tolerance mechanisms, e.g., binding to metalloproteins [42] or the precipitation of Cu complexes in globular bodies [43], were activated in response to low concentrations of Cu. Root elongation of both *P. pinea* and *P. pinaster* seedlings were completely inhibited within 3 days of exposure to 5 mM Cu reported by Arduin et.al., 1995 [44]. Under our experimental conditions Cu and Ba were much more toxic than lead and manganese to the roots of pigeon pea (*Cajanus cajan*). In all the experimental conditions with all heavy metals used in present investigation resulted to decrease in root elongation process. The high toxicity of Cu has been reported for several species including *Loliumperenne L.* [45] and *Brassica chinensis L.* [46]. Barbolani et al., 1986 [47] reported a higher and more rapid uptake of Cu than Cd in *Iris pseudacorus L.* Copper may be more phytotoxic than Cd because it is an essential element and may be absorbed and become involved in metabolic pathways more readily than Cd [48]. In some species of plants, high Cu sensitivity of root growth is related to disturbances of mitosis [49] and especially to damage to the cell membrane, which is often the first target of Cu toxicity [50]. Some authors have explained both effects as consequences of Cu toxicity on protein synthesis or activity [51]. DeVos et al., 1991 [52] suggested that Cu-induced damage to integral proteins, through the formation of disulfide links, resulted in increased cell membrane permeability and ion efflux.

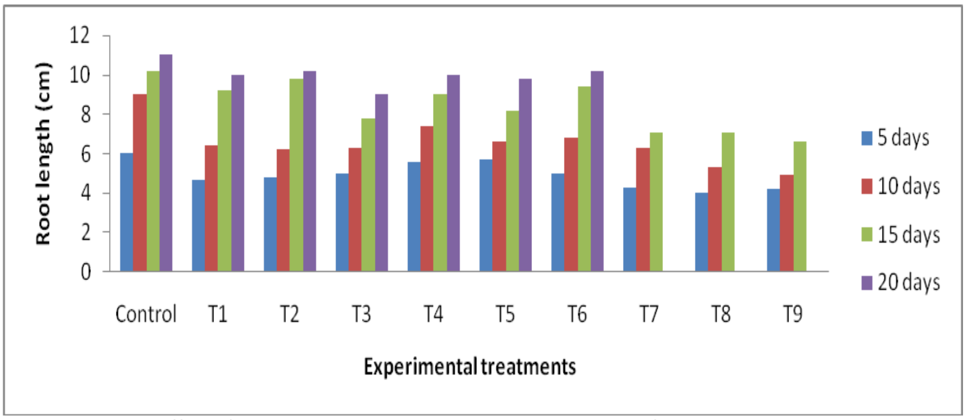

Figure-12: Effect of Copper (Cu) concentrations on root growth of pigeon pea (*Cajanus cajan*)

Fresh Weight

The effect of varying concentrations of barium on fresh weight of pigeon pea (*Cajanus cajan*) seedlings represented in figure-13. From the results it is observed that, barium promoted fresh weight of seedlings at lower concentrations during initial germination days i.e. 5th and 10th day of samplings. As growth period of crop increased the fresh biomass of pigeon pea (*Cajanus cajan*) increased with all treatments compared to control. The maximum fresh weight observed forT9 followed by T2, T6 and T7 at 20th day of experiment. The minimum fresh weight observed with T4 and T5 followed by T3 and T8.

The effect of varying concentrations of lead on fresh weight of pigeon pea (*Cajanus cajan*) seedling represented in figure-14. From the figure it is observed that, the fresh weight of seedlings were decreased as concentration of lead increase at initial growth periods i.e. up to 10 days of germination and seedling growth. The results on 15 and 20th day showed fresh weight of seedlings of T1 and T2 treatments were significantly greater than control treatment. From the results it can be concluded that duration of crop increases plant maybe construct a strategy to accumulate heavy metals in various tissues and organs of seedlings to overcome heavy metal toxicity.

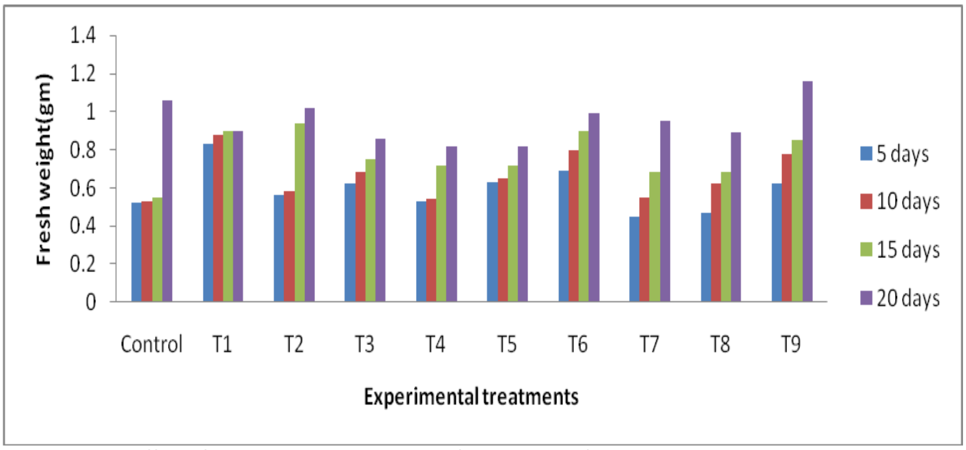

Figure-13: Effect of Barium concentrations on fresh weight of pigeon pea (*Cajanus cajan*) seedlings.

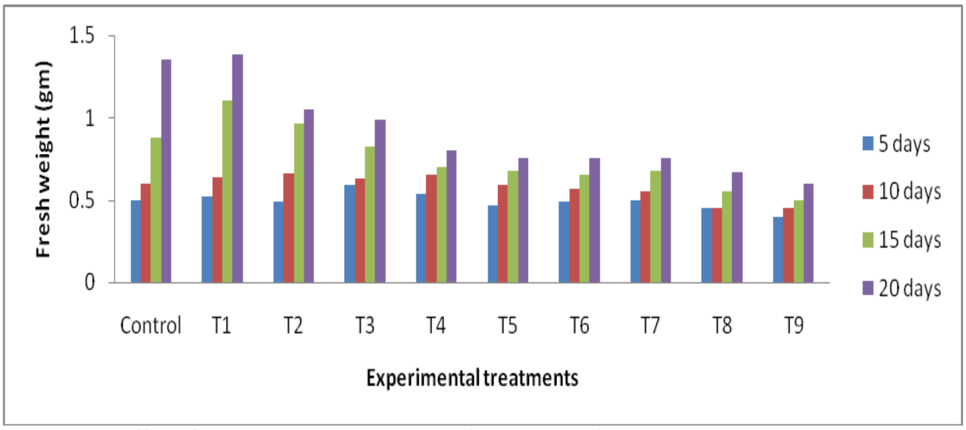

Figure-14: Effect of Lead (Pb) concentrations on fresh weight of pigeon pea (*Cajanus cajan*) seedlings.

The present investigation also concludes that the fresh weight of pigeon pea (*Cajanus cajan*) treated with various concentration of lead significantly greater than the seedlings treated with various concentrations of barium, which indicates tolerance levels of pigeon pea (*Cajanus cajan*) is greater towards lead toxicity.

The effect of varying concentrations of manganese on fresh weight of pigeon pea (*Cajanus cajan*) seedlings represented in figure-15. From the results it is observed that as 'Mn' concentration increases the fresh weight of pigeon pea (*Cajanus cajan*) seedlings decreased in all the treatments. The maximum fresh weight observed with control treatment and followed by T1 to T7 at 20th day of seedling growth. A few of investigations reveled that, higher concentrations of 'Mn' accumulate in plants and decrease the production rate of auxin and cytokines. Hence, there is no significant cell division took place in plant body leading lesser weight [38].

