UDC: 631.416.84/.88(497.781)”2012/2014”
Original scientific paper

CORRELATION BETWEEN THE CONTENT OF SEVEN HEAVY METALS IN CERTAIN SPECIES OF FUNGI AND THEIR CONCENTRATION IN SOILS NEAR TEPP OSLOMEJ-KICHEVO, NORTH MACEDONIA

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Abstract

Field research on 12 sites in the region of Kicevo valley during the period April 2012 to May 2014 was conducted at different distances from the Thermoelectric Power Plant (TEPP) Oslomej. The research included the determination of the total and extractable concentration of seven heavy metals: nickel (Ni), copper (Cu), zinc (Zn), iron (Fe), manganese (Mn), cadmium (Cd), and lead (Pb) in soil samples and fungi. A total of 15 species of fungi (Amanita rubescens, Amanita vaginata, Boletus aestivalis, Armillaria mellea, Cantharellus cibarius, Marasmius oreades, Russula cyanoxantha, Suillus granulatus and Suillus luteus, Gymnotus dryophillus, Laccaria laccata, Stereum hirsutum and Trametes hirsute, Amanita pantherina and Hypholoma fasciculare) were analyzed, 11 being terricolous fungi and four lignicolous fungi. In general, analyzed fungi species tend to accumulate Cd, Zn, Cu, and Ni (BAF >1). Most of the species tend to exclude Fe, Mn, and Pb (BAF<1). The highest bioaccumulation potential for Cd has A. pantherina as well as A. mellea, G. dryophilus, and R. cyanoxantha. The contents of total and extractable forms of heavy metals Ni, Cu, Zn, Fe, and Cd in soils were correlated with their content in fruiting bodies of Amanita pantherina.

Keywords: Heavy metals, correlation, fungi, concentration, coal mine

1. Introduction

Energy need in the Republic of North Macedonia is mainly satisfied by coal thermal power, including Thermal Power Plant "Oslomej" - Kichevo. It is known that the operation of these thermal power plants pollutes the air, soil, and water and through them and the animals, plants, and fungi [11].

Fungi in general are known to be potent accumulators of heavy metals from soils and thus were used as indicators for heavy metal pollution [4,12,13]. Compared to green plants, mushrooms can build up a large concentration of some heavy metals particularly Cd, Hg, Cu, and Pb [10].

Many mushroom species from the Kichevo valley (Republic of North Macedonia) are consumed by the human population regardless of the heavy metal content and uptake in the human body [7].

The main goal of this study was to evaluate the extent of heavy metal accumulation in the fruiting bodies of selected fungi about soil concentrations in a polluted area around thermoelectric power plant Oslomej (North Macedonia).

2. Materials and methods

Study area

Kichevo valley is situated in southwest Macedonia, surrounded by Bistra and Suva Gora mountains. Kichevo is an important industrial center in this part of Macedonia due to the coal mine Oslomej and the TEPP Oslomej. TEPP Oslomej provides for about 9% of the total electrical energy production in the Republic of North Macedonia.

Field collection of wild mushrooms and soil samples

The samples were collected at various distances from TEPP Oslomej in the period between 2012 and 2013 (exclusively from spring to autumn). In total, 256 samples of 15 species of wild mushrooms were collected: edible fungi *Amanita rubescens, Amanita vaginata, Boletus aestivalis, Armillaria mellea, Cantharellus cibarius, Marasmius oreades, Russula cyanoxantha, Suillus granulatus and Suillus luteus;* non-edible wild fungi: *Gymnotus dryophillus, Laccaria laccata, Stereum hirsutum, and Trametes hirsutum and poisonous fungi, such as Amanita pantherina and Hypholoma fasciculare.* Samples were collected from 12 localities at different distances (~0.5, ~3, and ~7.5 km) and orientations (north, west, east, and south) from TEPP Oslomej (Tab. 1). All the samples were collected in an Italian and Turkey oak forests (*Quercetum frainetto-cerris Horvat, 1954*). Dried samples are kept in the Macedonian Collection of Fungi (MCF). Soil samples from these localities at depth of 5-15 cm were also collected (in two replicates).

