Ecotoxicology and Environmental Safety 232 (2022) 113235

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Mercury in scarletina bolete mushroom (*Neoboletus luridiformis*): Intake, spatial distribution in the fruiting body, accumulation ability and health risk assessment

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ARTICLE INFO

Edited by Dr Fernando Barbosa

Keywords:
Mercury
Neoboletus luridiformis
Spatial distribution
Accumulation
Risk assessment

ABSTRACT

In the present work, we focused on two aspects of mercury (Hg) bioconcentration in the above-ground parts of *Neoboletus luridiformis*. In the first part, we monitored the bioconcentration potential of individual anatomical parts of a particular fruiting body and evaluated the obtained data by the spline interpolation method. In the second part, we focused on assessing the mercury content in 378 samples of *N. luridiformis* and associated samples of substrates from 38 localities with different levels of Hg content in Slovakia. From the obtained data of Hg content in samples of substrate and fungi, we evaluated ecological indicators (geoaccumulation index – *Igeo*, contamination factor – *Cf* a potential ecological risk – *PER*), bioconcentration indicators (bioconcentration factor – *BCF*; cap/stipe quotient – *Qc/s*) and health indicators (percentage of provisional tolerable weekly intake – *% PTWI* a target hazard quotient – *THQ*). Based on the Hg distribution results, the highest Hg content was found in the tubes & pores (3.86 mg/kg DW), followed by the flesh of cap (1.82 mg/kg DW). The lowest Hg content was in the stipe (1.23 mg/kg DW). The results of the *BCF* values indicate that the studied species can be included in the category of mercury accumulators. The results of the ecological indices representing the state of soil pollution pointed out that two localities (Malachov and Nižná Slaná) stood apart from all monitored localities and showed a state of an extremely disturbed environment. This fact was also reflected in the values of Hg content in the fruiting bodies of the studied mushroom species. In the case of the consumption of mushrooms from these localities, it can be stated that long-term and regular consumption could have a negative non-carcinogenic effect on the health of consumers. It was confirmed by the *%PTWI* (Malachov: 57.8%; Nižná Slaná: 53.2%) and *THQ* (Malachov: 1.11 Nižná Slaná: 1.02). The locality Čačín-Jelšovec is interesting from the bioconcentration characteristics point of view, where the level of environmental pollution was the lowest (Hg content in the soil was below the background value) compared to other localities, however, the *THQ* value was the highest (1.29).

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https://doi.org/10.1016/j.ecoenv.2022.113235
Received 12 October 2021; Received in revised form 18 January 2022; Accepted 21 January 2022
Available online 24 January 2022

1. Introduction

Edible wild mushrooms – macromycetes are an integral part of terrestrial ecosystems, especially forest ecosystems (Benchawattananon, 2016). Their tasks are to decompose organic matter into inorganic and thus to close the nutrient cycle in ecosystems (mainly saprophytic mushrooms) (Gadd, 2007) and, in the case of mycorrhizal fungi, in interaction via roots to enhance receiving of water and nutrients to the plant tissue. Plants on the other hands allow photosynthesis products to reach the fungus (Læssøe and Petersen, 2019). Edible wild mushrooms are an integral part of the human diet in many countries (Valverde et al., 2015). An important benefit of mushroom consumption is their nutritional value (Kalač, 2016), as well as the pharmacological effect of their substances (Türkekul et al., 2017). Mushrooms are characterized by specific physiology that among other things causes uptake of micro and macro elements to the mycelium and seasonal translocation into the above-ground parts (fruiting bodies) during the fructification process. The bioavailability and dynamics of the element uptake are influenced by many factors, such as environmental (pH of the substrate, the content and mobility of elements in the substrate and the organic matter content) and intrinsic (taxon, developmental stage, mycelium age, etc.) (Falandysz et al., 2020; Kokkoris et al., 2019; Urminská et al., 2013, Urminská et al., 2010, Urminská et al., 2004). This uptake can be considered problematic from a consumer (humans and animals) point of view, in areas that are contaminated by risk elements originating from anthropogenic or natural sources, due to toxic elements accumulated in fruiting bodies, as evidenced by several studies (Árvay et al., 2017, 2014; Falandysz et al., 2017; Kalač, 2016; Slávik et al., 2016; Záhorcová et al., 2016). Accumulation of Hg (both total and methyl Hg) in mushrooms is species-specific and influenced by the ecological groups of mushrooms (Rieder et al., 2011). This fact is supported by many studies that monitored bioconcentration characteristics of many different mushroom species in different conditions (Falandysz et al., 2015a, b, 2008, 2007; Kojta and Falandysz, 2016; Melgar, Alonso, and García, 2009; Ouzuni et al., 2009; Rzymski et al., 2016; Širić et al., 2017). Whereas mushrooms are consumed processed, certain risk elements can be lost or increased depending on the various culinary processes such as blanching, boiling and canning that have a positive effect on the final concentration of these pollutants in food (wet weight) but it can take out also nutritional ingredients. Braising, deep-oil wok frying, flat pan-frying, grilling can cause an increase of a pollutant (e.g., Hg, ^{137}Cs) in a cooked mushroom meal (wet weight) and thus the meal can contribute substantially to the intake of the pollutant. (Falandysz et al., 2021, 2019a, b). Blanching combined with other culinary processes can substantially decrease concentration of toxic elements such as Hg (Falandysz and Drewnowska, 2017). Chiocchetti et al. (2020) reported that the cooking of mushrooms reduced mainly the levels of As. The reduction of Hg, Cd, and Pb levels was lower.

Mercury (Hg) is one of the toxic elements especially for humans and animals (Pirrone et al., 2009) and it is found among the top ten chemicals or groups of chemicals of major public health concern (WHO, 2017). Large anthropogenic Hg emissions and its increased accumulation occurred with intensive industrial activities after Industrial Revolution. Since 1970s, the industrial activities connected to Hg production has gradually shifted from Europe and North America to Asia (Li et al., 2020). Although the use of Hg is currently severely limited, the human population is still exposed to health-threatening doses of Hg (Buchanan et al., 2015). Middle and low-income countries are facing increased exposure to Hg, mainly due to small-scale gold mining and burning of coal and toxic waste, lack of environmental regulation and limits in mobility and food choices (Anyanwu et al., 2018; Kampalath and Jay, 2015; Preker et al., 2016; UNEP, 2013). Prolonged exposure of the human body to Hg can lead to toxic effects on the immune, digestive and nervous systems, eyes and skin and to pulmonary, urinary, and reproductive problems (Rzymski et al., 2015; WHO, 2017). An example is a well-known fact that marine organisms contain significantly higher concentrations of Hg compared to freshwater organisms, and thus their regular and long-term consumption creates an increased risk of possible intoxication of consumers, especially in coastal areas (Barone et al., 2021, 2015; Mergler et al., 2007; Sulimanec Grgec et al., 2021). However, it should be noted that Hg in trace and/or ultra-trace quantity always occurs in food without health risk impact. From an ecotoxicological point of view, Hg is characterized as non-degradable contaminant with high bioaccumulative ability. Its natural content in the Earth's crust is 80 μg/kg (Gworek et al., 2016). The background value for the territory of Slovakia is 60 μg/kg (Šefčík et al., 2008). Its resources in the environment are of natural (geochemical anomalies, fires, etc.), and anthropogenic origin, as well. The main concern is areas affected by long-term human activity (mining and smelting activities, energy industry, burning, etc.) (Angelovičová and Fazekašová, 2014; Dadová et al., 2016; Demková et al., 2017a). The chemical form of risk elements in the environment is important and affects their toxicology. In the case of Hg, its organic compounds, especially monomethyl-Hg are the most toxic for living organisms (Ha et al., 2017; Mason and Benoit, 2003).

