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MUSHROOMS AS BIOMONITORS OF HEAVY METALS CONTAMINATION IN FOREST AREAS

GRZYBY JAKO BIOMONITORY ZANIECZYSZCZENIA TERENÓW LEŚNYCH METALAMI CIĘŻKIMI

Abstract: The aim of the research was to assess the level of contamination with heavy metals (manganese, iron, nickel, copper, zinc, cadmium and lead) in two forest areas selected in different places in Poland: the first one in the Swietokrzyskie Province (forests of the Staporkow Forest Division) and the second one in the Opolskie Province (forests of the Kup Forest Division). The degree of contamination of these forest areas with analytes was found using edible large-fruited mushrooms naturally occurring there - the research was carried out using passive biomonitoring method. Heavy metals in mushrooms (separately in stems and hats) as well as in soil samples were determined by atomic absorption spectrometry with excitation in flame (F-AAS). The obtained results were interpreted by assessing the degree of contamination of forest areas on the basis of concentrations of heavy metals in mushrooms. The obtained results indicate an increased accumulation of heavy metals in hats than in mushrooms stems. On the basis of the obtained data, significant contamination of forest areas with selected heavy metals was also found. This is confirmed by the possibility of using mushrooms as biomonitors in passive biomonitoring of forest areas, which are heavy metal accumulators. In the interpretation of the test results, the phytocumuling factor (*PF*) was also used. The degree of accumulation of heavy metals, from given forest areas - from soil to mushrooms - was assessed on the basis of determined *PF* coefficients. In addition, good bioavailability of the analysed analytes by mushrooms was found. Additionally, on the basis of the conducted studies, the possibility of mushroom consumption was assessed - they are not suitable for consumption due to the fact that the permissible concentration standards of heavy metals contained in mushrooms were exceeded.

Keywords: bioindication, passive biomonitoring, environmental contamination, atomic absorption spectrometry

Introduction

Emission of pollution is a consequence of human large industrial activity since the 19th century, which has resulted in considerable pollution of environment with heavy metals. Heavy metals are present in fauna and flora, penetrating various ecosystems; water and land biotopes are contaminated and, in time, trace elements get through to food chains [1]. The threat posed by heavy metals results directly from their migration in trophic chain: soil - plant - animal - humans, and the possibility of their accumulation in the last link of the chain, i.e. human body [2]. Heavy metals enter human and animal organisms most

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frequently with food, air or through the skin. The possibility to limit spreading of heavy metals exists in woods, thanks to their emission reducing characteristics they decrease quantities of heavy metals and suspended dust in the air, which humans breathe. The most toxic heavy metals, which are indispensable for growth and development of living organisms are cadmium, mercury and lead [2].

Bioindication is a method of environment condition assessment, mainly changes of pollution levels, on the basis of studies of living organisms reaction to changes in their environment. The method is mainly used in monitoring of industrial and communication type pollution [3]. The quantitative assessment of heavy metals charge in woodland areas is possible thanks to the application of biological methods, with the use of selected living organisms - bioindicators, which demonstrate bioaccumulation characteristics. The organisms which accumulate pollution in the form of trace elements, form one of the numerous groups of bioindicators used in assessment of various parameters of woodlands [4].

The aim of biomonitoring is the assessment of pollution distribution in the studied area. Active biomonitoring allows to detect, after certain time, pollution accumulated in an organism, which was taken before from an unpolluted area and exposed in a potentially pollutes location [5]. Passive biomonitoring is based on the analysis of chemical composition of the samples, collected for the study in their habitat [6].The main advantage of biomonitoring is low cost of environment samples collection and the fact that they accumulate bioaccessible forms of pollution [7].

Mosses, lichens, groundcover or various tree types are the most frequently used biomonitors in land, particularly woodland area ecosystems [8-15].

Mushrooms (*Fungi*) include approximately 14,000 of macrofungi species [16], of which at least 2000 species are edible [17]. It is estimated that there are currently 150,000 higher species of fungi, of which less than 10 % have been described [18]. Approximately 14,000 species of mushrooms, including 4500 of macrofungi have been described in Poland. Among the macrofungi, i.e. having value for consumers, there are approximately 1100-1400 edible species, 200-250 poisonous and 2850-3200 inedible and harmful species [19].

