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Chromium accumulation in Croatian wild edible mushrooms: an ecological and dietary risk assessment

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Akumulacija kroma u samoniklim jestivim saprotrofnim gljivama u Hrvatskoj: ekološka i prehrambena procjena rizika

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ABSTRACT

Wild edible saprotrophic mushrooms play a significant role in global diets due to their unique nutritional and medicinal properties. Saprotrophic mushrooms, which decompose organic matter, contribute to ecosystem nutrient cycling and offer various health benefits to humans. In recent times, rapid urbanization and industrialization have contributed to elevated levels of heavy metals, specifically chromium (Cr), in soil systems, which are later accumulated by saprotrophic mushrooms. In this study, an attempt was made to study the status of Cr contamination in soil and five saprotrophic mushroom species, i.e., *Agaricus campestris* L., *Armillaria mellea* [Vahl] P. Kumm., *Clitocybe inversa* [Sowerby] Vizzini, *Clitocybe nebularis* [Batsch] P. Kumm., and *Macrolepiota procera* [Scop.] Singer. around seven locations in central and coastal Croatia. Analysis using inductively coupled plasma optical emission spectroscopy (ICP-OES) showed significant levels ($P<0.05$) of Cr, predominantly in the caps compared to the stipes. Analysis across seven locations indicated that mushrooms and soil samples from central Croatian sites had higher Cr levels than those from coastal areas. Despite the elevated Cr levels, the bioaccumulation factor values and health risk indices, including the dietary intake of metal (DIM) and health risk index (HRI), were below the threshold limit of 1. This finding suggests no significant health risks from consuming these mushrooms. The findings are useful for assessing the potential risks associated with Cr contamination in edible mushrooms from central and coastal regions in Croatia.

Keywords: heavy metals, risk assessment, soil pollution, toxicity, wild edible mushrooms

SAŽETAK

Samonikle jestive saprotrofne gljive imaju značajnu ulogu u globalnoj prehrani zahvaljujući svojim jedinstvenim nutritivnim i ljekovitim svojstvima. Kao razlagači organske tvari, saprotrofne gljive doprinose kruženju hranjivih tvari u ekosustavu te pružaju brojne zdravstvene koristi za ljude. U novije vrijeme, ubrzana urbanizacija i industrijalizacija dovele su do povećanih koncentracija teških metala, osobito kroma (Cr), u tlu, koje saprotrofne gljive mogu akumulirati. Cilj ovog istraživanja bio je istražiti razinu onečišćenja kromom u tlu i u pet vrsta saprotrofnih gljiva – *Agaricus campestris* L., *Armillaria mellea* [Vahl] P. Kumm., *Clitocybe inversa* [Sowerby] Vizzini, *Clitocybe nebularis* [Batsch] P. Kumm. i *Macrolepiota procera* [Scop.] Singer – prikupljenih na sedam lokacija u središnjoj i obalnoj Hrvatskoj. Analiza provedena metodom optičke emisijske spektroskopije s induktivno spregnutom plazmom (ICP-OES) pokazala je statistički značajno više razine kroma (*P*<0,05) u klobucima u odnosu na stapke. Uzorci gljiva i tla s lokacija u središnjoj Hrvatskoj sadržavali su više koncentracije kroma u odnosu na one iz primorskih područja. Unatoč povišenim razinama, vrijednosti bioakumulacijskog faktora (BAF), dnevnog unosa metala (DIM) i indeksa zdravstvenog rizika (HRI) bile su ispod granične vrijednosti 1, što ukazuje na nizak zdravstveni rizik povezan s konzumacijom analiziranih gljiva. Dobiveni rezultati korisni su za procjenu potencijalnih rizika povezanih s kontaminacijom kromom u jestivim samoniklim gljivama iz različitih dijelova Hrvatske.

Ključne riječi: teški metali, procjena rizika, onečišćenje tla, toksičnost, samonikle jestive gljive

INTRODUCTION

Wild mushrooms are essential components of the ecosystem; they are life-sustaining mineral nutrients for plants, especially fiber and digestible proteins, besides many antioxidants (Sileshi et al., 2023). They are also the main sources of amino acids and bioactive compounds (Kalač, 2013). Wild mushroom species also play a crucial role in nutrient recycling through the decomposition of carbon and nitrogen sources found in decaying and dead plant materials (Mostafa et al., 2023). Due to the availability of numerous wild mushroom species worldwide, the evaluation of each species' composition is still limited to certain countries; this may hinder the nutraceutical and nutritional properties of many of them. Foraging is a collective tradition in many Central and Eastern European countries, among them Croatia, that mainly occurs during the rainy season. However, to date, the traditional use of wild edible mushrooms collected from Croatian forests is still unclear, with very little related research (Ninčević Runjić et al., 2024).

Wild mushrooms have a high potential to bioaccumulate potentially toxic elements (PTEs) from the environment they grow. Such elements can have deleterious effects on humans when ingested. This pushes us to assess the potentiality of associated human health risks. *A. campestris* was reported to bioaccumulate high concentrations of cadmium (Cd), chromium (Cr), mercury (Hg), lead (Pb), and nickel (Ni) in continental Croatian regions and natural parks (Širić et al., 2016). The collection of *A. mellea* mushrooms from the East Black Sea region revealed their high bioaccumulation of Hg (Demirbaş, 2002). Wang et al. (2017) mentioned the high bioaccumulation of Cd and Pb in *A. mellea* mushrooms collected from the Tumen River Basin, exceeding the safe limits set by the Chinese industrial standards. The compositional analysis of *C. inversa* samples collected from several Croatian regions depicted the bioaccumulation of Cd, copper (Cu), iron (Fe), Ni, Pb, and zinc (Zn) (Širić et al., 2017). However, further assessment of potential human health risks showed no toxicological risk. The health risk assessment of the consumption of *M. procera* mushrooms found in Southern Spain and Northern Morocco demonstrated some concerns related to high arsenic (As), Cd, Cr, and Hg levels (Barea-Sepúlveda et al., 2022). Moreover, these authors and Stefanović et al. (2016) both raised the need to avoid the consumption of this mushroom species in the "season of mushrooms" due to high Ni bioaccumulation.

