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THE APPLICATION OF Fe AND Cr(III) IN GROWING MEDIA AND ITS EFFECT ON PLANT GROWTH AND Cr(III) OXIDATION ON *Tagetes erecta*

Aplikasi Fe dan Cr(III) dalam Media Tanam dan Efeknya terhadap Pertumbuhan dan Oksidasi Cr(III) pada *Tagetes erecta*

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ABSTRACT
The oxidation of Cr(III) to Cr(VI) in the environment has a detrimental impact because it can change the form of non-toxic Cr(III) to Cr(VI), which is toxic to organisms. The study aimed to examine the effect of the application of iron (Fe) and trivalent chromium (Cr(III)) compounds in Tagetes erecta *growing media on growth and Cr(III) oxidation. Concentrations of Cr(III) 0, 100, and 500 mg L^{-1} and Fe 0, 3, 15, and 30 mg L^{-1} were applied to the growing media of* T. erecta *as the model plant. The growth and accumulation of Cr(VI) in plants were measured to determine the effect of Fe and Cr(III) treatment on growth and Cr(III) oxidation. The accumulation of Cr(VI) in the roots and shoots of* T. erecta *increased significantly due to the addition of Fe in the growing media treated with Cr(III). The highest accumulation of Cr(VI) in the roots and shoots of* T. erecta *found in the treatment of Cr(III) 500 mg L^{-1} and Fe 30 mg L^{-1}, were respectively 0.092 and 0.070 g L^{-1}. The addition of Fe in growing media containing Cr(III) increased plant height, root length, and shoot dry weight but decreased leaf number and root dry weight.* T. erecta *root biomass was more affected by the toxic impact of Fe than Cr(III). On the other hand, the inhibition of leaf formation was caused by the toxic effect of Cr(III) rather than Fe.*

Keywords: *chromium, ferrum, oxidation-reduction,* Tagetes erecta*, toxicity*

ABSTRAK
Oksidasi Cr(III) menjadi Cr(VI) di lingkungan mempunyai dampak merugikan, karena dapat mengubah bentuk Cr(III) dari tidak toksik menjadi bentuk Cr(VI) yang toksik bagi organisme. Tujuan penelitian adalah untuk mengkaji efek aplikasi senyawa besi (Fe) dan kromium trivalen (Cr(III)) dalam media tanam *Tagetes erecta* terhadap pertumbuhan dan oksidasi Cr(III). Konsentrasi Cr(III) 0, 100, dan 500 mg L^{-1} dan Fe 0, 3, 15, dan 30 mg L^{-1} diaplikasikan dalam media tanam *T. erecta* sebagai tanaman uji. Pertumbuhan dan akumulasi Cr(VI) pada tanaman diukur untuk mengetahui efek perlakuan dan mendeteksi terjadinya oksidasi Cr(III). Akumulasi Cr(VI) pada akar dan pucuk *T. erecta* mengalami peningkatan secara nyata akibat penambahan Fe dalam media tanam yang diberi perlakuan Cr(III). Akumulasi Cr(VI) pada akar dan pucuk *T. erecta* tertinggi dijumpai pada perlakuan Cr(III) 500 mg L^{-1} dan Fe 30 mg L^{-1} berturut-turut adalah 0,092 dan 0,070 g L^{-1}. Penambahan Fe dalam media tanam mengandung Cr(III) meningkatkan pertumbuhan tinggi tanaman, panjang akar dan berat kering pucuk, namun menurunkan jumlah daun dan berat kering akar. Biomassa akar *T. erecta* lebih dipengaruhi oleh efek toksik Fe dibandingkan Cr(III), sebaliknya penghambatan pembentukan daun lebih disebabkan oleh efek toksik Cr(III) daripada Fe.

Kata Kunci: kromium, besi, oksidasi-reduksi, *Tagetes erecta*, toksisitas

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INTRODUCTION

Heavy metals are environmental pollutants that need to be taken seriously because it's toxic, bioaccumulative, persistent, and non-biodegradable (Shadreck and Mugadza 2013). Chromium (Cr) is one of the heavy metal pollutants considered the most poisonous pollution. Moreover, its existence tends to increase from time to time. Chromium is not only sourced from nature, but it can also come from anthropogenic activities, especially from industrial activities that produce chromium-containing waste. Chromium is naturally abundant in serpentine soils (Cheng et al. 2011). Many industries and agricultural activities that have a lot of waste containing chromium pollutants are mining, metallurgy, metal plating (gilding), leather tanning, cement, textiles, paints and pigments, wood preservation, steel industry, canning industry, water cooling installations, and the use of pesticides and fertilizers (Narayani and Shetty 2013, Chebeir and Liu 2018, Sawicka et al. 2020).

Chromium has unique characteristics compared to other heavy metals because its mobility, solubility, reactivity, availability, and level of toxicity are determined by its oxidation state. Chromium in soil and water can be found in various oxidation states, including Cr(II) – Cr(VI), but only Cr(III) and Cr(VI) are the most stable. Cr(III) mainly comes from nature, while Cr(VI) comes from anthropogenic activities (Zayed and Terry 2003). Chromium can be beneficial or toxic to organisms depending on the concentration and oxidation state. Based on the level of toxicity, it was reported that Cr(III) has a toxicity 10 - 100 times lower than Cr(VI) (Butler et al. 2015, Lionel and Karunakaran 2017). Chromium in the form of Cr(III) is a nutritionally essential element needed by animals and humans for glucose and lipid metabolism, while Cr(VI) is toxic. Although Cr(III) is required by animals and humans, it has low toxicity. Nevertheless, based on several research results, it was reported that Cr(III) also showed cytotoxic effects on animal and human cells. Horie et al. (2013) reported that Cr(III) in the form of nanoparticles Cr(III) (Cr_2O_3) oxide decreased cell viability due to apoptosis in A549 lung carcinoma cells and human keratinocyte HaCaT cells. Tian et al. (2021) reported the toxic effect of Cr(III) in the form of $CrCl_3$ on embryonic development in mice.

Chromium is a heavy metal that is not essential for plants, but plants can absorb chromium well in Cr(III) and Cr(VI). Commonly the plants can absorb 3-5 times more Cr(VI) than Cr(III). Furthermore, most of Cr(III) is passively taken up, while Cr(VI) is actively absorbed by plants (Zayed and Terry 2003, Shanker et al. 2005, Sharma et al. 2020). Although the toxic effect of Cr(III) is lower than Cr(VI), it can inhibit plant growth in high concentrations. The toxic effects of Cr(III) (in the form of $CrCl_3$ and $KCr(SO_4)_2$) have been reported by many researchers on plants such as *Sorghum bicolor* (Kasmiyati et al. 2016), *Mentha* spp. (Barouchas et al. 2014), *Allium cepa* (Nematshahi et al. 2012), *Camellia sinensis* (Tang et al. 2012), and *Citrus aurantium* (Shiyab 2019).

Chromium in soil and water can be transformed from Cr(III) to Cr(VI) or vice versa through oxidation-reduction reactions. The content of organic matter, Fe and Mn, and redox potential and pH, are some factors that can affect chromium speciation in soil and water (Rajapaksha et al. 2013, Hausladen and Fendorf 2017, Varadharajan et al. 2017). Cr(III) in water and soil will form hydroxide compounds with Fe at certain pH conditions. The Cr(III)-Fe(III) hydroxide that formed in water and soil can be oxidized by oxygen and Mn oxide to Cr(VI) (Shadreck and Mugadza 2013, Chebier and Liu 2018). The availability of Fe in soil and water can affect the transformation of chromium. On the other hand, chromium in water and soil will affect the absorption of Fe nutrients by plants. Chromium has been reported to decrease Fe absorption in Spinacea oleracea (Gopal et al. 2009) and inhibit the activity of the Fe-reductase enzyme in alfalfa (Medicago sativa) roots (Shanker et al. 2005). The effect of Fe in the transformation of Cr(III) to Cr(VI) in soil and plant cells has not been widely studied and reported.

Tagetes erecta is one of the members of the Compositae family that has been widely reported to have potential as a remediation agent for heavy metal polluted environments. It also has significant growth and a well-developed root system. T. erecta is a non-edible ornamental plant that can grow in dry land and heavy metal toxicity stress areas. It can also absorb and accumulate various

heavy metals in roots and shoots (Bardiya-Bhurat et al. 2017, Coelho et al. 2017). The potential of T. erecta as a chromium phytoextraction agent has been widely reported, including in leather tanning waste (Miao and Yan 2013), artificial industrial waste (Hemalatha et al. 2017), textile waste (Parihar and Malaviya 2015), metal coating waste (Chitraprabha and Sathyavathi 2018), batik waste (Maryani et al. 2019), and lateritic soil contaminated with heavy metals (Madanan et al. 2021). The study aimed to examine the effect of the application of iron (Fe) and trivalent chromium (Cr(III)) compounds in T. erecta growing media on growth and Cr(III) oxidation.

