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# Potentially toxic element accumulation in wild mushroom rhizospheres and fruiting bodies with human health risk assessment in the Czech Republic

# Akumulace potenciálně toxických prvků v rhizosférách a plodnicích volně rostoucích hub a hodnocení rizik pro lidské zdraví v podmínkách České republiky

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## ABSTRACT

Potentially toxic elements (PTEs) loaded with fungal fruiting bodies consumption may lead to human health repercussions. This study was conducted to examine the PTE (Cu, Fe, Mn, Zn) concentrations in rhizospheres (10 cm) and in the dry matter of mushrooms collected from the Czech Republic, where wild mushroom harvesting is highly popular. Non-carcinogenic human health risk assessments were also performed. The results showed that Fe was the dominant element in both soils and mushroom fruiting bodies, followed by Mn, Zn, and Cu. Species-specific accumulation patterns were observed across the study sites, with higher element concentrations generally detected in mushrooms collected from roadside environments compared with the control locality. Differences in accumulation capacity were observed among mushroom species, indicating species-dependent uptake of potentially toxic elements. The non-carcinogenic health effects were highest in the rhizosphere of Švihov dam D1 (100%) and lowest in the rhizosphere of Ledenice (18.3%). The most affected area was Švihov dam D1, followed by Humpolec D1 and Ledenice. It was concluded that rhizosphere contamination of edible mushrooms could cause non-carcinogenic health issues, which could be avoided by avoiding areas in risk zones, such as those near highways or heavily trafficked roads.

**Keywords:** potentially toxic elements, mushrooms, food chain, bioconcentration, non-carcinogenic health risks assessment

## ABSTRAKT

Konzumace plodnic hub zatížených potenciálně toxickými prvky (PTE) může představovat riziko pro lidské zdraví. Cílem této studie bylo stanovit koncentrace PTE (Cu, Fe, Mn a Zn) v rhizosférické půdě (0–10 cm) a v sušině plodnic hub sbíraných v České republice, kde je sběr hub široce rozšířen. Součástí práce bylo hodnocení nekarcinogenního zdravotního rizika. Výsledky ukázaly, že železo (Fe) bylo dominantním prvkem v půdě i plodnicích, následované Mn, Zn a Cu. Byly zjištěny druhově podmíněné rozdíly v akumulaci prvků mezi lokalitami, přičemž vyšší koncentrace byly obecně zaznamenány u hub z lokalit v blízkosti komunikací oproti kontrolní lokalitě. Rozdíly v akumulační schopnosti byly potvrzeny například u druhů *Agaricus campestris* (pečárka polní), *Amanita rubescens* (muchomůrka růžovka) a *Xerocomellus chrysenteron* (suchohřib žlutomasý). Nejvyšší nekarcinogenní toxicita byla zjištěna v rhizosféře lokality Švihov – D1 (100 %), nejnižší v lokalitě Ledenice (18,3 %). Nejvíce zatíženou oblastí byla lokalita Švihov – D1, následovaná Humpolec – D1 a Ledenicemi. Kontaminace rhizosféry může vést ke zvýšenému přenosu PTE do jedlých částí hub a představovat nekarcinogenní zdravotní riziko. Riziko lze snížit vyhýbáním se sběru hub v blízkosti dálnic a silně frekventovaných komunikací.

**Klíčová slova:** potenciálně toxické prvky, houby, potravní řetězec, biokoncentrace, hodnocení nekarcinogenního zdravotního rizika

## INTRODUCTION

The global community is increasingly concerned about elevated levels of potentially toxic elements (PTEs) in the environment due to their potential adverse effects on human health (Garau et al., 2023). These elements, including cadmium (Cd), which is classified as a Group 1 carcinogen, cobalt (Co), copper (Cu), iron (Fe), lead (Pb), manganese (Mn), and zinc (Zn), are widely distributed in soil, water, air, and food systems (Xing, 2022). They originate from both natural processes, such as volcanic eruptions and rock weathering, and anthropogenic sources, including industrial operations, mining, agriculture, and urbanisation (Kowalska et al., 2022). The Czech Republic, located in Central Europe, has experienced substantial PTE contamination due to its history of industrialisation, mining activities, and emissions from coal-fired power plants and metal smelters (Marschalko et al., 2020).

Mushrooms are nutritionally valuable foods rich in protein, vitamins, minerals, and dietary fibre (Salami et al., 2016; Trujillo et al., 2021; Fernandes et al., 2021). In addition to their dietary value, some species contain bioactive compounds with therapeutic properties (Grimm et al., 2021). With the increasing awareness about the nutritional values and associated benefits, the trends for mushroom consumption are increasing worldwide, i.e., mushroom consumption was 1.0 kg per person in 1993 and 4.7 kg in 2013 per person and year (Royse et al., 2017). For instance, as many as 72% of families collect mushrooms at a yearly medium level of 7 kg per household in the Czech Republic (Kalač, 2013; Šišák, 1996). Due to increasing demand, the mushroom market is estimated to be worth 63 billion USD (Chakrabarti et al., 2019).

Mushrooms possess the distinctive capacity to take up PTEs from the contaminated rhizosphere, as they have a higher biomass production capacity (Asare et al., 2023). Because some species serve as indicators of environmental pollution, mushroom species exhibit varying levels of PTE uptake depending on their ecological preferences, anatomy, and physiology. *Agaricus* spp, *Boletus* spp, and *Laccaria* spp, which are commonly consumed mushrooms, have been shown to accumulate significant amounts of Cu, Fe, Mn, Cd, and Pb (Ali and Nasution, 2019; Bayouli et al., 2021). This has raised concerns about their safety for human consumption, particularly when they are produced in polluted regions. Mushrooms have profound cultural and economic significance in the Czech Republic, shaping its cuisine, traditional customs, and economy (Šišák, 1996; Procházka et al., 2023). Although mushrooms have both nutritional and cultural importance, the accumulation of PTEs in them poses a significant health hazard to those who eat them. Prolonged exposure to hazardous metals such as Cu, Fe, Cd, Mn, and Zn may result in serious health problems, In the present study, copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn) were selected because they are essential trace elements commonly present in soils and biological systems but may become toxic when accumulated at elevated concentrations. These elements are frequently associated with traffic emissions, industrial activities, and soil geochemistry, making them particularly relevant for environmental and food-safety assessments in the studied regions, such as neurological diseases, renal impairment, developmental abnormalities, and heightened susceptibility to cancer (Chakrabarti et al., 2019; Gwenzi et al., 2021). Despite the cultural and dietary importance of wild mushrooms in the Czech Republic, limited region-specific information is available linking rhizosphere contamination, metal accumulation in fruiting bodies, and associated human health risks. The scientific question addressed in this study is whether environmental contamination influences the accumulation of selected PTEs in wild mushrooms and whether this accumulation poses a measurable non-carcinogenic health risk to consumers.

The objectives of this study were to (i) determine the concentrations of Cu, Fe, Mn, and Zn in mushroom rhizosphere soils and fruiting bodies, (ii) evaluate the transfer of these elements using bioconcentration factors (BCFs), and (iii) assess potential non-carcinogenic health risks associated with mushroom consumption. It was hypothesised that roadside environments would exhibit higher PTE concentrations than the control locality, that mush-

room species would differ in their accumulation capacity, and that increased soil contamination would correspond to elevated health-risk indices.

