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Metals and Secondary Metabolites in Industrial Hemp Plants Grown in Contaminated Soil

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Abstract

Industrial hemp (*Cannabis sativa* L.) shows strong potential for phytoremediation due to its deep root system and high biomass; however, its ability to accumulate heavy metals raises concerns regarding its medicinal use.

The aim of the study was to determine and compare the presence of five metals (Cd, As, Pb, Zn, and Fe) in three different plant organs/tissues and in inflorescence extracts in order to assess their suitability for food or medicinal use. Zinc was included as an essential micronutrient for comparison with toxic metal accumulation.

The results demonstrate that hemp grown on contaminated soils accumulates elevated levels of cadmium and lead, primarily in the roots, while metal transfer to inflorescences and extracts remains limited. Genetic differences among breeding lines had little effect on metal uptake, indicating that cultivation for food, dietary supplement, or medicinal purposes should be restricted to uncontaminated soils in order to minimise health risks to patients and consumers.

Keywords

Hemp plant, cultivation, metals, secondary metabolites, contaminated soil

Introduction

Hemp (*Cannabis sativa* L. subsp. *sativa*) is a plant with a very wide range of applications across many sectors. In addition to its use in agriculture, and in the food, textile and cosmetics industries, its medicinal use has recently become a major focus of scientific research. Hemp contains more than 600 identified bioactive compounds, including terpenes, flavonoids, phenolic acids, sterols, and more than 120 cannabinoids, whose synergistic action contributes to its therapeutic potential.[1]

Cannabidiol (CBD) and delta-9-tetrahydrocannabinol (THC) are the two most abundant and extensively studied phytocannabinoids of the Cannabis plant. CBD is considered to have anti-inflammatory, anxiolytic, and analgesic effects, and it can be used in various forms, including extracts, capsules, and topical formulations. Unlike THC, CBD is not psychoactive and is therefore permitted for safe use in a wide range of products in many countries.[2]The THC molecule is used in medicine under strictly controlled conditions. Studies have shown its potential therapeutic use in the treatment of pain and neurological diseases such as epilepsy and multiple sclerosis, and its apparent anti-tumour activity in various types of cancer is the subject of numerous studies.[3,4] Due to its THC content, the Cannabis plant is also used as a recreational drug because of its psychoactive effects; consequently, legal regulations governing its use for these purposes vary from country to country.[5]

In addition to its medical and industrial importance, hemp has also been used in recent decades as a phytoremediation plant. Research has shown that, due to its deep root system, rapid growth, and ability to adapt to adverse soil conditions, hemp is capable of absorbing and accumulating heavy metals and organic pollutants from the soil.[6,7] Hemp is therefore useful in the remediation of contaminated agricultural land, mining, and industrial areas, and research is being conducted into its use in the revitalisation of polluted land and the improvement of soil quality.[8] Lead (Pb), cadmium (Cd), mercury (Hg) and arsenic (As) are the metals that hemp most often accumulates.[9]

Due to its ability to accumulate heavy metals, special attention should be paid to the control of hemp used for medicinal purposes. Bioactive compounds including cannabinoids are most often extracted from the flower and aboveground parts of the plant where heavy metals, especially Cd and As, may be present in significant concentrations. If these metals enter extracts during the extraction process, they may affect the purity and safety of medicinal preparations and therefore pose a serious health risk.[10,11]

As and Cd are known carcinogens.[12] Cd is nephrotoxic and, with long-term exposure, damages the kidneys and bone tissue, while As has genotoxic effects.[13] Pb affects the nervous and cardiovascular systems, while Hg, in addition

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to affecting the brain and the development of the nervous system, it affects the immune and digestive systems.[14,15]

Therefore, it is very important that hemp intended for human use is grown on clean and controlled surfaces, with strict laboratory control to ensure the safety of its use.

The aim of this study was to determine and compare the presence of five metals (Cd, As, Pb, Zn and Fe) in three different plant organs/tissues and flower extracts to assess their suitability for food or medicinal use, with Zn included as an essential micronutrient for comparison with toxic metal accumulation.

Experimental

2.1 Plant cultivation

Four distinct breeding lines of feminised hemp (*Cannabis sativa* L.) bred for high CBD content (G8K, G10K, MERK and CHK) were planted in two geographically distinct regions of Slovenia differing in soil metal contamination levels (Fig. 1). The experimental fields were planted in May and harvested in October in the growing season of 2024. The highly contaminated site (H) was located in the Celje Valley, an area known for historically elevated concentrations of Cd, with documented exceedances of national warning thresholds, as well as increased levels of Zn and Pb.[16,17] The same four breeding lines were also planted in a low-contamination site (L) near Brežice, where soils are not known to accumulate heavy metals above regulatory limits.

