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ORIGINAL PAPER

BIOCONCENTRATION OF CADMIUM (Cd), COPPER (Cu), LEAD (Pb) AND ZINC (Zn) IN *LACTARIUS SALMONICOLOR* IN THE WESTERN CARPATHIANS

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Abstract

As well as being part of the human diet, mushrooms are frequently used in bioindication. Mycorrhizal mushrooms, which live in symbiosis with different species of trees, can be used for accurate biomonitoring, including an analysis of related elements of the abiotic and biotic components of ecosystems. The study was conducted in the Jałowiec Range, which belongs to the Przedbabiogórskie Range in the Maków Beskids (the Western Carpathians). The aim of the research was to determine the content of cadmium, copper, lead and zinc in *Lactarius salmonicolor*. The research also aimed at determining the bioconcentration factor of the mentioned metals for the mushroom species. In addition, the suitability of *Lactarius salmonicolor* growing in the study area for human consumption was assessed. Samples of mushrooms (*Lactarius salmonicolor*) and soil were taken in autum 2015. Concentrations of Cd, Cu, Pb and Zn in the plant and soil material were determined by atomic emission spectrometry. In *Lactarius salmonicolor*, a significant correlation of the content of heavy metals in the mushrooms to the content of these metals in the soil was found for lead and copper (both for caps and stipes) and for zinc (for the soil to stipes ratio). The bioconcentration factor for caps and stipes of *Lactarius salmonicolor* was as follows: Cd>Zn>Cu>Pb. The relatively high cadmium content in the studied mushrooms may pose a risk to the consumer's health.

Keywords: mushrooms, bioconcentration, cadmium, copper, lead, zinc.

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INTRODUCTION

Wild growing, edible mushrooms are a valuable part of our diet, not only because of their taste but owing to a high content of carbohydrates, proteins, vitamins and minerals (Heleno et al. 2010, Kalac 2012, Reis et al. 2012). One of the problems associated with eating mushrooms is their capacity to bioconcentrate metal ions, which is influenced by environmental factors (Garcia et al. 1998), including mushroom life cycle, humus content in the substrate, substrate reaction, metal content in the substrate, age of fruiting bodies and of the mycelium (Srivastava, Shekhar 2006). Mushrooms have a much higher tendency to concentrate trace elements than agricultural crops, vegetables, fruit or animals (Hang et al. 2009, Mleczek et al. 2015, 2016). Therefore, their ability to accumulate high concentrations of heavy metals such as cadmium and lead (which are a toxicological risk to people whose diets include such mushroooms) is particularly dangerous. From the ecological point of view, mushrooms have a large effect on geochemical processes as they play a role in the circulation of elements and transformation of organic and inorganic substrates (Aloupi et al. 2012). According to numerous authors (Kula et al. 2011, Şen et al. 2012), owing to the mentioned capacity to accumulate trace elements and their strong connection to the collecting site, mushrooms can be bioindicators of the environmental pollution level. To identify the state of the environment, complete biomonitoring should include an analysis of related elements of the abiotic and biotic components of ecosystems.

The Przedbabiogórskie Range, which lies in the Maków Beskids (the Western Carpathians), comprises areas of high environmental value. It is not in close vicinity of any industry, busy roads or agglomerations, which is why it is not exposed to excessive emission of pollutants. Conditions occurring in this area make it an ideal destination for tourists, and plants and edible mushrooms which can be found there have been desired and collected for centuries. From a wide range of raw materials harvested in this area, special attention should be drawn to *Lactarius salmonicolor* (Wojewoda 2003). *Lactarius salmonicolor* is a mushroom collected for its unique taste; it is held in high regard by mushroom pickers because of its supreme “healthiness”, which arises from the fact that it appears late in the season (until November), when the activity of pests that prey on it is low. In Europe, *Lactarius salmonicolor* can be found mainly in mountainous areas, and in Poland – near the Pieniny Mountains, the Gorce Mountains and on Babia Góra Mountain (Skirgiełło 1998). It belongs to mycorrhizal mushrooms, forming symbiosis with *Abies alba*.

The aim of the research was to determine the content of cadmium, copper, lead and zinc in *Lactarius salmonicolor* grown in the Przedbabiogórskie Range (Poland). The research also aimed at determining the bioconcentration factor of the mentioned metals for the studied organisms and the suitability of *Lactarius salmonicolor* growing in the study area for human consumption.

