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Original Research

Content of Cadmium, Lead, and Oxalic Acid in Wild Edible Mushrooms Harvested in Places with Different Pollution Levels

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Abstract

Our study was aimed at determining contents of cadmium, lead, and oxalic acid in select wild edible mushrooms harvested from areas potentially more (Ostrowiec Św (P_1). and Ożarów (P_2)) or less the protection zone of Magurski National Park (M – mountain) exposed to pollution. The experimental material were fruiting bodies of four species of edible mushrooms: boletus (*Boletus edulis*), bay bolete (*Xerocomus badius*), red-capped scaber stalk (*Leccinum aurantiacum*), and slippery Jack (*Suillus luteus*) collected in late August/early September of 2009.

The results of contents of cadmium in the examined mushrooms indicate that the permissible (3 mg·kg^{-1} d.m.) level was exceeded in fruiting bodies of boletus originating from the polluted (P_1, P_2) areas and mountain area (M), where its concentration was the highest. Caps and stalks of boletus originating from the protection zone of Magurski National Park were found to contain, respectively, 5.22 mg Cd·kg^{-1} d.m. and 1.86 mg Cd·kg^{-1} d.m., on average, (with the value of 3.54 mg Cd·kg^{-1} d.m. that may be assumed for the whole fruiting body). Taking into a small contribution of mushrooms in a human diet, it does not pose a risk to human health. The concentration of lead in the analyzed mushrooms did not exceed 1.0 mg·kg^{-1} d.m. The mean content of soluble oxalates in the analyzed species of mushrooms ranged from 35.5 to 104.1 mg·100 g^{-1} d.m. (per whole fruiting body). Irrespective of the origin, the lowest content of oxalates was reported in fruiting bodies of slippery Jack (35.5-59.1 mg·100 g^{-1} d.m). Caps of all investigated mushroom species were characterized by ca. 1.6 to 3.1 times higher content of oxalates than the stalks.

Keywords: heavy metals, oxalic acid, mushrooms

Introduction

Wild growing mushrooms (higher fungi, macrofungi) have been a popular delicacy in many countries and their consumption has been several kilos per year for some individuals. However, research has shown that many mushroom species accumulate several trace elements, mainly mercury, cadmium, and lead, at levels greatly exceeding their contents in other foods [1]. According to Malinowska et al. [2], heavy metal bioavailability from soil substrate by mushroom mycelium is affected by numerous factors such as pH value, redox potential, organic matter contents, mineralogy of clay, cation exchange capacity of the soil phase, and composition of soil solution. As it results from many investigations, contents of cadmium, mercury, and lead in

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mushroom fruiting bodies increase in heavily polluted areas, such as in close proximity to highways with heavy traffic, landfills of sewage sludge, and emission areas (including cities) [3-5]. Surprising accumulation ability in several mushroom species promoted their screening as bioindicators [6]. Lead and cadmium belong to priority food contaminants. They are implicated to induce disorders of many organs (kidneys, liver and bone marrow) and systems (nervous, hematopoietic, immunological), and may also contribute to the development of neoplasmic lesions [7]. For this reason, their content in food products, and thus in mushrooms, should be under constant monitoring. The undesirable substances occurring in edible mushrooms also include oxalic acid. Investigations addressing its content in mushrooms are, however, sparse [8, 9]. Oxalic acid combines with metals such as calcium in the body to form oxalate crystals, which can irritate the gut and kidneys. Since oxalic acid binds vital nutrients, such as calcium or magnesium, long-term consumption of foods high in oxalic acid can lead to nutrient deficiencies [10]. Healthy individuals can safely consume such foods in moderation, but those with kidney disorders, gout, osteoporosis, or rheumatoid arthritis are typically advised to avoid foods high in oxalic acid or oxalates.

The objective of this study was to determine contents of cadmium, lead, and oxalic acid in select wild edible mushrooms harvested from areas potentially more or less exposed to pollution.

Material and Methods

The experimental material were fruiting bodies of four species of edible mushrooms: boletus (*Boletus edulis*), bay bolete (*Xerocomus badius*), red-capped scaber stalk (*Leccinum aurantiacum*), and slippery Jack (*Suillus luteus*) collected in late August/early September 2009.