Scarletina bolete – *Neoboletus luridiformis* (Rostk.) Gelardi, Simonini & Vizzini (Class: *Agaricomycetes*; Order: *Boletales*; Family: *Boletaceae*; Genus: *Neoboletus*) is a mycorrhizal mushroom that can be found in the whole northern hemisphere. It occurs primarily in coniferous forests (fir, spruce), and/or in deciduous forests (beech, oak). It is a typical genus of sub-montane and montane areas, growing from June to November. It is an edible mushroom with a typical flavor and aroma and characteristic blue colouration on the cut surface. However, it may cause stomach upset if it is undercooked. Therefore, the species is not considered edible in many countries. Edible wild mushrooms, especially *Boletaceae* spp., are very popular among pickers, which may pose a health risk especially in areas contaminated with risk elements (Slávik et al., 2016; Záhorcová et al., 2016). The current name of the species is used since 2014 (Gelardi et al., 2014). The species was earlier called differently, for example, *B. luridiformis* and *B. erythropus*. Based on the available resources, it is evident that the species went through many changes in nomenclature in recent decades. Discrepancies in the nomenclature were caused by the unavailability of modern molecular analytical methods that can separate some species previously considered as one. However, we still find these discrepancies in the literature (Urban and Klofac, 2015).

This work presents a novel approach to illustrate the translocation and spatial distribution of Hg in the mushroom fruiting body using an interpolation method. Furthermore, it focuses on monitoring the Hg content in the different anatomical parts of *N. luridiformis* collected from different locations in Slovakia characterized by varying degrees of Hg contamination. The degree of contamination of the monitored areas is determined by the contamination factor (C_f), index of geoaccumulation (I_{geo}) and potential ecological risk (*PER*). The level of Hg uptake from soil to the above-ground parts of the mushrooms is assessed by the bioconcentration factor for each anatomical part separately (BCF_{cap} and BCF_{stipe}). In addition to the interpolation, the quotient cap/stipe ($Q_{c/s}$) parameter was used to assess the level of mercury translocation within the fruiting body. The health risk resulting from the mushroom consumption is evaluated using the provisional tolerable weekly intake ($\%PTWI_{Hg}$) defined by the World Health Organization and target hazard quotient (*THQ*).

2. Materials and methods

2.1. Preparation of the N. luridiformis sample and Hg data interpolation

One sample of the whole *N. luridiformis* fruiting body intended for the determination of the Hg spatial distribution was carefully collected in a beech forest in Badín (48.666924° N; 19.098778° E), cleaned and transferred to the laboratory. On the same day, the sample was rinsed in deionized water, and cut along the central vertical line with a ceramic knife to obtain a 2 cm thick slice (Supplementary material Fig. 1S). Afterwards, the slice was cut into 1 × 1 × 2 cm prisms, each prism

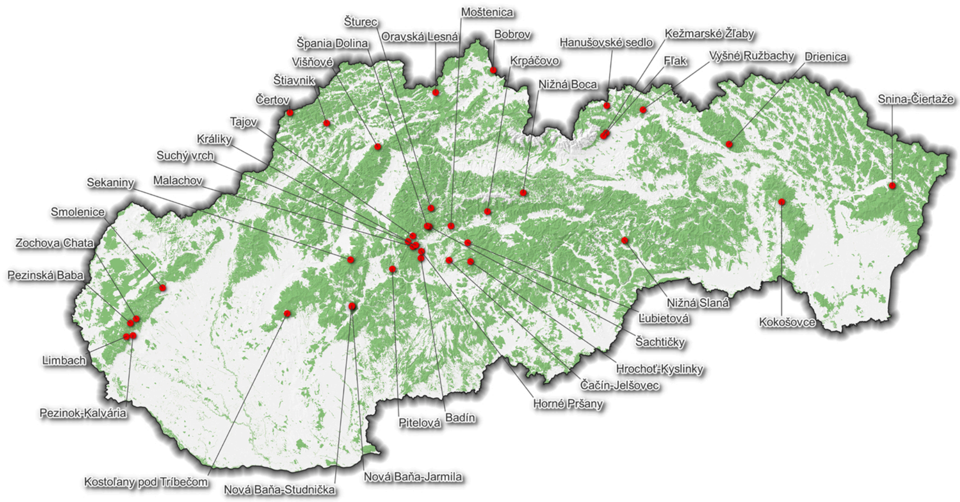

Fig. 1. Sampling locations in Slovakia.

representing a single subsample. Altogether, we have obtained 101 particular subsamples. The samples were then dried at 40 °C for ~ 24 h. After the drying, the samples were homogenized and stored before the analysis of Hg content by CV-AAS method. The shape of the fruiting body was traced on a grid paper to divide the whole shape into a grid of 1 × 1 cm cells. A point was placed inside each cell. Afterwards, the paper was scanned and the image uploaded into the open-source QGIS software (version 3.10). We created a vector polygon layer by tracing the shape of the fruiting body in the image and a point layer containing the points inside the grid. We merged the data of absolute Hg content in the individual subsamples with each of the points and then interpolated them using the multilevel B-spline interpolation.

2.2. Collection and preparation of experimental material from sampling areas

We collected samples of edible wild mushroom *Neoboletus luridiformis* (Rostk.) Gelardi, Simonini & Vizzini in the stage of full maturity (n = 378) from 38 locations in Slovakia during 2015–2019 (Fig. 1). These locations represent places with different degrees of the toxic load of mercury (geogenic but also anthropogenic origin) throughout Slovakia. The list of sites, the number of samples and the basic characteristics of the sampling points are given in Supplementary material Table 1 S. A different number of samples (n = 7 – 21) were obtained from the individual sites. Together with each mushroom sample, we also collected the associated soil/substrate sample (approximately 200 g) up to a depth of 0.10 m. After the collection, the mushroom samples were cleaned from dirt and temporarily stored in ventilated polyethylene boxes. The soil/substrate samples were taken from three points within 1 m of each collected mushroom. These samples were temporarily stored in re-sealable PE bags.

The mushrooms were further cleaned with deionized water, divided into the cap and the stipe and cut into thin slices in the laboratory the same day of the sample collection. Afterwards, the mushroom samples were dried in a laboratory oven with forced air circulation Memmert UF 110 m (Memmert GmbH & Co. KG, Schwabach, Germany) at 40 °C for ~ 24 h. After the drying, the mushroom samples were homogenized in the rotary homogenizer IKA A 10 basic (IKA-Werke GmbH & Co. KG, Staufen, Germany). Subsequently, the homogenized mushroom samples were stored in re-sealable PE bags prior to their analysis for Hg content. In the case of the soil/substrate samples, the samples were dried at room temperature for approximately 3 weeks, cleaned from debris, homogenized and sieved through a 2 mm sieve. The samples were then stored in paper bags until the analyses.

