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EFFECT OF ELEVATED CONCENTRATIONS OF CADMIUM ON HEAVY METAL(OID)S CONTENT, ANTIOXIDANT ACTIVITY AND CONTENT OF ROSMARINIC ACID OF LEMON BALM (*MELISSA OFFICINALIS* L.)

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ABSTRACT. Lemon balm (*Melissa officinalis* L.) is a well-established medicinal plant prized for its rich content of bioactive compounds. This study explored how artificial cadmium (Cd) contamination of soil affects the accumulation of heavy metal(oid)s, antioxidant activity, and the concentration of rosmarinic acid in lemon balm. At a soil Cd level of 20 mg/kg, plant growth was reduced, while the concentrations of most other elements, antioxidant activity, total phenolics, and flavonoids decreased, and the rosmarinic acid content increased slightly. In contrast, lower Cd levels (5 and 10 mg/kg) led to a modest increase in antioxidant activity and phenolic content. Cadmium accumulation in the aerial parts exceeded the WHO limit for medicinal plants. Although minor improvements in antioxidant properties were observed at lower Cd levels, and rosmarinic acid increased at 20 mg/kg, the health risks associated with Cd accumulation remain a concern. Therefore, monitoring Cd content in lemon balm grown on contaminated soils is essential to ensure its safe medicinal use.

Keywords: *lemon balm, cadmium, soil pollution, rosmarinic acid, antioxidant activity*

INTRODUCTION

Medicinal plants and their preparations are increasingly used today. In recent years, with the rapid flow of information thanks to the internet and media, it has become increasingly common to hear about the harmful effects

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of commercially available drugs. Consequently, people are turning more and more to the use of plants for treating various illnesses. The popularity of medicinal plants and herbal preparations is related to the fact that they are very accessible to humans, they have a relatively low price, as well as the fact that people around the world believe that their use, even if it does not result in the desired therapeutic effect, cannot lead to any unwanted side effects [1]. One of the problems that arises in the use of medicinal plants is the accumulation of heavy metal(oid)s in the parts of the plant used for preparing various preparations. For example, an analysis of medicinal herbs in Poland found an increased concentration of Cd [2], as well as an increased concentration of Cd in samples of thyme and chamomile in Spain [3].

Soil pollution with heavy metal(oid)s is one of the most important environmental problems today. Heavy metal(oid)s are naturally present in the soil. However, their increased concentrations in the soil are primarily the result of various human activities such as mining, smelting, disposal of industrial wastes, use of wastewater for irrigation, application of fertilisers and pesticides, *etc.* [4]. Unlike organic pollutants, heavy metal(oid)s are not biodegradable, so they are accumulated in the environment [5]. Plants growing in polluted soil can absorb heavy metal(oid)s, and if these plants are consumed by people or animals, the heavy metal(oid)s can have a harmful effect on them. For example, Cd and cadmium compounds are human carcinogens. Upon entering the human body, Cd is effectively retained and accumulates over a person's lifetime. Cadmium primarily hurts the kidneys, especially on the proximal tubular cells, which are its leading accumulation site. Cadmium also has an adverse effect on human bones, while increased exposure to Cd can disrupt normal lung function and lead to lung cancer [6,7].

Lemon balm (*Melissa officinalis* L.) is a perennial herbaceous plant originating from southern Europe and is now cultivated worldwide. The plant has a lemon-like scent and has been used for various purposes throughout the centuries. In medicinal applications, lemon balm leaves are used, which are collected just before flowering, or the essential oil obtained by steam distillation from fresh lemon balm shoots. Lemon balm is a good source of antioxidants and contains significant amounts of phenolic compounds. Lemon balm leaves contain flavonoid glycosides (quercitrin, rhamnocitrin, apigenin, and luteolin derivatives), phenolic acids, catechin tannins, triterpene compounds, and rosmarinic acid. The main components of the essential oil are the monoterpene aldehydes citral (geranial and neral). Important constituents of lemon balm essential oil also include geraniol, citronellol, linalool, geranyl acetate, β-caryophyllene, and β-caryophyllene oxide [8,9]. Lemon balm has antimicrobial, antispasmodic, antiviral, mildly sedative, and antithyroid effects. The leaf and essential oil are used in the form of infusions, extracts, and tinctures for

mild insomnia. Due to its pleasant aroma, lemon balm essential oil is also used in the perfume and food industries. Additionally, various lemon balm extracts and essential oils can be used for the treatment of herpes simplex viruses [9,10].