In comparison to other living organisms, mushrooms are little known and rarely used indicators of environmental changes in Poland and globally. Very few studies initiated attempts at environment monitoring with the use of mushrooms. However, these organisms are a common as well as important structural and functional element of many land ecosystems, as the most important link which participates in generation and mineralisation of soils (up to 90 % share in humus generation) [20]. Taking the above into consideration, one may distinguish the species with good bioindication characteristics, which specifically react to changes in environment [21]. Mushrooms can be used in monitoring of land ecosystems, woodlands in particular, as indicators of air and soil pollution and, among others, as bioindicators of environment pollution with heavy metals [22, 23].

The aim of the carried out research was to assess the level of pollution with the selected heavy metals: manganese, iron, nickel, copper, zinc, cadmium and lead of two wood areas located in the selected places in Poland, with the use of edible macrofungi, among others *Boletus edulis*, *Macrolepiota procera*, *Suillus luteus*. An analysis of the possibility to consume the mushrooms was carried out, based on the calculated values of phytocumuling factors for the determined analytes accumulated in fruiting bodies.

Materials and research methodology

The studies have been carried out in two provinces: in the woods of the Staporkow Forest Division (Swietokrzyskie Province) - area S and in the woods of Kup Forest Division (Opole Province) - area K. The Staporkow Forest Division manages 12 thousand hectares of woodlands. The region includes a hilly plateau, which is a fragment of north-eastern mesozoic edge of the Swietokrzyskie Mountains. The main part of the Forest Division is located in the area of the Nieklansko-Blizynskie Hills [24]. In the woods of the Staporkow Forest Division, near the village of Stary Janow, 12 measurement points were marked, where samples of mushrooms and the soil on which they grew, were collected (Fig. 1a).

