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LEAD CONTENT IN 15 SPECIES OF WILD FUNGI IN THE AREA OF THE THERMAL POWER PLANT OSLOMEJ (KICHEVO VALLEY, REPUBLIC OF NORTH MACEDONIA)

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Abstract

The main aim of this research paper is to analyze the content of lead (Pb) in fruiting bodies of 15 species of wild fungi. Fungi and soil samples were collected on 12 localities at three distances from the Thermal Power Plant Oslomej located in the Kichevo valley situated in the west part of the Republic of Macedonia.The samples of Fungi were collected in spring and autumn during three years period (2012-2014). The average content values of Pb were higher that the European standard of 2.00 mg·kg-1 in all of the analyzed species. The maximal values in some samples of edible species were up to five times higher (Marasmius oreades - 11.93, Russula cyanoxantha - 9.78, Armillariella mellea - 9.52, Amanita rubescens - 9.35, Cantharellus cibarius - 9.28 mg·kg-1, Boletus reticulatus - 8.86, etc.).

In general, statistical analyzes showed no direct correlation between Pb content in the fruiting bodies and total/extractable Pb in the soil samples. The discriminant analyzes proved that the Thermal Power Plant Oslomej has impact on the heavy metals content although direct and significant correlation between the distance from the power plant and Pb content in fruiting bodies was observed in only few species. Nevertheless, the Pb content in the wild fungi in Kichevo valley is very high and the use of the edible species should be controlled and monitored.

Keywords: mushrooms, fruiting body, lead, Thermal Power Plant-Oslomej, Kichevo

1. Introduction

Development of technology and industry in the world and in the country has a major impact in environmental pollution. Today, as a result of modern civilization, the world faces a serious problem of pollution with various harmful substances among which include heavy metals.

-Pollution is especially critical in the vicinity of: thermal power plants, mines, along roads, soil fed with fertilizers and pesticides, etc. landfills.

-The presence of trace elements in the soil may originate from different sources.
-In the region of Kichevo there are several industries, including Oslomej affecting soil, water and crops in this region.

Fungi are organisms that possess unique mycelium, through which perform absorption of heavy metals from the soil and their accumulation in the fruit bodies. For the concentration of heavy metals in fertile fungal fungi in the Kichevo region has studied and researched [10, 11,12].

It is continuing to analyze and determine the content of heavy metals in fruiting bodies of fungi. The local population of Kichevo region collects certain fungi for their own, and above all, for

economic purposes like, *Boletus sp. Chantharellus cibarius, Amanita caesarea, Morchella sp., Suillus sp*., and other comertial and edible mushrooms.

The aim of this paper is to analyze the content of lead (Pb) in fruiting bodies of 15 species of wild fungi. Fungi and soil samples were collected on 12 localities at three distances from the Thermal Power Plant Oslomej located in the Kichevo valley in the west part of the Republic of Macedonia.

Fungi were collected in spring and autumn during three years period (2012-2014). **Study area**

The research will include the determination of the above mentioned parameters in concentric circles around the power plant, mine tailings away from: first zone of 0.5-1.0 km, second zone 3-4 km and a third zone, over 7-8 km

North : v. Zhubrino, place Gorica and Ramnishte. Jagol Dolenci

East: Place Zadel near v. Srbica, v. Novoselo (Mountain Cheloica);

South: Osllomej, Mala Chuka (v. Crvivci) and mountain Krushino (near Kichevo);

West: Repetitor-Oslomej (near the source of pollution), v. Strogomishte and v. Zajas-Greshnica.

1. Materials and methods

1.1 Field collection of wild mushrooms

In total, 256 samples of 15 species of wild fungi were collected and analyzed. The determination of fungi was done in the Mycological laboratory at the Institute of Biology, Faculty of Natural Sciences and Mathematics , Skopje.