Analytical methods

All samples of fungi were ground and wet digested with HNO3 and H2O2. A sample of 0.5 g was measured on an analytical balance (0.0001 g) and transferred into a digestion flask. Immediately, 8ml of HNO3 was added followed by the addition of 4 ml of H2O2 (1 ml in one-hour intervals). The samples were digested at a temperature of 140˚C for 24 hours.

The total concentration of heavy metals in soils was determined by wet digestion of approximately 1 g dry soil sample in 10 ml of digestion solution HNO3:HCLO4:H2SO4=10:2:1 [1].

DTPA method was used for the determination of the extractable heavy metals content in soil samples [6],[9]. In a 100 ml plastic bottle 10 g of air-dried soil was added and then 20 ml of prepared DTPA reagent (pH = 7,2–7,3). This solution was then mixed on a horizontal shaker for 2 hours. The extracts were then filtered using Whatman 42 (slow filtration ashless), and the volume was then adjusted to 25ml with distilled water.

All of the analyzed heavy metals were determined by flame atomic absorption spectrometry on Agilent 55A. The concentration of heavy metals was calculated and corrected by subtracting the blanks. The Bio Accumulation Factor (BAF) was calculated as a metal concentration in fungi/metal total content in the soil.

3. Results and discussion

In this study, the dependence was analyzed between the content of heavy metals in the soil (Tab. 1) and the fruiting bodies of 15 examined fungi (Tab. 2). The relationship between metal concentration in fungi and soils is expressed through bioaccumulation factors (Tab. 3). The correlation was determined between the total and the extractable content of heavy metals in the soil and the content of heavy metals in each species (Tab. 4). The following text only highlights statistically significant relationships between soil and fungus contents.

Table 1. Total (T) and extractable (E) concentration (mg·kg-1) of heavy metals in soils of 12 sampling localities around TEPP Oslomej.

Locality	Distance	Orientation	Ni		Cu		Zn		Fe		Mn		Pb		Cd	
			T	E	T	E	T	E	T	E	T	E	T	E	T	E
1. Zhubrino	0.5	N	4.6	0.032	4.2	0.15	11.3	0.05	4726.9	6.3	72.2	1.7	17.6	0.06	0.17	0.003
2. Oslomej - W	0.5	W	10.3	0.102	7.7	0.16	24.1	0.07	8780.7	4.4	142.0	2.9	19.8	0.14	0.05	0.004
3. Oslomej - S	0.5	S	8.9	0.052	5.5	0.09	19.2	0.08	4691.6	4.3	105.9	5.1	18.1	0.21	0.05	0.005

4 Zhubrino. E	0.5	E	9.3	0.04	10.1	0.17	25.6	0.15	9376.4	3.1	177.4	4.3	19.3	0.06	0.05	0.003
5. Gorica	3.25	N	8.7	0.021	5.1	0.08	17.0	0.04	9399.0	3.2	171.3	1.8	17.3	0.05	0.05	0.002
6. Stragomishte	3.25	W	15.5	0.044	23.7	0.14	37.0	0.03	7590.7	3.4	110.8	3.8	16.8	0.23	0.05	0.002
7. Crvivci	3	S	19.1	0.100	13.3	0.13	39.9	0.10	6315.6	3.1	102.7	5.4	16.9	0.10	0.10	0.006
8. Zadel-Srbica	2.75	E	16.1	0.029	20.1	0.12	32.7	0.05	9455.2	2.6	160.7	0.9	39.2	0.02	0.12	0.003
9. Jagol-Dolenci	7.5	N	9.5	0.015	5.1	0.05	15.6	0.02	4792.3	2.0	111.4	5.7	17.4	0.05	0.05	0.001
10. Zajas	7.5	W	34.2	0.104	21.5	0.12	43.2	0.18	4085.9	3.8	101.3	1.1	17.2	0.12	0.07	0.002
11. Krushino	7.5	S	9.3	0.041	10.1	0.17	25.6	0.15	9376.4	3.1	177.4	4.3	19.3	0.06	0.05	0.003
12. Novo Selo	7.5	E	3.3	0.053	3.0	0.09	10.1	0.07	3690.7	4.9	54.5	2.5	16.8	0.14	0.07	0.003
Average			12.7	0.054	10.8	0.12	25.1	0.08	6627.7	3.7	119.1	3.2	19.7	0.11	0.08	0.003