Out of them, *Agaricus campestris* (Agaricaceae), commonly known as field or manure mushroom, is a saprophytic edible species very close and mistaken as the cultivated button mushroom (*A. bisporus*) with a homogenous stipe length and cap diameter. It has been reported that *A. campestris* holds good protein (4.73%), vitamin C (2.3 mg/100 g), and dry matter (11.1–13.6 g/100 g dry matter) levels (Rózsa et al. 2017; Zakhary et al. 1983). Moreover, good levels of anthocyanins (0.11 mg/g), glucose (29.4 µg/g), proline (149.6 µmol/g), and soluble protein (55.5 mg/g) were detected in *A. campestris* fruiting bodies (Turfan et al., 2019). Similarly, *Armillaria mellea* (Agaricaceae), commonly known as honey fungus, is a pathogenic wild species that grows on living trees and woody shrubs as well as on dead and decaying food materials. It generally parasitises the root systems of oak, beech, and coniferous trees, seriously threatening their survival. This mushroom species has a developed underground system consisting of rhizomorphs that are initiated from mycelium. (Kostić et al., 2017) mentioned the richness of *A. mellea* in ash (8.8 g/100 g dry weight), carbohydrates (81.2 g/100 g dry weight), fat (1.9 g/100 g dry weight), proteins (1.8 g/ 100 g dry weight), mannitol (free sugar), malic acid (organic acid), δ-Tocopherol, besides polyunsaturated fatty acids. According to the same authors, such a composition makes this mushroom emerge as a functional food rich in nutraceuticals. Other authors mentioned that the polysaccharides *A. mellea* encloses allow it to play a positive effect on learning and memory (Li et al., 2022). This makes such mushrooms a neuroprotective candidate for treating and/or preventing neurodegenerative diseases like Alzheimer's (An et al., 2017).

Likewise, *Clitocybe inversa* (Trichlomataceae), commonly known as *Paralepista inversa*, is a saprophytic mushroom with a 3–10 cm cap diameter. It grows naturally on humus-rich soils and compost under conifer needles (Erbiai et al., 2023). To date, few data have been reported regarding the nutritional value and medicinal properties of *C. inversa*. The latter can be considered as a form of *P. inversa*, which has been reported to contain around 15 amino acids and four fatty acids (mainly linoleic and palmitic acids) (Erbiai et al., 2023). According to the same authors, *P. inversa* showed a high antioxidant potential; however, no investigation was previously launched regarding *C. inversa* in the same terms. *Clitocybe nebularis* or *Lepista nebularis* (Trichlomataceae), commonly known as the clouded agaric or cloud funnel, is an abundant gilled fungus. Although it is reported to cause gastric issues in some cases, it has still been considered an edible species. Dizeci et al. (2021) mentioned that *C. nebularis* contains a large variety of phenolics, and thus has many antioxidative, antibiofilm, antimicrobial, and antiproliferative properties. Pohleven et al. (2016) reported that *C. nebularis* contains lectins conferring some therapeutic characteristics, i.e., antitumor and immunomodulatory properties, making it a promising medicinal mushroom. Similarly, *Macrolepiota procera* (Agaricaceae), commonly known as the parasol mushroom, can be found abundantly growing solitarily or in groups in well-drained soils and especially pasture lands. It is mainly known for its white spores and gills, and large cap, and can be easily identified when encountered. This mushroom species is mainly rich in carbohydrates, fiber, fatty acids, and protein (Yilmaz et al., 2013), and is low in fat (Aydin et al., 2017). This makes it a suitable substitute for meat and meat by-products with high antioxidant activity (Adamska and Tokarczyk, 2022).

Based on the hypothesis that wild edible mushrooms growing in different Croatian regions may accumulate Cr at varying levels depending on local environmental conditions, this study aimed to quantify Cr bioaccumulation in selected mushroom species and evaluate associated human health risks. Although the study included samples from the Continental, Istria, and Kvarner regions, the objective was not to compare regional differences but to assess overall contamination patterns. Differences between cap and stipe Cr concentrations were also examined to identify organ-specific accumulation, and while regional climatic variations exist, the focus remained on species-level accumulation and health risk rather than ecological comparisons.

MATERIALS AND METHODS

Study area

This study was conducted in seven locations in central and coastal Croatia (Figure 1). The three central locations (Trakošćan, Medvednica, and Petrova Gora) are mainly characterized by hilly terrain with mixed deciduous and coniferous forests dominated by beech, fir, spruce, and oak. These regions experience a continental climate, with average annual precipitation ranging from 900 to 1,200 mm and mean annual temperatures between 8 °C and 11 °C. Such climatic conditions, along with forest heterogeneity, have previously been reported to support rich fungal diversity, including both mycorrhizal and saprotrophic mushrooms (Širić et al., 2017; Mostafa et al., 2023).

The four coastal locations (Skrad, Krk, Labinština, and Motovun) experience Mediterranean to sub-Mediterranean climates with milder winters and average annual temperatures between 12 °C and 15 °C. Annual precipitation in these regions ranges from 1,000 to

