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Analysis of primary and secondary metabolites of *Celosia argentea* in the presence of heavy metal stress

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Abstract
Heavy metals are ever-present because of extreme use of their compounds in industrial applications and are very detrimental because of their non-biodegradable nature, long biological half-lives and their potential to accumulate in different body parts of plant. These accumulated heavy metals may cause serious issues in plants. The aim of present study was to assessed the effect of heavy metals (Cd and Zn) on the quantity of plant's primary and secondary metabolites like Total soluble sugar (TSS), Starch, Protein, Lipid, Phenols, Flavonoids, Alkaloids, Phytosterols. The plant species *Celosia argentea* (Silver cocks-comb) was castoff for the investigation. The different concentration of heavy metals in range of 5ppm, 25ppm and 50ppm were used. A set-up without heavy metal was used as control. The Primary and Secondary metabolites such as Total soluble sugar, starch, protein, lipid phenol, flavonoids, alkaloids and phytosterols were estimated after three months at mature stage of the plant. All metabolites are found in decreasing amount with increased concentration of heavy metals in treated plants.

Keywords: amaranthaceae, *Celosia argentea*, heavy metal stress, primary metabolites, secondary metabolites etc

Introduction

Heavy metals can be released into the environment by both natural and anthropogenic sources and remain in soil much longer than another section of the biosphere (Lasat, 2002) [15]. So Earth is being continuously contaminated through emissions from industrial areas, leaded gasoline, lead based paints, coal ignition deposits, wastewater irrigation, fertilizer and pesticide application, sewage sludge, disposal of heavy metal wastes, discharge of petrochemicals and atmospheric deposition (Kabata-Pendias and Pendias, 1989) [8].

Heavy metals are classified into two Groups *viz.* essential and non-essential metals. Essential ones are needed in trace amount for the optimal growth of plants they include Zn, Fe, Co, Mo, Ni. Excess supply of these metals causes toxic effects on growth and life cycle of plants (Forstner and Wittmann, 1981; Young and Blevin, 1981) [5, 23]. Non-essential heavy metals include As, Ag, Cd, Hg and Pb (Bryan, 1976) [2]. Heavy metals are lethal for both plant and animal health above certain concentration. If heavy metals present in soil above the safe limits the cause toxic impact on plants as they inhibit the vegetative growth and decrease the productivity (Liu *et al.*, 2003) [12].

In present investigation we are going to study about Zn and Cd heavy metals. Both the metals show maximum transfer to plants from soil because of higher transfer coefficient (Kloke *et al.*, 1984) [10].

Zinc (Zn) is an essential heavy metal which require in minimum concentration for plants and becomes toxic in excess quantity. It is directly and indirectly associated with the protein and involve in several biological activities so it plays a vital role in appropriate growth and development of plant.

Cadmium (Cd) is considered as an environmental pollutant and a carcinogenic element which is more available than other heavy metals for the plants. It is also recognized as an important pollutant due to its higher solubility in water and high toxicity (Pinto *et al.*, 2004) [19]. Cd can be easily absorbed by plants and transferred to upper plant parts and further in food chain (Vig *et al.*, 2003) [21]. Through food chain it may easily enter in the human diet. It can be easily accumulated in large amounts in the body of all organisms and alter physiological metabolism processes like photosynthesis, respiration, transpiration and nitrogen assimilation (Wang *et al.*, 2008) [22].

The present study is done on *Celosia argentea*, commonly known as silver cockscomb which belongs to the family Amaranthaceae. It is an herbaceous plant of tropical origin and known for its very brilliant colors and medicinal usage. *C. argentea* is patented for its cosmetic application such as skin whitening agent. It is also recognized as bio-sorbents and fertilizer (Pingale *et al.*, 2012) [18]. The purpose of the current work is to assess the impact of different concentrations of Zinc and Cadmium on the primary and secondary metabolites quantity in *Celosia argentea.* In the present study quantification of various metabolites were analyzed from treated and controlled plants. These plants were observed till the maturity stage after three months and whole plants was used for the study.

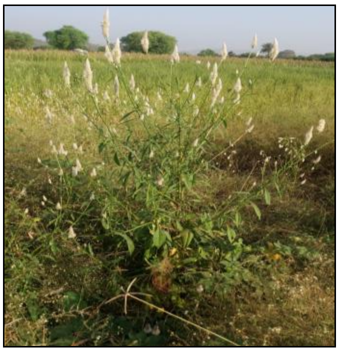

Fig 1: Photograph of *Celosia argentea*

Materials and Methods

Celosia argentea plant species of family Amaranthaceae were used as experimental plants for this study. Purchased chemicals like ZnSO4 and CdSO4 were used as the source of heavy metals in this experimentation. Both the salts were used in three different concentrations 5 ppm, 25 ppm, and 50 ppm (T1, T2 and T3). The test plants were grown in earthen pots of 10kg capacity. All pots which were used for experimental set-ups, were filled with pre-sieved and dried garden soil.

