**ARSENIC, CADMIUM AND MERCURY IN THE MACROLEPIOTA PROCERA (SCOP.) SINGER FRUITING BODIES**

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**ABSTRACT**

In this study, the content of As, Cd and Hg in fruiting bodies was determined. These three elements were determined in 94 Macrolepiota procera (Scop.) Singer samples (separately for caps and stems) and their corresponding soils/substrates collected from 8 different localities in Slovakia. Arsenic and cadmium were analyzed by Optical Emission Spectrometry with Inductively Coupled Plasma (OES-ICP), and mercury by Advanced Mercury Analyzer (AMA-254). In the soil/substrate As content varied from ND (notdetected) to 11.9, Cd from 0.66 to 22.9 and Hg from 0.02 to 0.28 mg kg−1 DW (dry weight), respectively. In fruiting body stems arsenic content varied from ND to 4.77, cadmium from ND to 5.96 and mercury from 0.03 to 2.83 mg kg−1 DW. In the caps, As content varied from ND to 13.0, Cd from ND to 19.8 and Hg from 0.04 to 4.00 mg kg−1 DW. After comparing obtained results with the EU limits, for As in mushrooms 6.6% (cap) of analyzed samples exceeded the limit value, while for Cd, 4.7% (cap), 2.0% (stem) of analyzed samples exceeded the limit value. Regarding the background values in the soils/substrates of selected elements in Slovakia only Cd exceeded the limits (though almost 82% of samples). Regarding Hg content, all analyzed samples (fruiting bodies and soils/substrates) did not exceed the limit value. The selected monitored localities in Slovakia have been contaminated with trace elements. Some of the analyzed mushroom samples exceeded EU limits, and as such, they can pose a risk to human health.

**Keywords:** Macrolepiota procera, Accumulation, Arsenic, Cadmium, Mercury

**INTRODUCTION**

Fungi are a very important part of ecosystems (Nnorom et al., 2020). They are a highly biodiverse group of organisms and play various roles in nature, the economy, environmental and food science, health, and are involved in soil mineral weathering, organic substrate decomposition and elemental recycling. For centuries, edible mushrooms have been collected from forests, or later cultivated, and consumed due to their nutritional value and unique flavor (Rasalanavho et al., 2020; Olah et al., 2020). Edible mushrooms, due to their chemical composition, constitute a food of nutritional value. They have low-fat content and high contents of minerals, essential amino acids, vitamins and fiber. Mushrooms are important sources of polysaccharides with immunomodulation properties. Also, mushrooms contain several natural phytochemicals with a wide range of positive health effects and medicinal benefits (Reis et al., 2020). Macrolepiota procera (Scop.) Singer, an edible wild-growing mushroom belongs to Agaricaceae family and is a saprobe. This species is widely distributed in the northern areas of Asia and across Europe. Saprobic mushrooms usually live on dead vegetable matter in forests, and they are the only multi-cellular organisms that can digest cellulose and lignin, the two major components of wood. According to that, mycorrhizal and saprobic mushrooms take part in a biogeochemical turnover of all mineral constituents in soil compartments or other substrates in which mycelium develops. It is known that M. procera is a good source of minerals, especially K, Mg and Se, as well as carbohydrates, proteins, and dietary fibers. However, this species is also an accumulator of toxic elements such as Cd, Hg and Pb (Durdić et al., 2020).

