

EFFECT INTERACTION OF SULFATE (SO_4^{2-}) DAN CHROMATE (CrO_4^{2-}) ON GROWTH AND Cr^{6+} ACCUMULATION IN *Tagetes erecta* L.

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ABSTRACT

Chromium is a heavy metal that is toxic, bioaccumulative, persistent, and cannot be decomposed in the environment. The Cr^{6+} ion is a chromium ion that has the highest toxicity among other chromium ions, so its presence in the environment is hazardous for plants, animals, and humans. Sulfur is an essential element for plants, whose presence can reduce Cr^{6+} concentrations in the terrestrial environment by inhibiting the uptake of Cr^{6+} by plants. *Tagetes erecta* is a bioremediation agent plant that can remediate land polluted with Cr^{6+} waste. In this research, we studied the growth of *T. erecta* in media polluted with Cr^{6+} and sulfate and its ability to accumulate of Cr^{6+} . Several parameters observe in this experiment, i.e. growth parameters, namely plant height, number of leaves, number of flowers measured at the end of the study, dry weight, root length, and Cr^{6+} concentration estimated using the diphenylcarbasid method. The results showed that the plant height of *T. erecta* was not significantly different, and sulfate addition did not affect plant height. The addition of sulfate affected the number of leaves and flowers, dry weight of root shoots, root length, and Cr^{6+} content in *T. erecta* polluted with Cr^{6+} . so that in these parameters, there was a significant difference in the addition of Cr^{6+} and sulfate on growth of *T. erecta* and its ability to Cr accumulation.

Keywords: Cr^{6+} , sulfate, bioaccumulation, *Tagetes erecta*

INTRODUCTION

Chromium is one of the heavy metals whose presence is hazardous to the environment because it has toxic, bioaccumulative, persistent properties and is unable to be bound in the background, and can accumulate in the human body through the food chain (Tya et al., 2016). The presence of chromium as a pollutant can be found in all phases of the environment, including water, soil, and air. The wood tanning industry is the industry that has the most significant contribution to Cr pollution in soil and water (Ramana et al., 2012). In addition, there are many other industries, such as rubber, cable, battery, metallurgy, electroplating, paint and pigment production, and Cr chemical production which are the cause of the increasing Cr pollutant (Nuraini et al., 2017)

The toxicity of chromium metal is highly dependent on its chemical stability. Among all the oxidation states of chromium, Cr^{3+} and Cr^{6+} are the most stable in aquatic and terrestrial environments (Augustynowicz et al., 2010). Cr^{6+} has high solubility and it is a potent oxidizing agent (Rahman et al., 2007), while Cr^{3+} has low solubility, so it can be seen that Cr^{6+} ions have higher toxicity than Cr^{3+} ions (Armadi and Oktavian 2009). Due to the stable nature of Cr^{3+} and Cr^{6+} , this causes plants easily absorb Cr^{3+} and Cr^{6+} . The absorption of Cr^{6+} by plants is more than Cr^{3+} because Cr^{3+} is absorbed passively through cation exchange sites in plant cell walls (Xu et al., 2021), while Cr^{6+} enters plant cells under sulfate transporters (Singh et al., 2013), where it occurs through the

mediation of an active process that requires the metabolic energy provided by the hydrolysis of ATP. The growth and development of plants can generally be disrupted by chromium. The toxic properties of chromium can cause decreased plant growth, changes in water content in leaves, damage to the root system (Schiavon et al., 2008), reduced biomass production, oxidative stress, changes in chloroplast structure, degradation of photosynthetic pigments, and reduced catalytic activity of antioxidant enzymes (Coelho et al., 2017). Several factors affect the storage and translocation of Cr in plants, including the length of exposure and the presence of other elements.

Sulfur is one of the elements that significantly affect the process of Cr absorption in plants. According to research (Kulczycki and Sacala, 2020), it was found that the application of element S can be an effective strategy to reduce side effects in plants grown in Cr-contaminated soil. The application of S significantly increases total biomass production and reduces Cr content in rice plants and maize, this is because the S element is not only an essential nutrient for plants but S element also plays a role in the mechanism of response and tolerance to biotic and abiotic stresses in the environment. According to Schiavon et al. (2008), the entry of Cr into plants involves sulfate transporters sulfate is a transporter in the process of accumulation of Cr, this is because sulfate has a chemical structure similar to Cr so that it can be reduced to bind and detoxify metals and metalloids (Freeman et al., 2004). In responding to

environmental pollution by Cr at this time, it is necessary to have a remediation process for land polluted with Cr waste so that Cr-polluted land can be reused. Several ways can be done, one of which is by using plants that can tolerate land polluted by Cr waste and can also act as an accumulator for Cr.

T. erecta (African marigold) is a dicotyledonous plant from the Asteraceae family and the *Tagetes* genus and can be a source of biological compounds (Coelho et al., 2017). This plant has the local name *kenikir*, which is usually used as an ornamental plant. The current use of *T. erecta* is not only an ornamental plant but also benefits detoxifying heavy metals in the soil. *T. erecta* is a plant with fast growth, high biomass production, a sound root system, and the ability to accumulate and tolerate various heavy metals (Clemens et al., 2002). *T. erecta* can be used as a phytoremediation agent for land polluted by Cr. The research of Meitiniarti et al. (2022), found three types of bacteria from the rhizosphere of *T. erecta*. that were resistant and reduced Cr^{6+} . Research Intansari et al. (2021), stated that the roots and shoots of the *T. erecta*. plant could accumulate 0.0992 mg L⁻¹ and 0.072 mg L⁻¹ Cr^{6+} in the growing media (soil and compost). This study aims to determine the growth of *T. erecta* in Cr^{6+} polluted media and its ability to accumulate of Cr^{6+} .

