Turkish Journal of Agriculture - Food Science and Technology, 10(10): 1845-1853, 2022
DOI: https://doi.org/10.24925/turjaf.v10i10.1845-1853.5200

Turkish Journal of Agriculture - Food Science and Technology

Available online, ISSN: 2148-127X | www.agrifoodscience.com | Turkish Science and Technology Publishing (TURSTEP)

The Effect of Leonardite on Chromium Toxicity and Growth of Cordes Rose

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ARTICLE INFO

Research Article

Received : 01/04/2022
Accepted : 25/08/2022

Keywords:
Chromium
Heavy metals
Hyper-accumulator
Ornamental plants
Phytoremediation

ABSTRACT

High concentrations of chromium in the soil have a toxic effect on the living organisms in the soil ecosystem. If chromium, which is not an absolute essential element, accumulates in plants, it causes structural changes, causing a decrease in plant growth and also high toxicity due to its accumulation in biomass. Use of plants to remove chromium (Cr) from contaminated soils, it is an environmentally efficient, cost-effective, modern, applicable technique. The different species of plant and ornamental plants are used in this technique. In this study, the Kordes shrub rose used in landscaping in our province, Cr phytoremediation capacity was evaluated by growing at contaminated soil with Cr. In the study, the different doses of Cr (0, 50, 100, 500, 1000 mg kg^{-1}) have been applied in Cr^{+3} and Cr^{+6} forms. In addition, two doses (0% and 3%) of leonardite were added to the pots to determine the effect on the developmental status of the plants and Cr uptake. In the study, plant height, number of branches, number of flowers, flower diameter, stem diameter, flower yield values and total wet and dry weight values at the end of the experiment were determined. At the end of the experiment, it was observed that generally developmental status of the plants was adversely affected at high Cr doses. Especially at 500 and 1000 mg kg^{-1} application doses was observed that the plants could not withstand Cr toxicity in a short time. It has been observed that plants treated with leonardite were healthier than those without. According to the data obtained at the end of the study, it was determined that the resistance of plant to high doses of Cr was low, but it showed better growth at 50 and 100 mg kg^{-1} doses.

Türk Tarım – Gıda Bilim ve Teknoloji Dergisi, 10(10): 1845-1853, 2022

Kordes Gülünde Leonarditin Krom Toksisitesi ve Büyüme Üzerine Etkisi

MAKALE BİLGİSİ

Araştırma Makalesi

Geliş : 01/04/2022
Kabul : 25/08/2022

Anahtar Kelimeler:
Krom
Ağır Metaller
Hiperakümülatör
Süs Bitkileri
Fitoremediasyon

ÖZ

Toprakta bulunan kromun yüksek konsantrasyonları toprak ekosisteminde bulunan canlılara toksik etki yaparlar. Mutlak gerekli bir element olmayan krom bitkilerde birikmesi durumunda; bitki büyümesinde azalmaya ve biyokütlede birikimi nedeniyle yüksek toksisiteye neden olarak, yapısal değişikliklere yol açar. Kirlenmiş topraklardan kromun (Cr) giderilmesinde bitki kullanımı; çevre açısından verimli, uygun maliyetli, modern, uygulanabilir bir tekniktir. Bu teknikte farklı bitki türleri ve süs bitkileri de kullanılmaktadır. Yapılan bu çalışmada ilimizde çevre düzenlemede kullanılan Kordes çalı gülünün; kromla kirlenmiş toprakta yetiştirilerek Cr alım kapasitesi değerlendirilmiştir. Çalışmada bitkilerin yetiştirildiği ortama farklı dozlarda krom (0, 50, 100, 500, 1000 mg kg^{-1}); Cr^{+3} ve Cr^{+6} formlarında uygulanmıştır. Ayrıca bitkilerin gelişim durumlarına ve Cr alımına etkisini belirlemek için saksılara iki dozda (%0 ve %3) leonardit ilave edilmiştir. Çalışmada bitki boyu, dal sayısı, çiçek sayısı, çiçek çapı, gövde çapı, çiçek verimi değerleri ile deneme sonunda bitki toplam yaş ve kuru ağırlığı değerleri belirlenmiştir. Deneme sonunda bitkilerin genel gelişim durumlarının yüksek Cr dozlarında olumsuz etkilendiği; özellikle de 500 ve 1000 mg kg^{-1} uygulama dozlarında bitkilerin kısa zamanda Cr toksisitesine dayanamadığı gözlenmiştir. Leonardit uygulanan bitkilerin uygulanmayanlara kıyasla daha sağlıklı olduğu gözlenmiştir. Çalışma sonunda elde edilen verilere göre bitkinin yüksek dozlardaki Cr'a dayanıklılığının düşük olduğu ama 50 ve 100 mg kg^{-1} dozlarında daha iyi gelişim gösterdiği belirlenmiştir.