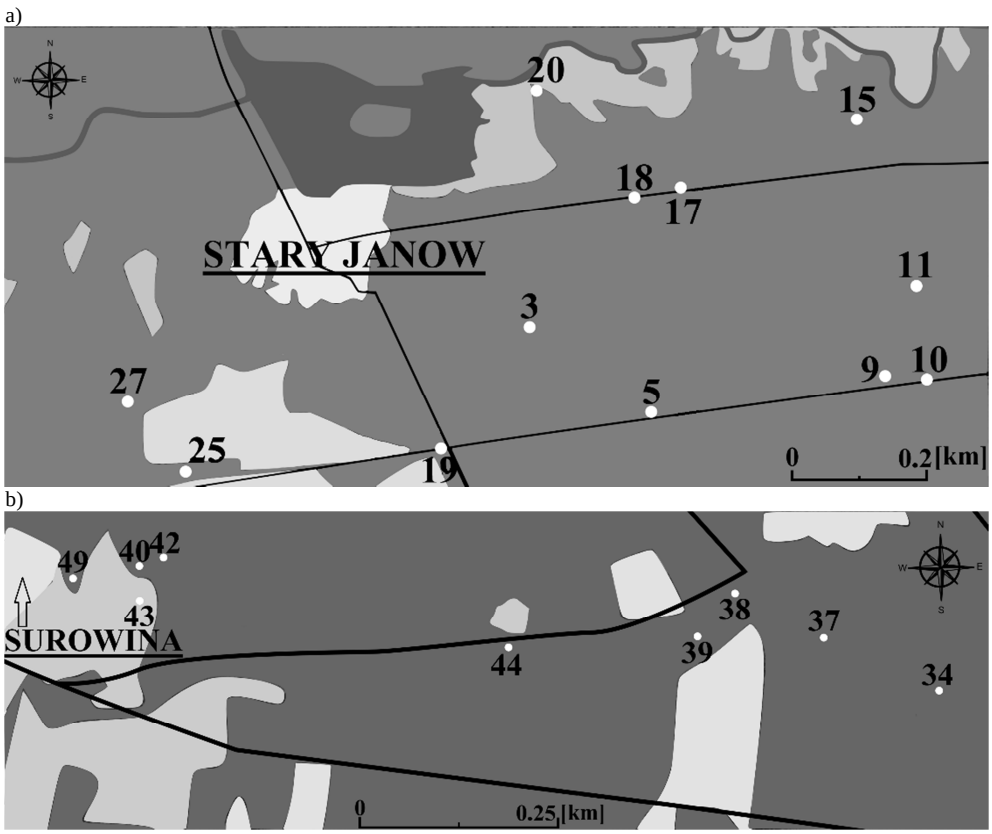

Fig. 1. Location of measurement points in the forests: a) of the Staporkow Forest Division, b) of the Kup Forest Division

The area of the Kup Forest Division is located within the Opole Province, in the following communes: Dobrzen Wielki, Lubniany, Murow, Pokoj, Popielow and Swierczow. The total area of the unit is approximately 20,670 hectares (including 19,960 hectares of woodlands). From the natural and geographical point of view, the Kup Forest Division woods are located mainly in the 5th Forest-Nature Silesian Area, in the 5th

Area of the Opolska Plain and Bory Stobrawskie mesoregion [25]. Samples from 9 measurement points, localised near a hamlet in the vicinity of the Brynica village, were taken in the area (Fig. 1b). Three edible mushrooms were picked from each measurement point. Biomonitoring studies were carried out during the period from September to November 2016, in the fall season.

In order to determine the level of heavy metals pollution in the woods of the Staporkow and Kup Forest Division, biomonitoring studies were carried out with the passive method, which determines heavy metal concentrations in the collected samples of the biota, which naturally exists in the study area. The study material consisted of soil and mushrooms (stems and hats) collected in the study area. Mushroom samples were cleaned from mechanical impurities, e.g. leaves and needles, divided into morphological parts and dried at room temperature to obtain dry mass.

Table 1

The instrumental detection limits (*IDL*) and instrumental quantification limits (*IQL*) for the spectrometer iCE 3500 [mg/dm^3] [26]

Metal	***IDL***	***IQL***
Mn	0.0016	0.020
Fe	0.0043	0.050
Ni	0.0043	0.050
Cu	0.0045	0.033
Zn	0.0033	0.010
Cd	0.0028	0.013
Pb	0.0130	0.070

Table 2

Comparison of measured and certified concentrations in BCR-482 *lichen*

Metal	**BCR-482 *lichen***		**AAS**		**Dev.****
	Concentration	**Uncertainty**	**Average**	**±SD***	
	[mg/kg d.m.]				[%]
Mn	33.0	0.50	31.70	0.68	–3.90
Fe	804	160	n.d.	n.d.	n.d.
Ni	2.47	0.07	2.16	0.32	–13
Cu	7.03	0.19	6.63	0.17	–5.70
Zn	101	2.20	95.1	2.30	–5.50
Cd	0.56	0.02	0.53	0.03	–5.30
Pb	40.9	1.40	38.2	1.00	–6.60

* - standard deviation, ** - relative difference between the measured (c_z) and certified (c_c) concentration $100\% \cdot (c_z - c_c)/c_c$, n.d. - not determined

Following collection, cleaning and drying, each sample was digested in the microwave digestion vessel Speedwave 4 made by Berghoff (DE). The averaged mushroom samples with the mass of 0.400 ±0.001 g d.m. (d.m. - dry mass) and soil with the mass of 0.500 ±0.001 g d.m. were dissolved in a mixture of nitric acid (V) 65 % and hydrogen peroxide (30 %) in the 5:3 proportion. Following the completed digestion process, the solutions were filtered into volumetric flasks of 25 cm^3 volume. The process temperature was 190 °C. MERCK company reagents were used to prepare solutions. Next, heavy metals concentrations (Mn, Fe, Ni, Cu, Zn, Cd and Pb) were determined in the digested samples, with the use of flame atomic absorption spectroscopy (F-AAS), using the atomic absorption spectrometer iCE 3500 made by Thermo Electron Corporation (USA). Table 1 presents the

limits of detection and the limits of quantification for flame atomic absorption spectrometer. The equipment was calibrated with the use of calibration standards from the company ANALYTIKA Ltd.

