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ORIGINAL PAPER

Cadmium hyperaccumulation potential of rosemary (*Rosmarinus officinalis*): Insights into nutrition uptake and volatile oil profiles

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Abstract

Cadmium (Cd) contamination is a critical environmental concern due to its persistence and toxicity. Phytoremediation provides an eco-friendly solution by using plants to accumulate and detoxify heavy metals. This study evaluated the Cd hyperaccumulation potential of rosemary (*Rosmarinus officinalis*), a medicinal-aromatic plant with ornamental value, by assessing agro-morphological traits, elemental uptake, and essential oil composition under Cd stress. In this regard, rosemary plants were exposed to five Cd doses (10, 20, 40, 80, and 100 mg kg^{-1}) in a randomized design with three replicates. The present findings of the study revealed that morphological traits were largely unaffected (p>0.05), indicating tolerance to Cd stress. However, significant responses with respect to the nutrient uptake were recorded, being concentration-dependent. Among the nutrients, nitrogen decreased by 55% (from 2.64% to 1.16%), magnesium by 24% (from 188.2 to 143.4 mg kg^{-1}), and calcium by 22% (from 355.7 to 278.1 mg kg^{-1}). Conversely, phosphorus increased by 34% (from 198.4 to 265.8 mg kg^{-1}), potassium by 5% (from 1561 to 1641 mg kg^{-1}), sodium by 24% (from 30.3 to 37.6 mg kg^{-1}), zinc by 83% (from 1.68 to 3.07 mg kg^{-1}), iron by 38% (from 34.8 to 48.0 mg kg^{-1}), manganese by 43% (from 1.09 to 1.56 mg kg^{-1}) and copper by 100% (from 0.46 to 0.92 mg kg^{-1}). Cadmium accumulation itself increased six-fold (from 0.12 to 0.73 mg kg^{-1}). Regarding the responses of the individual essential oil compounds, α-pinene, eucalyptol, camphene, D-limonene and camphor showed varying degrees of increase in response to stress. However, these compounds eventually declined as stress levels increased. These findings demonstrate that rosemary can tolerate Cd stress while effectively accumulating the metal, thus supporting its use as a Cd hyperaccumulator. Its dual value as both a phytoremediator and an essential-oil crop highlights its potential for the sustainable remediation of Cd-contaminated soils.

Keywords: heavy metal, rosemary, phytoremediation, hyperaccumulation

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INTRODUCTION

Since the early 20th century, uncontrolled population growth, improper urbanization, intensive industrialization, unsustainable agriculture, and unregulated mining have intensified environmental pollution, disrupting ecological balance and increasing the concentration of certain elements in some regions. The accumulation of these elements has led to widespread heavy metal contamination, now a major research focus. Such pollution harms ecosystems, causes medical problems in humans, and is difficult to remediate due to the long half-lives of heavy metals. Consequently, organisms exposed to these pollutants exhibit cytotoxic, genotoxic, mutagenic, and teratogenic effects (Dixit et al. 2015). In soils, heavy metals impair plant physiology and morphology, limiting growth and productivity, and in severe cases causing plant death (Shanker et al. 2005).

Heavy metals are chemically defined as transition metals with an atomic weight above 20 g mol^{-1} and a density over 5 g cm^{-3}, and biologically as metals or metalloids toxic to organisms even at low concentrations (La Rocca et al. 2009). Among the most significant heavy metals are Pb, Hg, Cd, As, and Cr, which enter the environment through natural geological processes and anthropogenic activities such as mining, metallurgy, paints, fertilizers, and fossil fuel combustion (Jaishankar et al. 2014).

Imbalances in macro- and micronutrient levels in air, soil, or water can disrupt living systems (Benavides et al. 2005). Heavy metals, being non-biodegradable, accumulate in the environment and transfer through trophic levels via bioaccumulation and biomagnification (Tchounwou et al. 2012), with aquatic ecosystems being especially vulnerable (Nagajyoti et al. 2010). Their toxic effects on humans depend on exposure level and duration: Pb impairs cognitive development in children (Needleman 2004), Hg causes neurotoxicity (Clarkson, Magos 2006), Cd damages kidneys, bones, and is carcinogenic (Godt et al. 2006), while As leads to skin lesions, cancers, and cardiovascular diseases (Naujokas et al. 2013). Overall, heavy metal pollution threatens agriculture, water quality, and public health, imposing long-term societal and economic burdens (Wuana, Okieimen 2011).

Efforts to prevent environmental contamination have highlighted the ability of certain plants to survive in heavy metal-polluted areas, leading to the development of phytoremediation, also called “green remediation” (Meagher 2000, McIntyre 2003). This ecological approach relies on plants’ capacity to uptake, detoxify, degrade, or immobilize contaminants, making it effective against both heavy metals (e.g., Pb, Cd, As, Zn, Ni) and organic pollutants such as PAHs, pesticides, and petroleum derivatives (Pilon-Smits 2005).

Phytoremediation is an eco-friendly technology that uses plants to remediate contaminated soil, water, and air by absorbing and sequestering heavy metals, pesticides, hydrocarbons, and other pollutants through metabolic processes (Türkoğlu 2006, Ali et al. 2013). Unlike conventional chemical or physical methods, it is cost-effective, minimizes secondary contamination, and reduces ecological impact. Additional benefits include site greening, biodiversity preservation, and sustainable restoration, particularly when native vegetation is used (Salt et al. 1998). Today, phytoremediation is applied in industrial waste rehabilitation, mine reclamation, agricultural land decontamination, wastewater treatment, and wetland restoration (Vangronsveld et al. 2009).

