Effect of Heavy Metals on Physiology and Biochemical Profiling in *Cyamopsis tetragonoloba L*

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Abstract:

In the present investigation effect of abiotic stress in the form of heavy metals was studied for their effect on biochemical parameters including seedling growth and fresh weight of *Cyamopsis tetragonoloba L*. For this seeds treated with different concentrations (10, 50, 100, 200, 500 and 1000 ppm) of Cu, Cd, Pb, Ni and Zn along with control. Maximum reduction in seed germination percentage was observed at 1000 ppm concentration. It decreased up to 40% in Cd, 60% in Pb 83% in Ni and Zn and 93% in Cu, whereas it was found to be 100% in the control. The shoot length at 500 ppm concentration was drastically reduced to less than one third of control in case of cadmium. At 500 ppm concentration of lead it was reduced to less than half of control. It was observed that heavy metal significantly reduced the fresh weight of seedling of *Cyamopsis tetragonoloba*. The percentage decreased in the fresh weight of seedling due to heavy metal application as compared to control. With increases in concentrations of heavy metals there was a gradual decrease in total chlorophyll content in *Cyamopsis tetragonoloba* except in respect of Zn and Cu up to 10 and 50 ppm concentration, respectively where it was superior to control. It was observed that Zn and Cu were less inhibitory to the amount of total carotenoid content in comparison to other heavy metals. In the control of carotenoid content was 0.37 mg/g fresh weight which decreased to 0.1 mg/g in Cd 0.20 mg/g in Pb, 0.23 mg/g in Ni 0.26 mg/g in Zn and Cu of fresh weight at 1000 ppm concentration. All biochemical parameters reduced at 1000ppm increasing from 10 ppm.

Keywords: *Cyamopsis tetragonoloba L*; abiotic stress; biochemical parameters; seedling growth; fresh weight.

Introduction:

Heavy metals such as copper, zinc, cadmium, nickel and lead are normal constituents of marine and estuarine environments. When additional quantities are introduced from industrial wastes or sewage they enter the biogeochemical cycle and, as a result of being potentially toxic, may interfere with the ecology of a particular environment. According to views of Chauhan *et al* (2000) it is necessary to prevent the environment though industrialization and development in agriculture are necessary to meet the basic requirement of people. In India, due to lack of sewage treatment plants, generally untreated sewage effluents are released either on
agricultural land for irrigation (Brar et al., 1997) or disposed of in nearby water bodies. In general, sewage effluents from industries (Volesky et al., 1994) and municipal origin contain appreciable amounts of plants nutrients (Sreeramu et al., 1994) and variable amount of metallic cations (Kansal et al., 1993) like Zn, Cu, Fe, Mn, Pb, Ni, Cd. Within the group of heavy metals, Cd is particularly toxic to the majority of plants and animals even at low concentrations (Shukla et al., 2007).

Studies revealed that heavy metals in their toxic concentration inhibit various stages of plant development specially the seed and seedling stages, physiological processes and biochemical processes. There has been rapid decline in crop productivity due to various abiotic stress present in water and soil. During the last few decades there is rapid increase in heavy metal contamination due to increased anthropogenic activities, rapid industrialization, modern agricultural practices like use of pesticides (Kavamura and Esposito, 2010) and release of sewage sludge which causes toxicity to the living organisms and thus causing disturbance its stability of nature.

There is an agreement among scientists about the effect of cadmium on chloroplast ultra-structure. In some earlier reports of Stoyanova and Chakalova (1990) it was established that cadmium, applied in toxic concentrations, disturbs the chloroplast envelope and the integrity of the membrane system and leads to increased plastoglobule number, changing the lipid composition and the ratios of the main structural components of thylakoid membranes.

Copper (Cu) is an essential micronutrient for higher plants involved in several metabolic processes including photosynthetic electron transfer (Haehnel, 1984). It is an essential cofactor involved in complex network machinery oxidative phosphorylation and mobilization. In excess, however, it inhibits growth and metabolism (Lidon and Henriques, 1991) due to many physiological constraints and by inducing the synthesis of a large number of enzymes like catalases and peroxidises.

