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Health risk assessment of heavy metals in basil (*Ocimum basilicum* L.) grown in artificially contaminated substrates

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Abstract: This study aimed to determine the levels of Cd, Cr, and Pb in basil (*Ocimum basilicum* L.) cultivated on artificially contaminated substrates and to assess their potential harmful effects on human health via the calculation of the target hazard quotients (THQ). A pot experiment was performed in a completely randomized design for each tested heavy metal. It included four contamination treatments (0, 20, 50, and 100 mg kg^{-1} for Cd, and 0, 100, 250, and 500 mg kg^{-1} for Pb and Cr). Concentrations of Cd, Cr, and Pb in plant samples were determined by atomic absorption spectroscopy. The results of this study showed that the concentrations of Cd, Cr, and Pb were several times higher in the roots than in the aboveground parts of basil regardless of contamination levels. These are desirable results because only aboveground parts of basil are used for medicinal purposes or consumption. The THQ values for Cd, Cr, and Pb observed in this study were lower than 1 regardless of contamination levels, indicating that the consumption of basil from the study site (up to 10 g per day) does not pose a risk to human health from the point of view of heavy metal investigated.

Key words: cadmium, chromium, lead, pollution

Ocena zdravstvenega tveganja s težkimi kovinami pri navadni baziliki (*Ocimum basilicum* L.) rastoči v umetno kontaminiranih substratih

Izvleček: Namen raziskave je bil določiti vsebnosti Cd, Cr, in Pb v navadni baziliki (*Ocimum basilicum* L.) rastoči na namensko onesnaženih tleh in in oceniti njihove potencialno škodljive učinke na zdravje ljudi preko izračuna potencialnega koeficienta tveganja (THQ). Za testiranje posameznih kovin je bil izveden lončni poskus s popolno naključno zasnovo. Poskus je obsegal štiri obravavanja kontaminacije (0, 20, 50, in 100 mg kg^{-1} za Cd, in 0, 100, 250, in 500 mg kg^{-1} za Pb in Cr). Koncentracije Cd, Cr in Pb v rastlinah so bile določene z atomsko obsorpcijsko spektroskopijo. Rezultati raziskave so pokazali, da so bile koncentracije Cd, Cr, in Pb večje v koreninah kot v nadzemnih delih bazilike, ne glede na stopnjo kontaminacije. Ti izsledki so zaželjeni, kajti za medicinske in prehrambene namene se uporabljajo le nadzemni deli bazilike. Vrednosti indeksa toksičnosti (THQ) za Cd, Cr, in Pb, pridobljeni v tej raziskavi, so bile manjše od 1, ne glede na stopnjo kontaminacijer, kar nakazuje, da je uživanje bazilike v dnevnem odmerku do 10 g na dan ne predstavlja rizika za zdravje ljudi glede na preučevane težke kovine.

Ključne besede: kadmij, krom, svinec, onesnaženje

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1 INTRODUCTION

Medicinal plants represent a special class of plants that have been used for therapeutic and medical purposes since ancient times. The use of medicinal plants is more easily available and accessible to the population compared to conventional medicine, and therefore, the demand for herbal remedies is growing worldwide and is expected to increase continuously (Ekor, 2014). However, due to the continuous increase of environmental pollution caused mainly by the urbanization, extensive use of chemical fertilizers and pesticides in plant production and rapid development of highly polluting industries, consumer concerns regarding the safety of their use have been highlighted (Łuszczki et al., 2019).