Many plant species are used in phytoremediation, including *Typha latifolia* (Vymazal 2011), *Pteris vittata* (Ma et al. 2001, Wei, Chen 2006), *Vetiveria zizanioides* (Chen et al. 2004), *Populus* spp. (Pulford, Watson 2003), *Helianthus annuus* (Dushenkov et al. 1995, Aybar 2022), *Brassica juncea* (Salt 1995), *Carthamus tinctorius* and *Ricinus communis* (Tütüncü 2018). However, research on ornamental plants in phytoremediation remains limited.

Ornamental plants can play a key role in reducing environmental pollution, as they are widely used in urban areas for landscaping and environmental management. Selecting species with phytoremediation potential can improve air quality, making the identification of hyperaccumulator ornamental plants essential (Özay, Mammadov 2013). *Rosmarinus officinalis* (rosemary), a drought-resistant evergreen shrub of the Lamiaceae family, is notable in this regard. Native to the Mediterranean region, it is widely used in landscaping due to its resilience, ease of cultivation, and pest resistance (Ambrose et al. 2016, Akkemik 2021).

The present study was designed to address the growing challenge of cadmium contamination by revealing the phytoremediation potential of *Rosmarinus officinalis*. Despite its dual economic and ecological value, this aromatic and ornamental plant has received limited attention in remediation research. Taken together, the present study provides an assessment of Cd accumulation, nutrient dynamics, and volatile oil composition in *R. officinalis* suffering from Cd stress. Studies dealing with sustainable, low-cost and eco-friendly strategies to mitigate heavy metal pollution are needed and this research topic deserves comprehensive investigations. In this regard, making the most of rosemary's resilience, its widespread use in landscaping and its valuable essential oil profile could be crucial. Collectively, this study systematically assessed how different cadmium (Cd) contamination levels affect the growth, elemental composition, and essential oil composition of rosemary. The primary objective was to evaluate its potential for use as a multifunctional phytoremediation agent in Cd-polluted soil.

MATERIALS AND METHODS

The experiment was conducted in 2022 using *Rosmarinus officinalis* (rosemary) as the test material. Uniform potted seedlings were procured from the Greenhouses of the Department of Plant Production, Faculty of Agriculture, Kilis 7 Aralık University, Türkiye.

Cadmium treatments

On May 25, 2022, rosemary (*Rosmarinus officinalis*) seedlings were transplanted into 8-liter pots containing a soil mixture of garden soil, perlite, and peat at a 3:1:1 ratio, prepared in consideration of the effective root depth of the plant. Each cadmium (Cd) treatment was established with three replicates (one plant per pot). Following transplantation, a one-month acclimatization period was allowed to ensure uniform plant establishment. On June 30, 2022, ethylenediaminetetraacetic acid (EDTA) was applied at 10 mmol kg^{-1} to enhance Cd bioavailability. One week later, cadmium nitrate $[Cd(NO_3)_2 \cdot 4H_2O]$ was administered to the pots at concentrations of 10, 20, 40, 80, and 100 mg kg^{-1}. To simulate natural soil contamination and ensure sufficient metal absorption, a 30-day stabilization period was observed before sampling. During this period, irrigation was carried out regularly and maintained at field capacity, following the method described by Bozcuk (2004).

Morphological measurements

In the harvested rosemary plants, agro-morphological parameters including plant height, leaf length and width, as well as the fresh and dry weights of leaves, stems, and roots were recorded. For biomass determination, the samples were initially air-dried under laboratory conditions for seven days and subsequently oven-dried at 65°C for 48 hours. After drying, both above- and below-ground tissues were weighed separately (Kacar, İnal 2010).

Mineral analysis in plant samples

Leaf samples were dried at 72°C, ground into fine powder, and 0.25 g subsamples were digested in a mixture of 9 ml nitric acid (HNO_3) and 3 ml hydrogen peroxide (H_2O_2) using a microwave oven at 200 W for 30 minutes. The digested samples were filtered and diluted to a final volume of 25 ml with deionized water. Mineral concentrations were quantified using an Atomic Absorption Spectrophotometer (AAS, Perkin Elmer 1100B, Waltham, MA, USA) for Ca, Mg, Fe, Mn, Zn, and Cu, while Na and K were measured with a Flame Photometer. Total nitrogen was determined using the Kjeldahl method with a Gerhardt Kjeldatherm digestion unit and Gerhardt Vapodest 20 S distillation system (Kacar, İnal 2010).

Essential oil analysis

The volatile oil components of the rosemary plants were identified using a GC-MS Headspace system (Agilent) (Column: HP-5 MS capillary, 30 m × 0.25 μm × 250 μm, Split ratio: 10:1). After being air-dried under room conditions, the rosemary leaves were ground into powder, and approximately 0.5 grams of plant material was used for the analysis. The identification of the components was performed by referencing the Wiley and NIST electronic libraries (Sarmoum et al. 2019).

Statistical analysis

The experiment was conducted in a completely randomized design with three replications, where rosemary plants were subjected to five cadmium (Cd) treatments (10, 20, 40, 80, and 100 mg kg^{-1}). Data were analyzed using one-way analysis of variance (ANOVA) to assess significant differences among treatment means. The Pearson's correlation coefficients (r) were calculated to evaluate relationships among the measured parameters. All statistical analyses were performed using SPSS version 18.0 (SPSS Inc., Chicago, IL, USA). In addition, advanced multivariate analyses including heatmap clustering, network plot analysis, and principal component analysis (PCA) were conducted using SRPlot and PAST software to visualize interrelationships and treatment effects more comprehensively.