Plants from both sites were harvested in October 2024. Three plant tissues - roots, inflorescences, and seeds - were collected and prepared for determination of metal content. For cannabinoid identification and quantification, only dried inflorescences from each breeding line in both experimental groups were analysed.

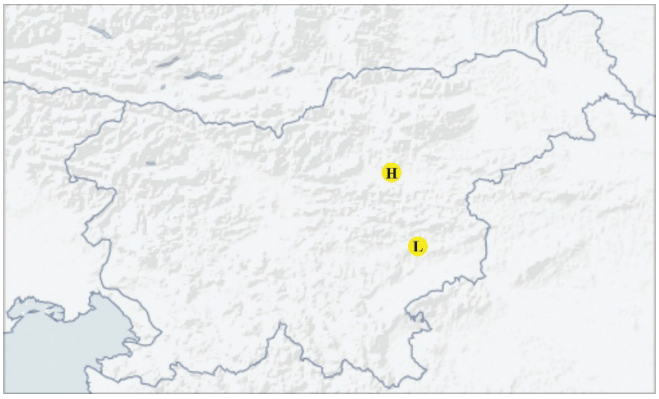

Fig. 1 – Map of two experimental plots in Slovenia (yellow dot). Highly contaminated area in Celje valley (H) and low-contamination site (L) near Brežice.

Slika 1 – Karta dviju eksperimentalnih parcela u Sloveniji (žuta točka). Visoko kontaminirano područje u Celjskoj dolini (H) i nisko kontaminirano područje (L) u blizini Brežica.

2.2 Plant sampling and analysis

For the metal analysis, the samples were prepared using aqua regia digestion prior to determination by atomic absorption spectroscopy (AAS) on an Agilent 240Z AA instrument (Agilent Technologies, Santa Clara, CA, USA) equipped with Zeeman background correction. Approximately 1 g of each homogenised plant sample was accurately weighed and transferred into Teflon digestion vessels. Ten millilitres of freshly prepared aqua regia were added to each vessel, after which the samples were incubated at 90 °C for 12 h to ensure complete digestion of the organic matrix and release of metal ions. Following digestion, the solutions were allowed to cool, then filtered to remove residual particulates. The resulting filtrates were quantitatively transferred and diluted with ultrapure water to a final volume of 50 ml, yielding solutions suitable for AAS analysis. AAS analysis was performed as described previously.

Aqua regia was prepared immediately before use by mixing concentrated nitric acid (HNO_3, 70 %; Sigma-Aldrich, Merck KGaA, Darmstad, Germany) and hydrochloric acid (HCl, 36.5–38 %; J.T. Baker, USA) at a 1 : 3 ratio. Although nitric acid–hydrogen peroxide digestion is commonly used for plant matrices, aqua regia digestion has also been applied for total metal extraction in plant tissues when multi-element determination is required, particularly in studies assessing total metal accumulation in phytoremediation plants.[18]

All analyses were performed in triplicate, and a procedural blank was included to ensure accuracy and to monitor potential contamination.

Calibration standards were prepared from certified stock metal solutions (1000 ± 4 mg l^{-1}; Sigma-Aldrich, Merck KGaA, Darmstad, Germany) for all analytes. All measurements were carried out at the Faculty of Pharmacy. Instrumental parameters used for metal determination are summarised in Table 1.

The limits of detection (LOD) for the analysed metals were determined based on three times the standard deviation of the blank signal. The limits of quantification (LOQ) were calculated as ten times the standard deviation of the blank. The LOD values for individual elements are presented in Table 1.

2.3 Soil sampling and analysis

Soil samples were collected from both experimental sites prior to planting to determine background metal concentrations. Composite soil samples were obtained from the topsoil layer (0–20 cm) at five randomly selected points within each plot, and homogenised to obtain representative samples. The samples were air-dried, sieved through a 2 mm mesh, and stored at room temperature until analysis.

Approximately 1 g of each soil sample was subjected to digestion using 10 ml of freshly prepared aqua regia. The digestion was performed at 90 °C for 12 h, after which the solutions were filtered and diluted with ultrapure water prior to metal determination using AAS.