Mining and other industrial activities contaminate the environment with toxic hazardous elements such as arsenic, cadmium, lead, copper and mercury. Compared to organic compounds, metals are not biodegradable and can bioaccumulate (causing them to enter the trophic chain) (Nnorom et al., 2020). Metal concentrations in mushrooms are considerably higher than those in crops, vegetables and fruits (Gebrelibanos et al., 2016). The accumulation of elements in mushrooms is affected by fungal (mushroom species, morphological part of the fruiting body, development stages, age of mycelium, and biochemical compositions) and environmental factors (e.g., presence of organic matter, pH, metal concentrations in substrates) (Nnorom et al., 2020). Since heavy metals could enter the food chain, for example, as a result of uptake by edible mushrooms, it is necessary to determine the levels of essential and non-essential metals and report possible contamination levels that could be the cause for health hazards (Gebrelibanos et al., 2016). Human exposure to toxic elements occurs through a variety of sources, which includes the consumption of contaminated foods and water as well as through the inhalation or dermal contact of air pollutants (Nnorom et al., 2020). Hazardous metals are toxic and their accumulation over time in human bodies could cause serious illnesses (Gebrelibanos et al., 2016). Cadmium (Cd) is a non-essential toxic heavy metal, an environmental toxicant, and toxic at a low concentration, and it has no known beneficial role in the human body (Kumar and Sharma, 2019). The intake of cadmium-contaminated food over years can result in the build-up of cadmium in different vital organs and tissues including the liver, kidneys, immune system, bone, and reproductive organs, but mostly in the kidney cortex, and if cumulative exposure is high enough, it may harm kidneys and bones and is a potent cause of cancer and cardiovascular diseases (Qasemi et al., 2019). Chronic As ingestion has been associated with varied clinical complications including skin lesions, diabetes mellitus, bronchitis, cardiovascular disease, peripheral neuropathy, adverse reproductive outcomes and hematological effects. Ingestion of high concentrations of As causes cancer of the skin, lung, urinary bladder, kidney and other malignancies (Sinha and Prasad, 2020). Mercury is highly toxic for living organisms and it has no role in biological functions (Shahid et al., 2020). Human exposure to Hg has been associated with numerous toxic effects on the immune, digestive and nervous systems, as well as on kidneys, lungs, eyes and skin. The WHO considers Hg as one of the top ten chemicals/substances of major public health concern (WHO, 2019).
This study is part of a comprehensive survey to investigate arsenic, cadmium and mercury contents in macromycetes and their correspondent substrates to understand more the contamination risk by risk elements and the risk to local consumers in Slovakia. This review report As, Cd and Hg concentrations in soil/substrate and M. procera from 8 different localities in Slovakia.

MATERIAL AND METHODS

Study areas, sampling and samples preparation

This study was carried out in Slovakia in 8 different localities (Figure 1). The basic characteristic of sampling areas is shown in Table 1. The identification of the sampling areas was performed using GPS coordinates. In total, 94 samples of fresh fruiting bodies (M. procera) directly after pickup, were cleaned up from any organic and inorganic debris with a ceramic knife and the bottom part of the stem was cut-off. Subsequently, they were divided into two parts – cap and stem and dried until DW at 40 °C in a laboratory heat-oven with forced air circulation (Memmert GmbH & Co. KG, Schwabach, Germany) for ~22 hours. Finally, they were pulverized in the rotary homogenizer (IKA-Weber GmbH & Co. KG, Staufen, Germany) and stored in polyethylene bags until further analysis. Soil/substrate samples were taken from the same location as mushrooms, from a depth of approximately 0.10 m. The samples were dried for several weeks under laboratory conditions and then sieved through a pore size of 2 mm and stored in paper bags until analysis.

Figure 1 Map of sampling sites in Slovakia

Samples analysis

For this study, we decided to determine three elements: arsenic, cadmium and mercury. Total mercury concentration was determined by cold-vapor AAS analyzer AMA 254 (Advanced Mercury Analyzer, Altec, Czech Republic), separately in 94 samples of caps, stems and soils/substrates (282 samples in total). The mineralization was performed by using the pressure microwave digestion system (Ethos One, Milestone, Italy) in 5 mL of concentrated nitric acid (69%, trace purity) and 1 mL of hydrogen peroxide (30%, trace purity) with the addition of 1 mL DI (deionized) water. The samples were prepared in triplicate and quantitatively analyzed by inductively coupled argon plasma emission spectrometry (ICP-OES) on an Agilent ICP-OES 720 (Agilent Technologies, USA). This method was used to determine arsenic and cadmium contents in samples.