## MATERIALS AND METHODS

### *Study sites*

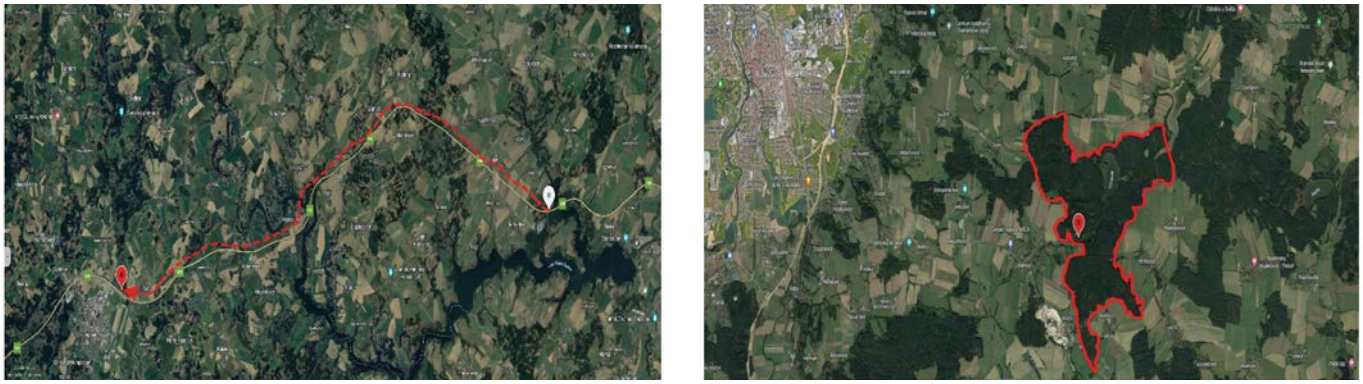
Nestled in the Czech Republic, the study sites, i.e., Ledenice (South Bohemia region), a control locality, and the highway D1 near Humpolec and the Švihov dam (Central Bohemian Region). Ledenice is situated roughly 10 kilometres southeast of České Budějovice (48.9733 N, 14.4809 E). The climate is humid continental, with warm summers and cold, snowy winters. The area is located at an elevation of around 450 meters above sea level. The Švihov dam is located 122 kilometres northwest of Ledenice and is a significant landmark in the region. This impressive water, constructed on the Želivka River, spans 142 hectares. The dam serves multiple purposes, including flood control, water supply for the Czech capital, Prague, and recreation. The locality near the D1 highway, close to Humpolec town, is located approximately 100 km northwest of Ledenice (Figure 1).

### *Sampling and samples preparation*

A total of 17 triplicated soil samples (n = 51) were collected from the rhizospheres (0 – 10 cm) of the different mushroom species, while mushrooms were also collected from the same sites belonging to other species. The collected species included *Amanita rubescens*, *Agaricus campestris*, *Leccinum versipelle*, *Leccinum scabrum*, *Imleria badia*, *Xerocomellus chrysenteron*, *Boletus edulis*, *Suillus grevillei*, and *Chlorophyllum rhacodes*. Sampling was done during the summer season of August 2023 – 2024. The collected soil samples were sieved through a 2 mm sieve to remove larger stones, debris, and wooden clogs, oven-dried at 105 °C for 8 hours and used for further analysis. For mushroom sampling, only the fruiting bodies were carefully collected by gently removing them from the soil surface using a knife. The underground mycelium was not collected or disturbed (n = 51). The collected mushroom samples were cleaned of dust and soil particles, sampled using a ceramic-blade knife, and then dried at laboratory temperature. After that, the dried mushrooms were ground to a powder using a laboratory mill and then used for further analysis.

### *Soil and mushroom sample analysis*

For assessing PTEs content in both soil and mushroom samples, 0.5 g of each sample with 10 ml of concentrated nitric acid (p.p.) was digested using a microwave reaction system MARS 5 (CEM Corporation, Matthews, North Carolina, USA). After 25 minutes of digestion time for cooling, the samples were filtered using 1.2 µm microfiber filters, then filled to 50 ml. The filtrate was then used to determine PTEs (Cu, Fe, Mn, and Zn) using an atomic absorption spectrophotometer (AAS) iCE 3500 series (Thermo Fisher Scientific, Cambridge, United Kingdom) operated in flame atomisation mode (Zeeshan et al., 2023). Deionised ultrapure water was produced by the Simplicity system (Millipore SAS, Molsheim, France).



(a) (b)

**Figure 1.** Map of soil and mushroom sampling locations in the Czech Republic, showing (a) sampling sites near the D1 Highway to Humpolec, and (b) a schematic map of sample collection sites in Ledenice

### *Bioconcentration factor (BCF)*

The bioconcentration factor (BCF) is an indicator of the concentration of contaminants in the surrounding environment relative to their concentration in humans, animals, or edible parts above ground. The BCF for this study was calculated through the following formula (Garg et al., 2014):

$$BCF = C_{mushroom} / C_{soil}$$

where $C_{mushroom}$ and $C_{soil}$ are PTE concentrations (in dry matter) in mushroom and soil (mg $kg^{-1}$).

### *Human health risks assessment*

A human health risk assessment (HRA) was developed to highlight the importance of selecting low-impact localities for mushrooming, focusing on heavy metal concentrations in mushroom bodies and topsoil. HRA was conducted following the LCIA principles and the harmonised life cycle impact assessment (LCIA) method Environmental footprint 3.1 / Characterisation model (Andreasi et al., 2023), an integrated method in the SimaPro Craft 10.2.0.0 (SimaPro, 2025), via the impact category of Human toxicity, non-cancer inorganics (HTNCi) expressed in CTUh (Comparative Toxic Unit for humans). The impact level reflects the Human toxicity and non-cancerous inorganic effects of anthropogenic Zinc concentrations in the fruiting body and in the subsoil beneath it. For the expression, the following characterisation factor was employed: component: soil, substance: Zinc (II), Compartment: soil, Subcompartment: industrial, CAS number: 023713-49-7, characterisation factor: 1.4458$E^{-6}$ CTUh $kg^{-1}$ Zinc (II). The results were related to one kilogram of dry matter (DM) of the fruiting body or subsoil under the fruiting body (CTUh per $kg^{-1}$ DM).

### *Statistical analysis and data representation tools*

All data were statistically analysed using MS Excel (Microsoft Corp., USA) and OriginPro 2024 (OriginLab Corp., USA). Descriptive statistics (mean, minimum, maximum, and standard deviation) were calculated. Differences among sampling sites were evaluated using one-way analysis of variance (ANOVA), and statistical significance was determined at $P < 0.05$. Pearson's correlation analysis was performed to assess relationships between PTE concentrations in soil and mushroom samples, and corresponding p-values were calculated. Principal component analysis (PCA) was conducted to identify patterns and associations among elements and sampling locations.

## RESULTS

### *Potentially toxic element concentration in the rhizosphere of the mushroom species*

Considerable variation in PTE concentrations was observed among rhizosphere soils and sampling sites (Table 1). Overall, the highest concentrations were generally recorded at the Švihov dam D1 and Humpolec D1 sites, while Ledenice exhibited lower levels. Copper concentrations ranged from 4 to 60 mg/kg, with the highest values detected in the rhizosphere of *Leccinum versipelle* at Humpolec D1. Iron showed the greatest overall abundance among the analysed elements, reaching a maximum of 42,700 mg/kg at Švihov dam D1. Manganese ranged from 6 to 1,330 mg/kg, with elevated levels mainly observed at impacted roadside sites. Zinc concentrations varied between 43 and 238 mg/kg, with the highest values recorded at Švihov dam D1. One-way ANOVA revealed significant differences ($P < 0.05$) in PTE concentrations among the three sampling sites, particularly for Fe and Mn, indicating spatial variability related to site characteristics.

### *Potentially toxic elements' concentration in edible mushrooms*

The analysis revealed notable variations in Cu contents among the studied species. Among the mushroom species, *Agaricus campestris* exhibited the highest Cu content (66 mg/kg) in samples belonging to the Švihov dam D1 area, followed by *Chlorophyllum rhacodes* (63 mg/kg) in the same area, while *Leccinum versipelle* (60 mg/kg) in the Ledenice area. Conversely, the lowest Cu content among the species examined was found in *Leccinum versipelle* (9.0 mg/kg), collected from the Humpolec D1 area.