Various concentration of heavy metals like 5ppm, 25ppm and 50ppm, were thoroughly mixed with respective soil in aqueous form to attain homogenized contamination. Untreated soil was used to grow control plants. A total of 3 pots were kept for each concentration. 10 healthy seeds of each test species were shown in each experimental set-up. The healthy seeds of *Celosia argentea* were collected from the nearby area of Ajmer (Rajasthan). The plants were grown using normal practice under field conditions. Any of fertilizers were not be added to soil for plant growth.

For the estimation of the various metabolites, standard protocols were utilized. The amount of total soluble sugars was estimated by the Loomis and Shull protocol (1937) [13]. For the estimation of starch quantity Mc Cready *et al* (1950) [16] protocol was used. Protein content of the test samples were determined using the protocol of Lowry *et al* (1951) [14]. Estimation of lipid was done according to Jayaraman (1981) [7]. For the quantification of secondary metabolites like flavonoids, alkaloids and phytosterols were used Subramanium and Nagarajan (1969) [20], Kogan *et al.* (1953) [11] and Kaul and Staba (1968) [9] respectively. To estimate total phenolic compounds in each test sample, the protocol of Bray and Thorpe (1954) [1] was used.

Statistical analysis

All the analyses were achieved in triplicates and the results were statistically evaluated and expressed as mean ± standard deviation (SD).

Results and Discussion

Plants are idyllic agents for remediation because of their unique properties. Plants have the potential to uptake and accumulate heavy metals in their parts. This process alters the overall fundamental composition of the plant like primary metabolites and secondary metabolites (Olajire and Ayodele, 2003) [17].

Effect of heavy metal stress were observed in primary and secondary metabolites of all plants when those were compared to plants grown as control without any heavy metal stress. The results of the study for primary and secondary metabolites are shown in the Table 1 and Table 2 respectively. These are graphically represented in figure 1 and 2 respectively.

Due to stress of heavy metals (Zn and Cd) the quantity of primary metabolites gets disturbed. TSS quantity were found to be maximum at Zn 5ppm and Cd 5ppm while minimum at Zn 50ppm and Cd 50 ppm. Quantity of Starch was maximum at Zn 25ppm and Cd 5ppm while minimum in Zn 50ppm and Cd 50ppm. Maximum proteins were found at Zn 25ppm and Cd 5ppm while minimum proteins were found at Zn 50ppm and Cd 50ppm. Lipid Content increases with Zn concentration and then decreases while increasing Cd concentration decreases lipid content. Phenols also increases with the concentration of Zn and then decreases. At Cd 5ppm concentration phenol were maximum while at Cd 50ppm, those were minimum in compare to control plants.

Heavy metals also affect the quantity of secondary metabolites of plants. Free flavonoids, bound flavonoids and total flavonoids were found to be maximum at Zn 25ppm, which is high from control treatment while minimum at Zn 50ppm. In the case of Cd, Free flavonoids, bound flavonoids and total flavonoids quantity decreases with the increasing quantity of Cd concentration. Maximum quantity of alkaloids was found at Zn 25ppm and Cd 5ppm while minimum at Zn 50ppm and Cd 50ppm. Phytosterols increases with Zn increasing concentration till 25ppm then decreases, while it was decreasing with the increased concentration of Cd in compare to control plants.

Results revealed that both heavy metals alter the quantity of primary and secondary metabolites. That study supports that the Cd alters chemical synthesis processes such as ammonification, nitrification, denitrification and microbiological process that affect the quantity of both primary and secondary products (Cojocaru *et al*., 2016) [4]. Cd leads to generate Reactive oxygen species (ROS) and oxidative stress so it can affect the performance of protein and lipids (Farooq *et al*., 2016) [6]. These altered amounts of TSS, Protein and lipid was found due to defense mechanism of plant from heavy metals (Zn and Cd).

Zinc and cadmium both have numerous chemical and physical similarities as they belong to II Group of the periodic table. They are usually found together in the ores and compete with each other for various ligands. Thus, interaction between Zn and Cd in the biological system in likely to be similar. The fact that cadmium is a toxic heavy metal and Zn is an essential element which makes this association interesting as it raises the possibility that the toxic effects of cadmium may be preventable or treatable by zinc (Chowdhury *et al*., 1977) [3].

