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Accumulation of radioisotopes and heavy metals in selected species of mushrooms

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ABSTRACT

Seven species of forest mushrooms from different regions of Poland (edible: *Imleria badia*, *Cantharellus cibarius*, *Xerocomus subtomentosus*, *Suillus luteus* and inedible by humans but being food for animals: *Paxillus involutus*, *Tylopilus felleus* and *Russula emetica*) were analyzed for radioisotope activity (Cs-137, K-40, Bi-214 and Pb-210) as well as concentrations of heavy metals (aluminum, chromium, cadmium, manganese, iron, lead, zinc, copper, nickel and mercury). The activity of radioisotopes was measured with a gamma spectrometer, while the concentrations of heavy metals were examined by microwave plasma – atomic emission spectrometry. The obtained results of the analyses were compared with the recommendations of the European Food Safety Authority concerning the consumption of the determined heavy metals and the European standards concerning the content of radioisotopes in food. The obtained results proved that the consumption of mushrooms may result in a significant exceeding of the consumption limits of cadmium, copper and Cs-137.

1. Introduction

Mushrooms are eukaryotic organisms classified as the kingdom of fungi. The term "mushroom" is used for the fruiting body (carpophore, mycocarp), mostly above ground, of higher fungi. The fundamental role of fungi in the environment involves organic and inorganic transformations, rock and mineral transformations, bio-weathering, element cycling, as well as interactions with metals and clays and mycogenic formation (Gadd, 2007). Mushrooms are valued due to their taste and aroma as well as their protein, vitamins and low-fat content. Consumption of wild-growing mushrooms has been high in many countries, mainly in central and eastern Europe. In Poland, according to the Chief Sanitary Inspectorate *(pol. Główny Inspektorat Sanitarny)*, a statistical Pole consumes a few kilograms of forest mushrooms per year (commercially available and self picked), although in a typically rural region this consumption can reach even 35 kg per year (Główny Inspektorat Sanitarny, 2018). In the Czech Republic, a yearly average level of forest mushroom consumption equals 7 kg per household (Šišák, 1996). The statistic mean of fresh mushrooms consumption per household yearly is 5.6 kg (Šišák, 2007), however, some consume more than 10 kg yearly. However, in the United Kingdom, the average intake of wild-growing mushrooms was estimated to be 0.12 kg fresh weight per capita (Román, Boa, & Woodward, 2006). Studies on the composition of the mushroom mostly focused on the following subject (Gast, Jansen, Bierling, & Haanstra, 1988): hazardous substances in which there might be a risk for mushroom consumers, using mushrooms in environmental monitoring, radioactive contamination before and after the Chernobyl accident, chemical forms of elements accumulated in the mushrooms and the influence of environmental pollution on accumulation on metallic elements. It seems that the main problem concerning mushrooms is that they can contain some hazardous elements, including toxic metallic elements (Cd, Hg, Sb), toxic metalloids (As), toxic non-metals (Se), other elements (Ag, Au, Rb, Zn, V) as well as some radionuclides, natural (^{40}K) and anthropogenic (^{137}Cs, ^{134}Cs, ^{90}Sr) (Cocchi, Vescovi, Petrini, & Petrini, 2006; Falandysz & Borovička, 2013; Falandysz, 2002; Gast et al., 1988; Tsvetnova & Shcheglov, 1994). Little is known of the influence of the mushroom processing on the changes in mineral composition of mushrooms and their bioavailability from

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mushrooms to man (Svoboda, Zimmermannová, & Kalač, 2000). Moreover, the levels of radiocaesium and hazardous materials can increase in tissues of animals eating mushrooms, for example wild ruminants such as roe deer, reindeer and wild boar (Švadlenková, Konečný, & Smutný, 1996). The ability to accumulate an element from a substrate is expressed by the bio-concentration factor (BCF), the ratio of the element content in the fruiting body to the content in an underlying substrate (both in dry matter [DM]). Macrofungi can accumulate extremely high concentrations of metallic elements even when they are growing above soils with low metal contents (Gast et al., 1988). Moreover, macrofungi have the ability to absorb various trace elements from soil and accumulate them in their fruit body. The ratio of an element in the fruit body to its concentration in soil, called the bioaccumulation factor (BAF), describes the ability of the mushroom to accumulate a particular element. Typical elements accumulated in macrofungi (BAF > 1) include: Au, Ag, As, Br, Cd, Cs, Cu, Hg, Rb, Se, V, Zn and Cl. Elements with typically low concentrations in macrofungi (BAF < 1) include Co, Cr, F, I, Ni, Sb, Sn, Th, U and rare earth elements (Gast et al., 1988). Many factors influence the bioaccumulation of trace elements in macrofungi. The most important ones are as follows: natural factors (bedrock geochemistry), metalliferous areas and environmental pollution, fungal lifestyle (concentration of Hg, Cd, Pb is usually greater in terrestrial saprotrophs than in ectomycorrhizal mushrooms). Bioaccumulation of metallic elements is highly species-specific, the accumulation process can be highly element specific. It should be also mentioned that to be defined as hyperaccumulator, the metal concentration in macrofungi should be at least 100 times greater than the values to be excepted in non-accumulating species on the same substrate (Borovicka, Řanda, Jelínek, Kotrba, & Dunn, 2007). Tolerable intake levels for trace elements recommended by the FAO/WHO are 0.015 mg/kg (provisional tolerable weekly intake) for arsenic, 0.007 for cadmium, 0.025 for lead and 0.005 for mercury (http://www.inchem.org/). Tolerable weekly intake for an adult of 70 kg would be filled with one portion of mushrooms containing 11.7 16.3 35.0 or 58.3 mg/kg DM of total mercury, cadmium, arsenic or lead (Kalač, 2010). This means that provisional tolerable weekly intake that equals 300 g of fresh mushrooms with dry mater content of 100 g/kg per meal were used.

As for the radioactive elements in mushrooms, the data on radioactivity levels are usually also given per dry matter (DM) content. The unit of one Bq (Becquerel) is the radioactivity unit concerning the number of decays per second on average. The maximum permitted levels of food radioactive contamination has been given (Council Regulation, EURATOM). Activity concentration, the activity per one unit of DM has its limits in food: 600 Bg/kg of fresh weight i.e. 6 kBq/kg DM for mushrooms (Kalač, 2001). Moreover, based on the Council Regulations the limits are as follows: the maximum permitted level of ^{137}Cs is 1.25 kBq/kg fresh weight (i.e. 12.5 kBq/kg DM for mushrooms; it was recommended by International Atomic Energy Agency [Intervention criteria in a nuclear or radiation emergency]). Some countries have established statutory limits for the hazardous metals in edible mushrooms. 0.2 and 0.3 mg/kg fresh matter for cadmium and lead have been valid in the EU (EEC Directive 2001/22/EC). The limits 5.0; 2.0 and 10 mg/kg DM were valid in the Czech Republic for mercury, cadmium and lead in wild-growing mushrooms, while 1.0; 1.0 and 3.0 mg/kg DM for cultivated ones. Moreover, the limits were formerly established also for arsenic, chromium, copper, iron and zinc.