RESULTS AND DISCUSSION

Changes in agro-morphological parameters and biomass of rosemary plants under different cadmium (Cd) levels

The growth and morphological traits of rosemary plants were not significantly affected by cadmium (Cd) stress across all measured parameters (>0.05) (Table 1). Plant height, leaf dimensions (lower, middle, and upper leaf width and length), and biomass components (fresh and dry weights of leaves, stems, and roots) showed only slight changes among the treatments. For instance, plant height varied between 33.47 and 35.70 cm by Cd doses, while fresh leaf weight ranged from 10.51 to 14.17 g. Similarly, fresh root weight varied between 2.25 and 3.08 g. These findings suggest that rosemary exhibits a high degree of tolerance to Cd exposure, maintaining stable morphological performance even under the highest applied Cd concentration (100 mg kg^{-1}).

Multivariate statistical analyses for agro-morphological parameters and biomass traits

In addition to ANOVA, we further performed a series of statistical analyses including correlation analysis, heatmap clustering, principal component

Table 1

Changes in agro-morphological parameters and biomass of rosemary plants under different cadmium (Cd) levels*

Treatments	Control	10 (mg kg^{-1})	20 (mg kg^{-1})	40 (mg kg^{-1})	80 (mg kg^{-1})	100 (mg kg^{-1})	*p*-values
Plant height (cm)	35.7	33.54	35.53	35.6	34.48	33.47	0.599
Lower leaf width (mm)	3.08	2.66	2.36	2.83	2.41	2.66	0.25
Lower leaf length (mm)	32.19	31.15	29.98	32.21	28.14	30.51	0.576
Middle leaf width (mm)	3.09	2.83	2.41	2.97	2.48	2.73	0.283
Middle leaf length (mm)	33.64	32.86	29.83	33.1	32.25	34.81	0.597
Upper leaf width (mm)	2.99	2.68	2.23	2.71	2.16	2.53	0.129
Upper leaf length (mm)	29.81	29.79	25.82	31.16	28.89	29.75	0.721
Fresh leaf weight (g $plant^{-1}$)	12.67	11.69	11.22	14.17	10.51	11.14	0.193
Dry leaf weight (g $plant^{-1}$)	6.11	5.95	6.05	7.11	5.66	6.15	0.166
Fresh stem weight (g $plant^{-1}$)	5.09	4.49	4	5.47	3.93	3.87	0.08
Dry stem weight (g $plant^{-1}$)	2.04	1.72	1.81	2.17	1.68	1.7	0.207
Fresh root weight (g $plant^{-1}$)	2.85	2.86	2.39	3.08	2.25	2.52	0.063
Dry root weight (g $plant^{-1}$)	1.08	0.93	0.92	1.09	0.81	0.91	0.261

* Blue shading indicates relatively higher values, while red shading indicates relatively lower values for each trait

analysis and network plot analysis in order to correlate, visualize and reduce the dimensionality of the parameters considered. In this regard, we first performed correlationa analysis. According to the correlation coefficients, there were several significant positive relationships among rosemary traits under cadmium stress (Fig. 1A). For isntance, lower leaf width correlated strongly

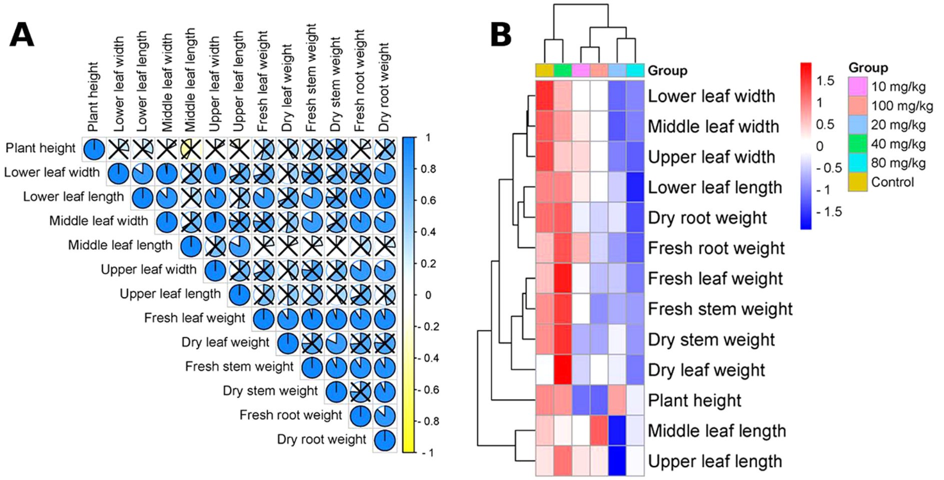

Fig. 1. Relationships among agro-morphological and biomass traits of rosemary under cadmium stress. **(A)** Correlation analysis showing pairwise associations between morphological parameters and biomass traits. Blue shading indicates positive correlations, while yellow indicates negative correlations; crosses denote non-significant relationships ($p > 0.05$). **(B)** Heatmap clustering analysis of agro-morphological and biomass traits corresponding to different cadmium treatments (0, 10, 20, 40, 80, and 100 mg kg^{-1}). The color gradient from red to blue represents relative trait values (high to low), and hierarchical clustering highlights grouping patterns among traits and treatments

with lower leaf length (r=0.846, p=0.034), middle leaf width (r=0.976, p<0.001), upper leaf width (r=0.968, p=0.002), and dry root weight (r=0.843, p=0.035). Lower leaf length also exhibited strong relationships with upper leaf width (r=0.909, p=0.012), fresh root weight (r=0.925, p=0.008), and dry root weight (r=0.941, p=0.005). Biomass traits were highly correlated, with fresh leaf weight strongly correlated with dry leaf weight (r=0.903, p=0.014), fresh stem weight (r=0.960, p=0.002), and dry stem weight (r=0.946, p=0.004). Similarly, there was a positive correlation between fresh root weight and dry root weight (r=0.868, p=0.025). Following correlation, we performed a heatmap clustering (Figure 1B). Accordingly, traits such as lower leaf width, middle leaf width, and upper leaf width exhibited relatively higher values under low to moderate Cd stress. However, middle leaf length values were highly pronounced in Cd-stress submitted plants. Concerning the treatment, control and 40 mg kg^{-1} clustered in the same group, while the others were clustered into two-sub groups.