Table 2. Average values and standard errors of heavy metals content (mg·kg-1) in 15 species of wild mushrooms from the Kichevo area

	Ni	Cu	Zn	Fe	Mn	Pb	Cd
Amanita pantherina	7.34 ± 3.13	16.08 ± 1.38	67.59 ± 5.23	391.99 ± 109.20	28.12 ± 2.94	6.20 ± 0.36	4.07 ± 0.48
Amanita rubescens	4.91 ± 1.60	18.13 ± 1.29	59.11 ± 3.55	174.43 ± 33.91	21.88 ± 2.47	5.83 ± 0.36	0.62 ± 0.09
Amanita vaginata	8.88 ± 3.16	36.77 ± 1.65	60.28 ± 3.18	389.32 ± 92.26	32.88 ± 5.13	6.74 ± 0.36	0.63 ± 0.09
Armillaria mellea	14.96 ± 7.39	11.04 ± 2.98	32.38 ± 8.01	945.84 ± 480.52	39.39 ± 8.50	5.4 ± 0.7	1.62 ± 0.21
Boletus aestivalis	4.12 ± 1.15	16.12 ± 1.97	59.82 ± 4.14	116.09 ± 23.92	29.68 ± 5.71	5.81 ± 0.47	0.58 ± 0.07
Cantharellus cibarius	14.64 ± 7.78	23.75 ± 2.25	32.36 ± 2.00	199.95 ± 82.69	28.29 ± 3.67	5.85 ± 0.36	0.21 ± 0.02
Gymnopus dryophilus	35.98 ± 22.56	21.51 ± 2.56	56.19 ± 4.27	339.61 ± 71.50	101.01 ± 14.71	6.16 ± 0.49	1.78 ± 0.16
Hypholoma fasciculare	2.13 ± 1.02	22.18 ± 2.10	34.12 ± 2.61	241.01 ± 53.34	33.61 ± 9.76	5.66 ± 0.57	0.63 ± 0.07
Laccaria laccata	22.61 ± 9.93	33.86 ± 1.50	52.39 ± 2.73	499.13 ± 118.42	40.24 ± 4.37	6.63 ± 0.59	0.64 ± 0.09
Marasmius oreades	6.57 ± 5.53	37.86 ± 3.32	62.72 ± 5.10	238.71 ± 77.21	29.64 ± 2.91	6.86 ± 0.66	0.31 ± 0.04
Russula cyanoxantha	6.95 ± 2.18	29.11 ± 2.11	43.68 ± 3.25	110.17 ± 16.84	22.86 ± 1.92	5.85 ± 0.45	1.35 ± 0.32
Suillus granulatus	6.41 ± 3.88	8.89 ± 0.98	47.38 ± 5.84	121.72 ± 28.21	13.23 ± 1.98	5.79 ± 0.50	0.31 ± 0.05
Suillus luteus	3.04 ± 2.04	8.00 ± 1.01	35.97 ± 2.24	152.33 ± 29.26	15.01 ± 1.89	5.38 ± 0.67	0.55 ± 0.30
Stereum hirsutum	4.67 ± 1.87	7.55 ± 0.89	24.8 ± 2.50	1015.14 ± 325.74	170.73 ± 18.75	6.14 ± 0.38	0.36 ± 0.03
Trametes hirsuta	10.84 ± 7.32	3.82 ± 0.89	17.13 ± 1.25	804.55 ± 261.71	135.04 ± 22.06	4.89 ± 0.72	0.30 ± 0.06