As far as the radioisotopes in mushrooms are concerned, the activity concentration is affected by several factors: mushrooms species, contamination of soils, time from the disaster, soil horizon from which a species takes nutrients and moisture. Several natural and anthropogenic isotopes can be found in mushrooms: ^{40}K, ^{210}Pb, ^{226}Ra, ^{234,238}U, ^{90}Sr, $^{239+240}$Pu, ^{238}Pu, ^{241}Am, ^{85}Sr, ^{60}Co and ^{226}Ra (Borovicka et al., 2007). One of the most important is K-40. Mushrooms contain between 1,5 and 117 g of potassium per kg DM (Borovicka et al., 2007) This element's concentration is the highest in mushrooms cap, lower in stipes that in gills or tubes in the spore-forming part and the lowest in spores. Radioactive K-40 is present at a constant level of 1.17×10^{-2}%. The activity concentration of K-40 is between 0.8 and 1.5 kBq/kg DM. Among anthropogenic isotopes in mushrooms coming from the nuclear weapons testing and the Chernobyl disaster, the most important are: Cs-137, Cs-134 (Anspaugh, Catlin, & Goldman, 1988; Langham, Anderson, & Alamos, 1959). The ability to accumulate Cs-137 from fallout was firstly reported by Gruter (Gruter, 1971). The mushrooms species differ in the ability to accumulate radiocesium (Borovicka et al., 2007). The activity of Cs-137 equals 1 kBq/kg DM, mushrooms do not accumulate Sr-90 or radioisotopes of plutonium at a significant level (Mascanzoni, 1992). The Chernobyl disaster released into environment about 3.8×10^{16} Bq from Cs-137 decay. Activity concentrations of Cs-137 and Cs-134 radiocaesium are distributed unevenly in the following order within the fruiting body: gills $>$ flesh or caps $>$ stripes (Heinrich, 1993). Very interesting information on the high accumulation of Cs-137 in norbadione A, the brown pigment of X. badius cap skin was reported (Aumann, Clooth, Steffan, & Steglich, 1989).

2. Hypotheses

The conducted research was aimed at determining the level of radioisotope and heavy metal contamination of mushrooms from the territory of the Republic of Poland in terms of consumption safety and establishing the relationship between soil contamination and the content of the tested substances in mushrooms. It should be emphasized that the undoubted advantage of this study is the simultaneous measurements of both the radioactivity of the selected mushrooms and the concentration of heavy metals in the research material. Moreover, a scientific novelty is the study of not only edible mushrooms, but also inedible ones, which may be an important part of the diet of forest animals. This is because venison consumption may be subject to additional risks due to the presence of radioisotopes and heavy metals. It should be also underlined that the observed bioaccumulation of ^{214}Bi in mushrooms, has not been reported in the world literature so far and also might be concerned as novel and unique.

Based on the information presented above, the authors of this study will check whether the consumption of mushrooms is associated with a high health risk related to the presence of radioactive isotopes and heavy metals in the samples. In addition the attempt to estimate the influence of radio geological background could influence the quality of collected forest mushrooms.

Table 1
Locations of the sampling points.

Name of the closest settlement	Collection date	Designation	Voivodeship (district)	Geographical coordinates
Jarosławiec	August 2019	J	Zachodniopomorskie (sławieński)	54°31′24.3″N 16°30′17.2″E
Gady	July 2019 – September 2019	G	Warmińsko-Mazurskie (olsztyński)	53°52′41.3″N 20°36′19.6″E
Ełk	September 2019	E	Warmińsko-Mazurskie (ełcki)	53°47′54.1″N 22°08′56.9″E
Przechlewo	August 2019 – September 2019	P	Kujawsko-Pomorskie (człuchowski)	53°49′54.4″N 17°13′49.1″E
Szumirad	August 2019	Sz	Opolskie (kluczborski)	50°50′16.4″N 18°12′53.1″E
Sowin/Łambinowice	August 2019	SL	Opolskie (nyski)	50°32′21.7″N 17°37′19.9″E

3. Materials and methods

The subject of the research is seven species of mushrooms common in Polish forests, including 4 species of edible mushrooms:

(a) *Imleria badia (Ib)*
(b) *Cantharellus cibarius (Cc)*
(c) *Xerocomus subtomentosus (Xs)*
(d) *Suillus luteus (Sl)*

and 3 species of inedible mushrooms:

(e) *Paxillus involutus (Pi)*
(f) *Tylopilus felleus (Tf)*
(g) *Russula emetica (Re)*

Based on the maps of the Chernobyl fallout on the territory of Poland (Fig. 1), six sites were selected from which samples of the aforementioned mushrooms were collected. The collection sites were around 10 km^2. These sites significantly differed from one another in the activity of Cs-137 in the soil. Cesium 137 isotope is an anthropogenic one. Its occurrence in the environment is the result of global radioactive fallout, resulting from test nuclear explosions carried out in the atmosphere in the middle of the 20-th century, and nuclear accidents, especially in Chernoby. Two of the above-mentioned sites are located in the so-called Opole anomaly, which is a distinctive area with clearly elevated levels of radiocaesium.

From each site, three to seven fruiting bodies of a given species were collected, as well as a soil sample as a representative sample for the entire area. The aim of the research was to analyze the same species of forest mushrooms from different areas. However, despite the selection of relatively popular species, it turned out that not all of them occur in each of the studied regions. In such cases, only the species that were present in the area were collected. Mushroom samples were cut and dried at 60°Celsius for about 10 h, then mushrooms of the same species from one area were triturated in a ceramic mortar, resulting in homogenized samples.

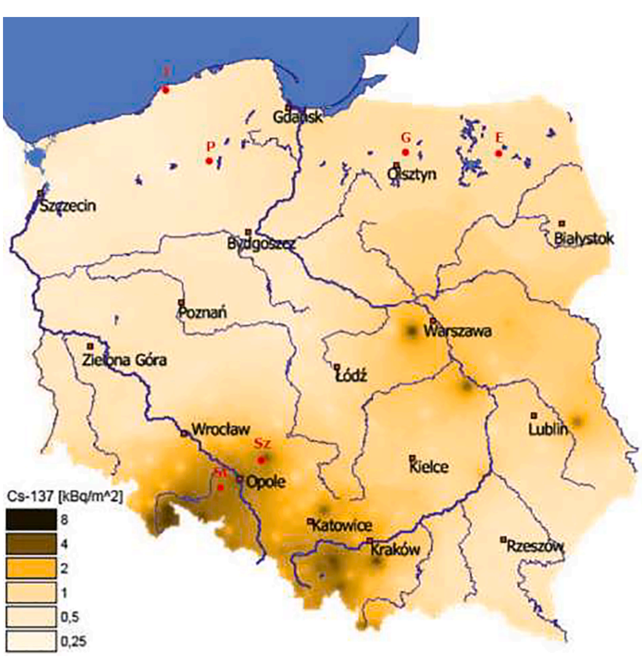

Fig. 1. Map of Poland's contamination with the Cs-137 isotope (Łukaszek-Chmielewska, Isajenko, Stawarz, Piotrowska, & Krawczyńska, 2017) with marked measurement points (markings in accordance with Table 1).