2.3. Determination of the water content

The water content was determined in individual anatomical parts of fruiting bodies of *N. luridiformis* using KERN DEB 160–3A moisture analyzer (KERN & Sohn GmbH, Balingern, Germany). The analysis was repeated 3 times for each anatomical part (tubes & pores, flesh of cap and stipe).

2.4. Determination of the total mercury content

The dried and homogenized mushroom and soil/substrate samples were analyzed to determine the Hg content by cold-vapor atomic absorption spectrometry (CV-AAS) using AMA-254 (AlTec spol. s r.o., Prague, Czech Republic) coupled with autosampler ASS- 254 (AlTec Ltd., Prague, Czech Republic). Quantitative Hg determination was performed at λ = 253.7 nm. The limit of detection (LOD) of Hg was 0.0011 mg/kg dry weight (DW) and the limit of quantification was 0.0031 mg/kg DW (Szaková et al., 2003). The weight of the analyzed samples ranged from 10 to 20 mg. To check the quality and assurance of the measurement, we analyzed two CRM materials, as well. ERM-CC 141: Loam soil (IRMM Geel, Belgium) and ERM-CE 278k: Mussel tissue (IRMM Geel, Belgium). Each CRM was measured 6 times, and in each series, we measured blank 3 times. Recovery of the studied reference materials, taking into account the current water content, was as follows: ERM-CC 141 – Loam soil (98.6%; the certified value was 0.083 mg/kg DW and the determined value was 0.0818 mg/kg DW) and ERM-CE 278k – Mussel tissue (101.4%; certified value was 0.071 mg/kg DW and the determined value was 0.072 mg/kg DW).

2.5. Bioconcentration factor – BCF and cap/stipe quotient– $Q_{c/s}$

The bioconcentration factor (*BCF*) was calculated to assess the level of transition and accumulation of Hg from soil/substrate to the fruiting body of *N. luridiformis* for each sample according to the following formula:

$$BCF = \frac{Hg_{ms}}{Hg_{ss}} \tag{1}$$

where: Hg_{ms} is the total content of Hg in mushroom samples (mg/kg DW) and Hg_{ss} is the total content of Hg in soil/substrate samples (mg/kg DW). $BCF < 1$ indicates excluder species, $BCF > 1$ indicates accumulators, and $BCF = 1$ indicates indicator species (Baker, 1981; Dryżalowska and Falandysz, 2014).

The cap/stipe quotient – Qc/s was evaluated to compare the level of

Hg translocation within the fruiting body and is calculated as follows:

$$Q_{c/s} = \frac{Hg_{cap}}{Hg_{stipe}} \tag{2}$$

where: Hg_{cap} is the mercury content in caps, and Hg_{stipe} is the mercury content in stipes.

2.6. Contamination factor – C_f

The level of soil contamination by Hg was determined by the contamination factor (C_f) (Hakanson, 1980) that is calculated using the following formula:

$$C_f^i = \frac{C_{0-1}^i}{C_n^i} \tag{3}$$

where: C_{0-1}^i is the measured Hg content in the soil samples and C_n^i is the background value of the Hg content in the soils that is 0.06 mg/kg according to Šefčík et al. (2008). The contamination factor values are divided into four categories: low contamination factor ($C_f^i<1$); moderate contamination factor ($1 \le C_f^i < 3$); considerable contamination factor ($3 \le C_f^i < 6$) and very high contamination factor ($C_f^i \ge 6$).

2.7. Index of geoaccumulation – I_{geo}

The index of geoaccumulation was used to quantify the degree of contamination of the studied areas by mercury. It is calculated as follows:

$$I_{geo}=log_2\ (Cn/1{\cdot}5\times Bn) \tag{4}$$

where: *Cn* is the Hg content in the soil samples and *Bn* is the background value (0.06 mg/kg DW) (Šefčík et al., 2008). According to Müller (1969) the I_{geo} values are divided into seven categories: background values ($I_{geo} \le 0$); uncontaminated ($0 < I_{geo} < 1$); uncontaminated or slightly contaminated ($1 \le I_{geo} < 2$); slightly contaminated ($2 \le I_{geo} < 3$); moderately contaminated ($3 \le I_{geo} <4$); strongly contaminated ($4 \le I_{geo} < 5$) and very strongly contaminated ($I_{geo} \ge 5$).

2.8. Potential ecological risk – PER

Potential ecological risk (*PER*) (Hakanson, 1980) evaluates the level of soil contamination by risk elements based on their toxic response in the environment (Chen et al., 2015). It is calculated as follows:

$$E_j^i = T_n^i \times C_f^i \tag{5}$$

$$\mathrm{PER} = \sum\nolimits_i^n E_j^i \tag{6}$$

$$\mathrm{PER_{Hg}} = E_j^i \tag{7}$$

where: *PER* is the potential ecological risk for the studied contaminant (Hg) in the soil/substrate samples; E_j^i stands for the ecological risk of Hg; C_f^i means the pollution factor of Hg and T_n^i is the biological toxic factor for Hg. Given that PER is calculated for multiple contaminants, in our case $PER_{Hg} = E_j^i$. The degree of ecological risk can be categorized as follows: $Ej < 40$: low risk; $40 \le Ej < 80$: moderate risk; $80 \le Ej < 160$: considerable risk; $160 \le Ej < 320$: high risk and $Ej \ge 320$: very high risk (Hakanson, 1980).

2.9. Health risk assessment

Since the studied species *N. luridiformis* is conditionally edible (after 20 min of heat-treatment), we identified the potential risk arising from its long-term regular consumption. To assess the health risk from the exposure to Hg, the percentage of the *Provisional Tolerable Weekly Intake – %PTWI* was used in this study. The *PTWI* was established to 0.004 mg/kg body weight (BW) for mercury (0.28 mg/adult person) (JECFA, 2010). The *%PTWI* was calculated as follows:

$$\%\mathrm{PTWI} = \frac{Hg\ \ in\ \ mushroom\ \ \times\ \ Intake}{\mathrm{PTWI(Hg)}} \times 100\ \ (\%) \tag{8}$$

where: *Hg in mushroom* is the measured concentration of Hg in the mushroom samples in mg/kg of fresh weight (FW), *Intake* stands for the consumption of the studied mushrooms (kg/week FW), *PTWI (Hg)* = 0.28 mg/adult person. If the detected value was greater than 100%, the consumption of mushroom samples from the area is potentially hazardous. The amount of the fresh weight was calculated based on the assumption that dry matter represents 10% in mushrooms (Kalač, 2010). There is a lack of data on the average consumption of wild edible mushrooms in Slovakia. The data used in this study are based on the statistics of consumption of "*Other vegetables including mushrooms*" in Slovakia in 2018 that is 0.23 kg per week (Statistical Office of the Slovak Republic, 2019).