RESEARCH METHODS

The research was conducted at the Biochemistry Laboratory, Faculty of Biology, Satya Wacana Christian University. The materials used in this study included *T.*

erecta plant seeds (2-3 weeks old) and planting media (mixture of soil and compost 1:1). The seeds and planting media were obtained in the Kopeng area, Semarang Regency, Central Java province. The tools used in this study include an oven to dry plants and a UV-Vis spectrophotometer to measure Cr^{6+} concentrations. The study was conducted experimentally using a completely randomized design (CRD) with two treatment factors, namely chromate with three concentrations, namely C0, C1 (50 mg L⁻¹), C2 (100 mg L⁻¹), and sulfate with three concentrations of S0 (0 mM L⁻¹, S1 (1 mM L⁻¹), S2 (2 mM L⁻¹). Each treatment with four replications, so the total sample used was 36 (Table 1).

Table 1 Experimental design with Cr^{6+} and SO_4^{2-} treatments

Cr^{6+} (mg L ⁻¹) treatments	SO_4^{2-} (mM L ⁻¹) treatments		
	0 (S0)	1 (S1)	2 (S2)
0 (C0)	C0S0	C0S1	C0S2
50 (C1)	C1S0	C1S1	C1S2
100 (C2)	C2S0	C2S1	C2S2

1. Preparation of *T. erecta* seedlings

Forty seedlings of *T. erecta* aged 2-3 weeks were obtained from Kopeng Village, Getasan District, located at the coordinates of -7.376397° South Latitude (LS) and 110.440711° East Longitude (BT). *T. erecta* seedlings were planted in polybags containing a mixture of soil and compost (1:1) as much as 0.5-1 kg and acclimatized for seven days to stabilize plant growth before being treated with chromate and sulfate.

2. Preparation of the treatment solution

Preparation of sulfate and chromate solutions according to the concentration treatment for chromate 0 (C0), 50 mg L⁻¹ (C1), and 100 mg L⁻¹ (2) and for sulfate 0 (S0), 1 mM L⁻¹ (S1) and 2 mM L⁻¹ (S2). The chromate

solution of 50 mg L⁻¹ (C1) was made by dissolving 0.186 grams of K₂CrO₄ in 1 liter of distilled water while for 100 mg L⁻¹ (C2) chromate solution as much as 0.373 grams of K₂CrO₄ was dissolved in 1 liter of distilled water. A sulfate solution with a concentration of 1 mM L⁻¹ (S1) was prepared by dissolving 0.121 grams of MgSO₄, and a concentration of 2 mM L⁻¹ by dissolving 0.241 grams of MgSO₄ in 1 liter of distilled water. Each plant was watered with as much as 30 ml of sulfate and chromate solution with the treatment as shown in Table 1 and the sulfate and chromate treatment was carried out simultaneously once a week.

3. Plant Growth Maintenance

The plants growth of *T. erecta* was maintained until the end of the study (8 weeks after the initial treatment solution was given), by watering with 50 mL of distilled water every day for each polybag, except when the treatment solution was applied. Plants are planted in a place exposed to direct sunlight. Control of pests and diseases, if any, is carried out mechanically.

4. Parameter measurement and data analysis

The parameters measured included plant growth and Cr⁶⁺ content in the roots and shoots of *T. erecta*. The plant growth including plant height, root length, number of leaves, number of flowers, and root and shoot dry weight of *T. erecta* was measured at the end of the reasearch.

Determination of Cr⁶⁺ content in the roots and shoots of *T. erecta* using the diphenylcarbasid method according to

Gheju et al. (2009) with modifications. The preparation of plant samples used the dry ashing method by washing the roots and shoots of the plants thoroughly and drying them in the oven for 48 hours at 80°C. The dried root and leaf samples were then crushed and incinerated using a furnace at 600°C for 6 hours. A total of 0.2 g of sample was used to mix with a solution of 2 M HCl and 1 M HNO₃ with a ratio of 1:1. The sample was filtered until clear, and the filtrate was put into a 10 mL volumetric flask then distilled water was added to a volume of 10 mL. The filtrate was then used to measure the Cr⁶⁺ content by taking 2.5 mL of the filtrate added 5 drops of concentrated H₂SO₄ and 0.1 mL of 5% diphenylcarbasid solution, incubated for 15 minutes. The absorbance measurement of Cr⁶⁺ content was measured at a wavelength of 540 nm using a UV-VIS spectrophotometer. The Cr⁶⁺ content was calculated using the standard Cr⁶⁺ curve.

The research data were statistically analyzed using a two-way ANOVA with SAS (Statistical Analysis System) version 9.1.3 to determine the effect of treatment on the parameters measured, followed by Tukey's Studentized Range (HSD) test to compare the means of all treatments. Statistical significance was considered at P < 0.05. All data reported in this research are mean of four replicates

RESULTS AND DISCUSSION

The effect of Cr and sulfate on the growth of *T. erecta*

The combination of Cr⁶⁺ and sulfate in the growing media has a significant effect on plant growth. The growth of *T. erecta*

showed a different response to the application of Cr^{6+} and sulfate. The addition of sulfate had a significant effect on root length, number of leaves and flowers, dry weight of roots and shoots and Cr^{6+} content of roots and shoots, but had no significant effect on the height of *T. erecta* grown on media containing Cr^{6+} .