Table 1 – AAS instrument parameters
Tablica 1 – Parametri AAS instrumenta

Metal	Wavelength / nm	Slit width / nm	Lamp current / mA	Lamp gain / %	Carrier gas	Limit of detection / $\mu g\,l^{-1}$
arsenic	193.7	0.5	10.0	65	Argon	0.22
cadmium	228.8	0.5	4.0	57	Argon	0.007
lead	283.2	0.5	10.0	42	Argon	0.06
iron	248.3	0.2	5.0	84	Argon	0.04
zinc	213.9	1.0	5.0	52	Argon	0.004

2.4 Soil pH determination

Soil pH was determined potentiometrically using a soil–water suspension. Air-dried and sieved soil samples were mixed with distilled water at a ratio of 1 : 5 (w/v), shaken for 30 min, and allowed to settle. The pH of the supernatant was measured using a calibrated Mettler Toledo pH meter (Hamilton, New Zealand) equipped with a glass electrode.

2.5 Sample preparation and HPLC UV/DAD analysis of cannabinoids

Samples of inflorescences from three different plants of each breeding line in each group were homogenised to a fine powder in order to form a single composite sample. Each sample was measured in duplicate in both groups. Cannabinoids were extracted from 500 mg subsamples of inflorescence or 50 mg of cannabis extract with a mixture of chloroform ($CHCl_3$, 99.4 %; Honeywell, Charlotte, NC, USA) and methanol (CH_3OH, 99.9 %; Sigma-Aldrich, Merck KGaA, Darmstad, Germany) at a ratio of 9 : 1 (v/v) using an ultrasonic water bath for 15 min. The extract solutions were then filtered through 0.45 µm filters.

The filtered solutions were diluted in a mobile phase with acetonitrile (CH_3CN, 99.9 %; Honeywell, Charlotte, NC, USA) and 0.1 % acetic acid (Carlo Erba Reagents S.r.l., Milan, Italy) at a ratio of 65 : 35 (v/v). Final solutions were analysed using an Agilent Technologies (Agilent Technologies Inc., Santa Clara, CA, USA) 1100 series HPLC chromatograph equipped with a Supelco Ascentis® Express (Sigma-Aldrich, Merck KGaA, Darmstad, Germany) 5 µm C18 column. The injection volume was 20 µl and the flow rate was 1.0 ml min^{-1}. The oven temperature was set to 25.0 °C and the wavelength of the detection signal (λ) was 220 nm. Cannabinoid content was determined by measuring 9 cannabinoids, using specific standards for each cannabinoid (Restek Corp., Pennsylvania, PA, USA) (not shown). The means of total values (mg g^{-1}) of three most frequently occurring cannabinoids are presented.

To calculate the total cannabinoid content (tCBD, tTHC, and tCBG), conversion factors of 0.877 for CBDA and THCA, and 0.878 for CBGA, were applied to convert the carboxylated forms (CBDA, THCA, and CBGA) into their corresponding neutral cannabinoids (CBD, THC, and CBG).

2.6 Different solvent extraction techniques

For all extraction procedures, inflorescences of the G8K breeding line were used. In the ethanol extraction, G8K samples from both regions were included, whereas in the remaining extraction techniques only samples originating from the highly contaminated region (Celje Valley) were used.

2.6.1 Ethanol extraction

Fifty grams of ground *Cannabis* inflorescences were soaked in 500 ml of cold 96 % ethanol (Carlo Erba Reagents S.r.l., Milan, Italy) and mixed by hand for three min in a freezer at −30 °C. After extraction, the plant material was separated from the solvent and subjected to vacuum filtration to remove any residual biomass. The filtrate was then evaporated using a heater to remove ethanol, and the resulting concentrate was used for the determination of cannabinoid and terpene potency as well as terpene profiling.

2.6.2 n-Butane extraction

A BHO StarterKit ADDIPURE (ADDITEQ s.r.o., Prague, Czech Republic) was used for butane extraction. Thirty grams of ground inflorescences were used to fill the extractor. ADDIPURE n-Butane (500 ml can, 99.39 % purity) was used as the extraction agent. Following extraction, a heat bath was used to evaporate the butane present in the extract. The resulting extract was then analysed to determine cannabinoid and terpene content, potency of obtained extracts, as well as terpene profile.

2.6.3 Drizzle Merlin 400

The Merlin400 is an automated kitchen extraction device designed for the preparation of medical-grade cannabis oil. For the extraction, 250 ml of 96 % ethanol (Carlo Erba Reagents S.r.l., Milan, Italy) and 30 g of ground inflorescences were used. The extraction was performed using the device's automated Protocol 1 to obtain the final extract.