We also performed a network plot analysis, providing an integrated visualization of the overall performance of the control and cadmium-treated groups (10, 20, 40, 80, and 100 mg kg^{-1}) based on agro-morphological traits (Figure 2A). Of the treatments, control and 10 mg kg^{-1} Cd grouped closely, while 20 mg kg^{-1} Cd was positioned distantly. The most distinct response was observed in 20 mg kg^{-1} Cd treatment. The 40 mg kg^{-1} treatment showed intermediate separation, whereas 80 and 100 mg kg^{-1} Cd clustered tightly. Based on the network plot, three main groups including control with low Cd, moderate Cd as a distinct outlier, and high Cd levels were found. We further carried out Principal Component Analysis (PCA) in order to reduce the dimensionality of the growth parameter data (Figure 2B). Accordingly, PC_1

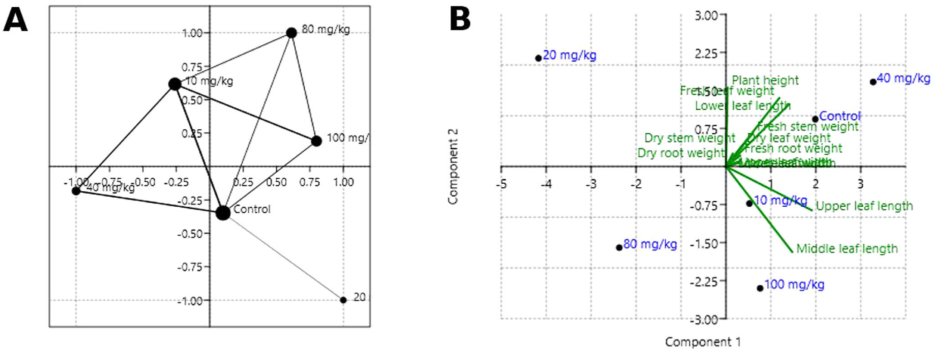

Fig. 2. Multivariate analysis of agro-morphological traits of rosemary under cadmium stress. (**A**) Growth associated-network plot showing relationships and proximities among treatment groups (Control, 10, 20, 40, 80, and 100 mg kg^{-1} Cd). The connecting lines illustrate the relative similarity in morphological responses across treatments. (**B**) Principal component analysis (PCA) biplot of treatments and agro-morphological traits. Treatments are distributed based on their association with trait variation, while vectors represent the contribution and direction of each trait to the principal components

has the largest eigenvalue (9.18) and explains most of the variance (approximately 70.59%), while PC_2, with an eigenvalue of 2.35, explains 18.08%. The first two components explain approximately 88.67% of the total variance. Such a high explained ratio can be a critical indicator of Cd effects on growth parameters. Regarding the scores of the treatments corresponding to the components, the first component (PC_1) was found to be positively associated with the control, while exhibiting the negative correlations with the 20 mg kg^{-1} and 80 mg kg^{-1} treatments. Although no significant differences were noted, according to ANOVA, the findings of PCA suggested that the Control group deviated significantly from the 20 mg kg^{-1} and 80 mg kg^{-1} treatments. In the same component, the 40 mg kg^{-1} treatment had the highest positive score, indicating that 40 mg kg^{-1} treatment was the most different from other treatments. In the case of the second component (PC_2), the 10 mg kg^{-1} treatment exhibited the highest positive score, while the 20 mg kg^{-1} treatment showed the most negative score in this component. According to the loadings of parameters, lower leaf width, lower leaf length, middle leaf width, fresh stem weight, and dry root weight contributed the most to PC_1, whereas plant height had lower loadings, indicating that they contributed less to PC_1. As in the case of PC_2, plant height, middle leaf length and upper leaf width were the most prominent contributing variables.

As given above, Cd-stress submitted plants did not demonstrate critical changes with respect to their morphological and biomass-related traits. These responses might be explained with the Cd's limited mobility in the xylem, and its delayed toxic effect (Chen et al. 2023, Kazachkova 2023). Significantly, the accumulation of Cd in roots is time-dependent, reaching its toxic levels over time (Zhang et al. 2005). In the present study, we have profilled the leaf mineral content, also recorded the changes in above-ground parts of the plants, in general. As reported by Wagner and Yeargan (1986), restricted mobility leads to greater accumulation in older leaves, while younger leaves show fewer symptoms. Taken Together, Cd stress effects may appear only after prolonged exposure, as in the cases of salt stress and drought stress (Waseem et al. 2011, Carvalho et al. 2021). Detoxification is of the critical biochemical strategies employed with respect to the defence system of the plants (Luo and Zhang 2021). In order to buffer and cope with the stress conditions such as Cd, vacuolar sequestration, binding to metal-binding proteins, and metabolic detoxification are of the crucial regulations provided by the plants (Clijsters et al. 1999). Such strategies can explain the no-visible symptoms observed in the present study. However, prolonged exposure disrupts photosynthesis, nutrient uptake, and hormone regulation (Shaari et al. 2022, Zulfiqar et al. 2022, Fan et al. 2023). As reported by Ayhan et al. (2005), plants suffering from contaminated environments often show root growth retardation, reduced height, abnormal root development, leaf narrowing, chlorosis, and necrosis. Additionally, following interaction of the plants with the heavy metals available in soil, impaired nutrient uptake and chlorophyll structure changes are observed, according to the report of Menge et al. (1978). Critically, it is worthy to note that the extent of metal uptake varies with plant species and ecological factors like temperature, humidity, soil pH, and texture (Stancheva et al. 2011).