Although the combination of Cr^{6+} and sulfate did not have a significant effect on plant height, plants treated with Cr^{6+} 50 mg L^{-1} and control showed a decrease in plant height with increasing concentrations of sulfate applied to *T. erecta* growing medium (Figure 1). *T. erecta* treated with the highest Cr^{6+} concentration of 100 mg L^{-1} with the sulfate addition of 0 mM L^{-1} and 1 mM L^{-1} showed an increase in plant height but not significantly. These results indicated that the addition of 2 mM L^{-1} sulfate was able to increase the tolerance of *T. erecta* to Cr^{6+} stress at a concentration of 100 mg L^{-1} , so

that it could maintain its growth and development. Sulfates are reported to be important for plants in dealing with heavy metal stress. Sulfate is not only an essential plant nutrient but is also involved in response mechanisms and tolerance to various heavy metal stress conditions (Kulczycki and Sacala, 2020). The application of sulfur in the form of sulfate and fertilizers containing S was reported to increase plant height or shoot length in *Brassica napus* under chromium stress conditions (Jahan et al., 2015) and *Solanum nigrum* under Cd stress conditions (Alatawi et al., 2022).

The combination of sulfate and chromate treatments had a significant effect on the number of leaves of *T. erecta* (Figure 2). Increasing levels of Cr (50 and 100 mg L^{-1}) in the growth medium reduced the number of leaves compared to control plants without Cr treatment. The addition of sulfate in the growing medium significantly increased the

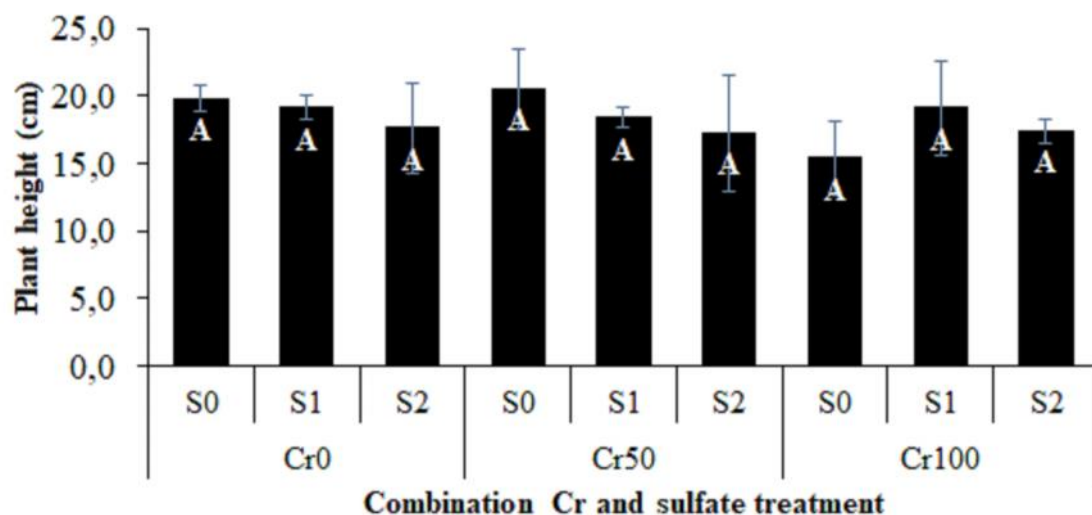


Figure 1. Plant height of *T. erecta* with combination treatment of Cr^{6+} in the form of K_2CrO_4 ($\text{Cr0} = 0$ mg L^{-1} , $\text{Cr50} = 50$ mg L^{-1} , and $\text{Cr100} = 100$ mg L^{-1}) and SO_4^{2-} in the form of MgSO_4 ($\text{S0} = 0$ mM L^{-1} , $\text{S1} = 1$ mM L^{-1} , and $\text{S2} = 2$ mM L^{-1}). The vertical bar on the column represent $\pm\text{SD}$, the same letter in column indicate were not significantly different at the 5% level of Tukey's test ($n=4$).

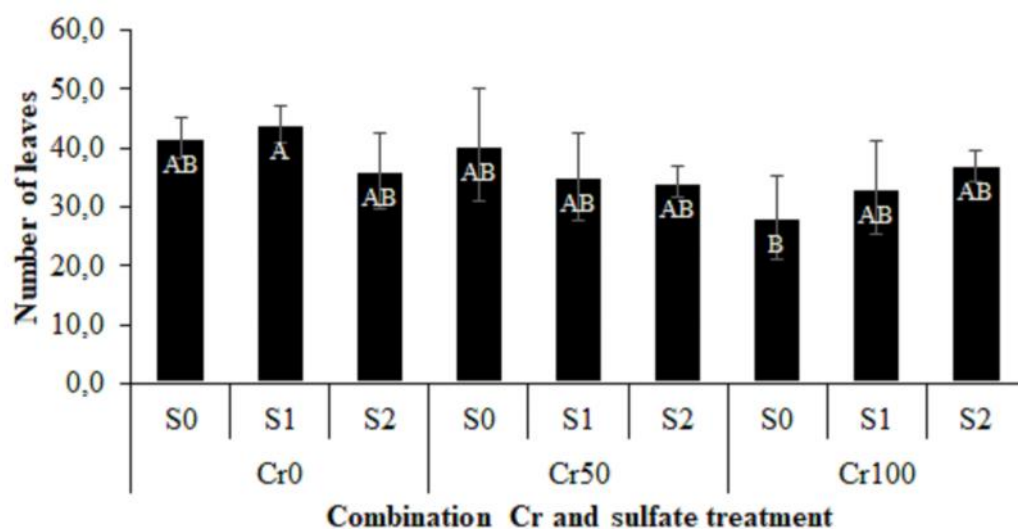


Figure 2 The number of leaves *T. erecta* with combination treatment of Cr^{6+} in the form of K_2CrO_4 ($\text{Cr0} = 0 \text{ mg L}^{-1}$, $\text{Cr50} = 50 \text{ mg L}^{-1}$, and $\text{Cr100} = 100 \text{ mg L}^{-1}$) and SO_4^{2-} in the form of MgSO_4 ($\text{S0} = 0 \text{ mM L}^{-1}$, $\text{S1} = 1 \text{ mM L}^{-1}$, and $\text{S2} = 2 \text{ mM L}^{-1}$). The vertical bar on the column represent $\pm\text{SD}$, the same letter in column indicate were not significantly different at the 5% level of Tukey's test ($n=4$)