2.7 GC-MS identification and quantification of terpenes

Terpenes were extracted from 100 mg of each sample by dilution in 7.9 ml of *n*-hexane, followed by sonication in an ultrasonic water bath for 15 min. The resulting solutions were centrifuged and filtered through 0.45 µm membrane filters prior to GC–MS analysis. Each sample was measured in duplicate.

Terpene analysis was performed using a gas chromatograph 6890 (GC; Agilent Technologies Inc., Santa Clara, CA, USA) with a 973 network mass detector (MS; Agilent Technologies Inc., Santa Clara, CA, USA). The analysis was performed using a HP-5MS UI, 30 m × 250 µm, film 0.25 µm capillary column (Agilent Technologies Inc., Santa Clara, CA, USA). The column temperature was initially 35 °C for 5 min, followed by temperature ramping from (35–150) °C at 5 °C min^{-1}, then to 250 °C at 15 °C min^{-1} (inlet: 250 °C; detector: 280 °C; split ratio 5 : 1; carrier gas: helium (flow rate: 1 ml min^{-1}). The mass spectrometer was set to the automatic scan mode of 50–550 amu. The ionisation voltage was set to 1200 V, and the ion source and quadrupole temperatures were set to 230 °C and 150 °C, respectively. The terpenoid content was determined from a standard calibration curve by measuring 21 terpenoids using specific standards (Restek Corp., PA, USA) (not shown). Terpenes were identified using the library (HPCH2205, Wiley7N in FENSC3).

2.8 Statistical analysis

Metal concentrations in soils and plant tissues were obtained from triplicate digestions/measurements ($n = 3$) and are reported as mean±SD. Cannabinoid and terpene determinations were performed as duplicate injections/measurements ($n = 2$) of composite samples, and are reported as mean±SD. For comparisons between the two cultivation sites (H *vs* L), Welch's t-test (two-sided) was used when the same matrix and breeding line were available at both sites; differences were considered statistically significant at $p < 0.05$. Because cannabinoid and terpene datasets were based on duplicate measurements ($n = 2$), the corresponding *p*-values should be interpreted as exploratory. Multiple-comparison adjustment was not applied; therefore, statistical conclusions were restricted to a small set of *a priori* comparisons (H *vs* L within a breeding line and matrix). Statistical analyses were carried out in IBM SPSS Statistics (IBM, USA, 2025).

3 Results and discussion

Total cannabinoid concentrations (tCBD, tTHC, and tCBG) were compared between the two cultivation sites for each breeding line (Table 2). Because cannabinoid determinations were based on duplicate injections of composite samples ($n = 2$), the statistical results are exploratory. A pronounced site effect was observed for the G8K line: tCBD was higher in the contaminated site H (79.6 ± 7.2 mg g^{-1}) than in site L (43.4 ± 5.4 mg g^{-1}; Welch's t-test, $p = 0.035$), and tTHC was likewise higher in H (3.0 ± 0.3 *vs* 1.3 ± 0.2 mg g^{-1}; $p = 0.031$). In the G10K line, tTHC was higher in H (2.8 ± 0.3 *vs* 1.4 ± 0.2 mg g^{-1}; $p = 0.042$), whereas the increase in tCBD (66.4 ± 6.9 *vs* 48.5 ± 4.3 mg g^{-1}) did not reach significance with $n = 2$ ($p = 0.111$). For MERK and CHK, site-related differences in tCBD and tTHC were not statistically supported at $p < 0.05$ under the present replication.

For tCBG, MERK showed lower concentrations in site H (2.0 ± 0.1 mg g^{-1}) than in site L (3.1 ± 0.1 mg g^{-1}; $p = 0.008$). The remaining breeding lines displayed no statistically supported H–L difference in tCBG with $n = 2$ ($p > 0.05$). Overall, the pattern suggests that site conditions may modulate cannabinoid profiles more strongly than breeding-line differences under the studied field conditions, but larger replicate numbers are required to robustly quantify these effects.

Table 2 – Total cannabinoid content (tCBD, tTHC and tCBG) of homogenised inflorescence samples from highly (H) and less (L) contaminated regions. Data are expressed as mean±standard deviation (SD).

Tablica 2 – Ukupni sadržaj kanabinoida (tCBD, tTHC i tCBG) u uzorcima homogeniziranih cvjetova iz visoko (H) i niže (L) kontaminiranog područja. Podatci su prikazani kao srednja vrijednost±standardna devijacija (SD).

