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Araştırma Makalesi / Research Article

Physiological Changes Due to Stress of Chromium and Lead in *Carthamus tinctorius* L.

Adnan AKCIN[1*], Tulay AYTAS AKCIN[2], Veli CELIKTAS[3]

Abstract

Heavy metal contamination has become a pressing environmental and public health concern, particularly in developing nations. Chromium (Cr) and lead (Pb) are ubiquitous environmental pollutants that pose substantial threats to ecological integrity and human health, even at sublethal concentrations. This study was conducted to elucidate the effects of Cr and Pb stress on photosynthetic pigments and proline content in *Carthamus tinctorius* L.cv. Zirkon. The findings revealed that Cr and Pb exposure caused a substantial reduction in chlorophyll a, chlorophyll b, total chlorophyll, total carotenoids, and proline content, while simultaneously increasing the Chl a/b ratio in heavy metal-stressed plants. A comparison of Cr and Pb exposure demonstrated that Cr exposure resulted in more pronounced damage compared to Pb exposure at equivalent concentrations. In response to both heavy metal stress, *C. tinctorius* L.cv. Zirkon plants displayed an increased accumulation of proline. These findings suggest that Cr and Pb exposure profoundly affects chlorophyll and proline content, leading to physiological alterations in *C. tinctorius* cv. Zirkon.

Keywords: Photosynthetic pigment**,** chromium, lead, proline, *C. tinctorius*.

Carthamus tinctorius L.'da Krom ve Kurşun Stresine Bağlı Fizyolojik Değişiklikler

Öz

Ağır metal kirliliği, özellikle gelişmekte olan ülkelerde önemli bir çevre ve halk sağlığı sorunu haline gelmiştir. Krom (Cr) ve kurşun (Pb), subletal konsantrasyonlarda bile ekolojik bütünlük ve insan sağlığı için önemli tehditler oluşturan ve her yerde bulunan çevresel kirleticilerdir. Bu çalışma, *Carthamus tinctorius* L. cv. Zirkon' da Cr ve Pb stresinin fotosentetik pigmentler ve prolin içeriği üzerindeki etkilerini ortaya koymak için yapılmıştır. Bulgular, Cr ve Pb uygulamasının klorofil a, klorofil b, toplam klorofil, toplam karotenoidler ve prolin içeriğinde önemli bir azalmaya neden olduğunu ve aynı zamanda ağır metal stresli bitkilerde Chl a/b oranını artırdığını ortaya koymuştur. Cr ve Pb uygulamasının karşılaştırılması, Cr uygulamasının eşdeğer konsantrasyonlarda Pb uygulamasına kıyasla daha belirgin hasara yol açtığını göstermiştir. Her iki ağır metal stresine yanıt olarak, *C. tinctorius* L.cv. Zirkon bitkilerinde artan bir prolin birikimi gözlenmiştir. Bu bulgular, Cr ve Pb uygulamasının klorofil ve prolin içeriğini önemli bir şekilde etkileyerek *C. tinctorius* cv. Zirkon'da fizyolojik değişikliklere yol açtığını göstermektedir.

Anahtar Kelimeler: Fotosentetik pigment, krom, kurşun, prolin, *C. tinctorius*.

[1,3]Amasya University, Suluova Vocational School, Crop and Animal Production, Plant Protection Program, Amasya, adnanakcin@hotmail.com veli.celiktas@amasya.edu.tr
[2]Ondokuz Mayıs University, Department of Biology, Faculty of Science, Samsun, Türkiye, taytas@omu.edu.tr

[*]Sorumlu Yazar/Corresponding Author
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1. Introduction

The rapid industrialization of the modern era has led to a surge in heavy metal contamination, making it one of the most pressing environmental concerns globally (Saud et al., 2022). Heavy metals accumulate in organisms at the lower levels of the food chain through bioaccumulation and are subsequently transferred across higher trophic levels, ultimately posing significant health risks to human populations (Patra et al., 2004a). The extensive release of heavy metals, such as chromium and lead, from both natural and human-induced sources has established them as major environmental pollutants (Steinberg, 2009).