A large number of studies on the topic of heavy metal(oid)s and medicinal plants emphasise the significance of researching the effects of soil pollution with heavy metal(oid)s on medicinal plants. For example there are studies about lemon balm, marigold etc. that investigate effect of soil pollution with heavy metal(oid)s on content of heavy metal(oid)s in these plants [11-13]. Also some studies investigate effect of soil pollution with heavy metal(oid)s on antioxidant activity and content of phenolic compounds in plants [14,15].

As lemon balm is one of the most commonly used medicinal plants worldwide, this study aims to investigate the impact of soil pollution with Cd on the heavy metal(oid)s content of lemon balm. Lemon balm is a good source of antioxidants, so another goal of this study is to examine the effects of soil pollution with Cd on the antioxidant activity of lemon balm extract, the content of total phenols, total flavonoids, as well as the content of rosmarinic acid, as one of the main bioactive components which is responsible for the medicinal properties of lemon balm.

RESULTS AND DISCUSSION

The soil used in this pot experiment was sampled in the vicinity of the city of Niš, Serbia, from a location where no agro-technical practices had been applied for many years to minimise the impact of these effects on the growth of lemon balm. The soil, belonging to the type silty clay loam, was sampled at a depth of about 20 cm, air-dried for three weeks, ground, and sieved. The obtained soil was mixed with special substrate Hawita Professional in a 1:1 (%v/v) ratio to improve soil quality. To create Cd-contaminated soil, the soil was treated with a solution of $Cd(NO_3)_2 \times 4H_2O$ in amounts to achieve soil with added Cd concentrations of 5, 10, and 20 mg/kg, and then mixed to ensure the Cd content in the soil was as uniform as possible. The artificial contaminated soil was placed in plastic pots with a diameter of 22 cm and then left to equilibrate, with daily mixing of the soil. After one month, four 3-week-old lemon balm seedlings (purchased from the Institute 'Dr. Josif Pancic', Belgrade) were planted in each pot. The pots were kept outdoors so that the conditions under which the lemon balm grew (temperature, sun exposure, humidity) were as close as possible to those in nature, and as much as possible the same for all pots from June to August (about seven weeks), after which the lemon balm was harvested, with the roots separated from

the above-ground parts, *i.e.*, the shoots. The height of the plants was measured, and all plant material was air-dried (indoors, on paper, covered with paper) until just before the analysis. To ensure sufficient quantities of material for the necessary analyses, each unit of the model system was replicated twice (two pots). Prior to analysis, the plant material and soil were combined into a single, representative sample for each unit of the model system. Labels on pots are Cd-0 for the referent pot, Cd-5, Cd-10, and Cd-20 for pots where Cd is added in amounts of 5, 10, and 20 mg Cd/kg of soil, respectively. Labels -S, -R, and -Sh represent soil, root and shoot samples, respectively.

To assess the potential effect of added Cd on the growth of lemon balm, the height of the plant was measured, and the results are presented in Figure 1 as the average length of all plants (four plants per pot) within a treatment. With the increase in Cd concentration in the soil, plant's height decreases. The results of the one-way ANOVA indicate that there is no statistically significant difference in the height of the plant between the uncontaminated soil and the soil treatments with 5 mg/kg and 10 mg/kg.

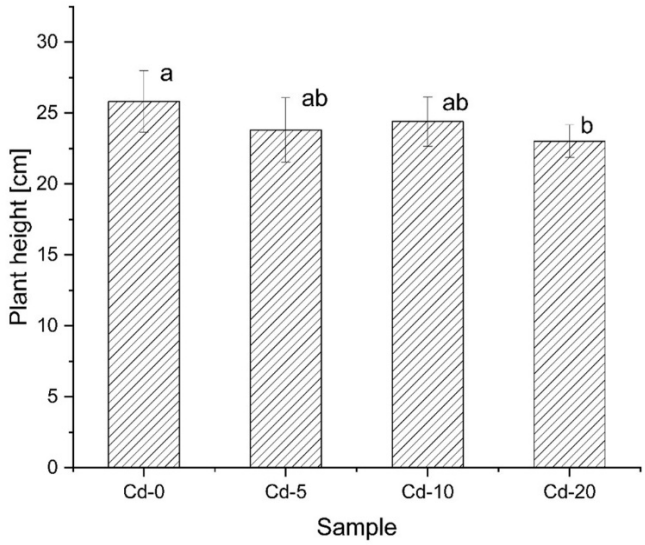

Figure 1. Effect of soil pollution with Cd on lemon balm plant height. *Vertical bar* represents standard deviation of mean height of eight plants per treatment. Bars marked with the same letter are not significantly different, according to the statistical test (α = 0.05)

A statistically significant difference is observed in the case of Cd addition at 20 mg/kg compared to uncontaminated soil. Namely, Cd, as a non-essential metal, has a toxic effect on plants at higher concentrations, which occurs through the induction of oxidative stress, changes in the permeability and integrity of the cell membrane, reactions with sulfhydryl groups, or the replacement of essential metals in enzymes and proteins [16].