Inorganic pollutants, such as heavy metals, are of great concern to public health because of their persistence and high toxicity (Jadaa & Mohammed, 2023). Cd, Cr and Pb are among the most dangerous heavy metals because they can cause adverse effects on human health even in small quantities (Isinkaralar et al., 2024). As a result, the abovementioned heavy metals represent a category of pollutants of particular interest for health risk studies. There are numerous scientific studies which have provided evidence that an excessive amount of Cd in the human body can damage the kidneys, liver, bones and heart, and in severe cases can even cause death (Fatima et al., 2019; Charkiewicz et al., 2023). Adverse health effects associated with Cr exposure include respiratory, gastrointestinal and cardiovascular damage (Shin et al., 2023), while exposure to Pb has been found to be associated with a high risk of brain and kidney damage and cardiovascular diseases (Bhasin et al., 2023). Considering these aspects, monitoring toxic heavy metals, particularly Cr, Cd and Pb, in plants intended for consumption or medicinal use has become imperative.

Basil (*Ocimum basilicum* L., Lamiaceae) is a well-known culinary and medicinal plant originating from South Asia, from where it is spread all over the world, especially around the Mediterranean Sea (Azizah et al., 2023). As a traditional herbal remedy, basil leaves have been used since ancient times to treat a wide range of respiratory and gastrointestinal ailments. In addition, basil is easy to grow and has a high harvest index, making it very popular among farmers and crop producers. On the one hand, this has resulted in increased production and consumption of basil on the global scene (Polyakova et al., 2015), but on the other hand, this has led to increased consumer concerns regarding the quality and safety of its use (Nadeem et al., 2022).

Given the fact that the information on the safety of basil is relatively limited, especially from the heavy metal contamination point of view, this study aimed to determine the levels of Cd, Cr, and Pb in different parts of basil plants grown on artificially contaminated substrates and to assess their potential harmful effects on human health *via* the calculation of the target hazard quotients (THQ).

Our hypotheses were: (1) levels of Cd, Cr and Pb in basil plants tend to increase with increasing their content in growing substrates, and (2) basil plants grown on artificially contaminated substrates would pose a risk to human health from the point of view of Cd, Cr and Pb contamination.

2 MATERIALS AND METHODS

2.1 EXPERIMENTAL SITE AND GREENHOUSE EXPERIMENTAL DESIGN

This study was carried out from mid-May to end of June 2024 in a naturally ventilated greenhouse at the experimental station of the Faculty of Agriculture and Food Sciences (Sarajevo, Bosnia and Herzegovina). On May 18, 2024, one-month-old basil plants (*Ocimum basilicum* 'Genovese Gigante', purchased from a local nursery), which exhibited minimal variation in size and appearance, were transplanted into plastic pots (12 cm diameter × 20 cm height, one plant per plot) previously filled with a commercial growing substrate artificially contaminated with heavy metals (Cd, Cr and Pb). The selected substrate composition was a mixture of black and white peat, coconut fibre, composted plant material and other organic

Table 1: Chemical properties of the growing substrate before adding the contaminants

Parameter	Measure unit	Value
pH H_2O	pH range	6.3
pH KCl	pH range	6.0
organic matter	%	56.5
available forms of potassium (K_2O)	mg 100 g^{-1}	37.6
available forms of phosphorus (P_2O_5)	mg 100 g^{-1}	43.2
Cd content	mg kg^{-1}	0.1
Cr content	mg kg^{-1}	4.7
Pb content	mg kg^{-1}	1.1
Cu content	mg kg^{-1}	13.4
Zn content	mg kg^{-1}	28.9
Mn content	mg kg^{-1}	125.9
Fe content	mg kg^{-1}	631.7
Ni content	mg kg^{-1}	2.8

material (wood fibre and compost) with 1 g fertilizer N-P-K 15-15-15 per litre of the substrate. Information on both substrate composition and physical-chemical properties was provided by the manufacturer. The chemical properties of the growing substrate are listed in Table 1.

From the point of view of chemical properties, the growing medium used in this study is suitable for basil growth and development. All of the tested heavy metals in the growing medium were found to be below the permissible limits recommended by the Food and Agriculture Organization of the United Nations (FAO). According to FAO, the permissible limits for Cd, Cu, Zn, Mn, Ni and Cr in agricultural soils are 3, 100, 300, 2000, 50 and 100 mg kg^{-1} dry mass, respectively (FAO, 1985).