MATERIAL AND METHODS

The research area consisted of the Jałowiec Range, which belongs to the Przedbabiogórskie Range, in the Maków Beskids. The research goal was reached by collecting samples of *Lactarius salmonicolor* from 17 sites (Figure 1).

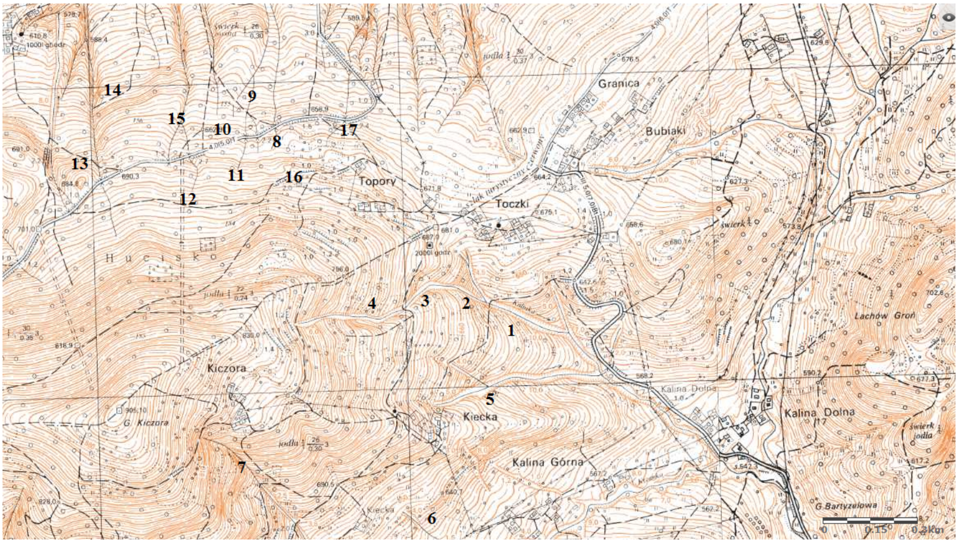

Fig. 1. Research area with established sample collection sites (1,2,3…)

Samples were collected in autnum 2015. A homogeneous area within a 50 m radius was used as a single collection site. A laboratory sample was a bulk sample consisting of approximately 100 sub-samples. A laboratory sample for each site weighed approximately 1000 g. The mushrooms were collected with a plastic knife. Out of the 17 collection sites, 16 were located along various mountain streams flowing through the Lachówka River basin (from the northern side) and the Skawica River basin (from the southern side). Simultaneously, soil samples were collected from the same sites, from a 0-20 cm layer. The soil was sampled with an Egner's stick. A bulk sample of the soil consisted of approximately 20 sub-samples. A laboratory sample was prepared by reducing a bulk sample to the mass of approximately 1000 g. Once the mushrooms were collected, they were washed with distilled water, and then stipes were separated from caps. All the collected samples were dried and homogenized before analyses. Soil organic carbon was determined using the elemental analysis method in a Vario MAX cube apparatus (Elemental Analysesysteme). Soil pH was determined with the potentiometric metod (pH-meter CP505 Elmetron). The laboratory samples of the mushrooms were subjected to dry mineralization in an open system. An analytical sample weighed 3 g. The material was digested in 6 ml of HNO_3 (14.4 M dm^{-3}) and

H_2O_2 (9,8 M dm^{-3}) (5:1 v/v ratio). The soil samples were mineralized in *aqua regia*. Concentrations of elements in the solutions thus obtained were determined by inductively coupled plasma atomic emission spectrophotometery (ICP-OES), on an Optima 7600 DV spectrometer manufactured by PerkinElmer. Wavelengths used to determine the concentrations of the elements as well as the detection limit for the methods are provided in Table 1. The correctness

Table 1

Parameters of the analytical method

Specification	Cd	Cu	Pb	Zn
Wavelengths (nm)	228.8	327.3	220.3	206.2
Detection limit (mg dm^{-3})	0.0027	0.0097	0.042	0.0059
Concentration in certified material (mg kg^{-1})	0.013	5.641	0.470	12.51
Measured (mg kg^{-1})	0.011	5.831	0.534	13.83
Recovery (%)	84.60	103.4	113.6	111.1

of analyses of the elements was verified using certified reference material (NIST-1515). Table 1 shows results of analyses of the reference material and an estimated value of recovery based on analyses conducted in 4 replications. Based on the results, bioconcentration factors of individual elements were calculated by dividing the concentrations of the elements in the dry matter of mushrooms used in the research by the concentration of these elements in the soil. In addition, a correlation coefficient between the concentration of each element in the soil and in the mushrooms was computed.