The same effect of Cd on the height of lemon balm was observed by Kilic and Kilic [13]. They also concluded that increased Cd concentration in the soil leads to decreased essential oil content in the leaves. In the case of soil pollution with a quantity of 30 mg Cd/kg, the essential oil content decreased by as much as 97% compared to lemon balm grown on uncontaminated soil. The same negative impact of Cd was noted in the case of sorghum [17]. However, literature data also indicate that an increase in Cd concentration in the soil leads to an increase in coriander height at lower Cd concentrations, followed by a decrease at higher Cd concentrations [18].

The pH of the used soil was 6.8, and the electrical conductivity was 0.48 mS/cm. In a study of the pH values of various soil samples in the Niš area, the pH ranged from 7.26 to 10.24, while conductivity levels were between 0.12 and 0.88 mS/cm, which is consistent with the obtained results [19].

Concentrations of analysed elements in soil samples are presented in Table 1. Among the analysed elements, Fe is the most abundant, while Cd is the least abundant. The content of all analysed elements is below the Maximum Allowed Concentration (MAC) [20]. The addition of Cd as nitrate salt to the soil resulted in an increase in the concentration of Cd in the soil to such an extent that the concentration of Cd was always higher than the MAC value (3 mg/kg): for the sample Cd-5-S by 1.97 times, for Cd-10-S by 4.08 times, and for Cd-20-S by 8.5 times.

Concentrations of analysed elements in roots and shoots are presented in Tables 2 and 3, respectively. In the roots of the lemon balm, the highest concentrations of Fe were found, while Pb and As, as non-essential and potentially toxic elements, were the least present. Soil contamination with Cd leads to an increase in Cd concentration in the roots. In the shoots of lemon balm, the order of the most abundant elements among the analysed ones is Fe > Zn > Mn > Cu, while Cd, As, Pb, and Cr are present in much lower amounts. This is consistent with the fact that the first-mentioned elements are essential metals necessary for plant growth, whereas the others are non-essential elements, some of them potentially toxic.

Table 1. Contents of elements ± SD (mg/kg of sample, dry mass) in soil samples

Element	Sample			
	Cd-0-S	**Cd-5-S**	**Cd-10-S**	**Cd-20-S**
As	8.9 ± 0.1	8.8 ± 0.2	8.18 ± 0.06	8.8 ± 0.3
Cd	0.77 ± 0.01	5.90 ± 0.06	12.26 ± 0.02	25.5 ± 0.2
Cr	39.5 ± 0.3	42 ± 2	37.7 ± 0.3	38.3 ± 0.4
Cu	23.8 ± 0.2	23.1 ± 0.9	23.5 ± 0.2	23.9 ± 0.3
Fe	14400 ± 70	14930 ± 20	13300 ± 100	13960 ± 80
Mn	372 ± 4	379.2 ± 0.9	332 ± 4	377.5 ± 0.6
Pb	23.45 ± 0.09	24.0 ± 0.3	21.89 ± 0.07	23.28 ± 0.09
Zn	27.0 ± 0.2	28.31 ± 0.09	25.39 ± 0.05	26.37 ± 0.06

Table 2. Contents of elements ± SD (mg/kg of sample, dry mass) in root samples

Element	Sample			
	Cd-0-R	**Cd-5-R**	**Cd-10-R**	**Cd-20-R**
As	1.0 ± 0.2	1.24 ± 0.06	1.18 ± 0.05	1.36 ± 0.04
Cd	0.19 ± 0.01	12.78 ± 0.09	50.8 ± 0.2	72.2 ± 0.2
Cr	5.00 ± 0.03	6.37 ± 0.06	5.18 ± 0.02	6.27 ± 0.03
Cu	10.75 ± 0.08	9.7 ± 0.1	15.20 ± 0.08	15.40 ± 0.03
Fe	2150 ± 10	2721 ± 4	2170 ± 30	2797 ± 5
Mn	38.6 ± 0.2	38.8 ± 0.2	30.0 ± 0.3	43.0 ± 0.4
Pb	2.50 ± 0.06	2.87 ± 0.04	2.499 ± 0.008	3.02 ± 0.03
Zn	28.26 ± 0.09	39.4 ± 0.2	40.9 ± 0.3	42.8 ± 0.3

Contamination of the soil with Cd resulted in a noticeable increase in the concentration of Zn and Cu in the roots (51% and 43%, respectively), a slightly lower increase in the case of As, Cr and Fe, and the least in the case of Mn and Pb. Increase of Pb and Cu content in roots of lemon balm under increasing concentration of Cd in soil was also observed previously [11]. Adamczyk-Szabela et al. [21] observed that the uptake of Zn in the root of lemon balm is inversely proportional to the concentration of Cd in the soil. The increase in the concentration of Cd in the soil also resulted in an increase in the concentration of Cd in the above-ground part of the plants. A similar

effect was observed by Arsenov et al. [22] in parsley and celery. The application of solutions of Cu to the soil also resulted in an increase in Cu concentration in the roots and above-ground parts of wheat [23].