Effect of cadmium stress on the macro- and microelement concentrations of rosemary plants

According to the results of the analysis of variance, cadmium (Cd) stress had a statistically significant effect on the elemental composition of rosemary leaves ($p<0.05$) – Table 2. Increasing Cd concentrations led to pronounced decreases in nitrogen (N), magnesium (Mg), and calcium (Ca) contents. In contrast, phosphorus (P), potassium (K), sodium (Na), zinc (Zn), iron (Fe), cadmium (Cd), manganese (Mn), and copper (Cu) contents exhibited significant increases with rising stress levels.

Multivariate statistical analyses for macro- and microelements considered for the study

Correlation analysis showed that nitrogen (N), magnesium (Mg), and

Table 2

Changes in macro- and microelement concentrations of rosemary plants under different cadmium (Cd) treatments*

Element	Control	10 (mg kg^{-1})	20 (mg kg^{-1})	40 (mg kg^{-1})	80 (mg kg^{-1})	100 (mg kg^{-1})
N	2.64±0.02 a	2.38±0.09 b	1.96±0.11 c	1.81±0.08 c	1.44±0.09 d	1.16±0.13 e
P	198.38±1.61 d	202.01±1.08 d	215.23±8.44 c	231.67±11.50 b	260.07±4.85 a	265.78±3.35 a
K	1560.96±0.57 d	1568.54±2.82 cd	1575.17±1.80 cd	1578.35±1.81c	1611.19±19.30 b	1640.94±1.16 a
Na	30.31±1.58 d	36.08±1.51 c	38.73±0.43 ab	39.22±0.23 a	36.87±1.37 bc	37.55±0.72 abc
Mg	188.21±2.82 a	174.92±7.86 b	161.02±1.27 c	157.16±2.50 c	149.51±0.84 d	143.39±3.08 d
Ca	355.67±4.38 a	334.88±11.82 b	313.31±1.89 c	306.24±6.88 c	288.23±2.00 d	278.07±6.07d
Zn	1.68±0.14 c	1.91±0.22 c	1.70±0.28 c	2.3±0.31b	2.91±0.13 a	3.07±0.05 a
Fe	34.77±3.93 c	46.37±4.37 ab	41.71±0.51 b	42.78±0.53 b	45.87±0.84 ab	48.00±1.38 a
Cd	0.12±0.04 e	0.33±0.06 d	0.49±0.03 c	0.56±0.03 c	0.67±0.03 b	0.73±0.02 a
Mn	1.09±0.04 e	1.39±0.05 d	1.72±0.03 b	1.98±0.14 a	1.85±0.05 ab	1.56±0.03 c
Cu	0.46±0.01 e	0.58±0.05 d	0.64±0.03 c	0.70±0.04 c	0.79±0.02 b	0.92±0.02 **a**

* Blue shading indicates relatively higher values, while red shading indicates relatively lower values for each trait. Values are means ± SE (n=3). Different letters within rows indicate significant differences according to the Tukey's test (p<0.05).

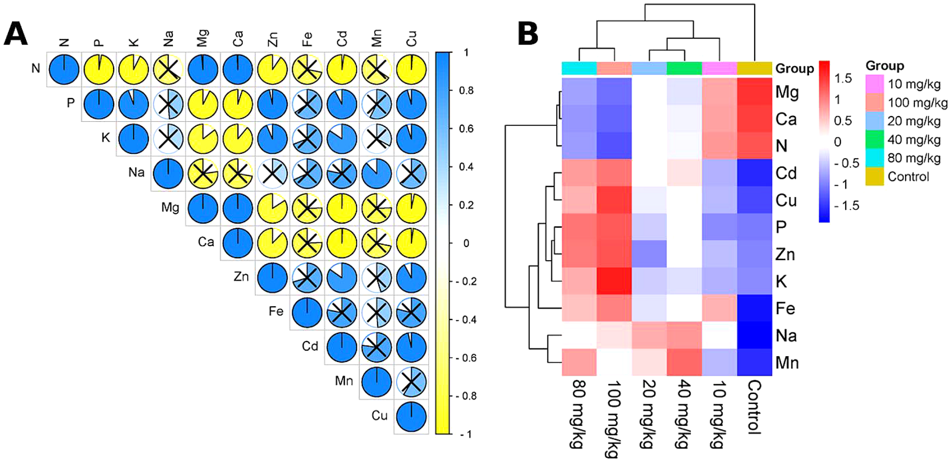

Fig. 3. Relationships among nutrients of rosemary under cadmium stress. **(A)** Correlation analysis showing pairwise associations between nutrients. Blue shading indicates positive correlations, while yellow indicates negative correlations; crosses denote non-significant relationships (p> 0.05). **(B)** Heatmap clustering analysis of nutrients corresponding to different cadmium treatments (0, 10, 20, 40, 80, and 100 mg kg^{-1}). The color gradient from red to blue represents relative trait values (high to low), and hierarchical clustering highlights grouping patterns among traits and treatments