Table 1 Sampling areas, number of samples and element contents in analyzed samples

| Locality  | n   | As (mg kg⁻¹ DW) | Cd (mg kg⁻¹ DW) | Hg (mg kg⁻¹ DW) |
|----------|-----|-----------------|-----------------|-----------------|
|          | Soil |  Cap            | Soil            | Cap            | Soil | Cap            |
|          | AVG | S.D.            | AVG | S.D.            | AVG | S.D.            |
|          | min-max |            | min-max |            | min-max |            |
| Hodruša-Hámeľ 16 | 4.04±5.40 | 0.94±1.35 | 2.71±2.72 | 10.6±7.11 | 0.53±0.91 | 2.09±4.23 | 0.07±0.03 | 0.63±0.39 | 1.06±0.68 |
| Kráľovce 20 | 7.38±2.34 | 1.21±0.81 | 1.82±1.27 | 5.84±1.19 | 1.27±1.55 | 2.41±3.47 | 0.07±0.05 | 0.73±0.23 | 0.88±0.30 |
| Lazy pod 12 | 4.15±2.08 | 0.39±0.52 | 0.54±0.87 | 5.21±1.25 | 0.66±0.48 | 1.36±1.11 | 0.09±0.02 | 1.46±0.64 | 2.28±0.84 |
| Makytov 8 | 0.94±7.95 | 0.13±1.37 | 0.22±2.82 | 3.19±3.79 | 0.29±1.84 | 0.34±0.87 | 0.06±0.13 | 0.67±0.83 | 1.22±1.00 |
| Nemečky 6 | 2.53±1.66 | 0.50±1.12 | 1.40±2.33 | 3.24±0.33 | 0.24±0.02 | 0.41±0.27 | 0.12±0.07 | 0.73±0.16 | 1.03±0.26 |
| Orlovica 8 | 2.84±1.87 | 0.68±1.16 | 3.98±4.23 | 3.74±0.26 | 0.06±0.06 | 0.69±0.17 | 0.07±0.00 | 0.79±0.22 | 1.43±0.41 |
| Tesare 15 | 5.91±2.54 | 0.35±0.54 | 0.49±0.54 | 6.00±1.42 | 0.84±0.32 | 1.60±0.57 | 0.13±0.01 | 1.06±0.46 | 1.93±0.66 |
| Zbyhov 9 | 2.79±9.85 | 0.41±1.48 | 3.30±1.49 | 0.05±0.11 | 0.61±1.35 | 0.81±1.66 | 0.11±0.15 | 0.07±1.76 | 1.06±3.26 |

Figure 3 Average values of As, Cd and Hg in caps, stems and soil/substrate from 8 localities in Slovakia.

Statistical analysis

XLSTAT was used to calculate the average (AVG), standard deviation (S.D.), minimum and maximum values for each sample (Addinsoft, 2014). Two-way analysis of variance (ANOVA) was used to determine the differences between localities and after, the data were subjected to a t-test to compare each locality with the average value. Pearson’s correlation coefficient was used to determine the statistical relationship between tested variables, soils/substrates and mushrooms, with a significant level p<0.05. All statistical operations were performed using R studio version 1.2.5033 (RStudio Team, 2020).

RESULTS AND DISCUSSION

Arsenic, cadmium and mercury contents in analyzed samples (mushrooms and soil/substrate) are shown in Table 1.

Arsenic (As)

The arsenic concentration in the soil/substrate varied from ND to 11.9 mg kg⁻¹ DW. The highest arsenic average concentration was measured at locality Kráľovce (7.38±2.34 mg kg⁻¹ DW) and the lowest in Ņemčeky (2.53±1.66 mg kg⁻¹ DW). In mushroom stems, the highest average arsenic concentration was detected just like in the soil/substrate (Kráľovce) and it was 1.21±0.81 mg kg⁻¹ DW. The highest (0.27±0.99 mg kg⁻¹ DW) was detected at locality Ľubov. Arsenic content in mushroom caps varied from ND to 13.0 mg kg⁻¹ DW. The highest average concentration was 3.98±4.23 mg kg⁻¹ DW (Orlovica) and the lowest 0.49±0.54 mg kg⁻¹ DW (Zbyhov).

The highest arsenic concentrations (for soil and cap) were measured at locality Kráľovce, which is following the fact that this locality is classified to the 2nd environmental region quality (regions with moderately disturbed environment) (Environmental regionalization of the Slovak Republic, 2016).