Table 1: Effect of heavy metal stress on quantity of various primary metabolites in *Celosia argentea* after maturation.

S. no.	Treatment of plant (ppm)	Total soluble sugar (mg/g)	Starch (mg/g)	Proteins (mg/g)	Lipids (mg/g)	Phenols (mg/g)
1.	Control	20±0.01	48.60±0.10	38.33±0.0	38±0.02	0.5±0.0
2.	$ZnSO_4$ (5ppm)	165±0.50	52.4±0.05	54±0.01	39±0.01	0.75±0.0
3.	$ZnSO_4$ (25 ppm)	122±1.0	60.6±0.02	62±0.01	47±0.05	1.0±0.0
4.	$ZnSO_4$ (50 ppm)	94±0.20	43.4±0.01	53±0.05	40±0.01	0.62±0.01
5.	$CdSO_4$ (5 ppm)	14±0.02	47.6±0.05	30±0.05	32±0.01	0.45±0.01
6.	$CdSO_4$ (25 ppm)	9±0.0	36±0.05	28±0.0	19±0.01	0.33±0.02
7.	$CdSO_4$ (50 ppm)	3±0.01	27±0.02	13±0.01	12±0.02	0.25±0.01

Table 2: Effect of heavy metal stress on quantity of various secondary metabolites in *Celosia argentea* after maturation.

S. no.	Treatment of plant (ppm)	Total flavonoids (mg/g)	Free flavonoids (mg/g)	Bound flavonoids (mg/g)	Alkaloids (mg/g)	Phytosterols (mg/g)
1.	Control	6.7±0.01	3.3±0.0	1.5±0.01	1.08±0.0	21.3±0.02
2.	$ZnSO_4$ (5ppm)	6.9±0.02	4.3±0.01	2.3±0.01	2.31±0.0	32±0.10
3.	$ZnSO_4$ (25 ppm)	7.38±0.05	5.2±0.02	2.7±0.03	2.68±0.01	32.5±0.05
4.	$ZnSO_4$ (50 ppm)	6.4±0.10	3.6±0.05	1.4±0.05	0.86±0.01	13.6±0.02
5.	$CdSO_4$ (5 ppm)	4.94±0.01	2.6±0.0	1.2±0.01	1.09±0.02	17±0.05
6.	$CdSO_4$ (25 ppm)	4.90±0.01	2.4±0.01	0.8±0.0	0.95±0.01	15.9±0.1
7.	$CdSO_4$ (50 ppm)	3.7±0.0	1.1±0.0	0.6±0.0	0.88±0.0	14.2±0.01

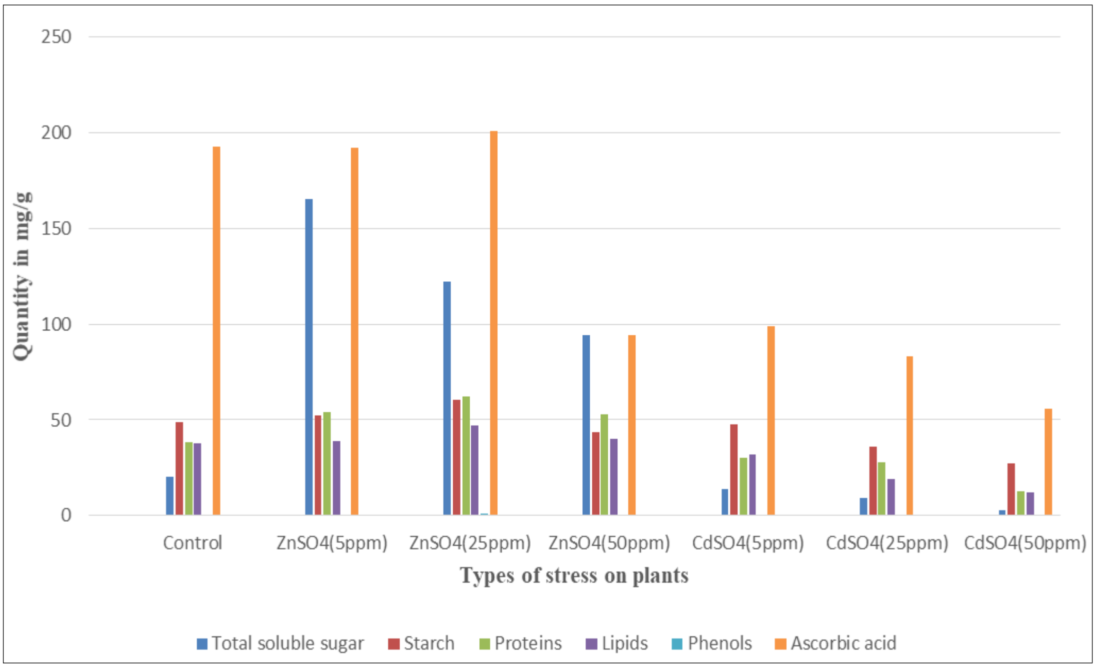

Fig 1: Effect of heavy metal stress on quantity of various Primary metabolites in *Celosia argentea* after maturation.

