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Influence of Heavy Metals (Cu, Cr, Zn) on Antioxidant Potential in *Emilia sonchifolia (L.) DC*

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Abstract - Heavy metal contamination poses significant threats to plant physiology and secondary metabolite production. This study investigated the effects of copper (Cu), chromium (Cr), and zinc (Zn) on the antioxidant defense system of *Emilia sonchifolia*, a medicinal plant with therapeutic potential. Hydroponically grown plants were exposed to metal concentrations ranging from 25–125 ppm for 30 days. Antioxidant responses were evaluated through DPPH radical scavenging assays and superoxide dismutase (SOD) activity measurements. Results revealed metal-specific and concentration-dependent effects. Cu exhibited a hormetic response, with peak antioxidant activity at 50 ppm (lowest IC50 = 4.77 µg/mL; highest SOD activity = +42% vs control), followed by decline at higher concentrations. Cr demonstrated consistently toxic effects, with antioxidant capacity progressively decreasing (IC50 increased 4.4-fold at 125 ppm) and SOD activity declining by 62% at highest exposure. Zn showed optimal enhancement at 50–75 ppm (IC50 = 5.87 µg/mL; SOD +33%), but reduced efficacy beyond 100 ppm. Two-way ANOVA confirmed significant interactive effects of metal type × concentration ($p < 0.001$). These findings demonstrate that while moderate Cu and Zn can stimulate antioxidant defenses, Cr is uniformly detrimental, providing critical insights for cultivating medicinal plants in metal-contaminated environments and assessing their pharmacological potential under stress conditions.

Key Words: heavy metal stress, oxidative stress, medicinal plants, hydroponics, hormesis, phytoremediation

1.INTRODUCTION

Emilia sonchifolia is a herbaceous species within the Asteraceae family, is commonly referred to as the lilac tasselflower or cupid's shaving brush and it is traditionally used in Ayurveda for treatment of fever and respiratory ailments due to its therapeutic and antioxidant properties.

With escalating environmental contamination—particularly from heavy metals such as copper (Cu), chromium (Cr), and zinc (Zn)—there is a pressing need to evaluate how such pollutants influence plant physiology and secondary metabolite synthesis. While Cu and Zn are essential micronutrients required for various biochemical functions, their accumulation beyond physiological thresholds can disrupt cellular homeostasis and impair endogenous defense mechanisms. In contrast, hexavalent chromium (Cr VI) is highly toxic, exerting deleterious effects even at minimal concentrations.

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging test and superoxide dismutase (SOD) activity are commonly used teqniqes to asses the antioxidant activity of plants. These methods provide insights into the plant's ability to neutralize reactive oxygen species (ROS), which tend to accumulate under stress conditions. Excessive ROS generation, typically a consequence of disrupted redox equilibrium, signifies the onset of oxidative stress, which may compromise cellular integrity and metabolic function.

To explore the specific impact of Cu, Cr, and Zn on *E. sonchifolia*, plants were cultivated under hydroponic system in controlled conditions— hydroponic culture helps to eliminates soil-mediated variability and ensures precise control over metal exposure. The experimental design incorporated graded concentrations of each metal (25–125 ppm), administered over a 30-day period. This range encompasses both sub-lethal and potentially phytotoxic doses, facilitating the identification of thresholds where antioxidant activity may be either upregulated or inhibited.

By quantifying DPPH radical scavenging efficiency and SOD enzyme activity, the study aims to elucidate how differing metal concentrations influence oxidative stress responses and antioxidant defense in *E. sonchifolia*. Notably, the findings are expected to shed light on the paradoxical role of heavy metals—as both stress inducers and possible elicitors of secondary metabolite production. Beyond the immediate scope, these results have broader implications. Understanding the metal tolerance and adaptive responses of medicinal plants such as *E. sonchifolia* contributes to optimizing growth conditions for phytopharmaceutical applications while simultaneously informing ecological strategies related to metal contamination. Moreover, this investigation may serve as a foundational reference for subsequent studies examining heavy metal interactions in other botanicals of pharmacological significance, particularly in the context of phytoremediation and sustainable herbal medicine practices