According to the EU limit value, the limit in edible mushroom (both, cap and stem) for As is 0.50 mg kg⁻¹. Compared with this, 6.6% of mushroom caps exceeded the limit, while all of the mushroom stems were below the limit value. For soil/substrate, the background value for As in Slovakia is 6.80 mg kg⁻¹ (Šefik et al., 2008) and compared with this, all measured soil/substrate samples did not exceed the background value.

Stefanovič et al. (2016) studied Ag, Se and As concentration in M. procera (n=41) and its soil/substrate (n=30) from Serbia. The content of total arsenic in caps of investigated M. procera ranged from below the detection limit (0.01 mg kg⁻¹ DW) to 3.40 mg kg⁻¹ DW. Average concentrations were ranging from 0.50±0.10 to 1.80±1.30 mg kg⁻¹ DW for caps and from 0.50±0.40 to 1.30±0.80 mg kg⁻¹ DW for stems. Vetter (2004) analyzed arsenic contents of 162 fruit body samples of 37 common edible mushrooms (Hungary). Notable arsenic contents were measured in different Agaricus, Lepista and Macrolepiota species. In Macrolepiota procera the average arsenic concentration was 4.84±5.67 mg kg⁻¹ DW. The highest arsenic levels were found in fruit bodies of Lactarius amethystea (147 mg kg⁻¹ DW) and Macrolepiota rhododes (42.6 mg kg⁻¹ DW), respectively. Their measured data demonstrated the role of the taxonomic position of the mushroom in arsenic uptake and content. The arsenic-accumulating capacity (ability) of macroscopic edible mushrooms were found in two orders of the class Basidiomycetes (Agaricales and Gasteromycetales). Some taxa of these orders can accumulate high amounts of arsenic and this phenomenon seems to be independent of their habitats.
As part of the statistical analysis, we used a boxplot for graphically depicting groups of numerical data through their quartiles. Figure 2 shows the relationship between locality and the analyzed sample. There is a strongly significant difference between locality and soil/substrate (p=2.2e-07). A strong difference is between two localities and soil: soil/Kráľovce and soil/Lozorno, while even though between soil/Tesáre there is a significant difference, it is very low. Lozorno is significantly lower, and Kráľovce is significantly higher in the proliferation group (Figure 2). Considering mushroom stems, we can say that there are no significant differences between locality/stem, since p=0.098. The only difference is between stem/Kráľovce and it is very low. Nemečky and Tesáre are significantly lower and Kráľovce is significantly higher in the proliferation group. The relationship between locality and cap showed that there is a low significant difference, p=0.016, but a strong significant difference was found in cap/Lazy pod Makytou and a very strong significant difference in cap/Zbyňov. From Figure 2 we can conclude that results from Orovnica are significantly higher and Lazy pod Makytou is significantly lower in the proliferation group. These boxplots statistically confirmed measured data.

Figure 2 Arsenic concentrations in mushrooms and soils/substrates, concerning localities. The dashed line presents the average concentration for soil, stem and cap samples, separately. Note: The lowest data point presents minimum data value; the highest data point presents maximum data value; the middle value of the dataset presents median, and dots out of the box are outliers.

Cadmium (Cd)

The Cd concentration in the soil/substrate varied from 0.66 to 22.9 mg kg\(^{-1}\) DW (Table 1). The highest cadmium average concentration was measured in Hodruša-Hámre (10.6±7.11 mg kg\(^{-1}\) DW) and the lowest in Lozorno (1.12±0.33 mg kg\(^{-1}\) DW). In mushroom stems, the highest average cadmium concentration was detected in Kráľovce (1.27±1.55 mg kg\(^{-1}\) DW) and the lowest (0.06±0.48 mg kg\(^{-1}\) DW) was detected in Orovnica. Cd content in mushroom caps varied from ND to 19.8 mg kg\(^{-1}\) DW. The highest average concentration was 4.94±6.30 mg kg\(^{-1}\) DW (Lozorno) and the lowest 0.41±0.27 mg kg\(^{-1}\) DW (Nemečky).