calcium (Ca) were strongly and negatively correlated with cadmium (Cd), copper (Cu), phosphorus (P), potassium (K), and zinc (Zn) – Figure 3A). The strongest negative associations were observed between Mg-Cd (r=-0.999, p<0.001) and Ca-Cd (r=-0.995, p<0.001). In contrast, Cd, Cu, P, K, and Zn exhibited strong positive correlations with each other, such as Cd-Cu (r=0.966, p=0.002) and P-Zn (r=0.965, p=0.002). According to the heatmap clustering, marked-reductions in leaf N, Mg, and Ca contents recorded under higher Cd doses (80 and 100 mg kg^{-1}), clustering together with negative values. Regarding Cd, Cu, P, K, Zn, Fe, Na, and Mn, we observed a significant progressive accumulation. Importantly, Control group was clearly separated from Cd stress-submitted groups. Additionally, the network plot analysis further revealed a clear dose-dependent discrimination among the treatments (Figure 4A). Of the treatments, Control and 10 mg kg^{-1} groups were closely related. On the other hand, 100 mg kg^{-1} was positioned distantly. Other doses including 20 and 40 mg kg^{-1} occupied transitional positions. Based on the responses of elements, 80 mg kg^{-1} formed a distinct cluster. This dose can be deduced as a threshold effect. We also observed a PCA-aided clear discrimination of the treatments (Figure 4B). Being consistent with the network plot analysis, the control and 10 mg kg^{-1} groups clustered together, indicating similar nutrient uptake, while the 100 mg kg^{-1} group was positioned distantly. Other doses were positioned in Component 1.

As given above, Cd stress significantly reduced the uptake or accumulation of N, Mg, and Ca, whereas it enhanced the uptake of other elements. That can be explained by Cd transport pathways interfering with N, Mg,

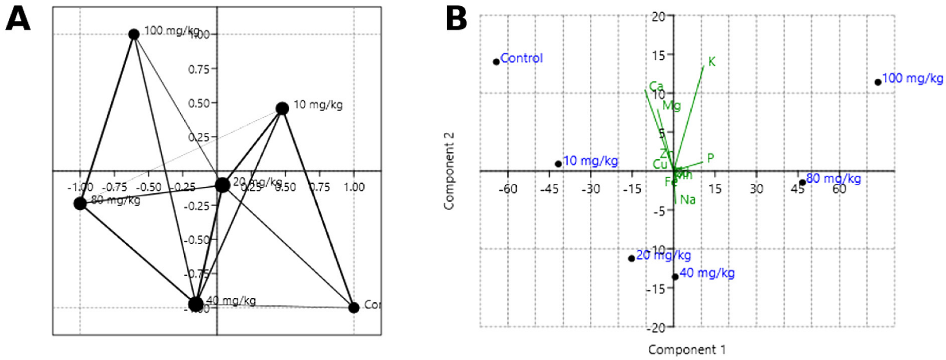

Fig. 4. Multivariate analysis of nutrient uptake under cadmium stress. (**A**) Nutrient associated-network plot showing relationships and proximities among treatment groups (Control, 10, 20, 40, 80, and 100 mg kg^{-1} Cd). The connecting lines illustrate the relative similarity in nutrient uptake responses across treatments. (**B**) Principal component analysis (PCA) biplot of treatments and nutrient uptake. Treatments are distributed based on their association with trait variation, while vectors represent the contribution and direction of each trait to the principal components

and Ca uptake. Furthermore, it can linked to stronger binding interactions facilitating Cd and Zn transport. Similar findings were reported by Kılıç (2018), who observed biomass reduction under Cd stress with citric acid but no change in Cd uptake. According to the report of Balcan (2014), rosemary has potential of being copper hyperaccumulator, despite physiological and morphological alterations under high Cu doses. Similarly, rosemary was also assayed for its lead (Pb) hyperaccumulation, accumulating Pb, with 2.25 mg kg^{-1} in stems and 24.85 mg kg^{-1} in roots at 100 mg kg^{-1} Pb exposure (Karabulut 2020).

Effect of cadmium stress on the volatile oil composition of rosemary plants

The volatile oil compounds of rosemary under Cd stress are shown in Table 3. Among the treatments, α-pinene was the dominant component, followed by eucalyptol, camphene, D-limonene, and camphor. Of the compounds, α-pinene reached the highest level at 10 mg kg^{-1} but declined with increasing stress. Camphene peaked at 40 mg kg^{-1}, but then exhibited a declining trend, while D-limonene showed reductions only at 100 mg kg^{-1}. Eucalyptol increased at 20 mg kg^{-1} and 40 mg kg^{-1} but declined at higher doses, with both eucalyptol and camphor reaching their maximum at 20 mg kg^{-1}.

Multivariate statistical analyses for volatile oil compounds identified in leaf samples of rosemary

According to the correlation matrix (Figure 5A), significant correlations were observed between verbenene and *p*-cymene, *β*-myrcene and eucalyptol, as well as 3-carene and camphor. We did not observe significant correlation

Table 3

Changes in volatile compounds of rosemary plants under different cadmium (Cd) treatments