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Introduction

Chromium (Cr) pollution in soil is a growing problem in sustainable agricultural production and food security. As known, one of the heavy metals, chromium is highly toxic for environment and human (Korkmaz et al., 2017). The main sources of total world reserves of Cr are Kazakhstan, South Africa, India and the United States (Dhal et al., 2013). Especially in the places where the Cr mine is processed and, in its surroundings, Cr pollution is intensely encountered. Cr concentrations in the soils around the Cr mine in northeastern Iran average 156.19 mg kg^{-1} (Solgi and Parmah, 2015); The average Cr content in arable land in China is 78.94 mg kg^{-1} and 1.26% of these areas are stated to be at high risk of Cr pollution (Zhang et al., 2016).

In Russia, the Cr concentration in the alluvial soil of the Egoshikha river floodplain ranges from 400 to 500 mg kg^{-1}, and Cr in the Danilikha River floodplain ranges from 600 to 1400 mg kg^{-1} (Vodyanitskii et al., 2009) and it is between 50-100 mg kg^{-1} in sodic podzolic soils where the Perm River is located (Vodyanitskii, 2009). In our country, the extractable Cr content in the Karamenderes Basin soils of Çanakkale region is between 0.001 - 0.037 mg kg^{-1} (Sümer et al., 2013). It has been stated that the wastewater of Trabzon province is well above the limits specified by the Water Pollution Regulation (Boran et al., 2004) and the average Cr concentration in the agricultural lands around Hatay Airport is 0 - 0.780 mg kg^{-1} (Özkan et al., 2017). While total Cr concentration is between 12.69 - 135.2 mg kg^{-1} in the areas irrigated with the water coming from the wastewater treatment system in Konya, it is between 16.37 - 19.31 mg kg^{-1} in agricultural areas (Akay et al., 2009).

Remediation of chromium-contaminated soil not only helps sustain agriculture, but also reduces its negative impact on the environment (Patra et al., 2018). Cr, which is not an essential element for plants, is a toxic heavy metal that causes morphological, physiological, biochemical and molecular toxicity in plants (Singh et al., 2013). Cr is taken up by plants through carriers of essential ions such as sulfate, and Cr uptake, transport and accumulation depend on the plant species (Oliveira, 2012).

The most stable and common forms of Cr in the biosphere are Cr (Cr^0), the trivalent Cr (Cr^{3+}), and the hexavalent Cr ($Cr^{6\ +}$) species (Oliveira et al., 2018). Chromium toxicity depends on the oxidation state, the dangerous effect is reduced by reducing Cr (VI) to Cr (III) (Vodyanitskii, 2009). While hexavalent chromium is an environmental pollutant, toxic, carcinogenic and mutagenic metal, the trivalent chromium form is less soluble in water and is even an essential nutrient (Hashmi et al., 2018). Chromium toxicity seriously affects plant growth and development. Cr is considered a carcinogen that enters the human body through inhalation or consumption of food products contaminated with chromium. Cr^{6+} is the most toxic and stable form of Cr in soil (Srivastava et al., 2021). Hexavalent Cr (Cr^{6+}) in chromium-contaminated soils is more toxic to plants than the trivalent Cr (Cr^{3+}). Cr retards growth, reduces the number of septal and spongy tissue cells in leaves (Han et al., 2004). The toxicity of chromium negatively affects seed germination, root elongation, growth and plant development. In addition, it inhibits nutrient absorption, water balance, chlorophyll production, cell division and causes genotoxicity (Shahid et al., 2017). It rearranges its genetic and transcriptional organization, when plants are exposed to Cr in order to better adaptation (Srivastava et al., 2021). In particular Cr^{6+} is one of the most toxic pollutants released into the soil through various human activities. It has many negative effects both on the plant system and on beneficial soil microorganisms (Ahemad, 2015).

Microorganisms can convert (Cr^{6+}) in the soil to (Cr^{3+}) or actively absorb it into their bodies through bioreduction and biosorption, while chromium is absorbed by plants and deposited in tissues, reducing the total chromium content in the soil (Guo et al., 2021). Soil physical, chemical, and microbial properties (such as pH, redox potential, and organic contents) control chromium speciation and its transfer from soil to plant (Shahid et al., 2017). Cr^{6+} affects the morphological, physiological and biochemical quality of plants at the molecular level (Kumar et al., 2019).