3.1. Measurement methodology

Many different analytical methods are used to analyze mushroom composition and contamination. Among them the most important are: atomic absorption spectrometry (AAS) coupled with various atomization techniques such as cold-vapor AAS (CV-AAS), flame AAS (F-AAS) and electrothermal AAS (ET-AAS), inductively coupled plasma-atomic emission spectrometry (ICP-AES) and inductively coupled plasma-mass spectrometry (ICP-MS), neutron activation analysis (NAA), photon activation analysis (PAA), atomic fluorescence spectroscopy (AFS). The last three are unable to determine very low levels of trace elements (Falandysz, 2013; Jarzyńska & Falandysz, 2011; Maihara, Moura, Catharino, Castro, & Figueira, 2008; Řanda & Kučera, 2004; Yin, Shi, Tian, Shen, Ji, & Zeng, 2012). However, in the presented research microwave plasma atomic emission spectrometry (MP-AES) was used to determine all heavy metals (except Hg) in the analyzed mushrooms and soil samples. The technique was considered sufficient to perform the planned analysis, since in most cases the results should be above the limits of detection and quantification (LOD/LOQ). Moreover, the cost of performing the analysis was identified as significantly lower than in case of the most often used ICP-OES and ICP-MS techniques, mainly because in case of MP-AES there is no need of a constant supply of argonium or helium to maintain the atomization system running. The microwave plasma atomization system is supplied with nitrogen which is generated with the use of a molecular sieve, which makes the technique significantly more eco-friendly. Moreover, the time of the analysis is much shorter than in case of using regular AAS systems, especially when determining a broad range of elements. It is worth mentioning that authors have no knowledge of the MP-AES system be used to perform heavy metals analysis as presented in the research paper. That is why microwave plasma atomization atomic emission spectrometry (MP-AES) was used to determine a wide spectrum of heavy metals and elements. Additionally, atomic absorption spectrometry with cold vapor atomization (CV-AAS) was used for mercury determination. Radioisotope measurements were carried out by gamma spectrometry, whereas the analysis of a wide spectrum of heavy metals was performed with the use of three analytical methods.

3.1.1. Gamma-ray spectrometry

The method of gamma spectrometry with the use of a germanium semiconductor detector was used to determine selected radionuclides. Due to the low mass of mushrooms, measurements were made in the geometry of a disc. Before the measurement, the supplied material was weighed with an accuracy of 0.0001 g and placed in a plastic cup 60 mm in diameter and 15 mm high. The measurement time was 24 h for each sample. The measurements were performed with the use of a Silena-Canberra spectrometer, equipped with a HPGe germanium crystal with an extended energy range, 87 cm^3 volume, relative efficiency of 17.5%, FWHM resolution of 1.83 keV (for the 1.33 MeV line) and the peak/Compton ratio of 46:1. Energy and efficiency calibration was performed using a standard source – a mixture of radioactive gamma isotopes covering the range of 88–1836 keV (source BW/Z-62/24/19). Gamma measurement is a direct measurement. After entering the appropriate data, the Genie2000 program (Canberra, version 2010) generated a report showing the activities of individual gamma radioactive radionuclides contained in the sample. The measurement uncertainty was defined as 1 sigma (one standard deviation).

3.1.2. Microwave plasma – atomic emission spectrometry (MP-AES)

The procedure for preparing samples for testing using MP-AES was as follows: 1 g of mushroom samples and 0.75 g of soil samples (each subjected to previous homogenization) were weighed to the nearest 0.0001 g. The samples were flooded with 8 cm^3 of nitric acid (V) at a concentration of 65% and subjected to mineralization using the Multiwave GO microwave mineralizer from Anton Paar. The mineralization was carried out in stages in order to reduce the likelihood of a sudden increase in pressure: heating to 100 °C for 15 min and maintaining the temperature for 5 min; heating to 120 °C for 5 min and maintaining the set temperature for the next 5 min; heating to 185 °C for 10 min and full mineralization for the next 10 min. The samples prepared in this way were transferred quantitatively into volumetric flasks to the volume of 10 ml, filtered and analyzed using the MP-AES 4210 from Agilent. Table S1 presents the validation parameters of the analytical procedure.

3.1.3. Cold-vapor atomic absorption spectrometry (CV-AAS)

The determination of mercury using the CV-AAS method did not require the mineralization of the samples. 0.1 g (to the nearest 0.0001 g) of each of the homogenized samples was weighed and placed in the thermally treated ceramic measuring boats. The samples were sprinkled with additive B (trademark). The additive B is a backfill based on activated aluminum oxides, the addition of which is intended to make the sample flame-retardant and to reduce the loss of mercury during the analysis due to the limitation of its evaporation at low temperatures (<100 °C) before the analysis itself. In each case, the uncertainty budget takes into account the uncertainty of weight and the uncertainty of repetition, calculated on the basis of the standard deviation.

3.1.4. Presentation method

The water content in fresh mushrooms can vary greatly (usually around 90% depending on the species, atmospheric conditions and the type of soil), the results were presented as dry mass. Most of the analysis results were presented in the form of box plots. All obtained results are contained in the Supplementary Materials. If the obtained parameter value was lower than the LOD, a value of half the LOD was assumed in order to create graphs. If the parameter value was in the range between LOD and LOQ, then the arithmetic mean of the two was taken. The first and third quartiles are presented on the box plots, while the inner lines show the medians of the results. The lower and upper error bars correspond respectively to the minimum and maximum measured parameter values for a given sample type.

4. Results and discussion

The obtained results were compared with the European standards concerning the content of radioisotopes in food and the recommendations of the European Food Safety Authority concerning the consumption of the determined heavy metals.

4.1. Radionuclide activity analysis

Prior to the analysis of isotope content, a radiological analysis of the soils was performed, from which fungal samples were collected. Detailed results of the determination of radionuclides in the samples are given in Table S2 in the additional materials. Isotope activities are given per kilogram of dry matter. The obtained results were processed statistically. The graph below (Fig. 2) presents the activity of radioisotopes in the soil. The areas selected for research turned out to be significantly different from each other not only in the activity of radiocaesium, but also in the activity of natural radioisotopes (K-40, Pb-210 and Bi-214). As predicted, the soil from the forests located in the Opole Anomaly (Sowin/Łambinowice and Szumirad) is characterized by a significantly increased activity of Cs-137. Also noteworthy is the 10-fold higher activity of Bi-214 in the soil from Jarosławiec in relation to the other samples. This is most likely caused by significant differences in the geological characteristics of the studied areas. The activity of the Pb-210 isotope in the tested samples showed the lowest differentiation, being the highest in the sample from Przechlewo.