Despite a long history of its beneficial use by humankind, lead has no known biological function in living organisms (Maestri et al. 2010) and is now recognized as a chemical of great concern in the new European REACH regulations (EC 1907/2006; Registration, Evaluation, Authorization, and Restriction of Chemicals). Moreover, lead was reported as being the second most hazardous substance, after arsenic, based on the frequency of occurrence, toxicity, and the potential for human exposure. The transfer of lead from polluted soils to plants was therefore widely studied, especially in the context of food quality, use in phytoremediation, or in biotesting (Uzu et al. 2009)

Ni as an essential micronutrient, which is required by urease for hydrolysing urea. Observed an improvement in plant growth and N uptake of Wheat (Triticum aestivum L.) from urea by Ni application on a calcareous soil (Singh et al., 1990). Several investigators have also shown beneficial effects of Ni on ureas activity and improving N use efficiency by plants in hydroponic studies. However, deficiency of Ni in soil has rarely been reported showed that Ni deficiency affects plant growth, plant senescence, nitrogen (N) metabolism and Fe uptake and may play role in disease resistance. Ni is essential for plants, but the concentration in the majority of plant species is very low (0.05-10 mg/kg dry weight). Further. With increasing Ni pollution, excess Ni rather than a deficiency, is more commonly found in plants. Toxic effects of high concentrations of Ni in plants have been frequently reported.

Zn2+ is an essential micronutrient for plants, and it is therefore essential for plant growth, development, and many metabolic processes being one of the major cofactors, together with iron and manganese, in numerous enzymes (Andreni et al., 2008). More than 1200 proteins are predicted to contain, bind, or transport Zn2+ (Hansch and Mendel, 2009).

The vernacular name of Cyamopsis tetragonoloba is guar which is derived from the Sanskrit word: GAU AAHAR which means cow fodder. In Sanskrit it is called as Gopali, Bengali – jharsim, Gujrati – Govar.
Telugu – Goorchikuarkai, Marathi – Gavar, Oriya – Guanrachuuum and Tamil as Kottavarai. Now it seems destined to assume a larger role among the domesticated plants that supply the gum, feed, food and other needs of man. Clusterbean commonly known as guar is an annual Kharif legume grown for green fodder, vegetable, green manuring and seed gum pureposes. Guar has diversified uses in explosive, textile, paper, oil well drilling mining, food and cosmetic industries etc. It has become an important industrial crop with great potential for foreign exchange. Traditionally, its green immature pods are used as a vegetable for human consumption, plant as fodder for livestock and seed for the extraction of gum which serves as a useful source of hydrocolloids for various uses and guar meal as feed for non-ruminants. Being a leguminous crop it is also used for soil improvement. Empty guar pods are better in forage quality than guar fodder. Guar seed consists of seed coat (14-17%), endosperm (35-42%) and embryo (43-47%). Guar seed has attained an important place in industry because of its galactomannan rich endosperm. The hull and embryo portion of guar seed are termed as guar meal.

Carotenoids–accessory photosynthetic pigments are fat-soluble tetraterpenoid molecules that are divided into no oxygen-containing carotenes (β-carotene) and oxygen-containing xanthophylls (lutein, astaxanthin, zeaxanthin) (Takaichi, 2011). Chlorophylls, carotenoids and phycobiliproteins can find applications in food, cosmetic and pharmaceutical products as coloring, antioxidant, food additive or therapeutic agents (Kuddus et al., 2013). Carotenoids help in prevention against harmful UV rays (Aust et al., 2005). Protein solubilisation depends on lot of factors from algal source like its chemical composition, their morphological and structural characteristics. Some proteins are difficult to solubilise because of their hydrophobic nature or because of the presence of a disulphide bond between protein molecules that induce the decreasing of protein solubility (Shen et al., 2008).

Carbohydrates are mainly represented by polysaccharides that include various soluble and physiologically active components. The release of polysaccharides in the pericellular space largely determines the course of allelopathic interactions and the processes of sorption, desorption, ion exchange, and cell protection from extreme influences (Lombardi and Vieira, 1999). The functional groups of extracellular polysaccharides can interact with heavy metals (copper, lead, cadmium, etc.), modifying their state, mobility, and toxicity in the aquatic environment (Lombardi and Vieira, 1999).