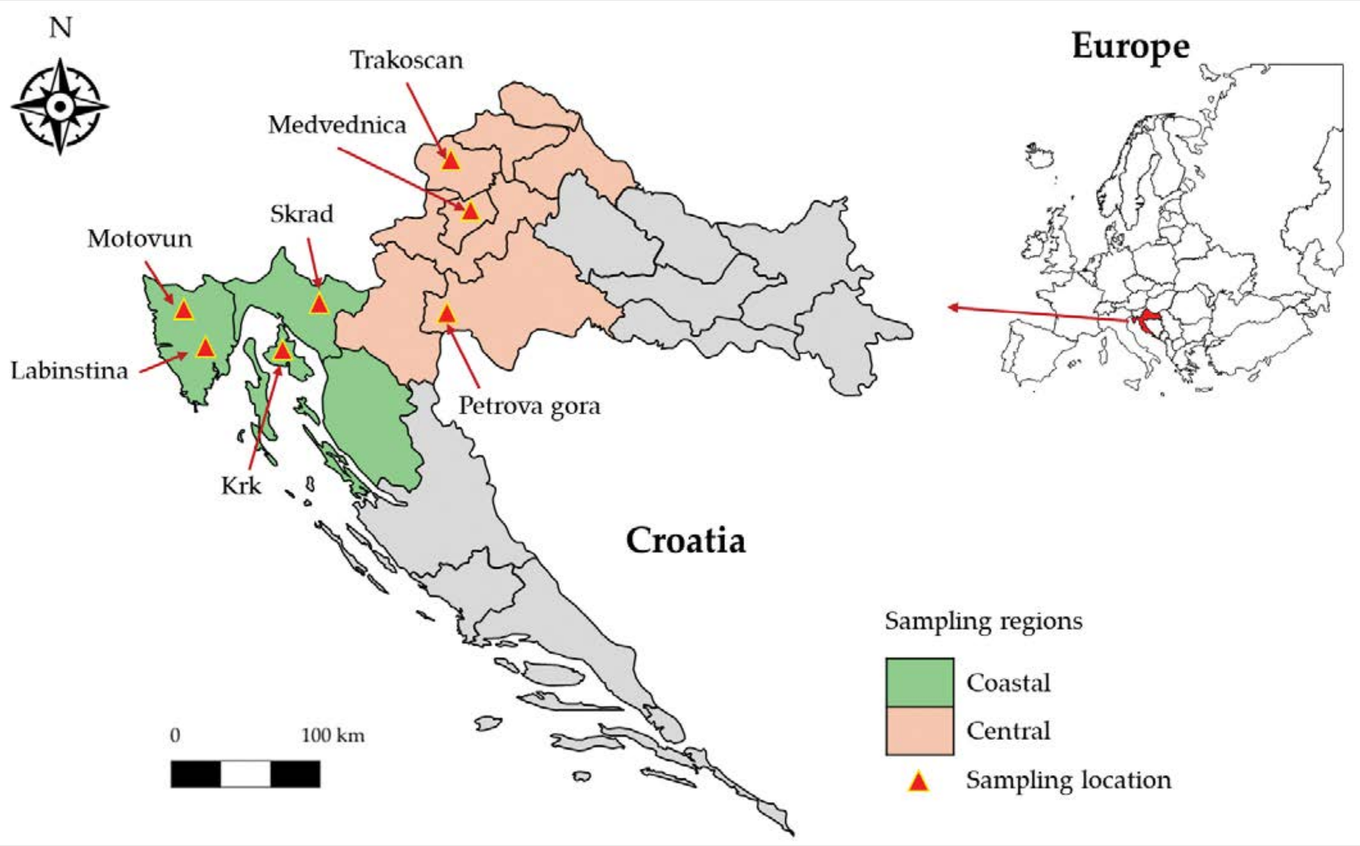


Figure 1. Map of study area showing sampling regions and sites

1,500 mm, with higher relative humidity throughout the year. Krk and Labinština, located on islands, host typical Mediterranean vegetation such as evergreen oak (Quercus ilex) and Aleppo pine (Pinus halepensis), along with various shrub species. These environmental conditions, along with field observations and previous reports, confirm that both regions offer favorable ecological niches for wild mushroom growth. Although detailed soil properties such as pH, grain size, organic carbon and nitrogen content, and electrical conductivity are important, these were not within the scope of the current study, which focused specifically on chromium contamination. However, soils at these sites are generally described as fertile and have been reported to support saprotrophic and truffle-forming mushrooms (Mleczek et al., 2022; Gucia et al., 2012).

Study design and sample collection

In this study, soil and mushroom samples were collected from seven locations across central and coastal Croatia from January to December 2021. We collected samples of five species of mushrooms: SP1: meadow mushroom (*Agaricus campestris* L.), SP2: honey fungus (*Armillaria mellea* [Vahl] P. Kumm.), SP3: funnel-shaped clitocybe (*Clitocybe inversa* [Sowerby] Vizzini), SP4: clouded agaric (*Clitocybe nebularis* [Batsch] P. Kumm.), and SP5: parasol mushroom (*Macrolepiota procera* [Scop.] Singer). These wild edible mushrooms are common in Croatia and are popular in local foods. However, proper identification is critical before consumption, as some species can be toxic. Additionally, certain mushrooms can accumulate hazardous heavy metals like Cr, posing health risks if consumed in large amounts. For each of the seven locations, ten replicate samples of both mushrooms and soils (n = 10) were collected to capture within-site variability and ensure statistical reliability of the results. Each soil sub-sample was collected approximately 5–10 cm from the base of the mushroom fruiting body to ensure spatial relevance while minimizing root zone disturbance. The collection of mushrooms was done using a sterile blade and tweezers to cut them near the volva, followed by separating the stipe and cap, which were placed in separate clean zip-lock plastic bags. After collection, the mushroom samples were gently cleaned with a brush to remove surface debris and then dried in a hot-air oven at 40 °C for 48 hours until a constant weight was achieved. The final moisture content of the dried samples was below 10%, which is consistent with standard practices for elemental analysis (Falandysz et al., 2015). Soil samples were taken from the top 15 cm of the soil surface using a shovel and placed in clean plastic bags. At each site, ten replicate soil samples (n = 10) were collected for analysis, corresponding to the locations of the ten mushroom samples to maintain spatial consistency. Collections were performed between 10:00 AM and 4:00 PM (GMT+2), with mushrooms identified morphologically based on features such as shape, size, color, appearance, texture, stems, gills, and spores, using the online identification guide. Each sample bag was labelled with information including the sample type, collection site and date and time of collection, and sample depth. The samples were promptly transferred to the laboratory and stored at 4 °C for further analysis.

