

PHYTOREMEDIATION OF CHROMIUM BY BAD BIRNBACH ROSES IN URBAN AREAS

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Abstract

Chromium is a heavy metal that is toxic to plants and other living things. It is known that plants can be used to remove heavy metals from the environment if they accumulate in the soil. Various ornamental plants are used in urban areas due to their decorative properties. In this study, the effectiveness of the Bad-Birnbach rose variety in removing excess chromium in the soil was investigated. To determine its effect on the vegetative and generative development states of roses and Cr uptake from the soil, different doses of Cr⁺⁶ (0, 25, 50 mg/kg soil) and 0-5-10-20 mmol EDTA were applied to the plant growth medium. At the end of the experiment, the dry weight of the stem and root decreased significantly with the applications of Cr⁺⁶. With EDTA application, the dry weight of roots and flowers increased. Cr concentrations in stem, root and rose are between 3.63-55.04, 14.53-314.77 and 2.31-13.44 mg/kg, respectively. Cr concentrations in the plant are above the permissible limit values and Cr⁺⁶ has accumulated significantly in the root zone.

Key words: chromium, EDTA, heavy metal, ornamental plants, phytoremediation.

INTRODUCTION

In recent years, chromium (Cr) has reached increasingly toxic levels in the environment as a result of various industrial and agricultural activities. Cr cause concern as an environmental pollutant (Zayed & Terry, 2003). In various countries such as China, Iran and Russia, Cr pollution is intensely encountered around the places where chromium mines are processed (Vodyanitskii, 2009; Solgi & Parmah, 2015; Zhang et al., 2016). In our country, it was found in the soils of the Karamenderes Basin (Sümer et al., 2013), in the wastewater of Trabzon province (Topcuoglu et al., 2002), in the agricultural lands around Hatay (Özkan et al., 2017) and in the areas irrigated with water from the wastewater treatment system in Konya (Akay et al., 2009), Cr concentrations are above the limit values.

In addition to various remediation methods, the phytoremediation technique is also recommended to remediate Cr-polluted soils and waters. The toxic effect of Cr in Cr³⁺ and Cr⁶⁺ forms depend on the oxidation state (Vodyanitskii, 2009). Cr⁶⁺ is the toxic and persistent form of Cr in the soil (Srivastava et

al., 2021). Cr retards plant growth (Han et al., 2004); seed germination, root elongation and plant development are negatively affected (Shahid et al., 2017).

Cleaning Cr-contaminated areas using plants is a good and environmentally friendly technology (Thakur et al., 2016). The use of ornamental plants in areas where pollution treatment is applied can be preferred in remediation studies due to their various advantages (Rocha et al., 2022). It has been determined that periwinkle and oleander flowers have the capacity to remove Cr and Pb minerals (Al-Anbari et al., 2018). Among the ornamental plants *Mirabilis jalapa*, *Impatiens balsamin* (I. Balsamin) and *Tagetes erecta* L., it was observed that *Mirabilis jalapa* was more effective in Cr uptake than the other two plants (Miao & Yan, 2013). It has been stated that marsh iris (*I. pseudacorus* L.) also retains Cr in its roots (Caldeas et al., 2012). Cr⁺⁶ stress causes early flowering in *Zinnia elegans* L. (Panda et al., 2020); *Cosmos bipinnatus* Cav. and *Celosia cristata* L. belongs to Asteraceae and Amaranthaceae family plants showed morphological changes when grown in Cr⁺⁶ polluted soils, and *C. bipinnatus* Cav. was stated to be able to adapt and withstand harsh

conditions (Karthik & Sharavanan, 2016). Various chelating substances can be used to increase the heavy metal uptake of plants (Saifullah et al., 2009). Thus, it is stated that some chemicals and various chelators will be effective to increase the effectiveness of the remediation study (Liu et al., 2009). Synthetic chelates such as ethylene diamine tetraacetate (EDTA), diethylene triamine pentaacetic acid (DTPA), and hydroxy ethylene diamine triacetic acid (HEDTA) are widely used chemicals that solubilize metals in soil (Sabir et al., 2014). Chelating substances dissolve heavy metals in the soil, making them available to the plant and allowing remediation plants to absorb more metal (Salt et al., 1998).