Materials and methods:
Certified seeds of *Cyamopsis tetragonoloba* L were obtained from Durgapura Agriculture Research Station, Jaipur. Seed were stored in glass stoppered bottles. After a preliminary selection for uniformity criteria (size and colour of seeds), the seed were surface sterilized with 0.1% HgCl2 for two minutes then washed with distilled water three times and then soaked for two hours in respective solutions of different concentrations (10,50,100,200,500,1000 ppm) of copper sulphate, cadmium sulphate, lead nitrate, nickel sulphate and zinc sulphate. Seeds soaked in distilled water for two constituted the controls. After the above treatments, seeds were removed and allowed to germinate in petri plates on filter paper soaked in each of the above metallic solution. Three replicates each of 10 seeds were kept for each concentration of every heavy metal. The filter paper was moistened with metallic solution. The experiments were carried out for ten days under laboratory conditions of temperature (25±2°C) and diffuse light.

On the day of termination of experiment germinated were counted, seedling growth parameters viz, shoot length, root length fresh weight of seedlings total chlorophyll and carotenoid were recorded along with various primary metabolites viz. Proteins, Lipids, Phenols, TSS, and Starch.

(i) Seed germination percentage:
The daily progress in germination were recorded of a period of 10 days. The criterion used for seeds germination were taken as emergence of stub through the seed coat.
(ii) **Shoot length and root length:**

The root and shoot length of 10 days old seedlings were measured in centimeters using a meter scale. Five seedlings were randomly selected from petri plates; growth of these seedlings of all the four varieties, both under control and treatment were determined after 10 days by measuring the lengths of root and of plumule (longer leaf).

(iii) **Fresh weight:**

For fresh weight determination, five seedlings from each treatment were weighted on an electric balance. Average values were calculated in g/seeding.

(iv) **Estimation of pigments:**

Chlorophyll ‘a+b’ (total chlorophyll) and total carotenoids were determined by the method of Kirk and Allen (1965).

**Biochemical Parameters**

All primary metabolites were done using established protocol viz. Proteins (Lowry et al., 1951), TSS and Starch (Dubois et al., 1951), Phenols (Bray and Thorpe, 1954), Lipids (Jayaram, 1981)

**Results:**

The data regarding the effect of heavy metals on seed germination percentage, shoot length, root length, and fresh weight of ten days old seedlings *Cyamopsis tetragonoloba* cv. RGC 936 are recorded.

**Effect of metal on seed germination:**

Seed germination was adversely affected by the treatment of heavy metals. There is gradual decreases in germination percentage with increases in concentration of heavy metals. Among germination percentage studies, the most toxic heavy metal was found to be cadmium. A significant reduction occur in seed germination at 200, 500 and 1000 ppm of Cd, 200, 500 ppm of Pb, 100 to 1000 ppm of Ni and 1000 ppm of Cu and Zn onwards in comparison to the control.

Maximum reduction in seed germination percentage was observed at 1000 ppm concentration. It decreased up to 40% in Cd, 60% in Pb 83% in Ni and Zn and 93% in Cu, whereas it was found to be 100% in the control. the statistical analysis of data showed very highly significant difference between the control and treatment and among chemicals no such differences were observed (Table 1).

**Table 1. Showing the Effect of Heavy Metals on Seed Germination (percentage) in *Cyamopsis tetragonoloba* (Values are means of three replicates each)**

| Name of chemical      | Cont. | Concentrations (ppm) |
|-----------------------|-------|----------------------|
|                        |       | 10       | 50       | 100      | 200      | 500      | 1000     |
| Cadmium Sulphate      | 86.6% | 73.3%    | 63.3%    | 60%      | 53.3%    | 63.3%    | 53.3%    |
| Lead Nitrate          | 86.6% | 60%      | 73.3%    | 63.3%    | 56.6%    | 63.3%    | 53.3%    |
| Nickel Sulphate       | 86.6% | 83.3%    | 73.3%    | 70%      | 63.3%    | 63.3%    | 63.3%    |
| Zinc Sulphate         | 86.6% | 76.6%    | 83.3%    | 70%      | 73.3%    | 80%      | 76.6%    |
| Copper Sulphate       | 86.6% | 76.6%    | 73.3%    | 63.3%    | 73.3%    | 60%      | 63.3%    |
Effect of heavy metals on shoot length:

Shoot length of seedling was highly affected by treatment of heavy metals. All the heavy metals, particularly at higher concentration, resulted in adverse effect on shoot length. It was observed after seven-eight days of treatment that shoot length of seedling decreased with increasing concentration of heavy metals. There was gradual increases in shoot length with increases in concentration up to 100 ppm of Cu and Zn. Above that there was gradual decreases in shoot length with increases in concentration with two metals. At 1000 ppm concentration of all the heavy metals the shoot length was 1.5 cm in cadmium, 2.3 cm in lead, 2.6 cm in nickel, 3.6 cm in zinc and 4.1 cm in copper which was very less as compared to control where it was 6 cms. A significant reduction starts in shoot length from 10 ppm of Cd, 100 ppm of Pb, 10 ppm of Ni, 200 ppm of Zn and Cu. The shoot length at 500 ppm concentration was drastically reduced to led than one third of control in case of cadmium. At 500 ppm concentration of lead it was reduced to less than half of control. Data were statistically analysed and found to be very highly significant among control versus treatment, among concentration, among chemicals except replicates where it was not significate (Table 2)

| Name of chemical       | Cont. | Concentrations (ppm) |
|------------------------|-------|----------------------|
|                        |       | 10       | 50       | 100      | 200      | 500      | 1000     |
| Cadmium Sulphate       | 4.86  | 4.16±0.08 | 4.78±0.09 | 4.99±0.1 | 4.43±0.08 | 4.63±0.08 | 4.71±0.09 |
| Lead Nitrate           | 4.86  | 4.83±0.05 | 4.99±0.08 | 4.46±0.06 | 4.13±0.04 | 4.42±0.05 | 4.38±0.05 |
| Nickel Sulphate        | 4.86  | 4.42±0.05 | 4.78±0.06 | 4.55±0.06 | 4.85±0.07 | 4.84±0.06 | 4.52±0.05 |
| Zinc Sulphate          | 4.86  | 5.47±0.14 | 5.66±0.2  | 5.39±0.18 | 5.45±0.19 | 5.66±0.2  | 5.32±0.2  |
| Copper Sulphate        | 4.86  | 4.91±0.08 | 4.85±0.08 | 4.36±0.07 | 4.52±0.05 | 4.65±0.06 | 4.54±0.04 |

Effect of heavy metals on root length:

With increasing concentration of heavy metals, the root length of seedling gradually in Cyamopsis tetragonoloba cv. RGC 936 in all the five metals. The highly significantly differences in root length were observed between control versus treatment, among concentration and among chemicals. All heavy metals particularly at higher concentration showed adverse effect on root length. The root length was 1.3 cms in Cd, 1.5 cms Pb, 1.6 cms in Ni, 2.0 cms in Zn and 2.3 cms in Cu at highest concentration of 1000 ppm of heavy metals and in control it was 5.2 cms. There was sharp decline in root length at 10 ppm concentration of Cd (3.0 cms), at 1000 ppm concentration Pb (1.5 cms) and Ni (1.6 cms). Subsequent to treatment by heavy metals in C. tetragonoloba cv RGC 936, root length increased up to 50 ppm in Ni and up to 100 ppm concentration in Zn and Cu, after which it gradually declined (Table 3).
Table 3: Showing the Effect of Heavy Metals on Root Length (cm) of Seedlings in *Cyamopsis tetragonoloba* (Values are means of three replicates each)

| Name of chemical       | Cont. | Concentrations (ppm) |
|------------------------|-------|----------------------|
|                        |       | 10       | 50       | 100      | 200      | 500      | 1000     |
| Cadmium Sulphate       | 3.52  | 3.88±0.02 | 4.19±0.06 | 4.32±0.06 | 3.55±0.01 | 3.40±0.01 | 3.50±0.02 |
| Lead Nitrate           | 3.52  | 4.14±0.07 | 4.61±0.06 | 4.39±0.05 | 4.17±0.04 | 3.63±0.03 | 4.37±0.06 |
| Nickel Sulphate        | 3.52  | 4.05±0.04 | 4.12±0.06 | 4.46±0.05 | 4.70±0.07 | 4.44±0.06 | 4.31±0.05 |
| Zinc Sulphate          | 3.52  | 4.04±0.04 | 3.79±0.03 | 4.07±0.04 | 4.22±0.05 | 4.51±0.06 | 4.33±0.07 |
| Copper Sulphate        | 3.52  | 3.63±0.03 | 3.50±0.03 | 3.07±0.02 | 3.27±0.03 | 3.60±0.03 | 3.50±0.03 |

