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Effect of Heavy Metal on Seed Germination and Biochemical Profiling in *Sorghum bicolor* (L.) Moench

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ABSTRACT

Fodder grasses like *Sorghum bicolor* play a vital role in livelihood generation in arid and semi-arid areas of Rajasthan in India. Cadmium pollution from industries can affect soils in farmlands thereby playing havoc with health of humans and livestock. The present study was undertaken to study the effect of Cadmium salts on seed germination percentage, seedling growth and fresh weight including various biochemical parameters of *Sorghum bicolor*. Seeds were treated with different concentrations (10, 50, 100, 200, 500 and 1000 ppm) of cadmium sulphate along with control for 10 days. On the 10th day results were noted for above parameters. Seed germination percentage was recorded maximum at 10 ppm followed by 100 ppm. Both shoot and root length showed progressive decline with cadmium treatment. Fresh weight of seedlings was observed better than control for 10 ppm dose level. Further maximum amount of proteins and total soluble sugars were observed at 500ppm dose level while starch and total phenols were found to be maximum at 100ppm dose level. However, in lipids there was constant decrease as compared to control with increase in treatment dose. Total chlorophyll was found to be maximum at 1000 ppm , carotenoids and MDA at in control. These results can be further developed as markers for field testing of cadmium polluted plants.

Introduction

Heavy metals such as copper, zinc 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, being potentially toxic, may interfere with the ecology of a particular environment. According to 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 (Sreeramulu *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 most 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 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. Cadmium is usually accumulated in roots sometimes beyond the limit (Prasad, 1995). In the last decades, in field, potted, and hydroponic trials, the capacity to accumulate and stabilize Cd in several crops, including sunflower (Turgut et al., 2005) was evaluated.

Indeed, some authors reported that Cd can cause many morphological, physiological and biochemical changes in sunflower plants (Turgut et al., 2005).

Sorghum is the fifth most important cereal crop in the world after wheat, rice, corn and barley. Sorghum outperforms other cereals under various environmental stresses and is thus generally more economical to produce. More than 35% of sorghum is grown directly for human consumption. The rest is used primarily for animal feed, alcohol and industrial products. The United States is the largest producer and exporter of sorghum, accounting for 20% of world production and almost 80% of world sorghum exports in 2001–2002 (USDA-FAS, 2003). World sorghum production was 57 million metric tons during this period.

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)

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(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).

LPO (Lipid Peroxidation) is oxidative deterioration of polyunsaturated lipids and it involves ROS and transition metal ions. It is a molecular mechanism of cell injury leading to the generation of peroxides and lipid hydroperoxides, which can decompose to yield a wide range of cytotoxic products, most of which are aldehydes such as malondialdehyde (MDA) and 4 hydroxynonenal. LPO is a highly destructive process that affects cellular organelles and causes them to lose biochemical function and/or structural integrity, which may lead to irreparable damage or cell death (Wiseman and Halliwell, 1996).

Materials and methods

Certified seeds of *Sorghum bicolor* (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% $HgCl_2$ 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 cadmium 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^{0}C) and diffuse light.

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

(i) Seed germination percentage

The daily progress in germination was 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 experimental plant, 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).

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

Name of chemical	**Cont.**	**Concentrations (ppm)**					
		10	50	100	200	500	1000
Cadmium Sulphate	80%	70%	45%	65%	55%	20%	10%

Table 2. Showing the Effect of Heavy Metals on Shoot Length (cm) of Seedlings in Sorghum (Values are means of three replicates each).

Name of chemical	Cont.	Concentrations (ppm)					
		10	50	100	200	500	1000
Cadmium Sulphate	14±0.87	14.1±0.88	8.9±0.62	8.7±0.60	5.5 ± 0.56	3.4 ± 0.21	1.3± 0.11

Table 3. Showing the Effect of Heavy Metals on Root Length (cm) of Seedlings in Sorghum (Values are means of three replicates each).

Name of chemical	**Cont.**	**Concentrations (ppm)**					
		10	50	100	200	500	1000
Cadmium Sulphate	15.8± 0.92	14.4± 0.91	5.7± 0.34	5.3± 0.32	2.3± 0.14	0.9± 0.08	0.15± 0.11

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

Name of chemical	**Cont.**	**Concentrations (ppm)**					
		10	50	100	200	500	1000
Cadmium Sulphate	0.155±0.008	0.170±0.010	0.121±0.002	0.096±0.001	0.100±0.0090.002	0.118±0.002	0.060±0.001

Table 5. Showing the Effect of Heavy Metals on Total chlorophyll content (mg/gdw).