ANOVA (Analysis of Variance) was used for statistical analysis of the results. The significance of differences between means from the objects was tested with the Tukey's test at a significance level of a = 0.05. The results were also described with linear regression.

RESULTS

The acidity and the concentrations of Cd, Cu, Pb and Zn in the soil were varied (Table 2). Taking into consideration effective regulations (Regulation 2002), the examined soils did not exceed the maximum permissible concentration of Cd, Cu, Pb or Zn. In the case of cadmium and lead, the determined concentrations of these elements corresponded to the natural concentrations of these elements in soil (Wyszkowska et al. 2013). The pH of the analyzed forest soils ranged from 4.22 to 5.67 (pH H_2O) and from 3.44 to 4.72 (pH KCl). The soils had a high content of organic matter (from 3.43 to 13.59%).

The mean concentrations of the metals in stipes and caps of *Lactarius salmonicolor* (Table 3) can be presented in the following order: Zn>Cu>Cd>Pb. Other authors have found similar results in their studies (Paraskevi

Table 2

Selected chemical properties of soil

Specification	pH H_2O	pH KCl	(%)	
			organic C	organic matter
Minimum	4.220	3.440	1.987	3.426
Maximum	5.679	4.720	7.884	13.593
Mean	5.076±0.3801*	4.140±0.370	4.244±1.631	7.317±2.803
Specification	(mg kg^{-1})			
	Cd	Cu	Pb	Zn
Minimum	0.351	4.875	13.762	30.637
Maximum	1.275	22.851	34.501	78.387
Mean	0.864±0.231	10.068±5.262	24.877±6.000	53.164±13.737

* ± Standard deviaton

Table 3

Concentrations of Cd, Cu, Pb and Zn in *Lactarius salmonicolor* (mg kg^{-1})

Specification	Stipes	Caps	Stipes	Caps
	Cd		Cu	
Minimum	0.528	1.351	6.737	7.537
Maximum	4.922	6.002	17.151	22.425
Mean	2.118^{a}±1.144*	3.492^{b}±1.194	10.028^{a}±2.530	11.225^{a}±3.707
	Pb		Zn	
Minimum	0.350	0.375	39.087	59.834
Maximum	3.862	4.425	76.375	98.162
Mean	1.904^{a}±1.087	2.096^{a}±1.229	60.081^{a}±11.596	78.297^{b}±10.834

* ± Stadard deviation, means followed by the same letter for the same element did not differ significantly at $a < 0.05$ according t-Tukey test.

et al. 2009). In the mushrooms studied in our experiment, the concentrations of these metals were higher in caps than in stipes (Table 3). In the case of cadmium and zinc, the significance of differences in their content in caps and stipes was confirmed statistically; as for lead and copper, only a tendency was observed. The ratio of metal concentrations in caps to stipes was: 1.10 for lead; 1.65 for cadmium; 1.12 for copper; and 1.30 for zinc. Transfer of these metals from the soil to the mushrooms was varied. Values of the bioconcentration factor (Table 4), which (similarly to that in plants) is described as the ratio of the concentration of a metal in mushrooms to its concentration in soil, both for stipes and caps, can be presented in the following order: Cd>Zn>Cu>Pb. The concentrations of the metals in the mushrooms showed a positive correlation with their concentration in the soil. In the case of lead, copper and zinc, the mentioned relationship was statistically significant (Figures 2, 4, 5). The highest value of the bioconcentration factor was record-