MATERIALS AND METHODS

Location and time

The research was conducted in the Suruhan area, Rogomulyo Village, Kaliwungu District, Semarang Regency, and Biochemistry Laboratory, Faculty of Biology, Universitas Kristen Satya Wacana, Salatiga. The study was conducted from January to April 2020.

Research material

In this study, the plant material used was three-week-old *T. erecta* seedlings obtained from Kopeng, Semarang Regency, and used as a model plant. The chemical compounds used as treatment were Cr(III) in the form of $CrCl_3$ and Fe in the form of FeEDTA. The other chemicals were DMSO, to analyze the chlorophyll content, a mixture of HNO_3, HCl, and H_2O_2 for wet destruction of plant samples, aqua regia (a mix of HNO_3 and HCl made in a 3:1 ratio), diphenylcarbazide, H_2SO_4, $K_2Cr_2O_7$ for measuring the content of Cr(VI) with diphenylcarbazide method. The chemicals used were obtained from the Biochemistry Laboratory, Faculty of Biology, Universitas Kristen Satya Wacana, Salatiga.

The tools used in this research include UV-VIS spectrophotometer (Shimadzu UV-mini 1240), oven, desiccator, blender, mortar and pestle, hot plate, vortex, digital scale, water bath, stirrer, fan, porcelain dish, thermometer, and universal indicator pH. Other tools were a set of vacuum filters and filter paper with a pore size of 0.45 micron, a separating funnel, and glassware (test tubes, Erlenmeyer, measuring cups, and measuring pipettes of various sizes).

Table 1. Experimental design with Cr(III) and Fe treatments

Cr(III) mg L^{-1} treatment	Fe (mg L^{-1}) treatment			
	0 (F0)	3 (F1)	15 (F2)	30 (F3)
0 (C0)	C0F0	C0F1	C0F2	C0F3
100 (C1)	C1F0	C1M1	C1F2	C1F3
500 (C2)	C2F0	C2F1	C2F2	C2F3

Experimental design

The study was conducted experimentally using a completely randomized design (CRD) with two factors. The first factor was the Cr(III) treatment in $CrCl_3$ with three concentrations, namely 0, 100, and 500 mg L^{-1}. The second was Fe treatment in the form of Fe EDTA with four concentrations, namely 0, 3, 15, and 30 mg L^{-1}. The research design can be seen in Table 1, and each treatment with five replications.

Growing media preparation

The growing media of *T. erecta* used was a mixture of soil and compost with a ratio of 1:1 obtained from the Bioflora Store, Jalan Imam Bonjol Km 2, Kecandran Village, Sidomukti District, Salatiga. About 2 kg of growing media was prepared in 14 × 28 cm polybag plastic which had a volume of 2–3 kg. The total number of growing media prepared was 60 polybags.

Preparation of plants

The seedlings of T. erecta, obtained from nurseries in the Kopeng, Semarang, Regency, were three weeks old. The seedlings were selected based on the uniformity of growth conditions in height and number of leaves. Each polybag containing growth media was planted with one seedling and then acclimatized for one week. The watering was performed every two days on T. erecta seedlings during the acclimatization period.

Application of Cr(III) and Fe

The application of Cr(III) and Fe in the growing media of *T. erecta* was given in the form of $CrCl_3$ and Fe EDTA compounds ($FeSO_4$ and EDTA) by dissolving them in distilled water with different concentrations according to the treatments. Cr(III) and Fe were carried out. Cr(III) and Fe were applied simultaneously by mixing the two compound solutions, and 100 mL of the mixed solution

was carried out to each planting medium according to the treatments. The application of Cr(III) and Fe solutions was carried out one day before and seven days after planting *T. erecta*. The application of Cr(III) and Fe EDTA after planting was repeated every seven days (once a week) for eight weeks (two months).

Maintenance of plant growth

The preservation of *T. erecta* plant growth was carried out for eight weeks after planting by watering with distilled water every two days, as much as 50 mL for each polybag, except when the treatment solution was applied. The plants were placed in direct sunlight. During planting, pests and diseases were controlled mechanically.

Parameter measurement and data analysis

The parameters measured included plant growth, photosynthetic pigment content, and Cr(VI) concentration in plant tissue. The growth of *T. erecta* was measured at the end of the experiment, including number of leaves, stem height, and root length measured with a ruler, and dry weight of roots and shoots was determined by oven at 80 ºC for 48 hours until a constant weight was reached. The content of chlorophyll and carotenoid pigments in the leaves at the end of the experiment was measured based on the method of Caesar et al. (2018), modified with DMSO solvent. The leaf samples about 0.04 g were cut into small pieces and soaked in 5 mL of DMSO solution in a dark bottle and incubated for 48 hours at room temperature. The solutions were filtered, and a UV-VIS spectrophotometer measured absorbance values at 480, 649, and 665 nm wavelengths. The chlorophyll and carotenoids contents were determined based on the following (Wellburn 1994):

$$Ca = 12{,}19A_{665} - 3{,}45A_{649}$$
$$Cb = 21{,}99A_{649} - 5{,}32A_{665}$$
$$Cc = (1000A_{480} - 2{,}14Ca - 70{,}16Cb)/220$$

Ca = chlorophyll a (µg mL^{-1})

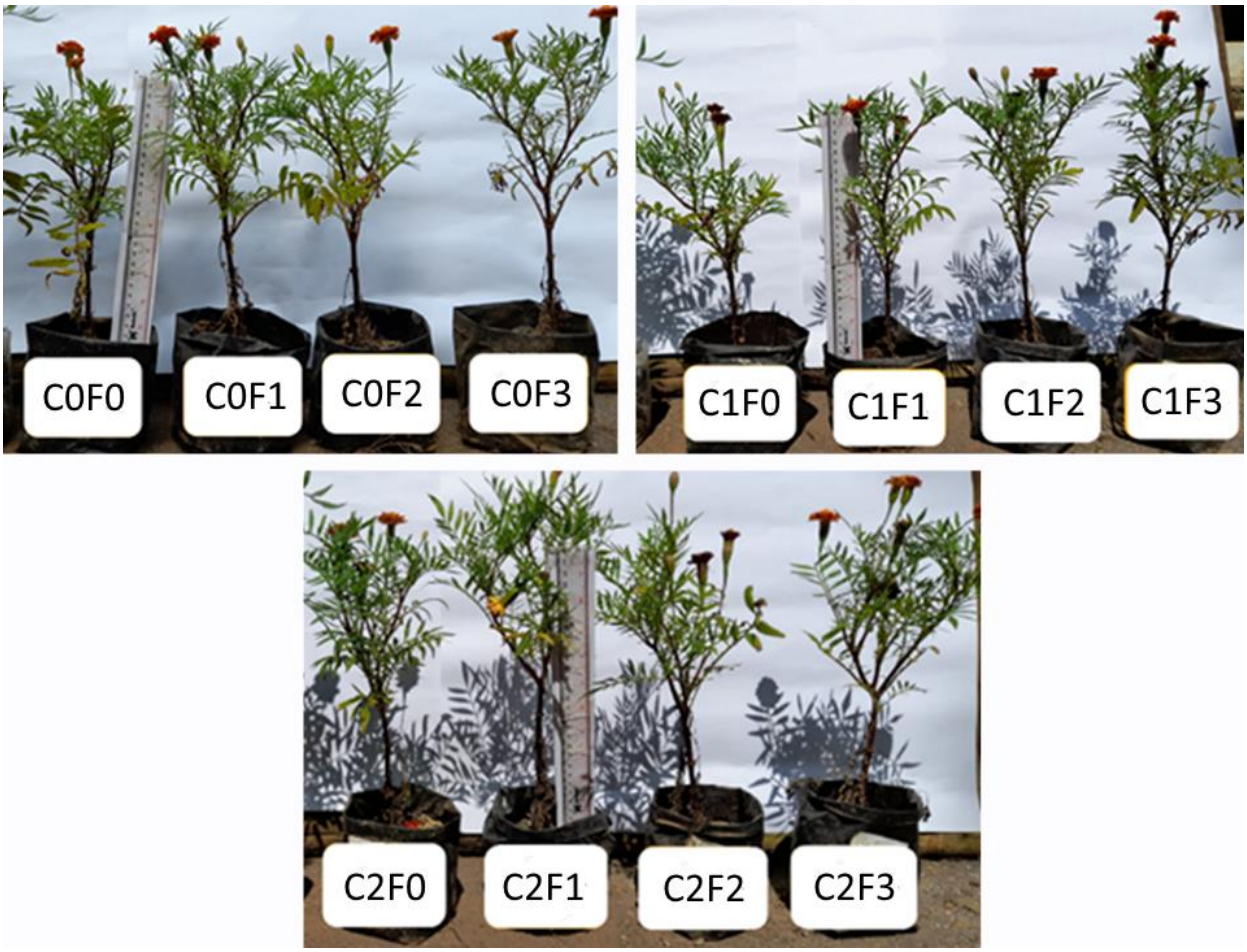

Figure 1. The plant growth of *T. erecta* on growing media with the application of Cr(III) in the form of $CrCl_3$ (C0 = 0 mg L^{-1}, C1 = 100 mg L^{-1}, and C2 = 500 mg L^{-1}) and Fe in the form of FeEDTA (F0 = 0 mg L^{-1}, F1 = 3 mg L^{-1} F2 = 15 mg L^{-1} and F3 = 30 mg L^{-1})