Chromium (Cr), a toxic heavy metal and the 21st most abundant element in Earth's crust, exhibits resistance to degradation in the environment (Cervantes et al., 2001). Chromium exists in multiple oxidation states, primarily trivalent (Cr (III)) and hexavalent (Cr (VI)), and its toxicological profile is significantly influenced by its oxidation state (Iyaka, 2009). Among its various oxidation states, Cr (VI) poses the greatest threat due to its high solubility, which facilitates its uptake by organisms and its ability to induce severe oxidative stress (Von Burg and Liu, 1993). Hexavalent chromium, which is highly soluble in water, easily penetrates cell membranes and triggers the formation of reactive oxygen species (ROS). These ROS directly interact with DNA, inducing DNA degradation and disrupting the cell cycle, ultimately leading to cell death (Beyersmann and Hartwig, 2008; Rodriguez et al., 2011). Chromium stress in plants manifests as stunted growth and development, reduced productivity, and alterations in anatomical features, ultimately culminating in plant death (Kasmiyati and Sucahyo, 2014). Visible symptoms of chromium toxicity in plants include impaired initial growth, stunted root development, leaf chlorosis, and diminished biomass production (Shanker et al., 2005; Arif et al., 2021; Saud et al., 2022).

Lead, a highly toxic heavy metal, poses significant health risks to humans (Flora et al., 2012; Mudgal et al., 2010). Unique physicochemical properties render lead resistant to biodegradation, leading to its persistent accumulation within the global environment (Mahaffey, 1990). Smelting, combustion of leaded gasoline, and application of lead-contaminated materials to land are major anthropogenic contributors to environmental lead contamination (Gupta et al., 2009; Grover et al., 2010). In the majority of plant species, the majority of absorbed lead accumulates in the roots, with only a minor fraction translocated to above-ground tissues, as observed in *Pisum sativum* (Małecka et al., 2008), *Lathryus sativus* (Brunet et al., 2009), *Zea mays* (Gupta et al., 2010), and *Allium sativum* (Jiang and Liu, 2010). Excessive accumulation of lead within plant tissues exerts a toxic effect on most plants, leading to a diverse range of detrimental consequences. These include reduced seed germination, stunted root elongation, decreased biomass production, inhibition of chlorophyll biosynthesis, impaired mineral nutrition, and disruption of enzymatic reactions (Sengar et al. 2008, Arias et al., 2010; Cenkci et al., 2010; Singh et al., 2010).

Safflower (*Carthamus tinctorius* L.), a member of the Asteraceae family, holds significant economic importance as an oilseed crop (Mahmoud et al., 2014). Historically, its cultivation has been driven not only by its oil-rich seeds but also by its vibrant flowers, traditionally utilized as natural food coloring and flavoring agents, and even in medicinal preparations (Dorado et al., 2004). Notably, safflower boasts a remarkable ability to withstand drought stress. This resilience stems from a combination of its deep root system, allowing for efficient water acquisition, and a short period of drought sensitivity, minimizing its vulnerability during dry spells (Angelova, 2016; Manvelian et al., 2021). Despite its documented metal tolerance (Baran and Ekmekçi, 2022; Tunçtürk et al., 2023) and potential for phytoremediation (Pourghasemian et al., 2019; Ciaramella et al., 2022) the precise physiological responses of *Carthamus tinctorius* cv. Zirkon to chromium (Cr) and lead (Pb) stress remain largely unknown. This study aims to elucidate the effects of Cr and Pb on the photosynthetic capacity of *C. tinctorius* cv. Zirkon, seeking to identify potential adaptive mechanisms that enable this species to survive in heavy metal-contaminated soils.

2. Materials and Methods

2.1. Growth Conditions and Experimental Design

C. tinctorius cv. Zirkon seeds were germinated in a perlite medium that was sterilized in sodium hypochlorite for ten minutes, washed with distilled water, and supplemented with Hoagland's solution. Thirty plants exhibiting uniform growth and development were selected for the experiment and carefully placed in 1 cm diameter holes drilled into foam sheets. To investigate the effects of chromium and lead, the plants were divided into four groups per treatment. Each pot contained 5 liters of pure water and 200 ml of Hoagland's solution. Plants were grown hydroponically for one week to acclimate to the growing medium. Except for the control group, 20 ml of a heavy metal solution containing chromium or lead at concentrations of 0.01, 0,10, and 0,20 mM was added to the respective treatment groups once a week. All groups received 200 ml of Hoagland's solution twice a week and were grown in a climate chamber for three weeks under controlled conditions: temperature of 22 °C, light-dark cycle of 18/6 hours, and light intensity of 200 μmol/m2 s. Plants were harvested when they reached the desired size and subjected to a comprehensive analysis.

