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Original Research

Contribution of Bioavailable Forms of Chosen Metals in Soil to Heavy-Metal Contamination of Wild Mushrooms

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Abstract

Certain species of mushrooms, owing to their capacity to accumulate some bioavailable heavy metals such as Cd and Pb, can contain several-fold higher amounts of metals than the soil (speciation forms) in which they grow. It seemed noteworthy to undertake studies concerning the content of bioavailable forms of metals contained in the soil of mushroom habitats.

The field part of our study was performed in forest ecosystems found in the Silesian and Żywiecki Beskid mountains as well as in the Silesian Upland region of southern Poland. Altogether, 147 mushroom habitats were monitored, with particular consideration paid to wild mushroom habitats. Metal content was measured using atomic absorption spectrometry (AAS) at 0.01 µgMe/g precision limit. Measurement accuracy was verified using certified reference materials (Wzormat, Warsaw, Poland). Speciation analysis was performed according to a method described by Rudd.

Our investigation has demonstrated differing capacities of various mushroom species to accumulate selected elements, depending on their occurrence form of bioavailable metals in the habitat. Certain mushroom species together with results speciation heavy metals in soil can be used to monitor metal levels, particularly those that exhibit significant correlations in the content of, for example, Co, Ni, Cr, or Pb.

Keywords: cadmium, lead, mushrooms, soil, speciation

Introduction

Mushrooms are valued foodstuffs because of their taste, fragrance, and nutritional qualities. At the same time, fruiting bodies of wild-growing mushrooms, thanks to their universal occurrence and accessibility, are of interest to toxicology researchers.

Mushrooms are a particularly valuable bioindicator of heavy metals' presence in forest ecosystems since they play an important role in circulation of elements and often show selectivity in accumulating heavy metals [1-4].

Wild-growing mushrooms, like other elements of the natural environment, are exposed to the imission of numerous metals that are naturally present or appear as a result of human activities. Some mushroom species, owing to their capacity to accumulate bioavailable amounts of some heavy metals, for example Cd and Pb, may contain several-fold higher amount of metals than soil in which they grow [5, 6]. Concentrations of metals differs significantly depending on mushroom species and is considerably higher than those

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observed in plants [6]. The level of metal content in mushrooms depends on the degree of metal pollution in the upper soil layer, amount of organic matter, soil pH, physiological condition, stage of mushroom growth, activity of certain enzymes, bioavailability, chemical form of the metal present in soil, and other factors [6, 7]. An essential factor determining concentration levels of specific elements accumulating in mushroom fruiting bodies is the quality or the degree of substrate (soil, subsoil) pollution, including content and bioavailability of both metals and non-metals [7, 8]. The content of various elements in soil affects the intensity of biological processes as well as deciding whether dietary intake of a given product is safe for consumers.

Considering the above, studies on the content of bioavailable forms of metals in soil of mushroom habitats seem justified.

Materials and Methods

Field studies were conducted in forest ecosystems lying in the Silesian and Żywiecki Beskid mountains, as well as in the Silesian Upland region of southern Poland. In total, 147 mushroom habitats were monitored.

Soil

Upper soil samples (0-14 cm layer) were collected from under the mushrooms' location. The samples were fragmented, air-dried, and passed through a sieve (1-mm eylet). Next, the samples (1 g) underwent sequential chemical analysis. Speciation analysis was performed according to Rudd [9]. Soil aliquots (1 g) were treated for 24 hours with the following solutions (40 ml solution per 1 g of soil):

- 1.0 M KNO_3 – for exchangeable form
- 0.5 M KF – for adsorbable form
- 0.1 M $Na_4P_2O_7$ – for organic combinations
- 0.1 M EDTA (pH=6.5) – for carbonate form
- 6.0 M HNO_3 – for sulfide form

The soil samples were treated with particular solutions, centrifuged for 5 min., and then washed with 10 mL distilled water and centrifuged again. The obtained extracts were acidified (up to 1%) with concentrated nitric acid.