**Table 1.** The contents of PTEs in the rhizosphere of the mushroom samples

| Sites | Species | Cu | Fe | Mn | Zn |
|---|---|---|---|---|---|
| | | (mg/kg of DM) | | | |
| Ledenice | *Xerocomellus chrysenteron* | 9.0 ± 0.96 | 5,650 ± 24 | 6.0 ± 0.21 | 55 ± 2.4 |
| | *Chlorophyllum rhacodes* | 4.00 ± 0.17 | 5,310 ± 45 | 872 ± 24 | 71 ± 12.5 |
| | *Suillus grevillei* | 20.0 ± 1.3 | 335 ± 22.3 | 144 ± 21.3 | 93.0 ± 4.7 |
| | *Amanita rubescens* | 7.00 ± 0.12 | 6,160 ± 34 | 884 ± 24.6 | 44.0 ± 4.5 |
| | *Imleria badia* | 4.00 ± 0.64 | 3,730 ± 56 | 490 ± 21.4 | 44.0 ± 4.6 |
| | *Leccinum scabrum* | 9.00 ± 0.78 | 6,820 ± 104 | 130 ± 21.5 | 53.0 ± 5.5 |
| | *Agaricus campestris* | 9.00 ± 0.41 | 10,300 ± 212 | 320 ± 23.5 | 62.0 ± 9.3 |
| | *Boletus edulis* | 6.00 ± 0.52 | 5,610 ± 121 | 175 ± 10.5 | 43.0 ± 6.8 |
| | *Leccinum versipelle* | 8.00 ± 0.36 | 8,260 ± 423 | 380 ± 103 | 52.0 ± 4.6 |
| Humpolec D1 | *Leccinum versipelle* | 60.0 ± 3.6 | 39,600 ± 306 | 1,330 ± 12.5 | 136 ± 11.4 |
| | *Leccinum scabrum* | 28.0 ± 1.5 | 21,500 ± 422 | 456 ± 23.5 | 98.0 ± 12.5 |
| | *Amanita rubescens* | 21.0 ± 0.79 | 19,200 ± 303 | 222 ± 10.6 | 102 ± 1.1 |
| | *Imleria badia* | 19.0 ± 1.3 | 18,400 ± 124 | 225 ± 12.5 | 107 ± 16.4 |
| | *Xerocomellus chrysenteron* | 18.0 ± 0.89 | 18,300 ± 343 | 180 ± 1.09 | 103 ± 11.45 |
| Švihov dam D1 | *Agaricus campestris* | 15.0 ± 1.2 | 42,700 ± 436 | 1,110 ± 101 | 238 ± 32.3 |
| | *Amanita rubescens* | 14.0 ± 1 | 32,500 ± 457 | 844 ± 45.3 | 137 ± 12.6 |
| | *Chlorophyllum rhacodes* | 25.0 ± 2.2 | 30,700 ± 436 | 557 ± 23.5 | 207 ± 46.7 |
| Mean | | 16.12 | 16,184.0 | 490.18 | 96.67 |
| Min | | 3.50 | 355.0 | 6.14 | 42.70 |
| Max | | 59.50 | 42,669.0 | 1334.0 | 237.80 |
| SD | | 13.43 | 13,247 | 387.01 | 56.79 |
| Skew. | | 2.03 | 0.74 | 0.77 | 1.24 |
| Kurtosis | | 4.42 | -0.71 | -0.52 | 0.75 |

The data are presented as average values of three replicate measurements ± standard deviation.

The Fe content was the highest among all the PTEs in this study; the maximum Fe was found in *Imleria badia* in the Humpolec D1 area at 1,887 mg/kg. Minimum Fe contents were found in the same area in *Xerocomellus chrysenteron* species, i.e., 137 mg/kg. The Mn content ranged from 6 – 267 mg/kg. The maximum Mn content (267 mg/kg) was found in *Xerocomellus chrysenteron* in the Ledenice area, while the minimum (7 mg/kg) was recorded in the same species in the Humpolec D1 area (Table 2).

**Table 2.** PTEs in edible mushroom species

| Sites | Species | Cu | Fe | Mn | Zn |
|---|---|---|---|---|---|
| | | (mg/kg of DM) | | | |
| Ledenice | *Xerocomellus chrysenteron* | 26.0 ± 1.1 | 577 ± 22.5 | 267 ± 20.2 | 92.0 ± 5.6 |
| | *Chlorophyllum rhacodes* | 55.0 ± 2.3 | 173 ± 13.5 | 73.0 ± 22.4 | 123 ± 11.4 |
| | *Suillus grevillei* | 20.0 ± 1.3 | 335 ± 21.2 | 144 ± 20.2 | 93.0 ± 3.6 |
| | *Amanita rubescens* | 21.0 ± 1.6 | 424 ± 32.6 | 129 ± 23.5 | 62.0 ± 3.4 |
| | *Imleria badia* | 18.0 ± 1.8 | 203 ± 54.6 | 110 ± 20.3 | 142 ± 3.5 |
| | *Leccinum scabrum* | 11.0 ± 0.98 | 236 ± 103 | 80.0 ± 11.5 | 91.0 ± 4.4 |
| | *Agaricus campestris* | 47.0 ± 3.4 | 1,370 ± 111 | 55.0 ± 7.5 | 88.0 ± 8.2 |
| | *Boletus edulis* | 27.0 ± 1.4 | 255 ± 120 | 66.0 ± 9.4 | 93.0 ± 5.7 |
| | *Leccinum versipelle* | 60.0 ± 2.5 | 868 ± 116 | 146 ± 12.4 | 135 ± 3.5 |
| Humpolec D1 | *Leccinum versipelle* | 9.00 ± 0.98 | 178 ± 12.5 | 8.00 ± 0.98 | 59.0 ± 20.3 |
| | *Leccinum scabrum* | 12.0 ± 1.1 | 601 ± 16.6 | 33.0 ± 2.5 | 106 ± 11.4 |
| | *Amanita rubescens* | 34.0 ± 2.5 | 1,890 ± 103 | 26.0 ± 2.5 | 105 ± 12.6 |
| | *Imleria badia* | 27.0 ± 3.1 | 1,890 ± 123 | 27.0 ± 1.5 | 117 ± 15.3 |
| | *Xerocomellus chrysenteron* | 29.0 ± 1.3 | 137 ± 18.7 | 7.00 ± 0.89 | 113 ± 10.4 |
| Švihov dam D1 | *Agaricus campestris* | 66.0 ± 3.6 | 1,910 ± 135 | 57.0 ± 9. 8 | 118 ± 4.6 |
| | *Amanita rubescens* | 21.0 ± 1.3 | 4,030 ± 456 | 128 ± 6.9 | 155 ± 11.5 |
| | *Chlorophyllum rhacodes* | 63.0 ± 3.50 | 1,610 ± 165 | 37.0 ± 6.9 | 106 ± 11.5 |
| Mean | | 32.13 | 981.27 | 81.88 | 105.78 |
| Min | | 9.30 | 137.0 | 6.50 | 59.0 |
| Max | | 66.40 | 4,026.0 | 266.90 | 155.0 |
| SD | | 18.93 | 1,033.10 | 66.48 | 25.41 |
| Skew. | | 0.65 | 1.59 | 1.23 | -0.02 |
| Kurtosis | | -0.95 | 2.29 | 1.43 | -0.28 |

The data are presented as average values of three replicate measurements ± standard deviation.