Effect of Heavy Metals on Fresh Weight of Seedlings:

It was observed that heavy metal significantly reduced the fresh weight of seedling of *Cyamopsis tetragonoloba*. The percentage decreased in the fresh weight of seedling due to heavy metal application as compared to control (Table 4).

Table 4: Showing the Effect of Heavy Metals on Fresh Weight (g) of Seedlings in *Cyamopsis tetragonoloba* (Values are means of three replicates each)

| Name of chemical       | Cont. | Concentrations (ppm) |
|------------------------|-------|----------------------|
|                        |       | 10       | 50       | 100      | 200      | 500      | 1000     |
| Cadmium Sulphate       | 0.357 | 0.385±0.02 | 0.390±0.03 | 0.398±0.03 | 0.351±0.01 | 0.386±0.03 | 0.368±0.02 |
| Lead Nitrate           | 0.357 | 0.365±0.02 | 0.387±0.03 | 0.354±0.01 | 0.342±0.01 | 0.348±0.01 | 0.345±0.01 |
| Nickel Sulphate        | 0.357 | 0.347±0.01 | 0.348±0.01 | 0.343±0.02 | 0.385±0.04 | 0.374±0.03 | 0.338±0.02 |
| Zinc Sulphate          | 0.357 | 0.443±0.04 | 0.453±0.05 | 0.466±0.04 | 0.446±0.04 | 0.427±0.03 | 0.456±0.04 |
| Copper Sulphate        | 0.357 | 0.330±0.03 | 0.367±0.02 | 0.338±0.02 | 0.347±0.01 | 0.34±0.02  | 0.337±0.02 |

Effect of Heavy Metals on total chlorophyll content:

With increases in concentrations of heavy metals there was a gradual decrease in total chlorophyll content in *Cyamopsis tetragonoloba* except in respect of Zn and Cu up to 10 and 50 ppm concentration, respectively where it was superior to control. Very highly significant results were recorded for control versus treatments.
and among various concentrations themselves. No such differences were obtained among various chemicals and among replicates. It was observed that Cd and Pb in comparison to Zn, Cu and Ni drastically reduced the total chlorophyll content at 1000 ppm concentration (Table 5).

**Table 5 Showing the Effect of Heavy Metals on Chlorophyll (g) of Seedlings in Cyamopsis tetragonoloba**  
(Values are means of three replicates each)

| Name of chemical | Cont. Concentrations (ppm) |
|------------------|---------------------------|
|                  | 10  | 50  | 100 | 200 | 500 | 1000 |
| Cadmium Sulphate | 0.022 | 0.006±0.001 | 0.011±0.001 | 0.012±0.002 | 0.014±0.002 | 0.005±0.002 | 0.007±0.003 |
| Lead Nitrate     | 0.022 | 0.019±0.004 | 0.019±0.004 | 0.020±0.005 | 0.003±0.001 | 0.007±0.002 | 0.010±0.003 |
| Nickel Sulphate  | 0.022 | 0.004±0.001 | 0.006±0.004 | 0.007±0.005 | 0.009±0.006 | 0.007±0.005 | 0.009±0.007 |
| Zinc Sulphate    | 0.022 | 0.006±0.001 | 0.005±0.001 | 0.004±0.003 | 0.003±0.002 | 0.019±0.004 | 0.018±0.004 |
| Copper Sulphate  | 0.022 | 0.015±0.005 | 0.006±0.002 | 0.002±0.001 | 0.003±0.002 | 0.005±0.002 | 0.009±0.002 |

**Effect of heavy metals on Carotenoid Content**

It was observed that Zn and Cu were less inhibitory to the amount of total carotenoid content in comparison to other heavy metals. In the control of carotenoid content was 0.37 mg/g fresh weight which decreased to 0.1 mg/g in Cd 0.20 mg/g in Pb, 0.23 mg/g in Ni 0.26 mg/g in Zn and Cu of fresh weight at 1000 ppm concentration, Cd and Pb caused the highest reduction in pigment content. Statically very highly significant results were observed between controls versus treatment and various chemicals among various concentrations whereas not significant results were notices among various chemicals and replicates (Table 6).