The highest cadmium concentration (in soil/substrate) was measured in Hodruša-Hámre and this is because the samples were collected from the mining area, but the highest cadmium concentration in caps was detected in Kráľovce, that is following the fact that this locality (Kráľovce) belongs to the 2nd environmental region quality (regions with moderately disturbed environment).
According to the EU limit value, the limit in edible mushrooms (both, cap and stem) for Cd is 0.50 mg kg\(^{-1}\). In concordance with this, almost 2.0% of mushroom stems and 4.7% of mushroom caps exceeded the limit. For soil/substrate, the background Cd value in Slovakia is 0.20 mg kg\(^{-1}\) (Šefík et al., 2008) and compared with this, almost 82% of samples exceeded the background value.

Arvay et al. (2014) analyzed 92 samples (12 species) of wild edible mushrooms in the Slovak Paradise National Park, that borders with a region of historical mining and processing of polymetallic ore, and they found out that the cadmium content in caps of analyzed mushroom exceeded statutory limits in 96% of the samples. Jančo et al. (2019) examined selected risk elements (Cd, Pb and Hg) contamination in 50 samples of M. procera. The cadmium content in mushroom caps varied from ND to 0.88 mg kg\(^{-1}\) DW and the highest average concentration was in Lozorno (4.94 mg kg\(^{-1}\) DW) and the lowest in Nemečky (0.41 mg kg\(^{-1}\) DW). Considering the admissible limit for Cd in edible mushrooms, 76% of samples exceeded the limit. The Cd content in stems varied from 0.21 to 3.67 mg kg\(^{-1}\) DW. When considering the EU limit, 54% of mushroom stem samples were above the limit.

Figure 3 shows the relationship between locality and the analyzed sample. There is a strongly significant difference between locality and soil/substrate (p=1.9e-05). A strong difference is between four localities and soil: soil/Orovnic, soil/Lozorno, soil/Nemečky and soil/Tesáre, while between soil/Hodruša-Hámre there is a low significant difference. Figure 3 shows that Lozorno is significantly lower, and Hodruša-Hámre is significantly higher in the proliferation group. Considering mushroom stems, we can say that there are no significant differences between locality/stem, since p=0.16, but there is a very strong difference between stem/Orovnic and stem/Nemečky. Orovnic is significantly lower and Hodruša-Hámre is significantly higher in the proliferation group. The relationship between locality and cap showed that there is no significant difference, pرف 2.5, but a very strong significant difference was found in cap/Nemečky and cap/Orovnic. From Figure 3, we can conclude that Lozorno is significantly higher and Nemečky is significantly lower in the proliferation group.

Mercury (Hg)

The mercury content in the soil/substrate varied from 0.02 to 0.28 mg kg\(^{-1}\) DW (Table 1). The highest Hg average soil concentration was measured at locality Zbyňov (0.13±0.01 mg kg\(^{-1}\) DW) and the lowest one in Lozorno (0.04±0.02 mg kg\(^{-1}\) DW). In mushroom stems, the highest average mercury concentration was detected at locality Lazy pod Makytou and it was 1.46±0.64 mg kg\(^{-1}\) DW and the lowest detected Hg content was 0.63±0.39 mg kg\(^{-1}\) DW (Hodruša-Hámre). Hg content in mushroom caps varied from 0.04 to 4.00 mg kg\(^{-1}\) DW. The highest average concentration was 2.28±0.57 mg kg\(^{-1}\) DW (Lazy pod Makytou) and the lowest 0.88±0.30 mg kg\(^{-1}\) DW (Kráľovce).

The highest mercury concentrations were measured at localities Zbyňov and Lazy pod Makytou, even though these localities belong to the 1st environmental quality region (regions with the undisturbed environment). This fact can be explained by the high ability of M. procera to accumulate mercury, even those concentrations which are considered to be negligible.

The EU limit value in edible mushroom (both, cap and stem) for Hg is 0.75 mg kg\(^{-1}\). In concordance with this, no mushroom cap/stems exceeded the limit. In Slovakia, the background mercury value in the soil is 0.06 mg kg\(^{-1}\) (Šefík et al., 2008) and all of the analyzed samples were below this limit.