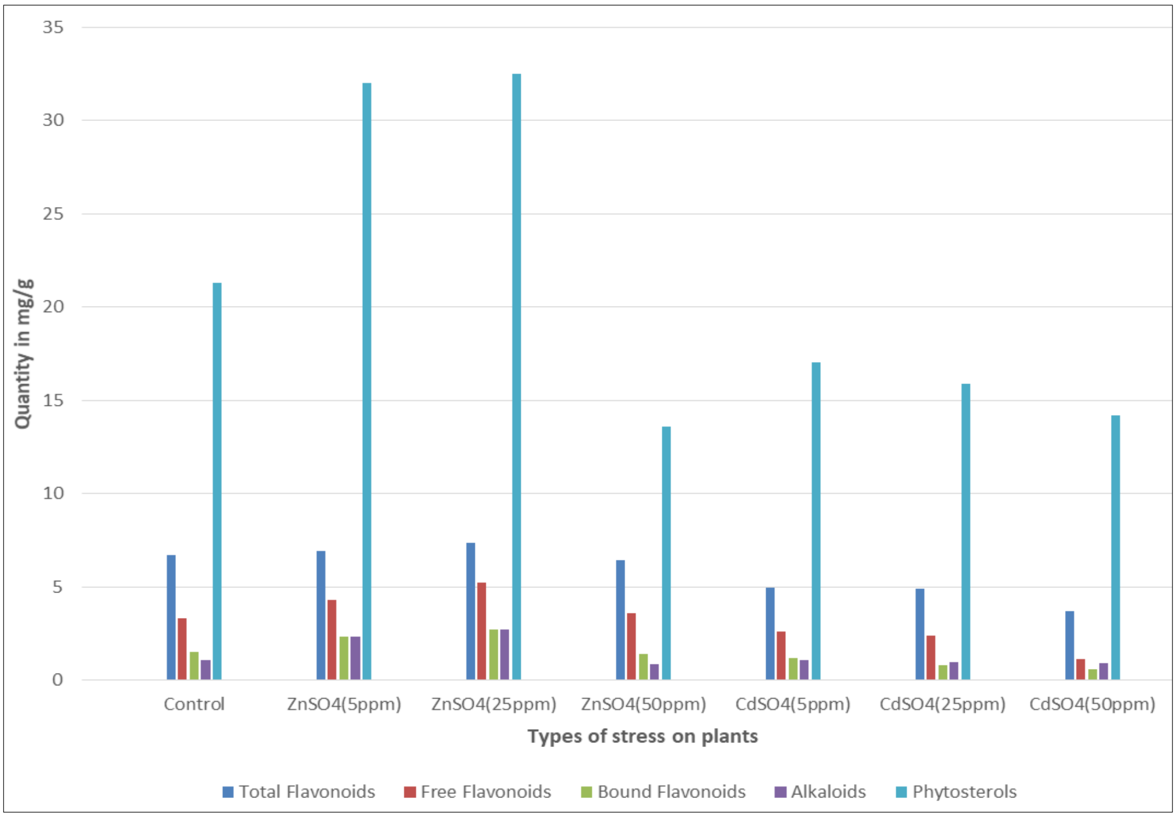

Fig 2: Effect of heavy metal stress on quantity of various Secondary metabolism in *Celosia argentea* after maturation.

Conclusion

Heavy metals accumulate in plant parts and alters their Phyto-constituents. In the present investigation Cd was found more phytotoxic metal as compared to Zn metal added individually in the soil. In this investigation we find that treatment of *Celosia argentea* plant with heavy metals (Zn and Cd) resulted in the altered quantity of TSS, Starch, Protein, Lipid and Phenols. Secondary metabolites also altered with greater concentration of heavy metals because of plant defense mechanism. So, we can conclude that Zn is essential metal for plant at low concentration but at increased concentration it becomes toxic. Cd is highly toxic then Zn metal.

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