Inflorescence samples	Region	tCBD / mg g^{-1}	tTHC / mg g^{-1}	tCBG / mg g^{-1}
MERK	L	59.2 ± 4.0	1.4 ± 0.1	3.1 ± 0.1
MERK	H	56.4 ± 3.5	2.1 ± 0.2	2.0 ± 0.1
G8K	L	43.4 ± 5.4	1.3 ± 0.2	1.8 ± 0.2
G8K	H	79.6 ± 7.2	3.0 ± 0.3	2.3 ± 0.2
CHK	L	53.4 ± 21.7	1.5 ± 0.6	3.2 ± 1.1
CHK	H	64.9 ± 5.4	2.5 ± 0.2	1.3 ± 0.1
G10K	L	48.5 ± 4.3	1.4 ± 0.2	3.0 ± 0.4
G10K	H	66.4 ± 6.9	2.8 ± 0.3	1.8 ± 0.3

Table 3 summarises total cannabinoid concentrations in extracts obtained from the G8K breeding line using different extraction techniques. Each extract was analysed as duplicate injections ($n = 2$). Because most extraction techniques were applied only to material from the contaminated site (H), the data primarily support descriptive comparison between techniques rather than formal statistical testing across methods.

For the ethanol extract, which was prepared from both sites, tCBD was higher in site H (648.1 ± 1.9 mg g^{-1}) than in site L (598.2 ± 2.5 mg g^{-1}; Welch's t-test, $p = 0.003$). tTHC showed a numerical increase in H (21.6 ± 0.7 *vs* 16.9 ± 1.8 mg g^{-1}) that was not statistically supported with $n = 2$ ($p = 0.133$), while tCBG was lower in H (15.2 ± 1.1 *vs* 25.9 ± 2.1 mg g^{-1}; $p = 0.045$). Across the H-site extracts, ethanol and n-butane yielded the highest tCBD values (648.1 ± 1.9 and 642.1 ± 3.4 mg g^{-1}, respectively), whereas Merlin400 produced lower tCBD (601.6 ± 1.8 mg g^{-1}). All analysed extracts contained substantial tTHC, indicating that careful product stand-

ardisation and, where required by regulation, dilution/blending are necessary to meet hemp-derived product thresholds.

Table 3 – Total cannabinoid content (tCBD, tTHC, tCBG) of extracts obtained from G8K breeding line with different extraction techniques from highly (H) and less (L) contaminated regions. Data are expressed as mean ± standard deviation (SD).

Tablica 3 – Ukupni sadržaj kanabinoida (tCBD, tTHC, tCBG) u ekstraktima dobivenim iz G8K linije s različitim tehnikama ekstrakcije iz visoko (H) i manje (L) kontaminiranog područja. Podatci su prikazani kao srednja vrijednost ± standardna devijacija (SD).

Sample name – extraction technique	Region	tCBD / mg g^{-1}	tTHC / mg g^{-1}	tCBG / mg g^{-1}
G8K – ethanol	L	598.2 ± 2.5	16.9 ± 1.8	25.9 ± 2.1
G8K – *n*-butane	H	642.1 ± 3.4	22.8 ± 2.1	16.5 ± 0.5
G8K – ethanol	H	648.1 ± 1.9	21.6 ± 0.7	15.2 ± 1.1
G8K – Merlin400	H	601.6 ± 1.8	21.5 ± 3.9	23.9 ± 1.1

Table 4 shows profile and concentrations of detected terpenes in extracts obtained using different extraction techniques from two regions. The results are expressed as the mean total in mg/g of extract, with standard deviations given in parenthesis. Each sample was analysed in duplicate. The dominant terpenes detected in the samples were sesquiterpenes and oxygenated sesquiterpenes, with β-caryophyllene, α-humulene, caryophyllene oxide, guaiol, and bisabolol present in all extracts. In contrast, low concentrations of ɣ-terpinene and linalool were observed only in the extract obtained using the Merlin400 extraction device, whereas α-terpinene was detected in both the Merlin400 and *n*-butane extracts; these compounds were not detected in the *n*-butane or ethanol extracts from either region.

A comparison between regions revealed that the ethanol extract from the H region exhibited a higher terpene content than the ethanol extract from the L region. This difference can be explained by the different terpene potency of the starting material (data not shown). Among the extraction techniques used in this experiment, the extract obtained using the Merlin400 device yielded the highest terpene concentrations across all analysed samples.