Table 2 shows heavy metals concentrations, determined in the certified reference materials as BCR-482 *lichen*, prepared by the Institute for Reference Materials and Measurements, Belgium.

Research results

Table 3 presents heavy metals concentrations determined in the soil and mushroom samples collected in the woods of Staporkow and Kup Forest Divisions. The values in bold, e.g. **2.19** are the concentrations, which exceed limit values for edible mushrooms [27].

Table 3

The values of heavy metals concentrations determined in the samples [mg/kg d.m.]

Number of the measuring point	Mn	Fe	Ni	Cu	Zn	Cd	Pb
Forests of the Staporkow Forest Division							
3 SOIL	36.9	4743	< 3.13	7.07	35.4	< 0.813	70.1
3 STEM	5.09	28.7	< 3.13	31.4	64.3	< 0.813	< 4.38
3 HAT	18.9	26.3	< 3.13	77.0	125	**2.19**	< 4.38
5 SOIL	27.0	3586	< 3.13	5.22	24.4	< 0.813	74.1
5 STEM	8.44	39.0	< 3.13	24.4	58.3	< 0.813	< 4.38
5 HAT	17.4	39.8	< 3.13	68.1	134	< 0.813	< 4.38
9 SOIL	29.0	4498	< 3.13	4.82	30.7	< 0.813	159
9 STEM	9.12	75.0	< 3.13	23.4	46.9	**2.31**	< 4.38
9 HAT	7.29	63.3	< 3.13	22.6	48.2	**3.14**	< 4.38
10 SOIL	174	11349	9.01	13.3	55.9	< 0.813	17.6
10 STEM	3.39	42.5	< 3.13	5.56	37.6	< 0.813	**7.11**
10 HAT	3.63	54.6	< 3.13	15.6	78.6	< 0.813	< 4.38
11 SOIL	22.8	3351	< 3.13	8.21	24.0	< 0.813	73.2
11 STEM	4.96	25.9	< 3.13	30.4	59.8	< 0.813	**5.06**
11 HAT	15.2	33.5	< 3.13	69.8	111	**3.00**	< 4.38
15 SOIL	37.4	9383	< 3.13	12.2	39.6	< 0.813	116
15 STEM	3.46	25.30	< 3.13	25.0	53.4	< 0.813	< 4.38
15 HAT	3.90	35.7	< 3.13	38.0	81.7	< 0.813	< 4.38
17 SOIL	23.5	8733	< 3.13	4.76	21.9	< 0.813	39.7
17 STEM	6.13	34.2	< 3.13	10.8	53.1	< 0.813	< 4.38
17 HAT	10.3	36.5	< 3.13	25.5	54.7	< 0.813	< 4.38
18 SOIL	46.8	7039	< 3.13	9.17	72.8	< 0.813	95.4
18 STEM	5.61	50.5	< 3.13	18.8	27.0	< 0.813	< 4.38
18 HAT	5.42	87.5	< 3.13	25.8	43.0	< 0.813	< 4.38
19 SOIL	46.8	4104	< 3.13	8.01	38.9	< 0.813	40.2
19 STEM	9.68	181	< 3.13	19.9	27.4	< 0.813	< 4.38
19 HAT	5.03	74.9	< 3.13	36.7	42.3	< 0.813	< 4.38
20 SOIL	121	15988	3.93	8.53	47.7	< 0.813	47.0
20 STEM	8.34	34.1	< 3.13	8.58	95.9	**3.17**	**23.0**
20 HAT	9.33	90.4	< 3.13	28.2	207	**14.6**	< 4.38
25 SOIL	40.4	6818	< 3.13	7.60	47.4	< 0.813	119
25 STEM	3.63	36.9	< 3.13	15.8	26.6	< 0.813	< 4.38
25 HAT	8.10	223	< 3.13	20.6	33.3	< 0.813	**22.2**
27 SOIL	17.9	3248	< 3.13	8.83	24.1	< 0.813	101
27 STEM	2.89	44.5	< 3.13	13.8	69.8	**4.70**	**19.4**