2.2. Determination of Photosynthetic Pigment and Proline Content

After three weeks of exposure to heavy metal contaminants, the concentrations of chlorophyll a (Chl a), chlorophyll b (Chl b), and carotenoids were evaluated using the method described by Lichtenthaler and Wellburn (1983). To quantify the pigment levels, leaf extracts prepared with 80% acetone were measured for absorbance at 663 nm, 644 nm, and 452 nm, corresponding to Chl a, Chl b, and carotenoids, respectively. Pigment content was then calculated according to the protocol of Lichtenthaler and Wellburn (1983).

Free proline levels in leaves were evaluated according to the method of (Bates et al., 1973). Briefly, homogenates were prepared from tissue type with 3% sulfosalicylic acid. They were then reacted with glacial acetic acid and ninhydrin to obtain a pink chromophore whose intensity was directly proportional to the concentration of free proline. This color intensity was then measured spectrophotometrically at 546 nm.

2.3. Statistical Analysis

Statistical analysis was conducted using SPSS software version 22.0, employing one-way ANOVA followed by Tukey's HSD test for post-hoc comparisons. Data were expressed as mean ± standard deviation (SD) based on three replicates. Differences were considered significant at $p < 0.05$ and denoted by distinct letters. This approach ensured robust evaluation of statistical significance and allowed for identification of meaningful variations among the groups.

3. Findings and Discussion

This study investigated the effects of varying chromium (Cr) and lead (Pb) concentrations on pigment content and the stress-responsive metabolite proline in *C. tinctorius* seedlings. Seedlings were exposed to four different concentrations of Cr and Pb (0, 0.01, 0.10, and 0.20 mM) and then analyzed for chlorophyll a (chl a), chlorophyll b (chl b), total chlorophyll, total carotenoids, proline, and the chl a/b ratio (Figures 1, 2). As shown in Figures 1and 2, both Cr and Pb stress significantly reduced the levels of chl a, chl b, total chlorophyll, and carotenoids, suggesting potential impacts on the photosynthetic apparatus.

Figure 1 clearly shows that increasing Cr concentrations have a progressively detrimental effect on the measured photosynthetic parameters. The chlorophyll a decreased by 60.56%, chlorophyll b by 71.93% and total chlorophyll by 64.68% ratio with increasing Cr applications (Figures 1 A-C). The decrease in total carotenoid content was 59.02% ratio (Figure 1 D). This suggests that Cr has an effective power to disrupt the photosynthetic mechanism of *C. tinctorius* cv. Zirkon seedlings, potentially leading to impaired growth and development. Several studies have reported Cr-induced inhibition of chlorophyll biosynthesis in various plants, including *Brassica napus* and *Nymphae alba* (Vajpayee et al., 2000; Afshan et al., 2015; Ali et al., 2018; Patra et al., 2004b). For example, Afshan et al. (2015) demonstrated that 100 and 500 µM Cr significantly reduced chlorophyll content in *B. napus*. Similarly, Vajpayee et al. (2000) observed reductions in both chlorophyll and protein content in *N. alba* treated with Cr concentrations exceeding 1 µM. Exposure to Cr stress triggers ultrastructural alterations in chloroplasts and other membrane-bound organelles, potentially leading to reductions in photosynthetic pigments (Zaheer et al., 2019). This depletion of chlorophyll, as documented by Chandra (2004), can be attributed to disrupted chlorophyll biosynthesis pathways. Furthermore, Cr-induced changes can lead to chloroplastic and ultrastructural damage, disrupting electron transport mechanisms and ultimately reducing photosynthetic efficiency. Exposure to toxic levels of Cr disrupts the photosynthetic machinery by altering the redox state of essential Cu and Fe heme groups, significantly reducing plant photosynthesis (Shahid et al., 2017). This impairment is further enhanced by Cr-induced overproduction of reactive oxygen species (ROS) through oxidative stress mechanisms (Ugwu and Agunwamba, 2020). Consequently, the combined effects of altered redox status and ROS overproduction lead to oxidative stress, ultimately resulting in reductions in plant growth, biomass and yield (Farid et al., 2019).

