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Potential of *Rosmarinus officinalis* for Phytoremediation of Soil Contaminated with Cadmium

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Abstract: In this study, the use of *Rosmarinus officinalis* was investigated to clear soil contaminated with Cd. The soil sample for pot experiment is taken from the old municipal waste storage in Mardin. The pot experiment conducted in the greenhouse. Increasing dose of Cd (0-5-10-20 mg kg^{-1} as $3CdSO_4.7H_2O$ form) and 0.005 mM citric acid applied to the soil and all pots were incubated for 30 days. The fertilizer (200 mg N kg^{-1}) applied before seedling and then plant seedlings transferred to the pots. The plants were grown for 45 days in the greenhouse. After harvest Cd, N, P, K, Mn, Fe, Cu, and Zn concentrations of plant samples were determined in ICP-MS. The highest Cd content (8.31 µg plant^{-1}) was obtained in 20 mg Cd kg^{-1} treatments. The result of the experiment was shown that the *Rosmarinus officinalis* was not suitable for pyhtoextraction of Cd-contaminated soils.

Key words: *Rosmarinus officinalis*, phytoremediation, cadmium, contaminated soil, decontamination

Biberiye (*Rosmarinus Officinalis*) Bitkisinin Fitoremediasyon Amaçlı Kullanım Potansiyeli

Özet: Bu çalışmada, Cd ile kontamine olmuş toprağı temizlemek için *Rosmarinus officinalis* bitkisinin kullanım potansiyeli araştırılmıştır. Toprak deneyi için toprak örneği Mardin'deki eski belediye atık depolama alanından alınmıştır. Denemeler serada saksıda kontrollü koşullarda yapılmıştır. Artan dozlarda Cd (0-5-10-20 mg kg^{-1} Cd, $3CdSO_4.7H_2O$ formunda) ve 0.005 mM sitrik asit uygulanan tüm saksılar 30 gün boyunca inkübe edilmiştir. Çimlenmeden önce gübre (200 mg N kg^{-1}) uygulanmış ve daha sonra fideler saksılara aktarılmıştır. Bitkiler, serada 45 gün boyunca yetiştirilmiştir. Hasattan sonra bitki örneklerinin Cd, N, P, K, Mn, Fe, Cu ve Zn konsantrasyonları ICP-MS'de belirlenmiştir. En yüksek Cd içeriği (8.31 µg bitki^{-1}) sitrik asitli ortamda 20 mg Cd kg^{-1} uygulamalarından elde edilmiştir. Denenenin sonucu, *Rosmarinus officinalis* bitkisinin Cd ile kirlenmiş toprakların fitoekstraksiyonu için uygun olmadığını göstermiştir.

Anahtar kelimeler: *Rosmarinus officinalis*, fitoremediasyon, kadmiyum, kirlenmiş toprak, dekontaminasyon

Introduction

Heavy metals, especially cadmium, are widely being considered as responsible for soil pollution, soil infertility and decreased crop production. Due to high cost of traditional engineering methods (Glass 2000), in order to remediate the soils that are already being polluted by heavy metals, recent studies are offering new methods such as phytoremediation (Shi and Cai 2009), that are more friendly to the environment and less costly. The phytoremediation terminology was firstly introduced in 1991 (Etim 2012) and can be defined as the usage of plants to remove toxic metal ions from the soil as a biological cleaning method and it is reliable, environmentally friendly and a long-term technology to clean contaminated soils (Blaylock 2000). In our country, studies on removal of organic and inorganic pollutants from the soil are very limited. Compared to other already developed countries, our country is still having a lot to achieve in order to prevent soil pollution. On the other hand, there are only few studies reporting the pollution level of the soil. It takes a lot of investment and time to get rid of the polluters from soil, which is the biggest limitation of such works (Çiftçi 2016). Soil pollution can occur by various factors originated from both nature or human influence. Domestic, industrial and agricultural activities are the most soil polluting factors (Türkoğlu 2006). Aim of this study is to determine the ability of rosemary plant to accumulate cadmium (Cd) using flowerpots in order to be able to use the soil after refining from contaminants as well as assessing the growth and phytoremediation potential of the rosemary plant while it is absorbing the Cd within the contaminated soil.