The analysis of the content of Cs-137 in mushrooms from different studied areas shows significant differences between the mushroom samples from the different sampling locations. In Fig. 3a, the mentioned contrasting values between sampling sites are presented in two graphs due to over 10-fold differences. It is also worth mentioning that mostly contaminated samples were obtained from Opole anomaly sampling sites (SL and Sz). The results are also characterized by significant differences in extreme values. In the case of areas belonging to the Opole Anomaly, the maximum values of Cs-137 activity were approximately 4000 Bq/kg and 18,000 Bq/kg DM (for Sowin/Łambinowice and Szumirad, respectively). In the same order, the medians were around 900 and 10,800 Bq/kg DM. As for the remaining areas, typical radiocaesium activities in the samples ranged from 200 to 500 Bq/kg, and the maximum values did not exceed 1400 Bq/kg.

There is a clearly visible variation in the activity of Cs-137 depending on the species of mushroom analyzed. In Fig. 3b over 10-fold differences between samples were also indicated. *Scaling was used to emphasize the significant differences between samples.* Statistically, the highest concentrations of radiocaesium were recorded in *Imleria badia*, which is consistent with previous studies (Byrne, 1988; Heinrich, 1993; Tsvetnova & Shcheglov, 1994), *Suillus luteus* and *Tylopilus felleus*. In the case of the last two mentioned species, the median activity of Cs-137 in samples of these mushrooms is slightly lower than in *Imleria badia*, which is consistent with the observations of Kammerer et al. al. (Kammerer, Hiersche, & Wirth, 1994). The result of the presented research, however, does not confirm the thesis put forward in the quoted paper about the increased accumulation of Cs-137 in *Paxillus involutus*. The lowest radiocaesium activity was recorded in *Xerocomus subtomentosus*.

Strong variability in the activity of Cs-137 is observed in mushrooms coming from the same area, which may result from the different affinities of individual species to this isotope, the ways in which the fungus nourishes, as well as from a very large variation in the level of contamination even within small areas.

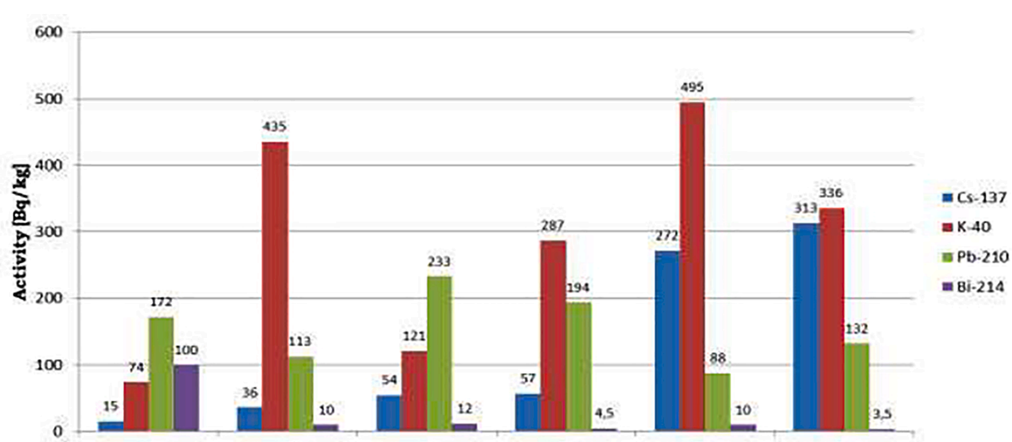

Fig. 2. Radiotope activity in tested soil samples. Designation: J - Jarosławiec, G – Gady, P – Przechlewo, E – Ełk, SL – Sowin/lambinowice, Sz – Szumirad.

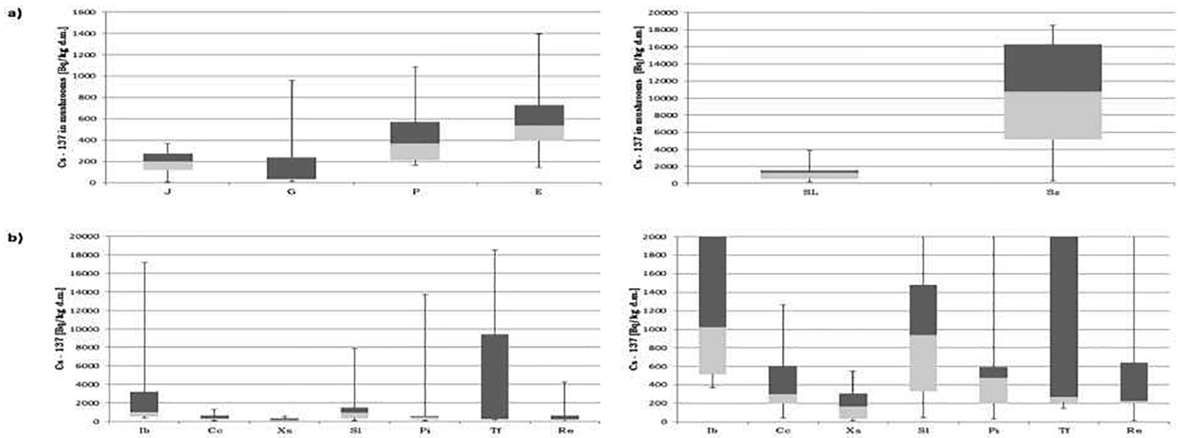

Fig. 3. Cs-137 activity in mushroom samples by area (a): J – Jarosławiec, G – Gady, P – Przechlewo, E – Ełk, SL – Sowin/Łambinowice, Sz – Szumirad; and species (b): Ib – Imleria badia, *Cc* – Cantharellus cibarius, Xs – Xerocomus subtomentosus, Sl – Suillus luteus, Pi – Paxillus involutus, Tf – Tylopilus felleus, Re – Russula emetica.

The maximum activity of radiocaesium in the tested samples in *Imleria badia* was approx. 17,000 Bq/kg DM, and typical results ranged from 500 to 1000 Bq/kg. It should be noted that the obtained results, especially in the Opole voivodeship, are still clearly higher than those presented in the studies conducted before 1986 (Kalač, 2001), both in Poland and in Europe as a whole. As it is known, over the years after the Chernobyl Catastrophe, a clear decrease in the activity of Cs-137 can be observed.