Bioremediation of Cr by plants may be the best technology today for cleaning chromium-contaminated areas, and these technologies are environmentally friendly (Risikesh et al., 2016). Chromium toxicity causes oxidative stress by inhibiting the activity of enzymes, targeting chlorophyll biosynthesis, cell membranes and biomolecules. It also causes plant growth delay, induction of chlorosis, and leaf wilt (Sharma et al., 2020). The increase in Cr^{6+} accumulation in plant parts negatively affected photosynthetic pigments and nitrogen metabolism, this situation also inhibited the growth of guar bean plants (Sangwan et al., 2014).

The use of ornamental plants in areas where pollution treatment is applied provides a high added value and tourism option, and also improves the landscape. These plants can be preferred in remediation studies due to their advantages such as high stress tolerance, rapid growth, high biomass, good root development, and not intended for animal and human food consumption (Rocha et al., 2021). As it is known, outdoor ornamental plants are plants that are generally used in parks, gardens, roads, active and passive green areas (Köksal et al., 2016). In the screening study conducted with Honeysuckle (*Lonicera nidita*), Magnolia (*Magnolia grandiflora*), Hydrangea (*Hydrangea macrophylla*), Rose (*Rosa Odaorata*) and Oleander (*Nerium oleander*), nutrient deficiencies at plants were determined (Işık and Adiloğlu, 2015). This may be due to the effect of heavy metal uptake by plants and the decrease in the intake of some nutrients due to the interaction between the elements (Sarwar et al., 2009; Cao et al., 2003; Prasad et al., 2016). In the study conducted to remove metals (Cr and Pb) using fan flower (Periwinkle) and oleander (Oleander), a high concentration of Cr was found in fan flower leaves (20.34 mg kg^{-1}) and in oleander roots (19.61 mg kg^{-1}). It was determined that the lead removal efficiency of both plants (94.36% in fan flower and 94.92% in oleander) was higher than that of Cr (91.08% in fan flower and 95.96% in oleander) (Al-Anbari et al., 2018). In the study conducted to evaluate chromium removal from aquatic tanneries sludge with different Cr concentrations for ornamental plants (*Mirabilis jalapa*, *Impatiens Balsamin* and *Tagetes erecta* L.), especially *Mirabilis jalapa* has been determined to be the most effective in removing Cr from tannery sludge (51.25 × 10^3mg kg^{-1}) (Miao and Yan, 2013). In the study carried out to examine the ability of tolerating and accumulating chromium and zinc of marsh iris (*I. pseudacorus* L.), which has an ornamental macrophyte plant with great potential for phytoremediation, Cr was mainly retained in the roots of plant and caused root damage more than zinc (Caldelas et al., 2012).

The aim of this study was determined to effect of leonardite on growth and Chromium toxicity in Cordes Rose. In the study, different forms of Cr (Cr^{3+} and Cr^{6+}) were applied to the soil at different doses. In the study, two applications were made leonardite (-) and leonardite(+) to determine the effectiveness of leonardite against plant growth and chromium toxicity.

In addition to determining whether Kordes rose grown in soils contaminated with Cr at different doses is a hyperaccumulator plant, it is also aimed to determine the potential of the dissolved humic substances to be released from leonardite to activate the chromium in chromium contaminated soil and its effect on the development of the Kordes rose.

Material and Method

Experiment Material

In the experiment was used the certified Kordes rose as a test plant. The roses were obtained from a certified production company in Türkiye. Sandy soil using for the experiment was obtained from the Directorate of Parks and Gardens of Konya Metropolitan Municipality. First of all, this soil was passed through a 4 mm sieve, and then was filled into 6-liter pots. Two different doses of Leonardite (0% and 3%) were applied to this soil (these doses calculated on a dry basis) and mixed homogeneously with the potting soil. The Cr, which is the subject of the experiment, was given to the pots in two different forms (Cr^{3+} and Cr^{6+}) at the doses indicated below:

Cr application: It was given at doses of 0-50-100-500-1000 mg Cr kg^{-1}, using $K_2Cr_2O_7$ (Cr^{6+}) and Cr $(NO_3)_3.9H_2O$(Cr^{3+}). Trial was carried out in 80 pots with 4 replications. After application of Cr, it was incubated to ensure the reaction of Cr with the soil for 2 months. At the end of the incubation period with Cr, the stem and roots of roses were pruned and immediately planted in pots.

The experiment was conducted in the greenhouse according to the factorial experimental design of random plots. The macro and micro nutrition requirements of plants were calculated according to the results of the soil analysis and applied to pots with the Hogland solution.