number of leaves in plants that were given Cr at high concentrations (100 mg L^{-1}) compared to plants without the addition of sulfate in the growing medium (S0Cr100). The number of leaves on the control plants and those given 50 mg L^{-1} Cr decreased significantly when the sulfate was added at different concentrations, except for the control plants when the sulfate was added at 1 mM L^{-1} . Control plants that were given the addition of 1 mM L^{-1} sulfate showed the highest number of leaves compared to plants with other treatments. Cr was reported to indirectly affect plant leaf parts, the presence of Cr (VI) greatly affected plant leaves, yellowing and wilting due to loss of petiole turgor, and reduced the number and size of leaves (Saleem et al., 2022). Dube et al., (2009), reported that Cr exposure affected the decrease in the number of leaves of the carrot plant. The addition of sulfur-fertilizer was reported to increase the number of

leaves of *Solanum nigrum* under Cd stress conditions (Alatawi et al., 2022). Kulczycki and Sacala (2020) reported that wheat's tolerance to Cr stress increased due to the addition of sulfur. The greater tolerance to Cr may be related to the high concentration of S in wheat. Under heavy metal stress, the essential nutrient sulfur is very important because of its role in detoxification.

The root length of *T. erecta* was significantly affected by the combination of Cr^{6+} and sulfate treatment. The addition of sulfate of 1 mM L^{-1} and 2 mM L^{-1} significantly reduced root length in control plants (without Cr^{6+}) and plants given Cr^{6+} of 50 mg L^{-1} , compared to plants that were not given sulfate (S0Cr0 and S0Cr50). In contrast, the root length of plants treated with high Cr^{6+} concentrations (100 mg L^{-1}) increased significantly with the addition of 1 mM L^{-1} and 2 mM L^{-1} sulfate treatment (Figure 3).

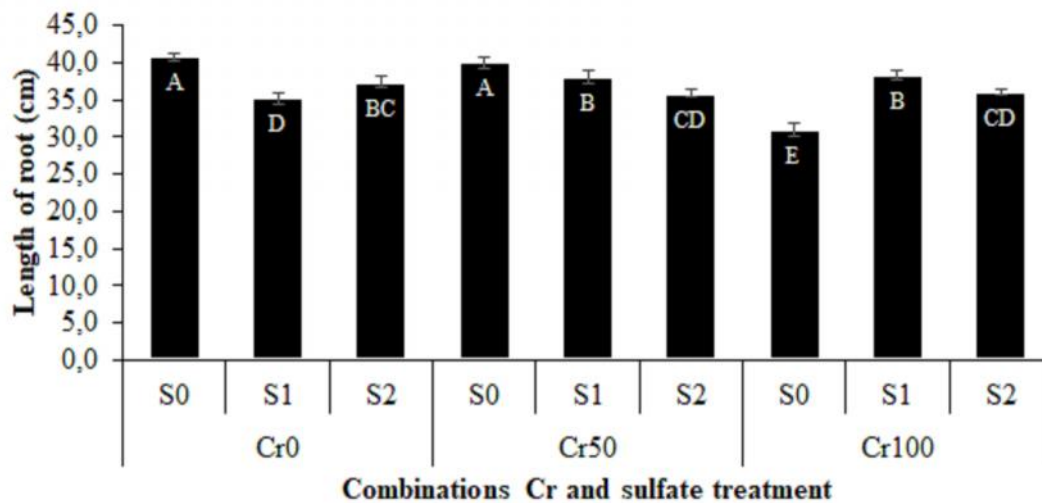


Figure 3. The root length of *T. erecta* with combination treatment of Cr^{6+} in the form of K_2CrO_4 ($\text{Cr0} = 0 \text{ mg L}^{-1}$, $\text{Cr50} = 50 \text{ mg L}^{-1}$, and $\text{Cr100} = 100 \text{ mg L}^{-1}$) and SO_4^{2-} in the form of MgSO_4 ($\text{S0} = 0 \text{ mM L}^{-1}$, $\text{S1} = 1 \text{ mM L}^{-1}$, and $\text{S2} = 2 \text{ mM L}^{-1}$). The vertical bar on the column represent $\pm\text{SD}$, the same letter in column indicate were not significantly different at the 5% level of Tukey's test ($n=4$).

These results indicate that under high concentrations of Cr^{6+} stress the addition of sulfate can increase the tolerance of *T. erecta* by increasing root growth, especially root length (Figure 4). The roots play a role in Cr uptake because they are the main organs for nutrient absorption, so they are also the main site of Cr toxicity in plants. Cr^{6+} of 2.5 mg L^{-1} was reported to reduce the root length

of *Pistia stratiotes* (Kakkalameli et al., 2018). A decrease in root length due to Cr^{6+} stress of 6, 12, 18, and 24 mg kg^{-1} was also reported in wheat (*Triticum aestivum*) (Ghani et al., 2015). The decrease in root length of *Brassica napus* sprouts due to Cr treatment of 40 and 160 ppm was reported by Jahan et al. (2015), and the decrease in root length in *Glycine max* was reported by



Figure 4. The root of *Tagetes erecta* with combination treatment of Cr^{6+} in the form of K_2CrO_4 ($\text{Cr0} = 0 \text{ mg L}^{-1}$, $\text{Cr50} = 50 \text{ mg L}^{-1}$, and $\text{Cr100} = 100 \text{ mg L}^{-1}$) and SO_4^{2-} in the form of MgSO_4 ($\text{S0} = 0 \text{ mM L}^{-1}$, $\text{S1} = 1 \text{ mM L}^{-1}$, and $\text{S2} = 2 \text{ mM L}^{-1}$).