Table 3. Bioaccumulation factors (BAF) for heavy metals in 15 species of wild mushrooms from the Kichevo area

Species	Ni	Cu	Zn	Fe	Mn	Pb	Cd
Amanita pantherina	2.43	1.92	4.55	0.20	0.25	0.55	55.44
Amanita rubescens	1.88	2.15	3.57	0.06	0.20	0.53	7.91
Amanita vaginata	2.46	4.21	3.65	0.12	0.30	0.62	8.44
Armilaria melea	1.00	1.02	1.23	0.23	0.29	0.47	24.49
Boletus aestivalis	1.19	1.92	3.98	0.04	0.29	0.54	8.61
Cantharellus cibarius	3.07	2.72	2.08	0.06	0.25	0.51	2.68
Gymnopus dryophilus	3.25	3.23	2.86	0.13	0.89	0.57	27.19
Hypholoma fasciculare	1.23	2.99	1.96	0.11	0.29	0.48	8.43
Laccaria laccata	7.35	4.04	2.98	0.15	0.33	0.59	9.39
Marasmius oreades	5.11	4.94	3.47	0.08	0.29	0.64	4.32
Russula cyanoxantha	1.51	3.28	2.57	0.04	0.21	0.53	24.66
Suilus granulatus	0.56	0.74	1.75	0.04	0.10	0.58	3.66
Suilus luteus	0.48	1.18	1.88	0.08	0.14	0.55	4.33
Trametes hirsuta	2.16	0.57	0.93	0.29	1.18	0.40	5.42
Average	2.49	2.60	2.82	0.12	0.40	0.55	14.03

Table 4. Correlation between heavy metals content in soils (T-Total, E-Extractable) and fruiting bodies of mushrooms

Species	Form	Formula	p	F	r	n
Manganese (Mn)						
A. pantherina	T	1/(a+b/X)	0,0317	5,48	0,49	19
A. vaginata	E	1/(a+b/X)	0,0039	10,36	0.59	20
C. cibarius	E	1/(a+b/X)	0.0463	4.85	-0.52	15
Lead (Pb)						
A. pantherina	T	a+bX	0,0297	5,7	0,512	18
L.laccata	T	a+b/X	0.0017	13.63	0.66	20
Zinc (Zn)						
A. vaginata	E	a+b·ln(X)	0,0112	7,98	0,554	20
H. fasciculare	T	a+b/X	0.0068	10.64	0.69	14
M. oreades	T	a+bX	0.0433	5.1	0.55	14
R. cyanoxantha	E	a+b/X	0.0055	9.3	0.53	26
Cadmium (Cd)						
B. aestivalis	T	1/(a+bX)	0.0381	5.17	0.51	17
H. fasciculare	T	a+bX	0.0427	5.14	0.55	14
M. oreades	T	1/(a+b/X)	0.0117	8.83	0.65	14
R. cyanoxantha	T	a+b/X	0.0204	6.17	0.45	26
S. luteus	T	1/(a+b/X)	0.015	8.98	0.71	11
Copper (Cu)						
G. dryophilus	T	1/(a+b/X)	0,040	11,30	0,64	18
S. granulatus	E	1/(a+b/X)	0.0168	7.93	0.65	13
S. luteus	T	1/(a+b/X)	0.0149	10.28	-0.77	9
Iron (Fe)						
G. dryophilus	E	a+b/X	0.0134	8.01	0.6	16
H. fasciculare	E	a+b/X	0.007	10.93	0.71	13
L. laccata	T	a+b/X	0.0019	13.24	0.65	20

S. luteus	T	a+bX	0.0118	10.54	-0.75	10

In general, analyzed fungi species tend to accumulate Cd, Zn, Cu, and Ni (BAF >1). Most of the species tend to exclude Fe, Mn, and Pb (BAF<1).