Before starting the experiment, the substrate was artificially contaminated by adding a solution containing heavy metals (Cd, Cr and Pb) and then mixed thoroughly to ensure a uniform distribution of the heavy metal solution. Cd, Cr and Pb were applied as $CdCl_2$, $K_2Cr_2O_7$ and $Pb(NO_3)_2$, respectively.

This study included four contamination treatments within each heavy metal tested i.e. 0, 20, 50, and 100 mg kg^{-1} for Cd, and 0, 100, 250, and 500 mg kg^{-1} for Pb and Cr, with three replications per treatment. Each contamination treatment consisted of five pots/plants, resulting in 180 pots in this study. Air temperature and relative humidity inside the greenhouse varied from 19 ± 3 °C to 29 ± 3 °C and from 60 % to 90 %, respectively. Air circulation was achieved by opening the roof vents and main door during the day. A green shade cloth was used to reduce heat build-up during hot days. Each plant/pot was regularly watered every other day. There was no fertilizers application during the investigation.

After two months of plant growth under these conditions, the experiment was terminated. The plants, i.e., leaves and roots of control and heavy metal-stressed basil plants, were harvested and then separated into roots and aboveground parts of plants. The fresh mass was recorded right after harvesting, whereas the dry mass was determined after being oven-dried at 60 °C until a stable weight was reached. Plant height was measured with a ruler, and the leaf area was computed based on the method outlined by Pandey and Singh (2011). The plant material for heavy metal analysis was dried in an oven at 80 °C to a constant mass, ground into powder using an electric blender, and kept dry in paper bags in a desiccator until analysis.

2.2 HEAVY METAL ANALYSIS

Wet digestion method was used to extract the heavy metals of the plant samples (Lisjak et al., 2009). In short, 1g of dry plant sample was placed in an Erlenmeyer flask and then 10 ml of HNO_3 and 4 ml of H_2SO_4 were added. The mixture was left overnight at room temperature and then heated on a hot plate for 2 h at 60 °C and then at 100 °C until the formation of brown fumes stopped (approximately 1 h). After cooling to room temperature, the mixture was filtered to the mark through quantitative filter paper (Whatman, No. 42) in a volumetric flask (25 ml) and diluted with deionized water.

Heavy metal concentrations (Cd, Cr, Pb) in plant samples were determined by atomic absorption spectrophotometry using the Shimadzu AA-7000 device (Shimadzu Instruments, Tokio, Japan). The standard and working solutions of investigated heavy metals were prepared on a daily basis by diluting the certified stock solutions (Merck, Darmstadt, Germany) with deionized water as necessary.

2.3 HEALTH RISK ANALYSIS

The potential human health risk of Cd, Cr and Pb through consumption of basil plants was assessed using target hazard quotient (THQ), which was described in detail by the USEPA (United States Environmental Protection Agency). In short, the THQ value describes the non-carcinogenic health risk posed by exposure to the respective toxic element. The following equation was used for THQ calculation (USEPA, 20111).

$$THO = \frac{c\ x\ IR\ x\ EF\ x\ ED}{ET\ x\ BW\ x\ RfD}$$

where is:

c - concentration of contaminant in analysed plant sample (mg kg^{-1})

IR – food (basil) dietary intake (0.01 kg/person/day)

EF – exposure frequency (365 days per year)

ED – exposure duration for adult (70 years)

ET – averaged exposure time (25550 days (EF x ED))

BW – body mass (for adults 70 kg, for children 32 kg)

RfD – the oral reference dose i.e. the highest level of contaminant at which no adverse health effects are expected (according to USEPA (2011) the RfD values for Cd, Cr and Pb are 0.001, 0.004 and 0.003 mg/kg/day, respectively.