List of fungi were collected and analyzed

1. *Amanita pantherina* (DC.) Krombh. (19 samples),
2. *Amanita rubescens* Pers. (22 samples),
3. *Amanita vaginata* (Bull.) Lam. (22),
4. *Armillaria mellea* (Vahl) P. Kumm. (10),
5. *Marasmius oreades* (Bolton) Fr. (14),
6. *Hypholoma fasciculare* (Huds.) P. Kumm. (14),
7. *Gymnotus dryophilus* (Bull.) Murrill (26),
8. *Boletus reticulatus (aestivalis)* (Paulet) Fr. (19),
9. *Suillus luteus* (L.) Roussel. *11p-(10),*
10. *Suillus granulatus* (L.) Kuntze (13)
11. *Laccaria laccata* (Scop.) Cooke. (20),
12. *Trametes hirsuta* (Wulfen) Pilát (8),
13. *Russula cyanoxantha* (Schaeff.) Fr. (26),
14. *Stereum hirsutum* (Willd.) Pers. (10),
15. *Chantharellus cibarius* Fr. (18)

Chemical analyzes were conducted at Laboratory for Plant Ecology at the Institute of Biology, Faculty of Natural Sciences and Mathematics, Skopje using Atomic Absorption Spectroscopy (AAS) – flame and graphite furnace.

1. All samples were ground and dried.
2. Sample of 0.5 g was measured on analytical balance (0.0001 g) and transferred into digestion flask.
3. Immediately, 8ml of HNO3 were added and 4 ml of H_2O_2 (1 ml in 1-hour intervals). The samples were digested on temperature of 160˚C during 24 hours.
4. All of the analyzed heavy metals were determined by flame atomic absorption spectrometry on Agilent 55A.
5. The concentration of heavy metals was calculated and corrected by subtracting the blanks.

2. Results and Discussion

The examination of the fruiting bodies of *Amanita pantherina* collected of sites Zhubrino-north (AN) ((9,42 and 7,45 $mg \cdot kg^{-1}$) and Goritza (BN) (8,93 and 8,23 $mg \cdot kg^{-1}$) determined the maximum values for the content of Pb. On the other hand, the minimum concentration for the content of Pb was obtained from the sample ext. Zadel-Serbica (BE) (3,50 $mg \cdot kg^{-1}$). The average value for the content of Pb in all tested samples was 6,20 $mg \cdot kg^{-1}$. the resulting values for the content of Pb in *A. pantherina* from all sites were relatively high and exceed European standards of MPC of the contents of Pb (2 $mg \cdot kg^{-1}$) in the case of *A. pantherina* no particular importance to the diet since it is about a poisonous fungus.

2.1 Key features of heavy metals

Border toxic concentration of Pb of products are consumed by humans is 2 mg · kg-1, while vegetables and fruits (subject pesticides) tolerated concentrations to 3 $mg \cdot kg^{-1}$ Pb (Mitrikeski et al., 2000, Tanevski & Gorgieski, 1994 [9,18].

European standards (regulations 1881/2006 and 466/2001)

Heavy metal	Cultivated fungi	Wild mushrooms
Pb	**0,3** $mg \cdot kg^{-1}$	**0,3** $mg \cdot kg^{-1}$

Table 1. Content of lead in fruiting bodies of fungi with maximum, minimum and average values

	Name of fungi	**Maximum**	**minimum**	**average**
1	*Amanita pantherina*	9.42mg.kg-1 Zhubrino-north (AN)	3.5mg• kg-1Zadel-Serbica (BE)	6.20 mg • kg-1
2	*Amanita rubescens*	9.35 mg • kg-1,Zadel-Serbica (BE)	2.35mg•kg-1Osllome-S (AS)	5.85 mg • kg-1
3	*Amanita vaginata*	10.91 mg • kg-1, Krushino (CS)	3.59 mg • kg-1, Zajaz, (CW)	6.74 mg • kg-1
4	*Armillaria mellea*	9.52 mg • kg-1 Krushino (CS)	2.50 mg • kg-1Drogom. (BW)	5.40 mg • kg-1
5	*Boletus reticulatus*	8.86 mg • kg-1 Zhubrine-N (AN)	1.50 mg • kg-1Novosele, (CS)	5.81 mg • kg-1
6	*Cantharellus cibarius*	(9,28 mg • kg-1 Oslomej-S (AS)	(3,35 mg • kg-1)Goritza (BN)	5.85 mg • kg-1
7	*Gymnopus dryophilus*	(9,48 mg·kg$^{-1,}$ Crvica (BS)	(2,25 mg • kg-1Krushinë (CS)	6,16 mg • kg-1
8	*Hypholoma fasciculare*	(8,25 mg • kg-1, Novoselle (CE)	0,45mg•kg-1 Goritza (BN)	5,66 mg • kg-1
9	*Laccaria laccata*	10,44 mg·kg^{-1}, Gorica (BN)	1,45 mg • kg-1,Zajas (CW)	6,63 mg • kg-1
10	*Marasmius oreades*	(11,93 mg • kg-1Drogom- BW	2,39 mg • kg-1	6,86 mg • kg-1
11	*Russula cyanoxantha*	9,78 mg • kg-1, Crvivci	1,25 mg • kg-1,Zadel-Serbica	5,85 mg • kg-1
12	*Suillus granulatus*	8,55mg• kg-1, Zhubrino (AN)	2,34 mg • kg-1,Zajaz (CW)	5,79 mg • kg-1
13	*Suillus luteus*	8,85 mg • kg-1. Zhubrino (AN)	1,19 mg • kg-1Novoselle (CE)	5,38 mg • kg-1
14	*Stereum hirsutum*	9,05 mg • kg-1 Ramnishte (CN)	4,24 mg • kg-1 Novoselle	6,14 mg • kg-1
15	*Trametes hirsuta*	(8,91 mg • kg-1 Oslome (AW)	2,99 mg • kg-1 Novoselle	4,89 mg • kg-1