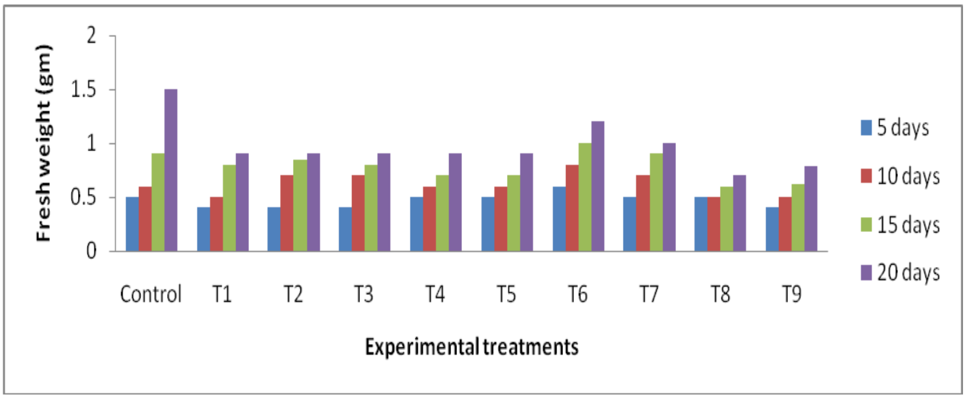

Figure-15: Effect of Manganese (Mn) concentrations on fresh weight of pigeon pea (*Cajanus cajan*) seedlings.

The effect of varying concentrations of copper on fresh weight of pigeon pea (*Cajanus cajan*) seedlings represented in figure-16. From the figure it is observed that there is no significant reduction in fresh weight of seedlings observed up to 10 days of germination. There is a decrease in fresh weight of seedling observed from 15th day. The maximum fresh weight observed with control treatment and followed T1 to T6 respectively. The seedlings of T7, T8 and T9 were dried due to heavy metal toxicity from 11th day onwards. Similar reports described by Arduin 1995 [44] in the study of influence of copper on root growth and morphology of *Pinus pinea* L. & *Pinus pinaster* seedlings. From the above data it can be concluded that 'Cu' showed adverse effect on pigeon pea (*Cajanus cajan*) when compared to barium, manganese and lead.

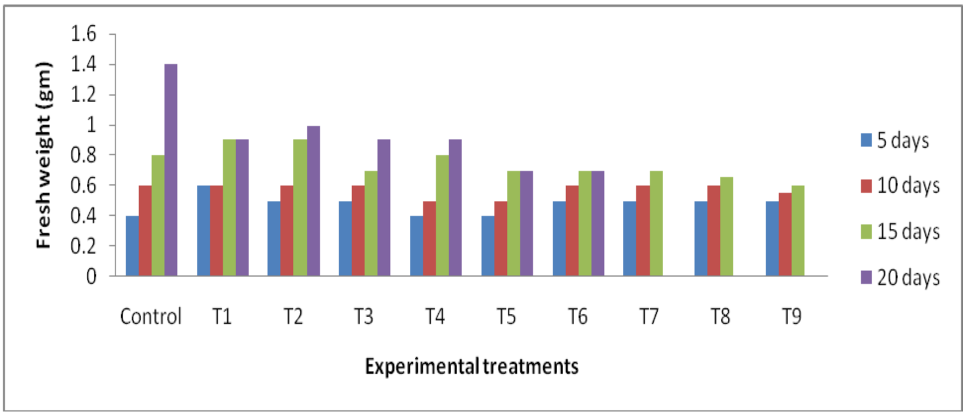

Figure-16: Effect of Copper (Cu) concentrations on fresh weight of pigeon pea (*Cajanus cajan*) seedlings.

Dry weight:

The impact of varying concentrations of barium (Ba), lead (Pb), manganese (Mn) and copper (Cu) on pigeon pea (*Cajanus cajan*) seedlings dry weight were represented in figure-17, 18, 19 and 20 respectively. From the results it is observed that the dry weight of seedlings shown significant increase with control treatment up to 15 days of crop growth period at all concentrations used in current investigation. Results can be concluded that increase in dry weight of seedlings maybe due to accumulation of heavy metals in various parts of seedlings which leads to more dry weight at initial crop growth periods. From the 20 and 25th day of seedling growth, it is observed that dry weights of seedling were decreased with increase of heavy metal concentration used in present investigation. The result also concludes that the seedling started phytoremediation mechanism (phyto stabilization, phyto volatilization) and decreased metal accumulation in plant body resulting decreased dry weight.

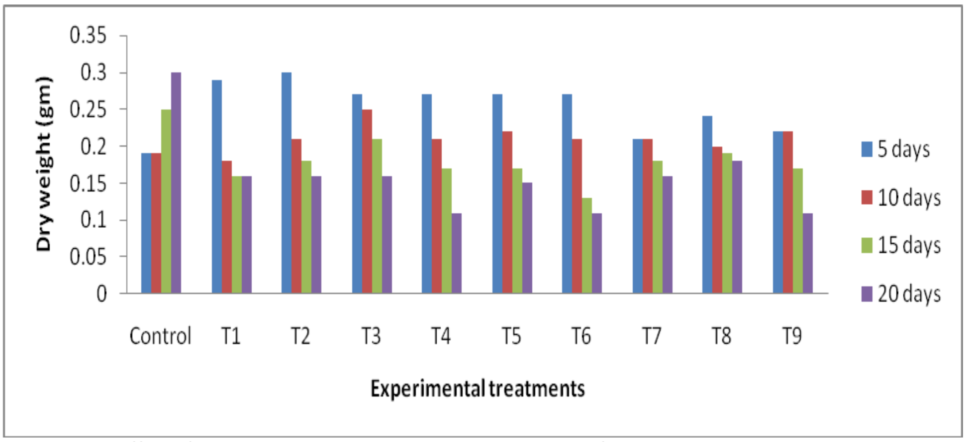

Figure-17: Effect of Barium (Ba) concentrations on dry weight of pigeon pea (*Cajanus cajan*) seedlings.

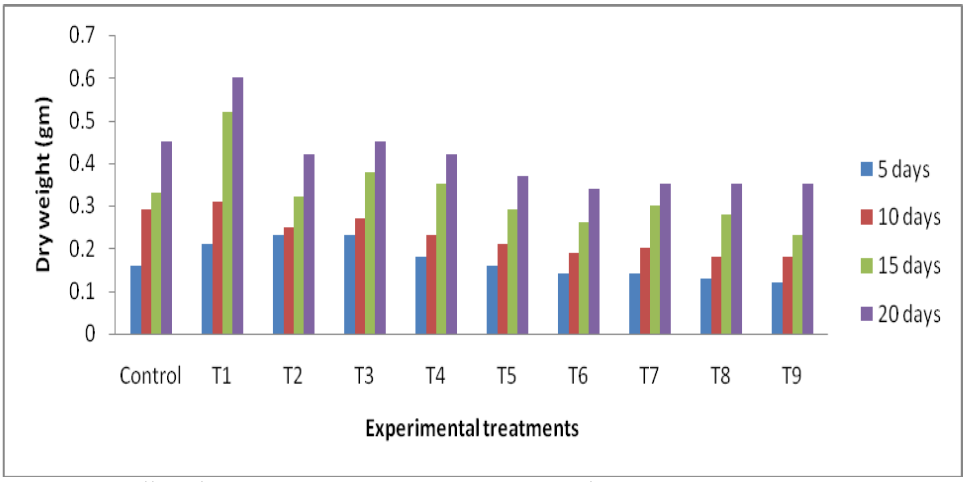

Figure-18: Effect of Lead (Pb) concentrations on dry weight of pigeon pea (*Cajanus cajan*) seedlings.