Table 2: Growth characteristics of basil plants cultivated on contaminated substrates

Treatments	Plant height (cm)	Leaf area (cm^2)	Fresh aboveground mass (g)	Dry aboveground mass (g)
Control	31.3 ± 6.2	8.5 ± 5.9	26.7 ± 2.7	3.7 ± 0.6
100 mg kg^{-1} of Cr	30.5 ± 6.3	8.6 ± 5.2	26.3 ± 4.1	3.5 ± 0.9
250 mg kg^{-1} of Cr	29.7 ± 6.2	8.4 ± 5.1	26.1 ± 3.7	3.2 ± 0.7
500 mg kg^{-1} of Cr	28.2 ± 7.6	7.7 ± 6.9	25.5 ± 2.1	2.9 ± 0.9
100 mg kg^{-1} of Pb	31.1 ± 5.9	8.2 ± 6.2	25.7 ± 6.3	3.1 ± 1.3
250 mg kg^{-1} of Pb	28.2 ± 7.9	7.9 ± 5.3	25.8 ± 3.9	3.1 ± 0.9
500 mg kg^{-1} of Pb	26.5 ± 7.2	7.3 ± 4.2	25.5 ± 2.5	3.0 ± 1.2
20 mg kg^{-1} of Cd	29.9 ± 5.9	8.5 ± 5.3	25.9 ± 7.2	3.4 ± 1.1
50 mg kg^{-1} of Cd	28.1 ± 6.3	8.2 ± 5.6	25.5 ± 6.5	3.3 ± 0.9
100 mg kg^{-1} of Cd	28.6 ± 5.2	7.7 ± 7.2	25.8 ± 4.2	3.4 ± 1.2
$LSD_{0.05}$	-	-	-	-

Any THQ value lower than the threshold value of 1 indicates that there is no risk of non-carcinogenic diseases through the consumption of the tested food.

2.4 STATISTICAL ANALYSIS

All data were analysed using Microsoft Excel software. The least significant difference (LSD) test at a 5 % probability level was performed to establish significant differences between treatment's means.

3 RESULTS

The basic growth characteristics of basil plants grown in substrates contaminated with Cr, Pb, and Cd are given in Table 2.

The basil plants grown in the substrates artificially contaminated with Cd, Cr and Pb, presented similar phenotypes comparing with the plants from the non-contaminated growing medium. No considerable difference in plant height, leaf area and fresh and dry aboveground mass among basil plants was observed during the exposure period, regardless of the level of substrate contamination. Furthermore, no signs of heavy metal toxicity were observed in basil plants.

Heavy metal concentrations (Cd, Cr, Pb) in roots and leaves of basil plants grown on contaminated substrates are shown in Table 3, 4 and 5. All results are expressed on a dry mass basis in mg kg^{-1}.

As expected, the concentrations of Cd, Cr and Pb were significantly higher in basil plants cultivated on contaminated substrates compared to control plants. The results also showed that the uptake of Cd, Cr and Pb by basil plants corresponded to the increasing level of soil contamination.

In this study, the concentrations of Cd, Cr, and Pb were several times higher in the roots than in the aboveground parts of basil plants, regardless of the level of

Table 3: Heavy metal levels in basil plants grown on Cd-contaminated substrates

Treatments	Roots	Leaves
0 mg kg^{-1} of Cd	$0.1 \pm 0.1^{d*}$	0.3 ± 0.1^{c}
20 mg kg^{-1} of Cd	58.7 ± 10.1^{c}	17.9 ± 5.7^{b}
50 mg kg^{-1} of Cd	145.3 ± 9.7^{b}	18.8 ± 7.9^{b}
100 mg kg^{-1} of Cd	223.7 ± 23.1^{a}	30.1 ± 5.8^{a}
$LSD_{0.05}$	13.1	6.5

*Averages denoted by the same letter in the same column indicate no significant difference ($p < 0.05$)