The results showed that all samples of the fruiting bodies of *Amanita rubescens* exceed the contents of Pb compared with the MPC (2,00 mg • kg^{-1}), even samples with the lowest content of Pb collected in loc. Oslomej-south (AS) (2,35 mg • kg^{-1}). However the highest value was registered in loc. Zadel -Serbica (BE) which is 9,35 mg • kg^{-1}. The average value for the content of Pb in all tested samples was 5,83 mg • kg^{-1} (Fig. 1,2; Tab.1).

Maximum content of Pb was found in fruiting bodies of *Amanita vaginata* collected in loc. Krushino (CS) (10,91 mg • kg-1), while the lowest values were obtained in samples in loc. Zajaz (CW) (3,59 mg • kg^{-1}). The average value for the content of Pb in all tested samples of *A. vaginata* was 6,74 mg • kg^{-1} (Fig. 1,2; Tab. 1).

Maximum value for the content of Pb was obtained in samples from *Armillariella mellea* collected in loc. Krushino (CS) (9,52 mg • kg^{-1}), while the minimum values were observed in samples loc. Strogomishte (BW) (2,50 mg • kg-1). The average value for the content of Pb in all tested samples of *A. mellea* was 5,40 mg • kg^{-1} (Fig. 1,2; Tab. 1).

According to the results, maximum values for the content of Pb was established in fruiting bodies of *Boletus aestivalis* collected of loc. Zhubrino-North (AN) (8,86 mg • kg^{-1}), while the minimum values were observed in samples ext. Novo Selo (CE) (1,50 mg • kg^{-1}). In all tested samples of *B. reticulatus* the average value for the content of Pb was 5,81 mg • kg^{-1} (Fig. 1,2 Tab. 1).

The highest content of Pb was found in the fruiting bodies of *Cantharellus cibarius* collected of loc. Oslomej-South (AS) (9,28 mg • kg^{-1}), while the lowest content was obtained in samples from loc. Goritza (BN) (3,35 mg • kg^{-1}). The average value for the content of Pb in all tested samples of *C. cibarius* was 5,85 mg • kg^{-1} (Fig. 1,2 Tab. 1).

The highest content of Pb was registered in the fruiting bodies of *Gymnopus dryophilus* collected of loc. Crvivci (BS) (9,48 mg • kg^{-1}), while the minimum values were obtained in samples from collected loc. Krushino (CS) (2,25 mg • kg^{-1}). According to the results, the average value for the

content of Pb in all tested samples of *G. dryophilus* (6,16 mg • kg^{-1}) exceeds the maximum allowable concentration for this heavy metal (Fig. 1,2; Tab. 1).

The highest content of Pb was obtained in fruiting bodies of *Hypholoma fasciculare* collected of loc. Novo Selo (CE) (8,25 mg • kg^{-1}), minimum values registered in the loc. Gorica (BN) (0,45 mg • kg^{-1}). The average value for the content of Pb in all tested samples is 5,66 mg • kg^{-1} (Fig. 1,2; Tab. 1).

Regarding the content of Pb, the fruiting bodies of *Laccaria laccata* maximum content is very high and it is registered in the loc. Goritza (BN) (10,44 mg • kg-1), the minimum value is registered in loc. Zajas (CW) (1,45 mg • kg^{-1}), while the average value for all tested samples of *L. laccata* was 6,63 mg • kg^{-1} (Fig. 1,2; Tab. 1).