Target Hazard Quotient – THQ was used to evaluate the long-term non-carcinogenic consumption of *N. luridiformis* samples from the studied sites. THQ takes into account several parameters that have a significant impact on consumer health (Antoine et al., 2017). THQ can be defined as the ratio of exposure to a toxic element and its highest reference dose at which no adverse health effects are expected (US EPA, 2016). Like *%PTWI*, this parameter is converted to 70 kg individual. If the THQ value is < 1 non-carcinogenic health effects are not expected. A THQ value > 1 indicates an increased probability of a harmful health effect. THQ was calculated according to the following formula:

$$\mathrm{THQ} = \frac{(Efr\ \times\ ED\ \times\ ADC\ \times\ C_E)}{RfDo\ \times\ BW\ \times\ ATn} \times 10^{-3} \tag{9}$$

where: *Efr* is the frequency of exposure (365 days); *ED* is the exposure duration (70 years); *ADC* is the average daily consumption of fresh mushrooms (33 g/day); C_E is the average Hg concentration in mushroom samples (mg/kg FW); *RfDo* is the oral reference dose for mercury (0.0003 mg/kg/day) (Kalač, 2019). *BW* is the average body weight (70 kg); *ATn* is the average exposure time (365 days × 70 years = 25 550 days) and 10^{-3} is the factor taking into account the conversion of units.

2.10. Statistical analysis

At first, all the obtained data were characterized by descriptive statistics for minimum and maximum values, median and standard deviation. Then, all the variables were tested for normality. The variables followed the Gaussian distribution according to the Kolmogorov-Smirnov test and the Shapiro-Wilk test. The analysis of variance (ANOVA) was performed to find the significant differences between the tested variables. Analysis of variance was performed using the RStudio software, version 1.2.5033 (RStudio, 2015). Descriptive statistics and normality tests were performed using the MS Excel and XLSTAT package program (Addinsoft, 2014).

3. Results and discussion

The mercury content in all analyzed samples is specified as the median ± standard deviation (min-max) in the dry matter unless stated otherwise (Supplementary material Table 2 S). The Hg content is rounded to three significant digits, except for the data in Supplementary material Table 2 S.

3.1. Mercury distribution in N. luridiformis fruiting body

It is generally known that some higher plants and particularly mushrooms (fungi) absorb large amounts of contaminants into the aboveground parts, fungi through mycelium during their development (fructification) (Ali et al., 2013; Gadd, 2007). A relatively few papers have been published on the detailed investigation of the distribution and translocation of elements in different parts of the fructifying organs of mushrooms using sophisticated visualization techniques, such as laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) (Kavčič et al., 2019; Zocher et al., 2018). This paper focuses on determining the distribution and translocation of mercury to individual above-ground parts of the studied species by the interpolation method described in Section 2.1. This visualization method of element translocation in mushrooms has not been previously used.

A graphical representation of the Hg content in the whole fruiting body of *N. luridiformis* is shown in Fig. 2. The detected Hg median content in all 101 partial samples was 1.54 ± 0.97 (0.08–4.32) mg/kg DW. The highest average Hg content was recorded in the tubes & pores (3.86 mg/kg DW). The average Hg content in the flesh of the cap was 1.82 mg/kg DW and the lowest average content was recorded in the stipe (1.23 mg/kg DW). Fungi from the *Boletaceae* family are characterized by such distribution, as evidenced by the findings of Zocher et al. (2018). Kavčič et al. (2019) used a sophisticated, however, expensive instrumental analytical method LA-ICP-MS to study the distribution of various chemical forms of Hg and Se in the fruiting bodies of three species (*B. edulis*, *B. aereus* and *S. pes-caprae*). Therefore, we assume they focused on small parts of the mushroom fruiting body. Based on the results obtained, they indicate that species of the genus *Boletus* accumulate significant amounts of Hg, especially in environmentally polluted areas. The highest Hg content was accumulated in the cap, especially in the hymenium (spore-forming part). Our results obtained by using the interpolation method on the whole cross-section of the mushroom fruiting body were similar. Although our method is not as detailed (significantly lower amount of the monitored points on the studied cross-section of the fruiting body sample), we also found the highest Hg content in the hymenophore and that the content increased in the radial direction. We also studied the water content in the individual anatomical parts that ranged as follows: flesh of cap: 86.0 ± 1.15%, tubes & pores: 78.5 ± 1.05% and stipe: 78.6 ± 0.98%. Water content in caps is in agreement with the findings of Falandysz et al. (2019b) who proved moisture in raw mushrooms of *Boletus* spp. caps ranging from 87.7% to 91.5%, however, content in stipe water content in our study was lower compared to the mentioned work, but in agreement with another study of *Boletus* spp. (Sari et al., 2017). Other surveys deal with mushroom water content in the entire fruiting body, resulting in general water content in *Boletus* spp. mainly between 86% and 92% (Kalač, 2013; Ouzouni and Riganakos, 2007).

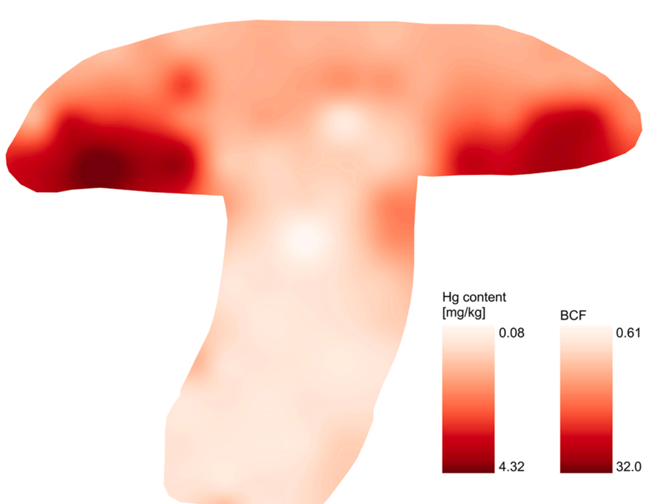

Fig. 2. Spatial distribution of Hg in *N. luridiformis* fruiting body and bioconcentration characteristics.

3.2. Bioconcentration characteristics of anatomical parts of *N. luridiformis* fruiting body

Mercury is one of the elements characterized by a relatively high bioconcentration potential ($BCF_{Hg} > 1$), particularly in mushrooms (Falandysz and Borovička, 2013). It is, of course, reinforced by other factors (bedrock geochemistry, fungal ecology, accumulation process, etc.). The content of mercury in the above-ground parts of fungi is strongly dependent on the species and depends on the developmental stage, anatomical part, total carbon and sulfur content in the substrate and the like (Nasr and Arp, 2011). Species of the genus *Boletus* are characterized by a relatively high bioconcentration capacity (Melgar, Alonso and García, 2009; Širić et al., 2016), which is also reflected in the results of this work. The bioconcentration potential of *N. luridiformis* had a relatively wide range of 11.4 ± 7.14 (0.61 – 32.0), depending on the anatomical part of the fruiting body. The highest BCF value was recorded in tubes & pores 18.1 ± 8.43 (4.22 – 32.0). In the flesh of cap, the value was 12.2 ± 2.17 (9.64 – 20.6) and the lowest BCF values were recorded in stipe: 6.25 ± 2.56 (0.61 – 14.7). These results are closely correlated with the findings of other authors, stating that the species belong to the group of accumulators (BCF > 1). It accumulates the highest contents of risk elements in tubes & pores and flesh of cap (whole cap) (Falandysz, 2017; Świslowski and Rajfur, 2018; Vogel-Mikuš et al., 2016; Zocher et al., 2018) due to the presence of more mercury-biding proteins and enzymes compared to the rest of the fruiting body (Melgar, Alonso and García, 2009) and/or increased physiological activity in these parts of the fruiting body (Falandysz and Drewnowska, 2015). These authors also studied the Hg content and bioconcentration potential of 28 species of edible wild mushrooms from Spain and found that the BCF highest values in the group of mycorrhizal species were in four species of the genus *Boletus* (126 – 421). Of course, BCF values are affected by several factors, including substrate pH, mycelium age, fructification time, water content, etc. (Aloupi et al., 2012). The chemical binding of Hg in Boletales order is highly dependent on the Hg content in the substrate. Falandysz et al. (2015b) found a highly significant correlation between Hg content in the fruiting bodies of various Boletales species and Hg content in the corresponding substrate. Boletales mushrooms from uncontaminated regions bind Hg to di-thiolate and di-selenol compounds and those from contaminated regions bind Hg to tetra-thiol and di-selenol ligands. Kavčič et al. (2017) reported that *Boletus* spp. contain more than 1% of sulfur (other mushroom species 0.1 – 0.5%). Therefore, due to the fact, that bonds between sulfur and mercury are high, the *Boletus* spp. accumulate more Hg than other mushroom species.