Table 4: Heavy metal levels in basil plants grown on Cr-contaminated substrates

Treatments	Roots	Leaves
0 mg kg^{-1} of Cr	$2.9 \pm 1.4^{d*}$	1.9 ± 1.8^{c}
100 mg kg^{-1} of Cr	73.7 ± 10.1^{c}	12.1 ± 1.9^{b}
250 mg kg^{-1} of Cr	394.2 ± 22.5^{b}	12.3 ± 7.7^{b}
500 mg kg^{-1} of Cr	625.9 ± 20.1^{a}	25.3 ± 17.2^{a}
$LSD_{0.05}$	15.9	9.4

*Averages denoted by the same letter in the same column indicate no significant difference ($p < 0.05$)

substrate contamination. These results strongly indicate that basil plants have the ability to significantly reduce the transfer of Cd, Cr and Pb from roots to aboveground parts. From the public health point of view, these results are extremely desirable because only aboveground parts of basil are used for medicinal purposes or consumption.

The target health quotient (THQ) was estimated to assess the human health risks posed by the intake of those potentially toxic elements (Cr, Cd, and Pb) from the consumption of the basil plants. The THQ values calculated for each tested heavy metal found in basil leaves are shown in Table 6.

Table 5: Heavy metal levels in basil plants grown on Pb-contaminated substrates

Treatments	Roots	Leaves
0 mg kg^{-1} of Pb	1.4 ± 0.3^{d*}	0.4 ± 0.2^{c}
100 mg kg^{-1} of Pb	90.9 ± 7.7^{c}	12.3 ± 2.1^{b}
250 mg kg^{-1} of Pb	369.4 ± 38.3^{b}	53.2 ± 46.1^{a}
500 mg kg^{-1} of Pb	1041.7 ± 111.1^{a}	75.3 ± 43.8^{a}
$LSD_{0.05}$	59.8	31.4

*Averages denoted by the same letter in the same column indicate no significant difference ($p < 0.05$)

Table 6: THQ values for Cd, Cr and Pb due to consumption of basil leaves

Treatment	Adult	Child	Treatment	Adult	Child	Treatment	Adult	Child
0 mg kg^{-1} of Cd	0.004	0.008	0 mg kg^{-1} of Cr	0.01	0.02	0 mg kg^{-1} of Pb	0.002	0.004
20 mg kg^{-1} of Cd	0.26	0.57	100 mg kg^{-1} of Cr	0.06	0.13	100 mg kg^{-1} of Pb	0.05	0.06
50 mg kg^{-1} of Cd	0.27	0.59	250 mg kg^{-1} of Cr	0.06	0.13	250 mg kg^{-1} of Pb	0.22	0.48
100 mg kg^{-1} of Cd	0.42	0.92	500 mg kg^{-1} of Cr	0.12	0.26	500 mg kg^{-1} of Pb	0.31	0.52

The THQ values of Cd, Cr and Pb for adults and children in all tested basil leaves were much less than 1, indicating no significant health risks associated with basil leaves consumption.

4 DISCUSSION

The basil plants that were grown in the substrates artificially contaminated with Cd, Cr and Pb showed similar phenotypic traits to those grown in uncontaminated media. Additionally, no indications of heavy metal toxicity were detected in the basil plants. On the basis of these findings, basil can be considered as plant species that have a high ability to grow successfully in a Cd, Cr and Pb rich growing medium. Similar finding was also reported by Dinu et al. (2020) and Hlihor et al. (2022).

Adaptation of basil or any other plant species to heavy metal stress conditions is a complex process and depends not only on the specific metals present, their speciation and amounts in the growing medium, but also on the plant genotype and substrate/soil physical and chemical properties (Rashid et al., 2023). In this regard, it is important to note that there is no adaptation to heavy metal stress in general, suggesting that tolerance to heavy metal stress is mainly related to the individual heavy metal. However, there is a growing body of studies that show that some plants exhibit combined tolerance to different heavy metals (Mehes-Smith et al., 2013; Viehweger, 2014). The results of the current study point to the conclusion that basil can be considered one of those plants.