The analysis of studies conducted in 1991 by Mietelski et al. (Mietelski, Jasińska, Kubica, Kozak, & Macharski, 1994) shows that the

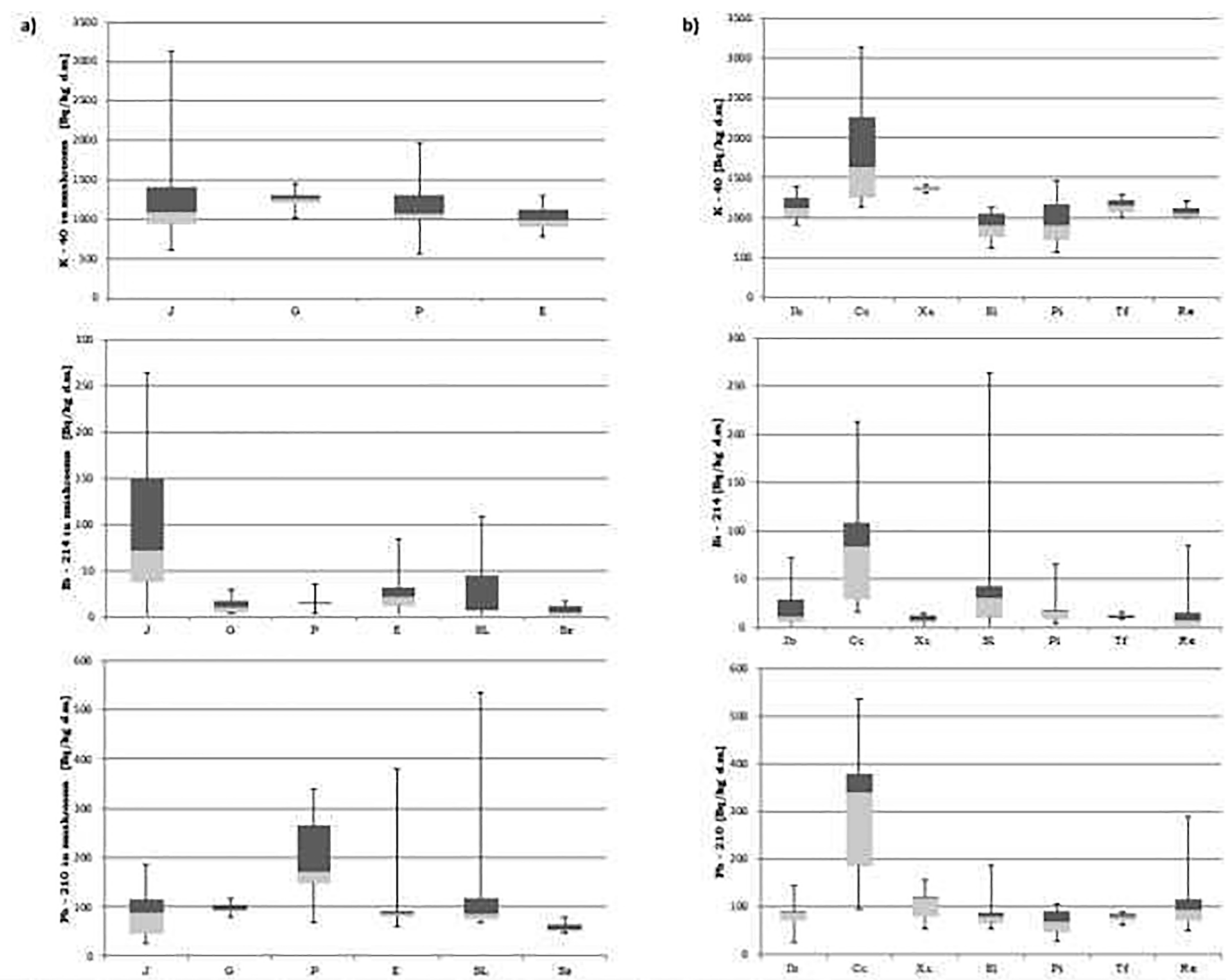

Fig. 4. Natural radioisotope activity in mushroom samples by area (a) and species (b) Designation: J – Jarosławiec, G – Gady, P – Przechlewo, E – Ełk, SL – Sowin/Łambinowice, Sz – Szumirad, Ib – Imleria badia, *Cc* – Cantharellus cibarius, Xs – Xerocomus subtomentosus, Sl – Suillus luteus, Pi – Paxillus involutus, Tf – Tylopilus felleus, Re – Russula emetica.

activity of radiocaesium in *Imleria badia* growing in Poland reached even 157,000 Bq/kg (with a dominant between 4000 and 7000 Bq/kg). As already mentioned, the maximum activity of Cs-137 in mushrooms admitted for consumption in Poland was determined legally at the level of 1250 Bq/kg fresh matter (Regulation of the Council of Ministers of 27 April, 2004), which would be approximately 12,500 Bq/kg DM., same as the European standard (Kirchner & Daillant, 1998), which means that the activity of this isotope exceeds the value allowed in the cited legal standard in three mushroom samples (*Imleria badia*, *Tylopilus felleus* and *Paxillus involutus*) from Szumirad.

The analysis results of the activity of natural radioisotopes in mushroom samples depending on the area are presented in the diagrams below (Fig. 4). The activity of the K-40 isotope in mushrooms turned out to be similar in all areas. Typical values ranged between 800 and 1600 Bq/kg DM. The diagram (Fig. 4a) presents the activities of K-40 only for four areas, for which reliable results with low uncertainty were obtained. Based on the analysis of radioactive potassium activity in individual species, also no significant differences in the obtained values were found between the species (Fig. 4b). Slightly higher activities were recorded for *Cantharellus cibarius*. Such an observation has not been made in any of the previous studies.

In the case of the Bi-214 isotope, the typical recorded activities did not exceed 50 Bq/kg DM. Significantly higher results were recorded for the samples from Jarosławiec (Fig. 4a). The median for this area was determined at 72 Bq/kg DM, and the highest activity was 264 Bq/kg DM. Based on the species analysis, it was shown that the *Cantharellus cibarius* and *Suillus luteus* have the highest affinity for Bi-214 among the species studied (Fig. 4b). So far, the accumulation of the Bi-214 isotope in European mushrooms has not been studied.

The activity of the Pb-210 isotope in samples from most areas was similar (Fig. 4a, median results in the range of 53–94 Bq/kg). Higher results were recorded for the samples from Przechlewo (median 172 Bq/kg). As in the case of bismuth, *Cantharellus cibarius* is characterized by an increased accumulation of the Pb-210 isotope compared to other species (Fig. 4b). So far, such an observation has not been made by other research teams. The median activity of radioactive lead in samples of *Cantharellus cibarius* is 380 Bq/kg DM, while for the remaining species it is 79–157 Bq/kg DM. Analyzing in general, without dividing into individual species of mushrooms, the obtained results of the analysis of Pb-210 activity are much higher than those presented so far by other teams (max. 36.5 Bq/kg in France (Kirchner & Daillant, 1998) and max. 2,52 Bq/kg in Poland (Szymańska, Falandysz, Skwarzec, & Strumińska-Parulska, 2018).