A very important parameter for the determination of Hg distribution in the fruiting body, and/or the ratio of bioconcentration potential between cap and stipe is $Q_{c/s}$. In general, the bioconcentration potential of the cap is higher than the rest of the fruiting body, as evidenced by our findings. In a set of 378 *N. luridiformis* samples, the Hg content cap/stipe ratio was relatively wide: 2.38 ± 1.28 (1.40 – 9.66). This wide range is, of course, caused by a number of environmental as well as physiological factors. If this parameter is higher than 1, it means that the content of Hg in the cap is higher than in the stipe. There were only 10 samples with a value lower than one in the whole set of samples (n = 378). It means that the high translocation capacity of Hg into the cap, in combination with the preference of cap consumption, creates an increased risk of possible Hg intoxication (Demková et al., 2021).

3.3. Mercury content in the soil/substrate samples from the studied locations

The average Hg content in Slovak soils is 0.06 mg/kg (Šefčík et al., 2008). The results show that Hg content had a very wide range of 3.07 ± 16.4 mg/kg DW (0.05 – 197) in all soil/substrate samples (n = 378) from the 38 monitored locations. The standard deviation indicates a nonparametric distribution of the whole set of values. In 7 cases, the Hg content was lower than the background value. This condition is caused by extreme differences in Hg content in soil/substrate samples. Two locations, namely Malachov and Nižná Slaná had significantly higher Hg contents ($p < 0.001$) compared to the other ones. The Hg content in these two locations was 47.4 ± 52.7 mg/kg DW (0.44 – 197) and 5.74 ± 3.09 mg/kg DW (3.43 – 12.9) in Malachov and Nižná Slaná, respectively (Supplementary material Fig. 2S and Supplementary material Table 2 S). The large differences among the studied locations are due to diametrically different geological characteristics of the area and different anthropogenic land use. Slovak legislation defines the limit value of Hg for soil 0.50 mg/kg DW (AoL, 2004). The limit was exceeded in 42 samples, while 57% of these samples were collected in Malachov and Nižná Slaná.

The localities in Malachov and Nižná Slaná are characterized by historical mining and metallurgical activities. Cinnabar (HgS), used to be surface-mined and processed in Malachov and this locality represented the richest Hg deposit in the world in the Middle Ages (Andráš et al., 2021).

From a long-term perspective, these activities represent the main source of contamination of this area. It is also confirmed by the findings of Dadová et al. (2016), who found the Hg content ranging from 2.0 to 416 mg/kg DW in the soil and technosoil from dumps surface ore field and 0.84 – 394 μg/L in groundwater and surface water samples in the locality Malachov – Veľká Studňa.

In the locality of Nižná Slaná, iron ore with mixtures of Hg, As and Pb was mined and processed in the 20th century. It led to significant contamination of all environmental components around the metal-working plant and tailing pond (Demková et al., 2020, 2019). Nowadays, it belongs to the most environmentally hazardous areas in Slovakia with the highest priority of reclamation and remediation. The tailing pond contains almost 5.5 million tons of sludge (Demková et al., 2020).

One of the most Hg contaminated areas is central Spiš (Rudňany), where cinnabar was mined and processed in the past. The level of Hg pollution in this area is documented by the findings of many authors (Árvay et al., 2017, 2014; Demková et al., 2017a, b; Musilová et al., 2016). There are many similar sites around the world affected by the mining and Hg processing activities. One of the most historically famous areas is Idrija (Slovenia) that was a rich and actively used source of Hg between the years 1490 – 1995. During that period, approximately 12 million tonnes of ore were mined and processed at that site, from which 153,000 tonnes of Hg were recovered. At present, the content of total Hg in the soils in the Idrija ranges from 8.40 to 415 mg/kg DW (Miklavčič et al., 2013). The values are partially comparable with the results from the Malachov. The extremely high Hg contents at these localities were recorded mainly in places that border with or are located directly on tailing ponds and/or landfills of the excavated tailings.

3.4. Ecological risk assessment

3.4.1. Contamination factor – Cf

The level of mercury contamination of soil in the selected sites in Slovakia was found serious. Based on the results of the contamination factor, 18% of the sites are considered as very highly contaminated, 42% as considerable contaminated while other sites were found moderately contaminated (Fig. 3). The highest values of mercury contamination factor were found mainly in the former mining areas such as Malachov ($C_f = 790$), Nižná Slaná ($C_f = 95.7$) and Špania Dolina ($C_f = 11.8$). In several previous studies, it has been confirmed that the quality of soils in former mining areas in Slovakia has long been threatened by high mercury content (Kimáková and Poráčová, 2020). As regards the release of risk elements into the environment, particularly problematic are the mining bodies, ore processing plants, and their surroundings (Demková et al., 2020). High mercury contents have also been detected in other environmental components of former mining areas, e.g. water resources (Dadová and Romančík, 2019; Dadová et al., 2016) or living organism's bodies (Andráš et al., 2021).

3.4.2. Index of geoaccumulation – Igeo

The index of geoaccumulation was used to evaluate the intensity of anthropogenic influence on the soil quality (Fig. 4). Based on the geoaccumulation index, background values of mercury were found at 4 sites. The highest number of sampling sites have been categorized as uncontaminated or slightly contaminated (13) and slightly contaminated (14). Five sites were evaluated as moderately contaminated. Consistent with the results of the contamination factor, soil quality status was the worst at former mining areas where very strong contamination was determined (Malachov: 7.55; Nižná Slaná: 5.85). On the other side, earlier studies have confirmed, that increased mercury content in Slovakia has also been recorded in localities with no direct influence of any anthropogenic factor and no geochemical origin (Kontrišová et al., 2010). It should be related to the ability of hazardous substances to be transported long distances from their sources (Turkyilmaz et al., 2018).