Name of chemical	**Cont.**	**Concentrations (ppm)**					
		10	50	100	200	500	1000
Cadmium Sulphate	0.188±0.011	0.112±0.002	0.134±0.003	0.141±0.004	0.146±0.004	0.150±0.005	0.157±0.006

Table 6. Showing the Effect of Heavy Metals Total carotenoids content (mg/gdw).

Name of chemical	**Cont.**	**Concentrations (ppm)**					
		10	50	100	200	500	1000
Cadmium Sulphate	0.035±0.0002	0.024±0.0001	0.026±0.0001	0.026±0.0001	0.029±0.0001	0.030±0.0001	0.031±0.0001

Results

All data regarding the effect of Cadmium on seed germination, root length, shoot length and fresh weight including various biochemical parameters are tabulated.

Effect of Heavy metals on Seed germination percentage

As compared to control, seed germination rate decreased with increase in dose level of heavy metal. Among various treatments best germination was observed at 10 ppm where germination rate was 70%. Minimum seed germination percentage was recorded at 1000 ppm which was only 10%.

Effect of Heavy metals on root length

Here we observed decrease in root length as compared to control. Among various treatments maximum length was observed at 10 ppm which was around 14.4cm while minimum length was at 1000ppm (0.15 cm). Overall length was reduced with increase in dose level of heavy metal.

Effect of Heavy metals on shoot length

It was observed that at 10 ppm level there was slightly increase in shoot length which was maximum as compared to control (14.1cm) while at all other concentrations shoot length was decreased. Minimum length (1.3cm) was observed at 1000 ppm level.

Effect of Heavy metals on fresh weight

Here also we observed increase in fresh weight at 10 ppm level as compared to control which was found to be maximum (0.170g) and while it reduced to minimum (0.060) at 1000 ppm.

Effect of heavy metals on Biochemical parameters

When various biochemical parameters were studied, there were some variations in their content. It was observed that protein content reduced constantly as compared to control. Similar scenario was observed in total soluble sugars maximum content was found to be at 500 ppm (3.17mg/gdw) and minimum at 1000 ppm (1.81mg/gdw). Further in starch the contents were reduced as compared to control. Among various stress maximum content was observed at 100 ppm (2.57mg/gdw) while minimum at 200 ppm (1.54mg/gdw). When phenols were quantified there was reduction in contents as compared to control. However maximum content was observed at 100 ppm (3.52mg/gdw) while minimum at 1000 ppm (1.89 mg/gdw).

There was decrease also in lipids content as compared to control. However, among various stress level maximum content was observed at 10 ppm (7.72mg/gdw) which was slightly lower than control and minimum in 500 ppm which was at par with 1000 ppm (6.59mg/gdw).

Among various bio pigments there was increase in chlorophyll level which was not observed in above parameters. As compared to control maximum content was observed at 1000 ppm (0.157mg/gdw) and minimum at 10 ppm (0.112) while in carotenoids there was constant increase in content with increase in treatment dose of heavy metal.

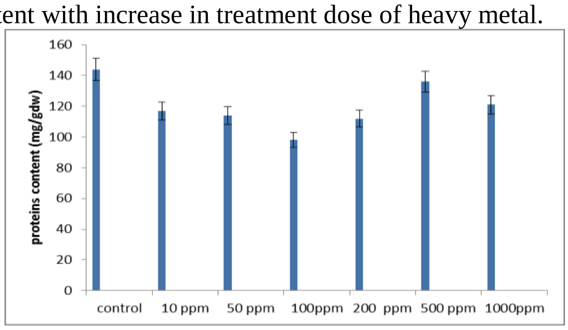

Fig. 1. Effect of cadmium stress at various doses in Protein content in *Sorghum bicolor* (L.)

Maximum carotenoid content was observed at 1000 ppm (0.031mg/gdw) and minimum at 10 ppm (0.024 mg/gdw). Finally, in Lipid peroxidation assay as compared to control the activity decreased significantly with increase in treatment of heavy metal stress.