Cr analysis using ICP-OES

The contents of Cr in mushroom and soil samples were analyzed using inductively coupled plasma optical emission spectroscopy (ICP-OES, ICP-OES, Optima 8000, Perkin Elmer, SAD, Waltham, MA, USA). For this purpose, a total of 1 g of mushroom sample (dry weight basis) was gently mixed with 10 mL di-acid mixture (1 part of $HClO_4$ and 3 parts of HNO_3) and subjected to overnight self-digestion followed by heat digestion on a hot plate as described by Mohamed et al. (Mohamed et al., 2020). Further, the digested sample was filtered through a Whatman no. 41 filter paper and added with 5% HNO_3 solution to make up the content of 50 mL for final analysis using ICP-OES. Similarly, 1 g soil sample was also digested and processed for Cr analysis. The Cr analysis was carried out after sample dilution in deionized water followed by calibrating the instrument using different working standards of Cr i.e., 0, 1, 5, 10, 50, and 100 µg/L. The matrix effect correction was achieved by using an internal standard i.e., yttrium (Y) at a concentration of 1 mg/L. The plasma conditions were adjusted as follows: RF power of 1500 W, plasma gas flow rate of 15 L/min, auxiliary gas flow rate of 0.5 L/min, and nebulizer gas flow rate of 1.0 L/min. The nebulizer and spray chamber types were of crossflow and Scott-type double pass, respectively. The sample was introduced into the instrument at an uptake rate of 1.5 mL/min with a rinse time of 30 seconds using deionized water. Finally, the detector parameters were adjusted as follows: wavelength for Cr detection of 267.716 nm (primary) and 283.563 nm (secondary), integration time of 3 seconds, and 3 replicates of each sample, respectively. The quality control (QC) was achieved by running 5% HNO_3 solution as a blank solution and running mid-range standards after every 10-sample analysis. The certified reference material (CRM) of Cr of known concentration was also run to test the accuracy of the results. The spile recovery rates ranged between 92-108% ensuring the precision of instrument results (Fathabad et al., 2018).

Data analysis

The bioaccumulation factor (BAF) of Cr uptake by selected saprotrophic mushroom species from soil was quantified based on the following equation (Eq. 1):

$$BAF = Cr_{(Mushroom)} / Cr_{(Soil)} \qquad (1)$$

where, $Cr_{(Mushroom)}$ and $Cr_{(Soil)}$ represent the concentration of Cr in the mushroom body (cap and stipe parts) and soil samples, respectively. The BAF index represents the heavy metal bioaccumulation potential of mushrooms. The BAF values approaching near zero indicate less bioaccumulation of heavy metal, while a value of 1 or above shows high bioaccumulation (Sun et al., 2017).

On the other hand, dietary intake modelling (DIM), a prevalent method for assessing the risk of heavy metal exposure through dietary intake, was also performed to understand potential risks associated with the consumption of Cr-contaminated mushrooms (Zhong et al., 2018). Also, the health risk index (HRI) was computed using DIM values to understand potential risk factors. For this purpose, DIM and HRI were calculated using the following models (Eqs. 2 and 3):

$$DIM = IR \times HMC \times CF/BW \tag{2}$$

$$HRI = DIM/RfD \tag{3}$$

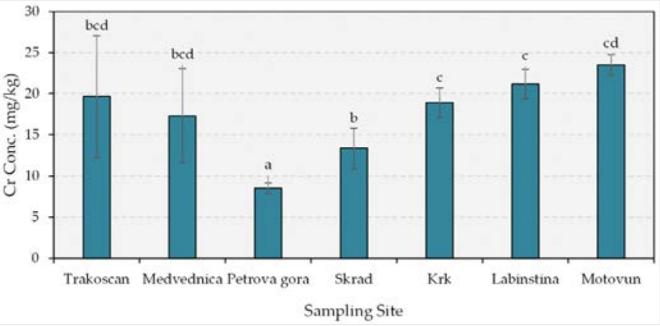
where IR, HMC, CF, and BW refer to the following parameters: ingestion rate (IR), heavy metal concentration (HMC), conversion factor (CF), and body weight (BW). IR represents the rate at which mushrooms are consumed per person per day, HMC indicates the concentration of Cr in mushrooms, CF is a factor used to convert dry to fresh mushroom weight, and BW is the average body weight of the consumer, which is 70 kg in this case. In the context of HRI, the RfD refers to the safe levels of Cr oral exposure for a lifetime, as outlined by Sarikurkcu et al. (2020).

To analyze the data obtained in this study, Microsoft Excel (version 2021, Microsoft Corp., Redmond, WA, USA) and OriginPro (version 2024, OriginLab, Wellesley Hills, MA, USA) software packages were used. Before analysis, the normality of each variable was assessed using the Shapiro-Wilk test, confirming the normal distribution and the appropriateness of parametric statistical tests. Data processing was done using principal component analysis (PCA) and hierarchical clustering to generate heatmaps. Statistical significance was set at $P<0.05$, i.e., 95% confidence interval.

RESULTS AND DISCUSSION

Concentration of Cr in soil samples

Table 1 outlines the Cr concentration in soil samples collected from different sites of both the Central and Coastal regions. Soil samples of the Central region showed an average range of 8.52–19.63 mg/kg Cr, whereas those of the Coastal region outlined a higher range of 13.34–23.49 mg/kg (Figure 2). However, a high standard deviation (SD) was observed in the case of Cr concentrations in soils from the central region, compared to relatively lower variation in coastal region soils. Low to moderate coefficients of variance (CV) (5.35–37.85%) in terms of Cr concentration among different selected sites were observed, which delineate a restricted uncertainty. Furthermore, the observation of the Kurtosis test's results revealed both negative and positive values (−1.78 to 1.33); this depicts a random symmetric distribution throughout the sampling sites of the studied regions. The same applies to the results of the Skewness test, which depicted both negative and positive values (−1.06 to 0.41).



$^{a-d}$ Different letters indicate significant difference among sampling sites based on Tukey's test at $P < 0.05$

Figure 2. Mean comparison of Cr concentration (mg/kg) in soil samples (n = 10) collected from central and coastal regions of Croatia

The results of the comparison of Cr concentration between soil samples revealed that Petrova gora soil was the least contaminated, whereas Trakoscan, Medvednica, and Motovun sites showed the highest contamination levels with comparable values (17–23 mg/kg; $P>0.05$). Heavy metal contamination (Pb, Zn, Cd, and Cu) of forest soils originating from an old French Mines was reported in Medvednica (Perković et al., 2017). Whereas tourism was previously found as the main anthropogenic source of soil contamination in Motovun (Mance et al., 2020).

