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Effect of heavy metals on the pigmentation and photosynthetic capability in *Jacobaea maritima* (L.) Pelser & Meijden

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Abstract

Photosynthesis is a fundamental process in plants that enables them to produce their own food. However, this process can be influenced by multiple factors including external factors such as sunlight, nutrients availability and gas concentrations. The present study aimed to investigate the effects of heavy metal stress on the plant *Jacobaea maritima* (L.) Pelser & Meijden. Three different heavy metals, namely cadmium, chromium, and lead, were applied to the plants at five concentrations ranging from 50-250 ppm (50, 100, 150, 200, and 250). The growth of the plants was observed, and several parameters including net photosynthetic rate (Pn), transpiration rate (E), leaf stomatal conductance (C), and the photosynthetic active radiation (PAR) were measured. The results revealed that the chlorophyll content was higher in the Cr150 concentration (5.47±0.4). The chlorophyll values for Pb-100 (9.4±0.35) and Pb-250 (9.8±0.26) were in close proximity to each other. The Cd-100 concentration showed the highest chlorophyll content. The net photosynthetic rate was least affected in Pb-150 (30.98±0.75), while Cr-100 (4.05±0.09) exhibited the greatest impact. Transpiration rate increased slightly in plants treated with Pb, but significantly decreased in Cd-treated plants. The Cr-50 concentration (0.19±0.02) showed the lowest transpiration rate. Leaf stomatal conductance was reduced significantly in all treated plants, with Cr-100 showing the least variation (2298.25±1.85). The photosynthetic active radiation capability was reduced in all treated plants, with Pb-treated plants exhibiting nominal reduction and Cd- and Cr-treated plants experiencing substantial reduction. Statistical analysis confirmed significant variations in the measured parameters following heavy metal treatment.

Keywords

Heavy Metals; *Jacobaea maritima*; Leaf stomatal conductance; Photosynthetic rate; Photosynthetic active radiation; Transpiration rate

Introduction

The plant *Jacobaea maritima*, also known as *Cineraria maritima,* belongs to the family Asteraceae and is commonly referred to as silver dust or dusty miller (1). It is an evergreen shrub that can grow up to 15-60 cm in height. The flowering season occurs from June to August, with seed ripening taking place from July to September (2). Insect pollination is commonly seen due to its hermaphrodite flowers. The leaves are slivery and tomentose. *Jacobaea maritima* is widely used in the homeopathic system of medicine for treating ophthalmological disorders, and is also cultivated as an ornamental plant in many countries (3).

Heavy metals have been shown to negatively affect photosynthesis in plants by damaging chloroplasts, interfering with photosynthetic enzymes, and disrupting chloroplast membranes (4). The accumulation of heavy metals indirectly impacts photosynthetic pigments and the photosynthesis process through changes in stomatal functioning, transpiration rate, and other factors (4). The uptake of heavy metals varies among different plant species, influenced by various factors, including translocation properties (5). Plant sensitivity to heavy metals involves a complex interplay of physiological and molecular mechanisms (4). Heavy metals can potentially influence plant growth patterns and metabolic activities, such as the production of photosynthetic pigments, proteins, and sugars (6). Under stress conditions, including heavy metal stress, plants produce pigments such as anthocyanins and carotenoids, which act as protective agents (7).

The main objective of the present study is to assess the impact of three heavy metals (cadmium, chromium, and lead) on the pigmentation of *Jacobaea maritima*, including chlorophyll, carotenoids, and xanthophylls. Additionally, the photosynthetic ability, transpiration efficiency, leaf stomatal conductance, and photosynthetic active radiation, which play an important role in many plant processes throughout the lifecycle.

The CI-710 SpectraVue is a powerful spectrophotometer equipped with a leaf probe attachment, onboard operating software, and a display screen. There are two broadband light sources attached in this device. One is positioned in the leaf clamp for transmissive measurements and the other is placed inside the case for reflective measurements. This is capable of taking readings in three main aspects, which are transmittance, absorbance, and reflectivity. While most spectrometers are used to measure transmission and absorption parameters, the CI-710 SpectraVue is particularly suitable for measuring photochemical reactions like photosynthesis, color analysis, and some of the optical properties like the thickness of the film, refraction index, etc. Reflectivity refers to the fraction of incident light reflected from the leaf sample, allowing for the measurement of absorption spectra of surfaces and opaque samples (8).