These results can be explained by the fact that the Merlin 400 device performs the extraction protocol under vacuum conditions, whereas this was not the case for the other extraction techniques used in this experiment. Temperature and pressure are key factors affecting the terpene profiles preserved in a sample. Under vacuum, the lower boiling point of the solvent minimises heat exposure of the plant matrix, which helps preserve more thermally labile, volatile terpenes in the extract.[19]

Metal concentrations in soils, plant tissues, and extracts are summarised in Table 5. The soil from the contaminated site (H) contained higher Cd and Pb concentrations than the soil from low-contamination site (L), whereas the L soil contained higher total As, indicating a metal-specific contamination pattern rather than uniform enrichment. Across the four breeding lines, no consistent genotype-dependent pattern was apparent from the mean values alone, suggesting that site conditions dominated uptake under the studied field settings. In inflorescences (dry flowers), Cd was detected at both sites for all lines (0.015 – 0.068 mg kg^{-1}) and did not differ significantly between sites ($p > 0.05$), while Pb and As were mostly below the detection limit. In roots, Cd and Pb concentrations were higher at site H than at site L for three lines (MERK: Cd $p = 0.001$, Pb $p = 0.031$; CHK: Cd $p = 0.037$, Pb $p = 0.04$; G10K: Cd $p = 8.20\text{e-}04$, Pb $p = 2.22\text{e-}04$), whereas the same trend was not statistically supported for G8K because of high within-sample variability (Cd $p = 0.138$, Pb $p = 0.228$).

Table 4 – Terpene profile data expressed as the mean total in mg g^{-1} of extract with standard deviations
Tablica 4 – Podatci o terpenskom profilu izraženi kao ukupni prosjek u mg g^{-1} ekstrakta sa standardnim devijacijama

Terpene present in the samples	G8K-Merlin (H) / mg g^{-1}	G8K-*n*-butane (H) / mg g^{-1}	G8K-ethanol (H) / mg g^{-1}	G8K-ethanol (L) / mg g^{-1}
α-Terpinene	0.07 ± 0.01	0.06 ± 0.01	ND	ND
ɣ-Terpinene	0.32 ± 0.41	ND	ND	ND
Linalool	0.40 ± 0.01	ND	ND	ND
β-Caryophyllene	3.93 ± 0.36	1.55 ± 0.22	0.78 ± 0.04	0.40 ± 0.01
α-Humulene	2.19 ± 0.17	0.99 ± 0.12	0.62 ± 0.02	0.61 ± 0.06
Caryophyllene oxide	1.54 ± 0.20	0.92 ± 0.13	0.89 ± 0.06	0.38 ± 0.04
Guaiol	2.65 ± 0.30	2.11 ± 0.30	1.62 ± 0.09	0.51 ± 0.03
Bisabolol	4.07 ± 0.41	3.69 ± 0.61	2.61 ± 0.17	1.17 ± 0.17

*ND – not detected

Arsenic was detected in roots from site H but remained below detection limits in roots from site L, despite higher total soil As at L. This suggests that As bioavailability (speciation/sorption) rather than total concentration, controlled uptake. Seeds generally contained low concentrations of Cd and Pb; however, Pb concentrations were higher at site L than at site H for MERK ($p = 0.006$) and CHK ($p = 3.19e\text{-}06$). Zn was detected in seeds from site L for G8K, MERK, and G10K but was not detected in the corresponding seeds from site H, consistent with preferential allocation of this essential micronutrient to reproductive tissues when available. Extracts contained only trace amounts of Cd, Pb, Fe, while As was not detected, indicating limited transfer of metals into the extractable fraction under the applied extraction conditions.

The results presented in Table 5 demonstrate that metal uptake and internal distribution in plant tissues were strongly governed by soil chemical conditions, particularly soil pH. The measured soil reaction (pH(H_2O) = 7.28 for

Table 5 – Metal concentrations [mg kg^{-1}] in different hemp plant parts and extracts. Data are expressed as mean ± standard deviation (SD).

Tablica 5 – Koncentracija metala [mg kg^{-1}] u različitim dijelovima i ekstraktima konoplje. Podatci su prikazani kao srednja vrijednost ± standardna devijacija (SD).