Cb = chlorophyll b (µg mL^{-1})
Cc = carotenoid (µg mL^{-1})
A = absorbance at certain wavelength

According to Gheju et al. (2009), the determination of the concentration of Cr(IV) in plant tissue that used the diphenylcarbazide method was applied with some modifications. Root and shoot samples were prepared using the wet destruction method. Samples of dried roots and shoots dried in the oven at 80 ºC for 48 hours were mashed using a blender and destroyed in an acid solution. A fine sample of 0.2 g was added to the acid solution mixture containing 3 mL HNO_3, 1 mL HCl, 0.5 mL H_2O_2 and heated on a hotplate in an acid chamber until the solution became clear. The clear sample solution was cooled and filtered, then the filtrate was put into a 10 mL volumetric flask and distilled water was added to a volume of 10 mL. Then the filtrate was ready to be used to measure Cr(VI) levels. A total of 2.5 mL of sample filtrate was added with 3 drops of concentrated H_2S0_4 and 0.1 mL of 5% diphenylcarbazide solution, vortexed and incubated for 15 minutes. Then the absorbance was measured using a UV-VIS spectrophotometer at a wavelength of 540 nm. The Cr(VI) content of the sample was determined using the standard Cr(VI) curve.

All data reported in this research are the mean of five replicates. The parameter measurement data were analyzed using the statistical package SAS (Statistical Analysis System) version 9.1.3. The two-way ANOVA was carried out to determine the significant effect of means treatments on the measured parameters, followed by the post hoc Tukey test to determine the critical difference between means of treatments. Statistical significance was considered at $P<0.05$.

RESULTS AND DISCUSSION

Plant growth

The application of Cr(III) and Fe in the growing media significantly affected plant growth (Figure 1). The growth of *T. erecta* showed a different response to the treatment of Cr(III) and Fe concentrations. The growth response of *T. erecta* to the application of Cr(III) and Fe was observed based on plant height, a number of leaves, root length, and dry weight of roots and shoots. Cr(III) and Fe increased the plant height, root length, and shoot dry weight, however decreasing leaf number and dry root weight.

The interactive effect of Cr(III) and Fe application on plant height of *T. erecta* showed different responses between treatments. *T. erecta* treated with 100 mg L^{-1} Cr(III) and 15 mg L^{-1} FeEDTA showed the highest plant height of 33.8 cm and was significantly different from other treatments. The most stunted plant height was found in *T. erecta* with FeEDTA treatment of 30 mg L^{-1} without Cr(III) treatment which reached 26.0 cm (Figure 2).

The application of Cr(III) and Fe in increasing plant height was found in the 100 mg L^{-1} Cr(III) treatment with 15 and 30 mg L^{-1} Fe and 500 mg L^{-1} Cr(III) with 3 mg L^{-1} Fe treatment. The inhibition of *T. erecta* plant height by Cr(III) was affected by the concentration of Fe applied in the growing medium and the opposite. It can be seen that the inhibition of plant height occurred at the application of the highest concentration of Fe (30 mg L^{-1}) without Cr(III) treatment and a concentration of Cr(III) 500 mg L^{-1} with 15 and 30 mg L^{-1} Fe. The inhibition of plant height growth by Cr(III) treatment in the form of $Cr(NO_3)_3 \cdot 9H_2O$ without interaction with other elements has been reported, including shallot (*Allium cepa*) with 200 mg L^{-1} treatment (Nematshahi et al. 2012), tea (*C. sinensis*) with a treatment of 600 mg kg^{-1} (Tang et al. 2012) and sour orange (*C. aurantium*) with a treatment of 200 mg kg^{-1} (Shiyab 2019). Based on the results of this study, it was shown that the addition of Fe was able to increase the height growth of *T. erecta* plants treated with Cr(III).

The number of *T. erecta* leaves was also significantly affected by Cr(III) and Fe treatment. The applications of Cr(III) of 100 and 500 mg L^{-1} with Fe of 3–30 mg L^{-1} reduced the number of plant leaves, whereas in control without Cr(III), a decrease in the number of leaves was found in the treatment of Fe of 15 and 30 mg L^{-1} (Figure 3). The most significant decrease in the number of leaves occurred in the treatment of Cr(III) 500 mg L^{-1} with Fe of 30 mg L^{-1}. The highest number of leaves was found in control plants without Cr(III) with the addition of 3 mg L^{-1} Fe, which reached an average of 26.8 leaves per plant, while the least was in the treatment of Cr(III) 500 mg L^{-1} and Fe of 30 mg L^{-1} with an average of 7.4 leaves per plant. The inhibition of leaf formation in plants treated with Cr(III) was caused by the inhibition of cell

division and differentiation due to the accumulation of Cr in the leaves (Shahid et al. 2017). A decrease in leaf number due to chromium toxicity has also been reported in *Citrullus plants* (Dube et al. 2009).

The dry weight of the shoots of *T. erecta* showed different results with the number of leaves in the application of Cr(III) and Fe (Figure 4). The dry weight of shoots of *T. erecta* without Cr(III) treatment (control) and high Cr(III) concentration (500 mg L^{-1}) decreased in line with the increasing concentration of Fe applied to the growing media. The most significant decrease in dry weight was found in plants with Fe treatment of 30 mg L^{-1} both in the 500 mg L^{-1} Cr(III) treatment and the control without Cr(III). The highest shoot dry weight with an average of

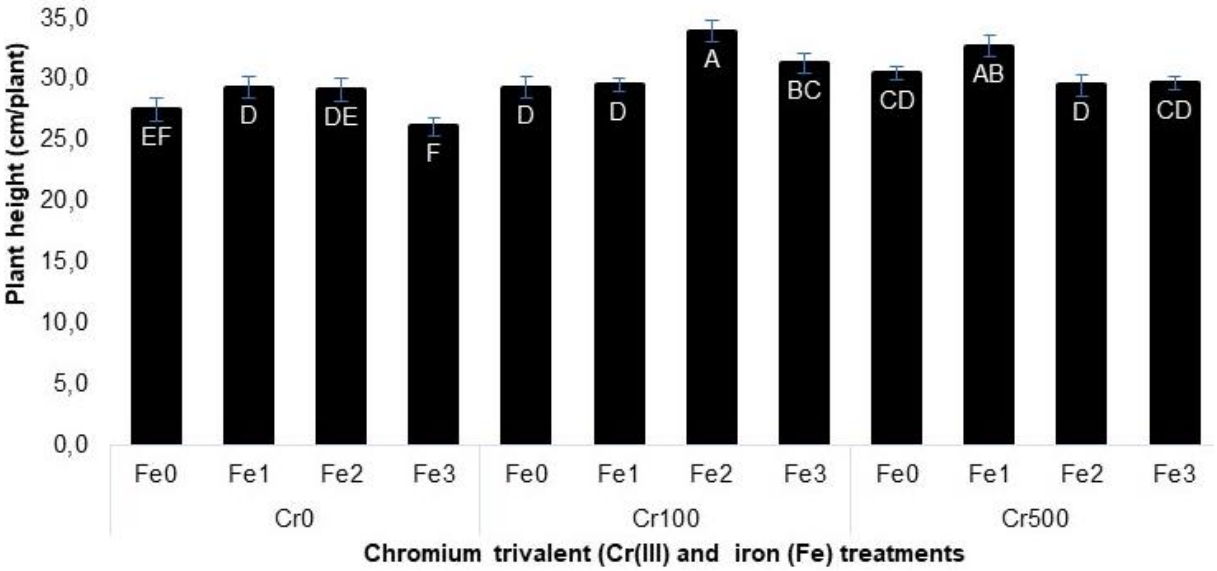

Figure 2. The plant height of *T. erecta* on growing media with the application of Cr(III) in the form of $CrCl_3$ (C0 = 0 mg L^{-1}, C1 = 100 mg L^{-1}, and C2 = 500 mg L^{-1}) and Fe in the form of FeEDTA (F0 = 0 mg L^{-1}, F1 = 3 mg L^{-1}, F2 = 15 mg L^{-1} and F3 = 30 mg L^{-1}). The vertical bars on the columns represents ±SD. Values with the same letter were not significantly different at the 5% level of Tukey's test (n = 5)