Similarly, Zn content ranged from 59 to 155 mg/kg. The maximum Zn contents (155 mg/kg) were found in *Amanita rubescens* species in the Švihov dam D1 area, while the minimum Zn contents (59 mg/kg) were found in the Humpolec D1 area. Overall, Fe had the highest content in soil (42,700 mg /kg) and in mushrooms (1887 mg/kg) among all the PTEs studied (Table 2).

### *Bioconcentration factors of PTEs*

The BCF is used to measure the extent of PTE accumulation in mushrooms relative to their concentration in the surrounding environment, such as soil. It is considered an essential indicator of PTE toxicity in mushrooms. It has four classes: BCF < 100, BCF = 100 – 1000, BCF = 1000 – 5000, and BCF > 5,000, ranging from low to very high health risks. It was recorded that BCF ranged from 0 to 43.5, indicating lower health risks. The Fe in *Leccinum versipelle* had the minimum (0) BCF, while Mn in *Xerocomellus chrysenteron* had the maximum BCF (43.5), indicating the maximum BCF from the studied metals and mushroom species (Table 3).

### *Health risks assessment*

Results revealed substantial variation across mushroom species and collection sites (Figure 2). The highest soil contamination was found under field mushrooms from Švihov ($3.44 \times 10^{-10}$ CTUh), while the lowest was recorded for blushing amanita and bay bolete from Ledenice ($6.30 \times 10^{-11}$ and $6.32 \times 10^{-11}$ CTUh, respectively). Regarding mushroom body contamination, the *Amanita rubescens* from Švihov exhibited the highest toxicity ($2.24 \times 10^{-10}$ CTUh), followed by the bay bolete from Ledenice ($2.05 \times 10^{-10}$ CTUh). In contrast, the lowest value was observed in *Amanita rubescens* from Ledenice ($8.89 \times 10^{-11}$ CTUh).

**Table 3.** Bioconcentration factors from the area studied

| Sites | Mushroom species | BCFs | | | |
|---|---|---|---|---|---|
| | | Cu | Fe | Mn | Zn |
| Ledenice | *Xerocomellus chrysenteron* | 2.83 | 0.10 | 43.5 | 1.67 |
| | *Chlorophyllum rhacodes* | 12.8 | 0.03 | 0.08 | 1.73 |
| | *Suillus grevillea* | 1.00 | 1.00 | 1.00 | 1.00 |
| | *Amanita rubescens* | 3.29 | 0.07 | 0.15 | 1.41 |
| | *Imleria badia* | 5.00 | 0.05 | 0.22 | 3.24 |
| | *Leccinum scabrum* | 1.21 | 0.03 | 0.62 | 1.74 |
| | *Agaricus campestris* | 5.48 | 0.13 | 0.17 | 1.43 |
| | *Boletus edulis* | 4.70 | 0.05 | 0.38 | 2.17 |
| | *Leccinum versipelle* | 7.77 | 0.11 | 0.39 | 2.59 |
| | *Leccinum versipelle* | 0.16 | 0.00 | 0.01 | 0.43 |
| | *Leccinum scabrum* | 0.43 | 0.03 | 0.07 | 1.08 |
| Humpolec D1 | *Amanita rubescens* | 1.64 | 0.10 | 0.12 | 1.03 |
| | *Imleria badia* | 1.40 | 0.10 | 0.12 | 1.10 |
| | *Xerocomellus chrysenteron* | 1.61 | 0.01 | 0.04 | 1.10 |
| | *Agaricus campestris* | 4.31 | 0.04 | 0.05 | 0.49 |
| Švihov dam D1 | *Amanita rubescens* | 1.54 | 0.12 | 0.15 | 1.13 |
| | *Chlorophyllum rhacodes* | 2.52 | 0.05 | 0.07 | 0.51 |

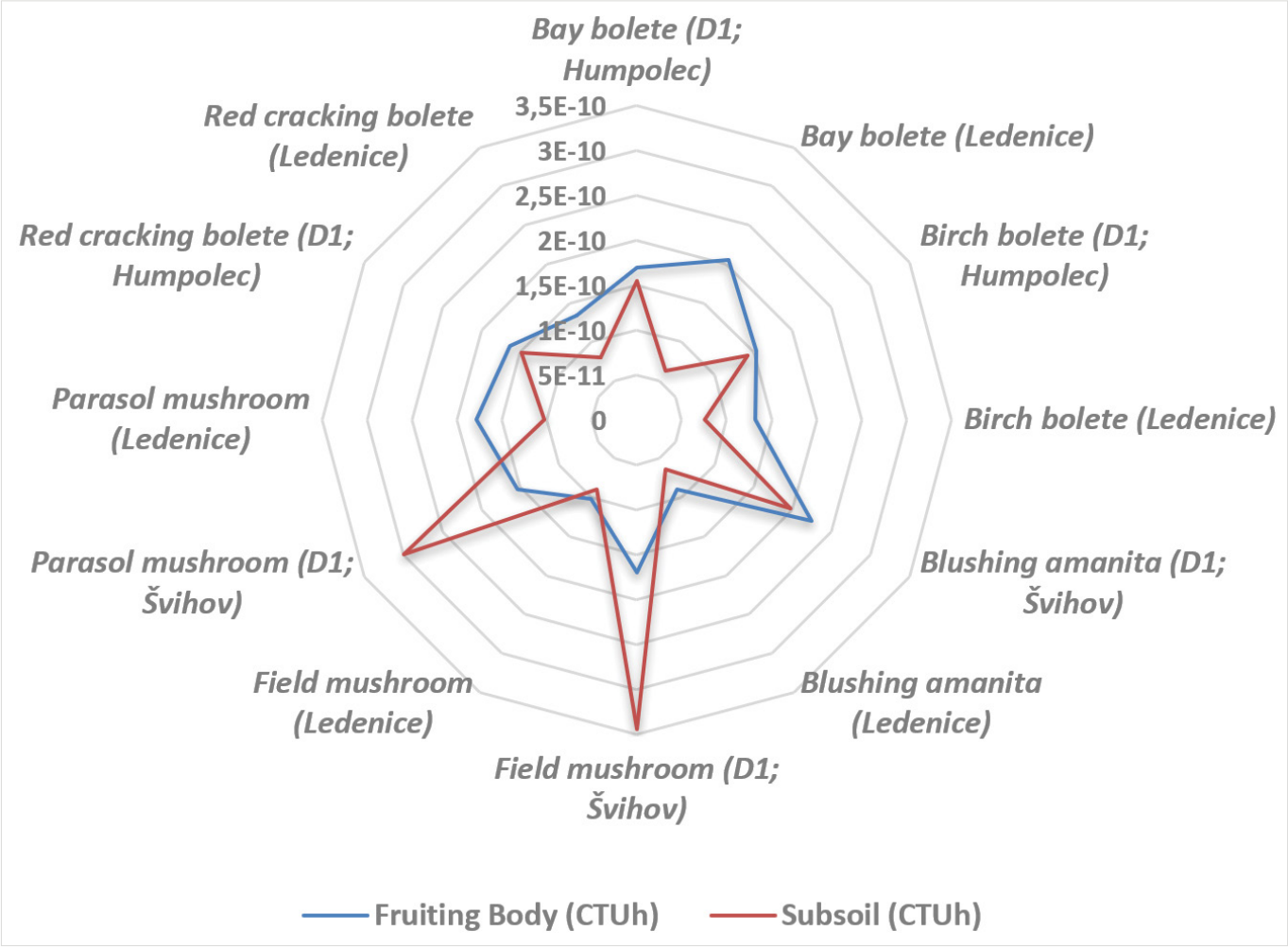



**Figure 2.** Human toxicity (non-cancer, inorganics) in soils and mushroom bodies from various wild mushroom species collected at three locations in the Czech Republic (D1; Humpolec, Ledenice, and Švihov), expressed in Comparative Toxic Units for humans (CTUh) using the Environmental Footprint 3.1 (adapted) characterisation method

Interestingly, certain mushrooms, such as bay bolete from Ledenice, showed high bioaccumulation despite low soil toxicity. In contrast, others, such as the field mushroom from Švihov showed moderate accumulation despite high soil contamination, indicating species-specific differences in inorganic uptake efficiency.