2 . Materials and Methods

Plant Material and Growing Conditions

Seeds of *Emilia sonchifolia* were collected from healthy, mature plants growing within the botanical garden of Sree Narayana College, Nattika, Thrissur, Kerala, India (10.4227° N, 76.1065° E). To ensure sterility, the seeds were surface-disinfected using a 1% sodium hypochlorite solution for five minutes, followed by multiple rinses with distilled water. Germination was initiated in plastic trays containing a mixture of soil and vermiculite in a 3:1 ratio. After ten days of growth, uniformly developed seedlings were carefully transferred to a hydroponic system for subsequent heavy metal exposure.

Experimental Setup

Plants were exposed to a gradient of metal concentrations—specifically, 25, 50, 75, 100, and 125 ppm—using salts of copper ($CuSO_4{\cdot}5H_2O$), chromium ($K_2Cr_2O_7$), and zinc ($ZnSO_4{\cdot}7H_2O$). These salts were dissolved in Hoagland nutrient solution to prepare metal-specific treatment media. A hydroponic setup was employed for a 30-day exposure period, during which the nutrient solutions were refreshed every three days to maintain consistent metal concentrations and nutritional balance. Control plants were grown exclusively in Hoagland medium, devoid of any added heavy metals. All treatments, including the control, were conducted in triplicate under controlled indoor environmental conditions.

Sample Collection

At the end of the 30-day treatment period, plants were harvested for biochemical assays. To eliminate surface contaminants, plant samples were rinsed thoroughly with distilled water and gently dried using tissue paper. Samples were then flash-frozen and stored at −80 °C until further analysis.

DPPH Radical Scavenging Assay

The antioxidant activity was calculated by the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method. About 50 µL of methanolic extract was mixed with 200 µL of 0.1 mM DPPH solution in methanol and incubated in the dark for 30 minutes. After the incubation absorbance was recorded at 517 nm. The percentage inhibition of DPPH was calculated using the following formula:

$$\text{Inhibition } \% = \frac{(A\ \text{control} - A\ \text{sample})}{A\ \text{control}} \times 100$$

where A control is the absorbance of the DPPH solution without the extract, and A sample represents the absorbance of the mixture containing the extract.

IC50 value of each metal treatments were calculated

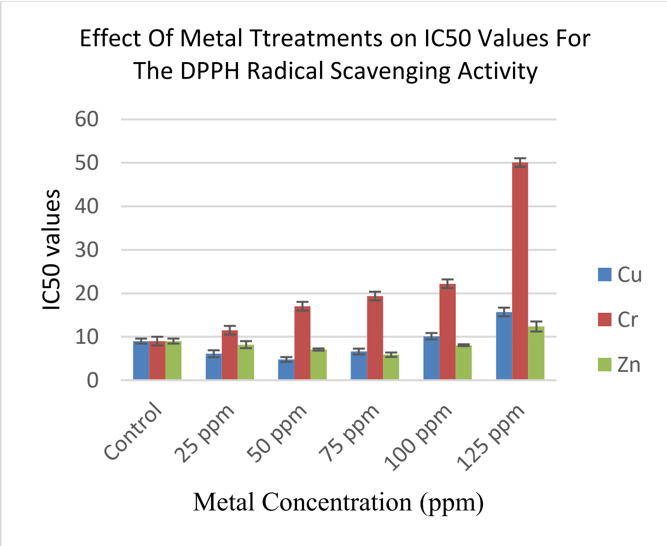
Enzymatic Antioxidant Activity (SOD)

Superoxide dismutase (SOD) activity was quantified using a spectrophotometric assay based on the inhibition of nitroblue tetrazolium (NBT) photoreduction. For this, 0.5 g of fresh leaf tissue from each treatment group and the control was homogenized in 5 mL of ice-cold 50 mM phosphate buffer (pH 7.8) containing 1 mM EDTA. The homogenate was centrifuged at 12,000 × *g* for 15 minutes, and the supernatant was used for the assay. Absorbance was recorded at 560 nm. One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of NBT reduction under the assay conditions.