Number of the measuring point	Mn	Fe	Ni	Cu	Zn	Cd	Pb
27 HAT	6.86	65.3	< 3.13	27.3	118	**8.20**	**13.6**
Forests of the Kup Forest Division							
34 SOIL	86.9	5585	6.22	13.4	71.7	< 0.813	136
34 STEM	8.71	36.1	< 3.13	25.8	113	< 0.813	**11.4**
34 HAT	11.3	83.0	< 3.13	77.7	179	**1.70**	**6.23**
37 SOIL	164	2544	4.43	13.0	119	0.885	84.0
37 STEM	53.0	56.0	< 3.13	22.8	58.3	< 0.813	**14.3**
37 HAT	23.0	47.2	< 3.13	37.0	102	**1.58**	**11.3**
38 SOIL	77.1	3677	3.93	14.8	95.1	0.840	85.1
38 STEM	14.1	37.5	< 3.13	31.6	84.7	< 0.813	**20.3**
38 HAT	38.5	43.8	< 3.13	64.2	146	< 0.813	**24.7**
39 SOIL	23.0	2397	< 3.13	5.10	57.1	< 0.813	50.9
39 STEM	20.0	132	3.29	7.26	26.0	**2.18**	**53.9**
39 HAT	22.8	149	4.28	22.7	84.5	**14.7**	**14.8**
40 SOIL	165	2641	4.48	5.69	80.3	< 0.813	41.3
40 HAT	19.2	99.9	< 3.13	123	202	**3.54**	**5.88**
42 SOIL	121	3642	4.52	6.91	83.5	< 0.813	46.1
42 STEM	20.7	167	< 3.13	9.40	82.4	< 0.813	**49.1**
42 HAT	38.2	118	< 3.13	27.1	204	**8.15**	**5.30**
43 SOIL	57.3	2213	< 3.13	4.03	58.0	< 0.813	37.0
43 STEM	5.58	35.9	< 3.13	3.29	36.5	< 0.813	**5.16**
43 HAT	8.30	31.9	< 3.13	12.4	98.2	**2.01**	< 4.38
44 SOIL	133	3717	4.96	7.48	90.1	< 0.813	44.4
44 STEM	16.9	201	< 3.13	3.00	63.4	**2.74**	**45.9**
44 HAT	17.0	184	< 3.13	11.1	127	**13.1**	**36.5**
49 SOIL	1333	11605	15.6	18.7	344	5.15	226
49 STEM	26.6	56.3	< 3.13	90.6	51.5	**1.23**	**24.4**
49 HAT	14.9	58.5	< 3.13	181	79.4	**4.89**	**12.3**

The graphs in Figures 2 and 3 present distribution of concentrations of the heavy metals determined in soil and morphological parts of edible mushrooms.

Table 4 presents the basic statistical parameters of concentrations of the heavy metals determined in the soil samples.

Table 4

The values statistical parameters of concentrations of the heavy metals determined in the analysed soil [mg/kg d.m.]

Forests of the Staporkow Forest Division							
Parameter	**Mn**	**Fe**	**Ni**	**Cu**	**Zn**	**Cd**	**Pb**
Average	52.0	5609	< 4.64	8.13	38.6	< 0.813	79.4
Minimum	17.9	134	< 3.13	4.76	21.9	< 0.813	17.6
Maximum	174	15988	9.01	13.3	72.8	< 0.813	159
Standard deviation	45.0	4956	-	2.53	14.7	-	38.8
Median	37.1	5461	< 3.43	8.11	37.2	< 0.813	73.7
Forests of the Kup Forest Division							
Average	240	4225	< 5.84	9.89	111	< 1.890	83.5
Minimum	23.0	2213	< 3.13	4.03	57.1	< 0.813	37.9
Maximum	1333	11605	15.6	18.7	344	5.15	226
Standard deviation	389	2788	-	4.87	84.3	-	58.5
Median	121	3642	< 4.50	7.48	83.5	< 0.86	50.9

