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The impact of heavy metals on the physiological responses in *Chaemocostus cuspidatus*

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Abstract

Chamaecostus cuspidatus (insulin plant) is a medicinally important plant used in several medicines and as dietary supplements. Leaves of this plant have been used to treat diabetes since ancient times. Photosynthesis is a crucial aspect of plant physiology, ultimately affecting plant growth and metabolite production. In the current study, the plant was grown in controlled polyhouse conditions and treated with three heavy metals (Pb, Cr, and Cu). Five different concentrations (Pb and Cr- 50, 100, 150, 200, 250 ppm and for Cu- 25, 50, 75, 100, 125 ppm) of each metal were used for the treatment. Non-destructive methods were used for the study of physiological aspects of plants. CI-340 Handheld Photosynthesis System and CI-710s SpectraVue Leaf Spectrometer were used to measure approx 10 different parameters. Photosynthetic active radiation (PAR) was highest in Cu 100 (52.733 ± 0.466) treated plants. The highest Net photosynthetic rate (Pn) values were observed in Cr 200 (38.65 ± 0.384). The transpiration rate (E) was found to be highest in Cu 125 (0.846 ± 0.0202). Total chlorophyll content (CPHLT) and Chlorophyll Content Index (CCI) were also measured, and it was found to be highest in Cu 75 (30.344 ± 0.262) and Pb 150 (11.979 ± 0.231), respectively. Water Band Index (WBI), Normalized Difference Vegetation Index (NDVI), Anthocyanin Reflectance Index 2 (ARI 2), and Carotenoid Reflectance Index 2 (CRI 2) were also measured and analyzed for all the treatment groups along with control for each set. Statistical analysis represents significant differences among all the treated and control plants. These indices represent plant physiology, growth, and vegetative health of plants. Further biochemical and metabolite level studies can be done to further understand the effect of heavy metals on plant growth and metabolite production.

Keywords

Anthocyanin Reflectance Index 2 (ARI 2); Heavy metals; Net photosynthetic rate (Pn); Photosynthetic active radiation (PAR); Water Band Index (WBI);

Introduction

Chamaecostus cuspidatus (syn. *Costus igneus* N.E.Br. and *C. cuspidatus* (Nees & Mart.) Mass) is a perennial herbaceous medicinal plant in the Costaceae family (1-3). *C. cuspidatus* commonly referred to as the "insulin plant," a native of South and Central America that was only recently brought to India as an herbal remedy for diabetes (4). The perennial herbaceous plant *Costus igneus* is erect and has long branches that dangle over the ground. Leaves are simple, alternating, oblong with parallel venation (5). With numerous pharmaceutically significant properties, including antiinflammatory, anti-diabetic, antibacterial, antifungal, hypolipidemic, hypoglycemia, and anti-cancer properties, *C. cuspidatus* is a considerable plant (6). Insulin plants' leaves are frequently taken as dietary supplements that can decrease blood glucose levels and help to treat diabetes mellitus. Other plant parts like stem, root, and rhizome also have several other bioactive compounds like lupeol, stigmasterol, quercetin, diosgenin, sapogenin, terpenoids, and tannins having medicinal properties (7). Except for these compounds, corosolic acid an important triterpenoid is also found in insulin plants, this corosolic acid works on lipid metabolism and regulation of blood glucose in cells and out of the bloodstream (5).

Heavy metals are considered the most dangerous environmental contaminants since they are non-biodegradable, mobile, and have definite effects on humans and other species. Due to numerous industrial, agricultural, and human activities, they accumulate in the environment. Plants grown in heavy metals contaminated soil not only disturb plant growth but also disturb plant physiological and biochemical responses. Heavy metal content in the groundwater of the Peenya industrial sector of Bangalore was suggested in one of the earlier case studies of the same area. Six eco-toxic heavy metal concentrations were found to be Cr>Fe>Pb>Cu>Ni>Cd (8). Heavy metals have been demonstrated to have a detrimental effect on photosynthesis in plants by destroying chloroplasts, interfering with photosynthetic enzymes, and rupturing chloroplast membranes (9). By altering transpiration rate, stomatal function, and other variables, the build-up of heavy metals indirectly affects photosynthetic pigments and the photosynthetic process (9). Various characteristics, such as translocation properties, influence the absorption of heavy metals differently in different species of plants (10). The intricate interaction between physiological and molecular pathways underlies plant susceptibility to heavy metals (9). The development of photosynthetic pigments, proteins, and sugars are just a few examples of the metabolic processes and growth patterns that plants may be affected by heavy metals (11). Plants develop protective pigments called carotenoids and anthocyanins in response to heavy metal stress (12).