Statistical Analysis

All experimental treatments were performed in triplicate. Results were expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was employed to determine statistically significant differences among treatment groups .Duncan analysis were done and visualization of Duncan analysis was done using GGplots, R studio was used to do all statistical analysis

Charts **-1:** Effect Of Metal Treatments on IC50 Values For The DPPH Radical Scavenging Activity



The antioxidant response of *Emilia sonchifolia*, assessed via the DPPH radical scavenging assay and expressed as IC_{50} values (the extract concentration required to neutralize 50% of DPPH radicals), revealed metal-specific and concentration-dependent trends under Cu, Cr, and Zn exposure (Table 1).

Copper (Cu) treatment elicited a biphasic (U-shaped) response. The antioxidant efficiency peaked at 50 ppm, where the lowest IC_{50} value of 4.77 µg/mL was recorded—indicating the strongest radical scavenging activity at this concentration. However, a gradual increase in IC_{50} was noted with further elevation in Cu levels, culminating in a value of 15.7 µg/mL at 125 ppm. This pattern suggests that while moderate Cu may enhance antioxidant responses, excessive levels likely trigger oxidative stress, thereby reducing the plant's scavenging efficiency.

In contrast, chromium (Cr) exposure led to a consistent and marked decline in antioxidant activity. IC_{50} values rose steadily from 11.5 µg/mL at 25 ppm to 50.07 µg/mL at 125 ppm. This linear increase indicates a dose-dependent inhibition of the plant's radical neutralization capacity, which may be attributed to Cr-induced disruption of antioxidant mechanisms and heightened oxidative burden.

Zinc (Zn) treatment exhibited a more complex pattern. At moderate concentrations (50–75 ppm), a noticeable enhancement in antioxidant activity was observed, with IC_{50} values dropping to 7.07 and 5.87 µg/mL, respectively. However, this trend reversed at higher concentrations, as the IC_{50} rose to 12.37 µg/mL at 125 ppm. These findings suggest that Zn, like Cu, may exert a beneficial effect on the plant's antioxidant system at low levels, but becomes detrimental when supplied in excess.

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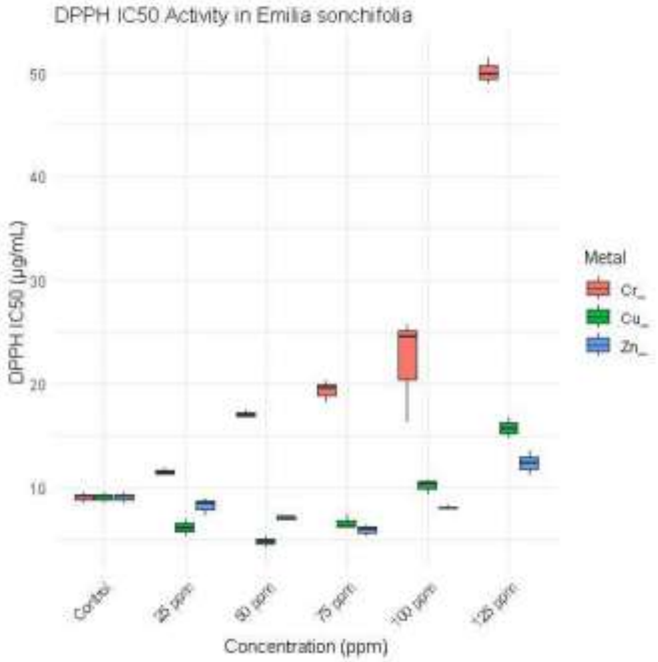
Table-1 ANOVA Result - Effect Of Metal Treatments on IC50 Values For The DPPH Radical Scavenging Activity

Source of Variation	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
Metal	2	2014.7	1007.3	508.69	< 2e-16
Concentration	5	2004.4	400.9	202.44	< 2e-16
Metal × Concentration	10	1593.5	159.4	80.47	< 2e-16
Residuals	36	71.3	2		