Maximum content was observed in control (0.09mg/gdw) while minimum at 200 ppm (0.019 mg/gdw) (Fig.1-6)

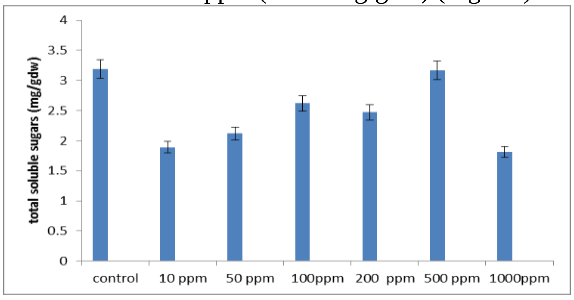

Fig. 2 Effect of cadmium stress at various doses in total soluble sugars in *Sorghum bicolor* (L.)

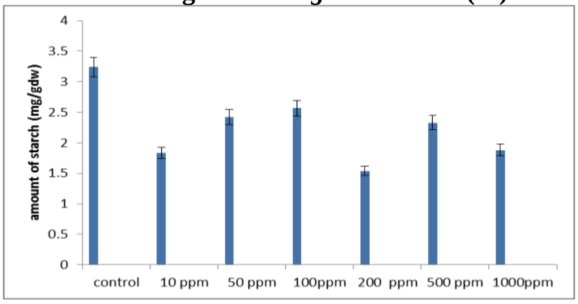

Fig. 3 Effect of cadmium stress at various doses in starch content in *Sorghum bicolor* (L.)

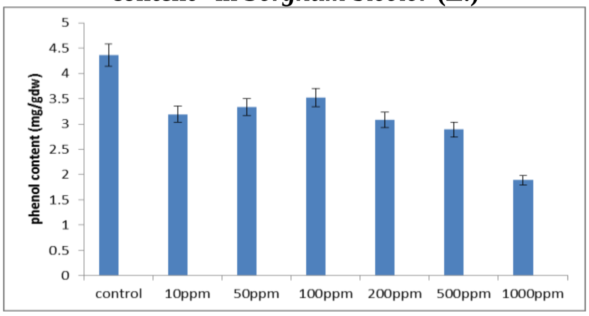

Fig. 4 Effect of cadmium stress at various doses in phenol content in *Sorghum bicolor* (L.)

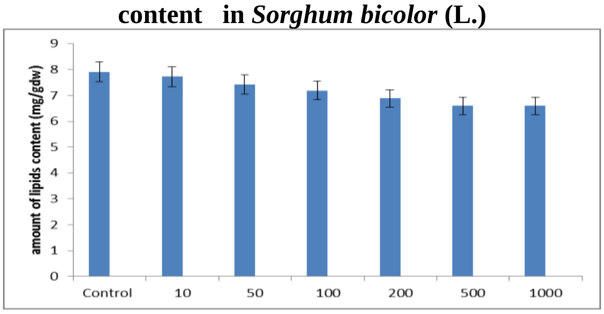

Fig 5. Effect of cadmium stress at various doses in Lipid content in *Sorghum bicolor* (L.)

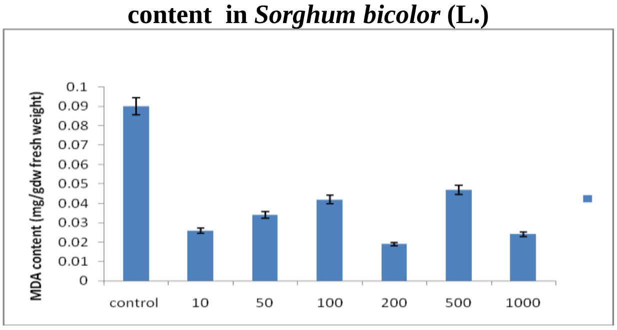

Fig. 6 Effect of cadmium stress at various doses in MDA content (mg/gdw fresh weight) in *Sorghum bicolor* (L.)

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).

Lipid peroxidation increased in rice treated with cadmium (Srivastava et al., 2014) and this was also seen to be the case in *Sorghum*. 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; Gupta et al., 2016). 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 bicolor* (L.) under cadmium, stress has been evaluated.

Miscanthus spp. has shown cadmium tolerance and may be used for phytoremediation of affected soils (Guo et. al, 2016). Growth of *Sorghum bicolor* (L.). under cadmium, stress has been evaluated and results indicate that metabolite concentrations vary in cadmium polluted plants. These changes in metabolite concentrations could be developed further as markers to be tested in field conditions. Sorghum also may be evaluated as a phytoremediation tool in contaminated soils.

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