Table 4

Bioconcentration factor for *Lactarius salmonicolor*

Specification	Stipes	Caps	Stipes	Caps
	Cd		Cu	
Minimum	0.665	1.698	0.529	0.549
Maximum	5.392	6.786	2.264	1.729
Mean	2.575^a±1.311	4.240^b±1.453	1.153^a±0.448	1.249^a±0.358
	Pb		Zn	
Minimum	0.014	0.023	0.733	0.823
Maximum	0.176	0.137	1.665	2.283
Mean	0.075^a±0.041	0.080^a±0.036	1.171^a±0.240	1.549^b±0.360

ed for cadmium (both in caps and stipes), without a significant correlation between its concentration in the soil and in the mushrooms (Figure 3). The examined mushrooms showed a high capacity to accumulate this element in stipes and caps. According to numerous authors (GAST et al. 1988, ARUGUETE et al. 1998), the capacity for excessive accumulation of cadmium is a genetically conditioned feature that is characteristic for mycorrhizal mushrooms. To understand this, it is necessary to explore the role of mycorrhizae for metal sequestration and movement in forest systems (transformation of organic and inorganic substrates). According to different authors (SHETTY et al. 1995, CHAUDHRY et al. 1999), mycorrhizae have been reported in plants growing on heavy metal contaminated sites, indicating that these mushrooms have evolved a tolerance for heavy metals and that may play a role in the phytoremediation of a polluted site (KHAN et al. 2000).

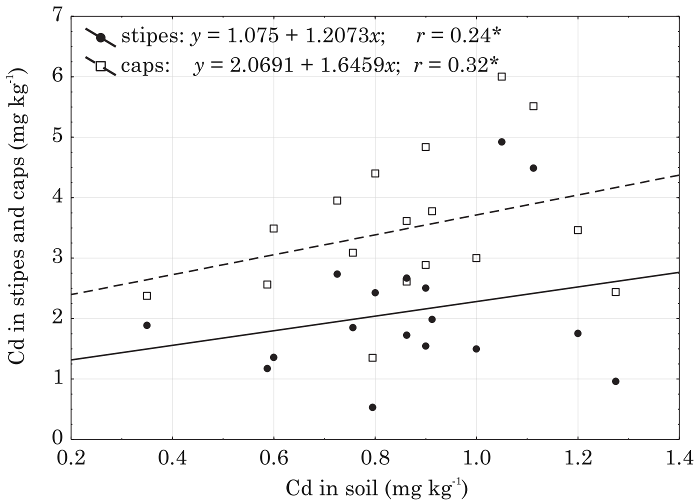

Fig. 2. Dependence of cadmium concentration in stipes and caps of *Lactarius salmonicolor* on the concentration of this element in the soil. * Not significant at importance level $a = 0.05$, $n = 17$

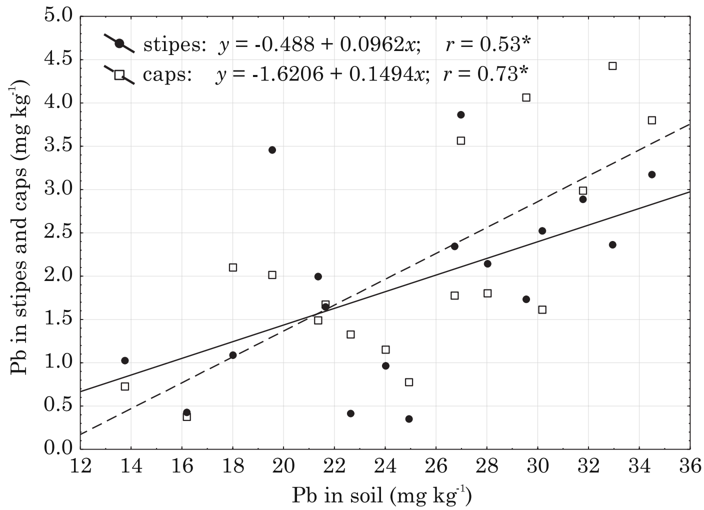

Fig. 3. Dependence of lead concentration in stipes and caps of *Lactarius salmonicolor* and on concentration of this element in the soil. * Significant at importance level $a = 0.05$, $n = 17$