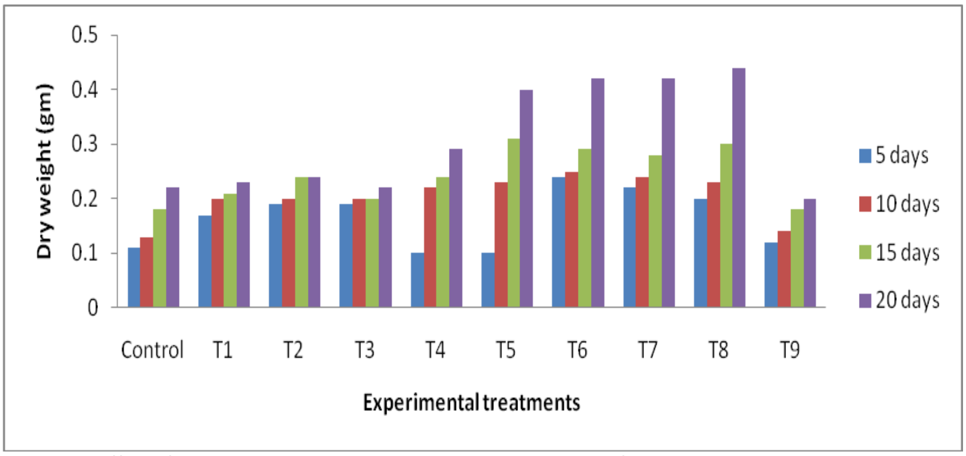

Figure-19: Effect of Manganese (Mn) concentrations on dry weight of pigeon pea (*Cajanus cajan*) seedlings.

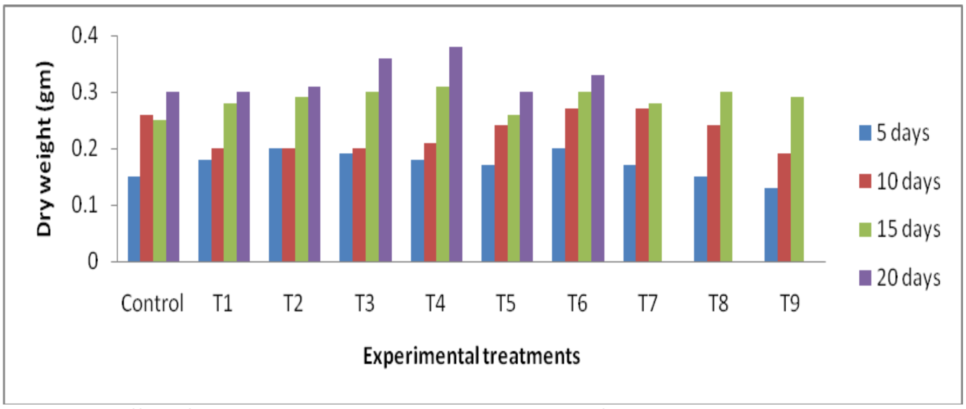

Figure-20: Effect of Copper (Cu) concentrations on dry weight of pigeon pea (*Cajanus cajan*) seedlings.

% Phytotoxicity:

% phytotoxicity of heavy metals (Ba, Pb, Mn & Cu) concentrations on pigeon pea (*Cajanus cajan*) was calculated according to formulae and results represented in figure-21, 22, 23 and 24 respectively. From the results it is observed that barium showed maximum % phtotoxicity at higher concentration (T7, T8 and T9). The toxicity levels are less at lower concentrations (T1, T2 and T3). The phytotoxicity significantly less in treatments T4, T5 and T6. Phytotoxicity of 'Mn' treated seedlings showed higher toxicity at lower concentrations and lower toxicity at higher concentrations confirming that 'Mn' is an essential nutrient which helps in photosynthesis process. Similar trend of % phytotoxicity followed in case of seedlings treated with various concentrations of copper. The seedlings treated with lead (Pb) showed maximum % phytotoxicity at all concentrations done in present investigation on comparison with other heavy metals. The above observed results concludes that lead (Pb) and barium (Ba) showed more phytotoxicity than manganese (Mn) and copper (Cu).

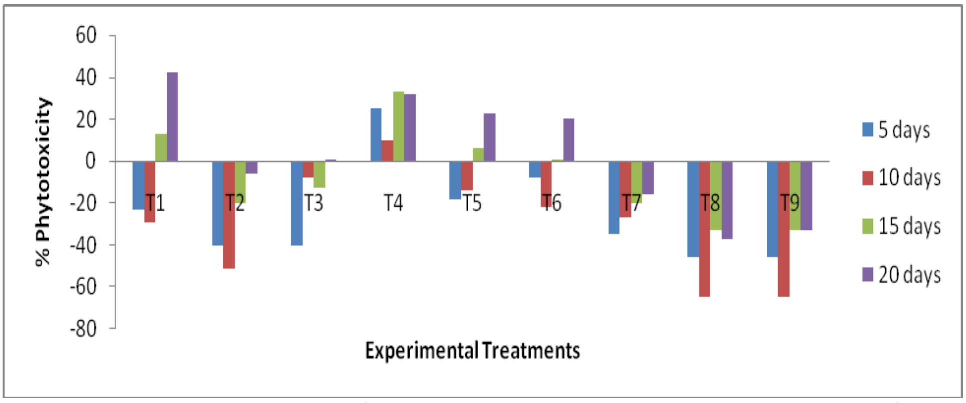

Figure-21: % Phytotoxicity on seedling of pigeon pea (*Cajanus cajan*) with various concentrations of barium

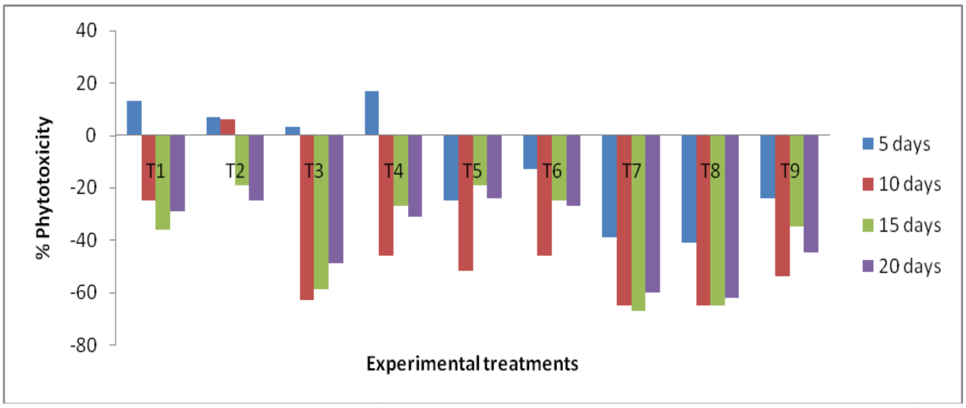

Figure-22: % Phytotoxicity on seedling of pigeon pea (*Cajanus cajan*) with various concentrations of lead

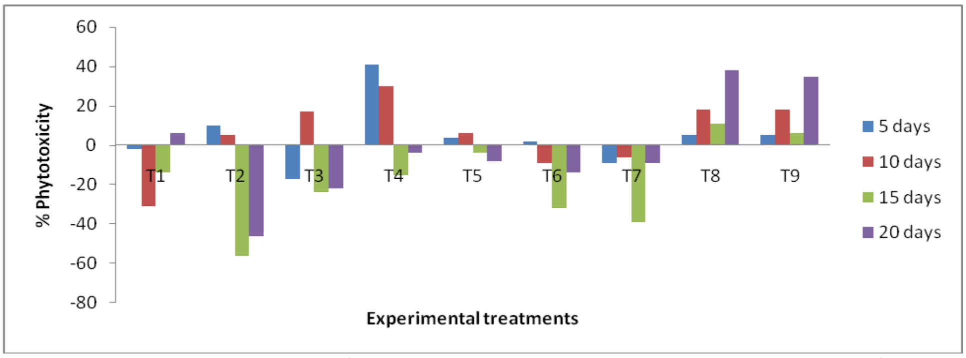

Figure-23: % Phytotoxicity on seedling of pigeon pea (*Cajanus cajan*) with various concentrations of manganese