Volatile oil	Control	10 (mg kg^{-1})	20 (mg kg^{-1})	40 (mg kg^{-1})	80 (mg kg^{-1})	100 (mg kg^{-1})
3-Thujene	0.142±0.02	0.15±0.02	0.22±0.01	0.26±0.04	0.27±0.02	0.28±0.02
α-pinene	40.45 ±4.17	44.09±4.67	36.49±1.95	35.68±2.41	34.85±1.87	33.81±0.45
Camphene	7.50±1.68	7.22±0.93	7.19±0.32	8.20±0.36	6.75±0.36	6.80±0.40
Verbenene	1.48±0.45	1.48±0.23	1.44±0.08	1.58±0.19	1.25±0.21	0.95±0.07
o-Cymene	0.23±0.03	0.24±0.02	0.29±0.04	0.39±0.03	0.41±0.02	0.29±0.02
β-pinene	1.88±0.12	1.93±0.07	1.33±0.03	1.48±0.15	1.64±0.07	1.73±0.09
3-Octanone	0.29±0.02	0.32±0.03	0.45±0.06	0.42±0.04	0.41±0.03	0.29±0.05
β-myrcene	3.52±0.52	3.55±0.62	4.02±0.17	4.33±0.10	3.99±0.32	3.44±0.30
α-phellandrene	0.85±0.09	0.85±0.10	0.83±0.06	1.04±0.06	1.04±0.16	1.06±0.11
3-Carene	1.47±0.16	1.74±0.08	1.83±0.07	1.24±0.15	1.25±0.07	1.23±0.05
α -terpinene	0.86±0.02	0.90±0.02	1.00±0.02	0.95±0.04	0.82±0.06	0.75±0.08
p-Cymene	2.94±0.34	3.08±0.11	3.13±0.10	3.08±0.11	2.93±0.13	2.44±0.15
D-Limonene	6.29±0.55	6.30±0.36	6.15±0.15	6.96±0.30	6.69±0.16	5.18±1.54
Eucalyptol	13.46±1.09	13.67±1.33	16.17±0.88	15.82±0.50	13.73±0.44	11.60±0.07
γ-terpinene	0.90±0.02	0.92±0.05	0.87±0.05	1.05±0.05	1.09±0.08	0.96±0.04
α- Terpinolene	0.99±0.11	1.08±0.06	1.14±0.09	1.08±0.13	1.13±0.09	0.76±0.52
Camphor	4.58±0.61	4.80±0.54	6.16±0.89	3.69±0.20	3.72±0.41	3.52±0.02
β-pinanone	0.15±0.01	0.15±0.02	0.14±0.01	0.15±0.02	0.15±0.01	0.16±0.17
endo-Borneol	0.73±0.08	0.78±0.03	0.65±0.06	0.65±0.01	0.60±0.07	0.45±0.03
3-Pinanone	0.45±0.06	0.49±0.04	0.55±0.06	0.62±0.04	0.59±0.11	0.59±0.06
D-Verbenone	0.87±0.08	0.87±0.05	0.87±0.10	0.84±0.08	0.78±0.1	0.82±0.03
Bornylacetate	0.32±0.09	0.26±0.00	0.29±0.01	0.27±0.02	0.29±0.01	0.26±0.03
Caryophyllene	0.19±0.01	0.18±0.00	0.19±0.02	0.18±0.00	0.19±0.00	0.18±0.04

* Blue shading indicates relatively higher values, while red shading indicates relatively lower values for each trait.

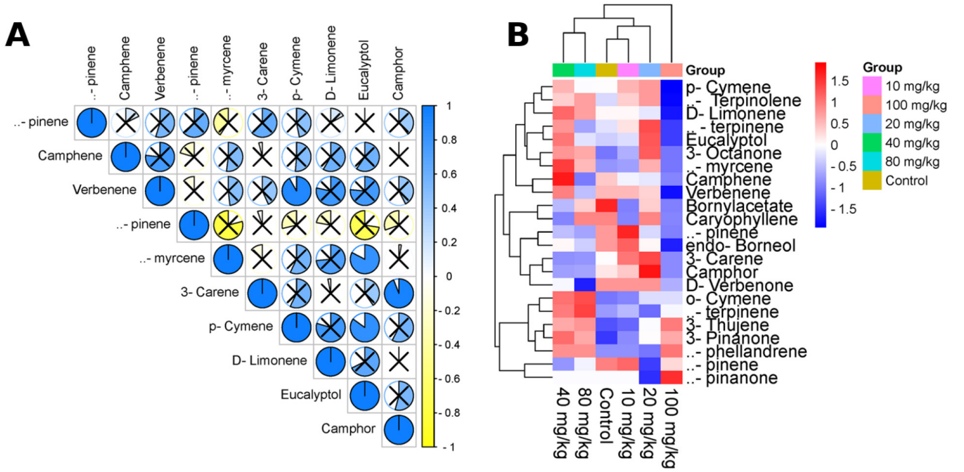

Fig. 5. Relationships among essential oil compounds of rosemary under cadmium stress. **(A)** Correlation analysis showing pairwise associations between nutrients. Blue shading indicates positive correlations, while yellow indicates negative correlations; crosses denote non-significant relationships (p> 0.05). **(B)** Heatmap clustering analysis of essential oil compounds corresponding to different cadmium treatments (0, 10, 20, 40, 80, and 100 mg kg^{-1}). The color gradient from red to blue represents relative trait values (high to low), and hierarchical clustering highlights grouping patterns among traits and treatments

among the major compounds (α-pinene, eucalyptol, camphene, D-limonene, and camphor) identified. According to the heatmap clustering (Figure 5B), 40 and 80 mg kg^{-1} clustered closely. The second cluster was composed of two sub-clusters. The first sub-cluster included Control and 10 mg kg^{-1} Cd and the second one comprised 20 and 100 mg kg^{-1} Cd. Of the compounds, *p*-cymene, terpinolene, and D-limonene showed strong accumulation under high Cd exposure, whereas α-pinene, camphor, and bornyl acetate were reduced. We further grouped the treatments for their total performance on volatile oil accumulation, reporting that Control, 10 mg kg^{-1}, 20 mg kg^{-1}, and 80 mg kg^{-1} treatments were positioned in close proximity, indicating high similarity in trait responses among these groups. On the other hand, the 40 mg kg^{-1} treatment was moderately separated. The 100 mg kg^{-1} group was completely positioned and isolated in the negative quadrant. In the context of PCA, we observed a clear discrimination of the treatments, indicating dose-dependent responses concerning the volatile oil compounds. Control and 10 mg kg^{-1} were associated with α- and β-pinene, while 20 mg kg^{-1} aligned with eucalyptol. The 40 mg kg^{-1} treatment grouped with β-myrcene and related terpenoids, whereas 100 mg kg^{-1} was strongly isolated, reflecting the most divergent profile, as also confirmed by other analyses.