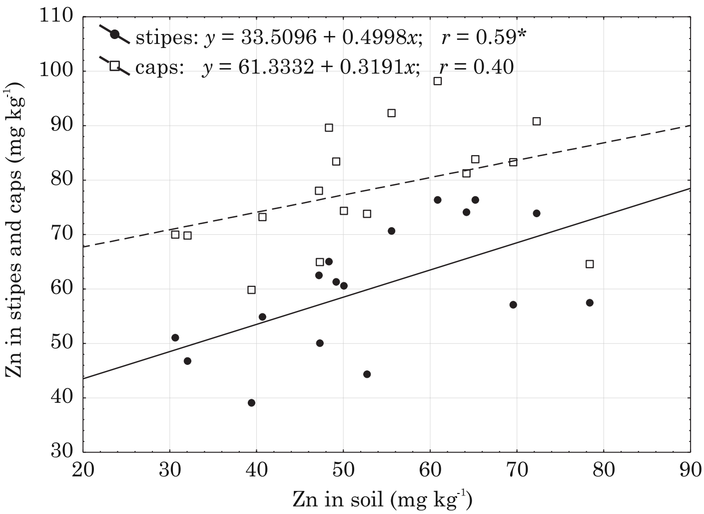

Fig. 4. Dependence of zinc concentration in stipes and caps of *Lactarius salmonicolor* on the concentration of this element in the soil. * Significant at importance level $a = 0.05$, $n = 17$

The mycelia of higher mushrooms can cover a relatively large area (up to several square meters), and their strong relation with soil, organic matter and roots of plants (particularly in the case of mycorrhizal mushrooms) creates perfect conditions for intensive exchange of nutrients (TURNAU et al. 2001, FALANDYSZ et al. 2012). For numerous species, the presence and concentrations of heavy metals in mushrooms are associated with their concentrations in soil in which these mushrooms grow. This applies to copper and zinc

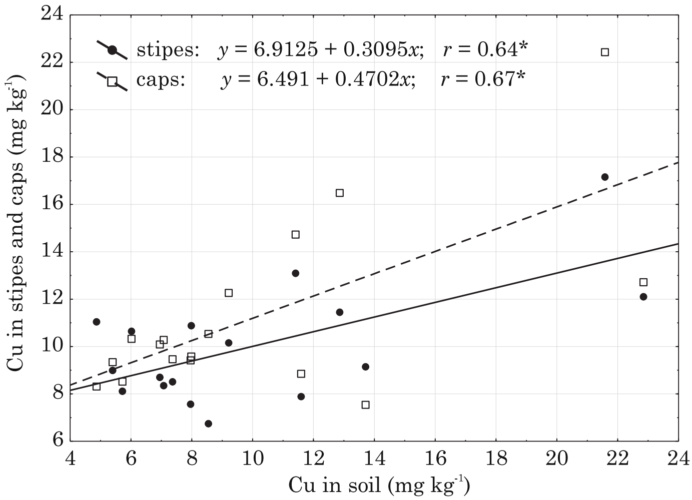

Fig. 5. Dependence of copper concentration in stipes and caps of *Lactarius salmonicolor* on the concentration of this element in the soil. * Significant at importance level α = 0.05, n = 17

as well (ALONSO et al. 2003, MELGAR et al. 2009). The occurrence and concentrations of heavy metals are also conditioned by other properties which characterize given soil, e.g. reaction, organic matter content. In acid soils, such as forest soils in the Maków Beskids, desorption of metals from soil to the soil solution is increased by soil saturation with H^+ ions, and depends on the specificity of a given metal. Copper uptake (stipes: r = -0.48; caps: r = -0.53 for α = 0.05) and zinc uptake (stipes: r = -0.63; caps: r = -0.62 for α = 0.05) by the studied mushrooms were significantly associated with the soil reaction. No such dependences appeared for the other studied metals. A positive correlation between the lead concentration in the studied parts of mushrooms and the organic matter content in the soil (stipes: r = 0.54; caps: r = 0.55 for α = 0.05) was observed in the present research.

A statistically significant correlation was found between zinc and copper concentration in caps (r = 0.69) and stipes (r = 0.65), and also between lead and zinc concentration in caps (r = 0.45). In their research, PARASKEVI et al. (2009) found similar relationships.