Compared to control conditions, increasing Cr concentrations significantly increased the chlorophyll a/b ratio. This positive correlation between the ratio and Cr concentration in plant leaves is clearly seen in Figure 1E. Chlorophyll a/b content was found to increase by 37.38%. Interestingly, this increase in the ratio has also been reported in other plant species such as *Spinacea oleracea* (Delfine et al., 1999) and is considered as a potential indicator of changes in the PSII-PSI ratio in leaves under stress conditions (Mahdi Hadif et al., 2015).

Plants utilize the accumulation of compatible osmolytes, such as proline, as a protective mechanism against various stress types. This accumulation helps maintain membrane stability and facilitate osmotic adjustment, enhancing the plant's resilience. Proline levels have been observed to increase in response to diverse biotic and abiotic stresses, as reported by Hayat et al. (2013) and Raza et al. (2023). While proline was previously considered the sole amino acid accumulating in stressed plants (Baskaran et al., 2009), recent studies have broadened this perspective. Rai et al. (2004) demonstrated a significant increase in proline content within *Ocimum tenuifolium* L. under Cr stress. This proline accumulation serves as a potent antioxidant, buffering the harmful effects of the metal. Proline accumulation in stressed tissues plays a crucial role in osmotic adjustment, further contributing to its use as a dependent marker for stress-tolerant genotypes (Baskaran et al., 2009).