EDTA is stated as the most effective chelate in extracting metals from soil (Hsiao et al., 2007). In the study where the solubility of Cr^{3+} in the presence and absence of EDTA was calculated as a function of pH, it was found that in the absence of the chelator, Cr^{3+} would form inorganic $\text{Cr}(\text{OH})_3$ complexes, and thus precipitated in solution between $\text{pH} > 3-4$. In the presence of EDTA, it was observed that Cr^{3+} maintained its solubility as a Cr^{3+} -EDTA complex in the pH range of 3-7.

Cr precipitation in the form of $\text{Cr}(\text{OH})_3$ could be prevented up to pH 9. Soluble Cr concentration and therefore the amount of plant-available Cr was five times higher in the presence of EDTA (Erenoglu et al., 2007). EDTA disrupts the concentration balance of Cr^{6+} in the liquid and solid phase of the soil and can form mobile compounds that can be absorbed by plants by chelating or coordinating some difficult-to-transport Cr (Ebrahimi, 2015). In such conditions, some resistant plants can still grow (Ram et al., 2019).

In this study, it was aimed to determine the Cr heavy metal uptake capacity of Bad Birnbach rose grown in soil contaminated with different concentrations of Cr. It was also aimed to determine the effectiveness of EDTA chelate in the solubility of Cr applied to the soil and in Cr uptake by the plant.

MATERIALS AND METHODS

Certified Bad Birnbach rose was used in the study. The roses were obtained from a certified production company in Turkey. The sandy soil

used in the greenhouse experiment was sieved through a 4 mm sieve and then filled into 6 litter pots. Chromium ($\text{K}_2\text{Cr}_2\text{O}_7$) in the Cr^{+6} form, which was the subject of the experiment, was applied to the pots at doses of 0, 25, 50 mg Cr kg^{-1} soil and 0-5-10-20 mmol EDTA chelate. After the Cr application, the pots were incubated for 2 months. At the end of the incubation period, bare-rooted rose seedlings were pruned in stem and root and immediately planted in pots. The experiment was carried out in the greenhouse in three replicates, according to the randomized plots factorial trial design. Macro and micronutrient need of plants were calculated according to soil analysis results and applied regularly using Hogland's solution (The content of the Hoagland solution is as follows: Fe-EDTA, H_3BO_4 , MnSO_4 , $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, Zn-EDTA, $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$, CaNO_3 , KH_2PO_4 , KNO_3 , MgSO_4 (16.50, 2.86, 1.29, 0.393, 1.33, 0.018, 590, 219.5, 443.5, 492 mg L^{-1}).

The soil used in the experiment was analyzed. The soil was slightly alkaline (pH 7.78) and sandy. It had an EC of $382 \mu\text{S cm}^{-1}$ (salt-free), CaCO_3 content was 4.96% (calcareous). The P concentration of soil was very little (1.76 mg kg^{-1}), Ca and Mg concentrations were sufficient (respectively 3972 and 207 mg kg^{-1}), and K concentration was little (71 mg kg^{-1}). The soil had 8.72 mg kg^{-1} available Fe (high), 1.62 mg kg^{-1} available Zn (sufficient), 1.06 mg kg^{-1} available Cu (sufficient), 0.80 mg kg^{-1} available B (little), and 4.27 mg kg^{-1} available Mn (little). Organic matter content was 0.38% (very little). Plant material: Bad Birnbach rose is a disease-resistant rose belonging to the Kordes' Klima-Rosen collection, which has awards from international rose competitions. It is a bushy vertical plant that can reach 50 cm in height and 50 cm in crown width. It is appropriate to plant 4 pieces per 1 m^2 in mass plantings. The average diameter of its flower is 4-5 cm. The plant, which is heat resistant, suitable for balcony boxes, semi-shades, hanging and growing in pots, is resistant to weather conditions (Anonymous, 2024). It was first introduced to the public in 2000 with its plump salmon and pink flowers (Anonymous, 2024a). This rose variety is widely used in pavements with heavy traffic and in various landscaping

applications in Konya, where the trial was carried out.