**Table 6 Showing the Effect of Heavy Metals on Carotenoid (g) of Seedlings in Cyamopsis tetragonoloba**  
(Values are means of three replicates each)

| Name of chemical     | Cont. Concentrations (ppm) |
|----------------------|---------------------------|
|                      | 10  | 50  | 100 | 200 | 500 | 1000 |
| Cadmium Sulphate     | 0.050 | 0.027±0.008 | 0.030±0.009 | 0.034±0.01 | 0.042±0.02 | 0.039±0.02 | 0.103±0.09 |
| Lead Nitrate         | 0.050 | 0.027±0.008 | 0.031±0.009 | 0.038±0.02 | 0.038±0.02 | 0.044±0.03 | 0.042±0.03 |
| Nickel Sulphate      | 0.050 | 0.029±0.009 | 0.033±0.009 | 0.039±0.01 | 0.050±0.04 | 0.057±0.05 | 0.064±0.06 |
| Zinc Sulphate        | 0.050 | 0.044±0.01  | 0.136±0.1   | 0.027±0.01 | 0.015±0.05 | 0.088±0.07 | 0.076±0.06 |
| Copper Sulphate      | 0.050 | 0.035±0.04  | 0.040±0.04  | 0.044±0.04 | 0.055±0.05 | 0.061±0.05 | 0.062±0.05 |
Effect of Heavy Metals on Protein:
A perusal of data showed that there was a sharp decline in protein contents at 1000 ppm of Ni and Zn. Nickel was found to be the least inhibitory among all heavy metals for protein contents whereas, Zn was found to be the most toxic followed by Pb. It was noticed that protein contents measured at 10 ppm concentration of all the heavy metals was lesser when compared to protein contents measured at 50 ppm concentration except in lead and zinc. The amount of protein contents measured at 10 ppm concentration was 16.3 mg/g weight in Pb, 14.6 mg/g weight in Zn, 14.7 mg/g weight in Cu and Ni which increased to 16.5 mg/g. Overall it was observed that proteins content showed some variations at 1000 ppm in all treatment doses of heavy metals (Table 7).

Table 7. Showing the Effect of Heavy Metals on Protein content (g) of Seedlings in *Cyamopsis tetragonoloba* (Values are means of three replicates each)

| Name of chemical      | Cont. | Concentrations (ppm) |
|-----------------------|-------|----------------------|
|                       | 10    | 50       | 100      | 200      | 500       | 1000      |
| Cadmium Sulphate      | 14.9  | 14.4±0.42 | 14.6±0.41 | 14.8±0.41 | 16.1±0.48 | 15.6±0.52 | 15.8±0.50 |
| Lead Nitrate          | 14.9  | 16.3±0.56 | 16.0±0.54 | 15.6±0.48 | 15.3±0.44 | 15.1±0.37 | 16.4±0.58 |
| Nickel Sulphate       | 14.9  | 16.5±0.61 | 16.6±0.62 | 15.9±0.48 | 16.7±0.53 | 16.2±0.52 | 17.1±0.61 |
| Zinc Sulphate         | 14.9  | 14.6±0.44 | 13.8±0.37 | 14.7±0.46 | 14.9±0.48 | 14.4±0.42 | 14.3±0.45 |
| Copper Sulphate       | 14.9  | 14.7±0.48 | 14.8±0.49 | 15.7±0.51 | 14.4±0.44 | 15.8±0.52 | 15.7±0.46 |

Effect of Heavy Metals on Total Soluble Sugars:
It was observed that Cd was the most inhibitory followed by Pb to total soluble sugars in comparison to other heavy metals. In control it was 0.95 mg/g weight. Total soluble sugars were at minimum at 1000 ppm concentration of Zn, 100 ppm of Cu and at all the concentrations of Cd. It was observed that at 1000 ppm concentration of all heavy metals reduced total soluble sugars as compared to control (Table 8).