Amin et al. (2014). Cr affects root growth as a result of inhibiting division and elongation of cell roots. The increase in root length due to the addition of sulfate in *T. erecta* growing medium under Cr^{6+} stress of 100 mg L⁻¹ may be due to increased cell division and elongation in the root meristematic region by sulfate (Chandel et al., 2002; Jahan et al., 2015).

The effect of the combination of Cr^{6+} and sulfate treatment on the number of flowers showed the same pattern as the effect on the number of leaves of *T. erecta*. The combination of Cr^{6+} and sulfate treatment significantly affected the number of flowers of *T. erecta* (Figure 5). The number of flowers on control plants and those treated with Cr^{6+} of 50 mg L⁻¹ decreased as the concentration of sulfate added to the growing medium increased, except for control plants (without Cr^{6+}) with 1 mM L⁻¹ sulfate (S1Cr0) which showed the highest number of flow-

ers (about 5 flowers/plant).

Flowering is a plant response to heavy metal stress. It has been reported by Aravindhana (2019) that exposure to Cr and Pb metals had an impact on increasing the number of flowers even though the flower size produced was much smaller than the flower size under Pb exposure. Figure 6 shows *T. erecta* plants and flowers formed in response to Cr stress and the addition of sulfate in the growing medium. In contrast to the result of this research, Ramana et al. (2013) reported that the application of Cr to other plants (*Aster novibelgii*, *Dahlia sambucifolia*, *Calendula officinalis*, and *Chrysanthemum indicum*) inhibited flower formation. Based on the research of Attanasova and Zapryanova (2014) on *Salvia splendens* was reported that exposure to a number of heavy metals (Pb, Cu, and Zn) resulted in delayed flowering and a much shorter flowering period and the flowers produced was very small and dam-

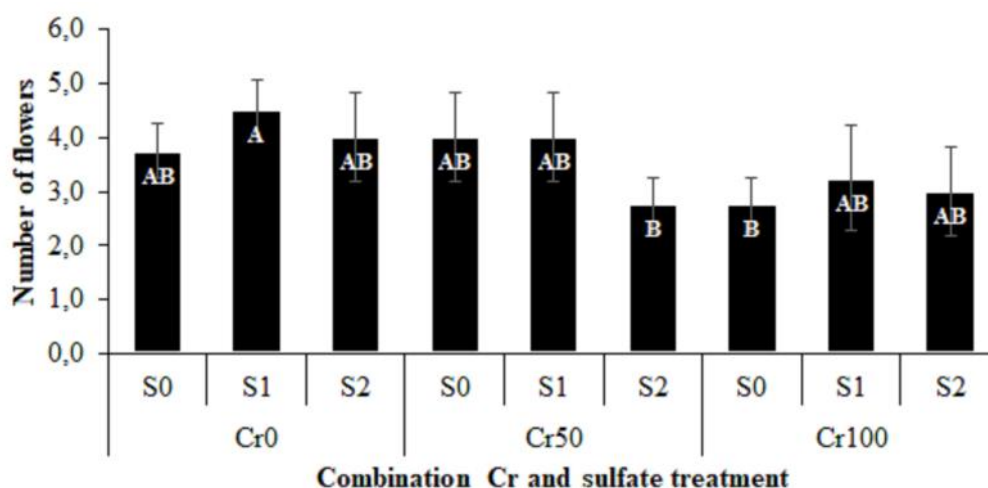


Figure 5. The number of flowers *T. erecta* with combination treatment of Cr^{6+} in the form of K_2CrO_4 ($\text{Cr0} = 0 \text{ mg L}^{-1}$, $\text{Cr50} = 50 \text{ mg L}^{-1}$, and $\text{Cr100} = 100 \text{ mg L}^{-1}$) and SO_4^{2-} in the form of MgSO_4 ($\text{S0} = 0 \text{ mM L}^{-1}$, $\text{S1} = 1 \text{ mM L}^{-1}$, and $\text{S2} = 2 \text{ mM L}^{-1}$). The vertical bar on the column represent $\pm\text{SD}$, the same letter in column indicate were not significantly different at the 5% level of Tukey's test ($n=4$).



Figure 6. The plant growth of *T. erecta* on media with combination treatments of Cr^{6+} in the form of K_2CrO_4 ($\text{Cr}0 = 0 \text{ mg L}^{-1}$, $\text{Cr}50 = 50 \text{ mg L}^{-1}$, and $\text{Cr}100 = 100 \text{ mg L}^{-1}$) and SO_4^{2-} in the form of MgSO_4 ($\text{S}0 = 0 \text{ mM L}^{-1}$, $\text{S}1 = 1 \text{ mM L}^{-1}$, and $\text{S}2 = 2 \text{ mM L}^{-1}$).

aged. Mansour et al., (2015) reported that exposure to heavy metal Cd had a significant negative effect on the number of *T. erecta* flowers. This may indicate that *T. erecta* is able to tolerate the presence of Cr^{6+} and produce flowers in response. On the other hand, the presence of sulfate inhibits the absorption of Cr^{6+} , so the presence of sulfate can suppress plant stress conditions due to Cr^{6+} toxicity (Xu et al., 2021).