One of the most prevalent and common hazardous metals in soil, lead harms plants' morphology, growth, and photosynthetic activities (13). Lead is considered a possible human carcinogen because of its low solubility in the soil due to its complexation with organic materials (14). Lead can also prevent ions from attaching to transporters, lowering the amount of vital nutrients plants absorb and move (15). The relationship between nutrient translocation and uptake is also impacted by chromium poisoning, which can alter plant nutrient content and stunt development (16). Plants under oxidative stress caused by chromium eventually lose their ability to synthesize photosynthetic pigment, which results in a growth decline. Elevated levels of chromium disrupt both the photosynthetic mechanism and the structure of the chloroplast (17). Copper is needed for photosynthetic creation of reducing power, which is required for CO2 fixation, respiration, lignification, and pollen development. In earlier investigations, Cu was discovered to be the most potent inducer of anthocyanins and highly poisonous. While Pb was the most potent inducer of carotenoids, it induced the least anthocyanins. According to the previous study (12), anthocyanins may be the primary antioxidants for Cu-stressed Arabidopsis, whereas carotenoids are the primary protective agents for Pb-stressed Arabidopsis.

Different plants react to heavy metal stresses in various ways depending on the types, concentrations, and duration of metal stresses. Therefore, the goal of the current study was to evaluate the effects of three distinct heavy metals (Pb, Cr, and Cu) on the physiological characteristics and pigment levels of *C. cuspidatus*, an important therapeutic plant. There has not been much research done on plant physiological aspects such as photosynthesis, leaf stomatal conductance, transpiration rate, and other stress-indicating indexes thus far, particularly on how exposure to metal shocks affects pigment levels and the vegetative health of the plant. This study will mainly focus on the impact of three different heavy metals on the photosynthetic active radiation, leaf stomatal conductance, transpiration rate, net photosynthetic rate, chlorophyll content, stress, and vegetative health-related indexes. These parameters give a clear idea about plant health, physiology, and pigment contents in a non-destructive manner.

Materials and Methods

2.1. Plant growth and preliminary soil and water analysis: One-month-old small plantlets of *C. cuspidatus* were collected from the University of Agricultural Sciences, GKVK, Bangalore. Before transplantation, the soil was mixed with organic manure in 3:1, sterilized using an autoclave, and dried. 3 kg sterilized soil was filled in pots, and preliminary soil and irrigated water analysis was done before giving any external heavy metal treatment. Soil was tested for pH, EC, Organic carbon, Available Nitrogen, Available Phosphorus, Available Potassium, Exchangeable Calcium, Exchangeable Magnesium, and Available Sulphur. The same soil and irrigated water were also tested for heavy metals before any external heavy metal treatment. Wet acid digestion of soil and water was carried out using the modified procedure (18). An Atomic Absorption Spectrophotometer (AAS) (Shimadzu, AA-6880, Japan) was used for the quantification of heavy metals present in soil and water. One-month-old plantlets were transplanted in pots.

2.2. Experimental design and Heavy metal treatment: Three heavy metals, Chromium (Cr), Copper (Cu), and Lead (Pb), were selected for the treatment. Based on WHO/FAO permissible limits for soil (Table 1), 5 heavy metal concentrations were selected (Table 2). Cr in the form of Chromium (III) chloride hexahydrate 97% AR salt, Cu in the form of Cupric sulphate EP (crystals) (Copper (II) sulphate pentahydrate salt], and Pb in the

Table 1. WHO/FAO permissible limit for heavy metal in soil, water, and food (30-31).