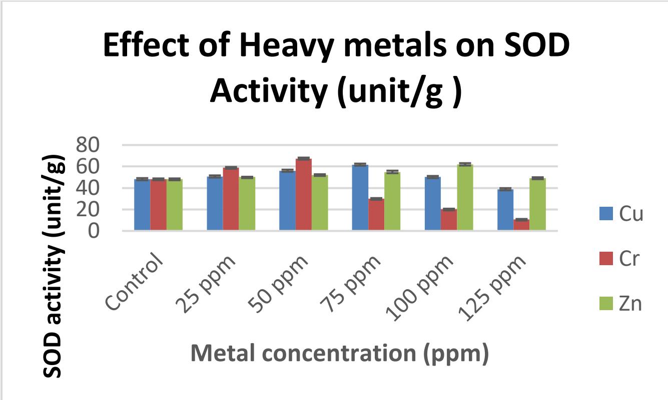
The ANOVA results reveal that all three factors—**metal type**, **concentration**, and their **interaction**—have a statistically significant effect on the IC50 values of the treated samples. The metal type showed a highly significant effect ($F = 508.69$, $p < 0.001$), indicating that different metals significantly influence the antioxidant capacity (IC50). Similarly, the concentration of the metals also had a significant effect ($F = 202.44$, $p < 0.001$), suggesting that varying concentrations of metals impact IC50 values differently. Importantly, the interaction between metal type and concentration was also significant ($F = 80.47$, $p < 0.001$), implying that the effect of metal concentration on IC50 varies depending on the type of metal used. The relatively low residual mean square (2.0) indicates that the model explains most of the variation in the data. Overall, these findings suggest that both the type and dose of heavy metals significantly affect the antioxidant response in the test samples, and their combined influence is not simply additive but interactive.

Charts -2 The Duncan multiple range test for Effect Of Metal Treatments on IC50 Values For The DPPH Radical Scavenging Activity



The Duncan analysis of DPPH scavenging activity (IC50) in *Emilia sonchifolia* revealed distinct concentration-dependent responses across the three metals (Cr, Cu, Zn). For **Cr**, antioxidant activity significantly decreased with rising concentrations, showing the highest IC50 at 125 ppm, indicating severe oxidative stress. **Cu** exhibited a biphasic response, with optimal scavenging at moderate concentrations (25–75 ppm) but reduced efficacy at higher doses (100–125 ppm), suggesting a threshold for beneficial effects. **Zn** demonstrated the strongest antioxidant enhancement at 75 ppm (lowest IC50), though this declined sharply at 125 ppm. The control group consistently showed intermediate activity, confirming that metal exposure significantly alters antioxidant capacity. These results highlight metal-specific toxicity thresholds and the potential for moderate Cu/Zn exposure to stimulate antioxidant defenses in *E. sonchifolia*.

Charts -3 : SOD Activity Response to Heavy Metal Stress in E.sonchifolia



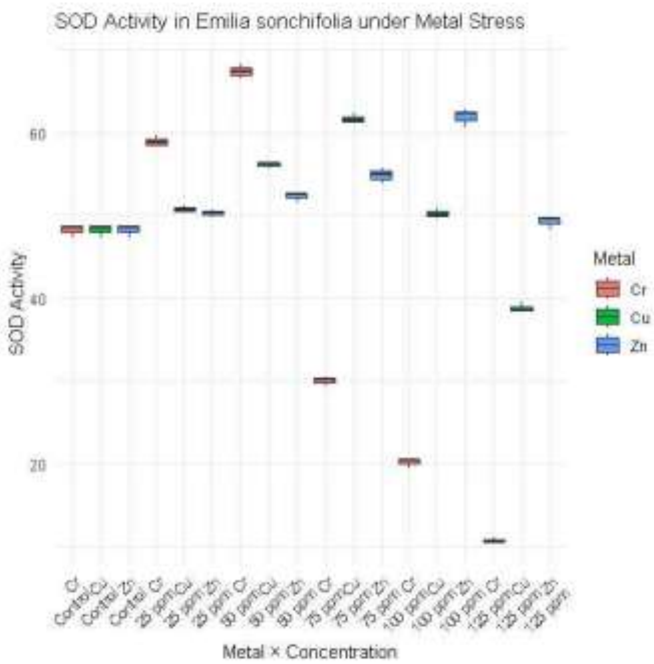
Superoxide dismutase (SOD) activity in *Emilia sonchifolia* exhibited distinct metal-specific responses to Cu, Cr, and Zn exposure. Copper stimulated SOD activity at moderate concentrations (peaking at 50 ppm with a 42% increase), but higher doses (100–125 ppm) suppressed activity by 15–22%, indicating a threshold for beneficial effects. Chromium consistently inhibited SOD across all tested concentrations, with reductions escalating from 18% (25 ppm) to 62% (125 ppm), demonstrating its potent toxicity. Zinc showed a biphasic effect, enhancing SOD activity by 25–33% at 50–75 ppm, but only marginally (8%) at 125 ppm, suggesting optimal Zn levels are critical for maintaining antioxidant defenses. These patterns highlight how metal type and concentration differentially regulate oxidative stress responses in *E. sonchifolia*.