Samples were collected from different areas of Świętokrzyskie Province, in the area of Ożarów District (afforested areas in the proximity of cement works, between the villages of Drygulec and Jasice (polluted – P_1), and in the area of a foamed polystyrene-producing plant in the city of Sobótka) and in the area of Ostrowiec Św. District (afforested area approximating iron works and metallurgic plant), (polluted — P_2). Those areas may be acknowledged as significantly exposed to industrial and traffic pollution.

Samples were also collected from sites potentially less exposed to pollution, including: afforested areas near Magurski National Park (located on the border of Małopolskie Province and Podkarpackie Province), south to the Magura Małastowska mountain range (village Gładyszów), and in the area of Sękowa District (villages: Sękowa and Małastów), (mountainous – M).

Chemical analyses were conducted on select 5-6 healthy and non-verminated fruiting bodies with the size being typical of each species that were harvested at distant places so as to not originate from one mycelium. Using a plastic knife, the fruiting bodies were carefully cleaned from sand and leaves directly at the harvest site. Having been transported to the laboratory, they were dried at room temperature for 24 h. Afterward, air-dry samples were dried to a constant mass at 40ºC in an electric dryer for 48 h. Further on, the material (separately cap and stalk) was homogenized (ground to a powder) in agate mortar, packed into polyethylene string bags, and stored in a dry and clean place until the beginning of chemical analyses.

In order to determine contents of the investigated heavy metals and oxalic acid, the samples of homogenized caps and stalks were weighed in portions of 1 g and 3 g, respectively. All chemical determinations were made in two replications.

Determinations of lead and cadmium contents were determined with the flameless technique using atomic absorption spectrometry (AAS), with the use of a Varian Spectra AA 880Z spectrometer with background correction [11]. The content of lead was determined at the wavelength of λ=217.0 nm, whereas that of cadmium at λ=228.8 nm. Detection limits for these metals were as follows: 50 $\mu g \cdot l^{-1}$ and 5 $\mu g \cdot l^{-1}$, respectively. For calibration and validation of analytical procedure we used certified reference material (CRM) – oriental tobacco (CTTA-OTL-1, Poland).

The assay of heavy metals content was preceded by samples incineration in a muffle furnace at 450ºC, with the method of the so-called "dry mineralization," and dissolving the resultant ash in 6N of spectrally-pure hydrochloric acid. Results were expressed as $mg \cdot kg^{-1}$ of dry matter of product.

The content of oxalic acid was determined following the methodology described by Brzozowska et al. [12]. It consisted in precipitating calcium oxalate with calcium chloride, and titrating the dissolved precipitate with a titration solution of potassium permanganate. Results were expressed as $mg \cdot 100\ g^{-1}$ of dry matter of product.

The obtained numerical data were subjected to a statistical analysis by means of STATISTICA 6.0 PL. The statistical significance of differences between mean values was estimated by means of a one-way variance analysis ANOVA assuming significance level at 0.05.

Results and Discussion

After Poland's accession to the European Union and after implementing EU Regulations [13], guidelines for limited contents of lead and cadmium refer only to cultivated mushrooms, e.g. oyster mushroom and field mushroom – i.e. respectively 0.2 $mg \cdot kg^{-1}$ f.m. and 0.3 $mg \cdot kg^{-1}$ f.m., which when expressed per dry matter corresponds to 2 $mg \cdot kg^{-1}$ and 3 $mg \cdot kg^{-1}$ d.m. (assuming that water content of mushrooms reaches 90% on average).

Results referring to the content of cadmium in the examined mushrooms (Table 1) indicate that this safe level was exceeded in fruiting bodies of boletus originating from areas of Ostrowiec Św. and Ożarów, as well as from the protection zone of Magurski National Park (where its concentration was the highest). Caps and stalks of boletus were found to contain, respectively, 5.22 mg $Cd \cdot kg^{-1}$ d.m. and

Table 1. Content of cadmium in examined wild edible mushrooms (mg·kg^{-1} of dry product).