Differences in the activities of Bi-214 and Pb-210 occurring in the same radioactive series may result from different solubilities of speciation forms containing the discussed isotopes and, consequently, differences in their migration in the natural environment. The limits on the content of radioactive isotopes in food apply only to these isotopes whose half-life is greater than 10 days, i.e. to Cs-137. Bi-214 has a half-life of about 20 min, so its content is not l regulated. Moreover, K-40 is an isotope excluded from the applicable limits. Therefore, the legal standards cover only the content of Cs-137 in food substances. According to the Regulations of the Council Ministry of August 20, 2003, the total limit of the content of the radioisotopes with a half-life of more than 10 days should not exceed 1250 Bq/kg.

An important aspect is the issue of the correlation of the activities of individual radioisotopes in mushrooms and in soil. The isotope activities given in the graphs included in Supplementary Materials (Fig. S1) are average values for all species. Certain relationships can be observed for the isotopes Cs-137 (correlation coefficient 0.607) and Bi-214 (correlation coefficient 0.9183). In the case of radiocaesium, its activity in mushrooms is many times (6–33 times) greater than in the soil. The ability of mushrooms to accumulate Bi-214 is lower (a maximum 6-fold increase in activity in mushrooms compared to the soil). Nevertheless, it is noticeable, as in each of the studied areas the activity of Bi-214 in mushrooms is higher than in the soil (although to varying degrees). The activity of K-40 in mushrooms does not depend on the activity of this isotope in the soil and is similar (on average 1000–1400 Bq/kg). A similar conclusion arises in relation to the Pb-210 isotope.

4.2. Detailed analysis of the results of heavy metal determination

The obtained results of determinations of selected metals in samples of mushrooms and soils collected from the collection sites of specific individuals were presented in box plots (Fig. 5). The results were compared with reference to the European Food Safety Authority's data on the recommended or maximum consumption of certain elements. Authors decided to present only European norms since the majority of the studied forest mushrooms are consumed in EU countries. These standards are as follows:

Al – 1 mg/kg body weight per week (European Food Safety Authority, 2008)
Cd – 0.0025 mg/kg body weight per week (European Food Safety Authority, 2012)
Cr - undefined (European Food Safety Authority, 2014)
Cu – 1.3–1.6 mg daily (European Food Safety Authority, 2015)
Fe – 11 mg daily (men), 16 mg daily (women) (European Food Safety Authority, 2015)
Hg – 1.3 μg/kg body weight weekly (methylmercury) and 4 μg/kg body weight weekly (inorganic mercury) (European Food Safety Authority, 2012)
Mn – 3 mg daily (European Food Safety Authority, 2013)
Ni – 0.0028 mg/kg body weight daily (European Food Safety Authority, 2015)
Pb - no doses considered safe (European Food Safety Authority, 2010)
Zn – 7.5–16.3 mg daily (European Food Safety Authority, 2014)

Aluminum concentrations in mushroom samples differed significantly depending on the species tested. Statistically the lowest values were recorded for *Tylopilus felleus* (median 12.32 mg/kg) and *Imleria badia* (median 40.20 mg/kg). The highest values were typically recorded in *Cantharellus cibarius* (median 114.73 mg/kg) and *Suillus luteus* (median 150.65 mg/kg). For the latter species, the maximum aluminum concentration of all the tested samples was recorded – 657.34 mg/kg. According to the recommendations, the maximum weekly aluminum intake is 1 mg/kg body weight. This means that a person with an average weight (70 kg) can safely consume up to 70 mg of aluminum per week, which in terms of the quantity of the tested edible mushrooms gives from 0.464 kg for dried *Suillus luteus* to 1.741 kg for dried *Imleria badia* (taking into account the median results). Such amounts far exceed the typical consumption of dried mushrooms, so the aluminum contained in the mushrooms does not seem to pose a risk to consumers.

Chromium concentrations in mushroom samples usually did not exceed LOQ = 0.81 mg/kg. A maximum value of 1.76 mg/kg was recorded in the *Cantharellus cibarius* sample (with a median of 0.88 mg/kg). Interpretation of the results is made difficult by the fact that the applied analytical method does not distinguish chromium (III), which is an important micronutrient, from chromate (VI), which is a carcinogenic factor and its consumption may lead to serious health consequences. However, it can be presumed that the vast majority of the total chromium contained in mushrooms is chromium in the + 3 oxidation state, because chromates (VI) are quickly reduced in contact with organic matter contained for example in the soil.

In the case of cadmium, the values were very diverse. On the one hand, one can point to species that accumulate cadmium to a very small extent, such as *Cantharellus cibarius*, *Paxillus involutus* and *Tylopilus felleus*. On the other hand, species such as *Imleria badia* and *Xerocomus subtomentosus* are characterized by increased accumulation of cadmium (medians 1.20 and 1.83 mg/kg respectively). High concentrations have also been recorded for *Russula emetica*. For this species a maximum value