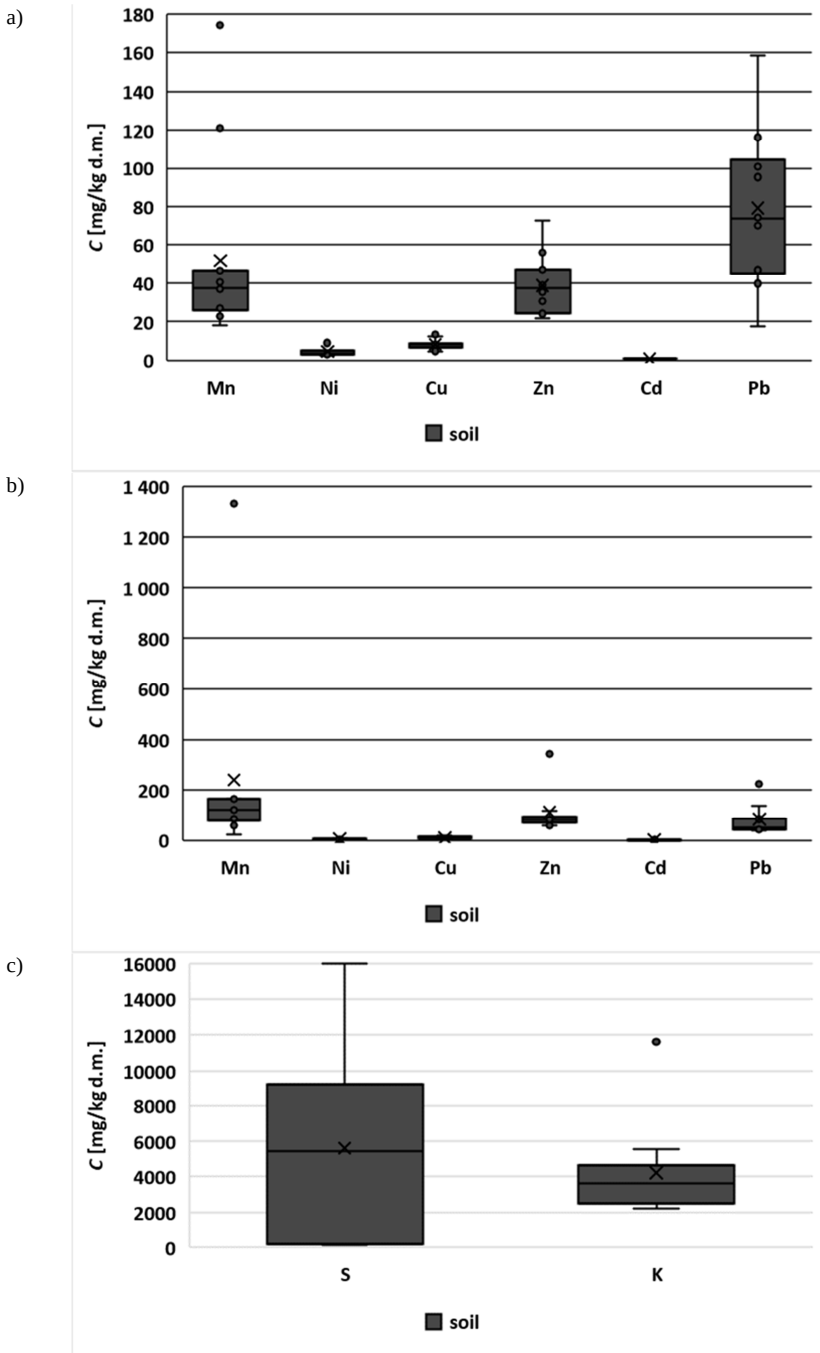

Fig. 2. Distribution of concentrations of selected heavy metals in the soil in: a) area S, in b) area K and c) distribution of concentration of iron for both areas