Plant material: Kordes rose is a pink- apricot colored, bush-shaped plant that can reach 60 cm in length and 50 cm in crown width. While it is suitable to be planted with 50 cm intervals in plantings to be made side by side, it is appropriate to plant 4-5 pieces per 1 m^2 in group plantings. The average diameter of the flower is 5 cm. Unlike other roses, it is a self-pruning rose because it does not leave buds. It is sufficient to prune once a year only at the beginning of spring. It is resistant to temperatures between +55°C and -42°C due to its rootstock (Anonymous, 2019).

Analyzes made in plants:

At the end of the research, the height of the plant in Kordes roses was measured by taking into account the distance from the root collar to the top. The number of branches in the plants in each pot (the number of large main branches emerging from the throat of the roots) and the number of flowers (counting the blooms on the plant from the time the plant grew until harvest) were recorded. The diameter of the flowers was measured using a digital caliper, and the flower bud was determined by recording the number of buds formed per plant from plant growth to harvest in each pot.

Stem diameter: The diameter of three random branches coming out of the throat of the root was measured with a digital caliper and averaged these values.

Flower yield: When the flowers bloomed in all pots, the weekly weight of the flowers taken from the plants in each pot was recorded and calculated by taking the total at the end of the experiment.

Leaf chlorophyll content: Five measurements were taken from the plants in each pot with a portable chlorophyll meter (Minolta SPAD-502, Osaka, Japan) and the average was taken.

In the experiment, after the plants were harvested, the fresh weights of the above-ground parts of the plants were taken. Then, in order to determine their dry weights, they were kept at 70°C for 2 days and their weights were determined by weighing them on a precision balance (Kacar and İnal, 2010).

Statistical Analysis: 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 Discussion

Some physical and chemical analysis results of the soils used in the experiment carried out under greenhouse conditions and some chemical properties of leonardite are given in Tables 1 and 2. The soil used in the experiment has a sandy texture, slightly alkaline pH, slightly saline and slightly calcareous. It also contains very low organic matter and medium inorganic nitrogen. The phosphorus, potassium, natrium values of the soil are low. The calcium, magnesium, iron and zinc content of the soil are high, Mn is less, Cu is sufficient, B is low.

According to the analysis of variance in the data obtained from the experiment, Cr doses, Cr forms, Cr dose*Cr form interaction, leonardite*Cr form interaction, Cr dose*leonardite*Cr form interaction affected statisticaly significantly on the values of plant dry weight, chlorophyll value, flower diameter ($P<0.05$). And also, the values of flower yield, number of flowers, plant height were significantly affected ($P<0.05$) from other applications and interactions, except for Cr dose (Table 3).

If the obtained data is analyzed separately, when the plant height is evaluated, the best height growth was observed in the application of 3% leonardite- 0 mg Cr^{+3} kg^{-1} (15.23 cm). Plant height decreased partially with increasing Cr doses, and it was observed that the negative effect of Cr was more effective in Cr^{+6} form ($P<0.05$). Plant height values varied between 9.31 and 15.23 cm (Table 4).

The number of branch and stem diameter of the plants did not change with leonardite and Cr applications. The number of branches is between 5.25 – 10.00 pieces/pot and the stem diameter values are between 0.45 - 0.62 mm (Table 4).

Table 1. Some physical and chemical properties of the soil used in the experiment

Properties	Unit	Values	Kullanılan yöntem
pH (1:2.5 soil:pure water)		7.78	(Jackson, 1973)
E.C. (1:5 soil:pure water)	(µS cm^{-1})	382.00	(Jackson, 1973)
$CaCO_3$ (%)	%	4.96	(Hızalan and Ünal, 1965)
Organic matter (%)	%	0.38	(Jackson, 1967)
Texture Class		Sandy	(Piper, 1966)
İnorganic N	mg kg^{-1}	50.93	(Subbaiah, 1956)
P		1.76	(Olsen, 1954)
Ca		3792.35	(Lindsay and Norvell, 1978)
Mg		206.70	(Lindsay and Norvell, 1978)
K		70.88	(Piper, 1966)
Na		23.79	(Lindsay and Norvell, 1978)
Fe		8.72	
Zn		1.62	
Mn		4.27	
Cu		1.06	
B		0.80	(Howe and Wagner, 1996)

Table 2. Some chemical properties of leonardite used in the experiment

Properties	Unit	Leonardite
pH		3.58
EC	(µS cm^{-1})	1385
Available Fe	mg kg^{-1}	0.188
Available Zn	mg kg^{-1}	7.93
Available Cu	mg kg^{-1}	0.17
Available B	mg kg^{-1}	1.56
Available Mn	mg kg^{-1}	46.94
Ni	µg kg^{-1}	0.49
Pb	µg kg^{-1}	0.05
Co	µg kg^{-1}	0.918
Cr	µg kg^{-1}	42.32
Cd	µg kg^{-1}	0.07
Humic fulvic acid	%	29.08