The remaining soil samples were quantitatively transferred to 50-mL crystallizers and treated with 5 mL concentrated HNO_3, followed by mineralization in a sand bath. Subsequently, to the obtained dry remainder 2 ml of concentrated HNO_3 was added, followed by 10 mL of distilled water and the whole was filtered into a 50-mL calibrated flask.

The extractants used allowed us to isolate metals in several forms (exchangeable, adsorbable, organic matter-bound, carbonate-bound, sulfide-bound) and the remainder.

Mushrooms

Thirty-seven mushroom species were analyzed. Collected samples were separated into caps and stalks, oven-dried to constant mass, and pulverized using an agatic mortar. Aliquots (1 g) of the powdered carpophores were treated with 5 mL concentrated nitric acid (V) (Supra pure, Merck) and left for 24 h. Next, the specimen samples were mineralized using a sand bath until completely dissolved. Consequently, the solutions were quantitatively transferred to 25-mL calibration flasks and filled with redistilled water.

Metal content was determined by atomic absorption spectroscopy (AAS) using Pye Unicam SP-9 apparatus at 0.01 μg/g precision level. Accuracy of the measurement method was 3-5%, depending on the element determined. Precision was verified using the method of standard addition as (standard reference materials from Wzormat, Warsaw, Poland) as well as SRM 1648 model standard (urban air pollution particulate) and reference material Mixed Polish Herbs (INCT-MPH-2). The results differed by ca. 3.8-5.0%.

Results

Among bioavailable forms of metals the exchangeable and adsorbable ones were singled out. A quantity of directly bioavailable chosen metal is determined by immission of soluble forms from total precipitation and the amount of easily soluble compounds of that particular element, depending on geochemical character of the substratum [10-12].

Speciation analysis of the soil was performed according to the method of Rudd [9]. Table 1 demonstrates the extent of changes in the content of particular metals in the exchangeable and adsorbable forms. Comparison of data from Table 1 reveals that mushrooms have at their disposal sizeable amounts of bioavailable elements and suggests that particular forest communities have been burdened with varying amounts of these elements. General average metal content in mushrooms is given in Table 2. Considering the average mushroom content of investigated metals, one can establish the following series of metals' occurrence:

- Silesian Beskid mountains:
  K>Fe>Ca>Mg>Na>Zn>Mn>Cu>Pb>Cr>Cd>Co>Ni
- Żywiecki Beskid mountains:
  K>Ca>Fe>Mg>Na>Zn>Mn>Cu>Pb>Cr>Cd>Co>Ni
- Silesian Upland:
  K>Ca>Mg>Zn>Fe> Na>Cu>Mn>Pb>Cd>Co>Cr>Ni

Regardless of the forest community location, unfavorable are relatively large amounts of Pb (on the order of 15 μg/g) and Cd (on the order of 5 μg/g). The results show that mushrooms from the studied areas can be a significant source of Cr supplementation to the consumers' diet (on average 12.4 μg/g for mushrooms from Silesian Beskid mountains and 12.7 μg/g for those from Żywiecki Beskid mountains). On the other hand, the lower content of Cr in mushrooms growing in the Silesian Upland regions (2 μg/g) could be explained by diversified dispersion of particulate matter in favor of a coarser one that contains lesser amounts of Cr [11]. Noteworthy is the comparison of maximum concentration of Cr in Silesian Beskid mountains (121 μg/g) with that in Żywiecki Beskid mountains (175 μg/g).

Table 1. The content span of examined metals present in exchangeable and adsorbable forms in soil originating from Silesian and Żywiecki Beskid mountains, as well as from the Silesian Upland region [µg/g].