3.4.3. Potential ecological risk – PER

The potential ecological risk index was computed to detect the ecological risk of mercury (Fig. 5). The results showed that most of the studied soil samples had considerable ecological risk (45%), 10 sampling sites had high ecological risk and very high risk was confirmed at 3 sampling sites. The moderate ecological risk was detected at 8 sampling

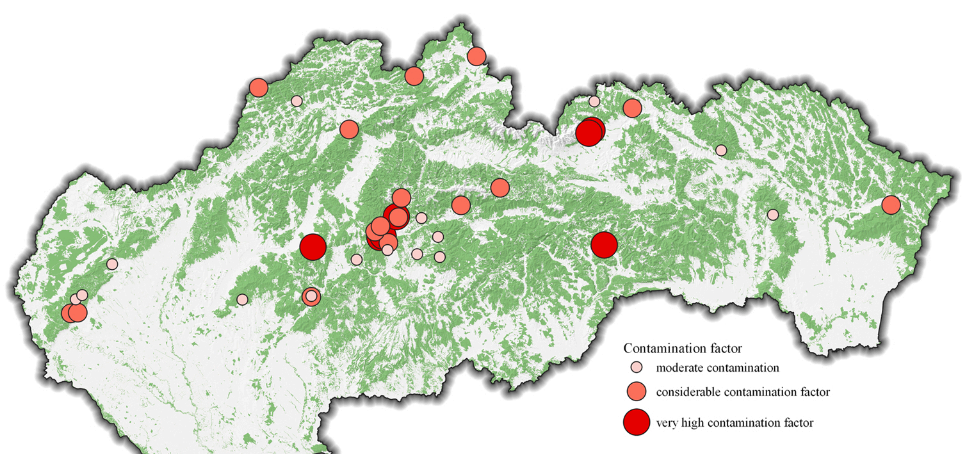

Fig. 3. Contamination factor (C_f) in soil/substrate samples from the studied locations.

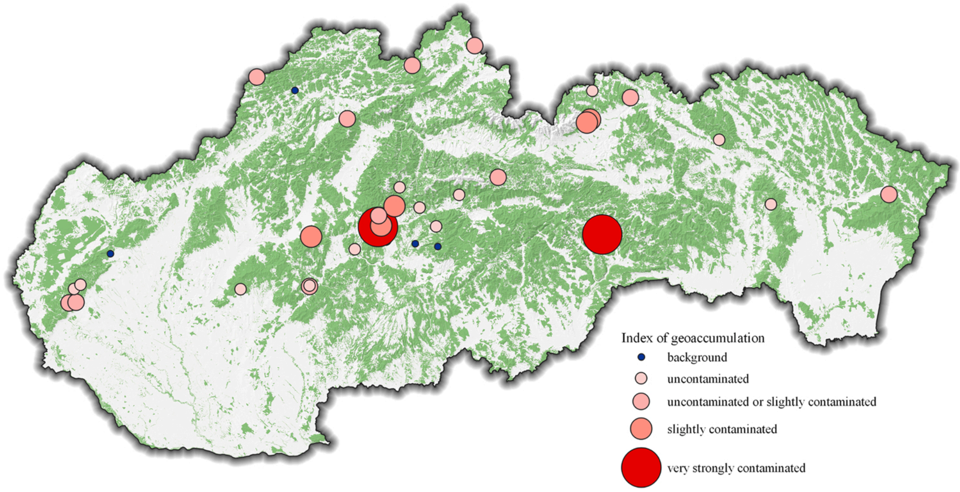

Fig. 4. Index of geoaccumulation (I_{geo}) in soil/substrate samples from the studied sites.

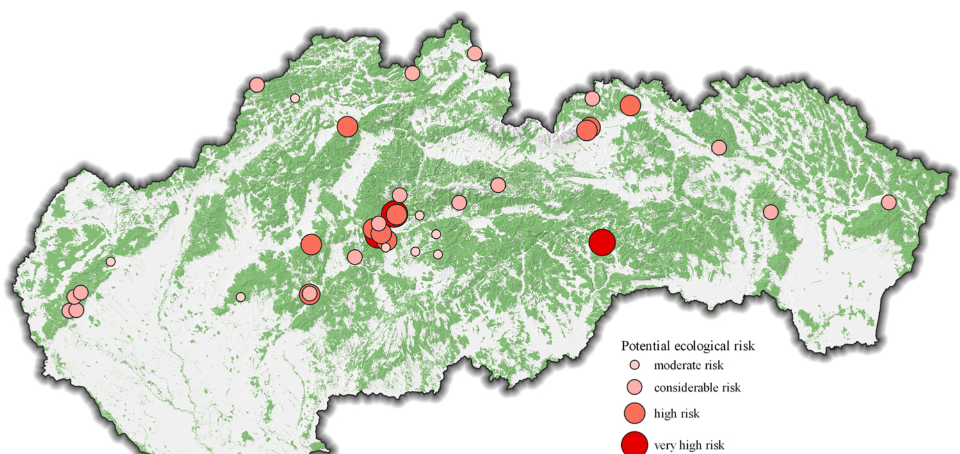

Fig. 5. Potential ecological risk (*PER*) in soil/substrate samples from the studied sites.

sites. The sites where the very high ecological risk was confirmed were Malachov, Nižná Slaná and Špania Dolina. The consequences of mercury environmental contamination are enormous, from the destruction of ecosystems, the death of aquatic or terrestrial animals to serious human diseases (Budnik and Casteleyn, 2019). Many of the former mining areas remain the most endangered because the environmental management of the mining bodies is insufficient or has completely failed (Demková et al., 2020).

3.5. Mercury content in the N. luridiformis samples from the studied areas

We have determined and compared Hg content in caps and stipes of 378 samples of *N. luridiformis* collected from the 38 studied areas in Slovakia. Individual areas are characterized by different levels of environmental pollution. The content of Hg in the above-ground parts of the studied species was in direct correlation to the Hg content in the soil/substrate. The median values of Hg content in the soil/substrate were higher than 5.00 mg/kg DW in two localities (Malachov and Nižná Slaná), while in the remaining 36 localities the median value ranged from 0.05 to 0.50 mg/kg DW.

The Hg contents in the cap and the stipe samples were 0.54 ± 7.41 (0.05 – 122) and 0.29 ± 4.22 (0.02 – 70.2) mg/kg DW, respectively (Fig. 6 and Supplementary material Fig. 3S). It is clear from the above data that the distribution of Hg content in the two main anatomical parts is nonparametric. This is caused by a large set of data representing different localities. Fig. 6 shows that the Hg content ranged from 0.05 to 0.5 mg/kg DW in 17 localities and from 0.5 to 5.0 mg/kg DW in other 17 localities. There were four localities where the Hg content was above 5 mg/kg DW.

From the whole set of mushroom samples ($n = 2 \times 378$) point of view, 216 cap samples were in the interval < 1 mg/kg; 61 cap samples 1 – 5 mg/kg; 28 cap samples > 5 mg/kg; 256 stipe samples < 1 mg/kg; 36 stipe samples 1 – 5 mg/kg and 9 stipe samples > 5 mg/kg. Melgar et al. (2009) studied 28 wild edible mushroom species and found out that all four *Boletus* spp. had the highest bioconcentration ability compared to other mycorrhizal species. The Hg content was > 1 mg/kg DW in all studied *Boletus* spp. samples ($n = 35$) except 2 cases.