On the other hand, the CI-340 is specifically designed to measure the photosynthesis in plant at a particular instance; it takes the parameters like stomatal conductance, transpiration rate, etc. into consideration for measuring the net photosynthesis. This system offers two options: an open system that takes in surrounding atmospheric air for circulation and a closed system that recirculates air within the chamber and system (9). The use of this instrument with a chamber that has a leaf area of 6.5 cm^2 has given clarity into how the movement of the gases and air and leaf temperature directly influence net photosynthetic rate and other parameters. Chromium toxicity primarily affects plant growth by inducing ultrastructural changes in chloroplasts and cell membranes, reducing photosynthetic pigments and nitrogen assimilation, and altering the activities of various enzymes (10). Chromium toxicity also affects the interaction between the nutrient translocation and their uptake, leading to change in the nutrient composition of plants and inhibited growth (11). Cadmium is a highly toxic heavy metal of significant concern to the environment, plants, animals, and humans. The excessive presence of cadmium is often attributed to the use of inexpensive contaminated phosphate fertilizers (12). Cadmium, which is nonessential for plant growth, exerts toxic effects such as leaf chlorosis, root decay, and growth inhibition (13). It exhibits a broad range of toxicity effects on plants during both vegetative and reproductive stages (14). Lead, one of the most widespread and common toxic metals in soil, adversely affects plants in various ways, including growth, photosynthetic processes, and morphology (15). Being limited in solubility in soil due to complexation with organic matter, lead is considered a potential carcinogen for humans (16). Lead can also inhibit the binding of ions to transporters, thereby reducing the absorption and transportation of essential nutrients in plants (17).

Materials and Methods

Collection of Plants

Jacobaea maritima (L.) Pelser & Meijden was purchased from the Centre of Medicinal Plants Research in Homeopathy (Central Council for Research in Homeopathy), Nilgiris district, Tamil Nadu, India, in November 2019. The same has been identified and supplied as *Cineraria maritima* (L.) L. *Cineraria* synonyms: *Jacobaea maritima* (L.) Pelser & Meijden, *Cineraria maritima* L. belongs to the (Asteraceae). A certificate for the purchase and identification (F.No. 6-7/2021-22/ CMPRH/Tech/253) of this plant has been issued by the Officer In-Charge Centre of Medicinal Plants Research in Homeopathy (Central Council for Research in Homeopathy) Emerald, Tamil Nadu, India (18).

Heavy metal treatment

The plants used in the study were subjected to heavy metal treatment, utilizing five different concentrations of Cd, Cr, and Pb. These heavy metals are considered nonessential for plant growth (19). The concentrations used were 50, 100, 150, 200 and 250 ppm, respectively. Cadmium chloride salt was used for Cd treatment, chromic sulfate salt for Cr treatment, and lead acetate salt for Pb treatment. A total of 15 pots were maintained for each metal, including triplicates and a control plant. The plants were three months old at thetime of cultivation from stem cuttings. Heavy metal solutions were applied to the plants every 7^{th} day, while regular water was provided in between to avoid sudden stress. The plants underwent five treatment cycles, and changes were observed accordingly (20). It is known that higher concentrations of heavy metals can have toxic effects on plant growth and development (19). Heavy metal stress inhibits various leaf enzymes involved in chlorophyll synthesis, resulting in a decrease in chlorophyll content (21). Previous studies have demonstrated the significant impact of heavy metal toxicity on seed germination, plant growth, and root elongation (7). Readings were taken after the completion of each treatment cycle, specifically one week after the cycle ended.

CI- 710s and CI-340

The CI-710s leaf spectrometer was employed to assess the variation in leaf pigments such as chlorophyll A, chlorophyll B, total chlorophyll, carotenoids, anthocyanins, and others (22). The equipment probe was attached to the fourth leaf of each plant to ensure consistent and accurate readings across all plants. Significant changes in the pigmentation index were observed as the concentration of heavy metals increased compared to the control plant. Negative values were obtained for pigments that are not typically present in plants. To capture readings with the instrument, the leaf of interest was placed in the leaf clamp, and the command was initiated from the instrument's screen.