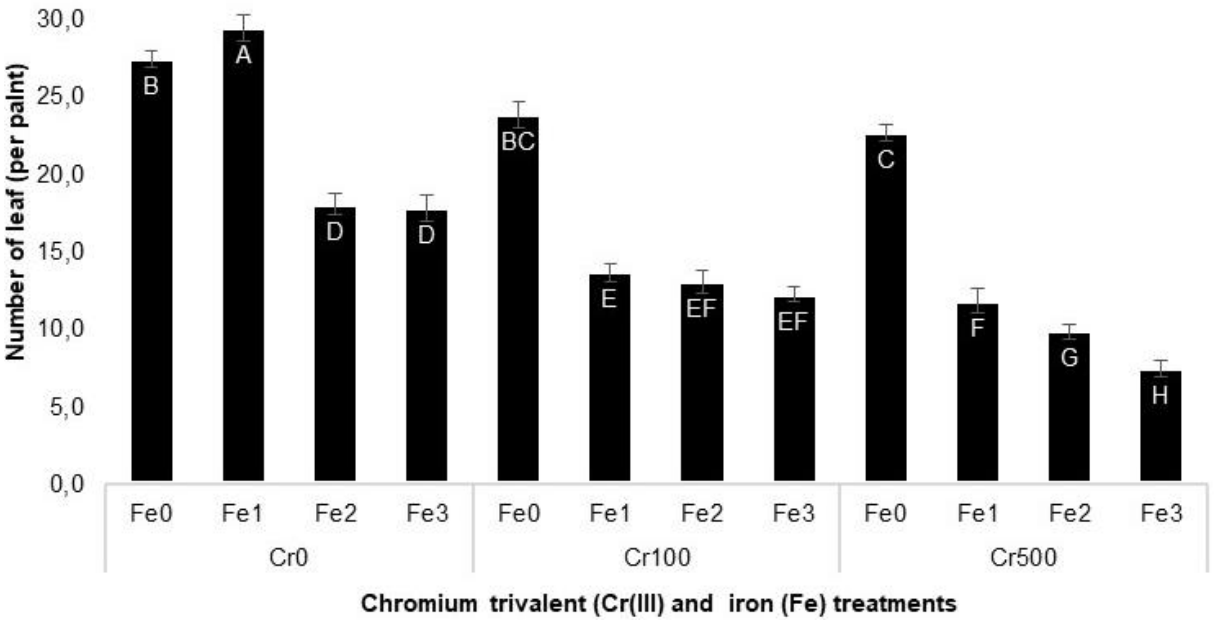

Figure 3. The **number** of *T. erecta* leaf on growing media with the application of Cr(III) in the form of $CrCl_3$ (C0 = 0 mg L^{-1}, C1 = 100 mg L^{-1}, and C2 = 500 mg L^{-1}) and Fe in the form of FeEDTA (F0 = 0 mg L^{-1}, F1 = 3 mg L^{-1}, F2 = 15 mg L^{-1} and F3 = 30 mg L^{-1}). The vertical bars on the columns represent ±SD. Values with the same letter were not significantly different at the 5% level of Tukey's test (n = 5)

3.29 g was shown in plants treated with Cr(III) 500 mg L^{-1} and Fe 3 mg L^{-1}, while the smallest was 1.75 g in treatment with Cr(III) 100 mg L^{-1} and without Fe treatment. The increase in shoot dry weight of *T. erecta* treated with Cr(III) 100 and 500 mg L^{-1} with the addition of Fe in the growing media showed the same response pattern with plant height (Figure 2).

The presence of Cr(III) in the growing media can affect the absorption of essential nutrients are needed by *T. erecta*, one of which is the absorption of Fe. The application of chromium in the soil can increase or decrease the uptake of essential nutrients by plants (Shanker et al. 2005). Chromium was reported to decrease Fe absorption in *Oryza sativa* (Sundaramoorthy et al. 2010). Dube et al. (2009) also reported inhibiting the absorption of essential nutrients S, P, and Fe in carrot (*Daucus carota*) shoots treated with 0.05 mM Cr stress in the growing medium. According to Shahid et al. (2017), antagonistic interactions between Cr and essential nutrients can occur due to interference in the soil and in plant tissues. In this study, the inhibitory effect of Fe absorption by Cr(III) could be restored through the application of Fe in the growing media so that the shoot biomass and plant height of *T. erecta* treated

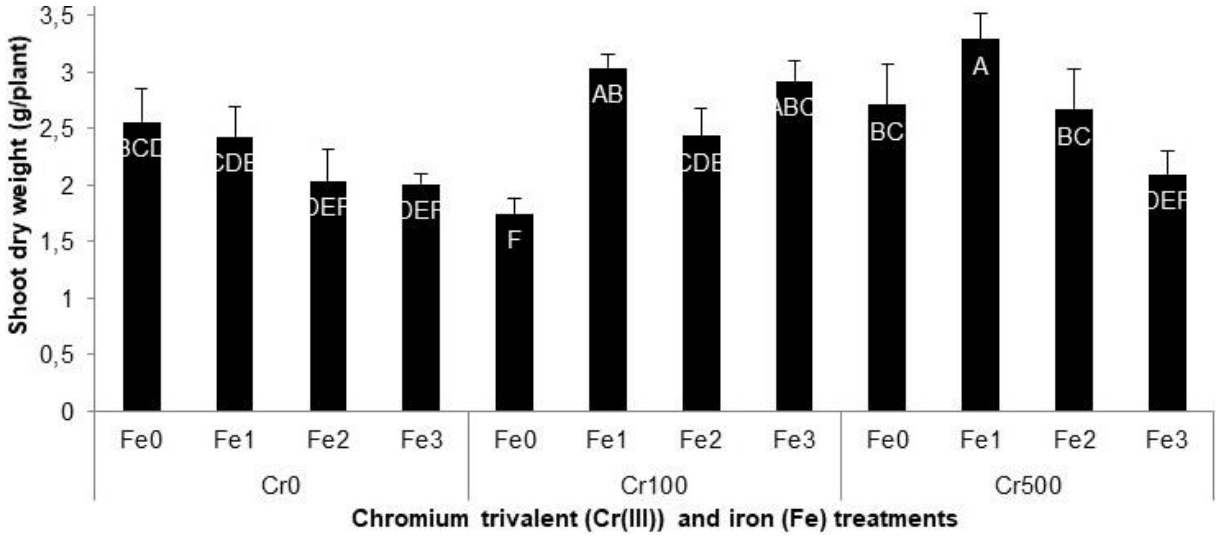

Figure 4. The shoot dry weight of T. erecta on growing media with the application of Cr(III) in the form of $CrCl_3$ (C0 = 0 mg L^{-1}, C1 = 100 mg L^{-1}, and C2 = 500 mg L^{-1}) and Fe in the form of FeEDTA (F0 = 0 mg L^{-1}, F1 = 3 mg L^{-1}, F2 = 15 mg L^{-1} and F3 = 30 mg L^{-1}). The vertical bars on the columns represent ±SD. Values with the same letter were not significantly different at the 5% level of Tukey's test (n = 5)