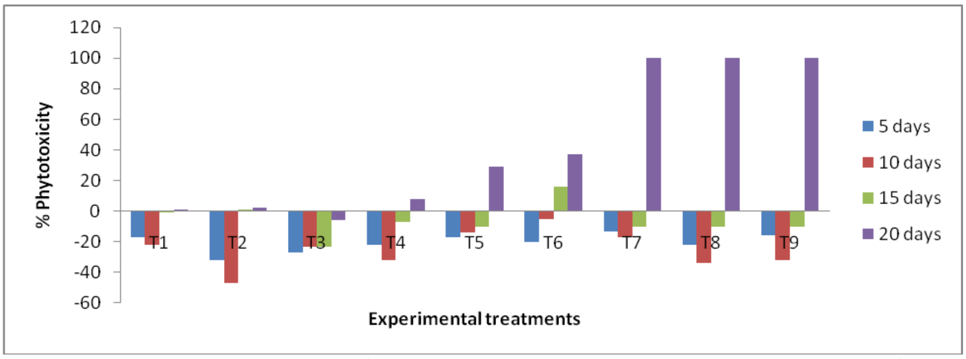

Figure-24: % Phytotoxicity on seedling of pigeon pea (*Cajanus cajan*) with various concentrations of copper

% Inhibition

% inhibition of heavy metals (Ba, Pb, Mn& Cu) concentrations on pigeon pea (*Cajanus cajan*) was calculated according to formula and results were represented in figure-25, 26, 27 and 28 respectively. From the results it is observed that less % inhibition observed with seedlings treated with barium and copper. As, plant developed mechanism to overcome heavy metal toxicity showed germination and growth. The % inhibition is high at lower concentrations and low at higher concentrations were observed with seedlings treated with manganese. The maximum % of inhibition rate was observed with seedlings treated with lead.

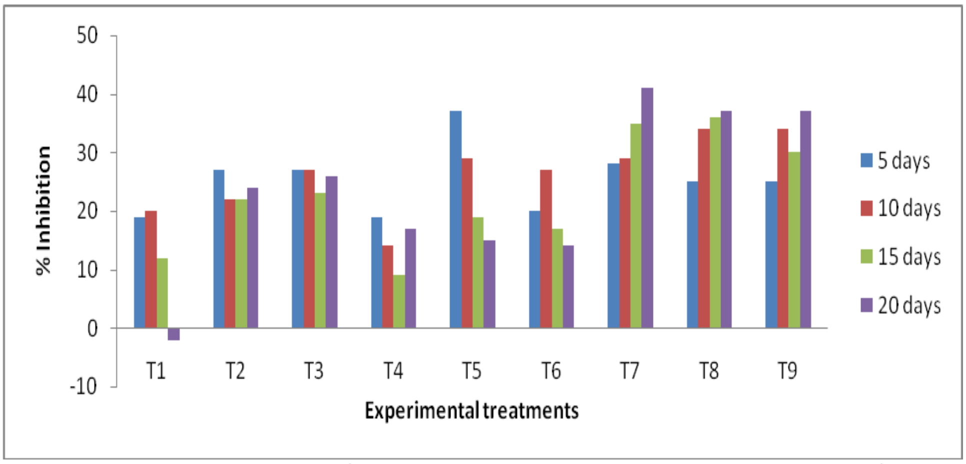

Figure-25: % inhibition on seedling of pigeon pea (*Cajanus cajan*) with various concentrations of barium

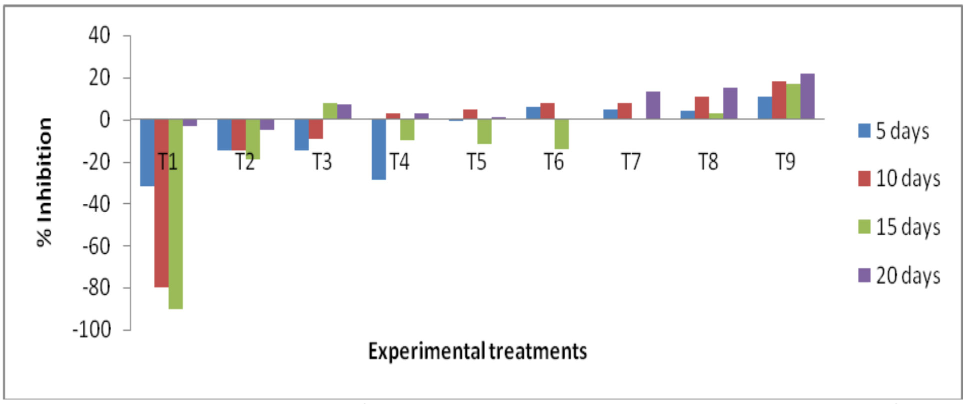

Figure-26: % inhibition on seedling of pigeon pea (*Cajanus cajan*) with various concentrations of lead

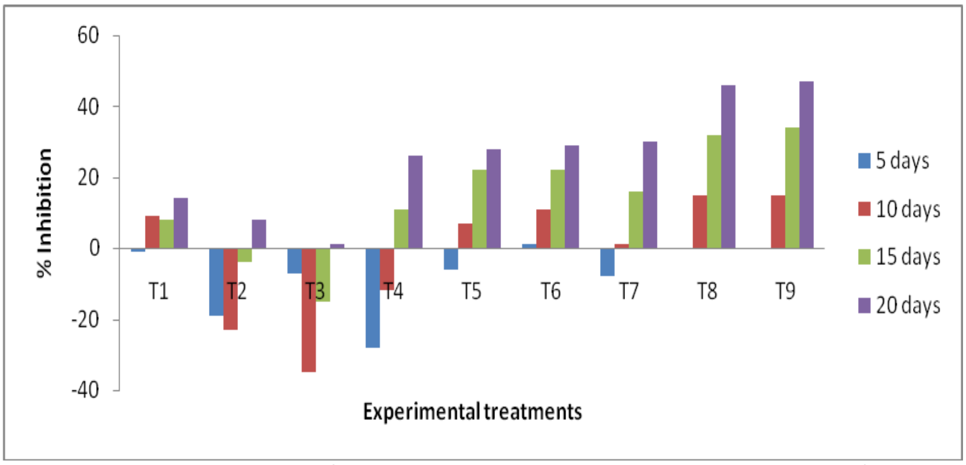

Figure-27: % inhibition on seedling of pigeon pea (*Cajanus cajan*) with various concentrations of manganese

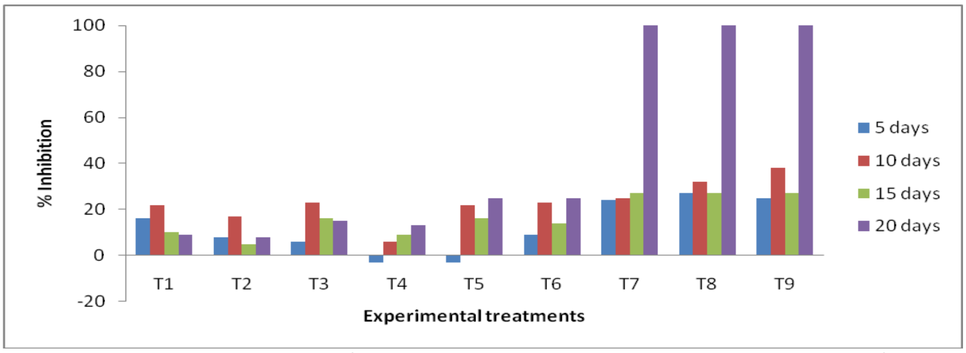

Figure-28: % inhibition on seedling of pigeon pea (*Cajanus cajan*) with various concentrations of copper

Seedling Vigor Index

Seedling vigor index of pigeon pea (*Cajanus cajan*) with various concentrations of heavy metals (Ba, Pb, Mn and Cu) were shown in figure-29, 30, 31 and 32 respectively. From the figures it is observed that seedling vigor index is decreased with increase in metal ion concentration. The same trend is followed in case, with all metal concentrations used in present investigation.