Material and Methods

Materials

Rosemary (*Rosmarinus officinalis*) plant was used as plant material in the study. As a Needle-tipped, small-leafed plant that belongs to *Lamiaceae* family. The aromatic plant, which is about 1-2 m in length, has a strong aroma such as *Camphor* or *Eucalyptus* odour, which does not foliage its leaves in winter (Çoban and Patır 2010). The rosemary plant used in the research was rooted for 3 months with the steel picking method and then the pot planting was done. The rosemaries used in the experiment were prepared in the greenhouse of Mardin Metropolitan Municipality Nursery and Green Areas Branch Directorate. The soil material for the pot experiment was taken from the old wild garbage landfill located in the Artuklu county, Mardin province, at a depth of 0-30 cm as reported by Jackson (1967). Test soil taken from a depth of 0-30 cm was passed through a 4 mm sieve after being dried and filled with a pot. The physical and chemical properties of the experimental soil taken from the solid waste landfill are given in Table 1.

Table 1. Some physical and chemical properties of test soil

Soil Properties	**Value**	**References**
Structure	Loam	(Bouyoucos, 1951)
pH	7.60	(Kacar, 1995)
Salt (%)	0.025	(Soil Survey Staff, 1951)
$CaCO_3$ (%)	33.1	(Loeppert and Suarez, 1996)
Organic substance (%)	1.94	(Kacar, 1995)
Organic Carbon (%)	1.13	(Kacar, 1995)
Total N (%)	0.10	(Bremner, 1965)
Available P (mg kg^{-1} P_2O_5)	4.12	(Olsen, 1954)
Available K (mg kg^{-1} K_2O)	30.9	(Sommers and Lindsay, 1979)
Extractable Cd with DTPA (mg kg^{-1})	3.02	(Sommers and Lindsay, 1979)
Extractable Fe with DTPA (mg kg^{-1})	34.7	(Sommers and Lindsay, 1979)
Extractable Cu with DTPA (mg kg^{-1})	33.1	(Sommers and Lindsay, 1979)
Extractable Mn with DTPA (mg kg^{-1})	17.7	(Sommers and Lindsay, 1979)
Extractable Zn with DTPA (mg kg^{-1})	97.1	(Sommers and Lindsay, 1979)

The Cd dose to be applied at the trial was determined by considering the averages and upper limits reported by (Lindsay 1979). In order to obtain a homogeneous distribution in the soil, 0.005 mM citric acid ($C_6H_8O_7$) was given in the form of Cd (0-5-10-20 mg kg^{-1}) $3CdSO_4.7H_2O$ at increasing doses prior to planting. Planting was successfully carried out covering the 60-65% of the soil capacity and they were left to be incubated under controlled conditions for a month.

Amount of the chlorophyll was determined according to the method reported by Wellburn (1994). 0.5g of fresh leaf was homogenized with 80% acetone and then centrifuged at 4^{o}C and 10.000 rpm for ten minutes. After that, chlorophyll-a, chlorophyll-b and total carotenoid levels were determined using a spectrophotometer by the wavelengths of 663.2 nm, 646.8 nm and 470 nm respectively.

As a result of the experiment, the plants that are being already grown for 45 days were harvested about 1 cm from the soil surface and the green parts (leaf and stem) were selected and washed with pure water, dried until reaching a constant weight at 65°C in the drying cabinet. The plant's dry weights were noted and then plants were milled in a grinding mill for plant analysis. Test results were evaluated as whole plant.

The total element concentrations (Cd, P, K, Fe, Mn, Cu and Zn) which will be evaluated after were determined by ICP-MS by dissolving the seeded plant samples in microwave (MarsXpress CEM) using nitric acid (HNO_3).

Data obtained as a result of the experiment were grouped by using Duncan test according to Bek (1986) using SPSS 22.0 statistical analysis program.