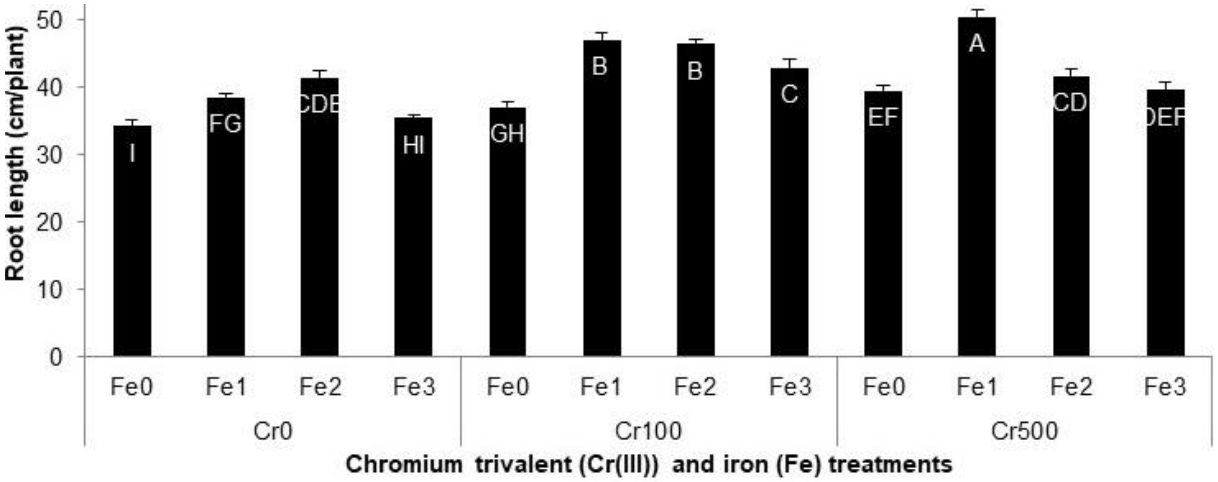

Figure 5. The **root** length of *T erecta* on growing media with the application of Cr(III) in the form of $CrCl_3$ (C0 = 0 mg L^{-1}, C1 = 100 mg L^{-1}, and C2 = 500 mg L^{-1}) and Fe in the form of FeEDTA (F0 = 0 mg L^{-1}, F1 = 3 mg L^{-1}, F2 = 15 mg L^{-1} and F3 = 30 mg L^{-1}). The vertical bars on the columns represent ±SD. Values with the same letter were not significantly different at the 5% level of Tukey's test (n = 5)

with Cr(III) did not decrease. On the contrary, the growth tended to show a different increase significantly compared to control plants.

The growth of *T. erecta* root length was significantly affected by the treatment of Cr(III) and Fe in the growing media (Figure 5). The

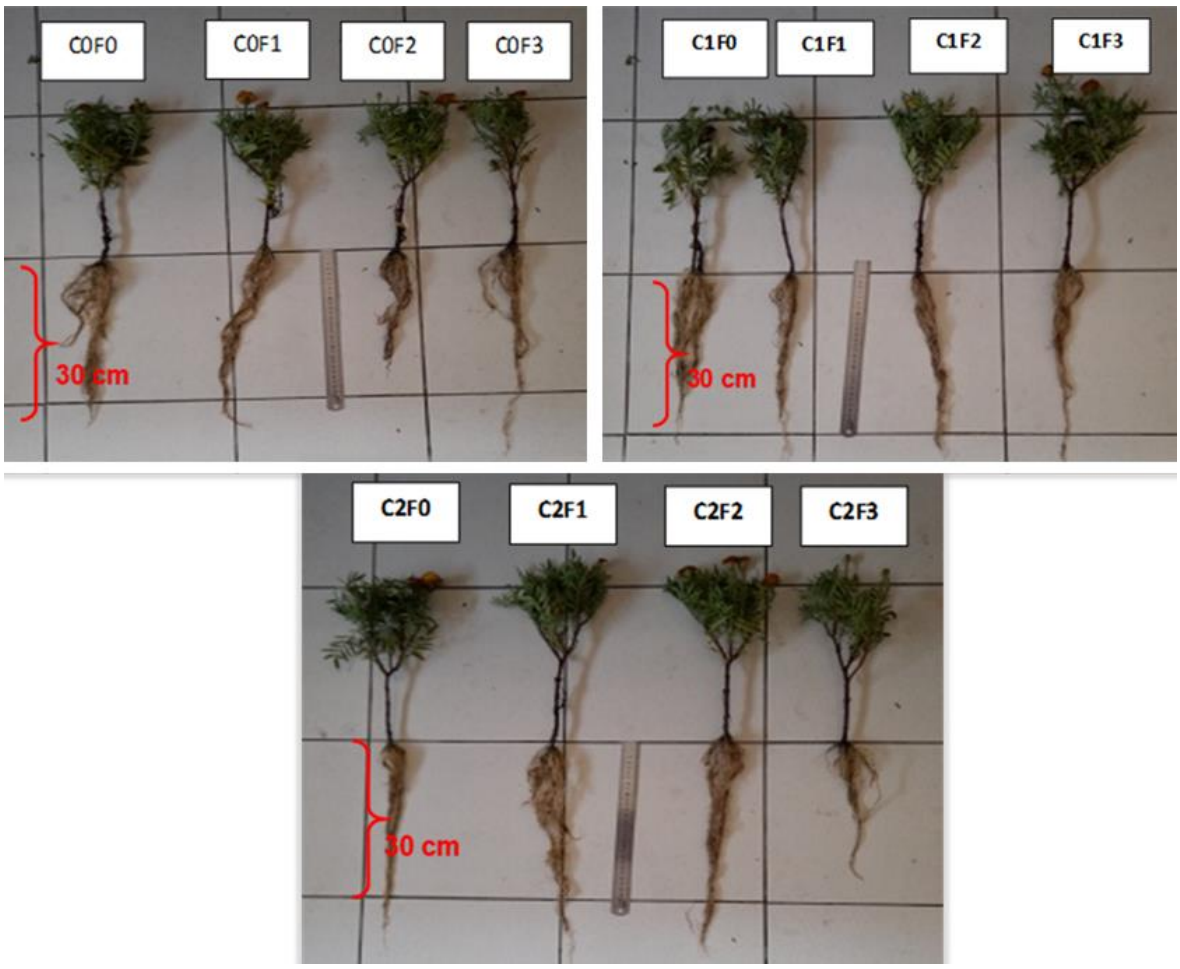

Figure 6. The growth of *T. erecta* roots on growing media with the application of Cr(III) in the form of $CrCl_3$ (C0 = 0 mg L^{-1}, C1 = 100 mg L^{-1}, and C2 = 500 mg L^{-1}) and Fe in the form of FeEDTA (F0 = 0 mg L^{-1}, F1 = 3 mg L^{-1}, F2 = 15 mg L^{-1} and F3 = 30 mg L^{-1})

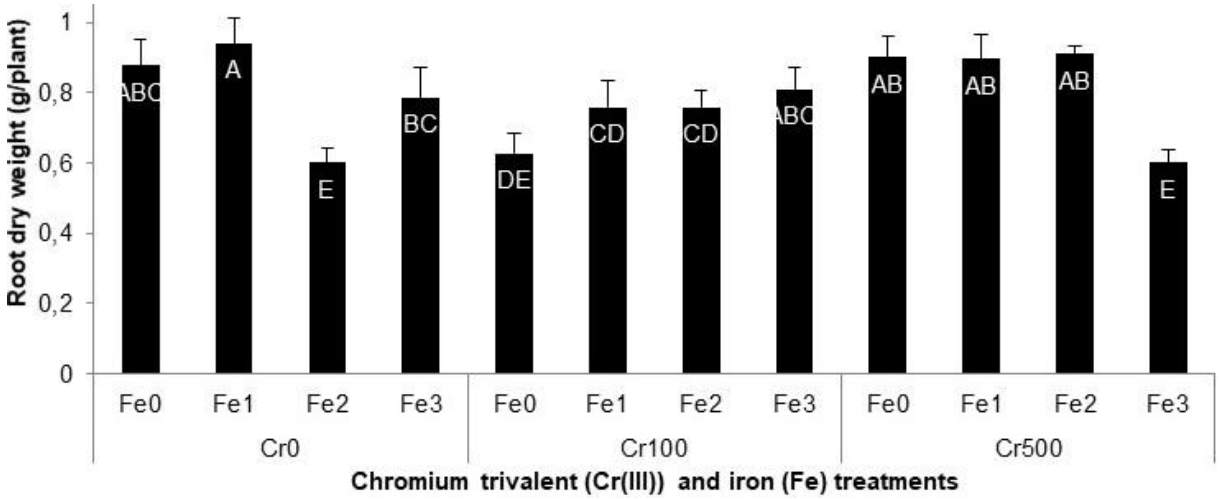

Figure 7. The root dry weight of *T. erecta* on growing media with the application of Cr(III) in the form of $CrCl_3$ (C0 = 0 mg L^{-1}, C1 = 100 mg L^{-1}, and C2 = 500 mg L^{-1}) and Fe in the form of FeEDTA (F0 = 0 mg L^{-1}, F1 = 3 mg L^{-1}, F2 = 15 mg L^{-1} and F3 = 30 mg L^{-1}). The vertical bars on the columns represent ±SD. Values with the same letter were not significantly different at the 5% level of Tukey's test (n = 5)

length of root that were treated using Cr(III) showed a different response from the control in the treatment of Fe application in growing media.