Sample ID	Part of the plant	Location	Cd / mg kg^{-1}	Pb / mg kg^{-1}	As / mg kg^{-1}	Zn / mg kg^{-1}	Fe / mg kg^{-1}
Soil		H	0.097±0.003	16.896±0.771	6.039±1.018	1.018±0.136	0.200±0.006
Soil		L	0.016±0.004	0.782±0.041	15.414±0.676	ND	0.074±0.003
G8K	*dry flowers*	H	0.068±0.010	0.372±0.046	ND	0.009±0.000	0.122±0.044
G8K		L	0.064±0.015	ND	ND	ND	0.092±0.001
MERK		H	0.015±0.006	ND	ND	0.185±0.035	0.104±0.002
MERK		L	0.019±0.006	ND	ND	0.113±0.021	0.108±0.000
CHK		H	0.063±0.001	ND	ND	ND	0.087±0.002
CHK		L	0.050±0.006	ND	ND	ND	0.087±0.004
G10K		H	0.039±0.012	ND	ND	0.163±0.014	0.103±0.004
G10K		L	0.020±0.003	0.377±0.014	ND	0.089±0.012	0.090±0.026
G8K	*roots*	H	0.150±0.060	1.090±0.724	0.314±0.100	ND	ND
G8K		L	0.068±0.010	0.372±0.046	ND	0.009±0.000	0.125±0.044
MERK		H	0.108±0.008	1.129±0.184	0.270±0.014	ND	ND
MERK		L	0.032±0.012	0.682±0.127	ND	0.021±0.000	0.162±0.016
CHK		H	0.121±0.022	0.916±0.126	0.366±0.169	ND	ND
CHK		L	0.060±0.004	0.594±0.033	ND	0.020±0.000	0.152±0.005
G10K		H	0.151±0.010	1.165±0.074	0.142±0.036	ND	ND
G10K		L	0.040±0.004	0.381±0.077	ND	0.009±0.000	0.077±0.001
G8K	*seeds*	H	0.026±0.001	0.259±0.013	ND	ND	0.167±0.003
G8K		L	0.019±0.007	ND	ND	1.771±0.049	0.225±0.021
MERK		H	0.011±0.000	0.064±0.005	ND	ND	0.108±0.004
MERK		L	0.033±0.027	0.027±0.000	ND	2.208±0.015	0.088±0.000
CHK		H	0.046±0.002	0.293±0.018	ND	ND	0.159±0.003
CHK		L	0.052±0.011	0.964±0.022	ND	0.020±0.001	0.158±0.007
G10K		H	0.014±0.000	0.185±0.013	ND	ND	0.207±0.012
G10K		L	0.034±0.007	ND	ND	1.635±0.030	0.247±0.002
MERLIN 400	*extract*	H	0.010±0.000	0.224±0.001	ND	ND	ND
n-BUTANE		H	ND	0.421±0.001	ND	ND	ND
CRYO-ETOH		H	0.030±0.001	0.515±0.000	ND	ND	0.029±0.000
CRYO-ETOH		L	ND	ND	ND	ND	ND

*ND – not detected

L and 7.30 for H) indicates a neutral to slightly alkaline environment at both sites, which is known to substantially reduce the mobility and phytoavailability of most trace metals. Given the similar pH values, soil reaction primarily explains the overall low metal translocation to flowers and extracts rather than site-specific differences in uptake.

At neutral to alkaline pH, metals such as Pb, Cd, Zn, and As exhibit decreased solubility due to enhanced adsorption onto clay minerals and Fe/Mn oxides, as well as precipitation as hydroxides and carbonate complexes.[20] These processes reduce dissolved metal concentrations in the soil solution and directly limit plant uptake.[21,22] The pronounced increase in pH after soil addition further suggests a high buffering capacity, which stabilises soil reaction and inhibits metal mobilisation even under acidic inputs.

The observed accumulation pattern (root > seed ≈ flower) is characteristic of plants grown in neutral soils. Lead was predominantly retained in root tissues, with very limited translocation to aboveground organs, reflecting its strong affinity for cell wall components and restricted xylem transport.[23] Cadmium, although more mobile than Pb, was also largely confined to roots, indicating effective physicochemical and biological barriers to translocation under the prevailing pH conditions.[24]