Heavy metals	Soil (ppm)	Water (ppm)	Plants (ppm)	Consumed medicinal plants (ppm)
Cr	**100**	0.1	1.3	2
Pb	**85**	0.05	2	10
Cu	**36**	2	10	3 (for edible plants)

However, the WHO limits have not yet been established for medicinal herbs for copper.

Table 2. Selected heavy metals and their respective concentration (ppm)

Cr	Pb	Cu
50	50	25
100	100	50
150	150	75
200	200	100
250	250	125

form of Lead nitrate salt was used for treatment. Plants were treated with 5 concentrations of each heavy metal. 3 replicates were designed for each heavy metal concentration along with control (18 plants for each set). A total of 20 doses of heavy metal treatment were given at the seven-day interval for five months.

2.3. Photosynthetic measurements using CI-340 Handheld Photosynthesis System: Plant photosynthesis was measured in real-time using the CI-340 portable device (19) data were collected between 8:30 and 9:30 am, when sun radiation is most beneficial for plant photosynthesis (20). Four different parameters such as Photosynthetic Active Radiation (PAR), Net Photosynthetic Rate (Pn), Transpiration Rate (E), and Leaf Stomatal Conductance (C) were considered for precise photosynthetic estimation (21).

2.4. Non-destructive plant stress measurements using CI-710s SpectraVue Leaf Spectrometer: Using integrated indices, the SpectraVue Leaf Spectrometer evaluates transmission, absorption, and reflection in addition to a wide range of additional plant stress and pigment indicators (22). Total Chlorophyll (CHPLT), Chlorophyll content index (CCI), Normalized Difference Vegetation Index (NDVI), Water Band Index (WBI), Anthocyanin Reflectance Index 1 (ARI 1), Anthocyanin Reflectance Index 2 (ARI 2), Carotenoid Reflectance Index 1 (CRI 1), Carotenoid Reflectance Index 2 (CRI 2) are some indices that are considered in the present study. CHPLT and CCI give a clear demonstration of chlorophyll content in plants. NDVI and WBI are the vegetative indices that give the idea about plant health without damaging the plant. The value of the NDVI scale is from -1 to 1. The standard range for green vegetation is 0.2-0.8 and the standard scale for green vegetation is 0.8-1.2. ARI 1, ARI 2, CRI 1, and CRI 2 are the stress-related indices. The value of ARI 1 ranges from 0 to more than 0.2. The standard range from green vegetation is 0.001-0.1. The value of the ARI 2 scale from 0 to more than 0.2. the common range from green vegetation is 0.001-0.1. This index, which measures greater anthocyanin concentrations in plants, is an adaptation of the ARI 1. It takes advantage of the absorption fingerprints of stress-related pigments by measuring reflectance in the visible spectrum. The value of CRI 1 ranges from 0 to more than 15. The Common range for green vegetation is 11. The value of the CRI 2 scale from 0 to more than 15. The Common range for green vegetation is 11. This index is an improvement over CRI 1, which performs better in regions with elevated concentrations of carotenoids. Higher CRI2 readings indicate greater concentrations of carotenoid relative to chlorophyll.

To measure the variance in leaf pigments, including carotenoids, anthocyanins, total chlorophyll, and chlorophyll A and B, the CI-710s leaf spectrometer was used (23). To provide precise and consistent readings across all plants, the equipment probe was affixed to the top third leaf of every plant.

2.5. Statistical analysis: All the measurements were done in triplicates, and One-way ANOVA was used in the statistical analysis to determine the significance of differences in every measured variable. A Duncan's Multiple Range Test (DMRT) was conducted, with a significance level of $p<0.05$. This post hoc analysis facilitates the identification of certain variations between mean pairings. The outcomes showed that every evaluated parameter responded significantly to the heavy metal treatment.

Results and Discussion

3.1. Preliminary analysis of soil and irrigated water: Soil sampling was done from the pots, and soil analysis was done at the Indian Institute of Horticultural Research (IIHR). Results are illustrated in Table 3. Preliminary heavy metal analysis was also done for soil and irrigated water, results are represented in Table 4. The soil used for the experiment was fertile and suitable for plant growth, and there were not many heavy metals in the experimental soil and irrigated water before any external heavy metal treatment.