The application of Fe on *T. erecta* growing media with Cr(III) treatment showed the roots growth were more significant than control plants without Cr(III) (Figure 6). The application of Fe in growing media increased root length in both control plants and those treated with Cr(III) 100 mg L^{-1} and 500 mg L^{-1}. The longest root length of 50.4 cm was found in the 500 mg L^{-1} Cr(III) treatment with 3 mg L^{-1} Fe, while the smallest was 34.2 cm in the control plant without Cr(III) and without the addition of Fe.

Besides affecting the shoots, the application of Cr and Fe also affected the roots of *T. erecta* plants. According to Shahid et al. (2017), roots as the main organ in nutrient absorption will be directly related to the absorption of Cr and Fe, and become the primary target for the toxic effects of Cr and Fe on plants. The toxic effect of Cr(III) on decreasing root length was reported in *C. aurantium* plants treated at 200 mg kg^{-1} (Shiyab 2019), while in tea plants (*C. sinensis*) at a concentration of 600 mg kg^{-1} (Tang et al. 2012).

In contrast to the results reported by previous researchers, in this study, the root length of *T. erecta* in Cr(III) treatment of 100 mg L^{-1} and 500 mg L^{-1} with the application of Fe increased significantly. The increase of root length in *T. erecta* was caused by the addition of Fe, which can reduce the toxicity of Cr(III) by reducing the effect of Fe deficiency on plants, or the addition of Fe in plant media was able to increase the tolerance of *T. erecta* to Cr(III). The inhibitory effect of Cr on root elongation can be caused by a decrease in root cells, a disturbance in the absorption of water and nutrients, and an increase in the length of the cell cycle (Sundaramoorthy et al. 2010)

The dry weight of *T. erecta* roots showed a response that supported the results of root length growth. The dry weight root in control plants without Cr(III) increased on Fe application at 3 mg L^{-1}, while Fe concentrations at 15 mg L^{-1} and 30 mg L^{-1} were decreased compared to the treatment without and using Fe at 3 mg L^{-1} (Figure 7). The application of Fe to the growing media of *T. erecta* with Cr(III) 100 mg L^{-1} significantly increased the dry weight of the roots compared to the application without the addition of Fe. In Cr(III) 500 mg L^{-1} treatment, *T. erecta* with Fe application of 3 mg L^{-1} and 15 mg L^{-1} showed that the dry weight of the roots was not significantly different from the control without the addition of Fe. In contrast, the Fe treatment of 30 mg L^{-1} decreased significantly compared to other treatments. The highest dry weight root of 0.94 g was found in *T. erecta* in the control treatment without Cr(III) with 3 mg L^{-1} Fe, and the lowest at 0.60 g in the control plant without Cr(III) and Fe 15 mg L^{-1}.

The decreasing root growth caused by heavy metal toxicity has been widely reported. Among heavy metals, the effect of Cr on root growth was reported to be higher than in other heavy metals, such as Cd and Pb (Shanker et al. 2005). Several researchers have reported the decreasing plant dry weight due to Cr(III) treatment. The roots dry weight of *Allium cepa* in the Cr(III) treatment at a concentration of 5 mg L^{-1} – 100 mg L^{-1} increased compared to the control. A concentration of 150 – 200 mg L^{-1} started to decrease (Nematshahi et al. 2012). Tea plant (*C. sinensis*) was also reported dry weight roots decreased at the same time with the increasing concentration of Cr(III), and the most significant decrease occurred at a concentration of 600 mg L^{-1} (Tang et al. 2012).

In contrast to the results of studies that have been previously reported, the results of this study indicate that the application of Fe in the growing media can reduce the inhibitory effect of root growth due to the toxicity of Cr(III) in *T. erecta*. This was indicated by the increase of root dry weight from plants that received Cr(III) 100 mg L^{-1} on Fe 3–30 mg L^{-1} application and plants with Cr(III) 500 m L^{-1} with Fe 3–15 mg L^{-1} application. The application of Fe in growing media to a specific concentration can inhibit the toxic effect of Cr on plants. According to Shanker et al. (2005), Cr(III) and Cr(VI) are absorbed by plants through different mechanisms. The uptake of Cr(III) and Cr(VI) is reported to inhibit the uptake of several other essential nutrients that have similar structures to Cr(III) and Cr(VI), namely Fe and S. Therefore, Fe application will push the interference effect in inhibiting the absorption of Fe by Cr(III), so plants did not run into Fe deficiency. Shanker et al. (2005) also reported that in deficient

plants, the addition of Cr(III) to a specific concentration could increase the dry weight of plant roots.

Content of photosynthetic pigments in leaves

Chlorophyll and carotenoids are essential pigments that play a role in photosynthesis, and the pigment content is very closely correlated with photosynthesis and growth. The content of total chlorophyll (Figure 8) and carotenoids (Figure 9) of *T. erecta* leaves was significantly affected by the application of Cr(III) and Fe in the growing media. The application of Cr(III) and Fe to the growing media of *T. erecta* showed the same effect on the total chlorophyll and carotenoid content. The total chlorophyll decreased in control plants without Cr(III) with 15 mg L^{-1} and 30 mg L^{-1} Fe application, while carotenoids did not significantly increase all Fe concentration treatments. The content of total chlorophyll and carotenoids in leaves plants treated with Cr(III) 100 mg L^{-1} increased with 3 mg L^{-1} and 30 mg L^{-1} Fe application compared to control plants without Cr(III) and without Fe addition. *T. erecta* plants grown on growing media with 500 mg L^{-1} Cr(III) treatment showed a decrease in total

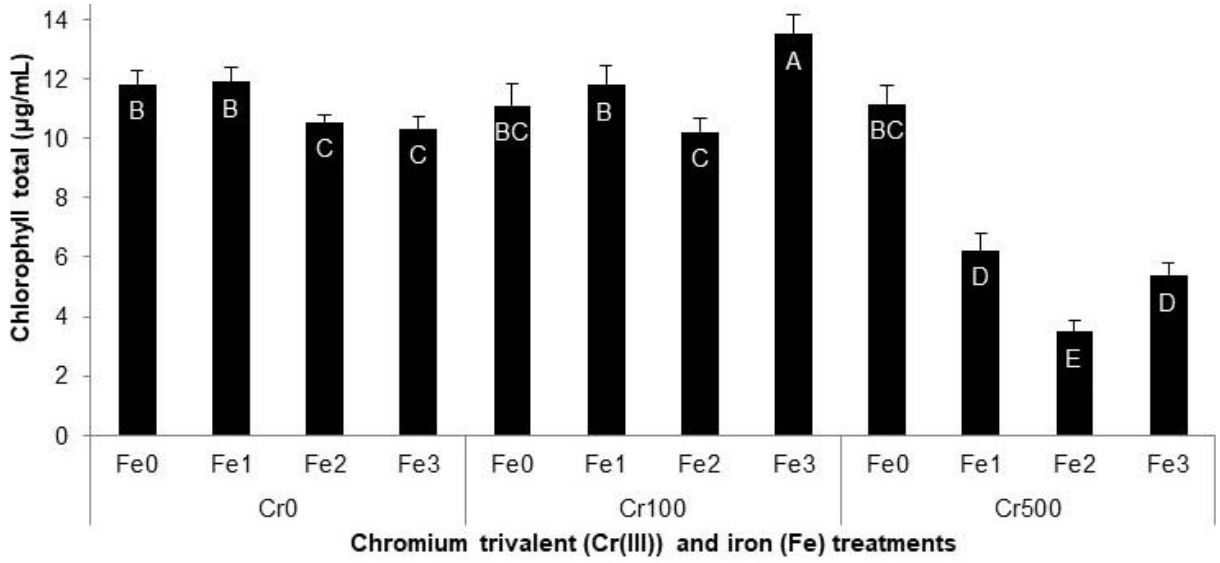

Figure 8. The total chlorophyll content of *T. erecta* on growing media with the application of Cr(III) in the form of $CrCl_3$ (C0 = 0 mg L^{-1}, C1 = 100 mg L^{-1}, and C2 = 500 mg L^{-1}) and Fe in the form of FeEDTA (F0 = 0 mg L^{-1}, F1 = 3 mg L^{-1}, F2 = 15 mg L^{-1} and F3 = 30 mg L^{-1}). The vertical bars on the columns represent ±SD. Values with the same letter were not significantly different at the 5% **level** of Tukey's test (n = 5)