Metal	Form	Silesian Beskid Mountains	Żywiecki Beskid Mountains	Silesian Upland Region
Pb	exchangeable	0.50-33.13	1.00-13.97	5.00-32.31
	adsorbable	1.00-25.09	1.00-6.50	1.00-19.48
Cd	exchangeable	0.15-2.08	0.15-0.51	0.63-0.87
	adsorbable	0.01-0.27	0.02-0.27	0.15-0,39
Mn	exchangeable	0.03-218.17	0.80-147.90	0.03-67.14
	adsorbable	0.03-0.80	0.03-2.42	0.027
Ni	exchangeable	0.06-3.81	0.06-3.35	0.06-2.21
	adsorbable	0.06-2.66	0.06-1.54	0.06-1.53
Fe	exchangeable	0.06-341.38	0.76-7.44	0.06-51.96
	adsorbable	0.76-297.15	0.06-118.63	0.06-174.23
Zn	exchangeable	0.01-394.64	2.94-24.49	84.89-94.67
	adsorbable	0.01-2.94	0.01-0.20	0.01-0.20
Co	exchangeable	0.048-2.48	0.048-1.55	0.048
	adsorbable	0.048	0.048	0.048
Cr	exchangeable	0.05-16.36	13.02-15.69	13.01-15.00
	adsorbable	1.05-15.69	0.05-13.69	8.35-11.66
Cu	exchangeable	8.70-22.05	6.03-13.95	11.30-13.29
	adsorbable	5.83-22.22	5.83-20.75	5.39-8.90

In general, elemental content levels in mushrooms growing in the studied areas are as follows: K – 10^4; Ca, Mg, Na, Fe – 10^3; Zn – 10^2; Mn – $6.1 \cdot 10^1$; Pb – $1.7 \cdot 10^1$; Cr – $1.2 \cdot 10^1$; Co, Ni – $2 \cdot 10^0$; Cd – $3 \cdot 10^1$ (µg/g).

Comparison of data found in Table 2 reveals that, besides elements with a known physiological role such as Na, K, Ca, Mg, Fe, and Zn, many mushroom species accumulate to a significant extent in elements originating from industrial emissions, both local and long-range.

The observed content levels of particular elements found in mushrooms depend, undoubtedly, upon bioavailable amounts of the former in respective habitats (Table 1), possible interactions at the accumulation stage, as well as genetically- and species-determined capacities to selectively accumulate some of them [1, 2, 13]. An example of mushrooms' different capacities to accumulate metals as a result of certain interactions between the latter is provided by correlation coefficients describing these relationships (Table 3). Previously reported properties of copper being an antagonist to such elements as Pb, Ni, or Cr can be confirmed in the case of Common Inkcap (*Caprinus atramentarius*) and Green Brittlegill (*Russula aeruginea*) mushrooms growing in both Silesian and Żywiecki Beskid mountains [14, 15]. In the region of Silesian Upland the discriminatory properties of copper have been subdued as a result of increased emission of these elements over forest communities in that area. The two mushroom species, chosen as examples, describe well the discriminatory properties of Ca vs. Cd and of K *vs*. Cd. In the two mountainous regions discriminatory properties were revealed in the case of Na *vs*. Ni and Cr. One should also note the antagonism existing between Mn *vs*. Pb and between Mn *vs*. Cr, as well as

Table 2. Average content of particular metals in the examined mushroom species growing in Silesian and Żywiecki Beskid mountains, as well as in the Silesian Upland Region [µg/g].

	Pb	Cd	Ni	Co	Cr	Mn	Zn	Fe	Cu	Na	K	Ca	Mg
Silesian Beskid Mountains													
N	331	332	315	141	199	332	332	332	332	332	332	332	332
Average	17.62	4.95	1.71	2.30	15.43	61.93	123.70	776.59	41.27	209.14	36,174.87	678.94	636.20
Minimum	1.25	0.03	0.17	0.24	0.89	0.00	3.00	0.78	0.95	0.90	9.94	0.26	3.25
Maximum	189.14	67.78	36.81	23.97	121.07	943.28	1,177.19	143,564.29	466.03	2,582.70	126,860.77	7,184.76	6,832.97
Żywiecki Beskid Mountains													
N	125	125	114	83	94	125	125	125	125	125	124	124	125
Average	13.44	3.79	2.21	2.65	12.74	73.60	137.61	356.88	44.29	166.70	34,291.53	419.79	274.83
Minimum	1.13	0.03	0.51	0.27	1.01	2.64	21.52	0.50	0.92	0.98	1,011.71	0.27	2.99
Maximum	192.36	41.18	16.77	13.21	174.93	387.81	1,157.81	9,879.08	371.90	1,266.84	103,012.08	2,325.67	1,250.57
Silesian Upland Region													
N	61	61	59	54	33	61	61	61	61	61	61	61	61
Average	15.44	5.04	1.41	3.02	2.01	43.59	207.19	129.71	43.64	120.40	34,475.31	1,003.81	661.15
Minimum	2.66	0.38	0.14	0.21	0.92	2.86	2.16	4.28	1.25	0.85	17.40	2.31	12.22
Maximum	227.09	54.88	15.14	30.19	3.40	378.94	2,031.12	628.18	122.37	887.64	152,862.38	19,954.95	6,173.69