The highest median Hg contents were found in the locations Čačín-Jelšovec (8.22 mg/kg DW), Malachov (7.03 mg/kg DW), Nižná Slaná (6.48 mg/kg DW) and Moštenica (5.20 mg/kg DW) (Supplementary material Table 2S). These four localities are characterized by their historical mining and metalworking activities. *N. luridiformis*, as well as other species of the Boletales order (*Boletus*, *Leccinum* and others) are generally characterized as accumulators, as evidenced also by our findings. However, it is worth mentioning the locality Čačín-Jelšovec, where the Hg bioconcentration factor was very high (cap: 117; stipe: 66.1) compared to other localities. Therefore, if the specific conditions are met, the BCF values are extremely high which is characteristic of the Boletales order (Kautmanová et al., 2021).

A lot of studies has focused on the content of mercury as well as other

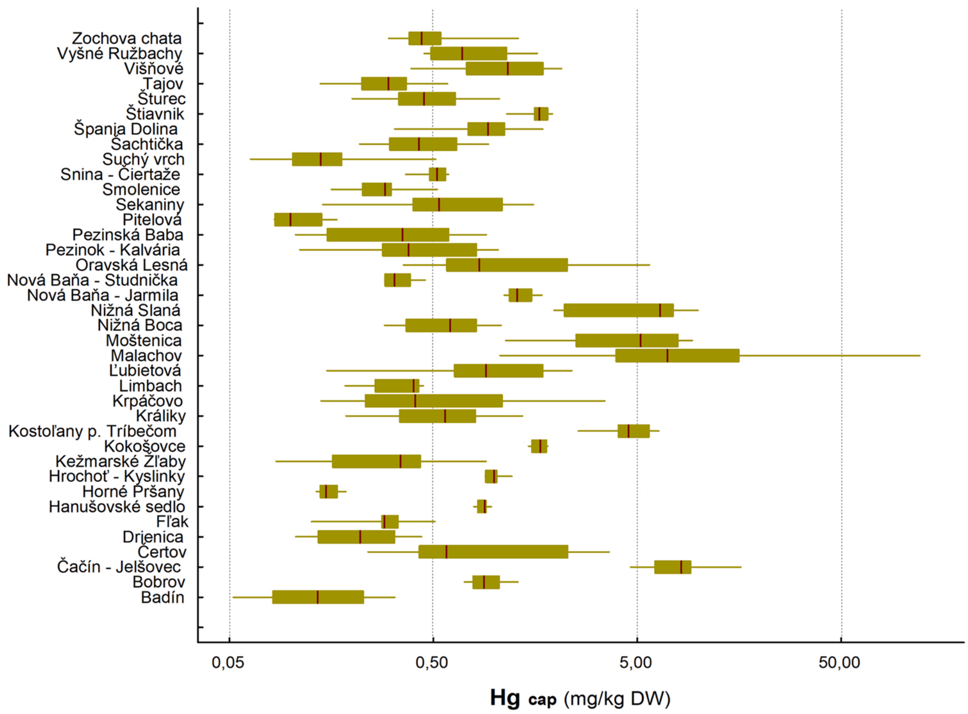

Fig. 6. Logarithmic representation of mercury content in samples of *N. luridiformis* caps.

toxic and potentially toxic elements in edible wild mushrooms. For example, Svoboda et al. (2000) analyzed 56 samples of 23 species of edible wild mushrooms from former cooper and mercury mining and processing areas in Slovakia. They recorded the highest Hg content in *Boletus reticulatus* (53.5 mg/kg DW). Similar results were published by Kalač et al. (1996). During 1990 – 1993, they analyzed 113 samples of 34 species of edible wild mushrooms collected from a catchment area of two metallurgical plants in eastern Slovakia where mercury and copper were mined and processed. The species that accumulated the most mercury were *Macrolepiota procera* (n = 10; 29.3 mg/kg DW) and *Boletus edulis* (n = 5; 32.4 mg/kg DW), and/or the genus *Boletus* in general and *M. procera* (n = 3; 119 mg/kg DW) in the locality Krompachy (processing of Cu-containing ores). Kautmanová et al. (2021) studied 52 samples of 39 wild mushroom species in three areas (Čučma, Medzibrod and Dúbrava) characterized by former mining of antimony. The Hg content results showed that *B. edulis* had the highest Hg content in comparison to all studies species (3.54 mg/kg DW in the locality Dúbrava).

3.6. Health risk assessment

It is well known that long-term consumption of some species of wild edible mushrooms characterized by increased or extremely high bioconcentration capacity may create an increased health risk in combination with other routes of mercury intoxication (Demková et al., 2020; Nowakowski et al., 2021; Sarikurkcu et al., 2020). In this paper, we focused on health risk assessment from two perspectives. We primarily focused on determining the level of health risk that results from the consumption of the entire fruiting body as such or its whole anatomical parts (cap and stipe). Some mushroom pickers prefer to eat caps and discard the stipes. The reason is utterly culinary (difference in the consistency, textural properties), however, if these practices are implemented for mushrooms collected in areas where the Hg content in the environment is increased, then in combination with the high bioconcentration potential of the observed species the mercury intoxication can occur much faster than if the whole fruiting bodies were consumed. In the case of consumption of the whole fruiting body, the diluting effect of the consumed mass occurs (the weight fraction of the cap and the stipe is approximately 3:1). The whole fruiting body contains approximately one-third to half the contents of Hg compared to the cap. The following can be concluded based on the evaluation of %PTWI and THQ:

- The values of the %PTWI in individual anatomical parts were as follows: 11.5% in the whole fruiting body, 31.7% in tubes & pores, 14.9% in the flesh of cap and 10.1% in the stipe. Therefore, the risk of consumption of individual anatomical parts decreases in the following order: tubes & pores > flesh of cap > stipe. Our findings correlate with the results of Zocher et al. (2018), who monitored the level of bioconcentration of major and trace elements between the different compartments of individual fruit bodies of *Suillus luteus*.
- The trend of the THQ values that assess the long-term non-carcinogenic effect of consumption of individual anatomical parts of *N. luridiformis* was similar to that of %PTWI, however, the values were twofold higher which might seemingly point to the higher health risk. The diversity in the resulting values of individual evaluation methods is caused by differences in the input data used in the calculations of these indicators. Therefore, it is appropriate to combine these parameters when drawing conclusions. THQ values have the following decreasing trend: tubes & pores (0.607) > flesh of cap (0.285) > stipe (0.193). The results show that the consumption of caps causes approximately 2.0 – 2.5 times and 1.5 – 2.0 times faster intoxication of the human body with mercury than the consumption of stipes ($Q_{c/s}$ = 2.36; n = 378) and the whole fruiting body, respectively. These findings were confirmed by Kavčič et al. (2019) who claimed that the removal of the spore-forming part from the fruiting bodies of *Boletus* spp. before consumption reduces up to 50% of Hg. From the health risk point of view, the total content of Hg is not as significant as its bioaccessibility. Toxicity of Hg for the consumers can be decreased by the complexation of Hg to Se (Kavčič et al., 2019, 2016). Chiocchetti et al. (2020) found that the risk associated with the intake of Hg (as well as Pb and Cd) is substantially reduced by gastrointestinal digestion influenced by the insoluble fibers (chitin and β-glucans) naturally present in the mushrooms.