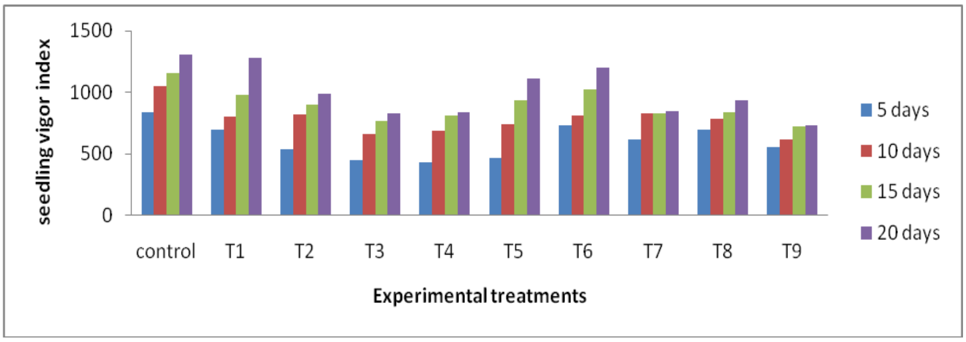

Figure-29: seedling vigor index with respect to barium treatments

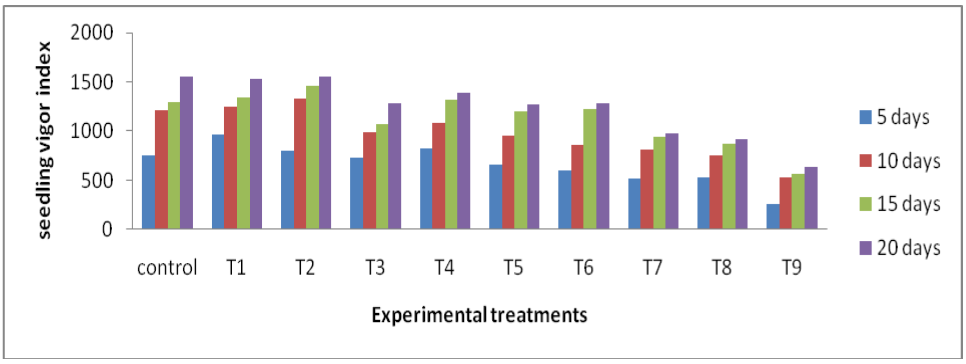

Figure-30: seedling vigor index with respect to lead treatments

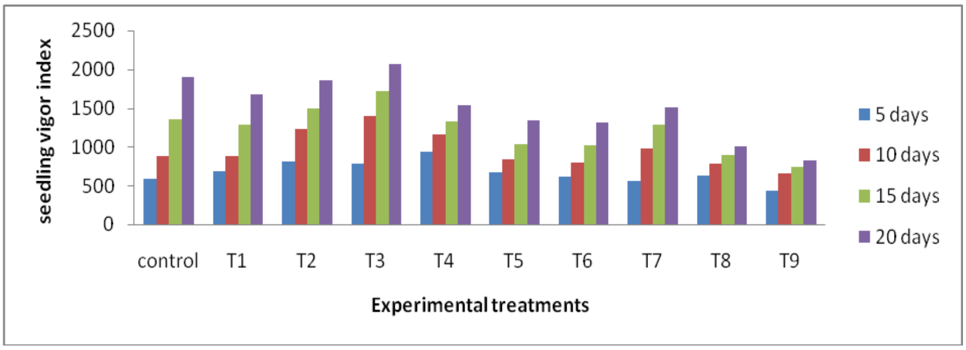

Figure-31: seedling vigor index with respect to manganese treatment

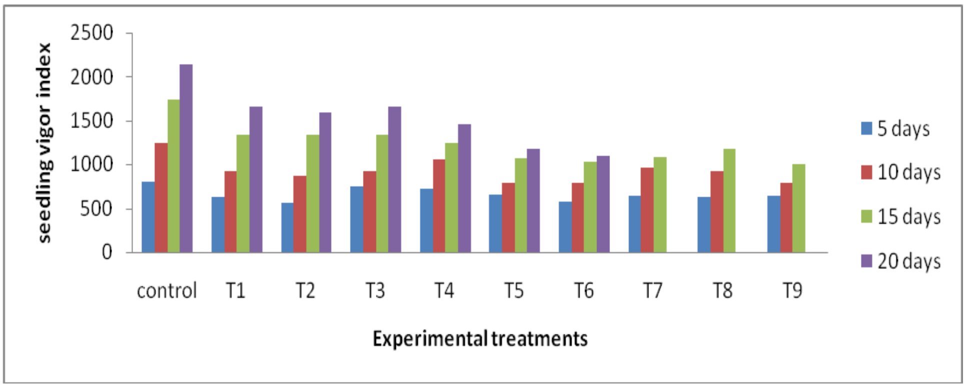

Figure-32: seedling vigor index with respect to copper treatment

Tolerance indices

The tolerance indices of pigeon pea (*Cajanus cajan*) with respect to various concentrations of heavy metals used in present study was calculated and represented in figure-33, 34, 35 and 36. From the figures it is observed that seedlings were more tolerant to heavy metals used in present investigation. The order of tolerance can be represented as Ba>Mn>Pb>Cu.