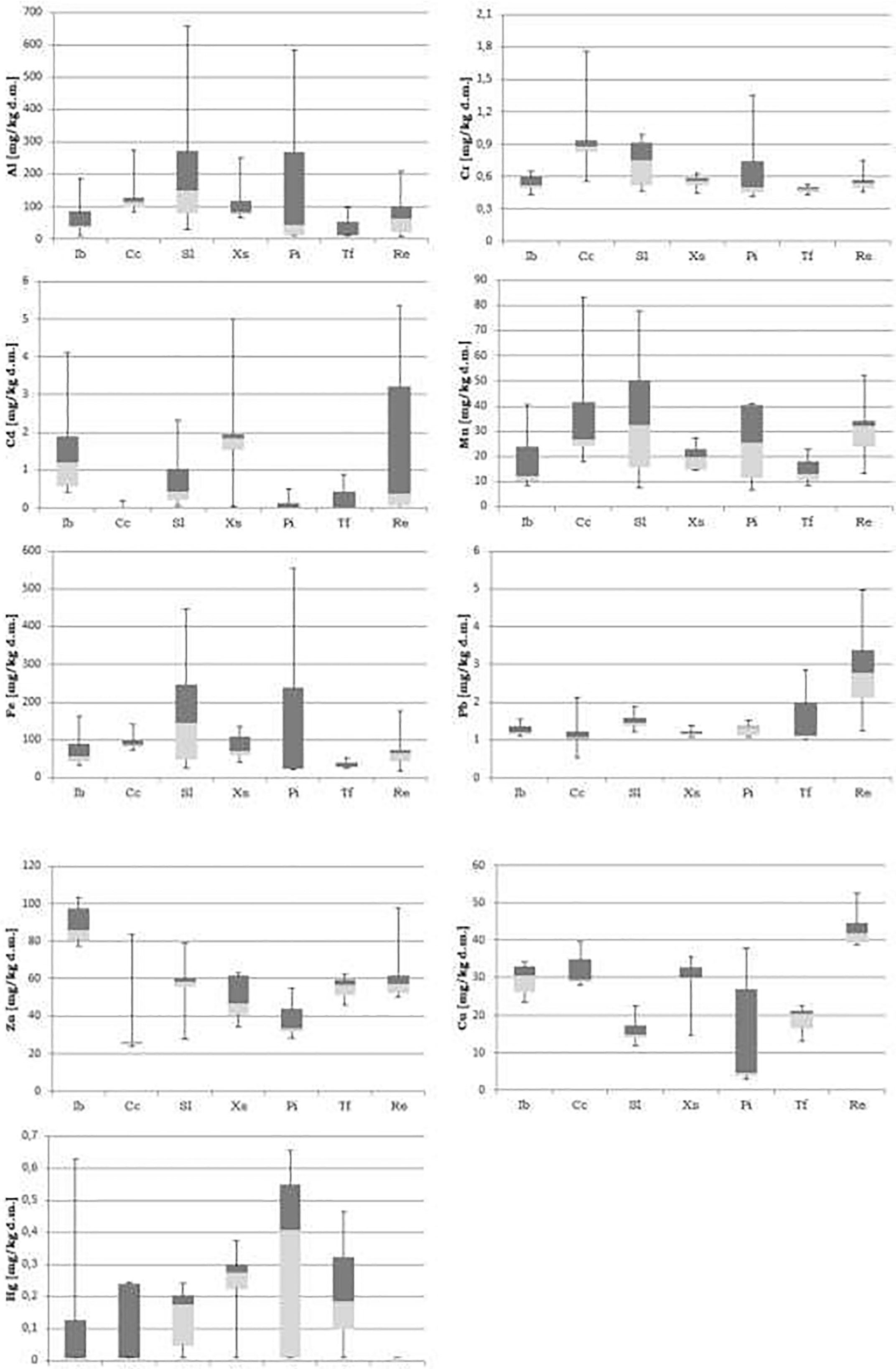

Fig. 5. Concentrations of analyzed heavy metals in mushrooms by species. Designation: Ib – Imleria badia, *Cc* – Cantharellus cibarius, Xs – Xerocomus subtomentosus, Sl – Suillus luteus, Pi – Paxillus involutus, Tf – Tylopilus felleus, Re – Russula emetica.

of 5.35 mg/kg has been recorded. According to EFSA recommendations, weekly cadmium consumption should not exceed 0.0025 mg/kg body weight. For the average person, the acceptable cadmium intake is 0.175 mg per week. Using simple calculations, it can be easily shown that even 145 g of dried *Imleria badia*, as well as 96 g of *Xerocomus subtomentosus* (based on medians), contain a dose of cadmium in excess of the permissible weekly consumption. With this in mind, it can be concluded that the cadmium concentration present in some species of the studied mushrooms poses a possible threat to health if consumed regularly in dried form. However, consuming fresh mushroom can be considered as relatively safe considering the concentrations of cadmium. In addition, the high concentration of cadmium often found in *Russula emetica* constitutes an indirect hazard when consuming meat from forest animals due to the phenomenon of bioaccumulation in the food chain.

The analysis showed moderate differences in manganese concentrations between the studied species of mushrooms. The median concentration of manganese in the mushroom samples ranged from 20 to 35 mg/kg. Slightly lower accumulation was found in *Imleria badia* and *Tylopilus felleus* (medians respectively 12.48 and 13.14 mg/kg). The highest value was recorded for *Cantharellus cibarius* – 83.10 mg/kg. The recommended daily intake of manganese is 3 mg, so it can be exceeded by consuming some species of mushrooms (especially *Cantharellus cibarius* and *Suillus luteus*). The recommended consumption of these species in dried form, in terms of manganese content, on the basis of the median was calculated to be 111 g and 92 g, respectively.

Significant differences in iron content depending on the species of the tested fungus were shown. The lowest values were obtained for *Paxillus involutus* (median 25.26 mg/kg) and *Tylopilus felleus* (median 29.27 mg/kg). The highest iron accumulation was found in samples of *Suillus luteus* (median 145.11 mg / kg), although the highest concentration of 553.96 mg/kg was recorded for *Paxillus involutus*. Assuming the daily iron requirement as 11 mg/kg for men and 16 mg/kg for women, it can be concluded that even 76 g and 110 g of *Suillus luteus* fulfills the daily iron requirement (for men and women, respectively).

The studied species of mushrooms were characterized by a similar ability to accumulate lead (Fig. 5). Typically, the values oscillated around 1–2 mg/kg. Slightly higher concentrations were reported for *Russula emetica* (median 2.78 mg/kg and a maximum concentration of 4.98 mg/kg). Because of the fact that lead is poisonous to humans and can affect people of any age. It can accumulate in the body, even low-level chronic exposure can be hazardous over time. Lead exposure is measured by testing for the level of lead in a person's blood. There is no identified safe blood lead level. Taking these facts into consideration, even the small amount of lead in the food you eat should be alarming.

Typical concentrations of zinc in the samples of most mushrooms ranged from 40 to 60 mg/kg. The lowest concentrations were recorded for *Cantharellus cibarius* (median 25.82 mg/kg), and the highest for *Imleria badia* (median 85.98 mg/kg). For the latter species, a maximum value of 103.50 mg/kg was recorded. Taking into account the recommended daily intake of zinc (7.5–16.3 mg), exceeding this value by consuming these mushrooms seems unlikely. This would require consuming between 190 mg for dried *Imleria badia* to 631 g for dried *Cantharellus cibarius* (assuming the top value of the daily requirement) in one day.

The lowest copper concentrations were recorded in the samples of the *Paxillus involutus*, *Tylopilus felleus*, and *Suillus luteus* (medians 4.51, 14.80 and 20.25 mg/kg, respectively), while the highest – in the samples of *Russula emetica* (median 41.83 mg/kg, maximum value 52.59 mg/kg). Edible mushroom species (with the exception of the aforementioned *Suillus luteus*) were characterized by typical copper concentrations of approximately 30 mg/kg. Comparing this value with the recommended copper intake (1.3–1.6 mg/day), it turns out that consumption of 42–55 g of dried mushrooms of these species fulfills the daily requirement for copper. Moreover, given the fact that the copper concentrations presented in this work are generally lower than in most studies in the literature, there is a real risk that consuming large amounts of mushrooms can lead to copper overdose.

Nickel in the vast majority of mushroom samples was not detected (below the LOD). No nickel concentrations above the lower limit of quantification (LOQ) were recorded in any of the samples.

Mercury content in the mushroom samples varied depending on the species, but was always at a low level. The lowest affinity for this element was found in *Russula emetica* (no values higher than the LOQ were recorded). The highest concentrations of mercury were recorded for samples of *Paxillus involutus* (median 0.408 mg/kg and maximum value 0.655 mg/kg). For the average person (weighing 70 kg), the maximum weekly mercury intake is 0.091–0.28 mg (depending on its form). The highest concentrations of mercury among edible mushroom samples were recorded for the *Xerocomus subtomentosus* (median 0.276 mg/kg), which means 330–1014 g of dried mushrooms must be eaten to exceed the recommended maximum values. This far exceeds the typical consumption of mushrooms, thus the health risk from mercury appears to be low.