Table 3. Contents of elements ± SD (mg/kg of sample, dry mass) in shoot samples

Element	Sample			
	Cd-0-Sh	**Cd-5-Sh**	**Cd-10-Sh**	**Cd-20-Sh**
As	0.50 ± 0.05	0.44 ± 0.05	0.21 ± 0.02	0.25 ± 0.03
Cd	0.09 ± 0.01	1.483 ± 0.001	1.736 ± 0.008	2.178 ± 0.007
Cr	4.178 ± 0.008	9.088 ± 0.007	18.1 ± 0.2	1.27 ± 0.05
Cu	10.54 ± 0.01	11.71 ± 0.03	12.9 ± 0.2	10.9 ± 0.2
Fe	882 ± 3	920 ± 10	345 ± 2	253 ± 3
Mn	30.1 ± 0.2	29.5 ± 0.2	33.7 ± 0.3	12.3 ± 0.2
Pb	0.83 ± 0.03	0.67 ± 0.03	1.24 ± 0.04	0.20 ± 0.04
Zn	39.0 ± 0.3	40.1 ± 0.3	55.2 ± 0.4	37.5 ± 0.3

An increase in the concentration of Cd in the soil was reflected differently in the concentrations of other elements in shoots. It is evident that at a Cd concentration of 20 mg/kg, the content of most other elements significantly decreases (As, Cr, Fe, Mn, Pb). Also, it is interesting to note that lower concentrations of added Cd (5 or 10 mg/kg) even enhance the uptake of some elements in shoots (Cr, Cu, Fe, Mn, Zn) probably as a defence mechanism of the plant. The increase in the concentration of Cd in the soil (20 mg/kg) also resulted in a decrease in Zn concentration in the above-ground part of lemon balm, as noted by Adamczyk-Szabela et al. [21]. In case of marigold [12] increase in Cd concentration in the soil led to a reduction of Zn content in marigold leaves and an increase in Zn content in the petals. The content of Cd in the above-ground part of the lemon balm in the reference sample is below some national limits for toxic metals in herbal medicines and products, as well as the WHO recommendations (0.3 mg/kg) [24], while in samples where the soil was artificially contaminated with Cd, this concentration was exceeded. This indicates that using lemon balm grown in soil contaminated with significant amounts of Cd could pose a potential health risk to users.

In order to assess the ability of a plant species to uptake, accumulate, and translocate heavy metal(oid)s, the following factors were calculated: BCF-Biological Concentration Factor as the ratio of element

concentration in root and soil; MR-Mobility Ratio as the ratio of element concentration in the above-ground part and the soil; TF-Translocation Factor as the ratio of element concentration in the above-ground part and the root and EF-Enrichment Factor as the ratio of element concentration in the plant part (root or shoot) from contaminated and control soil. The results obtained for Cd are presented in Table 4.

Table 4. Biological coefficients (factors) for assessing the potential of lemon balm according to the accumulation and translocation of Cd

Sample	BCF	MR	TF	EF_{root}	EF_{shoot}
Cd-0	0.25	0.12	0.40	/	/
Cd-5	2.16	0.25	0.12	66	16
Cd-10	4.15	0.14	0.03	263	18
Cd-20	2.83	0.08	0.03	373	23

From the values of BCF and EFs, it can be concluded that the lemon balm can uptake and accumulate Cd and the lemon balm from polluted areas can be harmful to consumers of this medicinal plant. Of the other determined elements, only Zn shows good accumulation in the roots (BCF 1.04-1.62), good accumulation in the above-ground part (MR 1.42-2.18) as well as good translocation through plant parts (TF 1.02-1.38). A slightly more pronounced accumulation in the roots is observed in the case of Cu (BCF up to 0.65), unlike other elements with BCF values no higher than 0.2. Additionally, Cu with MR values ranging from 0.44 to 0.55 exhibits a slightly better accumulation of the tested elements in shoots, unlike other elements with MR values ranging from 0.01 for Pb to 0.48 for Cr. Cr, Cu and Mn show a slightly more pronounced translocation from the roots to the aerial part.