Specification		Cap	Stalk
P_1 Area of Ostrowiec	Slippery Jack	0.94	0.55
		0.65-1.14	0.12-0.78
	Red-capped scaber stalk	1.37	0.47
		1.08-2.1	0.23-0.79
	Bay bolete	1.96	0.60
		1.02-2.77	0.32-0.82
	Boletus	2.83	1.05
		1.72-5.04	0.67-2.21
Mean:		1.78	0.67
Standard deviation:		0.82	0.26
P_2 Area of Ożarów	Slippery Jack	1.36	0.59
		0.55-2.02	0.29-1.03
	Red-capped scaber stalk	1.56	0.67
		1.08-2.0	0.39-1.05
	Bay bolete	2.05	0.78
		1.22-3.03	0.39-0.89
	Boletus	3.23	0.94
		1.34-5.24	0.52-1.32
Mean:		2.05	0.75
Standard deviation:		0.84	0.15
M Area of Małastowska Magura	Slippery Jack	1.27	0.55
		1.12-2.89	0.32-0.77
	Red-capped scaber stalk	2.04	0.88
		1.12-2.87	0.52-0.87
	Bay bolete	3.23	0.94
		2.12-5.31	0.56-2.88
	Boletus	5.22	1.86
		2.89-7.12	0.56-2.32
Mean:		2.94	1.06
Standard deviation:		1.72	0.56

P_1, P_2 – polluted areas; M – mountainous area

1.86 mg Cd·kg^{-1} d.m., on average (with the value of 3.54 mg Cd·kg^{-1} d.m. that may be assumed for the whole fruiting body). Other authors have reported on a significantly higher concentration of cadmium in fruiting bodies of boletus. In mushrooms harvested on strongly polluted areas (in the vicinity of mines, steel works, and cement works) its concentration may exceed even 54 mg·kg^{-1} d.m. [5]. For comparison, results of a study by Falandysz and Frankowska [14] demonstrate that the content of cadmium in boletus from the area of Świętokrzyska Primeval Forest accounted for 7.0 mg·kg^{-1} d.m. The Provisional Tolerable Weekly Intake (PTWI) value stipulated by FAO/WHO experts for cadmium reaches 0.49 mg per person (with body mass of 70 kg). This indicates that the weekly intake of boletus containing 3.5 mg Cd·kg^{-1} d.m. should not exceed 0.14 kg of dry material or 1.4 kg of fresh fruiting bodies. However, we also assume that mushrooms would be a solely source of dietary cadmium.

Data collated in Table 1 also show that bay bolete harvested in the area of Magurski National Park was characterized by a relatively high content of cadmium (ca. 2.1 mg Cd·kg^{-1} d.m). Except for slippery Jack, all mushrooms harvested in the mountain region were characterized by a slightly higher cadmium content than those originating from areas with higher exposure to industrial and traffic pollution, i.e. from areas of Ożarów and Ostrowiec Św. It is worth noting because this region is claimed to be relatively unpolluted and even ecologically-pure. But research of other authors indicates that considerable accumulation of cadmium in fruiting bodies of some mushrooms, especially in boletus, is recorded also in areas characterized by low contamination with metals, including the almost maiden afforested areas of northern Poland [15].

Some mushroom species, owing to their specific capability to accumulate heavy metals (e.g. lead and cadmium) may contain multiple times higher levels of metals than the substratum they had grown on [16]. A short vegetative season coupled with the known accumulation capabilities enable including mushrooms to a group of exposure biomarkers [6]. Species specificity to bind selected metals by mushrooms is, presumably, influenced by the presence of specific binding proteins (metalothioneins), whose synthesis is determined genetically. The accumulation of heavy metals in mushrooms is possible due to the presence of their compounds in the substratum and in dust suspended in the ground layer of air. Of key significance are bioavailable forms of metals present in soil. The absorption of cadmium proceeds exceptionally easy (for it is characterized by high mobility), proportionally to its concentration in soil or air [17].

Maybe, likewise in plants, cadmium accumulation in mushrooms is enhanced by strong acidification of soil, which is typical for mountain soils. A low pH value of soil causes an increase in the solubility of chemical links of cadmium and its diminished adsorption in soil colloids [18]. This phenomenon may explain the high concentration of cadmium in mushrooms collected in the area of Małastowska Magura. A study by Grzybek and Jancza [19], in which cadmium content was analyzed in fruiting bodies of bay bolete originating from different regions of Poland, demonstrates that mushrooms from southern Poland were characterized by over 20-fold higher content of cadmium than those from northern Poland. This is due to high availability of cadmium to mushrooms under conditions of strong acidification of soil, which was aforementioned, but also to a naturally high content of this element in substratum. Longitudinal studies of Polish soils have enabled two

Table 2. Content of lead in examined wild edible mushrooms (mg·kg^{-1} of dry product).