3.2. Photosynthetic indices: *C. cuspidatus* is a medicinally important plant and mainly its leaves are used as a dietary supplement to treat diabetes. Leaves are the primary organ for photosynthesis, stomatal conductance, and transpiration and this directly affects the plant growth and metabolite production which ultimately impacts the synthesis of most of the medicinally important bioactive compounds. *C. cuspidatus's* response to heavy metal treatment has led to notable variations in the plant's morphological and physiological traits. PAR was found to be highest in Cu 100 treated plants (52.733 ± 0.466), and PAR lowest values were observed in Cr 200 (12.933 ± 0.145) (Fig. 1). Net photosynthetic rate (Pn) values also varied

Table 3. Preliminary analysis of soil

Parameter	Unit	Values	General Optimum Range	Diagnosis
pH		6	6.50 – 7.50	Slightly Acidic
EC	dSm^{-1}	0.20	<1.00	Safe
Organic carbon	%	0.36	0.75 – 1.00	Low
Available Nitrogen	ppm	78.32	125 – 180	Low
Available Phosphorus	ppm	4.93	5- 10	Slightly lower than optimum
Available Potassium	ppm	60.00	62 – 125	Slightly lower than optimum
Exchangeable Calcium	ppm	1139.00	800 – 1500	Optimum
Exchangeable Magnesium	ppm	256.00	150 – 250	Slightly High
Available Sulphur	ppm	25.43	10 – 15	Slightly High

Table 4. AAS data of heavy metals present in soil and irrigated water before external heavy metal treatment

Sample	Cr (ppm)	Pb (ppm)	Cu (ppm)
Soil	1.0573	0.7253	0.6505
Water	0.2284	0.0763	0.1262

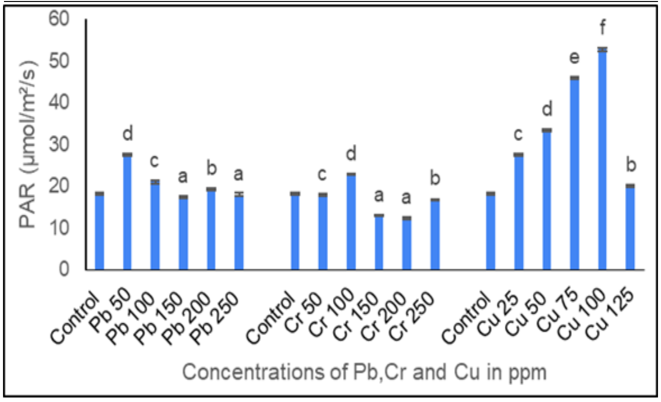

Fig 1. Photosynthetic active radiation (PAR) of Heavy metal treated plants. (Data represent mean values ± SE of 3 replicates; each experiment was repeated thrice. Means with common letters are not significantly different at P≤0.05 according to Duncan's multiple range test (DMRT). Pb: Lead, Cr: Chromium, Cu: Copper).

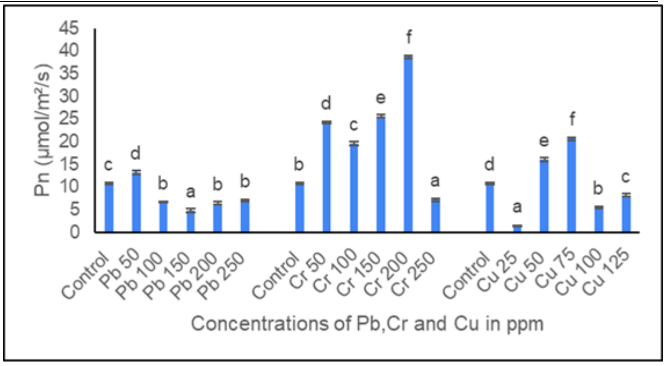

Fig. 2. Net photosynthetic rate (Pn) of Heavy metal treated plants (Data represent mean values ± SE of 3 replicates; each experiment was repeated thrice. Means with common letters are not significantly different at P≤0.05 according to Duncan's multiple range test (DMRT). Pb: Lead, Cr: Chromium, Cu: Copper).