Our previous study showed that the analyzed Macropleiotia procera from Central Slovakia (Banská Bystrica) had an average concentration of mercury 1.98 mg kg\(^{-1}\) (from 0.41 to 3.20 mg kg\(^{-1}\)) in the caps and 1.40 mg/kg (from 0.12 to 1.75 mg kg\(^{-1}\)) in stems. Another study showed that the mercury content in mushroom caps varied from ND to 0.40 mg kg\(^{-1}\) and from ND to 0.28 mg kg\(^{-1}\) in mushroom stems. The limit value for Hg in edible mushrooms was lower than the limit in all samples (Jančo et al., 2019; Arvay et al. 2014).

Míček et al. (2020) reported the content of 34 elements in four edible mushroom species: Boletus edulis, Inleria badia, Leccium scabrum and Macropleiotia procera, and associated soil collected from Polish forests between 1974 and 2019. In the soil, the mean content of As was 1.37 mg kg\(^{-1}\) DW (with the range of 0.19 to 2.31 mg kg\(^{-1}\) DW), of Hg 0.06 mg kg\(^{-1}\) DW (with the range of 0.05 to 0.08 mg kg\(^{-1}\) DW) and of Cd the mean content was 0.04 mg kg\(^{-1}\) DW (with the range of below detection limit to 0.21 mg kg\(^{-1}\) DW). In the fruiting bodies, As content varied from 0.09 to 1.51 mg kg\(^{-1}\) DW (mean value 0.61 mg kg\(^{-1}\) DW), Cd content from 0.04 to 0.64 mg kg\(^{-1}\) DW (mean value 0.25 mg kg\(^{-1}\) DW) and Hg content varied from 0.62 to 2.80 mg kg\(^{-1}\) DW (mean value 1.74 mg kg\(^{-1}\) DW). Falandybyst et al. (2017) analyzed the multi-elemental composition and associations between a group of 32 elements and 16 rare earth elements collected by mycelium from growing substrates and accumulated in fruiting bodies of Macropleiotia procera from 16 sites from the lowland areas of Poland. Cadmium and mercury are common constituents of M. procera and they occurred in caps at 2.10±2.40 mg kg\(^{-1}\) DW (arithmetic mean plus standard deviation) for cadmium and 2.00±0.50 mg kg\(^{-1}\) DW for mercury. According to them, if assume that Cd and Hg remain in the flesh of caps, when they are sautéed, roasted, fried in butter, grilled, or roasted with eggs, a single mushroom dish (100 to 300 g) certainly will provide an elevated quantity of each heavy metal (0.02–0.06 mg of Cd per capita; 0.02–0.06 mg of Hg per capita). Hence, frequent eating of M. procera caps is not recommended.

Figure 4 shows the relationship between locality and the analyzed sample. There is a strongly significant difference between locality and soil/substrate (p=7.8e-06). A very strong difference is between two localities and soil: soil/Zbyňov and soil/Lozorno, while between soil/Orovnic there is a low significant difference. From the plot above, we can notice that Lozorno is significantly lower, and Zbyňov is significantly higher in the proliferation group. Considering mushroom stems, we can say that there are strong significant differences between locality/stem, since p=3.8e-10, but there is only a low difference between three localities: stem/Kráľovce, stem/Hodruša-Hámre and stem/Lazy pod Makytou. Hodruša-Hámre is significantly lower and Lazy pod Makytou is significantly higher in the proliferation group.

A strong significant difference (p=1.8e-05) was found between locality and cap (Kráľovce-Krníšov and cap/Lazy pod Makytou). From Figure 4, we can conclude that Lazy pod Makytou is significantly higher and Krníšov is significantly lower in the proliferation group.
Depending on the fruiting body collection site, there can be a higher, lower, or significantly differentiated ability of mushrooms to accumulate individual elements/metals. However, the efficiency of element accumulation depends not only on their concentration in a substrate, but also on mushroom species, genus, or the families to which they belong (Mleczek et al., 2020b). The hypothesis that the increasing age of mycelium and a protracted interval between fructifications significantly elevate the contents of many elements in fruiting bodies and usually higher levels occur in caps than in stems (Kaláč, 2019). This is also in accordance with our measured data; the higher levels were detected in caps than in stems.