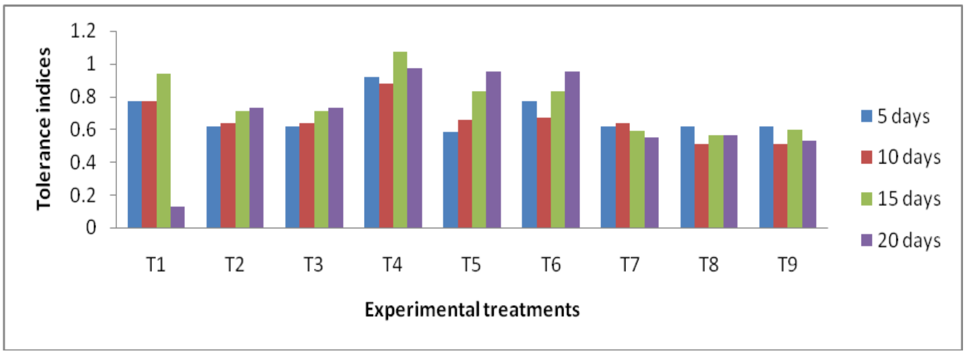

Figure-33: Tolerance indices with respect to barium concentrations

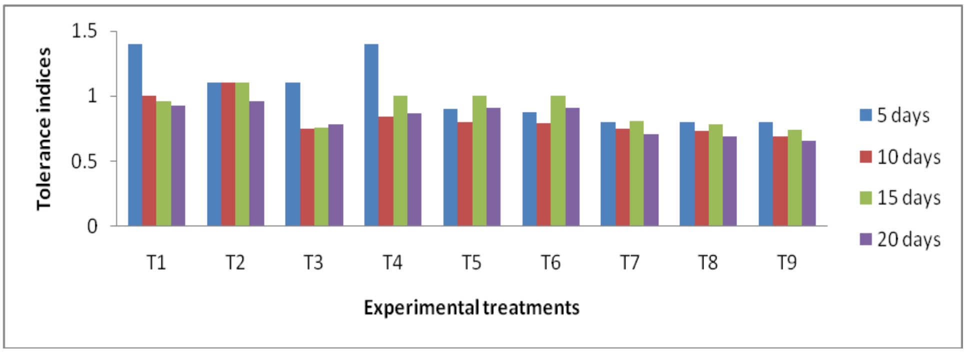

Figure-34: Tolerance indices with respect to lead concentrations

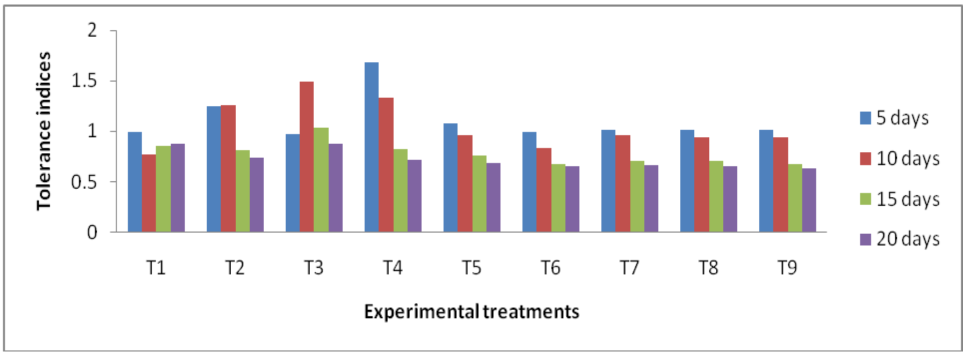

Figure-35: Tolerance indices with respect to manganese concentrations

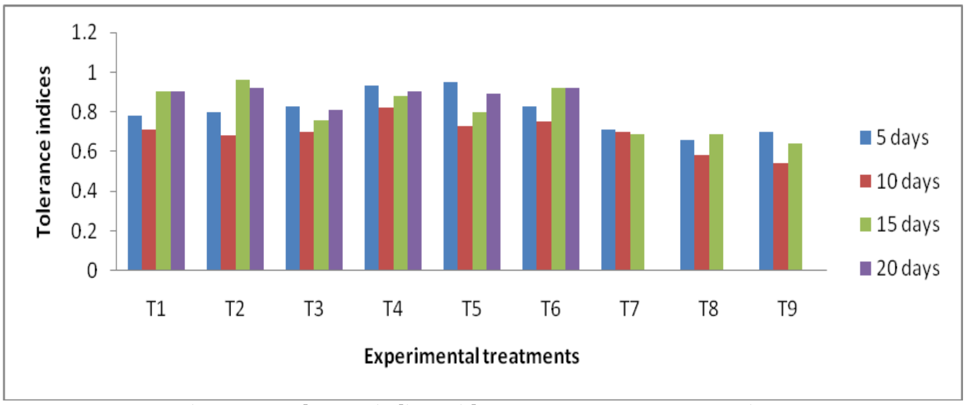

Figure-36: Tolerance indices with respect to copper concentrations

Seedling Growth Analysis

The seedling growth analysis parameters such as Relative Growth Rate (RGR- Table-3), Net Assimilation Rate (NAR), Leaf Area Ratio (LAR), Leaf Weight Ratio (LWR), Specific Leaf Area (SLA), Specific Leaf Weight (SLW), Leaf Area Duration (LAD) of pigeon pea under various concentrations of heavy metals used in present study were estimated and the metal concentrations showed decrease in growth with increase of concentration and crop growth periods, compared to control in all experimental conditions. The results were depicted in Table-4, 5, 6 & 7 respectively.

Biochemical Attributes

To find out the biochemical response of the pigeon pea (*Cajanus cajan*) against to different concentrations of heavy metals, the biomass allowed to check total carbohydrates (Anthrone method), total proteins (Biuret method) and peroxidase enzyme activity (O-dianisidine method) [4]. The results were illustrated in table-1. From the results it is observed that total carbohydrates of control treatment was 39.29 mg/g (mean) and the total sugar concentration was decreased with increase in heavy metal concentrations used in present investigation. The decrease ratio of carbohydrate concentration was very less in the seedlings treated with heavy metal Mn compare to other heavy metals, which indicating impact of Mn on carbohydrate metabolism is very less than other heavy metals. The highest reduction in total sugars concentration was observed with seedlings treated with various concentrations of copper, which concluding adverse effect of copper concentration on carbohydrate metabolism of pigeon pea (*Cajanus cajan*). The total protein content of pigeon pea (*Cajanus cajan*) was determined by biuret method and results shown in table-1. From the result it is observed that the total protein content is more in control treatment and concentration of protein is decreased with increase in metal concentration in all experimental cases except the seedlings treated with various concentrations of manganese (Mn). The protein concentration increased at lower concentrations of Mn and low level of protein were observed between T7 to T9 treatments with 6.89, 6.17 and 5.99 mg/g biomass of pigeon pea (*Cajanus cajan*). The sever affect on total carbohydrates and proteins were found at higher heavy metal concentration T7 to T9 treatments compare to control treatment. The peroxidase enzyme activity increased with increase in exposure concentration and crop growth duration. The increase in enzyme activity may be due to metal toxicity. The redox metal copper (Cu) can directly generate oxidative injury via undergoing Haber-Weiss and Fenton reactions, which leads to the aforementioned production of ROS or oxygen free radicals species in plants, resulting in cell homeostasis disruption, DNA strand breakage, defragmentation of proteins, or cell membrane and damage to photosynthetic pigments, which may trigger cell death [53,54]. The low active redox metal like lead (Pb) and barium, indirectly inflict oxidative stress via multiple mechanisms including glutathione depletion, binding to sulfhydryl groups of proteins [55], inhibiting antioxidative enzymes, or inducing ROS-producing enzymes like NADPH oxidases [56]. The basic criterion for the selection of these heavy metals for present study was based on their mode of action in biological system of plants, whether they are redox active or inactive metals in plant cells and it was proved by results.

Effect of heavy metal concentrations on chlorophyll contents.