Table 1. The concentration of Cr (mg/kg) in soil samples (each site is the average of n = 10 samples) collected from the Central and Coastal Regions of Croatia

Region	Site	Minimum	Maximum	Average	SD	CV	Kurt	Skew
Central	Trakoscan	11.32	30.14	19.63	7.43	37.85	−1.78	0.40
	Medvednica	10.12	26.10	17.27	5.76	33.34	−1.60	0.41
	Petrova gora	7.55	9.62	8.52	0.66	7.79	−0.64	0.01
Coastal	Skrad	9.52	16.38	13.34	2.43	18.23	−1.23	−0.18
	Krk	15.10	21.11	18.89	1.78	9.41	1.33	−1.06
	Labinstina	18.50	23.50	21.17	1.80	8.52	−1.34	−0.15
	Motovun	21.36	25.10	23.49	1.26	5.35	−0.94	−0.26

SD: standard deviation; CV: coefficient of variance; Kurt: Kurtosis; Skew: Skewness

Concentration of Cr in wild edible mushroom species

Table 2 outlines the concentration of Cr in both the cap and stipe parts of the studied mushroom samples. Cr concentration in SP1 (*A. campestris*) cap and stipe showed the following decreasing order in the studied sites: Trakoscan > Labinstina > Medvednica > Krk > Motovun > Petrova gora > Skrad. SP2 (*A. mellea*) showed the following respective decreasing Cr cap and stipe bioaccumulation order in selected sites: Medvednica > Petrova gora = Skrad > Labinstina > Krk > Motovun > Trakoscan and Motovun > Petrova gora > Medvednica > Krk > Labinstina > Skrad > Trakoscan. SP3 (*C. inversa*) showed the following decreasing Cr cap and stipe bioaccumulation order in selected sites: Medvednica > Petrova gora > Krk > Labinstina > Skrad > Motovun > Trakoscan. SP4 (*C. nebularis*) showed the following respective decreasing Cr cap and stipe bioaccumulation order in selected sites: Medvednica > Trakoscan > Petrova gora > Skrad > Krk > Labinstina > Motovun and Medvednica > Trakoscan > Petrova gora > Krk > Skrad > Labinstina > Motovun.

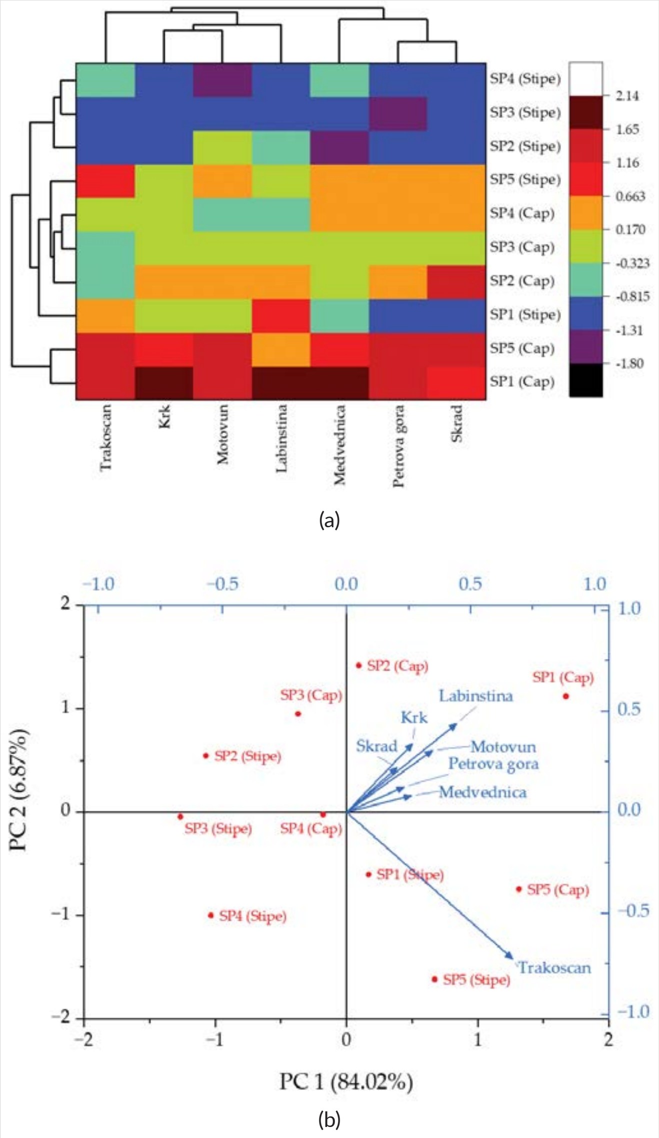
SP5 (*M. procera*) showed the following respective decreasing Cr cap and stipe bioaccumulation order in selected sites: Traoscan > Medvednica > Motovun > Petrova gora = Labinstina > Krk > Skrad and Trakoscan > Medvednica > Petrova gora > Motovun > Labinstina > Skrad > Krk. In brief, the Trakoscan site showed the highest Cr bioaccumulation in SP1 and SP5 cap and stipe parts. Whereas the Medvednica site outlined the highest Cr bioaccumulation in the SP2 cap part and both the cap and stipe parts of SP3 and SP4. This makes the mushrooms of the Trakoscan and Medvednica sites the most Cr-polluted among the studied sites.

Hierarchical component analysis (HCA) and principal component analysis (PCA) are generally performed to reveal grouping and similarities between studied samples (Granato et al., 2018). Herein, the observation of the HCA biplot (Figure 3a) revealed high similarities between SP1 (cap) and SP5 (cap) in terms of Cr bioaccumulation and between SP2 (stipe), SP3 (stipe), and SP4 (stipe) for the same. Whereas the PCA biplot (Figure 3b) demonstrated a high similarity between most studied sites in terms of Cr bioaccumulation in SP1 (cap) and SP2 (cap) – a species-dependent factor rather than a soil-dependent one. PCA results show that the first two components explain 71.3% of the total variance (PC1 = 44.8%, PC2 = 26.5%). The plot reveals species- and site-specific Cr accumulation patterns. SP5 (Cap and Stipe) and SP1 (Cap) are major contributors to positive PC1 values, indicating higher Cr accumulation, especially at Trakošćan. In contrast, SP2–SP4 (Stipes) group negatively on PC1, reflecting lower Cr uptake. The proximity of sites like Medvednica and Petrova Gora suggests similar accumulation trends, while Trakošćan is an outlier in its Cr bioaccumulation profile.