Pearson’s correlation test

Pearson’s correlation test (significant level p<0.05) was used to determine the relationship between the variables, in our case: analyzed samples (mushrooms and soils/substrates) and selected risk elements (As, Cd and Hg) (Figure 5). Regarding arsenic content, between stem/cap was found a positive weak correlation. Between soil/stem there was no correlation found and in soil/cap there was found a weak negative correlation. This shows that the detected As content in the fruiting bodies is not from the soil/substrate, but yet arsenic was passed from the stem to the fruiting body tissues.

Relationship between As, Cd and Hg contents in mushrooms (caps/stems) and soils/substrates

Health risk assessment

Based on the total mercury content determined in the studied biological samples and their average weekly consumption, Provisional Tolerable Weekly Intake - PTWI (%) was estimated. The percentage of PTWI assesses the health risk from exposure to the toxic element. To determine the percentage of PTWI for Hg derived from the consumption of the studied mushroom species, concentration levels and consumption levels were combined. The %PTWI was calculated for the whole fruiting body using concentration data obtained for each anatomic part. The percentage excess of tolerable weekly intakes per person weighing 70 kg for Hg is 0.28 mg person per week and for Cd is 0.44 mg person per week (JECCA, 2011) and the average consumption of “Other vegetables and mushrooms” in Slovakia was 0.23 kg person per week in 2018 (Statistical Organization of Slovak Republic, 2019). It was calculated as follows:

\[
\text{PTWI (\%) = \left( \frac{\text{element content in BS}}{\text{element content in PTWI}} \right) \times 100}
\]

where: BS is the measured concentration of a selected element (Hg) in the biological sample (mg kg\(^{-1}\) of fresh weight in mushrooms). If the detected value was greater than 100%, the consumption of mushroom samples from the area is potentially hazardous.

The PTWI for Hg is 300 µg (equivalent to 5 or 0.7 µg kg\(^{-1}\) body weight (BW) per day), and a reference dose is 0.3 µg kg\(^{-1}\) BW per day (JECCA 1978; US EPA 2005). PTWI for cadmium is 7 µg kg\(^{-1}\) BW (equivalent to 1 µg kg\(^{-1}\) BW per day) (European Food Safety Authority EFSA 2009). Results indicated that %PTWI using mean Hg concentration values ranged between 7.30 and 18.7 %PTWI for mushrooms caps and in the range 5.10–12.0 %PTWI for mushroom stems. For Cd, %PTWI ranged between 2.20 and 26.0 %PTWI for mushroom caps, and in the range of 0.30 and 6.60 %PTWI for mushroom stems. None of the analyzed samples did exceed the recommended daily intake.

Caps of *M. procera* can be large and are highly valued by the mushroom pickers, and all findings considered here can be used. Some individuals from the local countryside populations and the cottage house owners or the camp tourists, temporarily, eat them even several times a week during the harvesting season. Hence, in a good “mushroom” year an abundance of this species will lead to a short time exposure of fanciers to elevated toxic elements (e.g. Cd and Hg) doses that exceed the PTWI (Gucia et al., 2012).

CONCLUSION

Wild-growing mushrooms are consumed as a delicacy in many countries. Extensive research has been carried out to investigate the accumulation of three selected toxic elements (As, Cd and Hg) in edible wild-growing *Macrolepiota procera* and its corresponding soil/substrate. This research was carried out in 8 different localities in Slovakia. This study showed that the analyzed fruiting bodies and soil/substrate samples were contaminated with all of the selected elements and as such, they can pose a threat to human health. Statistical analysis confirmed the transport of trace elements (especially Hg) from the soil to the mushroom (stem/cap).

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Figure 4 Mercury concentrations in mushrooms and soils/substrates, concerning localities. The dashed line presents the average concentration for soil, stem and cap samples, separately. Note: The lowest data point presents minimum data value; the largest data point presents maximum data value; the middle value of the dataset presents median, and dots out of the box are outliers.

Figure 5 Relationship between As, Cd and Hg contents in mushrooms (caps/stems) and soils/substrates
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