The highest content of Pb was found in the fruiting bodies of *Marasmius oreades*, collected of loc. Strogomishte (BW) (11,93 mg • kg^{-1}), the highest value for this element (Pb) than any other examined samples from all sites. Also in the same locality was found the presence of the sample with the lowest content of Pb (2,39 mg • kg^{-1}). The average value for the content of Pb in the fruiting bodies of all tested samples of *M. oreades* was 6,86 mg • kg^{-1} (Fig. 1,2; Tab. 1). It is important to note that in all tested samples of *M. oreades* were found higher values for the content of Pb in terms of maximum allowable concentration for Pb in fungi.

After the results obtained for the content of Pb in the fruiting bodies of *Russula cyanoxantha* concluded that the highest value is registered in the loc. Crvivci (BS) and the same amount of 9,78 mg • kg^{-1}, while the lowest value was registered in loc. Zadel-Serbica (BE) and it amounted to 1,25 mg • kg^{-1}. The average value for the content of Pb in all tested samples of *R. cyanoxantha* was 5,85 mg • kg^{-1} (Fig. 1,2; Tab. 1)

According to the results of Pb content in fruiting bodies of *Suillus granulatus*, the highest values were registered in loc. Zhubrino (AN) and it was 8,55 mg • kg^{-1} Very high value is registered in the lock. Krushino (CS) and it was moving 8,38 mg • kg^{-1}, while the minimum value is registered in the loc. Zajaz (CW) (2,34 mg • kg^{-1}). The average value of all samples tested was 5,79 mg • kg^{-1} (Fig. 1,2; Tab. 1).

According to the results of Pb content in fruiting bodies of *Suillus luteus*, the highest value was 8,85 mg • kg^{-1} registered in the lock. Zhubrino (AN) while the minimum value is registered in the loc. Novo Selo (CE) (1,19 mg • kg^{-1}. The average value for the content of Pb in all tested samples of *S. luteus* was 5,38 mg • kg^{-1} (Fig. 1,2; Tab. 1).

The content of Pb in the fruiting bodies of *Stereum hirsutum* ranged from peak levels registered in the lock. Level (CN) (9,05 mg • kg-1) to minimum values registered in the lock. Novo Selo (CE) (4,24 mg • kg^{-1}). The average value for the content of Pb in all tested samples of *S. hirsutum* was 6,14 mg • kg^{-1} (Fig. 1,2; Tab. 1).

In terms of content of Pb in the fruiting bodies of *Trametes hirsuta*, the highest value was obtained in samples from ext. Oslomej Repetitor (AW) (8,91 mg • kg^{-1}), while the lowest was observed in samples ext. Novo Selo (CE) (2,99 mg • kg^{-1}). The average value for the content of Pb test specimens of *T. hirsuta* was 4,89 mg • kg-1 (Fig. 1,2; Tab. 1).

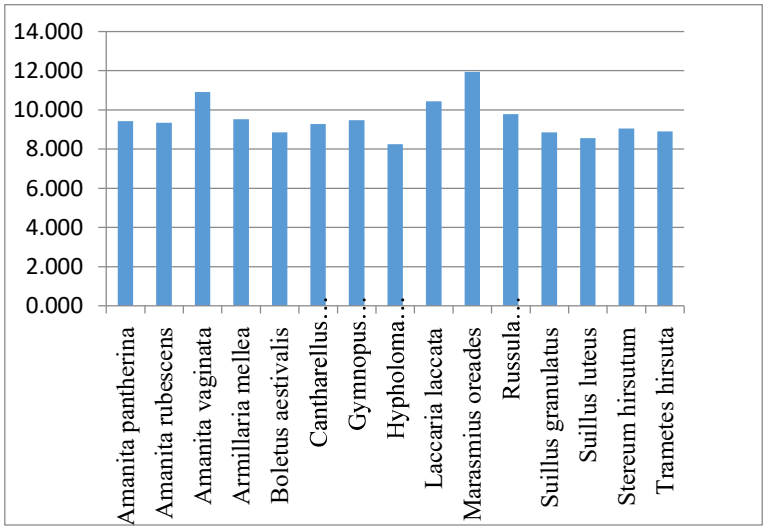

Figure 1. Maximum values of the lead content in the fruiting body of 15 wild fungi