After bloomed throughout the experiment, flowers were collected regularly weekly, and their fresh and dry weights were recorded. At the end of the experiment, flower yield (taking into account the applications in all pots) were calculated according to the values obtained. Harvesting was done, when the flowering rate of the plants decreased, after the 10-month development period. During the harvesting process, the roots, stems and rose parts were weighed separately, and their fresh weights were taken. Then, to determine the dry weights, the root, stem and flower parts were kept at 65°C for 2 days and their weight was determined by weighing on a precision scale.

Cr concentration in stem, flower, and root samples:

Cr analysis in plants was done using the modified wet burning method (Zheljazkov & Nielsen, 1996; Hseu, 2004). Cr concentrations were determined with the ICP-OES (Agilent Technologies (SPS3) 5100 model) device. 1000 mg/L (Merck, New Jersey, USA) was used as internal standard. STD IPE 952 (grass (mixture)/Poaceae) standard certified plant sample was used to verify the readings by the elemental analysis. The concentration of Cr for IPE 952 was 3810 $\mu\text{g kg}^{-1}$.

The amount of Cr removed from the soil by plant parts was calculated according to the following formula:

Cr removed by plant organ ($\mu\text{g kg}^{-1}$) = [(plant organ Cr concentration (mg/kg) \times plant organ dry weight (g/pot)/1000]*1000.

The translocation factors (TF) were calculated as the ratio of plant aerial part metal concentration to root metal concentration (Ghosh & Singh, 2005; Zurayk et al., 2002).

$TF = MS/MR*100$

were:

- MR = Cr concentration in root (mg kg^{-1})
- MS = Cr concentration in stem (mg kg^{-1})

Tolerance index (ToI) was calculated by dividing the dry weight of the trial plant to the dry weight of the control plant.

The bioaccumulation factor (BCF) for Cr was calculated according to Zayed et al. (1998).

Bioaccumulation factor (BCF) = Cr concentration in plant/ Cr concentration applied to the plant initially

Cr concentration in plant = (Stem Cr concentration*stem weight) + (Flower Cr concentration*flower weight) + (Root Cr concentration*root weight).

The data obtained as a result of the pot experiment were carried out with the MINITAB 18 statistical package program and the significant differences were subjected to the Tukey multiple comparison test.

RESULTS AND DISCUSSIONS

It was observed that the effect of Cr and EDTA applications on the fresh and dry weights of the stem, root and flower parts of the Bad Brinbach rose was statistically significant ($p < 0.05$) (Table 1 and Figure 1). Both fresh and dry weight values of stems and roots were higher in the Cr control application compared to the 25 mg kg^{-1} Cr and 50 mg kg^{-1} Cr applications. Also flower dry weight was higher (5.34 g) in the 25 mg kg^{-1} Cr application compared to the control and 50 mg kg^{-1} Cr applications. When the effect of EDTA applications was examined, there was no statistical difference in both fresh and dry weights of the stem and root compared to the control. In general, application of 20 mmol EDTA was reduced the stated weight data. When the effect of Cr*EDTA interaction was examined, the highest data was in the Cr0*E10 application in the stem wet weight (37.71 g). In generally, increasing doses of Cr caused a decrease in stem and root weights. Increasing doses of EDTA at Cr0 control application reduced stem and root weight. Flower dry weight, the average value (5.34 g) increased in the 25 mg kg^{-1} Cr application compared to the control (3.22 g), while it decreased in the 50 mg kg^{-1} Cr (4.08 g) application. When the Cr*EDTA interaction was examined, no statistical difference was generally observed between EDTA applications. The highest flower dry weight (8.98 g) was obtained only in the Cr25E10 application. Cr generally negatively affected the development of the plant.

The effect of EDTA and Cr applications on the stem, root and flower Cr concentration values were presented in Table 2 and Figure 2. When

the Figure 2 was examined, the highest Cr concentrations were generally in the root (14.53 - 314.77 mg kg⁻¹). The root was followed by the stem (3.63 - 55.04 mg kg⁻¹) and flower parts (2.31-13.44 mg kg⁻¹). Between stem, root and flower chromium concentrations with increasing Cr applications were observed significant differences. Similarly, Cr*EDTA interaction was also statistically significant (p < 0.05). While the average Cr concentration in the stem is 6.61 mg kg⁻¹ in the control, it is 40.86 mg kg⁻¹ at the 25 mg kg⁻¹ Cr dose. The Cr concentration of the root increased with increasing Cr doses (p < 0.05), and the highest value was observed at the 50 mg kg⁻¹ Cr dose (275.8 mg kg⁻¹). The Cr concentration of the flower at the 25 mg kg⁻¹ Cr (8.54 mg kg⁻¹) was higher compared to control and 50 mg kg⁻¹ Cr applications.