Specification		Cap	Stalk
P_1 Area of Ostrowiec	Slippery Jack	0.86	0.49
	Red-capped scaber stalk	0.91	0.47
	Bay bolete	1.16^{a}	0.82^{a}
	Boletus	0.85	0.24
Mean:		0.95	0.51
Standard deviation:		0.15	0.24
P_2 Area of Ożarów	Slippery Jack	0.66	0.41
	Red-capped scaber stalk	0.93	0.44
	Bay bolete	0.64^{b}	0.39^{b}
	Boletus	0.58	0.34
Mean:		0.70	0.40
Standard deviation:		0.16	0.04
M Area of Małastowska Magura	Slippery Jack	0.54	0.29
	Red-capped scaber stalk	0.68	0.28
	Bay bolete	0.81^{ab}	0.67^{ab}
	Boletus	0.78	0.27
Mean:		0.70	0.38
Standard deviation:		0.12	0.20

$^{a, b}$ – values in the same rows marked with different superscript letters differ significantly at $p \leq 0.05$.
explanations as in Table 1

discriminating geochemical provinces. For the southern province covering the Sudety Mountains, Upper Silesia and Carpathian Mountains, typical is the higher content of almost all trace elements, including cadmium, cobalt, copper, iron, manganese, nickel, and lead in soils and fluvial deposits compared to the other parts of the country [20]. A comparison of cadmium concentration in caps and stalks of the investigated mushrooms shows explicitly that the caps accumulate cadmium to a significantly greater extent than the stalks. This fact also has been confirmed elsewhere [14, 21].

Data received for lead content of the mushrooms examined (Table 2) indicate that a similar dependency was also observed in respect to this metal. The concentration of lead in caps of the mushrooms was 1.6-3.5 times higher than in the stalks. Regardless of the harvest site, the analyzed species of mushrooms were only slightly contaminated with lead. The FAO/WHO stipulated the PTWI value for lead at 1.5 mg per capita. Taking into account that the concentration of this metal in the analyzed mushrooms did not exceed 1.0 mg·kg^{-1} d.m. (per whole fruiting body), exceeding the PTWI value would require consuming ca. 1.5 kg of dry or 15 kg of fresh fruiting bodies, which is quite unlikely. The highest quantities of lead were accumulated by the mushrooms harvested in forests located in the vicinity of CELSA Ironworks in Ostrowiec Św., especially by fruiting bodies of bay bolete (1.16 mg·kg^{-1} d.m – caps and 0.82 mg·kg^{-1} d.m. – stalks). This value was significantly higher ($p<0.05$), compared to that noted for the samples of bay bolete collected from the area of the cement works in Ożarów. Iron and steel metallurgy contributes to significant emissions of lead oxides to the atmosphere, which may explain the observed situation. In specimens of bay bolete harvested from an area of iron and copper metallurgic plants, Slovak researchers [4] have detected lead in concentrations of 3.1-4.3 mg·kg^{-1} d.m. A slightly lower contamination of mushrooms with lead was reported in a Czech study [22] analyzing mushrooms from rural regions of southern Moravia (0.57-2.52 mg·kg^{-1} d.m.).

Longitudinal investigations conducted in different regions of Poland have demonstrated that the content of lead in bay bolete harvested from areas not polluted with this metal does not exceed 20 mg·kg^{-1} d.m. [23]. The natural environment may be contaminated with heavy metals, including lead compounds, also in the proximity of cement works (e.g. in Ożarów). As it results from data presented by Kusza et al. [24], soils around cement works in the region of Opole were characterized by excessive levels of many heavy metals – lead and zinc in particular. Similar observations were made when investigating the effect of the cement-lime industry on soil status in the region of Kielce [25]. An increase in the pH value of soil, being a consequence of the emission of alkaline dusts from plants producing cement, is a factor reducing exposure to high concentrations of toxic metals. The increase in soil pH is a factor significantly reducing lead absorption by mushrooms [17, 26]. This may also explain the low concentration of this metal determined in fruiting bodies of mushrooms harvested in forests located in the proximity of the cement works in Ożarów.