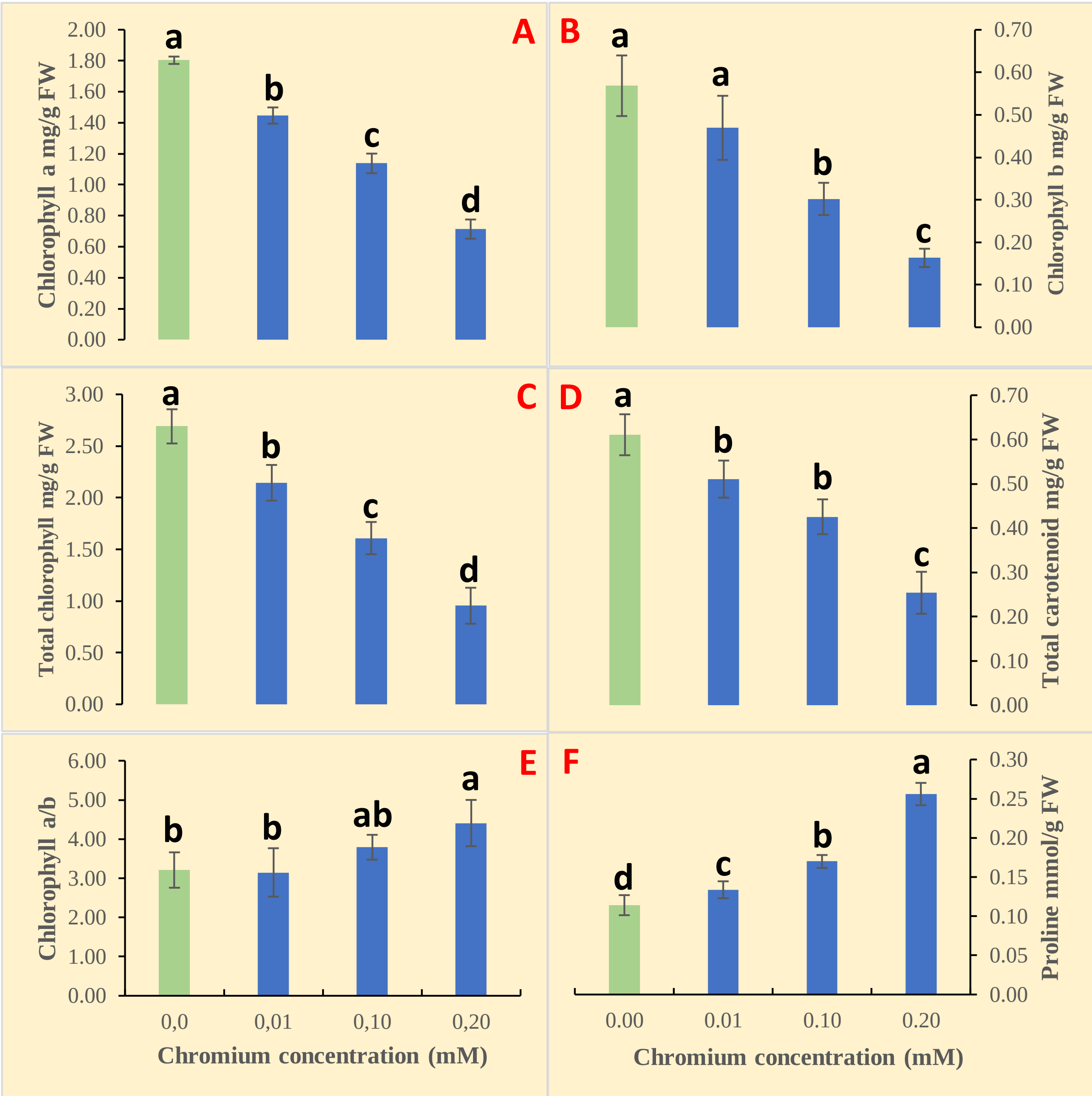

Figure 1. The effect of Cr on the photosynthetic pigments, carotenoids, and proline content of *Carthamus tinctorius* L. cv. Zirkon was studied at various concentrations. All values are the mean of three replicates ± SD. Error bars marked with different letters indicate significant differences ($p<0.05$). A: Chlorophyll a, B: Chlorophyll b, C: Total chlorophyll, D: Total Carotenoid, E: Chlorophyll a/b, F: Proline.

However, some studies suggest that proline accumulation in response to metal stress might indicate sensitivity rather than a purely defensive mechanism (Alvarez et al., 2022; Cia et al., 2012). Our findings support the established correlation between proline levels and heavy metal stress, highlighting its potential utility as a stress indicator. As shown in Figure 1 F, the amount of proline increased by 136.36% with increasing Cr concentration. According to the results of the study, the detrimental effect of Cr, especially on the chl a/b ratio and proline content, increased significantly with increasing Cr concentrations and showed more effect than Pb heavy metal (Figure 1 E, F). This differential toxicity may be attributed to the enhanced transport of Cr to leaves compared to Pb, resulting in a higher accumulation of Cr within the foliage. This indicates that Cr is more effect than Pb in disrupting the photosynthetic mechanism of *C. tinctorius* seedlings.