Hierarchical cluster analysis (Ward's method with squared Euclidean distance) on standardized data was used to group analysed elements according to their similarities of uptake by lemon balm shoots and roots (Figure 2.). Arsenic, iron, and lead form a single cluster because the fact that soil doping with Cd at a concentration of 20 mg/kg results in the highest concentrations of these elements in the root and the lowest concentrations in the aerial part. Manganese appears as a separate cluster, connected to the cluster formed by As, Fe, and Pb, but its concentration in the root is the lowest at the 10 mg/kg treatment, unlike As, Fe, and Pb. Chrome and zinc form one cluster because their content in the aerial part is much higher when the soil is doped at 10 mg/kg compared to other treatments. Cadmium and copper form a separate cluster, which indicate a possible

similarities in the mechanisms of uptake and translocation of these two elements in the lemon balm grown under such conditions. Certainly, the dendrogram shows the specifics of this plant species in the context of uptake and translocation of the examined elements from the roots to the aerial part.

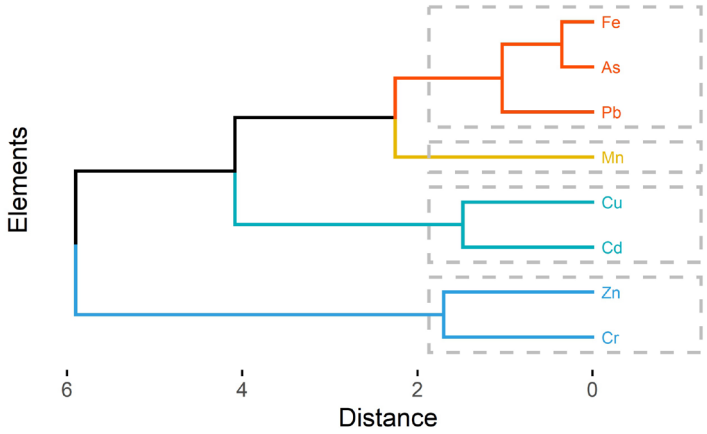

Figure 2. Hierarchical cluster analysis of analysed elements in lemon balm roots and shoots

The results of determining the total phenolic content (TPC), total flavonoid content (TFC), rosmarinic acid content, and antioxidant activities are shown in Table 5.

Table 5. The total phenolic content, the total flavonoid content, the rosmarinic acid content and antioxidant activities

Sample	TPC [mg/g gallic acid eq.]	TFC [mg/g catechin eq.]	Rosmarinic acid [mg/g]	DPPH [µmol/g trolox eq.]
Cd-0-Sh	69 ± 3^{a}	78 ± 2^{a}	26.9 ±0.1^{b}	380 ± 20^{ab}
Cd-5-Sh	70 ± 1^{a}	76 ± 2^{a}	26.2 ± 0.2^{b}	390 ± 20^{ab}
Cd-10-Sh	74 ± 3^{a}	77 ± 2^{a}	26.0 ±0.1^{b}	440 ± 30^{a}
Cd-20-Sh	65 ± 3^{a}	70.1 ± 0.4^{b}	31.4 ± 0.3^{a}	330 ± 20^{b}

*The contents of elements marked with the same letter for two or more samples within one column do not statistically differ significantly (α=0.05).

The one-way ANOVA results show: there is no statistically significant difference between all samples with regard to TPC; TFC noticeably decreases at Cd concentration of 20 mg/kg; antioxidant activity increases with the increase in Cd concentration up to a concentration of 20 mg/kg, when it noticeably decreases; the content of rosmarinic acid apparently does not depend on the concentration of Cd until a concentration of 20 mg/kg when it increase significantly. Adamczyk-Szabela et al. [21] noticed that the addition of Cd to the soil (1 mg/kg and 6 mg/kg) led to an increase in total phenols content in lemon balm. Similar effects were observed in beans [25], in chamomile grown in Hoagland's solution with the addition of Ni, where TPC first decreased at the lowest Ni concentration and then increased [26] and in the species *Drimia elata* where treatment with Cd at 2 mg/kg led to an increase in TPC, but at 5 and 10 mg/kg treatments, a decrease was observed [27]. In coriander, increasing the concentration of Cd in the soil in the same amounts as in this study led to an increase in total phenols, with the highest total phenol content observed in the 20 mg/kg soil treatment, while soil contamination with Pb at 100 mg/kg first led to a decrease in TPC, and at treatments of 200 and 400 mg/kg, an increase in total phenol content was observed [18]. Analysis of corn leaves for total phenols content [28] shows that soil contamination with Cd, Cu, and Pb leads to an increase in total phenols compared to the reference unit. In case of basil (*Ocimum basilicum* L.). increased Cd content in the soil resulted in higher TPC and TFC up to amount of 100 mg/kg Cd in soil and then further increase of Cd content in soil results in TPC and TFC decrease [14]. In the species *Erica andevalensis*, increasing the concentration of Cd in the soil initially did not have a significant effect on total phenolic compounds. However, at the 5 mg/kg soil treatment, a maximum was reached. Then at the 50 mg/kg soil treatment, total phenols decreased again, which may be explained as that Cd in higher concentrations in soil inhibits some methabolics paths which have impact of synthesis of phenolics compounds The total flavonoid content increased with the increase in Cd concentration in the soil but at a concentration of 50 mg/kg, there was a slight decrease in flavonoid content [15].