### *Pearson's correlation*

Pearson's correlation analysis was conducted to evaluate the relationships among PTE concentrations in both rhizosphere soils and mushroom tissues (Figure 3). In soils (Figure 3a), Cu did not show significant correlations with Fe, Mn, or Zn. However, Fe exhibited significant positive correlations with Mn ($r = 0.82$, $P < 0.05$) and Zn ($r = 0.91$, $P < 0.05$).

In mushroom samples (Figure 3b), stronger inter-element relationships were observed. Cu and Fe showed significant positive correlations with Mn ($r = 0.99$ and $r = 0.89$, respectively, $P < 0.05$) and Zn ($r = 0.71$ and $r = 0.86$, respectively, $P < 0.05$). Additionally, Mn and Zn were significantly correlated ($r = 0.71$, $P < 0.05$).

### *Principal component analysis*

The principal component analysis of the parameters, i.e., PTEs in soil samples, indicated that the first principal component explained 42.7% and showed a positive correlation with all PTEs. The second principal component explained 25.7% of the total variance and showed a correlation with other PTEs, with other PTEs with loading factors of 1.85 (Figure 4a, b).

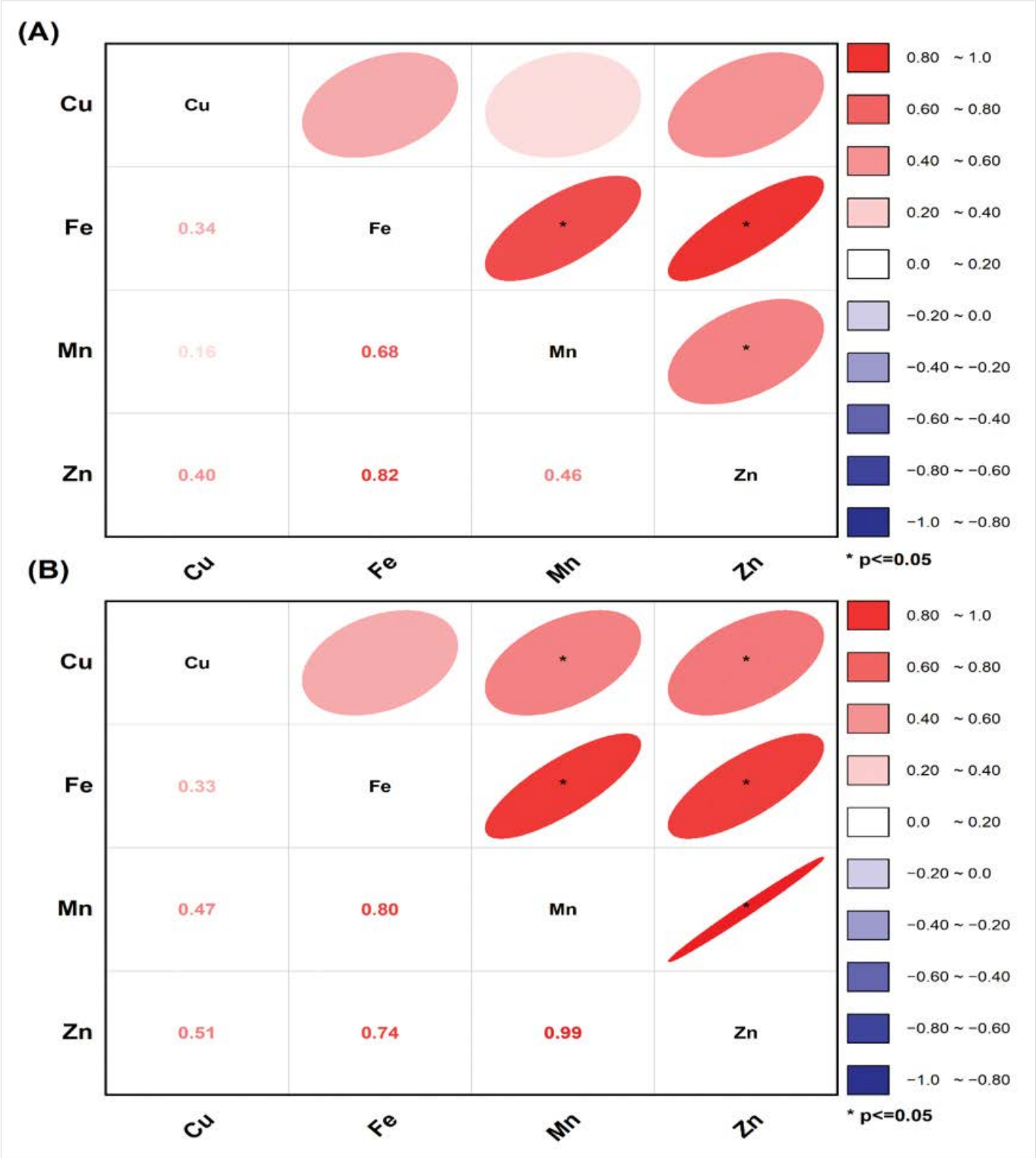



**Figure 3.** Pearson correlation matrices showing relationships among PTE concentrations in (a) rhizosphere soils and (b) mushroom fruiting bodies collected from the study area (Pearson correlation coefficients (r) are displayed; statistically significant correlations are indicated at $P < 0.05$)