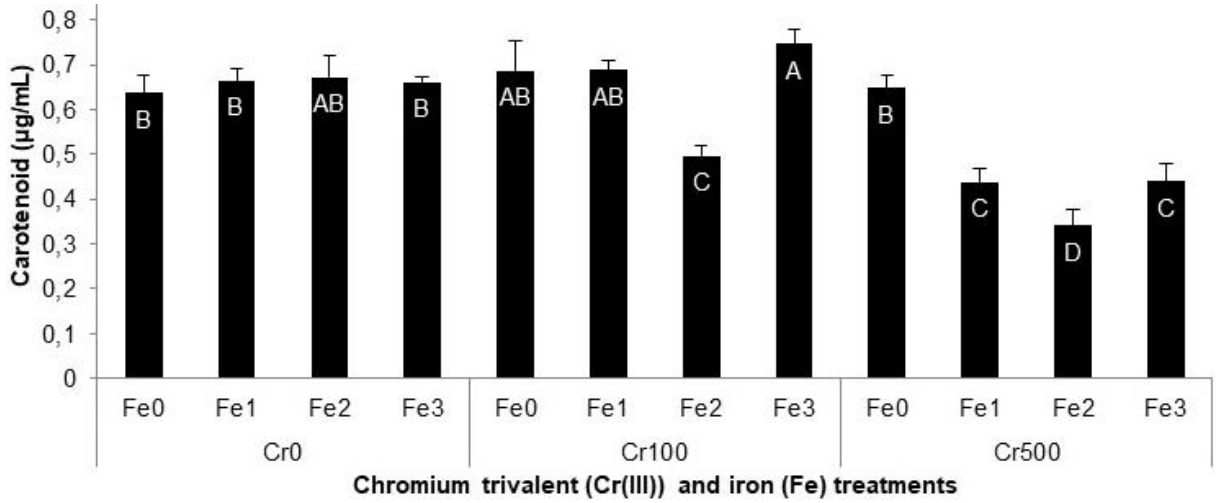

Figure 9. The carotenoid content of *T. erecta* on growing media with the application of Cr(III) in the form of $CrCl_3$ (C0 = 0 mg L^{-1}, C1 = 100 mg L^{-1}, and C2 = 500 mg L^{-1}) and Fe in the form of FeEDTA (F0 = 0 mg L^{-1}, F1 = 3 mg L^{-1}, F2 = 15 mg L^{-1} and F3 = 30 mg L^{-1}). The vertical bars on the columns represent ±SD. Values with the same letter were not significantly different at the 5% level of Tukey's test (n = 5)

chlorophyll and carotenoids with the addition of Fe at all treatment concentrations. The highest total chlorophyll and carotenoid content was 13.52 g mL^{-1}, shown in plants treated with Cr(III) of 100 mg L^{-1} and Fe of 30 mg L^{-1}, while the smallest was 3.48 g mL^{-1} in the treatment Cr(III) is 500 mg L^{-1}, and Fe is 15 mg L^{-1}.

Almost all heavy metals affect the photosynthetic apparatus and photosynthetic processes, which can impact decreasing growth, plant productivity. They can even cause cell and plant death (Shahid et al. 2017). The decreasing content of photosynthetic pigments chlorophyll (a, b, and total) and carotenoids caused by the toxicity of heavy metals Cr(III) and Cr(VI) has been reported in various species of plants including *C. sinensis* (Tang et al. 2012), *Triticum sativum* (Tripathi et al. 2015), *Salvinia minima* and *S. rotundifolia* (Pardo et al. 2016), and *C. aurantium* (Shiyab 2019). Based on this study, it was shown that the effect of Cr(III) was stronger in influencing the biosynthesis of photosynthetic pigments in *T. erecta* than Fe. This is supported by the results showing the most significant reduction in chlorophyll and carotenoid pigments content showed in the 500 mg L^{-1} Cr(III) treatment with all Fe concentrations. Shanker et al. (2005) reported that the content of photosynthetic pigments in plants subjected to Cr stress was highly correlated with Fe absorption. Cr stress in plants has a negative effect on Fe absorption and affects the biosynthesis of photosynthetic pigments. In this study, the effect of Cr(III) stress on the photosynthetic pigment content of *T. erecta* could be reduced by adding Fe up to 30 mg L^{-1} in the Cr(III) treatment of 100 mg L^{-1}. Nevertheless, the reduction in the effect of Cr(III) stress by Fe did not occur in the Cr(III) treatment of 500 mg L^{-1}.

The decrease in photosynthetic pigment content on *T. erecta* plants at 500 mg L^{-1} Cr(III) stress was also indicated by the appearance of chlorosis on the leaves. The chlorosis symptoms observed on leaf shoots indicate the occurrence of Fe deficiency. Shanker et al. (2005) stated that chlorosis in plants under heavy metal stress is generally caused by low levels of Fe in plants. This is due to mobilization or inhibition of Fe absorption. Heavy metal Cr affects the absorption of Fe through the inhibiting reduction of Fe(III) to Fe(II) by the enzyme Fe(III) reductase in roots or competing with Fe(II) at the absorption site. Dey and Mondal (2016) reported that Cr(III) and Cr(VI) stress can inhibit photosynthetic pigment biosynthesis through the degradation of the enzyme aminolaevulinic acid dehydratase, which is a crucial enzyme in chlorophyll biosynthesis. The inhibition of photosynthetic pigment synthesis is caused by the competition of Cr with Fe and Mg for absorption and transportation to leaves. The inhibition of Cr on Mn and Ca absorption causes damage to WOC (water oxidizing centers) in photosystem II (Rodriguez et al. 2012) and the replacement of Mg ions on the active sites of various enzymes (Shahid et al. 2017).

Cr(VI) content in roots and shoots

Chromium in the soil can be transformed from one oxidation state to another. Cr(III) can be transformed into Cr(VI) or otherwise through an oxidation-reduction reaction. The existence of oxidant compounds influences the reaction of oxidation Cr(III) in soil and water. Some environmental factors that directly affect the oxidation of Cr(III) to Cr(VI) are Mn and oxygen, while Fe, pH, organic matter, and microorganisms are indirectly (Oliveira 2012, Hausladen and Fendorf 2017). In this study, *T. erecta* plants were grown on growing media using Cr(III) treatment and were detected to contain Cr(VI) in their root and shoot tissues. These results indicated that the Cr(III) applied in the growing media will oxid to Cr(VI). This oxidation Cr(III) process may occur in soil media or plant tissues.

The levels of Cr(VI) in shoots and roots of *T. erecta* were significantly influenced by the application of Cr(III) and Fe in the growing media. The Cr(VI) levels in the roots were higher than in the shoots for all treatments. The levels of Cr(VI) in the shoots of *T. erecta* that were treated with Cr(III) increased in line with the increasing concentration of Fe applied to the growing media (Figure 10). Plants treated with Cr(III) 100 mg L^{-1} and 500 mg L^{-1} with the addition of Fe at 15 mg L^{-1} and 30 mg L^{-1} had Cr(VI) levels in the shoots higher than without and with 3 mg L^{-1} Fe. The detection of Cr(VI) in shoots and roots of *T. erecta* treated with Cr(VI) is suspected of having a close relation to the increase of H_2O_2 as the consequence of oxidative stress (Shanker et al. 2005).

The shoots of *T. erecta* accumulated less Cr(VI) than the roots. These results also showed that *T. erecta* could translocate Cr absorbed by the roots to the shoots. The maximum absorption of Cr occurs in the roots, while in other vegetative organs and generative are absorbed less (Moncekova et al. 2016). Generally, chromium is translocated very limitedly to the shoots (less than 1/100th of the concentration in the roots) because most of the Cr is sequestered in the vacuoles of root cells to detoxify and tolerate Cr toxicity (Shanker et al. 2005).