with the heavy metal treatment, its highest value observed in Cr 200 (38.65 ± 0.384) and lowest was observed in Cu 25 (1.436 ± 0.043). The entire range for Pn is demonstrated in Fig. 2. PAR mainly depends on active radiation received on the chlorophyll membrane. Net photosynthetic rate which not only on radiation but also another factor in the photosynthesis process. Possibly excessive radiation harms photosynthetic organs, which lowers the activity of associated enzymes involved in photosynthesis and reduces the ability of mesophyll cells for photosynthetic processes (24). Low radiation may support Photosynthesis as results demonstrate for the Cr 200 plant where with the least PAR values highest Pn was observed. In a hybrid poplar, the photosynthetic rate dropped as the content of heavy metals increased. Increased levels of heavy metals also substantially impacted transpiration rates (9). In St. John's wort, the leaf net photosynthetic rate (Pn) rose when the PPF (different light intensity) and/or CO_2 concentration increased. The plants produced in the HH-treatment group had the most significant value of Pn. As Pn increased, so did the amounts of hypericin and pseudohypericin in the leaf tissues (25). Variation in Transpiration rate (E) was observed in Cu-treated plants. Its maximum value was observed in Cu 125 (0.846 ± 0.0202). Least transpiration rate was observed in Cu 50 (0.08 ± 0.005) (Fig. 3). Leaf stomatal conductance (C) was found to be highest in Pb 250 (39.756 ± 0.284), and its lowest value was also observed in Pb-treated plants only in the concentration of Pb 50 (6.253 ± 0.209) (Fig. 4). Under both individual and combined stress, the net photosynthetic rate was inhibited more when Pb and Cd contents increased. The intercellular CO_2 concentration can be utilized to identify stomatal versus nonstomatal effects. Previous research revealed that stomatal restriction is not the primary factor impacting Davidia involucrate's photosynthesis since the intercellular CO_2 concentration increased and the transpiration rate increased initially before decreasing with increasing Cd concentrations (26). Photosynthesis in plants is reliant on light, which influences several processes such as absorption capacity, enzymatic activity, stomata opening, and the growth of the photosynthetic apparatus. Stomatal conductance influences transpiration rate which ultimately affects primary metabolite production and ATP synthesis through photosynthesis (27).

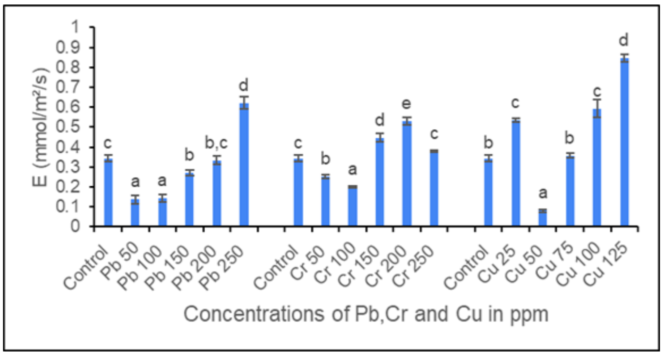

Fig. 3. Transpiration rate (E) of Heavy metal-treated plants (Data represent mean values ± SE of 3 replicates; each experiment was repeated thrice. Means with common letters are not significantly different at P≤0.05 according to Duncan's multiple range test (DMRT). Pb: Lead, Cr: Chromium, Cu: Copper).