In general, plant tolerance to heavy metal stress can be achieved by ‘avoidance mechanisms’ through which plants can restrict the heavy metal uptake into the body and/or by ‘tolerance mechanisms’ based on the hyperaccumulation of heavy metal ions within the plant body without negative effects on its growth and development (Yan et al., 2020).

Given the fact that the Cd, Cr, and Pb concentrations in this study were several times higher in the roots than in the above-ground parts of basil plants, regardless of the level of substrate contamination, it can be assumed that basil plants possess very effective mechanisms to absorb Cd, Cr and Pb from growing medium and then accumulate them in roots through various mechanisms including metal binding to cell walls and sequestration and compartmentalization of heavy metal ions in root cells, mainly in vacuoles. This finding was in line with findings in earlier studies where it was found that basil accumulates more Cd, Cr and Pb in its roots than in other plant parts (Adamczyk-Szabela et al., 2017; Lycas et al., 2022; Ur Rahman et al., 2024). However, these results are opposite to those obtained in study of Jena and Gupta (2012). In their study, the leaves of basil plants were found to contain higher concentrations of heavy metals, including Cr, than roots. According to the authors, the reason for this is the atmospheric deposition of pollutants by both dry and wet deposition.

In this study, the levels of Cd, Cr and Pb in basil plants grown on contaminated substrates increased with increasing the level of substrate contamination and ranged from 17.9 to 30.1 mg kg^{-1}, from 12.1 to 25.3 mg kg^{-1} and from 12.3 to 75.3 mg kg^{-1}, respectively. These values were much higher than the acceptable levels established by World Health Organization (WHO). According to WHO, the maximum acceptable levels for Cd, Pb and Cr in foodstuffs are 0.2 mg kg^{-1}, 0.3 mg kg^{-1} and 2.3 mg kg^{-1} dry mass, respectively (WHO/FAO, 2007). From this point of view, consumption of basil leaves from the studied site cannot be considered safe for human health.

However, in the case of THQ, the results showed the opposite; the mean THQ values of Cd, Cr, and Pb for adults and children were lower than 1, suggesting no significant human health risks through consumption basil from studied site. The disparity in the results can be explained by the fact that assessing the potential health risks associated with heavy metal in foodstuffs strongly depends on the average daily dietary intake (Rai et al., 2019). Given that basil and spices are consumed in amounts several times smaller than other food ingredients, the THQ values obtained in this study seem reasonable and logical. In this regard, it is important to note that the health risks of consuming heavy metals through spices increase sharply with increasing dietary spice intake.

However, the quantification of spice intake at the individual level is a difficult task and represents a great challenge because the frequency and quantity of spice intake depend considerable on the type of spice, form in which it is used, and consumers' consumption preferences (Siruguri & Bhat, 2015). In this study, the average daily intake of spices per person was estimated to be 10.0 g/person/day, since this value is mainly used in scientific studies to calculate THQ (Tefera & Teklewold, 2021; Ezez et al., 2024; Oladeji et al., 2024). If this data is relevant, it seems there is no human health risks associated with consumption of basil plants from the study site (up to 10 g per day). However, consumption of these plants in an amount higher than 10 g per day could potentially have a negative impact on human health, from a toxicological point of view. These findings strongly suggest that the knowledge of the consumption pattern regarding spices has a remarkable relevance in human health risk assessment.

5 CONCLUSIONS

Cd, Cr, and Pb concentrations were several times higher in the roots than in the above-ground parts of basil regardless of contamination levels, suggesting that basil possesses very effective mechanisms to prevent their transport from roots to other parts of plants. These are desirable results because only aboveground parts of basil are used for medicinal purposes or consumption. The THQ values for Cd, Cr, and Pb observed in this study were lower than 1 regardless of contamination levels, indicating that the consumption of basil from the study site (up to 10 g per day) does not pose a risk to human health from the point of view of heavy metal investigated.

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