The α-pinene, eucalyptol, camphene, d-limonene, and camphor volatile oils identified in the plant showed variable increases due to the presence of stress; however, as the stress level increased, a decline was observed. It is suggested that this reduction may be due to the negative impact of stress on

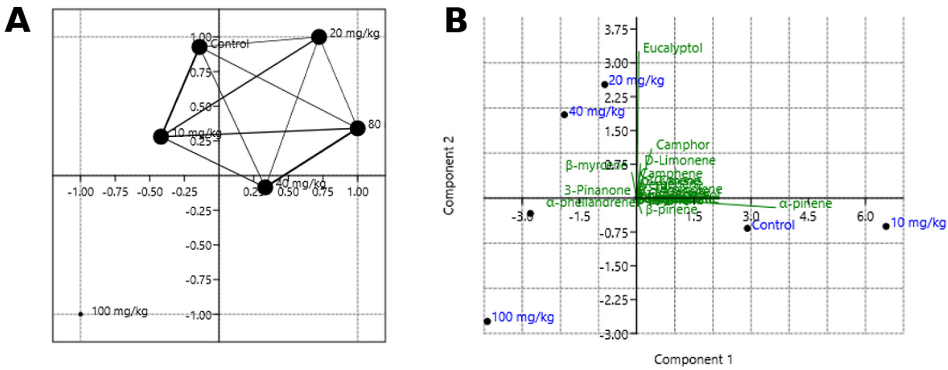

Fig. 6. Multivariate analysis of volatile oil compounds under cadmium stress. (**A**) volatile oil compounds associated-network plot showing relationships and proximities among treatment groups (Control, 10, 20, 40, 80, and 100 mg kg^{-1} Cd). The connecting lines illustrate the relative similarity in volatile oil compounds responses across treatments. (**B**) Principal component analysis (PCA) biplot of treatments and volatile oil compounds. Treatments are distributed based on their association with trait variation, while vectors represent the contribution and direction of each trait to the principal components

intracellular functions. Furthermore, it has been reported that attributing this variable, volatile oil profile, solely to stress might not be accurate, as other environmental conditions or stress factors that the plant experiences could also influence this outcome. Indeed, a study conducted in Bulgaria involved growing lavender and mint in an area with pollution and in a location 400 m away from the polluted site. The results showed a decrease in leaf yield of mint grown away from pollution, while no changes in herbage yield or essential oil content were observed in lavender (Zheljazkov, Nielsen 1993). In another study examining the changes in phenolic content under 100 μM concentration of Cd stress in rosemary, an increase in phenolic compound levels was observed (Abayhan et al. 2022).

Highlights and limitations of the present study

In the present study, we observed that rosemary (*Rosmarinus officinalis*) tolerated cadmium (Cd) stress without significant morphological changes but exhibited critical changes in nutrient uptake and volatile oil compound profile. Our findings support the role of rosemary as a candiate for a hyperaccumulator and a phytoremediation species. Despite the promising findings, the study has several limitations, having ben based on short-term, pot-based experiments under controlled conditions, focusing only on Cd and volatile oil compounds. Taken together, further research is necessary in order to assess long-term effects, multi-contaminant interactions, and field-scale applicability.

CONCLUSIONS

Phytoremediation plants, which are increasingly being used as an effective and inexpensive method for cleaning areas contaminated with heavy metals, have gained a growing interest in recent years. However, as the mechanisms of phytoremediation are still not fully understood, it is essential to utilize current technologies and genetic sciences to exploit this potential of plants more effectively. Many plants have been evaluated over time for their phytoremediation capabilities and their applicability, but studies involving ornamental plants are fewer compared to other plant groups. The potential of ornamental plants to improve air quality and reduce environmental pollution, particularly in phytoremediation applications, has become more important in contemporary times. Research on species of ornamental plants that can be used for phytoremediation is crucial for tapping into this potential. In this study, the agro-morphological characteristics, elemental uptake, and volatile oil composition of rosemary plants exposed to different Cd doses were investigated, along with the phytoremediative effects of rosemary in cleaning Cd-contaminated soils. The research concluded that the Cd content in the plant increased depending on the applied Cd dose; however, this effect was not lethal. Therefore, it can be concluded that rosemary, a plant with high potential for ornamental use, could be considered a Cd hyperaccumulator for preventing or remediating environmental pollution. Nevertheless, since data regarding the long-term consequences of Cd toxicity were not identified, further research is required to assess its long-term use.

Author contributions

Y.A.B. – conceptualization, methodology, investigation, data curation, writing-original draft preparation, D.D. – conceptualization, methodology, investigation, data curation; supervision, writing-original draft preparation; writing-review & editing, M. K. - Conceptualization; software; data curation; writing-original draft preparation, F.Y.K. – conceptualization, software, data curation, writing-original draft preparation, writing-review & editing. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

The authors ensure that they have neither professional nor financial connections related to the manuscript sent to the Editorial Board.

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