In the case of toxic metals, lead as well as cadmium, their concentration in the mushrooms did not exceed acceptable standards established by the European Commission (2006) for food products. Values presented in the WHO standards (2017) are more rigorous. Taking into account the daily food ration in the amount of 300 g fresh product (one meal), which contains 30 g dry matter (KALAC, SVOBODA 2000, SVOBODA et al. 2000), it was found that in mushrooms from 16 out of 17 collecting sites (Figure 6) the concentration of cadmium in caps was so high that their consumption in the aforementioned dose in a daily diet of a 60 kg adult person would cause excessive daily intake of cadmium that was established by the WHO (2017). As for the

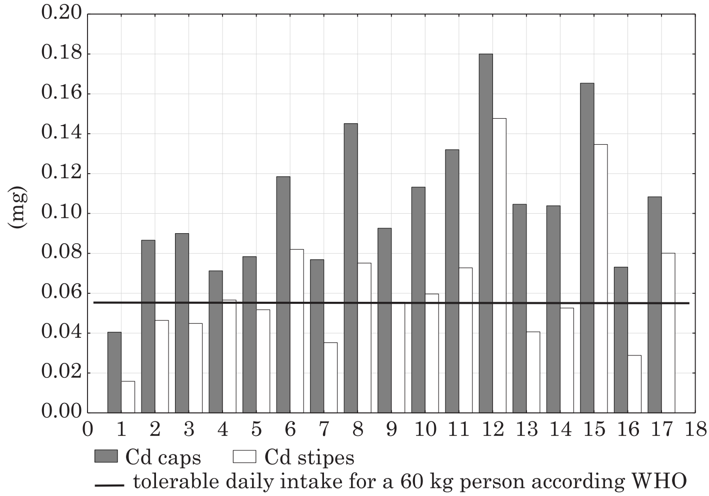

Fig. 6. Daily cadmium intake (mg) by a 60 kg adult person together with one meal (300 g fresh product), depending on the mushroom collection site, compared to tolerable standard (Aloupi et al. 2012, WHO 2017)

stipes, such a relationship was found in mushrooms from 6 sites. In the case of lead, the WHO (2017) does not publish provisional tolerable daily (weekly) intake for this element because it did not believe establishing a value that would be " safe for health" was possible (Anderson et al. 2017). A report of the U.S. Agency for Toxic Substances and Disease Registry (ATSDR) states that the average daily intake of lead from food sources in the general population is approximately 0.056 mg day^{-1} (ATSDR 2007). In comparison, the maximum daily lead intake with a food ration of mushrooms in this research has reached a level of 0.134 mg (Figure 7).

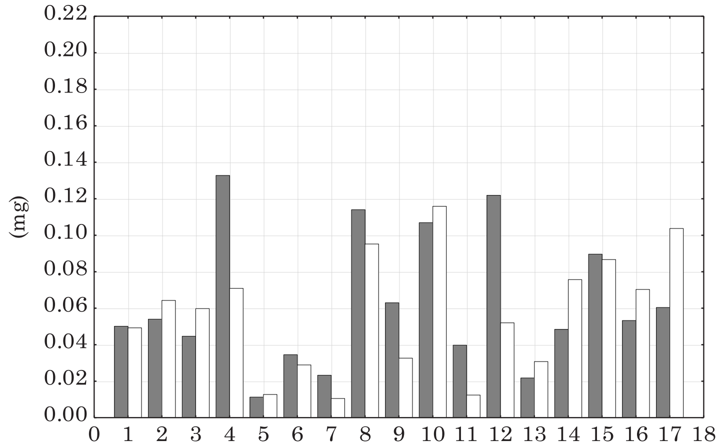

Fig. 7. Daily lead intake (mg) by a 60 kg adult person together with one meal, 300 g fresh product, (Aloupi et al. 2012, WHO 2017)

CONCLUSIONS

1. A statistically significant relationship between the copper and lead content in the soil and the concentrations of these metals in *Lactarius salmonicolor* was observed for both caps and stipes, and with respect to zinc – in the case of the soil to stipes ratio.

2. In the case of *Lactarius salmonicolor*, a significant correlation of the concentrations of heavy metals in the mushrooms with the concentrations of these metals in the soil was found for lead and copper (both for caps and stipes) and for zinc (in the case of the soil to stipes ratio).

3. The bioconcentration factor for caps and stipes of *Lactarius salmonicolor* was as follows: Cd>Zn>Cu>Pb. *Lactarius salmonicolor* caps had a higher concentration of the metals than stipes.

4. With a bad diet, the relatively high cadmium and lead concentrations in the analyzed mushrooms may pose a risk to the consumer's health.

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