The effect of various heavy metal concentrations on photosynthetic pigments of pigeon pea (*Cajanus cajan*) leaves were determined on 20th day (Table-1). The photosynthetic pigment chlorophyll-a, chlorophyll-b and total chlorophyll of pigeon pea (*Cajanus cajan*) were decreased with increase in heavy metal concentrations except seedlings treated with manganese. The mean amount of plant pigments of pigeon pea (*Cajanus cajan*) leaves treated with barium, lead, manganese and copper depicted in table- 3. From the results it is observed that chlorophyll-a in pigeon pea (*Cajanus cajan*) decrease from 0.76 to 0.592 with various concentration of barium, 0.873 to 0.392 with various concentrations of lead and 0.801 to 0.112 with respect to the seedlings treated with various concentrations of copper. Similarly the chlorophyll-b in pigeon pea (*Cajanus cajan*) leaves were decreased significantly from 0.207 to 0.169 with various concentrations of barium, 0.195 to 0.110 with various concentrations of lead treatments and 0.196 to 0.110 with respect to the seedlings treated with various concentrations of copper. This decrease in pigment content indicates that the chlorophyll synthesis system and chlorophyllase activity affected by the exposure to heavy metals (Ba, Pb and Cu) concentrations for longer period. The depletion in chlorophyll content under heavy metal stress may reduce the enzyme functions involve in the chlorophyll biosynthesis pathway. There is no adverse affect found in chlorophyll pigment reduction in the leaves of in pigeon pea (*Cajanus cajan*) hence, Mn is an essential micronutrient that plays a pivotal part in many metabolic and growth processes in plants including photosynthesis, respiration, and the biosynthesis of enzymes such as malic enzyme, isocitrate dehydrogenase, and nitrate reductase [57]. It is also a cofactor required for multiple plant enzymes, for example, Mn-dependent superoxide dismutase (MnSOD) [58]. Furthermore, manganese is involved in carbohydrate and nitrogen metabolism, synthesis of fatty acid, acyl lipids, and carotenoid as well as hormonal activation [58-60]. The contribution of manganese to the functionality of photosystem II (PSII) especially during the course of splitting of water molecules into oxygen [61] and its role in the protection of PSII from photo damage are of significant importance [62]. Mn^{2+} is the most stable and soluble form of manganese in the soil environment [63]. However, lower soil pH, less soil organic matter, and decreased redox potential increase the availability or toxicity of Mn^{2+} to plants [63, 64]. Contrary to some elements such as aluminum or copper, there is a tendency for manganese to easily translocate form roots to the upper parts of plants. This mobility is the reason why symptoms of Mn toxicity are first visible in aerial organs of plants [65]. The appearance of visual features in plants affected by Mn toxicity varies with the type of plant species, plant age, temperature, and light level [66-68]. The symptoms may include crinkled leaves [69], darkening of leaf veins on older foliage [70], chlorosis and brown spots on aged leaves [71], and black specks on the stems [72]. Mn toxicity has been associated with a decreased CO_2 assimilation but unaffected chlorophyll (Chl) level in *Citrus grandis* seedlings [73] and depleted Chlorophyll content in pea (*Pisum sativum* L.) and soybean (Glycine max L.) [74-76], indicating diversity among plant species in response to Mn excess [77].

Table -1: Effect of heavy metals (Ba, Pb, Mn and Cu) on biochemical properties (carbohydrates, proteins and POD enzyme activity of pigeon pea (*Cajanus cajan*) seedling.

T.No	Total Sugars (mg/g)				Total Proteins (mg/g)				POD enzyme activity (mg/g)			
	Ba	Pb	Mn	Cu	Ba	Pb	Mn	Cu	Ba	Pb	Mn	Cu
Control	37.96	39.09	42.84	37.28	7.99	7.98	8.90	7.90	8.59	8.57	9.08	9.12
T1	12.81	34.01	42.01	10.87	6.42	3.21	9.92	2.15	12.57	8.72	9.09	18.75
T2	15.87	37.09	42.08	9.98	5.12	2.76	9.54	3.21	15.54	9.02	9.08	20.45
T3	18.12	36.55	40.91	8.91	4.31	2.87	9.24	4.02	18.34	9.22	9.19	24.34
T4	17.02	31.32	40.09	7.00	3.98	2.45	8.91	3.76	20.54	9.43	9.25	32.13
T5	11.01	25.61	38.92	5.24	3.58	2.21	8.42	3.18	26.13	9.72	9.53	40.21
T6	11.57	20.12	38.00	3.06	3.34	1.76	7.67	2.56	29.45	9.90	9.55	45.62
T7	9.71	11.01	37.91	2.81	3.00	1.25	6.89	1.78	32.75	10.12	9.64	45.68
T8	8.92	10.05	37.52	Dried	3.00	0.98	6.17	Dried	36.62	10.52	9.65	Dried
T9	6.67	8.12	35.48	Dried	3.00	0.65	5.99	Dried	37.64	10.28	9.64	Dried

Table -2: Effect of heavy metals (Ba, Pb, Mn and Cu) on chlorophyll content (a, b, total) of pigeon pea (*Cajanus cajan*)

T.No	Chlorophyll-a				Chlorophyll-b				Total Chlorophyll (a+b)			
	Ba	Pb	Mn	Cu	Ba	Pb	Mn	Cu	Ba	Pb	Mn	Cu
Control	0.980	0.977	0.990	0.985	0.351	0.358	0.432	0.338	1.331	1.335	1.422	1.323
T1	0.768	0.873	0.998	0.801	0.207	0.195	0.256	0.196	0.968	1.068	1.254	0.997
T2	0.805	0.805	1.06	0.794	0.243	0.188	0.337	0.184	1.048	0.984	1.397	0.978
T3	0.854	0.798	1.42	0.364	0.285	0.170	0.404	0.176	1.139	0.968	1.824	0.540
T4	0.899	0.625	1.49	0.361	0.287	0.168	0.438	0.167	1.186	0.793	1.928	0.528
T5	0.739	0.558	1.56	0.288	0.207	0.155	0.485	0.158	0.94	0.713	2.045	0.446
T6	0.612	0.528	1.78	0.259	0.176	0.138	0.499	0.112	0.78	0.666	2.279	0.371
T7	0.600	0.449	1.92	0.112	0.170	0.120	0.518	0.110	0.77	0.569	2.438	0.222
T8	0.600	0.411	1.98	Dried	0.170	0.112	0.526	Dried	0.77	0.523	2.506	Dried
T9	0.592	0.392	1.99	Dried	0.169	0.110	0.588	Dried	0.76	0.502	2.578	Dried

Table-3: Relative Growth Rate (RGR) of pigeon pea (*Cajanus cajan*) under various concentrations of heavy metals (Ba, Pb, Mn and Cu)

T.No	Relative Growth Rate (RGR)											
	Ba			Pb			Mn			Cu		
	10th day	15th day	20th day	10th day	15th day	20th day	10th day	15th day	20th day	10th day	15th day	20th day
Control	0.01	0.15	0.16	0.10	-3.23	-3.23	-1.35	-2.39	-2.39	0	0	0
T1	-0.09	0.02	-0.06	0.09	-2.75	-2.75	-1.03	-1.42	-1.42	0	0	0
T2	0.01	0.07	0.08	0.02	0.05	0.05	-1.23	-1.22	-1.22	0	0	0
T3	-0.05	0.01	-0.04	0.03	0.08	0.08	-1.06	-1.40	-1.40	0	0	0
T4	-0.03	0.003	-0.01	0.09	0.06	0.06	-1.63	-1.22	-1.22	0	0	0
T5	0.21	0.02	-0.04	0.06	0.05	0.05	-1.41	-1.04	-1.04	0	0	0
T6	-0.13	0.05	0.02	-0.02	0.07	0.07	-1.08	-3.38	-3.38	0	0	0
T7	0.04	-0.01	0.02	0.06	0.09	0.09	-0.93	-1.07	-1.07	0	0	0
T8	0.04	0.003	0.03	0	0.07	0.07	-2.78	-1.37	-1.37	0	0	0
T9	0.003	-0.03	-0.03	0.09	0.1	0.1	-1.08	-1.48	-1.48	0	0	0

Table -4: Growth analysis & physiological response of Pigeon pea/ red gram (*Cajanus cajan*) under different concentrations of Barium (Ba).