4.3. *Correlations of heavy metal concentrations in mushrooms and soil*

The relationship between the concentration of heavy metals in soil and their concentration in fungi was determined. Correlation plots are presented in Supplementary Materials (Fig. S2). The highest correlation was observed for chromium ($R^2 = 0.9696$). Significant correlations can also be noticed for cadmium, copper, lead, iron and aluminum. There was no statistically significant relationship for zinc, manganese and mercury. It is worth noting that in the case of copper and cadmium, the concentrations of these metals in fungi are significantly higher than in soil, which proves the accumulation of these elements in fungi. Moreover, the BCF values were calculated and presented in Table S4 in the Supplementary Materials section.

4.4. *Collective analysis of heavy metal content in mushrooms*

Comparing the results of the conducted research with the results of other groups (Kalač, 2010) (looking generally, without division into individual species), it can be concluded that the contents of aluminum, cadmium, iron, lead, manganese, zinc, chromium and mercury recorded in mushroom samples in the present research are similar to the results obtained in previous studies. The determined concentrations of copper and nickel turned out to be unusually low. Typical concentrations of copper recorded in the previously conducted studies ranged from 20 to 100 mg/kg, and in the present study the median results ranged from 4.5 to 41.8 mg/kg. In the case of nickel, the studies showed different concentrations, from trace amounts to even 58.6 mg/kg. In the present study, the nickel concentrations turned out to be too low to be able to determine them (which was already mentioned in subsection 4.2.9.)

Brzostkowski et al. (Brzostowski, Falandysz, Jarzyńska, & Zhang, 2011) conducted analyses of the content of selected elements in *Paxillus involutus* collected in various places in Poland. For most metals, the results obtained in the present study are consistent with the cited work. Significant differences are found only in the case of zinc concentrations, which were on average about 5–7 times higher than those determined in the present study.

Studies on the accumulation of heavy metals in *Imleria badia* collected in various areas of northern Poland in the years 1993–1998 were carried out by Malinowska et al. (Malinowska, Szefer, & Falandysz, 2004). The results obtained by this team are consistent with those presented here for most metals, with the exception of zinc (on average twice as high in the studies by Malinowska et al.) And cadmium (slightly higher concentrations recorded in the present study).

In comparison with the results obtained by Chojnacka and Falandysz (Chojnacka & Falandysz, 2007), in *Xerocomus subtomentosus*, the concentration of Cd was determined at an average two-fold higher level. Moreover, in relation to the results of the cited work, there are significant discrepancies in the registered concentrations of lead and zinc.

Chojnacka and Falandysz obtained lead concentrations at the level of 0.3 mg/kg, which is much lower than the value determined in this study (median 1.17 mg/kg). Conversely, in the case of zinc, the content of this element in the samples of *Xerocomus subtomentosus* recorded in the present study was on average twice lower.

The accumulation of some elements in *Suillus luteus* and *Cantharellus cibarius* was investigated by Karmańska and Wędzisz (Karmańska & Wędzisz, 2010). While in the case of copper content, the results of the analyses carried out by Karmańska and Wędzisz are consistent with those presented in this publication, the differences concerning iron, zinc and cadmium are significant. The analyses conducted as part of the present study showed significantly lower concentrations of cadmium in both these species of mushrooms and lower concentrations of zinc in *Cantharellus cibarius*. Conversely, higher iron concentrations were observed in samples of *Suillus luteus*.

In the case of the *Tylopilus felleus* and the *Russula emetica*, no extensive research in this field has been conducted in Poland so far. There are reports of studies on the accumulation of single elements, e.g. lead in *Russula emetica* (Kwapuliński et al., 2009). The result of these studies confirms the bioaccumulation capacity of lead by this species of mushrooms, which was observed in the present study.

5. Conclusions

The presented work is a significant supplement to the current knowledge on the accumulation of radioisotopes and heavy metals in mushrooms, by confirming some observations obtained in previous studies and observing numerous previously unobserved phenomena. As an example, extensive measurements of Bi-214 activity have been made in European mushroom samples for the first time. The results of the measurements show a slight accumulation of this radioisotope by mushrooms and no health risk from Bi-214 associated with the consumption of forest mushrooms. It is also important to familiarize mushroom consumers with other potential threats. Nowadays, 34 years after the Chernobyl Disaster, there are still areas in Poland (in the vicinity of Opole) that are relatively heavily contaminated with the Cs-137 isotope. Due to conducted research the ability of wild-growing mushrooms to accumulate the Cs-137 radioisotope from the soil was confirmed. The results of the analyses of the activity of this isotope indicate that national and European standards have been significantly exceeded in terms of food safety in the case of samples from the vicinity of mentioned Opole Anomaly (Sowin/Łambinowice and Szumirad). It is also worth mentioning that activities of the Pb-210 isotope in the samples of mushrooms are significantly higher than the previous literature reports would suggest. However, It was impossible to pinpoint the exact reason for the noticeable changes in some elements concentrations through the years. It is assumed that different radiogeological specificity could be a critical factor. Moreover, comparing the ^{210}Pb activity results in mushroom samples and the content of the stable ^{208}Pb isotope, it can be stated that some species of mushrooms (e. g. *Cantharellus cibarius*) show a greater tendency to accumulate radioactive isotopes, while others (*Tylopilus felleus and Russula emetica*) more readily accumulate stable isotopes. Furthermore, the high accumulation capacity of some heavy metals in mushrooms, in particular cadmium and copper, was confirmed. The concentration of Cd in mushrooms is particularly high and can pose a health risk if they are consumed regularly in a dried form. Clear correlations were observed between the concentrations of most of the tested substances in the soil and the concentrations of these contaminants in mushrooms (Cs-137, Bi-214, Al, Cd, Cr, Fe, Cu, Pb). Such correlations were not found for K-40, Pb-210, Mn, Zn and Hg. As a final conclusion, it is worth considering organizing broad research on heavy metals and radioisotopes in forest mushrooms especially in radiogeological anomalies areas like Opole. The problem should be treated as a crucial issue in regions of Eastern Europe where forest mushroom collection seems to be especially popular.

CRediT authorship contribution statement

Oskar Ronda: Conceptualization, Resources, Investigation, Visualization, Writing - original draft. **Elżbieta Grządka:** Conceptualization, Methodology, Writing - review & editing. **Iwona Ostolska:** Methodology, Investigation, Formal analysis. **Jolanta Orzeł:** Methodology, Investigation, Formal analysis. **Bartłomiej Michał Cieślik:** Conceptualization, Methodology, Writing - review & editing, Supervision.

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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Appendix A. Supplementary data

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