Table 3. Variance Analysis table showing the effects of Cr and leonardite applications on some growth parameters obtained from Kordes rose

Applications	Number of flower buds (number/pot)	CSC	Plant dry weight (g/pot)	Plant fresh weight (g/pot)	Plant height (cm)
Cr	ns	*	*	ns	ns
Leo	*	*	ns	ns	*
Cr Forms	*	*	*	*	*
Cr*Leo	ns	*	ns	ns	ns
Cr*Cr Forms	-	*	*	-	-
Leo*Cr Forms	*	*	*	-	*
Cr*Leo*Cr Forms	*	*	*	*	*

Applications	Number of Branches (number/pot)	Stem diameter (mm)	Flower yield (g/pot)	Flower number (number/pot)	Flower diameter (mm/pot)
Cr	ns	ns	ns	ns	*
Leo	ns	ns	*	*	*
Cr Forms	ns	ns	*	*	*
Cr*Leo	ns	ns	*	ns	*
Cr*Cr Forms	-	-	*	*	*
Leo*Cr Forms	-	-	*	*	*
Cr*Leo*Cr Forms	-	-	*	*	*

(*P<0.05, ns- not significant). CSC: Chlorophyll Spad content

Table 4. The effects of Cr and leonardite applications on some growth parameters obtained from Kordes rose

L	CF	CR	Plant height (cm)	Number of Branches (number/pot)	Stem diameter (mm)
0	3	0	13.81 ± 0.34^{a-c}	5.25 ± 1.50	0.54 ± 0.06
		50	14.42 ± 1.44^{ab}	7.50 ± 1.29	0.46 ± 0.03
		100	14.79 ± 2.94^{a}	6.50 ± 1.73	0.55 ± 0.05
		500	11.77 ± 0.90^{a-c}	7.25 ± 3.50	0.52 ± 0.06
		1000	10.47 ± 1.93^{a-c}	6.25 ± 0.95	0.46 ± 0.10
	6	0	14.16 ± 3.37^{a-c}	5.25 ± 0.95	0.54 ± 0.09
		50	11.04 ± 0.46^{a-c}	6.25 ± 2.63	0.54 ± 0.03
		100	9.72 ± 0.84^{bc}	5.25 ± 1.25	0.56 ± 0.11
		500	11.33 ± 1.28^{a-c}	7.50 ± 2.38	0.50 ± 0.04
		1000	11.32 ± 0.60^{a-c}	9.50 ± 1.29	0.51 ± 0.07
3	3	0	15.23 ± 2.30^{a}	6.25 ± 1.50	0.54 ± 0.03
		50	13.13 ± 1.04^{a-c}	6.75 ± 1.89	0.55 ± 0.04
		100	14.60 ± 3.48^{ab}	6.50 ± 1.29	0.56 ± 0.04
		500	10.93 ± 2.11^{abc}	7.25 ± 1.50	0.53 ± 0.00
		1000	10.83 ± 1.85^{a-c}	7.75 ± 2.21	0.54 ± 0.11
	6	0	10.78 ± 2.73^{a-c}	8 ± 1.41	0.46 ± 0.02
		50	9.31 ± 1.53^{c}	6.75 ± 1.25	0.62 ± 0.04
		100	13.10 ± 1.15^{a-c}	8.25 ± 0.95	0.51 ± 0.06
		500	12.28 ± 0.73^{a-c}	10 ± 1.82	0.51 ± 0.33
		1000	9.47 ± 1.33^{c}	7 ± 3.36	0.45 ± 0.04