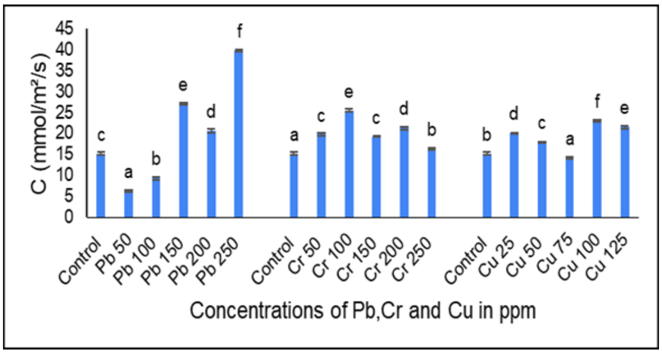

Fig. 4. Leaf stomatal conductance (C) of Heavy metal treated plants (Data represent mean values ± SE of 3 replicates; each experiment was repeated thrice. Means with common letters are not significantly different at P≤0.05 according to Duncan's multiple range test (DMRT). Pb: Lead, Cr: Chromium, Cu: Copper).

3.3. Leaf Spectrometer indices: Plants utilize carotenoids and chlorophyll, two pigments involved in photosynthetic energy conversion, to convert solar radiation into chemical energy. Plants can produce their nutrients because of these pigments. When the concentration level rises, chlorophyll and other pigment level also vary. In terms of how metals affect pigments, it is well known that high concentrations of heavy metals impair photosynthetic machinery and encourage the loss of photosynthetic pigments. In general, certain metals prevent plants from producing chlorophyll and carotenoid pigments. However, they have a more significant effect on chlorophyll production than on carotenoids (12). Total chlorophyll content (CPHLT) was found to be highest in Cu 75 (30.344 ± 0.262). The lowest value for CPHLT was observed in Cu 25 (14.659 ± 0.069) (Fig. 5). The Chlorophyll Content Index (CCI), an indicator for chlorophyll, was found to be highest in Pb 150 (11.979 ± 0.231). CCI lowest values were observed in Cr 50 (5.422 ± 0.234) (Fig. 6). In a previous study, Ferreyroaa et al., 2017 observed the effect of lead in *Brassica napus* plant, and they observed that at the Flowering stage, the presence of Pb increased chlorophyll content when compared to the control (28). At the Physiological Maturity stage, low Pb concentration caused a decrease in chlorophyll content, but at high Pb soil concentration, there were no discernible changes between the two groups. Lead is a hazardous metal. Plant roots take it up, store it, and sometimes even move it to the aerial biomass. Pb disrupts mineral nutrition and inhibits the functioning of numerous enzymes, impacting plants' growth, ultrastructure, and photosynthetic characteristics. Water band index (WBI) is an indicator and index for vegetative health and canopy stress analysis of plants. The highest value of WBI was observed in the control plant (1.196 ± 0.0024), and its lowest value was observed in Cu 125 (1.126 ± 0.0005) (Fig. 7). Data from WBI indicates that with the increasing heavy metal concentration plant vegetative health declined and its stress level increased. NDVI is another plant index that also represents the vegetative health of the plants its highest value was observed in Cu 100 (0.919 ± 0.002) its lowest value was found in Pb 150 (0.739 ± 0.0054) (Fig. 8). WBI is an indicator of the vegetative health of plants and WBI is found to be low in all the treated groups when compared with control.

Depending on the plant's kind, concentration, and species, metals can also increase the production of carotenoid pigments. Metal stress can cause the production of anthocyanins. Carotenoid Reflectance Index 2 was found to be highest in Cu 50 (0.077 ± 0.0003). CRI 2 lowest value was observed in control (0.0134 ± 0.0005) (Fig. 9). The highest value of Anthocyanin Reflectance Index 2 (ARI 2) was observed in Cu 50 (0.473 ± 0.003), and its lowest value was observed in Cu 75 (0.223 ± 0.0039) (Fig. 10). CRI 2 and ARI 2 both are found to be higher in all the treated groups when compared with the control. Both these indices are the indicator of stress and justify the heavy metal stress in all the samples. The anthocyanin pigment which gives plants their red, purple, and blue