For the measurement of real-time photosynthesis in plants, the CI-340 handheld system was utilized, specifically recording readings between 8:30-9:30 am when solar radiation is optimal for plant photosynthesis (23, 22). Recent studies have highlighted the strong correlation between atmospheric CO_2 levels and the net photosynthetic rate (Pn) (24). Environmental factors such as CO_2 concentration and light intensity not only impact photosynthesis but also have the potential to influence metabolite production (25). The stomatal conductance of the plant was also analyzed, as it plays a crucial role in various plant processes including organic matter accumulation, metabolism, nutrient absorption, and water use (26). Stomata are considered potential indicators of environmental pollution and climate change due to their characteristics. Both pieces of equipment were utilized at the appropriate time and location on the plant to ensure accurate and reliable results.

Statistical analysis was conducted using one-way ANOVA to assess the significance of variations in all measured variables. Duncan's Multiple Range Test (DMRT) was performed, considering $p<0.05$ as the threshold for significance. This post hoc test helps identify specific differences between pairs of means. The results revealed that all measured parameters exhibited significant variations in response to the heavy metal treatment (27).

Result

The treatment of heavy metals in *Jacobaea maritima* has resulted in significant differences in various morphological and physiological characteristics of the plant. The CI-340 instrument was used to measure the net photosynthetic rate (Pn), transpiration rate, leaf stomatal conductance, and photosynthetic active radiation. The highest Pn was observed in the Pb-150 ppm concentration (30.98±0.5), while the least was observed in Cr-250 ppm (0.75±0.12) (Figure 1). Transpiration rate was found to be high in all concentrations of Pb and significantly lower in all concentrations of Cd (Figure 2). Leaf stomatal conductance was highest in Cr-100 ppm (2298.25±0.54), while it was negligible in other concentrations and heavy metals (Figure 3). Photosynthetic active radiation was higher in Pb-treated plants compared to Cd and Cr-treated plants, except for Cr-250 ppm (63.66±0.45) (Figure 4). These results confirm the impact of heavy metal treatment on the mentioned plant characteristics.

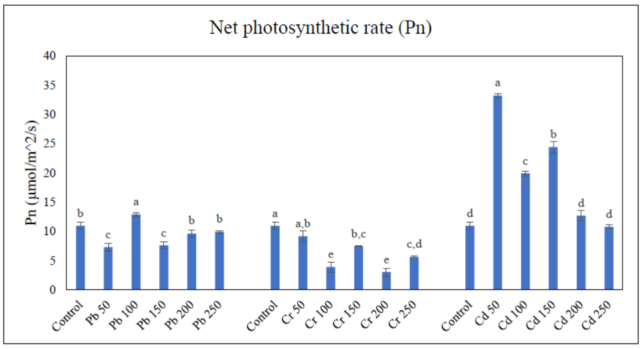

Figure 1. **Net Photosynthesis Rate (Pn)** in Heavy Metal Treated Plants
a – showing the highest value and e – showing the least value

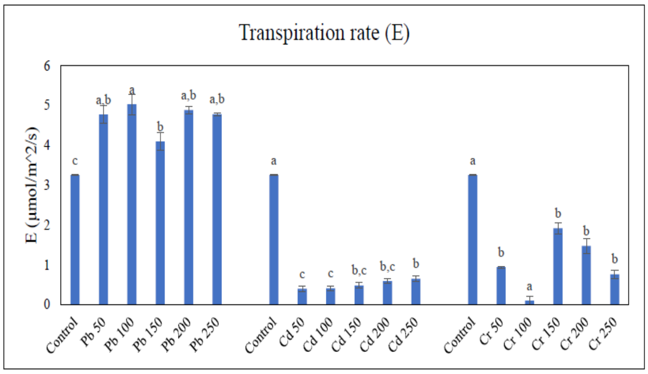

Figure 2. **Transpiration Rate (E)** in Heavy Metal Treated Plants
a – showing the highest value and c – showing the least value