L	CF	CR	Number of flower buds (number/pot)	Flower number (number/pot)	Flower diameter (mm/pot)	Chlorophyll Spad content
0	3	0	6.75 ± 1.70^{a-d}	11 ± 2.82^{ab}	4.34± 0.28^{a}	50.01 ± 7.88^{ab}
		50	2.5 ± 1.73^{b-d}	2 ± 0^{c-e}	4.60 ± 0.65^{a}	50.19 ± 6.59^{ab}
		100	13.5 ± 1.29^{a}	11.5 ± 5.07^{a}	4.29 ± 0.16^{a}	48.20 ± 8.93^{ab}
		500	0.75 ± 1.50^{cd}	0 .5± 1.00^{e}	0 ± 0^{c}	0 ± 0^{c}
		1000	1.25 ± 1.50^{cd}	0 ± 0^{e}	0 ± 0^{c}	0 ± 0^{c}
	6	0	10.75 ± 6.60^{a}	6.75 ± 1.50^{a-c}	3.80 ± 0.96^{ab}	54.96 ± 4.50^{a}
		50	0.50 ± 0.57^{cd}	0 ± 0^{e}	0 ± 0^{c}	48.55 ± 0^{ab}
		100	0 ± 0^{d}	0 ± 0^{e}	0 ± 0^{c}	0 ± 0^{c}
		500	0 ± 0^{d}	0 ± 0^{e}	0 ± 0^{c}	0 ± 0^{c}
		1000	0 ± 0^{d}	0 ± 0^{e}	0 ± 0^{c}	0 ± 0^{c}
3	3	0	8 ± 1.41^{a-c}	12 ± 4.55^{a}	4.55 ± 0.90^{a}	55.82 ± 4.98^{a}
		50	1.50 ± 0.57^{bcd}	4 ± 0^{cde}	0 ± 0^{c}	39.85 ± 10.78^{b}
		100	9 ± 9.27^{ab}	6 ± 0.82^{b-d}	4.94 ± 0.38^{a}	54.84 ± 6.20^{a}
		500	1.75 ± 1.70^{b-d}	0 ± 0^{e}	0 ± 0^{c}	0 ± 0^{c}
		1000	1.5 ± 3.00^{b-d}	0 ± 0^{e}	0 ± 0^{c}	0 ± 0^{c}
	6	0	2.75 ± 4.19^{b-d}	2.75 ± 2.50^{de}	2.47 ± 1.65^{b}	52.93 ± 7.05^{ab}
		50	0 ± 0^{d}	0.75 ± 0.95^{e}	0.68 ± 0^{c}	45.09 ± 3.73^{ab}
		100	0.50 ± 0.77^{cd}	0 ± 0^{e}	0 ± 0^{c}	46.20 ± 1.24^{ab}
		500	0 ± 0^{d}	0 ± 0^{e}	0 ± 0^{c}	0 ± 0^{c}
		1000	0 ± 0^{d}	0 ± 0^{e}	0 ± 0^{c}	0 ± 0^{c}

L: Leonardite %; CF: Cr forms; CR: Cr (mg kg-^{1}); (According to the Tukey test, there is a $p<0.05$ difference between the values shown with different letters in the same column)

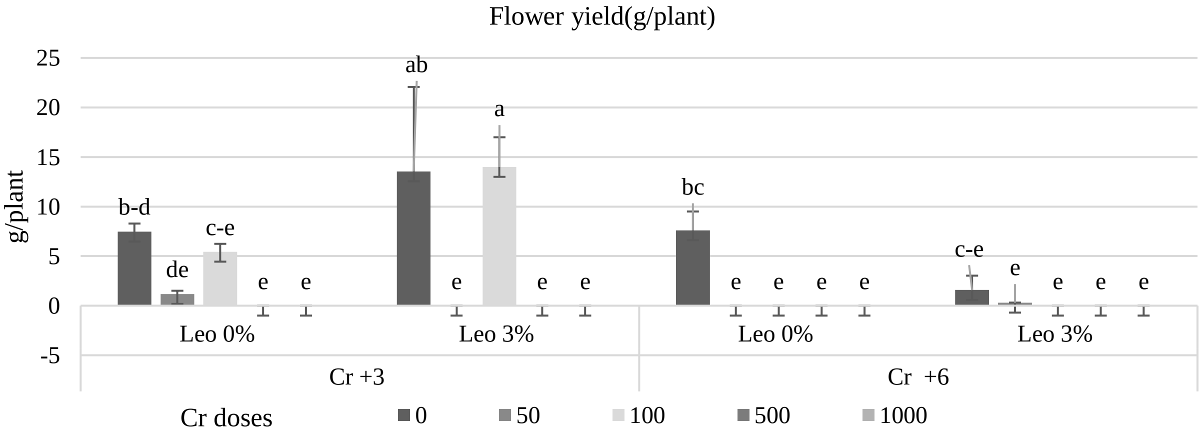

Figure 1. The effects of Cr and Leonardite applications on flower yield ($P<0.05$)
(According to the Tukey test, there is a $P<0.05$ difference between the values shown with different letters)