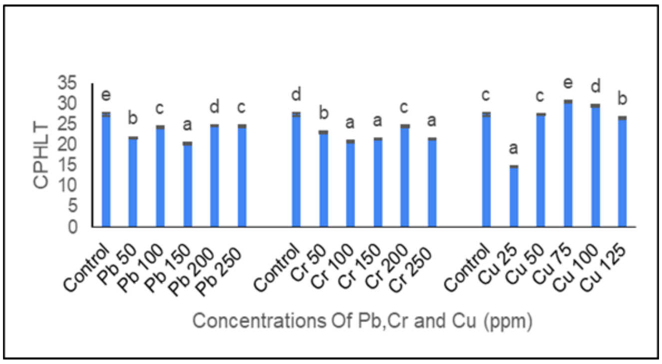

Fig. 5. Total Chlorophyll Content (CPHLT) in Heavy Metal Treated Plants (Data represent mean values ± SE of 3 replicates; each experiment was repeated thrice. Means with common letters are not significantly different at P≤0.05 according to Duncan's multiple range test (DMRT). Pb: Lead, Cr: Chromium, Cu: Copper).

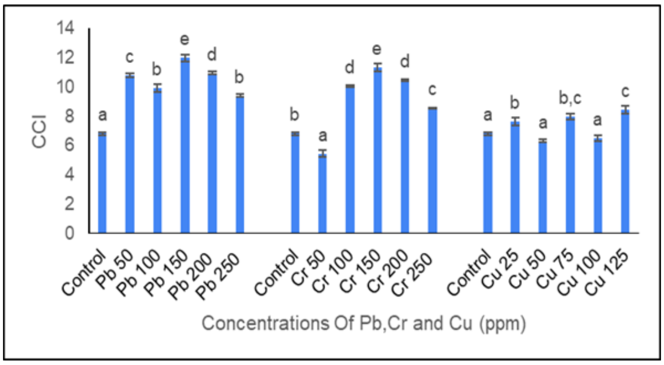

Fig. 6. Chlorophyll Content Index (CCI) of Heavy Metal Treated plants (Data represent mean values ± SE of 3 replicates; each experiment was repeated thrice. Means with common letters are not significantly different at P≤0.05 according to Duncan's multiple range test (DMRT). Pb: Lead, Cr: Chromium, Cu: Copper).

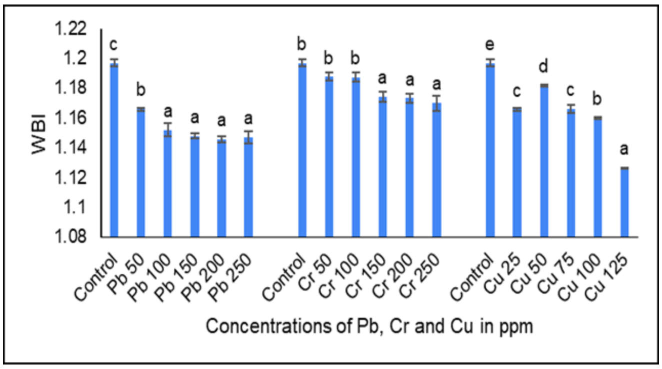

Fig. 7. Water band Index (WBI) of Heavy Metal Treated Plants (Data represent mean values ± SE of 3 replicates; each experiment was repeated thrice. Means with common letters are not significantly different at P≤0.05 according to Duncan's multiple range test (DMRT). Pb: Lead, Cr: Chromium, Cu: Copper).

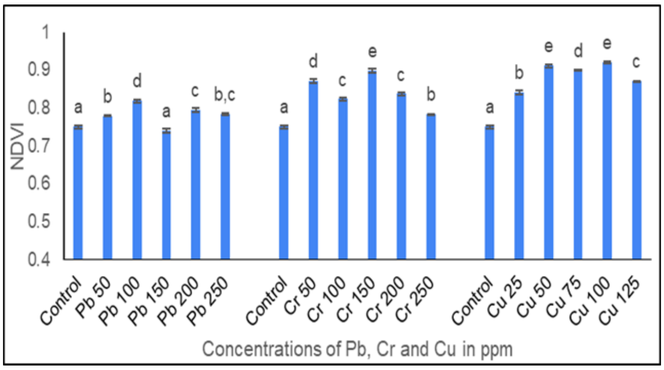

Fig. 8. Normalized Difference Vegetation Index (NDVI) of Heavy Metal Treated Plants (Data represent mean values ± SE of 3 replicates; each experiment was repeated thrice. Means with common letters are not significantly different at P≤0.05 according to Duncan's multiple range test (DMRT). Pb: Lead, Cr: Chromium, Cu: Copper).