T. erecta biomass determined based on the dry weight of roots and shoots showed different responses to the combination of Cr^{6+} and sulfate treatments. Root and shoot dry weight were significantly affected by the combination of Cr^{6+} and sulfate treatment. The root dry weight of control plants without Cr^{6+} and those given Cr^{6+} of 50 mg L^{-1} significantly decreased with increasing concentration of sulfate given in the growing

medium. In contrast, the root dry weight of *T. erecta* plants treated with Cr^{6+} of 100 mg L^{-1} increased significantly with the addition of 1 mM L^{-1} and 2 mM L^{-1} sulfate in the growing medium (Figure 7). The greatest Cr^{6+} stress effect on root dry weight reduction was shown in the Cr^{6+} treatment of 100 mg L^{-1} . Meanwhile, plants treated with Cr^{6+} of 50 mg L^{-1} without the addition of sulfate showed the highest root dry weight of 0.69 g , when compared to other treatments.

The combination of Cr^{6+} and sulfate treatment showed a significant effect on shoot dry weight. Cr^{6+} 100 mg L^{-1} had a greater effect on the dry weight of plant shoots than Cr^{6+} 50 mg L^{-1} for all concentrations of sulfates given. Control plants (without Cr^{6+}) and those given 50 mg L^{-1} Cr^{6+} without sulfate and with 1 mM L^{-1} sulfate showed the highest shoot dry weight,

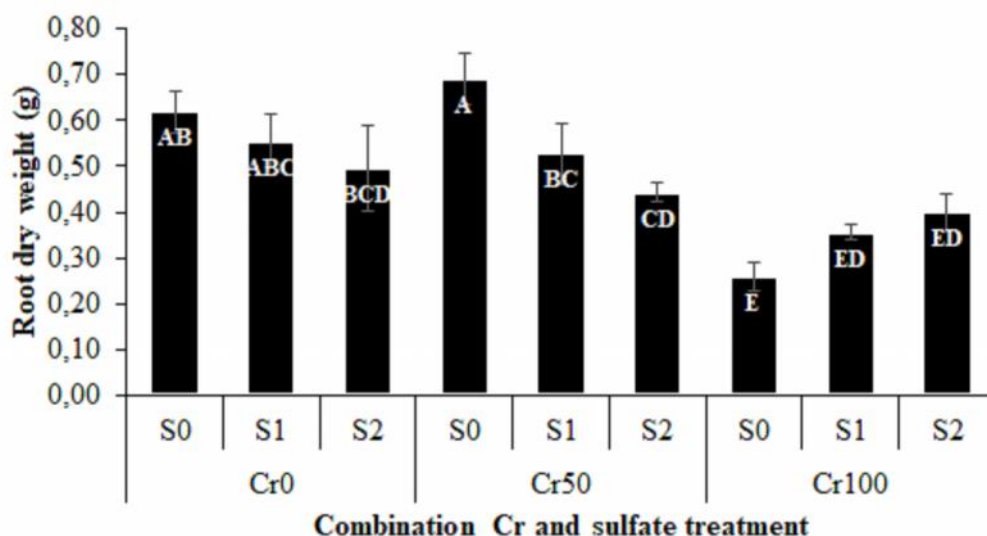


Figure 7. The root dry weight of *T. erecta* with combination treatment of Cr^{6+} in the form of K_2CrO_4 ($\text{Cr0} = 0 \text{ mg L}^{-1}$, $\text{Cr50} = 50 \text{ mg L}^{-1}$, and $\text{Cr100} = 100 \text{ mg L}^{-1}$) and SO_4^{2-} in the form of MgSO_4 ($\text{S0} = 0 \text{ mM L}^{-1}$, $\text{S1} = 1 \text{ mM L}^{-1}$, and $\text{S2} = 2 \text{ mM L}^{-1}$). The vertical bar on the column represent $\pm\text{SD}$, the same letter in column indicate were not significantly different at the 5% level of Tukey's test ($n=4$).

where as in the sulfate treatment of 2 mM L^{-1} , shoot dry weight decreased significantly (Figure 8). The shoot dry weight of plants treated with $\text{Cr}^{6+} 100 \text{ mg L}^{-1}$ showed no significant difference for all sulfate treatments, but shoot dry weight was lower

compared to control plants without Cr^{6+} and $\text{Cr}^{6+} 50 \text{ mg L}^{-1}$. The lowest shoot dry weight was found in plants treated with $50 \text{ mg L}^{-1} \text{Cr}^{6+}$ and 2 mM L^{-1} sulfate, with a shoot dry weight of 1.35 g .