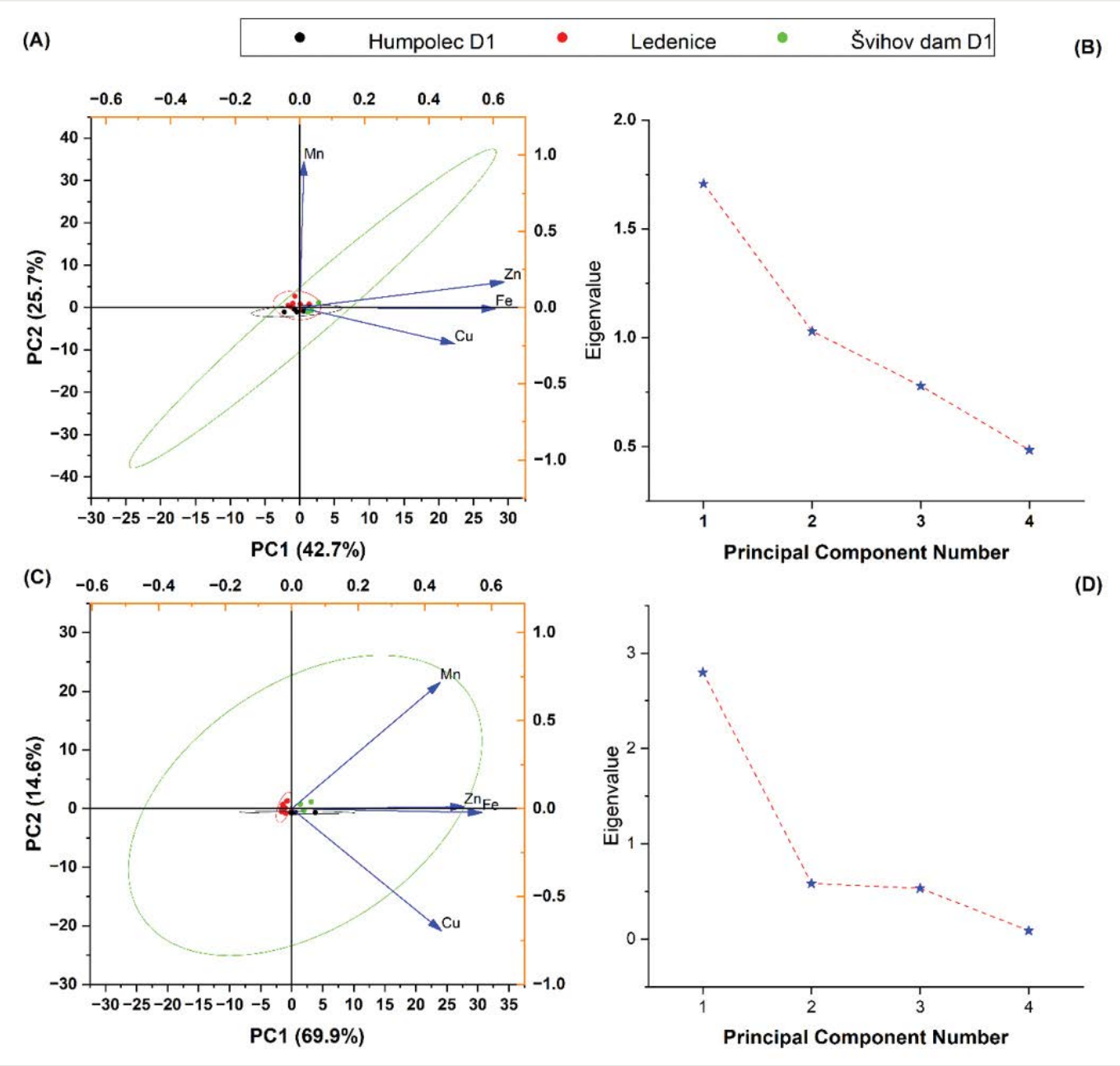



**Figure 4.** Principal component analysis (PCA) of PTE concentrations across sampling sites: (a) PCA biplot for rhizosphere soils, (b) scree plot for soil PCA, (c) PCA biplot for mushroom fruiting bodies, and (d) scree plot for mushroom PCA (Percentage variance explained by each principal component is indicated)

The principal component analysis of mushroom species and sites showed that the first principal component accounted for 69.9% and was positively correlated with all PTEs. The second principal component explained 14.6% of the total variance and showed correlations with Mn and Fe, with loading factors of 1.50 and 2.85 (Figure 4c, d). The analysis results show that there were significant influences from the sites and their correlation that could impact them.

## DISCUSSION

### *The concentrations of PTEs in the rhizosphere and mushroom species*

This study assessed PTEs (Cu, Fe, Mn, and Zn) in the rhizospheres of different mushroom species and their edible portions to evaluate human health risks associated with mushroom consumption, with a focus on the regions of Ledenice, Humpolec, and the Švihov dam in the Czech Republic. The acceptable range of Fe in soils is 2–300 mg/kg (Chandrasekara et al., 2024). The markedly higher Fe concentration observed at Švihov dam D1 (42,700 mg/kg) suggests combined effects of natural geochemical background and anthropogenic inputs, including traffic emissions, brake wear, and atmospheric deposition (Aftabtalab et al., 2022; Krachler and Krachler, 2021). Chandrasekara et al. (2024) reported similar higher contents of Fe in Sri Lanka, i.e., 339 mg/kg in paddy soils, while Skorbiłowicz et al. (2021) reported up to 4,605 mg/kg in roadside soils of Poland. Moreover, industrial activities in nearby regions may also contribute to higher Fe content in soils after atmospheric deposition and accumulation (Simiele et al., 2020). The Fe content of mushrooms growing in the respective areas was also higher, i.e., 137 – 4,030 mg/kg, with the highest value in *Amanita rubescens* from the Švihov dam D1 area. It is well established that PTEs accumulate in mushrooms with higher body mass ratios (Peters et al., 2018). Moreover, higher PTEs in the soils are also a factor that increases PTE uptake by mushrooms (Shojaei et al., 2021). The higher Fe in the mushrooms could be due to similar reasons, as Fe content was found to be high in the study areas, as reported by Oyetayo et al. (2021), who reported up to 417.6 mg/100 g of mushrooms. In another study, Gałgowska and Pietrzak-Fiećko (2020) reported up to 58.90 mg/kg Fe in *Cantharellus cibarius* species.

Copper concentrations reached 59.5 mg/kg in the Humpolec D1 region, exceeding the commonly recommended threshold of 20 mg/kg for uncontaminated soils (Caetano et al., 2016). This elevation may be associated with brake pad abrasion and vehicular emissions (Shabbir et al., 2020), indicating the influence of traffic-related contamination. Fagnano et al. (2020) reported Cu contents up to 217 mg/kg in soils of Naples, Italy. Similarly, Saleem et al. (2020) reported 2,153 mg/kg Cu in Hubei, China. Similar to Cu in soil, mushroom species also contained higher levels of Cu. Ronda et al. (2022) reported up to 52.59 mg/kg in *Russula emetica* mushroom species in Polish forest soils. The Mn content in the rhizosphere ranged from 6.0 to 1,330 mg/kg, with the highest content found in the Humpolec D1 region. This region is near the highway, and traffic-related sources include Mn in brake materials, diesel particulate matter, and infrastructure corrosion (Shukla et al., 2021). Its concentration should be 1.0 mg/kg in soil (Rashed et al., 2023), but Mn concentrations were much higher (1,334 mg/kg) in the study area. Similar results were previously reported by Monjardin et al. (2022), who found Mn contents up to 34,000 mg/kg in soils after flooding near a mining area. Wang et al. (2020) reported Mn concentrations up to 70.96 mg/kg in soils from Tianjin, China, due to road traffic and particulate matter. With higher Mn content in soils, the mushroom species also accumulated Mn, with 7 – 267 mg/kg Mn detected; *Xerocomellus chrysenteron* had the highest Mn content in the Ledenice area. Similar concentrations were reported by Sithole et al. (2022) in *Pleurotus ostreatus* species, i.e., 40.12 mg/kg in South Africa. The Zn content was also found to range from 43 to 238 mg/kg in the study area. Zn contents in soils should be within the range of 150 – 300 mg/kg (Rahman et al., 2021), but concentrations of 238 mg/kg in the Švihov dam D1 area indicate moderate Zn toxicity in the rhizosphere. Similar results were reported by Rahman et al. (2021), i.e., 329.70 mg/kg near roadside areas in Dhaka, Bangladesh, and 122.40 mg/kg in Hamedan, Iran (Hosseini et al., 2020). The Zn in mushroom species ranged between 59 and 155 mg/kg, which is also higher than the recommended Zn in the edible portion of the mushrooms. These higher Zn contents are transferred from the soil, and these higher Zn levels in mushrooms have previously been reported by Sithole et al. (2022), who detected 77.65 mg/kg Zn in *Pleurotus ostreatus*. Haro et al. (2020) also reported that Zn concentrations in Granada, Spain, were 363 mg/kg in Tricholoma equestre, a mushroom species.

Although BCF values were generally below 100, indicating low to moderate accumulation potential, elevated soil concentrations at roadside sites contributed to higher calculated non-carcinogenic toxicity values. The highest Mn BCF (43.5) corresponded with increased hazard indices at impacted sites, demonstrating that even moderate bioconcentration can contribute to measurable exposure risks when environmental concentrations are elevated.

### *Human non-carcinogenic health effects*

The human non-carcinogenic effects from mushroom consumption were higher in soils than in mushrooms. It was noted that the non-carcinogenic probability was the highest through the rhizosphere of *Agaricus campestris* from the Švihov dam D1 location, followed by the rhizosphere of *Chlorophyllum rhacodes* of the same area. In contrast, minimum with the rhizosphere of *Boletus edulis* from the Ledenice area, concluding that higher Zn concentration in rhizospheres can cause greater non-carcinogenic effects on humans. However, it was not at levels that would cause HNCT, but it could be toxic if its concentration continues to increase in the affected areas.