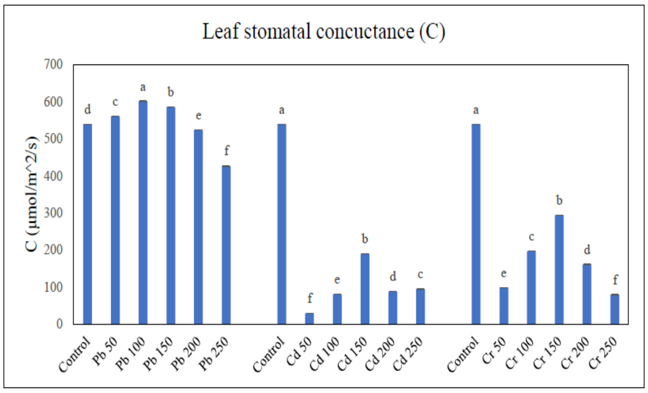

Figure 3. **Leaf Stomatal Conductance (C)** in Heavy Metal Treated Plants
a – showing the highest value and f – showing the least value

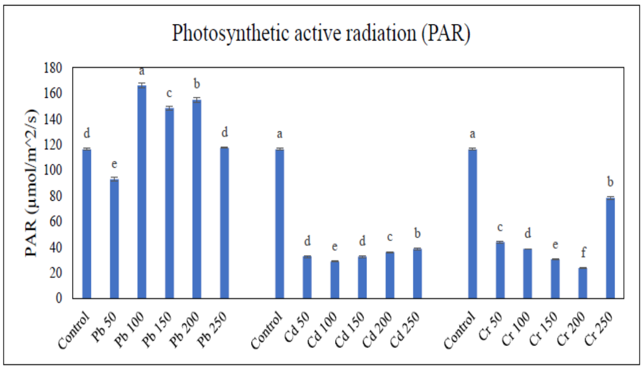

Figure 4. **Photosynthetic Active Radiation (PAR)** in Heavy Metal Treated Plants a – showing the highest value and e – showing the least value

The CI-710s leaf spectrophotometer was used to measure the variation in the chlorophyll pigmentation in the leaves. The chlorophyll showed a significant variation from the control plant to the different heavy metal-treated plants. In chromium treated plants, Cr-150 ppm (5.47±0.25) showed the highest amount of chlorophyll, whereas the plant treated with Cr-50 ppm (0.69±0.12) showed the least chlorophyll. In plants treated with Cd, Cd -100 ppm displayed the highest chlorophyll content, whereas Cd-250 ppm showed the least. In Pb-treated plants, Pb-100 ppm and Pb-250 ppm showed high chlorophyll content, while others exhibited substantial differences. Overall, Cr-150 ppm, Cd-100 ppm, and Pb-100 ppm and 250 ppm demonstrated positive results in chlorophyll content in *Jacobaea maritima* (Figure 5).

Among the heavy metals, only Cr-250 ppm showed a slight increase in anthocyanin levels (≥0.13), while the least was observed in Pb-250 ppm (0.046±0.08) (Figure 6). All other heavy metal treatments at different concentrations resulted in a reduction in anthocyanin levels. Similar trends were observed for carotenoids. Cr-250 ppm (0.0025±0.0011) showed a slight increase in carotenoid levels, while the least was observed in Cd-250 ppm (<0.003). In general, heavy metal treatments at different concentrations led to reduced carotenoid levels (Figure 7).

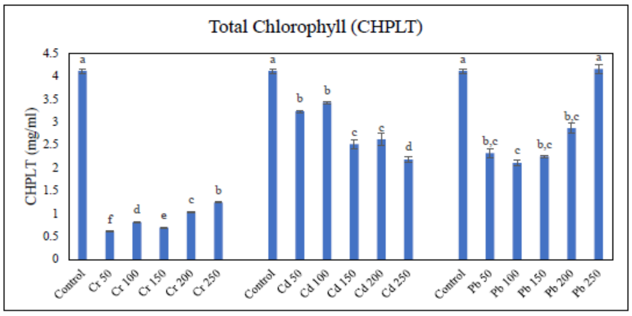

Figure 5. Chlorophyll values in Heavy Metal treated Plants
a – showing the highest value and e – showing the least value