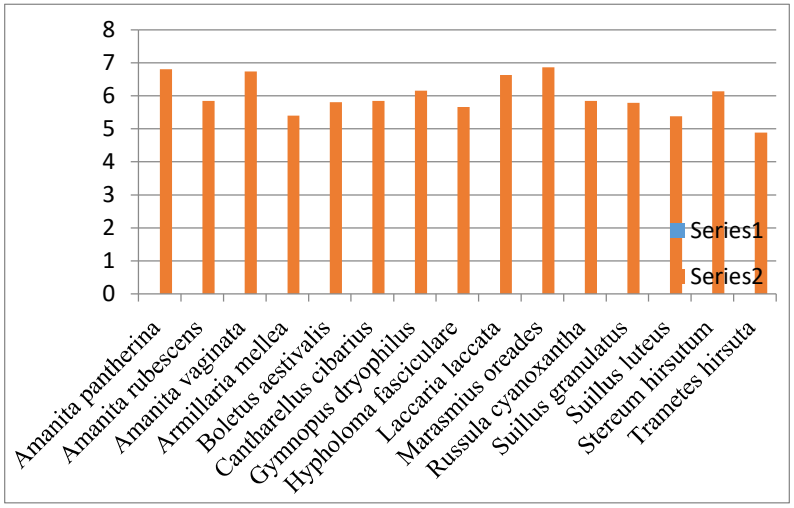

Figure 2. The average values of the lead content in the fruiting body of 15 wild fungi

The highest content of Pb was found in the fruiting bodies of *Amanita pantherina* collected of loc. Zhubrino village (AE) (9,42 mg • kg^{-1}), while the lowest content was obtained in samples from loc. Zadel(BE) (3,50 mg • kg^{-1}). The average value for the content of Pb in all tested samples of *A. pantherina* was 6.20 mg • kg^{-1} (Fig. 1,2 Tab. 1).

The main Pb content (5.83 mg•kg^{-1}) in all tested samples of *Amanita rubescens* is significantly higher compared to the studies of Radulescu et al., 2010a [14] for samples of this species collected from the Dambovita County (Romania) region, (0.68 mg•kg^{-1}). Also, Cocchi et al., 2006 [2] found relatively low Pb content (1.25 mg•kg^{-1}) in samples of *A. rubescens* from several regions in Italy.

Also, the main value for the content of Pb (6.74 mg•kg^{-1}) in the fruiting bodies of *Amanita vaginata* was almost identical (6.17 mg•kg^{-1}) as well as in samples collected from Konya, Turkey (Dogan et al., 2006) [4]. Previous research by Radulescu et al., 2010b [15] and Cocchi et al., 2006 [2] showed lower Pb content in samples of *A. vaginata* collected from different regions in Romania (1.93 mg • kg^{-1}) and Italy (1.25 mg • kg^{-1}).

According to the results obtained, the main value of Pb content in the fruit bodies of *Armillariella mellea* was 5.40 mg • kg^{-1}. In terms of these values, lower Pb content (from 2.36 mg • kg^{-1} to 4.17 mg • kg^{-1}) was found in samples collected from different regions of Turkey [4], Italy [2] and Romania [14]. Particularly low Pb content was found in samples of *A. mellea* collected from western Greece (0.49 mg • kg^{-1}) [13] and western Black Sea, Turkey (0.05 mg • kg^{-1}), [7].

The obtained results for the content of Pb (5.81 mg • kg^{-1}) in the fetal bodies of the *Boletus reticulatus* are in accordance with the research of Svoboda et al. 2000 [17] for the content of this metal (6.0 mg • kg^{-1}) in samples from some European countries. Similar values for the content of Pb (from 2.00 mg • kg^{-1} to 5.00 mg • kg^{-1}) in *B. reticulatus's* fruiting bodies were found in previous studies, [5, 19]. On the other hand, particularly low Pb content (0.74 mg • kg^{-1}) was found in samples from the *B. reticulatus* collected from some regions in Italy [2].

The main value for Pb content in the fetal bodies of *Cantharellus cibarius* was 5.85 mg • kg^{-1}. These values are within the content of Pb (from 0.49 mg • kg^{-1} to 7.9 mg • kg^{-1}) in samples of *C. cibarius* collected from different regions in Norway, [16]. Compared with the results of this study, previous studies show lower values for Pb content in samples from some unpolluted regions in the province of Ciudad Real, Spain (4.86 mg • kg^{-1}), [1], as well from some European countries (3.13 mg • kg^{-1}), [17];. Particularly low Pb content (0.04 mg kg^{-1}) was found in samples from the western part of the Black Sea, Turkey [7].