The Cr(VI) levels in the roots increased significantly in line with the increase in the concentration of Fe applied (Figure 11). The highest rate increase in Cr(VI) levels in roots was found in the addition of Fe 30 m L^{-1} in all Cr(III) treatments and control plants without Cr(III). The increased Cr(VI) levels in plants given Fe indicated that Fe played a role in the availability of Cr(VI) for plants. According to Chebeir and Liu (2018), the presence of Fe in soil and water plays an indirect in the Cr oxidation process. Fe and Cr(III) in the soil will form hydroxide Cr(III)-Fe(III) in the form of

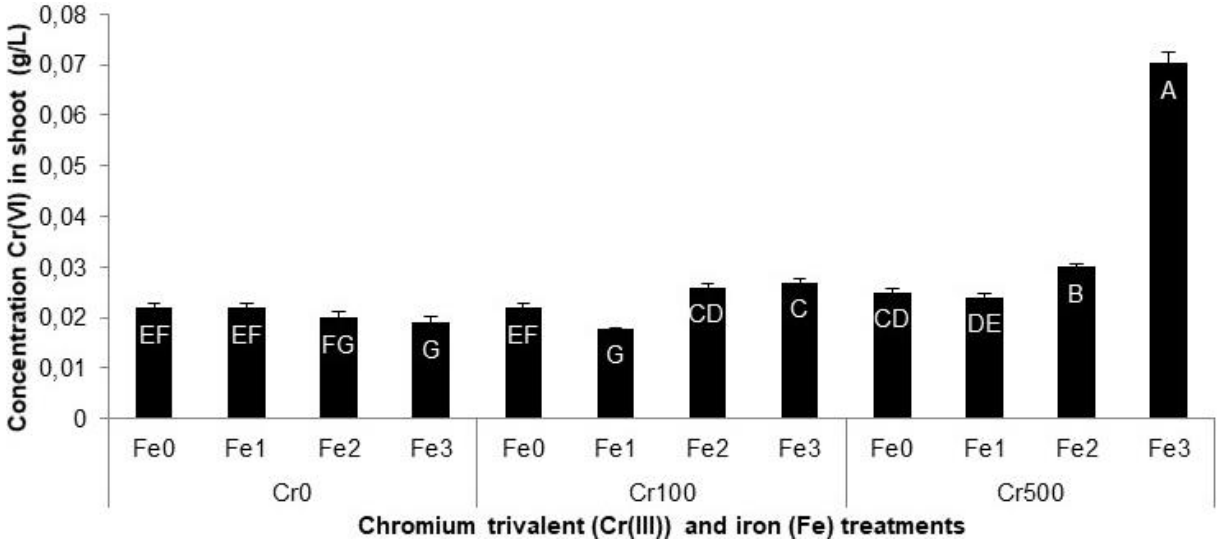

Figure 10. The levels of Cr(VI) in *T. erecta* shoots on growing media with the application of Cr(III) in the form of $CrCl_3$ (C0 = 0 mg L^{-1}, C1 = 100 mg L^{-1}, and C2 = 500 mg L^{-1}) and Fe in the form of FeEDTA (F0 = 0 mg L^{-1}, F1 = 3 mg L^{-1}, F2 = 15 mg L^{-1} and F3 = 30 mg L^{-1}). The vertical bars on the columns represent ±SD. Values with the same letter were not significantly different at the 5% level of Tukey's test (n = 5)

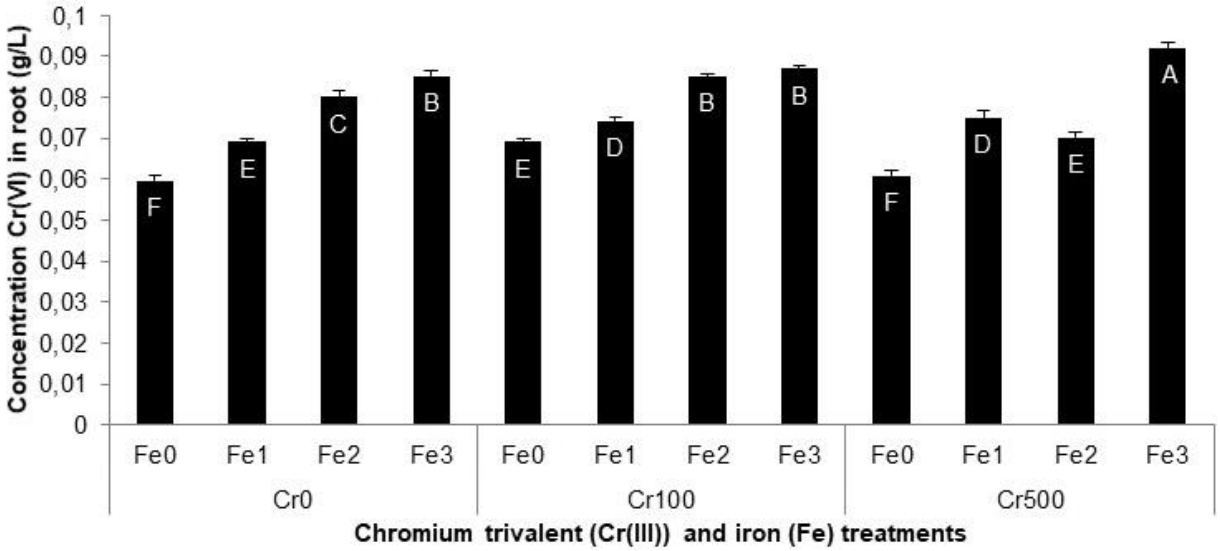

Figure 11. The levels of Cr(VI) in *T. erecta* roots on growing media with the application of Cr(III) in the form of $CrCl_3$ (C0 = 0 mg L^{-1}, C1 = 100 mg L^{-1}, and C2 = 500 mg L^{-1}) and Fe in the form of FeEDTA (F0 = 0 mg L^{-1}, F1 = 3 mg L^{-1}, F2 = 15 mg L^{-1} and F3 = 30 mg L^{-1}). The vertical bars on the columns represent ±SD. Values with the same letter were not significantly different at the 5% level of Tukey's test (n = 5)

$Fe_xCr_{(1-x)}(OH)_3(s)$, which can be oxidized to be Cr(VI) if there is oxygen and oxide Mn(IV) in high concentrations. Chromium in plant cells can have a reduction or oxidation reaction depending on the chemical form of Cr (Shanker et al. 2005). However, the process and mechanism of Cr(III) to Cr(IV) oxidation in plant cells has not been widely known and reported. Therefore, it is still necessary to do further research on the internal factors in plant cells that play a role in the process and mechanism of Cr(III) oxidation.

T. erecta plants grown on media without Cr(III) treatment also accumulated Cr(VI) in roots and shoots for all Fe treatments. These results indicated that the planting media contains Cr(VI) from the soil or compost. Although shoots and roots of control plants had Cr(IV), the levels were lower than those treated with Cr(III). These results indicated that Cr(III) applied in the growing media of *T. erecta* was oxidized. Thereby it increased the availability of Cr(VI), which plants absorb.

The plants of *T. erecta* treated with Cr(III) 500 mg L^{-1} and Fe 30 mg L^{-1} accumulated Cr(VI) in the roots and shoots is higher than other treatments. The levels of Cr(VI) in the roots and shoots of plants treated with Cr(III) concentrations of Cr 500 mg L^{-1} and Fe 30 mg L^{-1} were 0.0922 mg L^{-1} and 0.072 mg L^{-1}. The high accumulation of Cr(VI) in the shoots and roots of *T. erecta* with Cr(III) treatment of 500 mg L^{-1} and Fe 30 mg L^{-1} caused the most significant inhibition of growth and biosynthesis of photosynthetic pigments compared to other treatments. This indicates that even though Cr(III) is reported to have lower toxicity than Cr(VI), at high concentrations, it can be toxic and oxidate if the environmental factors are supported. According to Hausladen and Fendorf (2017), soil contains a lot of reactive Mn oxides, organic acids, and having a low pH supports the oxidation reaction of Cr(III) to Cr(VI).

CONCLUSION

The Fe application in the form of FeEDTA at 3-15 mg L^{-1} in the growing media was able to increase the tolerance of *T. erecta* to Cr(III) at 100-500 mg L^{-1}. The growth of plant height, root length, and shoot dry weight were increased with the application of Fe in growing media containing Cr(III), while the number of leaves and root dry weight decreased. The most significant growth of stem height, root length, and shoot dry weight were 33.8 cm, 50.4 cm, and 3.29 g respectively. It was found under Cr(III) stress of 100–500 mg L^{-1} with Fe application of 3–15 mg L^{-1}. The decrease in root biomass of *T. erecta* was more influenced by the toxic effect of Fe than Cr(III), whereas the inhibition of leaf formation was caused by the toxic effect of Cr(III) than Fe. The highest and lowest root dry weight were found in control plants without Cr(III) with Fe application of 3 mg L^{-1} and 15 mg L^{-1} with values of 0.94 g and 0.60 g continuously. The addition of 30 mg L^{-1} FeEDTA in *T. erecta* growing media treated with 500 mg L^{-1} Cr(III) increased the toxic effect of Cr on chlorophyll and carotenoid biosynthesis. The application of Fe and Cr(III) in the growing media increased Cr(VI) levels in the roots and shoots of *T. erecta*. The (VI) levels in the roots were higher than in the shoots. Fe is suspected of influencing the oxidation of Cr(III) to Cr(VI) in soil media or plant structure. The role of Fe in the Cr(III) oxidation process in soil and plant structure is not widely known and still needs to be studied deeply.

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