A similar increase in flavonoid content was observed in beans [25]. In chamomile, where the effect of Ni on flavonoid content was studied, the initial increase in Ni concentration led to a decrease in flavonoid content, followed by an increase with further Ni concentration increases [26]. In *Drimia elata*, soil treatment with 2 mg/kg of Cd)led to a slight increase in total flavonoid content [27]. However, higher concentrations of 5 mg/kg and 10 mg/kg caused a significant reduction in flavonoids. Similar trends were observed in *Gynura procumbens* [29] and *Gynura pseudochina* [30].

Since lemon balm is a good source of antioxidants responsible for its medicinal properties, it is crucial to assess the impact of soil contamination with Cd on its antioxidant activity. With the increase in Cd concentration in the soil, the antioxidant activity increased up to 10 mg/kg Cd in the soil. Although the antioxidant activity increased, which could potentially enhance the medicinal properties of lemon balm, the problem is that at such Cd concentrations in the soil, the lemon balm absorbs Cd in its aerial parts in amounts exceeding the allowable limits for medicinal plants, which may pose a risk of Cd toxicity to people using lemon balm grown in Cd-contaminated soil. When the soil was treated with 20 mg/kg Cd, there was a decrease in the antioxidant activity of lemon balm. A similar effect of soil contamination was observed in basil [31].

Rosmarinic acid is one of the most important phenolic compounds in lemon balm, known for its various medicinal effects, including the treatment of herpes simplex virus [32]. The initial increase in Cd concentration in the soil led to a decrease in the content of rosmarinic acid. However, when the soil was treated with 20 mg/kg Cd, the concentration of rosmarinic acid increased. However, since at this Cd concentration in the soil the amount of Cd in the aerial part of lemon balm exceeds the permissible levels, the use of lemon balm grown in contaminated soil, even though the rosmarinic acid content increased, is not recommended due to the high Cd content.

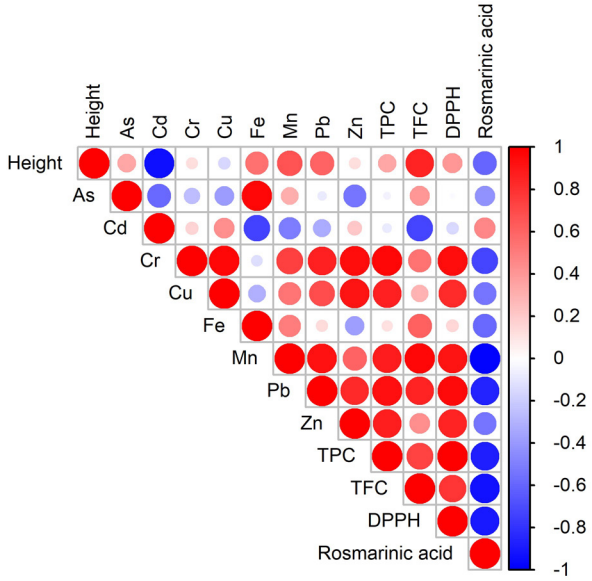

Figure 3. Correlation matrix (Pearson correlation)

A similar effect of Cd on the rosmarinic acid content was observed in *Abelmoschus esculentus* L. grown under hydroponic conditions, where the addition of 50 mg/l Cd led to a decrease in rosmarinic acid content, while at 100 mg/l Cd, there was an increase in rosmarinic acid content [33].

In order to see relationship between analysed items in lemon balm shoots correlation matrix with Pearson correlation coefficients are performed and results are presented on Figure 3.

As shown in Figure 3. Cd content is negatively correlated with most of the analysed parameters. Higher concentrations of Cd in shoots of lemon balm are strongly correlated negatively with lemon balm height and most of the other determined elements. It's interesting that there is some small positive correlation between Cd concentration in shoots and rosmarinic acid concentration. Also, there is a high positive correlation between DPPH, TPC and TFC, which is expected because phenolic compounds are the main bioactive compounds with antioxidant activity.