Despite elevated total arsenic concentrations in soil at site L, As levels in plant tissues were low or not detected. This indicates strong immobilisation of arsenic in soil, likely through adsorption of arsenate onto iron oxides, a process that is particularly effective at near-neutral pH values.[25,26] The presence of measurable As in roots from the H site, despite lower total soil As, suggests differences in arsenic speciation or competitive sorption rather than pH-driven effects alone. In contrast, zinc showed relatively higher concentrations in seeds, especially at site L.[27,28] The measured Zn concentrations in soils were relatively low compared to typical background values reported for agricultural soils. This may reflect site-specific geochemical conditions and the analytical extraction procedure used in this study. As an essential micronutrient, Zn is actively transported and preferentially allocated to reproductive tissues to support seed formation. This biologically regulated translocation can result in elevated Zn concentrations in seeds even when soil Zn availability is limited.[29,30]

Overall, the findings indicate that the neutral to slightly alkaline soil pH (7.28 at site L and 7.30 at site H) effectively limits metal solubility, reduces root-to-shoot translocation, and minimises accumulation in flowers and seeds. Consequently, the potential for metal transfer into economically important plant parts and derived extracts is low under the studied conditions.

These results align with established soil-plant interaction models and emphasise soil pH as an important parameter in evaluating metal bioavailability and environmental risk.

4 Conclusion

The results of this study demonstrate that hemp cultivated in contaminated areas can accumulate elevated levels of cadmium and lead, particularly in the roots, highlighting both its phytoremediation potential and the associated risks related to plant contamination. Differences among breeding lines did not markedly influence metal uptake, indicating that genetic selection alone is insufficient to mitigate accumulation under contaminated conditions. Although metal transfer to flowers and extracts was limited under the studied conditions, cultivation for food, supplement, or medicinal applications should preferentially be conducted on uncontaminated soils to minimise potential consumer exposure.

5 Study limitations

This study focused on total metal concentrations in soil and plant tissues, and did not include detailed characterisation of soil properties such as organic matter content, carbonate levels, or metal speciation, which may influence metal bioavailability. The investigation was conducted at two field areas during a single growing season, and therefore does not account for potential interannual variability or broader pedoclimatic conditions. Additionally, metal uptake was evaluated for a limited number of hemp breeding lines, and the observed patterns may not fully represent the behaviour of other genotypes. Future studies incorporating a wider range of soil characteristics, bioavailable metal fractions, and multi-season trials would further refine the understanding of metal transfer in hemp.

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List of abbreviations
Popis kratica

CBD	– cannabidiol – kanabidiol
THC	– tetrahydrocannabinol – tetrahidrokanabinol
Pb	– lead – olovo
Cd	– cadmium – kadmij
Hg	– mercury – živa
As	– arsenic – arsen
Zn	– zinc – cink
AAS	– atomic absorption spectrometry – atomska apsorpciona spektrometrija
tCBD	– total cannabidiol – ukupni kanabidiol
tTHC	– total tetrahydrocannabinol – ukupni tetrahidrokanabinol
tCBG	– total cannabigerol – ukupni kanabigerol
CBDA	– cannabidiolic acid – kanabidiolna kiselina
THCA	– tetrahydrocannabinolic acid – tetrahidrokanabinolna kiselina
CBGA	– cannabigerolic acid – kanabigerolna kiselina
G8K, G10K, MERK, CHK	– breeding lines of hemp – uzgajane sorte konoplje

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SAŽETAK

Metali i sekundarni metaboliti u industrijskoj konoplji uzgojenoj na kontaminiranom tlu

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Industrijska konoplja (*Cannabis sativa* L.) ima važan potencijal za fitoremedijaciju zbog svojeg dubokog korjenova sustava i visoke biomase, ali njezina sposobnost akumuliranja teških metala može predstavljati ograničenje u medicinskoj primjeni. Cilj istraživanja bio je utvrditi i usporediti prisutnost pet metala (Cd, As, Pb, Zn i Fe) u tri različita biljna organa/tkiva i ekstraktima cvijeta da bi se procijenila prikladnost za upotrebu u hrani ili lijekovima, pri čemu je Zn uključen kao esencijalni mikronutrijent za usporedbu s akumulacijom toksičnih metala. Rezultati su pokazali da konoplja uzgojena na kontaminiranom tlu akumulira kadmij i olovo, ponajprije u korijenu, dok je prijenos metala u cvijet i ekstrakt ograničen. Genetske razlike među uzgojenim linijama imale su mali utjecaj na unos i akumulaciju metala. Rezultati ukazuju na to da bi uzgoj konoplje za hranu, dodatke prehrani ili medicinsku upotrebu trebao biti ograničen na nekontaminirana tla da bi se smanjio rizik za potrošače.

Ključne riječi
Konoplja, uzgoj, metali, sekundarni metaboliti, kontaminirano tlo

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