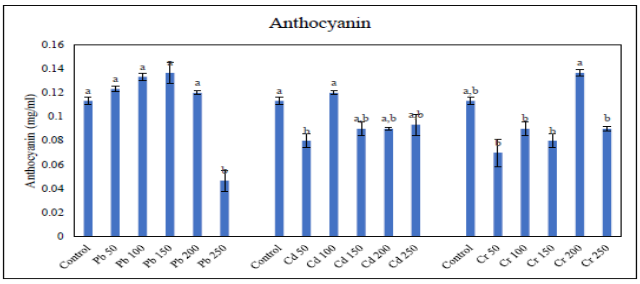

Figure 6. Anthocyanin values in Heavy Metal Treated Plants
a – showing the highest value and b – showing the least value

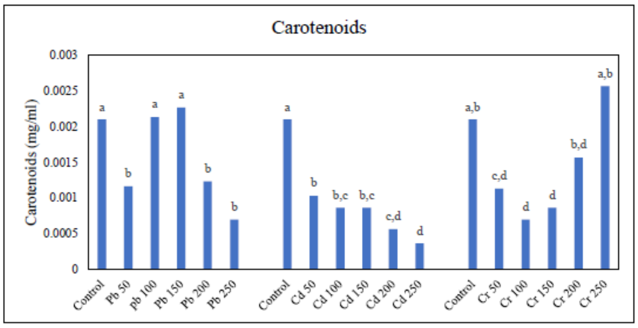

Figure 7. Carotenoid values in Heavy Metal Treated Plants
a – showing the highest value and d – showing the least value

The analysis of variance (ANOVA) was performed using SPSS version 22 software, and Tukey and DMRT tests were used to compare the means. A significance level of $P<0.05$ was considered significant. The results revealed that all measured parameters showed significant variations with the treatment of heavy metals.

Discussion

In medicinal plants, especially those where the aerial parts, such as leaves, are utilized for medicinal purposes, the rate of photosynthesis and the presence of various pigments play a crucial role in the production of secondary metabolites, which ultimately affect the efficacy of the medicine. *Jacobaea maritima*, a plant with medicinal uses in the homeopathic system, predominantly relies on the aerial parts, particularly the leaves. The presence of heavy metals has been shown to impact pigmentation and chlorophyll content in *J. maritima*. Differences in pigment levels can influence overall plant development and processes, which, in turn, can lead to variations in the percentage of metabolites. Heavy metal contamination alters the chemical composition of plants, thereby affecting the quality and efficacy of natural plant products. Research conducted by Rai et al. (27) suggests that chromium can reduce chlorophyll content and directly inhibit photosynthesis.

According to Rai et al. (28), heavy metals reduce the accumulation of photosynthetic pigments in leaves. Cadmium, for example, can decrease chlorophyll content, inhibit Rubisco activity, and impair both Photosystem I and Photosystem II (28). Souri et al. (29) have indicated a high probability of heavy metals causing severe damage to chloroplast structure. Carlson et al. (30) have also demonstrated that heavy metals can reduce the net photosynthetic rate in plants. Wahid et al. (31) suggested that cadmium can induce adverse effects in plants, including chlorosis, necrosis, and inhibited plant growth. Parmar et al. (32) stated that enzymatic inhibition and degradation contributes to the overall loss of chlorophyll content caused by cadmium. Rai et al. (33) found that increasing concentrations of chromium result in a reduction of chlorophyll a, chlorophyll b, and carotenoids. Panda et al. (34) indicated that chromium does not play a direct role in plant metabolism but its accumulation in plant tissues can reduce pigment content and cause ultrastructural modifications in chloroplasts and cell membranes. According to Ferreyroa et al. (35) lead inhibits enzyme activity and disrupts mineral nutrition in plants.

Conclusion

The research conducted on the plant species *J. maritima* reveals that exposure to heavy metal stress can lead to a decline in pigmentation and photosynthetic efficiency, ultimately affecting the plant's growth and lifespan. The presence of heavy metals, such as cadmium, chromium, and lead, has been shown to have significant impacts on the photosynthetic capability and pigment levels in plants. These findings highlight the importance of understanding and mitigating the effects of heavy metal contamination in plants, especially those with medicinal uses, to ensure the quality and efficacy of natural plant products.

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Authors' contributions

WJKS did the preliminary work with plants and instruments including the statistical analysis. JX has guided and helped in the usage of the instrument and the 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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