The results showed that the content of photosynthetic pigment significantly decreased with increasing Pb concentrations in comparison with the control (Figure 2). In their study, Dogan et al. (2021) determined that lead (Pb) exposure inhibited the production of photosynthetic pigments and phenolic compounds in *Carthamus tinctorius.* Sayyad et al. (2009) and Angelova (2016) have shown that the root system of *C. tinctorius* exhibits a remarkable ability to accumulate high levels of lead (Pb). Ciaramella et al. (2022) reported that the highest concentration of lead (Pb) among the different organs of the *C. tinctorius* plant was detected in the root tissues. The intense Pb accumulation in the roots can be considered an adaptive mechanism developed by the plant to survive in environments contaminated with Pb. This way, the increased retention of Pb in the roots limits its translocation to other parts of the plant, thereby minimizing the physiological damage caused by Pb. Al-Chami et al. (2015) determined that when safflower plants were grown in soil contaminated with 5 and 10 mg L^{-1} of Pb, no significant impact was observed on all productivity parameters. However, the same study found that when the lead concentration was increased to 25 mg L^{-1}, plant growth was completely inhibited. It was also reported that safflower has potential as an effective agent for phytoremediation of soils contaminated with heavy metals (Angelova, 2016). Researchers have also documented the detrimental effects of lead (Pb) on chlorophyll content in various plant species. Liu et al. (2012) and Bhatti et al. (2013) observed a decrease in chlorophyll levels in rice and wheat, respectively, upon exposure to Pb. Further exploring the mechanism, Sharma and Dubey (2005) reported elevated chlorophyllase activity, an enzyme that breaks down chlorophyll, in Pb-treated plants, suggesting its role in chlorophyll degradation. This finding aligns with the recognition by Ernst (1998) of the link between Pb-induced inhibition of chlorophyll synthesis and the visible symptom of chlorosis, a yellowing of leaves due to chlorophyll loss. Similarly, Ewais (1997) provided further evidence, demonstrating that Pb exposure negatively impacted both growth and chlorophyll content in three weed species: *Chenopodium ambrosoides, Digitaria sanguinolis,* and *Cyperus difformis*.This observed reduction in chlorophyll is likely attributed to Pb's inhibition of essential nutrient uptake by plants, as suggested by Khan et al., (2019). Furthermore, Pb may directly inhibit the enzymes responsible for chlorophyll synthesis, contributing to the observed decrease in chlorophyll levels. Pb exposure can lead to a decreased rate of photosynthetic pigment accumulation through two potential mechanisms. Firstly, elevated ROS generation induced by Pb can cause peroxidation of chloroplast membranes, hindering pigment synthesis (Malar et al., 2014). Secondly, Pb disrupts the uptake of essential elements like magnesium, potassium, calcium, and iron, crucial for chlorophyll biosynthesis. This disruption leads to dysfunctional photosynthesis as Pb substitutes divalent cations, further inhibiting pigment production (Gopal and Rizvi, 2008). The observed reductions in photosynthetic pigments under different heavy metal stress in *C. tinctorius* support these proposed mechanisms (Al Chami et al., 2015;Tunçtürk et al., 2023;Smaoui et al., 2023). Baran and Ekmekçi (2022) also found that nickel stress significantly reduced photosynthetic activity by approximately 50% in some wild and cultivated safflower species. The results of our study is consistent with the earlier research demonstrating the inhibitory effects of heavy metals on plant growth and development. According to the results of the research, the most remarkable changes in photosynthetic pigments in Pb heavy metal were observed in chlorophyll b and chlorophyll a/b ratio, unlike Cr. In Pb, chlorophyll b ratio decreased by 67.16% and chlorophyll a/b ratio decreased by 9.04% (Figure 2 B, E).