When Cr*EDTA interaction was examined, the Cr concentration in the stem was higher than Cr25*E10 and Cr50*E10 applications (55.04 and 53.73 mg kg⁻¹, respectively) compared to other applications. The Cr concentration in the root was higher at the 50 mg kg⁻¹ Cr dose compared to the control and 25 mg kg⁻¹ Cr doses, and the highest values were obtained in the Cr50*E10 and Cr50*E20 treatments (314.77 and 312.99 mg kg⁻¹, respectively) (p < 0.05) (Figure 2).

When the effect of EDTA and Cr applications on the amount of chromium removed from the soil by stem, root and flower parts is examined, the highest Cr removed from the soil was from the root and the values were between 0.348-6.111 mg kg⁻¹ (Table 3). The root was followed by the Cr values removed from the soil by the stem (0.071-0.749 mg kg⁻¹) and flower parts (0.007-0.121 mg kg⁻¹).

Considering the average values, the amount of chromium taken up by the root increased significantly with increasing Cr doses (p < 0.05). Similarly, in the Cr*EDTA interaction, there were statistically significant differences in the amount of Cr taken up by the root (p < 0.05).

When the average amount of Cr taken by the stem was examined, the values at 50 mg kg⁻¹ Cr and 25 mg kg⁻¹ Cr doses are higher than the control (0.514 and 0.492 mg kg⁻¹, respectively). In the Cr*EDTA interaction, the amount of Cr taken up by the stem increased with increasing doses of EDTA, up to the 10 mmol dose, and the highest values were in Cr50*E10 and Cr25*E10 applications (p < 0.05). EDTA application positively affected the amount of Cr taken from the soil. The values for the amount of Cr taken from the flower varied between 0.007-0.121 mg kg⁻¹, and the highest values were observed in 25 mg kg⁻¹ Cr applications.

Transfer Factor, tolerance index and Bioaccumulation Factor (BCF) values were presented in Table 4. In our Bad Brinbach rose, TF values are TF > 1 and generally varied between 9.88 and 55.98. The highest values were at the Cr0 control dose and there were significant differences between the values (p < 0.05). Tolerance index (ToI) values also varied between 42.85 and 100 (p < 0.05). While the ToI value decreased with increasing Cr doses, it increased with increasing EDTA doses. The plant's BCF factor values were >1, but when these values were taken into consideration, it was noteworthy that the plant was more resistant to the 25 mg kg⁻¹ Cr dose than the 50 mg kg⁻¹ Cr dose (p < 0.05) (Figures 3, 4 and 5).

Table 1. The effect of Cr and EDTA applications on the dry weight of the stem, root and flower of Bad Brinbach rose (Different letters indicate comparison of mean values according to Tukey test; p < 0.05)

Cr and EDTA doses	Stem wet weight (g)	Stem dry weight (g)	Root wet weight (g)	Root dry weight (g)	Flower dry weight (g)
Cr0	32.59 a	17.55 a	70.54 a	32.70 a	3.22 c
Cr25	24.94 b	11.85 b	38.24 b	19.03 b	5.34 a
Cr50	29.50 ab	13.86 b	36.71 b	16.41 b	4.08 b
E0	31.31 ab	15.93 a	45.56 b	23.33 ab	3.79 b
E5	27.82 ab	14.22 ab	48.99 ab	22.44 ab	3.88 b
E10	32.62 a	16.53 a	55.60 a	26.27 a	6.07 a
E20	24.29 b	10.99 b	43.85 b	18.80 b	3.12 b

Table 2. The effect of the application of Cr and EDTA on the concentration of Cr in the stem, root and flower of Bad Birnbach rose (Different letters indicate comparison of mean values according to Tukey test; $p < 0.05$)