Table 8. Showing the Effect of Heavy Metals on Total soluble sugars (g) of Seedlings in *Cyamopsis tetragonoloba* (Values are means of three replicates each)

| Name of chemical      | Cont. | Concentrations (ppm) |
|-----------------------|-------|----------------------|
|                       | 10    | 50       | 100      | 200      | 500       | 1000      |
| Cadmium Sulphate      | 0.95  | 1.5±0.06  | 1.55±0.06 | 1.75±0.07 | 1.75±0.07 | 1.4±0.04  | 1.6±0.06  |
| Lead Nitrate          | 0.95  | 1.95±0.08 | 0.7±0.03  | 0.6±0.05  | 3.9±0.08  | 3.05±0.04 | 2.8±0.03  |
| Nickel Sulphate       | 0.95  | 1.6±0.56  | 1.6±0.57  | 1.75±0.6  | 1.8±0.71  | 1.7±0.62  | 1.55±0.54 |
| Zinc Sulphate         | 0.95  | 0.3±0.03  | 1.15±0.44 | 0.9±0.34  | 4.15±1.1  | 2.1±0.88  | 0.2±0.03  |
| Copper Sulphate       | 0.95  | 1.85±0.78 | 2.9±1.02  | 1.3±0.87  | 2.4±0.98  | 4.05±1.18 | 1.15±0.96 |
Effect of Heavy Metals on Starch:

In starch in control was 2.75 mg/g weight. It was observed that the heavy metal Cd was found to be the most suitable and Pb most inhibitory for starch among all the heavy metals. In Ni starch was found to be more than control upto 100 ppm concentration. A slightly reduction in starch was observed from 10 ppm to 1000 onwards in treatment doses of all heavy metals. Highest amount of starch (3.5 mg/g weight) was observed at 500 ppm concentration of Cd (Table 9).

Table 9. Showing the Effect of Heavy Metals on starch (g) of Seedlings in *Cyamopsis tetragonoloba* (Values are means of three replicates each)

| Name of chemical | Concentrations (ppm) |
|------------------|----------------------|
|                  | 10 | 50 | 100  | 200 | 500 | 1000 |
| Cadmium Sulphate | 2.75 | 3.15±0.2 | 3.2±0.31 | 3.3±0.34 | 3.3±0.34 | 3.5±0.35 | 3.25±0.33 |
| Lead Nitrate     | 2.75 | 2.75±0.18 | 2.75±0.18 | 2.75±0.18 | 2.76±0.18 | 2.5±0.15 | 2.77±0.18 |
| Nickel Sulphate  | 2.75 | 2.8±0.19 | 2.85±0.19 | 2.5±0.16 | 2.7±0.17 | 3.0±0.22 | 2.45±0.19 |
| Zinc Sulphate    | 2.75 | 2.65±0.14 | 2.7±0.19 | 2.7±0.19 | 2.7±0.19 | 2.7±0.19 | 2.7±0.19 |
| Copper Sulphate  | 2.75 | 2.75±0.21 | 2.75±0.21 | 2.75±0.21 | 2.75±0.21 | 2.75±0.21 | 2.75±0.21 |

Effect of Heavy Metals on Phenols:

A perusal of data showed that there was a sharp decline in phenol contents at 1000 ppm of all heavy metals. Nickel was found to be the least inhibitory among all heavy metals for phenols contents whereas, Ni was found to be the most toxic followed by Cu. It was noticed that phenol contents measured at 10 ppm concentration of all the heavy metals was lesser when compared to contents measured at 50 ppm concentration. The amount of phenols contents measured at 10 ppm more in all treatment doses and decreased when dose level reached to 1000 ppm. Maximum amount was observed in 100 ppm of Ni (Table 10).