Results

It is observed that cadmium applications are statistically insignificant ($p \leq 0.01$) in terms of dry weight of plants, chlorophyll-a, chlorophyll-b and carotenoid amounts are not statistically significant (N.I.).

Table 2. The effect of different Cd applications on citric acid and citric acid-free environments on the mass of *Rosmarinus officinalis* (g), dry mass (g), chlorophyll-a (μg g^{-1}), chlorophyll-b (μg g^{-1}) and carotenoid (μg g^{-1}).

	Dose	Dry Mass	Chlorophyll-a	Chlorophyll-b	Carotenoid
(-) Citric Acid	Cd_0	1.63 a	25.7 a	15.0 a	-2.40 b
	Cd_5	1.54 ba	24.8 a	13.2 a	-1.53 ba
	Cd_{10}	1.51 cb	25.6 a	12.4 a	-1.26 ba
	Cd_{20}	1.44 dc	26.2 a	10.6 a	-0.77 ba
(+) Citric Acid	Cd_0	1.41 ed	27.3 a	13.5 a	-1.58 ba
	Cd_5	1.36 ed	25.8 a	10.5 a	-0.80 ba
	Cd_{10}	1.33 fe	27.2 a	10.6 a	-0.73 ba
	Cd_{20}	1.25 f	26.8 a	7.11 a	0.10 a
	F	19.3**	1.54 $^{N.I.}$	1.43 $^{N.I.}$	1.59 $^{N.I.}$

(**): p≤0.01 statistically significant within error bounds
(N.I.): not statistically significant

The dry masses of citric acid with different doses of Cd were between 1.25-1.63 g $plant^{-1}$ and the highest amount with 1.63 g $plant^{-1}$ were observed in the group of Cd_0, which is a citric acid-free application, and the dry masses exhibited a decrease with respect to the Cd doses as compared to the control group (Table 2). Kalınbacak et al. (2012) found that the plants cultivated in the pots with addition of 0, 5, 15, 30 and 45 mg Cd kg^{-1} to soil were negatively affected by Cd toxicity and decreased dry mass in wheat as Cd amount increased. Daghan et al. (2012) reported that when transgenic tobacco plants were used to treat soil contaminated with Cd (0, 0.2, 0.4, 0.6, and 1.6 mg Cd kg^{-1}) with phytoextraction, growing and dry mass of plant will be reduced with increased dose of Cd.

It was determined that the highest value (27.3) on the application of citric acid with various cadmium doses varied between 24.8-27.3 for chlorophyll-a in the Cd_0 group with citric acid application and the highest value (15.0) with various cadmium doses varied between 7.11-15.0 for chlorophyll-b in the Cd_0 group with citric acid-free application (Table 2).

Eren and Mert (2017) reported that chlorophyll amounts in the leaves of *Inula helenium, Physalis angulata* and *Verbascum thapsus* plants were reduced compared to control plants in the soil which Cd had been applied at increasing doses (0-5-10-20 and 40 mg Cd kg^{-1}). Amirjani (2012) reported that wheat was adversely affected in general and decreased the amount of chlorophyll (chlorophyll-a, b and a + b) in studies of increased Cd doses on wheat plant effects. Zengin and Kirbag (2007) reported that decreasing the chlorophyll-a, chlorophyll-b, total pigment I and II amounts of Cd (0, 0.05, 0.06 and 0.08 mM Cd) applied bean seedling compared to the control groups with investigating the effects of Cd, Cu, Hg and Pb on the pigment amounts of bean seedling.

It is stated by Zengin and Munzuroglu (2005) that heavy metals led to the formation of free radicals, leading to the oxidative destruction of lipids of the tilacoid membrane, thereby increasing the degradation of chlorophyll and inhibiting its synthesis. It was also found that cadmium applications were important at the level of p≤0.01 in terms of N%, P% and K% in *Rosmarinus officinalis* plant (Table 3).

Table 3. Effects of N%, P% and K% on rosemary plants with different Cd applications in citric acid and citric acid-free environments.