### *Public health and environmental implications*

From a public health perspective, these findings suggest that mushroom harvesting should preferentially occur in areas distant from highways and industrial sources. Regular monitoring of roadside soils and wild mushrooms may help prevent long-term exposure, and public awareness regarding contamination risks could support safer foraging practices in the Czech Republic.

## CONCLUSIONS

This study evaluated the concentrations of potentially toxic elements (Cu, Fe, Mn, and Zn) in the rhizosphere soils and fruiting bodies of wild mushrooms to assess potential non-carcinogenic human health risks associated with their consumption. Iron was the dominant element in soils, reaching up to 42,700 mg/kg, followed by Mn, Zn, and Cu. Elevated concentrations were also detected in mushroom tissues, with species-specific accumulation patterns observed. *Agaricus campestris* showed the highest Cu concentration, *Amanita rubescens* accumulated the highest Fe and Zn levels, and *Xerocomellus chrysenteron* exhibited the highest Mn concentration. Non-carcinogenic toxicity values were highest at the Švihov dam D1 site and lowest at the control site in Ledenice, indicating increased exposure potential in roadside environments. Overall, Švihov dam D1 represented the most impacted locality, followed by Humpolec D1, while Ledenice showed comparatively lower contamination levels.

## REFERENCES

Aftabtalab, A., Rinklebe, J., Shaheen, S. M., Niazi, N. K., Moreno-Jiménez, E., Schaller, J., Knorr, K. H. (2022) Review on the interactions of arsenic, iron (oxy)(hydr) oxides, and dissolved organic matter in soils, sediments, and groundwater in a ternary system. Chemosphere, 286, 131790. DOI: https://doi.org/10.1016/j.chemosphere.2021.131790

Ali, E. S., Nasution, A. S. (2019) Methods of reducing inorganic fertilizers with Aspergillus fungi on *Oryza sativa*. International Journal of Scientific Engineering and Science, 3 (5), 14-17.

Andreasi, B. S., Biganzoli, F., Ferrara, N., Amadei, A., Valente, A., Sala, S., Ardente, F. (2023) Updated characterisation and normalisation factors for the Environmental Footprint 3.1 method. Publications Office of the European Union. Available at: https://data.europa.eu/doi/10.2760/798894 [Accessed 13 July 2026]

Asare, M. O., Száková, J., Tlustoš, P. (2023) Mechanisms of As, Cd, Pb, and Zn hyperaccumulation by plants and their effects on soil microbiome in the rhizosphere. Frontiers in Environmental Science, 11, 1157415. DOI: https://doi.org/10.3389/fenvs.2023.1157415

Bayouli, I. T., Bayouli, H. T., Dell'Oca, A., Meers, E., Sun, J. (2021) Ecological indicators and bioindicator plant species for biomonitoring industrial pollution: Eco-based environmental assessment. Ecological Indicators, 125, 107508. DOI: https://doi.org/10.1016/j.ecolind.2021.107508

Caetano, A. L., Marques, C. R., Gonçalves, F., Da Silva, E. F., Pereira, R. (2016) Copper toxicity in a natural reference soil: ecotoxicological data for the derivation of preliminary soil screening values. Ecotoxicology, 25 (1), 163-177. DOI: https://doi.org/10.1007/s10646-015-1577-7

Chakrabarti, A., Campbell, B. L., Shonkwiler, V. (2019) Eliciting consumer preference and willingness to pay for mushrooms: a latent class approach. Journal of Food Distribution Research, 50 (1), 46-62.

Chandrasekara, C., Rajapaksha, I., Dissanayake, S., Kadupitiya, H., Sirisena, D., Chandrajith, R., Suriyagoda, L. (2024) Effects of climate, soil and water source on the distribution of bioavailable iron in lowland paddy soils of Sri Lanka. Applied Geochemistry, 160, 105875. DOI: https://doi.org/10.1016/j.apgeochem.2023.105875

Fagnano, M., Agrelli, D., Pascale, A., Adamo, P., Fiorentino, N., Rocco, C., Ventorino, V. (2020) Copper accumulation in agricultural soils: Risks for the food chain and soil microbial populations. Science of the Total Environment, 734, 139434. DOI: https://doi.org/10.1016/j.scitotenv.2020.139434

Fernandes, T., Garrine, C., Ferrao, J., Bell, V., Varzakas, T. (2021) Mushroom nutrition as preventative healthcare in Sub-Saharan Africa. Applied Sciences, 11 (9), 4221. DOI: https://doi.org/10.3390/app11094221

Gałgowska, M., Pietrzak-Fiečko, R. (2020) Mineral composition of three popular wild mushrooms from Poland. Molecules, 25 (16), 3588. DOI: https://doi.org/10.3390/molecules25163588

Garau, M., Castaldi, P., Pinna, M. V., Diquattro, S., Cesarani, A., Mangia, N. P., Vasileiadis, S., Garau, G. (2023) Sustainable restoration of soil functionality in PTE-affected environments: biochar impact on soil chemistry, microbiology, biochemistry, and plant growth. Soil Systems, 7 (4), 96. DOI: https://doi.org/10.3390/soilsystems7040096

Garg, V. K., Yadav, P., Mor, S., Singh, B., Pulhani, V. (2014) Heavy metals bioconcentration from soil to vegetables and assessment of health risk caused by their ingestion. Biological trace element research, 157 (3), 256-265. DOI: https://doi.org/10.1007/s12011-014-9892-z

Grimm, D., Kuenz, A., Rahmann, G. (2021) Integration of mushroom production into circular food chains. Organic Agriculture, 11 (2), 309-317. DOI: https://doi.org/10.1007/s13165-020-00318-y

Gwenzi, W., Tagwireyi, C., Musiyiwa, K., Chipurura, B., Nyamangara, J., Sanganyado, E., Chaukura, N. (2021) Occurrence, behavior, and human exposure and health risks of potentially toxic elements in edible mushrooms with focus on Africa. Environmental Monitoring and Assessment, 193 (5), 302. DOI: https://doi.org/10.1007/s10661-021-09042-w

Haro, A., Trescastro, A., Lara, L., Fernández-Figares, I., Nieto, R., Seiquer, I. (2020) Mineral elements content of wild growing edible mushrooms from the southeast of Spain. Journal of Food Composition and Analysis, 91, 103504. DOI: https://doi.org/10.1016/j.jfca.2020.103504

Hosseini, N. S., Sobhanardakani, S., Cheraghi, M., Lorestani, B., Merrikhpour, H. (2020) Heavy metal concentrations in roadside plants (*Achillea wilhelmsii* and *Cardaria draba*) and soils along some highways in Hamedan, west of Iran. Environmental science and pollution research, 27 (12), 13301-13314. DOI: https://doi.org/10.1007/s11356-020-07874-6

Kalač, P. (2013) A review of chemical composition and nutritional value of wild-growing and cultivated mushrooms. Journal of the Science of Food and Agriculture, 93(2), 209-218. DOI: https://doi.org/10.1002/jsfa.5960

Kowalska, J. B., Nicia, P., Gąsiorek, M., Zadrożny, P., Węgrzyn, M. H., Waroszewski, J. (2022) Are natural or anthropogenic factors influencing potentially toxic elements' enrichment in soils in proglacial zones? An example from Kaffiøyra (Oscar II Land, Spitsbergen). International Journal of Environmental Research and Public Health, 19 (20), 13703. DOI: https://doi.org/10.3390/ijerph192013703

Krachler, R., Krachler, R. F. (2021) Northern high-latitude organic soils as a vital source of river-borne dissolved iron to the ocean. Environmental science & technology, 55 (14), 9672-9690. DOI: https://doi.org/10.1021/acs.est.1c01439