Days	Control						Treatment 1					
	LAR	LWR	SLA	SLW	LAD	NAR	LAR	LWR	SLA	SLW	LAD	NAR
10	278	0.33	1039	0.0024	33	0	168	0.33	576	0.0046	28	0
15	194	0.33	631	0.0017	37	0	290	0.33	772	0.0012	40	0
20	134	0.33	102	0.02	22	0	190	0.33	167	0.008	15	0
	Treatment -2						Treatment-3					
	LAR	LWR	SLA	SLW	LAD	NAR	LAR	LWR	SLA	SLW	LAD	NAR
10	204	0.33	441	0.0043	22	0	120	0.33	406	0.0046	22	0
15	155	0.33	509	0.0019	30	0	180	0.33	489	0.0021	30	0
20	152	0.33	65	0.03	16	0	150	0.33	230	0.004	15	0
	Treatment -4						Treatment- 5					
	LAR	LWR	SLA	SLW	LAD	NAR	LAR	LWR	SLA	SLW	LAD	NAR
10	94	0.33	293	0.005	14	0	145	0.33	446	0.0066	21	0
15	154	0.33	439	0.008	25	0	233	0.33	623	0.0004	31	0
20	195	0.33	115	0.01	15	0	237	0.33	297	0.01	21	0
	Treatment -6						Treatment-7					
	LAR	LWR	SLA	SLW	LAD	NAR	LAR	LWR	SLA	SLW	LAD	NAR
10	88	0.33	221	0.0146	20	0	154	0.33	205	0.009	21	0
15	171	0.33	527	0.0026	30	0	141	0.33	431	0.0022	30	0
20	149	0.33	122	0.04	15	0	227	0.33	158	0.01	16	0
	Treatment -8						Treatment-9					
	LAR	LWR	SLA	SLW	LAD	NAR	LAR	LWR	SLA	SLW	LAD	NAR
10	132	0.33	481	0.004	18	0	112	0.33	351	0.0496	14	0
15	160	0.33	467	0.0021	31	0	160	0.33	451	0.0025	30	0
20	178	0.33	217	0.004	16	0	184	0.33	294	0.003	15	0

Table -5: Growth analysis & physiological response of Pigeon pea/ red gram (*Cajanus cajan*) under different concentrations of Lead (Pb).

Days	Control						Treatment 1					
	LAR	LWR	SLA	SLW	LAD	NAR	LAR	LWR	SLA	SLW	LAD	NAR
10	217	0.30	738	4.12	23	0.1	189	0.30	620	5.08	19	0
15	158	0.31	478	2.83	31	0	134	0.32	460	3.19	36	-0.01
20	123	0.32	492	3.09	29	0	122	0.32	385	1.38	48	-0.01
	Treatment -2						Treatment-3					
	LAR	LWR	SLA	SLW	LAD	NAR	LAR	LWR	SLA	SLW	LAD	NAR
10	206	0.30	713	4.36	24	0	228	0.31	696	5.93	24	-0.05
15	213	0.31	682	4.56	34	-0.01	195	0.32	587	2.63	28	-0.01
20	161	0.32	484	2.76	32	-0.01	139	0.32	451	3.04	29	-0.01
	Treatment -4						Treatment- 5					
	LAR	LWR	SLA	SLW	LAD	NAR	LAR	LWR	SLA	SLW	LAD	NAR
10	236	0.30	832	4.22	24	0	226	0.31	734	4.35	29	0
15	158	0.31	499	2.96	25	0	167	0.32	518	2.52	28	0
20	123	0.32	374	3.06	29	0	140	0.33	420	3.25	32	0
	Treatment -6						Treatment-7					
	LAR	LWR	SLA	SLW	LAD	NAR	LAR	LWR	SLA	SLW	LAD	NAR
10	166	0.31	561	6.18	29	0	242	0.33	727	4.18	24	0
15	180	0.32	542	2.59	25	0	191	0.32	600	1.69	28	0
20	134	0.32	405	2.91	29	0	112	0.32	342	4.02	28	0
	Treatment -8						Treatment-9					
	LAR	LWR	SLA	SLW	LAD	NAR	LAR	LWR	SLA	SLW	LAD	NAR
10	241	0.32	730	4.62	18	0.	288	0.29	1093	3.47	42	-0.07
15	170	0.30	548	1.97	25	0.04	185	0.31	585	1.76	44	-0.04
20	93	0.30	293	6.57	28	0.06	109	0.33	328	3.78	28	-0.03

Table -6: Growth analysis & physiological response of Pigeon pea/ red gram (*Cajanus cajan*) under different concentrations of Manganese (Mn).

Days	Control						Treatment 1					
	LAR	LWR	SLA	SLW	LAD	NAR	LAR	LWR	SLA	SLW	LAD	NAR
10	290	0.3	850	0	34	-14	120	0.3	300	0	15	-13
15	155	0.3	367	0	22	-22	188	0.3	600	0	36	-18
20	169	0.3	393	0	24	-36	256	0.3	950	0	36	-30
	Treatment -2						Treatment-3					
	LAR	LWR	SLA	SLW	LAD	NAR	LAR	LWR	SLA	SLW	LAD	NAR
10	172	0.3	417	0	25	-20	142	0.3	300	0	18	-17
15	183	0.3	533	0	32	-29	156	0.3	500	0	30	-29
20	148	0.3	447	0	25	-38	225	0.3	720	0	39	-52
	Treatment -4						Treatment- 5					
	LAR	LWR	SLA	SLW	LAD	NAR	LAR	LWR	SLA	SLW	LAD	NAR
10	132	0.3	313	0	25	-24	82	0.3	188	0	15	-14
15	158	0.3	767	0	23	-24	119	0.3	700	0	21	-20
20	174	0.3	2864	0	31	-28	140	0.3	462	0	31	-32
	Treatment -6						Treatment-7					
	LAR	LWR	SLA	SLW	LAD	NAR	LAR	LWR	SLA	SLW	LAD	NAR
10	187	0.3	363	0	29	-20	206	0.3	343	0	24	-21
15	140	0.3	438	0	35	-35	113	0.3	314	0	22	-22
20	164	0.3	568	0	37	-50	135	0.3	358	0	28	-28
	Treatment -8						Treatment-9					
	LAR	LWR	SLA	SLW	LAD	NAR	LAR	LWR	SLA	SLW	LAD	NAR
10	262	0.3	660	0	33	-28	322	0.3	73	0	29	-28
15	130	0.3	467	0	28	-27	152	0.3	517	0	31	-30
20	308	0.3	906	0	43	-28	190	0.3	593	0	32	-31

Table -7: Growth analysis & physiological response of Pigeon pea/ red gram (*Cajanus cajan*) under different concentrations of Copper (Cu).

Days	Control						Treatment 1					
	LAR	LWR	SLA	SLW	LAD	NAR	LAR	LWR	SLA	SLW	LAD	NAR
10	196	0.3	599	0	19	0	153	0.3	528	0	17	0
15	227	0.3	335	0	36	0	241	0.3	739	0	33	0
20	189	0.3	621	0	29	0	235	0.3	731	0	38	0
	Treatment -2						Treatment-3					
	LAR	LWR	SLA	SLW	LAD	NAR	LAR	LWR	SLA	SLW	LAD	NAR
10	228	0.3	801	0	23	0	262	0.3	591	0	24	0
15	231	0.3	727	0	35	0	183	0.3	694	0	35	0
20	187	0.3	615	0	32	0	234	0.3	799	0	35	0
	Treatment -4						Treatment- 5					

Table 7 to be continued...

	LAR	LWR	SLA	SLW	LAD	NAR	LAR	LWR	SLA	SLW	LAD	NAR
10	217	0.3	531	0	19	0	262	0.3	979	0	23	0
15	214	0.3	204	0	36	0	183	0.3	564	0	26	0
20	181	0.3	565	0	40	0	234	0.3	1980	0	29	0
	Treatment -6						Treatment-7					
	LAR	LWR	SLA	SLW	LAD	NAR	LAR	LWR	SLA	SLW	LAD	NAR
10	169	0.3	558	0	18	0	369	0.3	904	0	23	0
15	231	0.3	697	0	35	0	240	0.3	823	0	31	0
20	207	0.3	634	0	41	0	170	0.3	515	0	31	0
	Treatment -8						Treatment-9					
	LAR	LWR	SLA	SLW	LAD	NAR	LAR	LWR	SLA	SLW	LAD	NAR
10	193	0.3	668	0	22	0	325	0.3	3289	0	23	0
15	102	0.3	310	0	17	0	319	0.3	1042	0	31	0
20	162	0.3	513	0	29	0	327	0.3	1078	0	38	0

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