	Dose	% N	% P	% K
(-) Citric Acid	Cd_0	2.21 a	0.19 e	4.02 d
	Cd_5	2.19 a	0.25 ed	6.80 c
	Cd_{10}	1.97 cb	0.30 dc	7.45 c
	Cd_{20}	1.90 c	0.30 dc	7.44 c
(+) Citric Acid	Cd_0	2.09 ba	0.41 ba	8.42 cb
	Cd_5	1.91 c	0.25 ed	10.1 ba
	Cd_{10}	1.98 cb	0.43 a	6.77 c
	Cd_{20}	1.84 c	0.35 cb	11.2 a
	F	9.74**	16.6**	19.5**

(**): p≤0.01 statistically significant within error bounds

The effect of citlric acid application on the amount of N by different Cd applications was highest with 2.21% N in the Cd_0 group in which citric acid-free treatment, was highest with 0.43% P in the Cd_{10} group in which citric acid treatment, and was highest with 11.2% K in the Cd_{20} group in which citric acid treatment was applied (Table 3).

Gouia et al. (2000) reported that the activity of nitrate reductase and nitrite reductase, enzymes of N metabolism, decreased nitrate assimilation of plants under Cd stress. Daghan et al. (2013) noted a significant reduction in N, P and K concentrations of green parts of plants with increasing doses (0, 5 and 10 mg Cd L^{-1}) of cadmium. Ciećko et al. (2004) stated that using different doses of Cd (0, 10, 20, 30, and 40 mg Cd kg^{-1}) led to a decrease in the K concentration of the corn plant for 10 and 20 mg Cd kg^{-1} applications compared with the control plant that increased in 30 and 40 mg Cd kg^{-1} usages, a decrease in the K concentration in the hay section of oat plant compared to the control group and a decrease in the K concentration of the bean plant was for 0, 20 and 40 mg Cd kg^{-1} applications compared with the control plant increased in 30 mg Cd kg^{-1} usage. As can be seen on Table 4, application of cadmium is statistically significant ($p \leq 0.01$) in terms of the amounts of Mn, Fe, Cu and Zn in the plants.

Table 4. Effects of different Cd applications on the amount of Mn, Fe, Cu and Zn in rosemary plants in citric acid and citric acid-free environments.

	Dose	**Mn**	**Fe**	**Cu**	**Zn**
		(mg kg^{-1})			
(-) Citric Acid	Cd_0	7.09 d	224 e	4.86 c	17.7 d
	Cd_5	13.4 b	439 a	6.13 b	21.8 c
	Cd_{10}	11.7 cb	344 c	4.83 c	16.8 ed
	Cd_{20}	18.2 a	390 b	7.19 a	28.7 a
(+) Citric Acid	Cd_0	16.5 a	408 ba	6.16 b	24.2 cb
	Cd_5	11.3 cb	375 cb	3.70 d	10.9 f
	Cd_{10}	10.3 c	287 d	6.39 b	25.2 b
	Cd_{20}	12.5 cb	397 ba	4.80 c	13.9 fe
	F	22.7**	33.0**	35.1**	41.1**

(**): $p \leq 0.01$ statistically significant within error bounds

It was determined that the effect of citric acid usage on the amount of Mn in different Cd applications was the highest in the control Cd_0 group with 16.5 mg Mn kg^{-1} and usage on the amount of Fe in different Cd applications was the highest as 439 mg Fe kg^{-1} for the Cd_5 group, which is the one has citric acid-free application (Table 4).

Hernández et al. (1998) reported that 10 and 50 µM Cd applied plants showed a decrease in concentrations of Mn and Fe in plant roots and shoots according to their concentrations in the control group of their studies with pea plants. It was determined that the amount of Cu was increased to 7.19 mg Cu kg^{-1} and the effect on Zn amount was highest as 28.7 mg Zn kg^{-1} for Cd_{20} group which has citric acid-free application with the effect of citric acid application with increasing usage of Cd (Table 4). Wu et al. (2004) reported that the concentration of Fe, Cu and Zn in the suprasellar area of the plant decreased with the application of Cd (1 and 10 mM Cd) on the cotton plant. Cadmium applications seem to be statistically significant at $p \leq 0.01$ in terms of Cd concentration and Cd content in plants (Table 5).