Table-2 ANOVA Result - SOD Activity Response to Heavy Metal Stress in E.sonchifolia

Source	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
Metal	2	1937	968.5	1968	<2e-16
Concentration	5	3440	688	1398	<2e-16
Metal × Concentration	10	5396	539.6	1097	<2e-16
Residuals	36	18	0.5		

The ANOVA results for SOD activity in *Emilia sonchifolia* under different metal treatments reveal highly significant effects of metal type, concentration, and their interaction on SOD activity levels ($p < 0.001$ for all). The main effect of metal ($F = 1968$) and concentration ($F = 1398$) indicates that both the type of metal (Cu, Cr, Zn) and the level of concentration significantly influence antioxidant enzyme activity. Additionally, the very high F-value for the interaction term ($F = 1097$) suggests a strong interactive effect, meaning that the impact of each metal on SOD activity depends on its concentration. The low residual variance further confirms that the model explains the majority of the variation in the data, highlighting the sensitivity of SOD activity to both metal stress and dosage levels.

Charts -4 : The Duncan multiple range test for SOD Activity Response to Heavy Metal Stress in E.sonchifolia



The Duncan multiple range test for SOD activity under different metal treatments reveals statistically significant differences among the combinations of metals and concentrations. The highest SOD activity was observed in plants treated with Cr at 50 ppm (67.40), forming a distinct group ("a"), indicating a strong stimulatory effect of Cr at this concentration. This was followed by Zn at 100 ppm and Cu at 75 ppm, which also significantly increased SOD activity and formed group "b", suggesting moderate enhancement. Treatments with Cr at 25 ppm, Cu at 50 ppm, and Zn at 75 ppm showed progressively lower activity and belonged to separate statistical groups ("c", "d", "e"). Control treatments for all metals (Cr, Cu, Zn) clustered together in group "i", confirming similar baseline SOD levels in the absence of metal stress. Interestingly, the lowest activities were seen in Cr at 100 ppm and Cr at 125 ppm, which formed distinct lower groups ("l" and "m"), indicating possible oxidative stress and enzyme inhibition at higher Cr concentrations. Overall, the interaction between metal type and concentration plays a significant role in modulating SOD activity, with Cr showing both the most stimulatory and most inhibitory effects depending on the dose.

3. CONCLUSIONS

This study demonstrates that *Emilia sonchifolia* exhibits distinct, concentration-dependent antioxidant responses to Cu, Cr, and Zn stress, with Cu and Zn showing hormetic effects at moderate concentrations (25-75 ppm) through enhanced DPPH scavenging and SOD activity, while Cr consistently suppressed antioxidant capacity across all tested levels. The biphasic responses to Cu and Zn reveal an optimal range for beneficial stress priming, beyond which oxidative damage occurs, whereas Cr's linear toxicity highlights its greater phytotoxic risk. These findings provide critical insights for cultivating *E. sonchifolia* in metal-contaminated environments, suggesting its potential for phytoremediation at sub-toxic metal levels while cautioning against Cr exposure. The results further underscore the importance of species-specific metal thresholds in medicinal plants, with implications for both ecological restoration and the sustainable production of plant-based therapeutics under abiotic stress conditions.

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