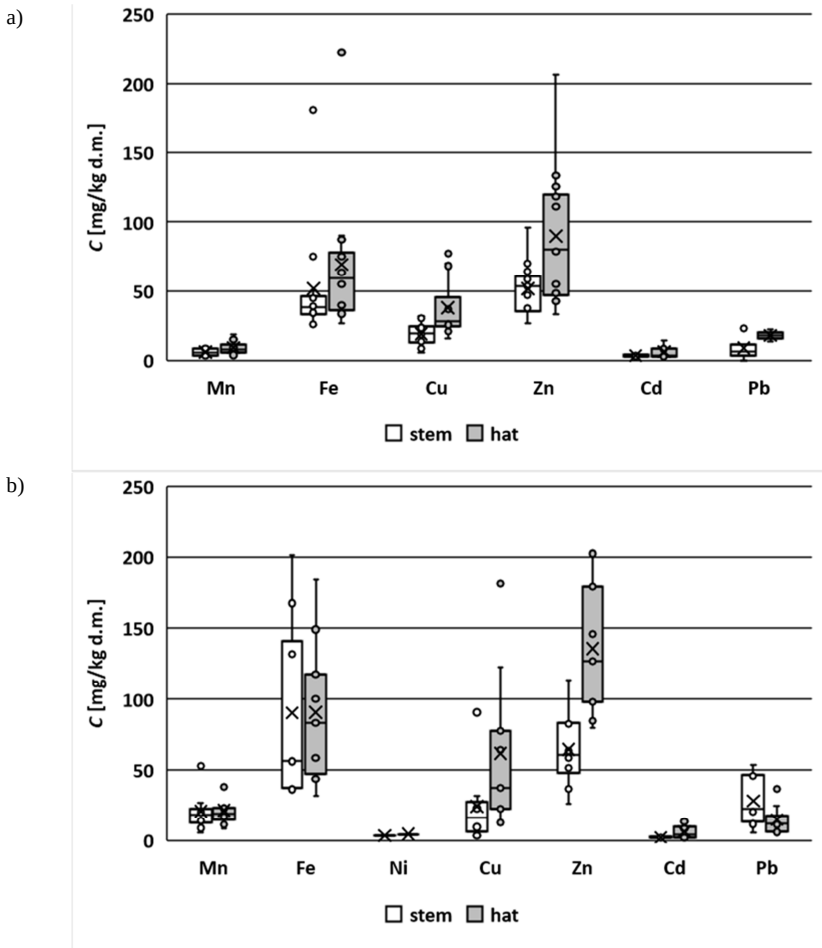

Fig. 3. Distribution of concentrations of selected heavy metals in the morphological parts of edible mushrooms in: a) area S and b) area K

The analysis of the results of carried out research demonstrated that the soil collected from both study areas shows much higher concentrations of Mn, Fe and Pb, in comparison to anatomical parts of mushrooms collected in these areas. For other determined elements, the content of analytes in soil was lower than the concentrations recorded in mushrooms. Total content of Ni, Cu, Zn, Cd and Pb determined in the analysed soils did not exceed the permissible quantities for those types of soils, in line with the regulatory arrangements [28]. On the basis of the analysis of heavy metals concentrations in soil, it should be stated that the area located in Opole Province is more polluted with the selected analytes. This can be justified by the very location of measurement points, which are close to the provincial road No. 461.

Heavy metals accumulation in fruiting bodies of mushrooms is a result of dry and wet deposition, however, atmospheric aerosol may also include pollution from soils in the form

of dust. In the case of mushrooms, translocation from soil, through mycelium to fruiting body is an important transport channel. The input for the above mentioned sources of metals depends of climate and meteorological conditions and is difficult to assess.

The analysis of heavy metals concentration accumulated in mushrooms allows to state that the material collected in the woods of the Kup Forest Division was more polluted. The concentrations of all elements were higher, in comparison to the mushrooms collected in the Swietokrzyskie Province. Nickel was determined only in the mushrooms collected in the areas of the Kup Forest Division. Higher concentrations of heavy metals in the mushrooms collected from the Kup Forest Division confirm translocation of heavy metals from the soil, which was also more polluted in that area.

The next stage of the studies included an analysis of heavy metals concentration in morphological parts of mushrooms. Higher concentrations of zinc, cadmium and copper were determined in hats than in stems of the mushrooms collected from both study areas. The results of the carried out analyses confirm the results of other authors [29]. In the case of other elements, concentrations determined in stems and hats are comparable or the concentration in stem is higher than the one in hat.