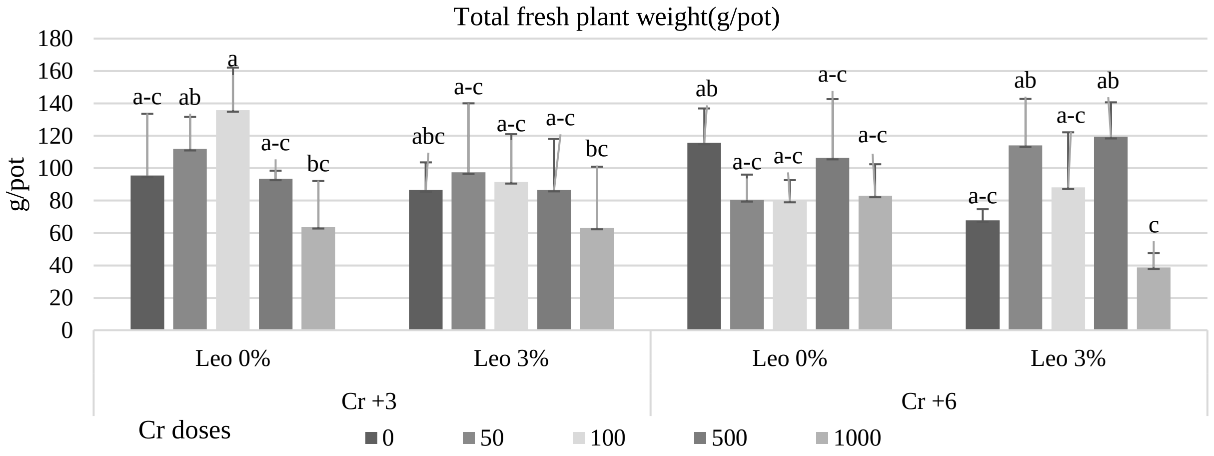

Figure 2. The effects of Cr and Leonardite applications on total fresh plant weight
(According to the Tukey test, there is a P<0.05 difference between the values shown with different letters)

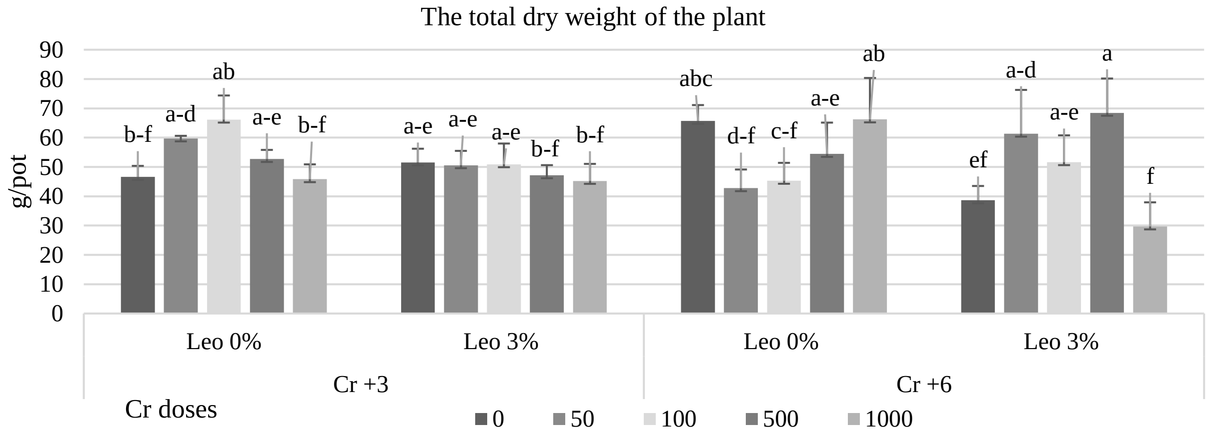

Figure 3. The effects of Cr and leonardite applications on the total dry weight of the plant
(According to the Tukey test, there is a P<0.05 difference between the values shown with different letters)

The values of flower bud number are between 0-13.5 pieces/pot, and these values decreased with increasing Cr doses. It was observed that these values decreased even more in the Cr^{+6} form compared to the Cr^{+3} form. When leonardite * Cr dose*Cr form interaction is evaluated, the number of flower buds (13.5 mm) was found to be the highest in 100 mg kg^{-1} Cr $^{+3}$ - 0% leonardite application (Table 4).

The number of flowers showed significant differences with leonardite, different Cr forms and leonardit*Cr doses*Cr form interaction ($P<0.05$) (Tables 3 and 4). The highest number of flowers was obtained in 0 mg/kg Cr^{+3} - 3% leonardite application (12 pieces/pot). In general, the number of flowers decreased with increasing Cr doses, and almost no flowers were formed in the plants from 100 mg kg^{-1} Cr dose. Flower formation in the trivalent form of Cr is higher than in the hexavalent form. It was observed that the application of leonardite also affected the formation of flowers positively, and the development of the plant is better against the toxicity of Cr.