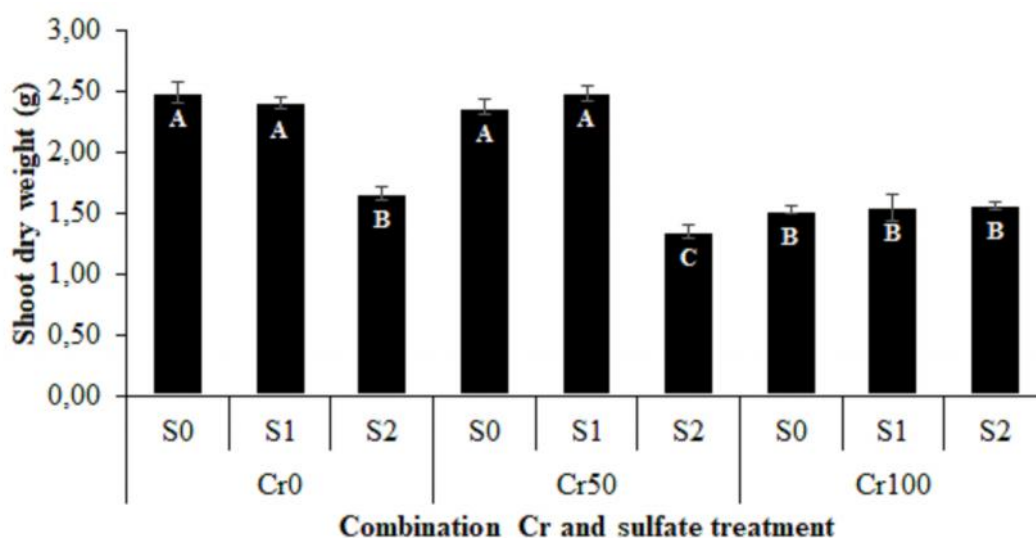


Figure 8. The shoot dry weight of *T. erecta* with combination treatment of Cr^{6+} in the form of K_2CrO_4 ($\text{Cr0} = 0 \text{ mg L}^{-1}$, $\text{Cr50} = 50 \text{ mg L}^{-1}$, and $\text{Cr100} = 100 \text{ mg L}^{-1}$) and SO_4^{2-} in the form of MgSO_4 ($\text{S0} = 0 \text{ mM L}^{-1}$, $\text{S1} = 1 \text{ mM L}^{-1}$, and $\text{S2} = 2 \text{ mM L}^{-1}$). The vertical bar on the column represent $\pm\text{SD}$, the same letter in column indicate were not significantly different at the 5% level of Tukey's test ($n=4$).

The high concentrations of Cr^{6+} reduced the dry weight of *T. erecta* roots and shoots. According to Sundaramoorthy et al. (2010) absorption and accumulation of Cr^{6+} by plants will reduce plant biomass because Cr^{6+} is toxic and inhibits the processes of carbohydrate and nitrogen metabolism, and reduces protein synthesis. Singh et al., (2020) reported that high concentrations of Cr^{6+} affected the total dry biomass of the *Cicer arietinum*. Ramana et al (2013) reported the effect of Cr stress on reducing biomass in various types of plants (*Aster novibelgii*, *Dahlia sambucifolia*, *Calendula officinalis*, and *Chrysanthemum indicum*). Based on the results of the study, it was shown that Cr^{6+} stress had a greater effect on root dry weight than shoot dry weight of *T. erecta*. Chitraprabha and Sathyavathi (2018) reported a greater reduction in root dry weight than shoot dry weight of *T. erecta* under Cr stress conditions originating from electroplating industrial waste. Santana, et al (2012) reported that the root and shoot dry weight of *Genipa americana* decreased significantly under the Cr^{6+} stress condition. Based on the results of the research of Kulczhicky and Sacala (2020) was reported that the application of S-fertilizer helps rice and corn plants to maintain biomass under Cr stress conditions. In this study, increasing sulfate tends to decrease the root and shoot biomass of *T. erecta*. Adequate fertilization with mineral fertilizers such as sulfur is essential for plants that are under heavy metal stress conditions including Cr^{6+} . Sulfur is necessary for proper plant growth and development. Aside from being a regulator, sulfur also has

a defense function in response to environmental stress and participates in the biosynthesis of some defense metabolites. Cysteine is a sulfur-containing amino acid that has a central role in the heavy metal detoxification process, because it is used to synthesize glutathione, phytochelatins, and metallothioneins (Kulczhicky & Sacala, 2020).

Cr content in the roots and shoots of *Tagetes erecta*

The Cr^{6+} treatment combined with the addition of sulfate in the *T. erecta* growing medium significantly affected the Cr^{6+} content in the roots and shoots. The Cr^{6+} content in the roots is higher than in the shoots. The Cr^{6+} content in the roots showed significant differences between treatments (Figure 9). The Cr^{6+} treatment of 50 mg L^{-1} and the control without Cr^{6+} treatment combined with three sulfate concentration treatments significantly increased the Cr^{6+} content in *T. erecta* roots. Meanwhile, the Cr^{6+} treatment of 100 mg L^{-1} and the addition of 1 mM L^{-1} sulfate (Cr100S1), caused a significant decrease in the Cr^{6+} content of the roots. The highest Cr^{6+} content in the roots of *T. erecta* was shown at the treatment concentrations of Cr^{6+} 50 mg L^{-1} and 100 mg L^{-1} with the addition of 2 mM L^{-1} sulfate of 0.020 mg .

The Cr^{6+} content of the shoots showed a different pattern from the Cr^{6+} content of the roots (Figure 10), and the Cr^{6+} content in the shoots was lower than that of the roots for all treatments. Plants that were under Cr^{6+} stress (50 mg L^{-1} and 100 mg L^{-1}) had higher shoot Cr^{6+} content than control plants. A

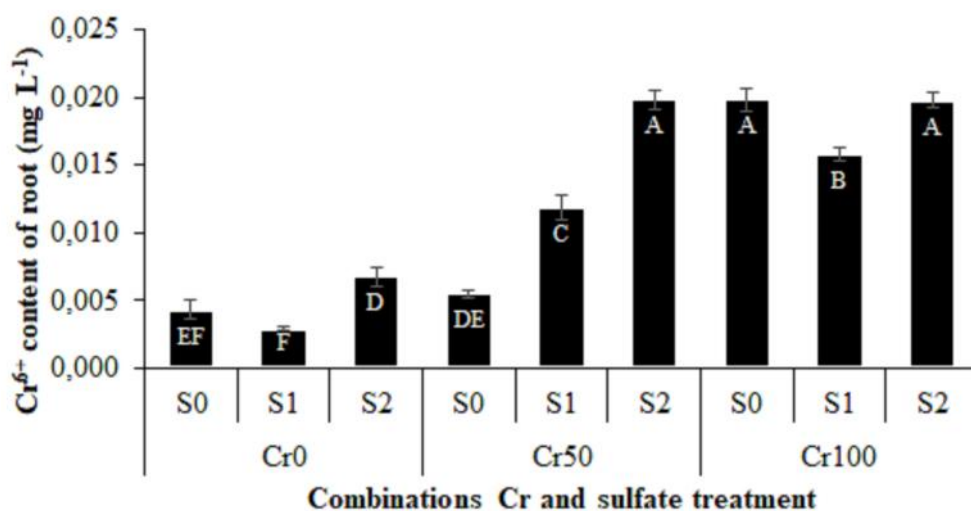


Figure 9. Cr⁶⁺ content of root *T. erecta* with combination treatment of Cr⁶⁺ in the form of K₂CrO₄ (Cr0 = 0 mg L⁻¹, Cr50 = 50 mg L⁻¹, and Cr100 = 100 mg L⁻¹) and SO₄²⁻ in the form of MgSO₄ (S0 = 0 mM L⁻¹, S1 = 1 mM L⁻¹, and S2 = 2 mM L⁻¹). The vertical bar on the column represent ±SD, the same letter in column indicate were not significantly different at the 5% level of Tukey's test (n=4).