Marschalko, M., Vicherek, P., Vicherková, M., Yilmaz, I., Kubáč, J., Popielarczyk, D., Yang, S. (2020) Soil contamination by tar in the alluvial sediments: case study of the brownfield remediation project in the Czech Republic. Environmental Earth Sciences, 79 (2), 52. DOI: https://doi.org/10.1007/s12665-019-8791-1

Monjardin, C. E. F., Senoro, D. B., Magbanlac, J. J. M., de Jesus, K. L. M., Tabelin, C. B., Natal, P. M. (2022) Geo-accumulation index of manganese in soils due to flooding in Boac and Mogpog Rivers, Marinduque, Philippines with mining disaster exposure. Applied Sciences, 12 (7), 3527. DOI: https://doi.org/10.3390/app12073527

Oyetayo, V. O., Ogidi, C. O., Bayode, S. O., Enikanselu, F. F. (2021) Evaluation of biological efficiency, nutrient contents and antioxidant activity of *Pleurotus pulmonarius* enriched with Zinc and Iron. Indian Phytopathology, 74 (4), 901-910. DOI: https://doi.org/10.1007/s42360-021-00410-7

Peters, D. E., Eebu, C., Nkpaa, K. W. (2018) Potential human health risk assessment of heavy metals via consumption of root tubers from Ogoniland, Rivers State, Nigeria. Biological trace element research, 186 (2), 568-578. DOI: https://doi.org/10.1007/s12011-018-1330-1

Procházka, J., Vinkler, P., Jójárt, K., Szenes, Z., Gruszczak, A., Kandrik, M. (2023) One threat-multiple responses: Countering hybrid threats in V4 countries. Obrana a strategie, 23 (1). DOI: https://doi.org/10.3849/1802-7199.23.2023.01.049-073

Rahman, M. S., Kumar, P., Ullah, M., Jolly, Y. N., Akhter, S., Kabir, J., Begum, B. A., Salam, A. (2021) Elemental analysis in surface soil and dust of roadside academic institutions in Dhaka city, Bangladesh and their impact on human health. Environmental Chemistry and Ecotoxicology, 3, 197-208. DOI: https://doi.org/10.1016/j.enceco.2021.06.001

Rashed, H. O., Jahangir, M. M. R., Hashem, M. A., Ferdous, J., Kader, M. A., Solaiman, Z. M., Hoque, T. S. (2023) Critical Limit of Manganese for Soybean in Soils of Three Agro-Ecological Zones of Bangladesh. Sustainability, 15 (23), 16410. DOI: https://doi.org/10.3390/su152316410

Ronda, O., Grządka, E., Ostolska, I., Orzeł, J., Cieślik, B. M. (2022) Accumulation of radioisotopes and heavy metals in selected species of mushrooms. Food chemistry, 367, 130670. DOI: https://doi.org/10.1016/j.foodchem.2021.130670

Royse, D. J., Baars, J., Tan, Q. (2017) Current overview of mushroom production in the world. In: Edible and medicinal mushrooms: technology and applications, pp. 5-13. DOI: https://doi.org/10.1002/9781119149446.ch2

Salami, A. O., Bankole, F. A., Olawole, O. I. (2016) Effect of different substrates on the growth and protein content of oyster mushroom (*Pleurotus florida*). International Journal of Biological and Chemical Sciences, 10 (2), 475-485. DOI: https://doi.org/10.4314/ijbcs.v10i2.2

Saleem, M. H., Fahad, S., Khan, S. U., Din, M., Ullah, A., Sabagh, A. E., Hossain, A., Llanes, A., Liu, L. (2020) Copper-induced oxidative stress, initiation of antioxidants and phytoremediation potential of flax (*Linum usitatissimum* L.) seedlings grown under the mixing of two different soils of China. Environmental Science and Pollution Research, 27 (5), 5211-5221. DOI: https://doi.org/10.1007/s11356-019-07264-7

Shabbir, Z., Sardar, A., Shabbir, A., Abbas, G., Shamshad, S., Khalid, S., Natasha, Murtaza, G., Dumat, C., Shahid, M. (2020) Copper uptake, essentiality, toxicity, detoxification and risk assessment in soil-plant environment. Chemosphere, 259, 127436. DOI: https://doi.org/10.1016/j.chemosphere.2020.127436

Shojaei, S., Jafarpour, A., Shojaei, S., Gyasi-Agyei, Y., Rodrigo-Comino, J. (2021) Heavy metal uptake by plants from wastewater of different pulp concentrations and contaminated soils. Journal of cleaner production, 296, 126345. DOI: https://doi.org/10.1016/j.jclepro.2021.126345

Shukla, A. K., Behera, S. K., Prakash, C., Tripathi, A., Patra, A. K., Dwivedi, B. S., Trivedi, V., Rao, Ch. S., Chaudhari, S. K., Das, S., Singh, A. K. (2021). Deficiency of phyto-available sulphur, zinc, boron, iron, copper and manganese in soils of India. Scientific reports, 11 (1), 19760. DOI: https://doi.org/10.1038/s41598-021-99040-2

SimaPro. (2025) SimaPro Craft 10.2.0.0 [software]. Amersfoort, The Netherlands: PRé Sustainability. Available at: https://simapro.com/ [Accessed 25 May 2026]

Simiele, M., Lebrun, M., Miard, F., Trupiano, D., Poupart, P., Forestier, O., Scippa, G. S., Bourgerie, S., Morabito, D. (2020) Assisted phytoremediation of a former mine soil using biochar and iron sulphate: Effects on As soil immobilization and accumulation in three Salicaceae species. Science of the Total Environment, 710, 136203. DOI: https://doi.org/10.1016/j.scitotenv.2019.136203

Šišák, L. (1996) The importance of forests as a source of mushrooms and berries in the Czech Republic. Mykologický sborník, 73, 98-101.

Sithole, S. C., Agboola, O. O., Mugivhisa, L. L., Amoo, S. O., Olowoyo, J. O. (2022) Elemental concentration of heavy metals in oyster mushrooms grown on mine polluted soils in Pretoria, South Africa. Journal of King Saud University-Science, 34 (2), 101763. DOI: https://doi.org/10.1016/j.jksus.2021.101763

Skorbiłowicz, M., Skorbiłowicz, E., Rogowska, W. (2021) Heavy metal concentrations in roadside soils on the Białystok-Budzisko route in northeastern Poland. Minerals, 11 (11), 1290. DOI: https://doi.org/10.3390/min11111290

Trujillo, K. G., Sánchez, J. E., Guillén, K., de la Rosa, A. P. B., Liedo, P. (2021) Edible mushroom protein content and antioxidant capacity increase the longevity of Anastrepha ludens fruit flies. International Journal of Medicinal Mushrooms, 23 (6). DOI: https://doi.org/10.1615/intjmedmushrooms.2021038305

Wang, S., Ji, Y., Zhao, J., Zhang, L., Li, Y. (2020) Sources and exposure risk of trace elements for traffic policemen in roadside locations. Transportation Research Part D: Transport and Environment, 78, 102176. DOI: https://doi.org/10.1016/j.trd.2019.11.005

Xing, L. (2022) Determination of toxic elements in food by ICP-MS using AOAC method 2015.01. Spectroscopy, 37 (10), 7-12. DOI: https://doi.org/10.56530/spectroscopy.zv7982s5

Zeeshan, N., Farooqi, Z. U. R., Ahmad, I., Murtaza, G., Jamal, A., Saifullah, Radicetti, E. (2023) Trace metals in rice grains and their associated health risks from conventional and non-conventional rice growing areas in Punjab-Pakistan. Sustainability, 15 (9), 7259. DOI: https://doi.org/10.3390/su15097259