Table 2. The concentration of Cr (mg/kg) in the cap and stipe parts of five mushroom species samples (n = 10) collected from central and coastal regions of Croatia

Mushroom spp.	Part	Variable	Sampling Sites						
			Trakoscan	Medvednica	Petrova gora	Skrad	Krk	Labinstina	Motovun
SP1	Cap	Mean ± SD	1.56±0.05	1.23±0.39	0.93±0.27	0.73±0.19	1.06±0.21	1.49±0.26	1.03±0.23
		Range	1.49–1.63	0.67–1.89	0.61–1.42	0.53–1.16	0.74–1.40	1.14–1.91	0.70–1.39
		Median	1.56	1.27	0.93	0.67	1.09	1.50	1.06
	Stipe	Mean ± SD	1.10±0.05	0.74±0.36	0.47±0.23	0.34±0.15	0.59±0.21	0.99±0.26	0.56±0.20
		Range	1.03–1.17	0.24–1.39	0.19–0.86	0.16–0.60	0.25–0.92	0.62–1.41	0.27–0.87
		Median	1.10	0.75	0.49	0.33	0.65	0.98	0.57
SP2	Cap	Mean ± SD	0.72±0.05	0.85±0.21	0.79±0.14	0.79±0.17	0.75±0.17	0.76±0.23	0.73±0.24
		Range	0.66–0.79	0.71–1.28	0.57–1.04	0.57–1.09	0.54–1.03	0.54–1.25	0.39–1.15
		Median	0.70	0.76	0.79	0.82	0.70	0.69	0.66
	Stipe	Mean ± SD	0.28±0.08	0.44±0.20	0.48±0.14	0.34±0.13	0.43±0.14	0.41±0.16	0.51±0.12
		Range	0.19–0.39	0.28–0.89	0.34–0.73	0.19–0.57	0.20–0.63	0.22–0.79	0.34–0.70
		Median	0.27	0.38	0.43	0.34	0.45	0.41	0.47
SP3	Cap	Mean ± SD	0.54±0.05	0.88±0.26	0.66±0.27	0.58±0.20	0.64±0.15	0.59±0.15	0.56±0.16
		Range	0.47–0.64	0.69–1.41	0.31–1.13	0.34–0.94	0.43–0.90	0.42–0.84	0.32–0.86
		Median	0.54	0.76	0.57	0.50	0.61	0.54	0.55
	Stipe	Mean ± SD	0.26±0.05	0.62±0.28	0.38±0.30	0.30±0.22	0.37±0.17	0.31±0.17	0.28±0.18
		Range	0.18–0.36	0.42–1.20	0.00–0.90	0.04–0.69	0.14–0.65	0.12–0.58	0.01–0.60
		Median	0.25	0.50	0.29	0.21	0.33	0.26	0.27
SP4	Cap	Mean ± SD	0.78±0.06	0.98±0.19	0.77±0.35	0.64±0.22	0.61±0.29	0.54±0.13	0.42±0.09
		Range	0.69–0.86	0.65–1.31	0.31–1.38	0.36–1.05	0.15–0.99	0.31–0.76	0.30–0.57
		Median	0.78	0.97	0.80	0.58	0.70	0.56	0.43
	Stipe	Mean ± SD	0.51±0.06	0.74±0.21	0.50±0.38	0.37±0.24	0.40±0.21	0.25±0.14	0.13±0.10
		Range	0.42–0.60	0.38–1.10	0.00–1.17	0.06–0.81	0.14–0.75	0.01–0.50	0.00–0.29
		Median	0.52	0.72	0.53	0.30	0.42	0.27	0.13
SP5	Cap	Mean ± SD	1.66±0.04	1.09±0.40	0.93±0.32	0.79±0.31	0.86±0.33	0.93±0.23	0.97±0.25
		Range	1.62–1.73	0.43–1.64	0.43–1.37	0.44–1.28	0.43–1.36	0.66–1.36	0.45
		Median	1.66	1.24	0.88	0.80	0.85	0.89	1.02
	Stipe	Mean ± SD	1.48±0.04	0.90±0.37	0.80±0.35	0.66±0.22	0.63±0.33	0.68±0.25	0.74±0.27
		Range	1.43–1.55	0.36–1.45	0.31–1.33	0.30–1.06	0.14–1.15	0.39–1.15	0.15–1.03
		Median	1.48	1.02	0.80	0.69	0.59	0.63	0.79

SP1: *A. campestris*, SP2: *A. mellea*, SP3: *C. inversa*, SP4: *C. nebularis*, and SP5: *M. procera*; SD: standard deviation



SP1: *A. campestris*, SP2: *A. mellea*, SP3: *C. inversa*, SP4: *C. nebularis*, and SP5: *M. procera*

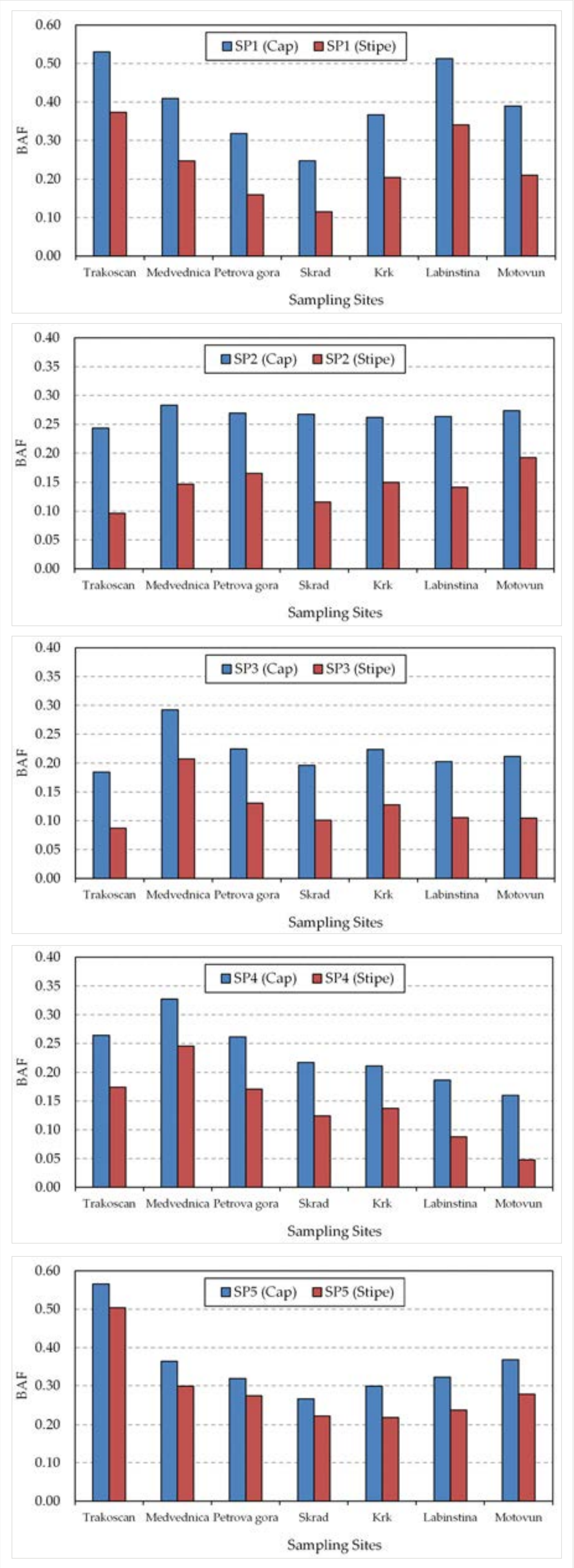
Figure 3. HCA (a) and PCA biplot (b) of Cr (mg/kg) in cap and stipe parts of five mushroom species samples (n = 10) collected from central and coastal regions of Croatia