In order to define the capacity of the analysed mushrooms to accumulate bioaccessible forms of heavy metals from the soil, phytocumuling factor *PF* [30] was set:

$$PF = \frac{C_m}{C_s} \tag{1}$$

where: C_m - average concentration of metal in mushroom [mg/kg d.m.]; C_s - average concentration of metal in soil [mg/kg d.m.].

PF coefficient values were interpreted [31]:

$PF \leq 0.01$ - no accumulation,

$PF \leq 0.10$ - low degree of accumulation,

$PF \leq 1.00$ - medium degree of accumulation,

$PF > 1.00$ - high degree of accumulation.

Table 5 presents the values of phytocumuling factor *PF* set for edible mushrooms collected from two study areas.

Table 5

Values of the *PF* coefficient for the mushrooms from the Staporkow and Kup Division areas

Staporkow area							
***PF* [-]**	**Mn**	**Fe**	**Ni**	**Cu**	**Zn**	**Cd**	**Pb**
stem/soil	0.11	0.01	-	2.33	1.34	4.27	0.17
hat/soil	0.18	0.01	-	4.66	2.33	7.84	0.23
mushroom/soil	0.29	0.02	-	7.00	3.67	12.1	0.40
Kup area							
***PF* [-]**	**Mn**	**Fe**	**Ni**	**Cu**	**Zn**	**Cd**	**Pb**
stem/soil	0.09	0.02	0.56	2.45	0.58	1.09	0.34
hat/soil	0.09	0.02	0.73	6.24	1.22	3.29	0.18
mushroom/soil	0.18	0.04	1.29	8.69	1.80	4.38	0.52

- not determined

The analysis of the values of the *PF* coefficient for the mushrooms collected from the area of the Staporkow Forest Division, it was confirmed that nickel was not accumulated. Mushrooms also accumulated little Fe (also those collected from the Kup Forest Division). The mushrooms collected from two study areas accumulated manganese and lead in

medium degree and Cu, Zn and Cd in high degree. On the basis of the data presented in Table 3, it should be stated that hats of the mushrooms collected from both study areas were more polluted with heavy metals than their stems.

Taking into consideration the affinity to biochemical structures of the selected edible mushrooms, heavy metals can be ordered in the following way:

$$Cd > Cu > Zn > Pb > Mn > Fe$$

The analysis of *PF* coefficient values demonstrated that mushrooms most effectively accumulated such metals as cadmium, copper and zinc. Other authors obtained similar results [32-34].

Conclusions

Biomonitoring studies have an important role in the assessment of environment condition, among others, the level of heavy metals pollution. Biomonitoring with the use of mushrooms is a forward looking type of environment monitoring, used in assessment of pollution level of woodlands with, among others, heavy metals. The analysis of concentrations of the analytes bound in mushroom fruiting bodies provides information not only about the environment pollution level but also allows to assess, whether mushrooms are suitable for human consumption, taking into consideration the content of substances harmful to humans.

The following conclusions were drawn on the basis of the carried out research, with the use of edible mushrooms fruiting bodies:

1. In line with the Polish standards of soil quality, in the soils from both study areas, the allowable concentrations of Ni, Cu, Zn, Cd and Pb were not exceeded [28].
2. Higher concentrations of the determined analytes in hats than in stems, of edible mushrooms collected from two study areas, were detected for copper, zinc and cadmium.
3. Mushrooms fruiting bodies are characterised with good bioaccumulation of heavy metals from soils, which was confirmed by the determined values of *PF* coefficient (Table 5). Intense accumulation of Cd, Cu and Zn was confirmed.
4. On the basis of the analysis of heavy metals concentrations accumulated in soil and mushrooms it should be stated, that the area of the Kup Forest Division is more polluted with trace elements and edible mushrooms from both areas are not suitable for human consumption, due to exceeded standards [27], e.g. the maximum level of edible mushrooms pollution with Pb is 0.1 mg/kg f.m. (fresh mass), according to the standard, whereas the value of 53.9 mg/kg d.m. was determined in the mushrooms collected in the Kup Forest Division.
5. Edible mushrooms are a sensitive biomonitor of heavy metal pollution level in woodlands.

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