Cr doses	Stem Cr (mg kg ⁻¹)	Flower Cr (mg kg ⁻¹)	Root Cr (mg kg ⁻¹)
Cr0	6.61 c	4.47 c	16.11 c
Cr25	40.86 a	8.54 a	171.2 b
Cr50	37.00 b	6.83 b	275.8 a
E0	22.88 b	5.39 b	123.49 c
E5	24.39 b	7.65 a	155.73 b
E10	39.31 a	8.87 a	172.97 a
E20	26.05 b	4.53 b	165.34 ab

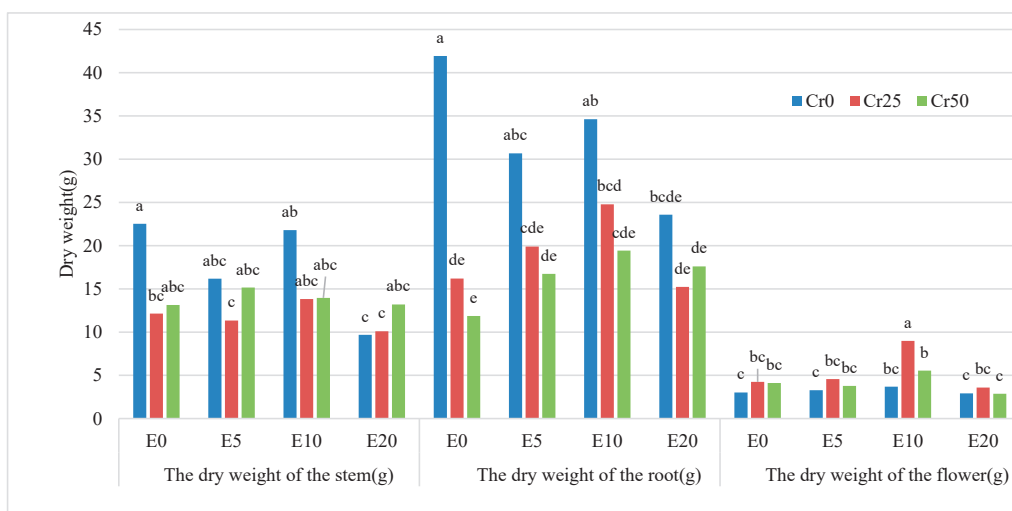


Figure 1. The effect of Cr and EDTA applications on the dry weight of the stem, root and flower of Bad Birnbach rose (Different letters on the bar plots indicate comparison of mean values according to Tukey test; $p < 0.05$)

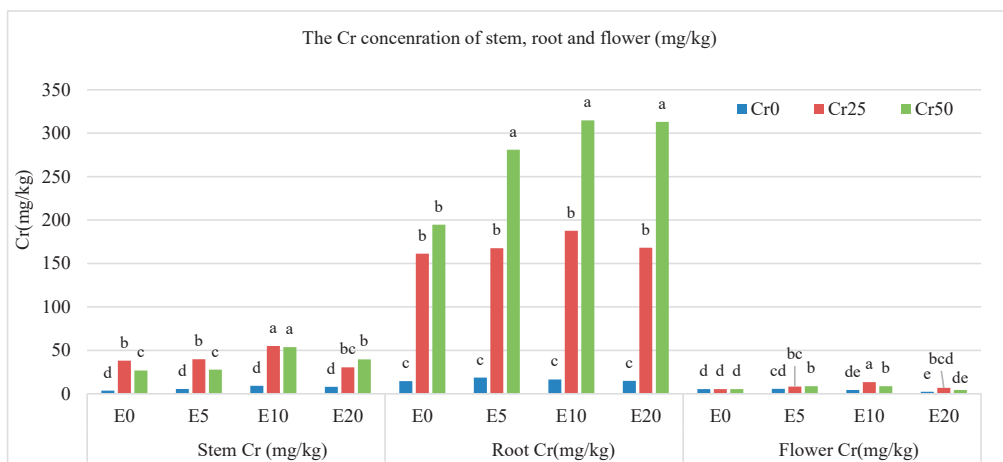


Figure 2. The effect of the application of Cr and EDTA on the concentration of Cr in the stem, root and flower of Bad Birnbach rose (Different letters indicate comparison of mean values according to Tukey test; $p < 0.05$)