CONCLUSIONS

Soil pollution with Cd negatively affects lemon balm growth by reducing plant height. Lemon balm grown on Cd-contaminated soil may pose a risk to human health, as the concentration of Cd in aerial parts exceeds the WHO-recommended limits for medicinal plants. Cadmium affects both total phenolic content (TPC) and total flavonoid content (TFC), with low concentrations promoting an increase, while higher levels cause a decline. At a concentration of 20 mg/kg Cd, antioxidant activity decreases despite an increase in rosmarinic acid content. Based on BCF and enrichment factors, lemon balm shows the ability to uptake and accumulate Cd, especially in shoots, which reinforces concerns about its safety when grown in polluted soils. Among other elements, only Zn showed efficient accumulation and translocation (BCF, MR, and TF > 1), while Cu and Cr demonstrated moderate accumulation and mobility. Future research should focus on monitoring of Cd transfer into herbal preparations and exploring methods to reduce Cd uptake for safe medicinal use, as well as methods to increase uptake for potential phytoremediation applications.

EXPERIMENTAL SECTION

Analysis of soil pH and electric conductivity of soil

Approximately 10 g of dry soil was weighed and transferred into a 100 ml Erlenmeyer flask. Then, 10 ml of deionised water was added, and the mixture was shaken for one hour on a shaker. Afterwards, the liquid was carefully decanted,

and the pH value was measured using a pH meter (Hanna Instruments, USA) and the electrical conductivity was measured using a conductometer (Hanna Instruments, USA) [34].

Analysis of heavy metal(oid)s content in soil and plant samples

The content of heavy metal(oid)s was determined using optical emission spectrometry with inductively coupled plasma (ICP-OES, iCAP 6300 Duo, Thermo Scientific, Cambridge, UK). The preparation of samples was performed using microwave digestion (ETHOS EASY, Milestone, Bergamo, Italy). The EPA3051a method, provided in the instrument's application note, was used for soil sample preparation. Approximately 0.5 g of soil was weighed and transferred into teflon vessels. To each vessel, 9 ml of concentrated nitric acid and 3 ml of concentrated hydrochloric acid were added. The digestion program was as follows: within 20 minutes, a temperature of 180 °C was reached and then maintained for 10 minutes. After 20 minutes of cooling, the contents were quantitatively transferred to a 50 ml volumetric flask and diluted to the mark with deionised water [35]. Approximately 0.35 g of plant material (root and above-ground part) was weighed, placed and transferred into vessels. Then, 3 ml of concentrated hydrogen peroxide and 5 ml of concentrated nitric acid were added. The digestion program was as follows: within 20 minutes, a temperature of 190 °C was reached and then maintained for 10 minutes. After digestion, the contents of the vessels were transferred to a 25 ml volumetric flask and diluted to the mark with deionised water [36]. Concentrations of eight heavy metal(oid)s (As, Cd, Cr, Pb, Mn, Cu, Zn, Fe) are determined by the ICP-OES method of external calibration curve. Working wavelengths were selected considering the relative intensity of emission, presence of spectral interferences, corrected peak (signal-to-background ratios) under the following instruments parameters: 1150 W (generator RF power), 50 rpm (peristaltic pump speed), 100 rpm (flushing pump speed), 12 L/min (cooling gas flow), 0.5 L/min (auxiliary gas flow), 0.5 L/min (nebulization gas flow), 5 s (pump stabilization time), 30 s (sample uptake delay), axial direction plasma observation and three trials for each measurement. Selected wavelengths, correlation coefficient (r), limits of detection (LOD=3σ/m) and limits of quantitation (LOQ=10σ/m), where σ is standard deviation of blank and m is slope of the calibration line, are presented in Table 6. The satisfactory relative standard deviation of the reproducibility of the sample preparation by microwave digestion (a sample of the aerial part of the plant untreated with Cd prepared six times) ranges from 2.21% for Mn to 5.90% for Cd, except for As with a value of 14.72%.

Table 6. Wavelengths, correlation coefficients, limits of detection, and limits of quantification for determined elements

Element	Wavelength [nm]	r	LOD [mg/kg]	LOQ [mg/kg]
As	189.042	0.999990	0.1307	0.3960
Cd	214.438	0.999958	0.0055	0.0183
Cr	267.716	0.999924	0.0605	0.2016
Cu	324.754	0.999997	0.0592	0.1974
Fe	259.940	0.999859	0.0972	0.3241
Mn	257.610	0.999992	0.0054	0.0182
Pb	220.353	0.999983	0.2220	0.7400
Zn	202.548	0.999823	0.0087	0.0291

Analysis of total phenolics, total flavonoids, antioxidant activity and rosmarinic acid content

Approximately 0.5 g of dried plant material was weighed and placed in an Erlenmeyer flask. To each flask, 50 ml of 70% methanol was added, and then the flasks were placed on a shaker for extraction, which was carried out for 2 hours at room temperature. After that, the liquid extract was separated from the solid residue by centrifugation in plastic tubes at 3000 rpm for ten minutes. The extract was then filtered through 0.45-micron microfilters and stored in a refrigerator until analysis.