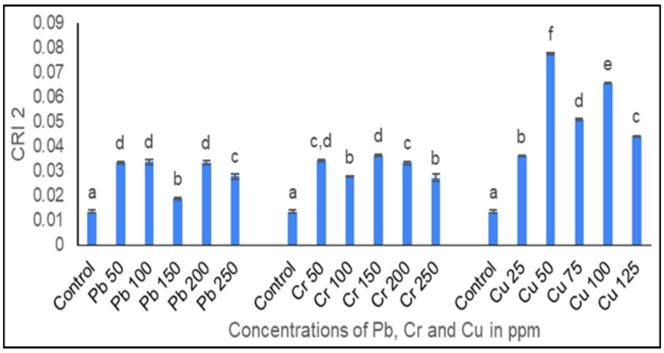

Fig. 9. Carotenoid Reflectance Index 2 (CRI 2) of Heavy Metal Treated Plants (Data represent mean values ± SE of 3 replicates; each experiment was repeated thrice. Means with common letters are not significantly different at P≤0.05 according to Duncan's multiple range test (DMRT). Pb: Lead, Cr: Chromium, Cu: Copper).

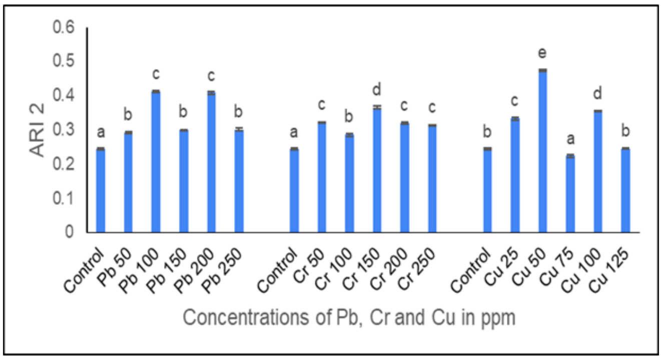

Fig. 10. Anthocyanin Reflectance Index 2 (ARI 2) of Heavy Metal Treated Plants (Data represent mean values ± SE of 3 replicates; each experiment was repeated thrice. Means with common letters are not significantly different at P≤0.05 according to Duncan's multiple range test (DMRT). Pb: Lead, Cr: Chromium, Cu: Copper).

hues, is especially sensitive to the ARI. Increased anthocyanin concentrations in plant tissues may be shown by higher ARI values. In a similar vein, carotenoid content affects the CRI, with higher green levels in the CRI corresponding to higher carotene concentrations. Furthermore, the primary determinant of indices such as NDVI is chlorophyll (29).

Conclusion

The present research conducted on *C. cuspidatus* clearly shows the effect of heavy metals on plant growth, photosynthetic ability, and pigment content in plants. The rate of photosynthesis was observed higher in some treated groups, possibly a defense mechanism for plants to elevate the stress condition. WBI values indicate the vegetative health and stress levels in treated groups. Anthocyanin and carotenoid pigments were also believed to an indicators for stress, which was found to be higher in all the treated plants when compared to the control. Without harvesting or harming the plant, this study clearly shows how heavy metals affect photosynthetic processes and other indices. It also provides insight into the physiological reactions of plants under stress conditions. These results emphasize how crucial it is to comprehend and lessen the impacts of heavy metal pollution in plants, particularly those used medicinally, to protect the effectiveness and purity of natural plant-based products. To further clearly understand the impact of heavy metals on plants detailed biochemical, antioxidant, and bioactive compound estimation can be done in future research. Genetic-level studies can give a clear insight into physiological and biochemical responses under stress conditions.

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Authors' contributions

VD did the preliminary work with plants and instruments including the statistical analysis. MBT has guided and helped in the usage of the instrument and the data analysis. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None.

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