Table 5. Effects of cadmium applications on the concentration of Cd in plants and the amount of Cd content in plants

	Dose	**Cd (mg kg^{-1})**	**Cd (µg plant^{-1})**
(-) Citric Acid	Cd_0	0.58 e	0.95 d
	Cd_5	2.02 d	3.11 c
	Cd_{10}	4.99 b	7.52 a
	Cd_{20}	5.32 b	7.62 a
(+) Citric Acid	Cd_0	1.05 e	1.49 d
	Cd_5	3.34 c	4.56 b
	Cd_{10}	5.83 ba	7.73 a
	Cd_{20}	6.63 a	8.31 a
	F	87.9**	63.9**

(**): $p \leq 0.01$ statistically significant within error bounds

In Table 5, influences of the difference Cd and citric acid applications on the Cd concentration were demonstrated. They were changed in the range of 0.58-6.63 mg Cd kg^{-1}. Highest value was determined as 6.63 mg Cd kg^{-1} for Cd_{20} group which was treated with citric acid application. On the other hand, lowest value was obtained as 0.58 mg Cd kg^{-1} by Cd_0 control group, which has citric acid-free application.

The effect of increasing Cd during citric acid application, on the amount of Cd was measured 0.95-8.31 μg plant^{-1}, while the highest amount was in the Cd_{20} group with citric acid application, resulted with 8.31 μg plant^{-1}. On the contrary, the lowest value was determined as 0.95 μg plant^{-1} for the Cd_0 group, which had citric acid application (Table 5).

Jiang et al. (2003) reported that different applications of Cd-containing soil at different doses reduced the accumulation of Cd in the roots of the mustard plant but increased it in the part above the soil, and for some applications, especially EDTA, it has reduced the amount of Cd deposition at the roots. This phenomenon has been explained by the fact that the movement of Cd from the root of the plant was increasingly facilitated to the parts above the soil.

Zhang et al. (2014), investigated the application of different ratios of Cd (15,30,60,100 mg Cd kg^{-1}) to two different grass plants and reported high amount of Cd accumulation within the roots and green parts of the plants.

Conclusion and Suggestions

The most pronounced response of plants to heavy metals in increasing doses was observed as reduction in the amount of biomass they produce. Additionally, the biomass was found to be decreased for the plant pots treated with citric acid application compared to ones which have citric acid-free application. In terms of body mass and dry mass, among the rosemary plant that is being treated with cadmium and citric acid application along with the Cd_{20} application, the lowest dry mass was measured as 1.25 g plant^{-1}.

Chlorophyll levels during the increased doses of Cd applications were found to be decreasing compared to the control groups in few applications such as citric acid, Cd (except for chlorophyll-a in Cd application without citric acid) and Cd without citric acid. It has been also found that the concentration for the Cd_{20} and citric acid applications of cadmium was 5.32 and 6.63 mg Cd kg^{-1}, respectively. Additionally, for the plants which were treated with Cd_{20} applications were found to possess 7.62 μg Cd plant^{-1} content while the other pots that are treated by citric acid including 8.31 μg Cd of plant^{-1}.

As a conclusion, it has been observed that the increased Cd content had a bad influence on the chlorophyll and biomass amount which were resulted with a diminished in terms of growth rate for rosemary plant. It has been also concluded that the heavy metal application has different effects on the intake of macro and micro nutrients of plants depending on types of the elements. Therefore, rosemary plants have no potential to be used in phytoremediation treatment of Cd-contaminated soils. Future studies are required to examine extended period of time and also reveal the accumulation of Cd within the roots.

The results obtained with citric acid application were also determined by this study and indicating that the rosemary plant was more abundant in soil with heavy metal (Cd) pollution. Since the rosemary plant used in the study is also used as a spice, the metal content of the rosemary plant, especially for the ones growing in dirty soil, should not be consumed as spice. Additionally, since the rosemary plant can be used as medical aromatic plant or as a spice, it's oil also needs to be controlled in order to determine any kind of possible toxic metal content.

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