The second part of this chapter focuses on the evaluation of the health risk indicators in connection to the studied sites that represent a wide range of different environmentally loaded areas of Slovakia (Fig. 4). Based on the results it is possible to state the following:

- When assessing the risk of consumption of *N. luridiformis* samples from 38 sites, the mean values of %PTWI showed that the riskiest localities are Čačín-Jelšovec (cap: 67.5%, stipe: 31.9%), Malachov (cap: 57.8%, stipe: 28.6%) and Nižná Slaná (cap: 53.2%, stipe: 21.8%). Based on the level of Hg soil contamination, the most interesting site is Čačín-Jelšovec, where despite the high values of %PTWI (38.1 – 132%), the content of Hg in the soil is slightly above the background value (0.06 – 0.11). This finding is in direct correlation with the highest BCF value found in the samples from this site compared to all monitored sites. In the case of Malachov and Nižná Slaná, the situation is different, as these are sites burdened by historical mining and metallurgical activities (Bobro et al., 2004; Fazekašová and Fazekaš, 2020; Musilová et al., 2021), as proved by the values of *Cf* (Fig. 3) and *Igeo* (Fig. 4). It is also reflected in the %PTWI values. In the samples from Malachov, the values ranged from 8.71% to 998% and almost 30% of the samples had the %PTWI value higher than 100%. In the case of Nižná Slaná, despite the high contents of Hg in the soil, the translocation of Hg to fruiting bodies was not so high that the %PTWI value would exceed 100%. The %PTWI values for all studied sites are shown in Supplementary material Table 2S.
- Target hazard quotient includes more parameters than %PTWI in the evaluation. Although this parameter is primarily used in atmospheric risk assessment (US EPA, 2014), it finds application in the assessment of risk from food consumption (Antoine et al., 2017; Barone et al., 2015; Petroczi and Naughton, 2009). THQ values ranged widely which is, of course, closely correlated with the values of %PTWI (Supplementary material Table 2S). The average THQ value > 1 was recorded in three sites and only in the caps, namely: Čačín-Jelšovec (cap: 1.29, stipe: 0.61), Malachov (cap: 1.11, stipe: 0.57) and Nižná Slaná (cap: 1.02, stipe: 0.42) (Fig. 7). However, a more detailed look at the individual sites shows that partial values were exceeded in four localities, namely: Malachov (11 of 17), Nižná Slaná (5 of 9), Čačín-Jelšovec (3 of 11) and Kostoľany pod Tríbečom (1 of 10). In the last-named site, the mean value of THQ_{cap} was almost 12 times higher than the mean value of the whole set (n = 378), despite the fact that the geoaccumulation index defines this area as uncontaminated (Fig. 4). It was reported that increased BCF levels occur at sites where the Hg content in the substrate is low or at the level of the background value and vice versa, in places where the Hg content in the soil is high or extreme, the BCF values are at the level of single digits. Such findings were published by Falandysz et al. (2012) in the case of *Imleria badia* (formerly *Xerocomus badius*) and Dryżałowska and Falandysz (2014) in the case of *Xerocomellus chrysenteron*.

4. Conclusion

The paper focused on two main study areas. The first one aimed at the monitoring of Hg content and its distribution in individual anatomical parts of *N. luridiformis* and the second one at the evaluation Hg content in the soil/substrates samples and ecological risk assessment in the studied areas, evaluation of Hg content in mushroom samples and a health risk arising from the long-term regular consumption of the mushrooms in the studied areas.

Our study showed that an interpolation method can be a good alternative to expensive methods of element content visualization in the fruiting bodies of mushrooms. The BCF values indicate that *N. luridiformis* can be classified as a Hg accumulator.

Two locations, namely Malachov and Nižná Slaná had significantly higher Hg contents in the soil/substrate samples compared to the other ones.

The content of Hg in the fruiting bodies was in direct correlation to the Hg content in the soil/substrate.

The results of the %PTWI and THQ values showed that the health risk resulting from the long-term regular consumption of the studied mushroom species decreases in the following order: tubes & pores > flesh of cap > stipe. The health risk resulting from long-term consumption of mushrooms from the studied locations is in direct correlation with the level of the environmental load caused mainly by anthropogenic activity.

CRediT authorship contribution statement

Július Árvay: conceptualization, formal analysis, validation, sample collection, writing – original draft, project administration, methodology. **Martin Hauptvogl:** Visualization, Reviewing. **Lenka Demková:** Conceptualization, Sample collection, Writing. **Ľuboš Harangozo:** Writing, Reviewing, Sample collection. **Marek Šnirc:** Sample collection, Writing, Statistical analysis. **Lenka Bobuľská:** sample collection. **Jana Štefániková:** writing, data analysis. **Anton Kováčik:** writing, sample collection. **Silvia Jakabová:** sample collection, data analysis. **Ivona Jančo:** sample collection and preparation, Hg analysis. **Vladimír Kunca:** sample collection, sample identification, reviewing. **Dubravka Relić:** reviewing and writing.

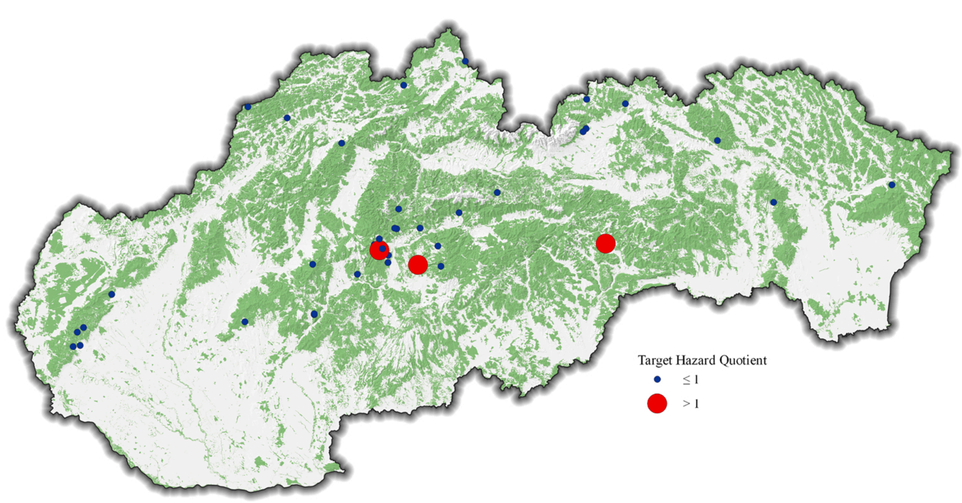

Fig. 7. Target hazard quotient in mushroom (cap) samples from the studied sites.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Acknowledgment and funding

The author would like to thank the following grants: VEGA 1/0591/18, VEGA 1/0326/18 provided by Ministry of Education, Science, Research and Sport of the Slovak Republic; contract No: SK-SRB-18-0038 provided by Slovak Research and Development Agency and contract No: 337–00-107/2019–09/17 provided by Ministry of Education, Science and Technological Development of Serbia. Special thanks go to MSc. Ďuršová, Mr. Pavlík, MSc. Vídenský and Dr. Šišková for their assistance in the mushroom sampling.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2022.113235.

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