Table 3. The effect of Cr and EDTA applications on the amount of Cr removed by the stems, roots, and flowers of Bad Birnbach rose (Different letters indicate comparison of mean values according to Tukey test; $p < 0.05$)

Cr and EDTA doses	Stem removed Cr (mg kg ⁻¹)	Flower removed Cr (mg kg ⁻¹)	Root removed Cr (mg kg ⁻¹)
Cr0	0.110 b	0.015 c	0.524 c
Cr25	0.492 a	0.052 a	3.294 b
Cr50	0.514 a	0.029 b	4.666 a
E0	0.299 b	0.021 bc	1.852 c
E5	0.323 b	0.030 b	2.882 b
E10	0.566 a	0.062 a	3.776 a
E20	0.300 b	0.015 c	2.802 b
Cr0xE0	0.081 d	0.016 cde	0.610 d
Cr0XE5	0.089 d	0.019 cde	0.572 d
Cr0XE10	0.199 cd	0.016 cde	0.566 d
Cr0XE20	0.071 d	0.007 e	0.348 d
Cr25xE0	0.464 abc	0.023 cde	2.627 c
Cr25XE5	0.451 abc	0.039 bc	3.336 bc
Cr25XE10	0.748 a	0.121 a	4.649 ab
Cr25XE20	0.303 bcd	0.025 bcde	2.561 c
Cr50xE0	0.351 bcd	0.022 cde	2.318 c
Cr50XE5	0.428 abc	0.033 bcd	4.738 ab
Cr50XE10	0,749 a	0,048 b	6,111 a
Cr50XE20	0,527 ab	0,013 de	5,497 a

Table 4. The effect of Cr and EDTA applications on Transfer Factor, Tolerance index and Bioaccumulation Factor of Bad Birnbach rose (Different letters indicate comparison of mean values according to Tukey test; $p < 0.05$)

Cr and EDTA doses	Transfer Factor	Tolerance index	BCF
Cr0	41.55 a	79.25 a	0
Cr25	23.78 b	53.68 b	8.82 a
Cr50	13.36 c	50.91 b	6.39 b
E0	20.83 b	63.82 ab	6.37 c
E5	21.23 b	60.08 b	7.49 b
E10	34.15 a	72.44 a	8.89 a
E20	28.71 ab	48.79 c	762 b

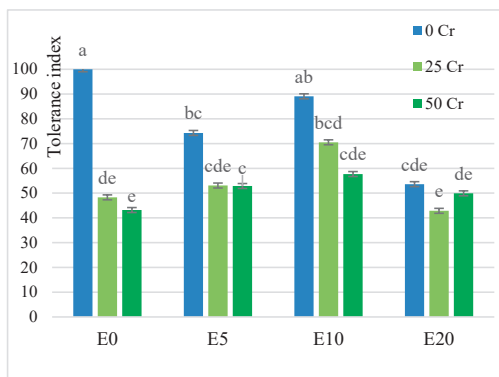


Figure 3. The effect of the application of Cr and EDTA on the tolerance index (Different letters indicate comparison of mean values according to Tukey test; $p < 0.05$)

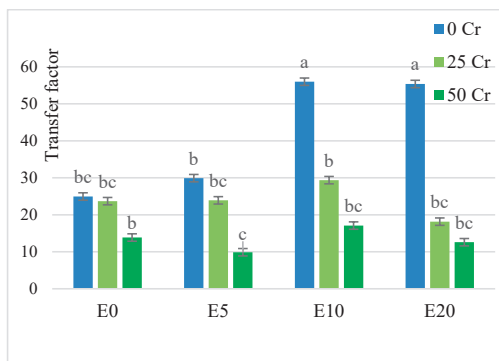


Figure 4. The effect of the application of Cr and EDTA on the transfer factor (Different letters indicate comparison of mean values according to Tukey test; $p < 0.05$)

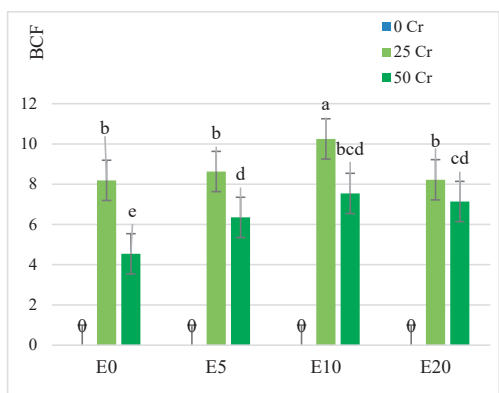


Figure 5. The effect of the application of Cr and EDTA on BCF (Different letters indicate comparison of mean values according to Tukey test; $p < 0.05$)