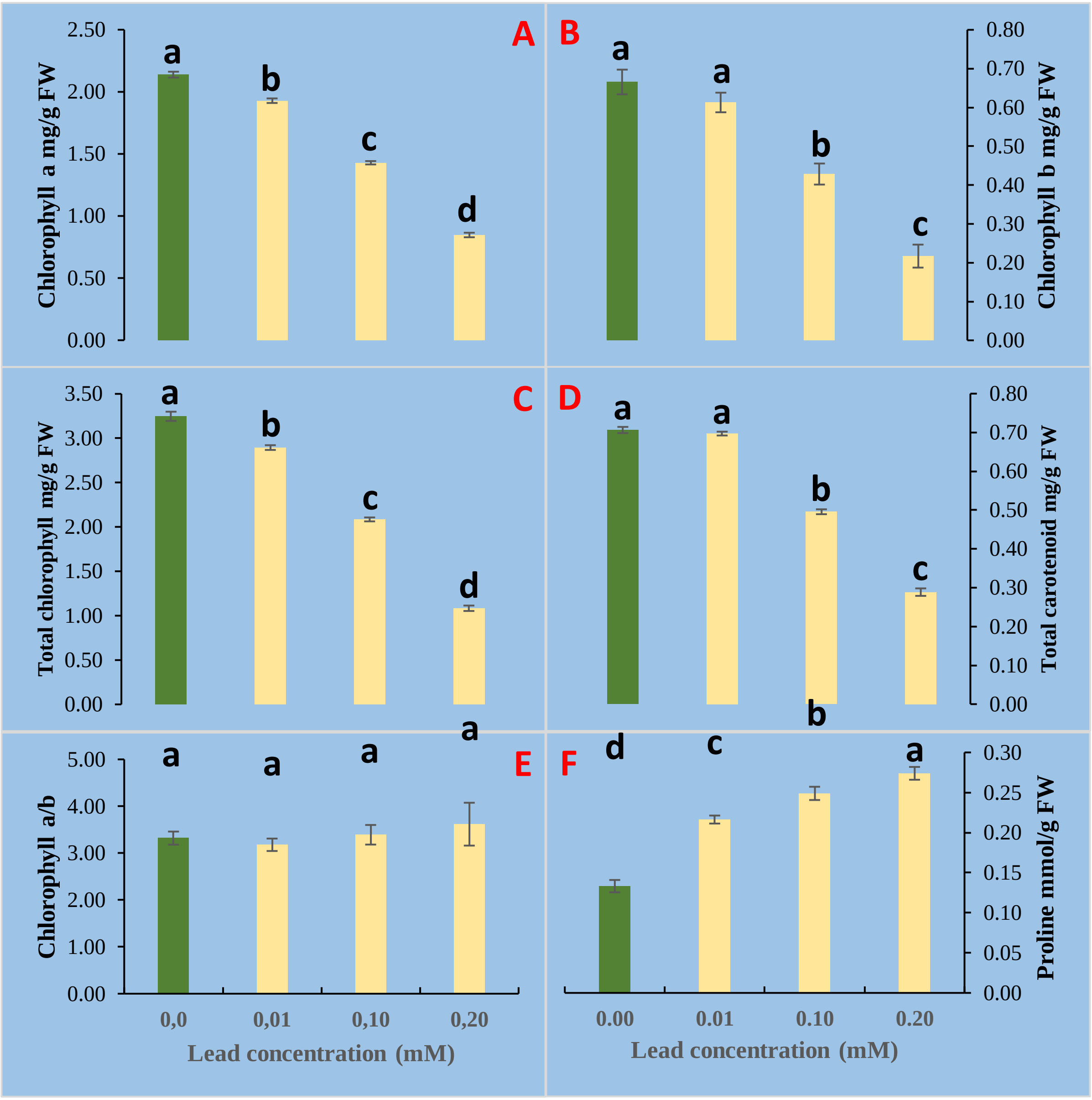

Figure 2. The effect of Pb on the photosynthetic pigments, carotenoids, and proline content of *Carthamus tinctorius* L. cv. Zirkon was studied at various concentrations. All values are the mean of three replicates ± SD. Error bars marked with different letters indicate significant differences ($p<0.05$). A: Chlorophyll a, B: Chlorophyll b, C: Total chlorophyll, D: Total Carotenoid, E: Chlorophyll a/b, F: Proline.

Our investigation revealed a biphasic response in proline content following Pb exposure, with an initial increase at lower concentrations and a subsequent decline at higher concentrations. This observation aligns with established knowledge, as proline accumulation is a well-documented metabolic response of plants to various stress conditions, including heavy metal exposure (Signorelli, 2016). The results concerning proline accumulation during Pb exposure are in accordance with observations made in other plant species. Our results show that the proline content in *C. tinctorius* cv. Zirkon increased by 107.69% with increasing Pb concentrations (Figure 2 F). This proline build up likely arises from a Pb-induced decline in plant water potential. Proline's contribution to water balance maintenance is crucial for lead tolerance, as demonstrated by Piršelová et al. (2015) and Signorelli (2016).

4. Conclusions

In this study, we examined how chromium (Cr) and lead (Pb) concentrations impact the photosynthetic processes in *C. tinctorius* cv. Zirkon seedlings. Our research demonstrates that both Cr and Pb stress significantly impair the photosynthetic process in *C. tinctorius* cv. Zirkon. Increasing concentrations of Cr and Pb progressively reduced chlorophyll and carotenoid content. Notably, the decrease in chlorophyll b and chlorophyll a/b ratio was more pronounced under Cr stress than under Pb stress, indicating a potentially greater impact of Cr on these specific photosynthetic pigments. Exposure to both Cr and Pb stress triggered an increase in proline content within the plants. This finding contributes to a deeper understanding of the physiological responses and potential mechanisms underlying Cr and Pb toxicity in plants.

Authors' Contributions

All authors contributed equally to the study.

Statement of Conflicts of Interest

There is no conflict of interest between the authors.

Statement of Research and Publication Ethics

The author declares that this study complies with Research and Publication Ethics.

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