In terms of Pb content, samples of *H. fasciculare* collected from the province of Ciudad Real, Central Spain showed lower content of the investigated metal (3.50 mg • kg^{-1}) compared with the results of this study, [1].

The content of Pb in the fruiting bodies of *Marasmius oreades* was 6.86 mg • kg^{-1}, which is a significantly higher value compared to the results of Uzun et al., 2011, [20] for samples from the provinces of Bingol and Selim, Turkey (0.01 mg • kg^{-1}). On the other hand, in some European countries high values (28.7 mg • kg^{-1}) were obtained for the content of Pb in samples from *M. oreades*, [17]. To highlight is the high maximum content of Pb in the fruiting bodies of this fungus registered in the locus. Strogomishte (BW), which is 11.93 mg • kg^{-1}.

According to the results obtained, the main value for the Pb content of the examined samples of *Russula cyanoxantha* was 5.85 mg • kg^{-1}, which is a significantly higher value (0.044 mg • kg^{-1}) compared to the collected samples from Russia, [8]. To highlight is the high maximum content of Pb in the fruiting bodies of this fungi registered in the loc. Crvica (BS) and the same is 9.78 mg • kg^{-1}.

The obtained results for the mean value for the content of Pb (5.79 mg • kg^{-1}) in the fruiting bodies of *Suillus granulatus* are significantly higher compared to the studies of Cocchi et al. 2006 (1.32 mg • kg^{-1}) for samples collected from different regions in Italy, [2].

The main value of Pb content in the fruiting bodies of *Suillus luteus* was 5.38 mg • kg^{-1}. In accordance with the obtained results, Svoboda et al., 2000 [17] found almost identical content of Pb (5.47 mg • kg^{-1}) in samples from Slovakia. Significantly lower Pb content (1.23 mg • kg^{-1}) compared with the results of this study was recorded in samples from different regions of Italy (Cocchi et al., 2006) [2]. In addition, low Pb content (from 0.01 mg kg^{-1} to 0.04 mg kg^{-1}) was recorded in samples of *S. luteus* collected from different regions in Turkey (Konuk et al., 2007 ; Uzun et al. 2011) [7,20].

According to the results obtained, the mean value for Pb content in the fertile bodies of *Stereum hirsutum* was 8.07 mg • kg-1. Compared to the results obtained, Karaman and Matavul, 2005 [6] found significantly lower Pb content (1.93 mg • kg-1) in samples from *S. hirsutum* collected in Fruska Gora, Serbia.

3. Conclusion

- High contentration of Pb was found in certain wild edible fungus such as: *Marasmius oreades* (11.93 mg·kg^{-1}, mg *Amanita vaginata*, 10.91 mg·kg^{-1}, *Laccaria laccata* 10.44 mg·kg^{-1} *Cantharellus cibarius 9.28* mg·kg^{-1} *Russula cyanoxantha 9.78* mg·kg^{-1} *Armillariella mellea* 9.52 mg·kg^{-1}.
- Low values of lead content in the fruiting body of 15 wild type fungi
- Samples of these fungi are not recommended to eat, especially those collected adjacent (first zone) and that contents very high concentrations of heavy metals such as lead (Pb).
- Although in almost all types of concentration was exceeded only in very rare specimens of these species that does not exceed the maximum allowable concentration for lead.
- According to the results of the contents of the studied heavy metals in soils and fruiting bodies of fungi collected can be concluded that the environment of Oslomej a contaminated area as a result of the increased use of coal and the introduction of high-tech industrial processes. Environmental pollution in the vicinity of Oslomej takes place through the release of heavy metals from the processing of coal in soil and water, resulting in the accumulation of metals in fungi from the group macromycetes. The results are consistent with previous research by which concluded that the environment of Oslomej is significantly contaminated with heavy metals.
- **Lead** is a highly **poisonous** metal affecting almost every organ in the body. ... Severe damage to the brain and kidneys, both in adults and children, were found to be linked to exposure to heavy **lead** levels resulting in death. In pregnant women, high exposure to **lead** may cause miscarriage.

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