In various studies, it has been reported that leonardite is effective in improving the plant growth media (Lao et al., 2005; Madejón et al., 2010) and reduces their bioavailability by adsorbing metals (Lao et al., 2005; Zeledón- Torunõ et al., 2005; Doulati, 2011; Dovlati et al., 2020).

The values of flower diameter showed significant differences with all applications ($P<0.05$) (Tables 3 and 4). Flower diameter values of control and 50 mg kg^{-1} Cr applications were similar in generally. But we couldn't take flower diameter at 500 and 1000 mg kg^{-1} Cr applications, because flower formation could not be occured at applications. The plant could not resist against increasing Cr doses (500 and 1000 mg kg^{-1} Cr). It is observed that the Cr^{+3} form has fewer toxic effects against the Cr^{+6} form. The highest flower diameter value is at the application of 100 mg kg^{-1} Cr $^{+3}$ - 3% leonardite (4.95 cm).

When the chlorophyll spad values were examined, it showed significant differences with all applications ($P<0.05$) (Table 3). Chlorophyll values were measured in the control, chromium 50 and 100 mg/kg applications, but the measurement could not be made because of the plant died from Cr toxicity at 500 and 1000 mg/kg Cr doses. Chlorophyll spad values range from 0.00 to 55.82 (Table 4). Flower yield values were adversely affected by increasing Cr doses and Cr forms. Although more positive results are obtained in flower yield with Cr^{+3} form applications compared to Cr^{+6} applications, the effect of 3% leonardite is better than compared to controls. However, when Figure 1 is examined in general, it is noteworthy that 500 and 1000 mg kg^{-1} Cr applications do not produce flowers in the plant and generally yields cannot be obtained.

Significant difference was observed only between Cr application forms in plant fresh weight values ($P<0.05$). In Cr^{+3} applications compared to Cr^{+6} applications, the above-ground fresh weight of the plant is higher, and it was observed that it decreased partially with increasing Cr doses (Table 3, Figure 2). Significant differences were observed in Cr application forms, Cr doses, triple interactions of these applications with leonardite in plant dry weight values ($P<0.05$). With increasing Cr doses, plant dry weight decreased compared to the control. These decreases were mostly observed in 3% leonardite-1000 mg kg^{-1} Cr application (Table 3, Figure 3).

When the effect of hexavalent chromium ($K_2Cr_2O_7$) on the ornamental plant *Zinnia elegans* (L.) was examined, the plant height, chlorophyll value, number of mature flowers opened, number of capillary roots decreased at high Cr concentration (75 mg/kg) (Panda et al., 2020). In the study carried out with different ornamental plant species such as daffodil, chrysanthemum, aster flower and dahlia, above 10 mg/kg of Cr concenration showed toxic effect for plants and accordingly there was a serious decrease in the growth of plants. High levels of Cr (25 mg/kg) show that at chrysanthemum increases the mortality of plants (Ramana et al., 2013).

In our study, Kordes roses survived up to 100 mg kg^{-1} Cr dose, however at higher doses, the plant growings first regressed due to toxicity and then the plants died. It has been observed that the crown of thorns (*Euphorbia milli*) plant, which is an ornamental shrub, can tolerate Cr applied up to 75 mg when used in the improvement of soil contaminated with Cr. Although this plant is not classified as a hyperaccumulator, when Cr uptake and translocation efficiency are evaluated, it has been determined that over 80% of Cr is transported from roots to shoots and the plant is effective for remediation of low or moderately contaminated soils (ie up to 50 mg/kg soil) (Ramana et al., 2015). The values of plant height, fresh and dry weight of the vinca (*Vinca rosea*) increased at low concentration (10-30 mg kg^{-1} Cr) in chromium-contaminated soil, however it was stated that it decreased in high Cr pollution (70 mg/kg Cr) (Ehsan et al., 2016).

Conclusions

At the end of the experiment, it was observed that the plants treated with leonardite were healthier than the plants that were not applied. Hexavalent Cr, its effect more negative compared to trivalent Cr on plant height, number of branches, number of flowers, flower diameter, stem diameter, flower yield. At the end of the experiment, it was determined that the general developmental status of the plants was adversely affected by high Cr doses; It was observed that plants could not withstand toxicity in a short time, especially at 500 and 1000 mg kg^{-1} application doses. According to the data obtained at the end of the study, although the plant's resistance to high doses of Cr is low, it has been determined that it provides resistance to 50 and 100 mg kg^{-1} Cr doses and shows better growth.

Acknowledgment

Project financially supported by the BAP Coordinator of Selcuk University of Türkiye (Proje No. 21401051)

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