DISCUSSIONS

In this study, increasing doses of chromium reduced the dry weights of stems, roots and flowers. It was observed that 5-10-15 mmol application doses of EDTA did not change these data, while 20 mmol EDTA application decreased it ($p < 0.05$) (Table 1). In various studies on Cr toxicity, it had been determined that the Cr^{+6} form was more toxic, more mobile, and soluble than the Cr^{+3} form (Sunitha et al., 2014; Oliveira, 2012; Sępniewska & Wolińska, 2005). Although the symptoms seen in plants in case of Cr^{+6} toxicity were various, it is generally stated that plant growth is inhibited and the development of some plant organs was limited (Were et al., 2017).

In this study, Bad Birnbach roses did not lose their vigour, but overall, there were partial decreases in stem root and flower weights compared to the control. Considering the concentration of chromium in plant organs, the concentration values were listed in root>stem>flower parts respectively. EDTA applications increased the Cr concentration in the plant (Table 2). A similar situation was valid for the amount of Cr taken from the soil. The plant accumulated the most Cr in its roots, followed by the stem (Table 3). In a similar study, it was stated that Cr accumulation was mostly in the roots of four bamboo species (Were et al., 2017).

The bioconcentration factor is considered as the ratio of the metal concentration in the aboveground part of the plant to the metal concentration in the soil, and the translocation factor (TF) is the ratio of the metal concentration in the stem part of the plant to the metal concentration in the root part. These two factors must be >1 in accumulator plants. Tolerance index (ToI) is the ratio of the dry weight of the plant, which indicates its growth status, to the dry weight of the control plant (McGrath & Zhao, 2003, Turner et al., 1991). The TI value (Ghosh & Singh, 2005; Zurayk et al., 2002), which calculates the transport of heavy metal from the root zone of the plant to the above-ground stem parts, is greater than 1 in the study plant. In the study, the highest BCF value was observed at the 25 mg kg^{-1} Cr dose and the highest tolerance index was observed at the 50 mg kg^{-1} Cr dose. In EDTA applications,

the highest TI, ToI and BCF values were observed in 10 mg kg⁻¹ applications.

Similarly, in case of heavy metal stress, increasing doses of EDTA applications increased TI values in the *Echinochloa crus galii* plant (Ebrahimi, 2013). In another study, 5 mmol EDTA kg⁻¹ of soil dose was the most appropriate dose for remediation of heavy metal (Ali & Chaudhury, 2016). In this study, considering the average TF values, it was noteworthy that Bad Brinbach rose may be a Cr hyperaccumulator. There are various studies in which plants with TF >1 is classified as hyperaccumulators (Arshad et al., 2008; Faridah et al., 2017; Aghelan et al., 2022). It has been stated that *Vinca rosea* L. plant has chromium levels between 10 and 60 mg kg⁻¹, plant height, fresh and dry weight values decreased at high doses of Cr, and TF value is greater than 1 at 30 and 60 mg kg⁻¹ Cr concentrations (Ehsan et al., 2016).

It will provide an advantage, because of this plant is not included in the human and animal food chain and this plant can be used in regions with Cr pollution up to 50 mg kg⁻¹. It is known that growing plants consumed as food, especially in soil contaminated with Cr, has various health risks. In the restoration of areas exposed to various heavy metal pollution, the advantage of the plant is that it reduces Cr pollution in the soil and can be used visually in landscaping. More research is needed on such ornamental plants in the phytoremediation technique to be used in the improvement of such areas.

CONCLUSIONS

The study showed that the Bad Birnbach rose variety can tolerate Cr-polluted environments and remove chromium from the soil. The plant can easily grow in areas with heavy traffic, on roadsides, on pavements and median strips, without requiring special care. It is a species that can be used to reduce areas contaminated with low and medium levels of Cr. The plant can be recommended for phytostabilization of Cr-contaminated areas.

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