Table 3. Co-occurrence of chosen metals in certain mushroom species.

	Cd	Ni	Co	Cr	Mn	Zn	Fe	Cu	Na	K	Ca	Mg
Common Inkcap, *Coprinus atramentarius* (Silesian Beskid Mountains)												
Pb	-0.82	0.95	0.96	0.82	-0.35	-0.86	0.61	-0.90	-0.73	0.63	0.96	0.71
Cd		-0.85	-0.77	-0.93	0.45	0.78	-0.90	0.79	0.92	-0.93	-0.71	-0.18
Ni	-0.39		0.99	0.94	-0.62	-0.97	0.77	-0.75	-0.88	0.77	0.97	0.60
Cr	0.82	0.94	0.87		-0.74	-0.94	0.95	-0.63	-0.99	0.94	0.82	0.28
Green Brittlegill, *Russula aeruginea* (Żywiecki Beskid Mountains)												
Pb	-0.68	-0.50	0.39	0.60	-0.66	0.73	0.64	-0.51	-0.90	-0.92	0.30	-0.86
Cd		0.03	0.06	0.16	0.05	-0.57	-0.12	0.94	0.45	0.34	-0.90	0.87
Ni	0.03		0.23	-0.77	0.95	-0.84	-0.02	-0.30	0.82	0.61	0.34	0.02
Cr	0.16	-0.77	0.43		-0.91	0.48	0.61	0.38	-0.79	-0.85	-0.58	-0.15
Green Brittlegill, *Russula aeruginea* (Silesian Upland Region)												
Pb	-0.06	-0.96	0.24	-0.80	-0.35	-0.48	-0.17	0.02	-0.84	-0.25	-0.12	-0.76
Cd		-0.22	0.96	0.64	-0.92	0.90	0.99	1.00	-0.49	-0.95	-0.98	0.69
Ni	-0.22		-0.49	0.61	0.59	0.23	-0.11	-0.29	0.96	0.51	0.39	0.55
Cr	0.64	0.61	0.4		-0.27	0.91	0.72	0.57	0.36	-0.37	-0.49	1.00

between Mn *vs*. Cd (in the Silesian Upland region). Comparing data put together in Table 3, one can single out elements that have a common source of origin. Among them are undoubtedly Cd, Co, and Cr, since for these elements the greatest number of directly proportional correlations was found. This means, in addition, that these elements are preferentially accumulated in mushrooms if only their bioavailable resources are significant. For example: in Common Inkcap changes in Co content are directly proportional to those of Pb, Ni, and Cr (0.87-0.99). For that mushroom species an equally good example of correlation is provided by Cr and Cd *vs*. Pb and Ni (0.82-0.94), whereas in Green Brittlegill Cr correlations concerned Cd and Ni (0.61-0.64). It has to be mentioned that the authors of this report have been gathering data concerning metal accumulation in other mushroom species and that the results confirm detailed observations made for common inkcap and for Green Brittlegill.

Conclusions

1. This study has demonstrated varying capacities of different mushroom species to selectively accumulate chosen elements. Furthermore, that ability depends on the presence of directly bioavailable forms of the element in soil at mushrooms' sites.
2. In the mushroom species, studied physiologically important elements revealed distinct antagonist properties to elements of industrial pollution origin, which points to mushrooms' discriminatory properties against such elements.
3. Certain species of mushroom speciation elements in soil can be used for metal contamination biomonitoring, especially those for which there are significant correlations concerning occurrence of such metals (Co, Ni, Cr, and Pb).

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