The total polyphenol content (TPC) in the above-ground part of lemon balm was analysed using the Folin-Ciocalteu method. A 0.1 ml portion of the extract, prepared as described above, was transferred to a 10 ml volumetric flask. Next, 0.5 ml of Folin-Ciocalteu reagent was added, and after 5 minutes, 2 ml of saturated Na_2CO_3 solution was added. The flask was filled with deionised water to reach a total volume of 10 ml and left in the dark for 30 minutes. The absorbance was then recorded at 760 nm using a UV-VIS PerkinElmer Lambda 15 spectrometer, with deionised water serving as the blank. For calibration, standards of gallic acid solutions were prepared by adding 0.5 ml of Folin-Ciocalteu reagent and 2 ml of saturated Na_2CO_3 solution to a properly weighted amount of gallic acid, adjusting the volume with deionised water to achieve final concentrations ranging from 1 to 9 µg/ml. After 30 minutes in the dark, the absorbance of the standards was measured, and a calibration curve was generated: $A = 0.04385 + 0.10517C_{gallic\ acid}$ [37,38]. The total polyphenol content of the extracts was expressed as milligrams of gallic acid equivalents per gram of dry lemon balm sample (mg GAE/g).

For the determination of total flavonoids content (TFC), a 0.1 ml portion of the lemon balm extract was placed into a 10 ml volumetric flask, followed by the addition of 0.3 ml of 5% $NaNO_2$. After 5 minutes, 1.5 ml of an $AlCl_3$ solution was added, and 5 minutes later, 2 ml of 1M NaOH was added. The flask was brought to a final volume of 10 ml with deionised water. Absorbance was measured at 510 nm, using deionised water as a blank. A calibration curve was established using catechin working solutions (1–10 mg/l) prepared from a 0.5 mg/ml stock solution. The curve demonstrated linearity, following the equation $A = 0.03612 + 0.00491C_{(catechin)}$. Using this equation, the total flavonoid content was determined and expressed as milligrams of catechin equivalents per gram of dry sample (mg CE/g) [39].

To prepare the sample, 0.5 mL of the extract was diluted to 50 ml using 70% (v/v) methanol. Antioxidant activity was assessed using the DPPH method, as outlined by Brand-Williams et al. [40], with slight modifications. A DPPH solution at a concentration of 1×10^{-4} mol/l in methanol was prepared. From this diluted DPPH solution, 5.0 ml was transferred to a 10 ml volumetric flask, and 0.5 ml of the diluted extract was added. The flask was then filled up with methanol to a final volume of 10 ml. After 30 minutes, the colour change of the DPPH radical was measured spectrophotometrically at 520 nm. A calibration curve was established using Trolox solutions, based on the reduction in absorbance, which represented the DPPH radical scavenging activity. The antioxidant activity of the samples was expressed as micromols of trolox equivalents per gram of dry lemon balm (μmolTE/g).

To determine the content of rosmarinic acid, the method by Mašković et al. [41] with some modifications was used (Agilent 1200 system, Agilent Technologies,Santa Clara, CA, USA). The analytical column employed was a Zorbax Eclipse XDBC18 column (5 μm, 4.6 × 150 mm, Agilent Technologies, Santa Clara, CA, USA). The mobile phase was delivered at a constant flow rate of 0.8 ml/min over a total run time of 40 minutes. Solvent A consisted of 5% formic acid in deionised water, while Solvent B was composed of 5% formic acid in 80% acetonitrile. The gradient program was as follows: 0% B for the first 10 minutes, increasing to 25% B from 10 to 20 minutes, 25–40% B between 20 and 30 minutes, 40–70% B from 30 to 35 minutes, and 70–80% B during the final 5 minutes of the analysis.

Statistical analysis

Statistical analysis is performed using R. The normality of obtained values is tested with the Kolomogorov-Smirnov test. For correlation between elements in shoots, the corrplot package was used, for hierarchical clustering the factoextra and dentextend packages were used and for one-way ANOVA

and Tukey posthoc test, the dplyr and multicompView packages were used. For making graphs OriginLab and R were used.

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