Generally, wild mushroom species bioaccumulate more PTEs in their cap rather than their stipe (Dowlati et al., 2021). However, the bioaccumulation level of such elements can be species- and/or soil-dependent (Kokkoris et al., 2019). Earlier, it was reported that *A. campestris* (SP1) bioaccumulates more PTEs in its cap rather than its stipe (Širić et al., 2017), which conforms with the findings of this study. Nowakowski et al. (2021) mentioned that *A. mellea* (SP2) bioaccumulated higher Cd concentrations in its cap than its stipe; a similar finding was revealed in the current investigation. Being scantily investigated, no information is available in terms of PTE bioaccumulation in the cap and stipe of *C. inversa* (SP3) and *C. nebularis* (SP4). It is worth mentioning that some soil properties, e.g., pH and organic carbon, might not have a significant effect on the bioaccumulation of PTEs by *M. procera* (SP5). While other factors, i.e., soil C:N ratio, can play a crucial role in the translocation of such elements from soil to mushroom stipe and cap (cap > stipe) (Mleczek et al., 2022). However, hyperaccumulation of some PTEs in *M. procera* cap has raised concerns regarding its safe consumption without health risk pre-assessment (Gucia et al., 2012).

BAF of Cr in wild edible mushroom species

In this study, the BAF of Cr in selected wild edible saprotrophic mushroom species was found to be below 1, as shown in Figure 4. In particular, the SP5 mushroom showed the highest values of BAF in the cap parts, while SP3 showed the lowest BAF values in the stipe parts, respectively. Overall, the BAF of Cr in cap parts of all mushroom species was identified as SP5 > SP1 > SP4 > SP3 > SP1, while for stipe parts the order was as follows: SP5 > SP1 > SP4 > SP3 > SP2. The differences in BAF values between the cap and stipe parts of the mushrooms suggest distinct accumulation mechanisms within the fruiting body of fungi (Zhu et al., 2019). This differential accumulation could be due to varying metabolic activities or structural differences between the cap and stipe, influencing Cr uptake and storage by selected mushroom species (Zhu et al., 2019). In terms of sampling sites, Trakošćan and Labinština exhibited high BAF values for SP1 and SP5, whereas Medvednica showed high BAF values for SP2, SP3, and SP4. These site-specific differences in Cr bioaccumulation likely reflect a combination of environmental, biological, and geochemical factors. For example, the elevated BAF values at Medvednica may be associated with localized Cr enrichment from historical mining activity or atmospheric deposition (Perković et al., 2017). However, due to the absence of site-specific soil properties (e.g., pH, organic matter, C/N ratio) and geochemical background data, it is not possible to determine whether the observed patterns result from lithogenic or anthropogenic Cr sources. This limitation restricts a mechanistic understanding of uptake processes and should be addressed in future studies through comprehensive soil and rock profiling. The soil in Medvednica may contain elevated levels of Cr due to both natural geological formations and anthropogenic activities, thereby its bioavailability in the soil directly impacts its uptake by mushrooms (Perković et al., 2017). Also, this area might be more contaminated with Cr as compared to other non-contaminated areas exhibiting significantly higher BAFs for chromium. The mobility and bioavailability of heavy metals, including chromium, are strongly influenced by soil pH. While pH measurements were not conducted in this study, previous research indicates that forest soils in the central Croatian regions tend to be slightly acidic (pH 5.0–6.5), which may enhance Cr solubility and uptake by fungi (Perković et al., 2017; Mleczek et al., 2022). In contrast, soils in the coastal Mediterranean areas are often neutral to slightly alkaline (pH 6.8–7.5), potentially reducing Cr mobility. These regional pH differences may partially explain the observed variability in BAF values and should be investigated further in future studies.

The bioaccumulation of Cr in saprotrophic mushrooms has been extensively investigated. However, there is a lack of studies focusing on Croatian mushrooms in this context. A study by García et al. (2013) found that 22 species of edible mushrooms collected from Lugo province (Galicia, NW Spain) showed BAF values of Cr ranging from 0.09 to 0.34, which is in good agreement with those observed in the current study. Similarly, Mleczek et al. (2015) also found that the BAF values of Cr accumulation approached >1 in three edible mushrooms (*Leccinum scabrum*, *Boletus edulis*, and *B. badius*) collected from southwest Poland. Another report by Paluch et al. (2017) showed Cr bioaccumulation in *Lactarius salmonicolor* (L.) mushrooms collected from forest ecosystems dominated by *Abies alba* (silver fir trees). They reported that the BAF of Cr was highest among other elements due to its high availability and uptake by mushrooms. Thus, the results of this study suggest targeted research on Croatian mushrooms to understand local Cr bioaccumulation patterns.