significant decrease in the Cr⁶⁺ content of shoots was found in plants treated with Cr⁶⁺ of 100 mg L⁻¹ for all sulfate applications. A significant increase in the Cr⁶⁺ content of shoots was found in plants treated with Cr⁶⁺ of 50 mg L⁻¹ with the addition of 1 mM L⁻¹ and 2 mM L⁻¹ sulfates. The control plants

without Cr and sulfate (Cr0S0) showed the highest Cr⁶⁺ content of 0.009 mg.

Cr is the most immobile element in plant roots compared to other heavy metals. Many studies have reported that the most accumulation of Cr occurs in the roots

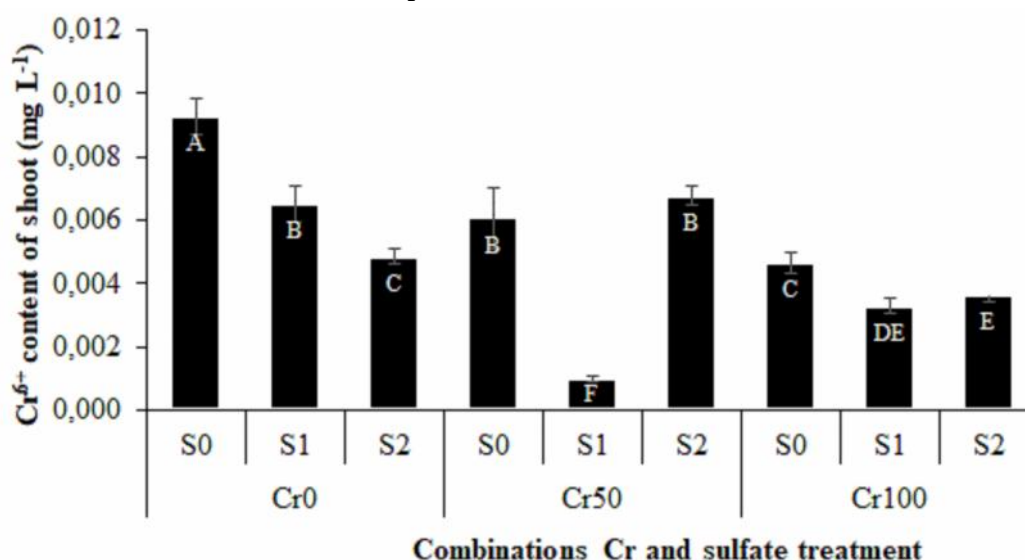


Figure 10. Cr⁶⁺ content of shoot *T. erecta* with combination treatment of Cr⁶⁺ in the form of K₂CrO₄ (Cr0 = 0 mg L⁻¹, Cr50 = 50 mg L⁻¹, and Cr100 = 100 mg L⁻¹) and SO₄²⁻ in the form of MgSO₄ (S0 = 0 mM L⁻¹, S1 = 1 mM L⁻¹, and S2 = 2 mM L⁻¹). The vertical bar on the column represent ±SD, the same letter in column indicate were not significantly different at the 5% level of Tukey's test (n=4).

compared to other parts of the plant (Shahid et al., 2017). Ram et al. (2019) reported that Cr accumulation was higher in roots than in shoots in *Napier hibrida*, and Cr accumulation in roots and shoots increased with increasing concentrations of Cr^{6+} in the soil, and the maximum increase occurred at the highest concentrations. Cr was found to accumulate in roots compared to leaves and stems (Srivastava et al., 2021).

Cr is structurally similar to sulfate and the interaction between the two mineral elements is antagonistic (Ulhasan et al., 2019). The availability of sulfate in the growing medium minimizes Cr^{6+} uptake by plants (Oliveira, 2012), because uptake of Cr^{6+} by plant cells has been reported to occur via sulfate carriers (membrane transporters) (Singh et al., 2013). However, based on the results of Oliviera et al. (2016) reported that Cr^{6+} did not reduce sulfur concentrations in *Pteris vittata*, possibly the plant roots absorbed sulfur faster than Cr^{6+} . The fact that Cr^{6+} does not affect sulfur concentrations suggests that sulfate may not compete with Cr^{6+} uptake and translocation or that *P. vittata* possesses a unique sulfate transporter that does not compete with Cr^{6+} uptake.

CONCLUSIONS

Based on the results of research that has been carried out, it can be concluded the interaction of Cr^{6+} and sulfate did not have a significant effect on plant height in *Tagetes erecta* but gave significantly different results on the number of leaves and flowers, dry weight of roots and shoots, root length, and Cr content in shoot and root of *Tagetes erecta*.

The application of 1 mM L⁻¹ and 2 mM L⁻¹ sulfate was able to increase plant height, number of leaves, root length, number of flowers, root dry weight, and Cr^{6+} content of *T. erecta* roots under Cr^{6+} stress conditions of 100 mg L⁻¹. *T. erecta* plant roots grown on Cr polluted media of 50 mg L⁻¹ and 100 mg L⁻¹ accumulated Cr^{6+} greater than the shoots at all concentrations of sulfate applied.

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