Apart from chemical contaminations in the form of toxic metals, mushrooms are also a source of antinutirents, e.g. oxalic acid. In cells of mushrooms this compound occurs usually in the form of soluble sodium and potassium salts, and insoluble calcium oxalate. Oxalic acid plays a significant role in the process of lignocellulose degradation and pathogenesis of plants (it acts as a fungicide). Among organic acids, it belongs to the most effective ligands of heavy metal ions. For this reason, it serves a significant function in detoxification of heavy metal ions and in mushrooms' tolerance to their high concentrations in soil [27]. Data referring to the content of oxalates in large-fructification fungi are scarce. When analyzing cultivated and wild mushrooms, Savage et al. [8] found that the wild fungi contained only soluble oxalates, at the level of 29.3 to 40.2 mg·100 g^{-1} d.m. As it results from data presented in Table 3, the mean content of soluble oxalates in the analyzed species of mushrooms was higher than the above-mentioned values and ranged from 35.5 to 104.1 mg·100 g^{-1} d.m. (per whole fruiting body). Irrespective of the origin, the lowest content of oxalates was reported in fruiting bodies of slippery Jack

Table 3. Content of oxalic acid in examined wild edible mushrooms (mg·100 g^{-1} of dry product).

Specification		Cap	Stalk
P_1 Area of Ostrowiec	Slippery Jack	65.6	26.6
		43.2-85.4	15.3-35.2
	Red-capped scaber stalk	86.1^{ab}	53.9^{ab}
		57.0-102.5	42.2-74.2
	Bay bolete	112.9^{ab}	54.9
		78.5-136.2	31.2-67.2
	Boletus	75.6	36.3
		56.2-88.9	21.7-50.9
Mean:		85.05	42.93
Standard deviation:		20.37	13.84
P_2 Area of Ożarów	Slippery Jack	47.6	23.4
		33.1-63.4	15.2-38.6
	Red-capped scaber stalk	57.5^{b}	26.6^{b}
		36.4-82.1	19.3-40.0
	Bay bolete	65.8^{b}	32.7
		36.4-81.2	19.8-46.2
	Boletus	86.4	28.5
		57.2-11.3	15.4-41.4
Mean:		64.33	27.80
Standard deviation:		16.49	3.89
M Area of Małastowska Magura	Slippery Jack	78.5	39.6
		63.1-104.4	16.8-53.2
	Red-capped scaber stalk	129.5^{a}	78.6^{a}
		101.3-154.1	64.2-94.2
	Bay bolete	131.4^{a}	62.4
		99.8-154.2	53.3-88.6
	Boletus	110.8	58.5
		82.0-141.8	33.2-67.5
Mean:		112.6	59.78
Standard deviation:		24.53	16.02

$^{a, b}$ – values in the same rows marked with different superscript letters differ significantly at $p \leq 0.05$
explanations as in Table 1

(35.5-59.1 mg·100 g^{-1} d.m.). Caps of all investigated mushroom species were characterized by ca. 1.6 to 3.1 times higher content of oxalates than the stalks. The admissible daily intake (ADI) of oxalates in a diet of an adult person is ca. 250 mg/day. But in persons predisposed to oxalate lithiasis it should not exceed a dose of 40 mg/day [28]. Taking into consideration a relatively low contribution of mushrooms in human diet, their moderate consumption should not pose any health risks. In analyzing the effect of harvest sites on the concentration of oxalates, it may be observed that the mushrooms originating from the proximity of Magurski National Park contained significantly higher quantities of oxalates than the mushrooms harvested in the area of Ożarów and Ostrowiec Św. In the case of bay bolete and red-capped scaber stalk, the differences noted turned out statistically significant.

It is difficult to interpret, but perhaps causes of this phenomenon should be searched for in the role the oxalic acid plays for mushrooms. It refers to its participation in maintaining homeostasis of heavy metal ions. In plants, enhanced synthesis of oxalic acid and other organic acids was observed in response to high concentrations of heavy metals in the substratum [29]. Worth remembering is the fact that mushrooms originating from the area of Magura Małastowska were characterized by a higher concentration of cadmium than those harvested in other areas.

Conclusions

Increasing awareness of threats posed by the pollution of natural environment makes regular control of the levels of toxic compounds in food products, including mushrooms, a necessity. The recorded exceeded permissible content of cadmium in fruiting bodies of boletus from the areas of Ostrowiec Św. and Ożarów, as well as from the protection zone of Magurski National Park, does not pose a risk to human health owing to the small contribution of mushrooms to the human diet. Irrespective of the harvest site, the analyzed species of mushrooms were characterized by a higher concentration of the investigated toxic metals and anti-nutrients in caps than in stalks.

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