SP1: *A. campestris*, SP2: *A. mellea*, SP3: *C. inversa*, SP4: *C. nebularis*, and SP5: *M. procera*

Figure 4. Bioaccumulation factor (BAF) of Cr (mg/kg) in cap and stipe parts of five mushroom species samples (n = 10) collected from the Continental, Istria, and Kvarner Regions of Croatia

Health risk of Cr in wild edible mushroom species

In edible mushrooms, Cr poses a significant hazard that could affect consumer health due to its potential toxicity, such as carcinogenicity, genotoxicity, organ toxicity, allergic reactions, etc. The potential toxicity of Cr can be estimated based on DIM and HRI studies as adopted in several previous studies (Dimitrijević et al., 2023). This study found that the DIM values of Cr varied considerably across sampling sites and between mushroom parts. In general, cap tissues exhibited higher DIM values than stipes. The DIM values ranged from 0.0031 to 0.0404 mg/kg/day, with the highest exposure associated with SP5 (*M. procera*, cap) at Trakošćan and the lowest with SP4 (*C. nebularis*, stipe) at Motovun. These variations reflect species-specific uptake patterns as well as site-related differences in Cr availability. The corresponding HRI values followed a similar trend but remained below the critical threshold of 1, indicating no immediate health risk from dietary intake of these mushrooms under typical consumption rates. Table 3 shows the DIM and HRI results of Cr in the cap and stipe parts of five mushroom species collected from the central and coastal regions of Croatia.

Table 3. DIM and HRI results of Cr (mg/kg) in cap and stipe parts of five mushroom species samples (n = 10) collected from central and coastal regions of Croatia

Mushroom spp.	Part	Index	Sampling Sites						
			Trakoscan	Medvednica	Petrova gora	Skrad	Krk	Labinstina	Motovun
SP1	Cap	DIM	0.0378	0.0298	0.0226	0.0178	0.0256	0.0361	0.0251
		HRI	0.0568	0.0447	0.0339	0.0267	0.0384	0.0542	0.0376
	Stipe	DIM	0.0266	0.0180	0.0114	0.0083	0.0143	0.0240	0.0135
		HRI	0.0400	0.0269	0.0171	0.0125	0.0215	0.0360	0.0203
SP2	Cap	DIM	0.0174	0.0206	0.0192	0.0193	0.0183	0.0186	0.0176
		HRI	0.0261	0.0310	0.0288	0.0289	0.0275	0.0279	0.0264
	Stipe	DIM	0.0069	0.0107	0.0117	0.0083	0.0105	0.0100	0.0124
		HRI	0.0103	0.0161	0.0176	0.0125	0.0157	0.0149	0.0186
SP3	Cap	DIM	0.0132	0.0213	0.0160	0.0141	0.0156	0.0143	0.0136
		HRI	0.0198	0.0319	0.0240	0.0212	0.0235	0.0214	0.0204
	Stipe	DIM	0.0062	0.0151	0.0093	0.0073	0.0089	0.0074	0.0067
		HRI	0.0094	0.0226	0.0139	0.0109	0.0134	0.0111	0.0101
SP4	Cap	DIM	0.0189	0.0238	0.0186	0.0156	0.0148	0.0131	0.0103
		HRI	0.0283	0.0358	0.0279	0.0235	0.0221	0.0197	0.0154
	Stipe	DIM	0.0124	0.0179	0.0122	0.0089	0.0096	0.0062	0.0031
		HRI	0.0187	0.0268	0.0182	0.0134	0.0144	0.0093	0.0046
SP5	Cap	DIM	0.0404	0.0265	0.0227	0.0192	0.0209	0.0227	0.0237
		HRI	0.0606	0.0397	0.0340	0.0287	0.0314	0.0340	0.0355
	Stipe	DIM	0.0360	0.0218	0.0195	0.0161	0.0153	0.0166	0.0179
		HRI	0.0539	0.0327	0.0292	0.0241	0.0230	0.0249	0.0268

SP1: *A. campestris*, SP2: *A. mellea*, SP3: *C. inversa*, SP4: *C. nebularis*, and SP5: *M. procera*

However, cap parts showed relatively high DIM values as compared to stipe parts. Herein, SP5 showed the highest DIM values (0.0404) at the Trakoscan site, whereas the lowest in SP4 (0.0031) at the Motovun site, respectively. On the other hand, the HRI values were highest at Trakoscan, i.e., 0.0606 (cap part of SP5), while the lowest was observed as 0.0046 in the stipe of SP4, respectively. Overall, the trends of DIM and HRI indices showed no significant health risk associated with the consumption of selected mushroom species, as the values did not exceed the permissible level of 1. The results suggest that while there are detectable levels of Cr in the mushrooms, the exposure levels do not pose a significant health risk under the current consumption patterns. However, continuous monitoring and further studies are recommended to ensure that Cr levels remain safe and to assess the potential long-term health effects of chronic exposure.

Health risk studies have been vital for identifying any potential health hazards related to the ingestion of food and food products. Recently, several researchers have reported that the use of DIM and HRI studies helped assess the health hazards of heavy metals, including Cr, in wild as well as cultivated mushroom species. A study by Qiu et al. (2024) found that black fungus (Auricularia heimuer) mushrooms in China showed significant health risk index values for heavy metal bioavailability using Monte Carlo simulation-based modelling. Similarly, Chukwuka et al. (2023) also reported that wild mushrooms growing in Nigeria showed significant health risks for several heavy metals, which need urgent attention for wild foragers. Also, Dimitrijević et al. (2023) found that five wild edible mushroom species, i.e., Macrolepiota mastoidea, M. konradii, *M. procera*, Suillus collinitus, and Cuphophyllus pratensis, showed significant values of HRI when tested using an ICP-OES instrument. Thus, health risk studies are crucial for assessing potential hazards associated with mushroom consumption and for implementing effective environmental monitoring and pollution control measures.

CONCLUSIONS

The study demonstrated species- and site-specific variability in Cr accumulation among selected wild edible saprotrophic mushrooms, with higher concentrations typically observed in caps compared to stipes. Central Croatian sites generally exhibited elevated Cr levels relative to coastal areas. These findings showed the need for incorporating species traits, site history, and environmental factors in future risk assessments. Also, the bioaccumulation factor values and health risk indices, such as dietary intake of Cr metal (DIM) and health risk index (HRI) values, were below the threshold limit of 1, suggesting no significant health hazard associated with the consumption of selected mushrooms. Overall, this study provides insight into the risk of Cr contamination in wild edible saprotrophic mushrooms of central and coastal Croatia. The results provide a foundation for long-term biomonitoring and public health evaluations related to heavy metal exposure through wild mushroom consumption. Moreover, investigating the biochemical and molecular mechanisms underlying Cr uptake and accumulation in different mushroom species and their various parts is highly recommended for future studies.

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