Table 10. Showing the Effect of Heavy Metals on Phenols (g) of Seedlings in *Cyamopsis tetragonoloba* (Values are means of three replicates each)

| Name of chemical | Concentrations (ppm) |
|------------------|----------------------|
|                  | 10 | 50 | 100  | 200 | 500 | 1000 |
| Cadmium Sulphate | 3.35 | 3.3±0.26 | 3.3±0.26 | 3.35±0.26 | 3.35±0.26 | 3.1±0.24 | 3.2±0.24 |
| Lead Nitrate     | 3.35 | 3.25±0.25 | 3.27±0.28 | 3.3±0.29 | 3.25±0.25 | 3.45±0.33 | 3.45±0.33 |
| Nickel Sulphate  | 3.35 | 3.45±0.33 | 3.55±0.38 | 3.7±0.41 | 3.6±0.39 | 2.95±0.28 | 2.8±0.27 |
| Zinc Sulphate    | 3.35 | 3.2±0.31 | 3.2±0.31 | 3.25±0.32 | 3.2±0.32 | 3.25±0.25 | 3.2±0.24 |
| Copper Sulphate  | 3.35 | 3.45±0.38 | 3.45±0.37 | 3.25±0.32 | 3.2±0.20 | 3.25±0.22 | 3.2±0.24 |
Effect of Heavy Metals on Lipids:

Here also similar observations were made. Though contents reduced from 10 to 1000 ppm at dose level of each heavy metal but there was not so variation when contents were measured individually (Tables 11)

Table. 11. Showing the Effect of Heavy Metals on Lipids (g) of Seedlings in *Cyamopsis tetragonoloba* (Values are means of three replicates each)

| Name of chemical       | Cont. | Concentrations (ppm) |
|------------------------|-------|----------------------|
|                        |       | 10       | 50       | 100      | 200      | 500      | 1000     |
| Cadmium Sulphate       | 3.33  | 3.33±0.2  | 3.33±0.2 | 3.33±0.2 | 3.33±0.2 | 6.66±1.0 | 3.33±0.2 |
|                        |       | 6        | 6        | 6        | 6        | 7        | 7        |
| Lead Nitrate           | 3.33  | 6.66±1.0  | 3.33±0.2 | 10.0±2.2 | 6.66±1.0 | 3.33±0.2 | 3.33±0.2 |
|                        |       | 2        | 7        | 4        | 2        | 6        | 6        |
| Nickel Sulphate        | 3.33  | 6.66±1.0  | 6.66±1.0 | 3.33±0.2 | 3.33±0.2 | 3.33±0.2 | 3.33±0.2 |
|                        |       | 2        | 2        | 7        | 7        | 27       | 7        |
| Zinc Sulphate          | 3.33  | 3.33±0.2  | 3.33±0.2 | 3.33±0.2 | 3.33±0.2 | 3.33±0.2 | 3.33±0.2 |
|                        |       | 6        | 6        | 6        | 6        | 6        | 6        |
| Copper Sulphate        | 3.33  | 3.33±0.2  | 3.33±0.2 | 3.33±0.2 | 6.66±1.0 | 3.33±0.2 | 3.33±0.2 |
|                        |       | 7        | 7        | 7        | 2        | 28       | 7        |

Discussion:

All organisms living in the intertidal zone are subject to recurring, harsh changes in the environment associated to life in the interface of terrestrial and marine habitats. These changes include, but are not limited to, mechanical strain, biotic attacks, pollution, temperature, light (UV), desiccation, changes in salinity, and control the distribution of organisms in the intertidal zone (Davison & Pearson, 1996). Stress, and in particular heavy metal is known to have several effects on plant cells that lead to the activation of stress signaling pathways, which include changes in cell turgor, to a certain extent changes in cell volume (Xiong and Zhu, 2002). Membrane proteins such as receptor-like kinases, stretch-dependent ion (calcium) channels and redox-mediated systems (Kacperska, 2004) have been identified as osmosensors which activate downstream signaling cascades. The inhibitory effect of heavy metals has been studied by number of scientist (Solanki et al., 2011; Kalaikandhan et al., 2014). Copper (Cu), Cadmium(Cd) and zinc (Zn) are trace elements essential for normal plant growth, but at higher concentrations they become toxic and can interfere with numerous biological processes (Vaillant et al., 2005). The phytotoxicity of Cd is indicated by decrease in growth and development, metabolism and an induction of oxidative damage in various plant species such as *Phaseolus vulgaris* (Cakmak and Marshner, 1993) and *Brassica juncea* (Prasad et al., 1999). Growth of *Sorghum vulgarae* L under cadmium, stress has been evaluated.

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