BAF for Ni varied from 0.48 to 7.35. The highest potential for bioaccumulation has L. laccata, and M. oreades followed by G. dryophilus and C. cibarius. These species have much higher values for Ni in their fruiting bodies than the total concentration of Ni in the soil. The lowest BAF was recorded in S. granulatus and S. luteus (Tab. 3). Cu is another element that fungi tend to accumulate in their fruiting bodies. The highest values for BAF were determined in M. oreades, A. vaginata, and L. laccata. S. luteus is the only species that excludes Cu from the soil. Almost all the analyzed species accumulate Zn, as well (BAF > 1), except T. hirsuta. Both Fe and Mn are excluded by fungi, hence BAF is considerably less than 1. Only T. hirsuta (1.18) and G. dryophilus have higher BAF for Mn (0.89). All the analyzed fungi exclude Pb (BAF<1) and have similar values for BAF (Tab. 3). In the case of Cd, all the analyzed species of fungi have BAF>1 which means that all of them accumulate Cd in higher concentrations than present in the soil. The highest bioaccumulation potential for Cd has A. pantherina. Significantly high potential for Cd was recorded in A. melea, G. dryophilus, and R. cyanoxantha.

A significant correlation was found between the total Mn and Pb content of soils and their content in the fruiting bodies of A.pantherina (Tab. 4). It is important to note that there was no significant positive correlation between the content of Cd in the soil with the content in the fruiting body of this species, although high accumulation values were recorded here.

According to the obtained results, a statistically significant correlation was established between the content of the extractable forms of Mn and Zn in the soil and their content in the fruiting bodies of Amanita vaginata (Tab. 4). This species has similar BAFs for most of the heavy metals. It has higher BAF for Cu than the other two Amanita species (Tab. 3).

The content of total and extractable forms of all tested heavy metals was not significantly correlated with their content in the fruiting bodies of the tested samples of Amanita rubescens. This species has lower BAFs for all heavy metals compared to A. pantherina and A. vaginata.

All three analyzed species of fungi of the genus Amanita showed considerable ability to accumulate heavy metals from the soil, which is particularly expressed in the example of A. pantherina and Cd [8]. In the case of species of the genus Amanita, we found a greater number of statistically significant relations. It is also interesting that in all three species of fungi a correlation was found between the content of Zn and Mn between the soil and the fruiting bodies.

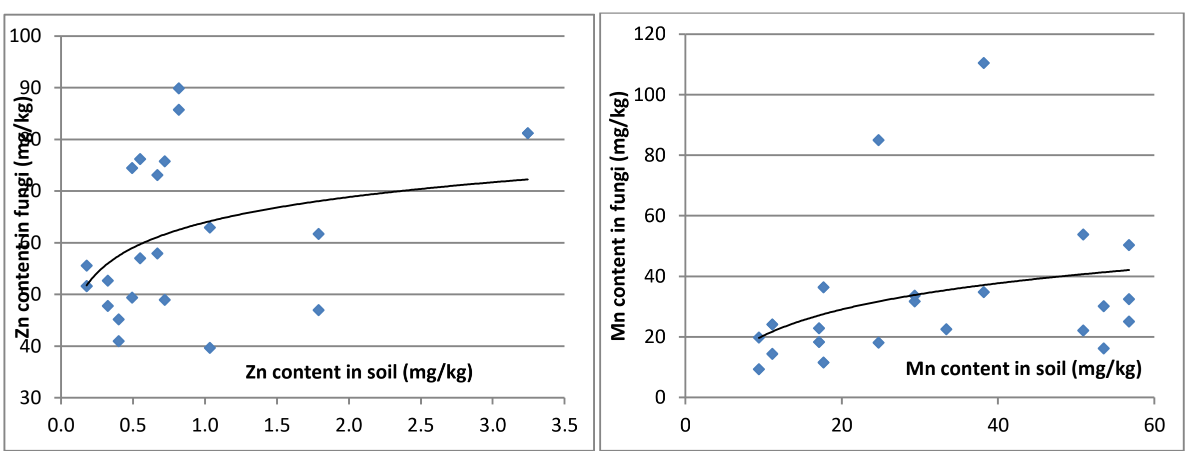

Fig1. Correlation between the content of Zn (left) and Mn (right) in the fruiting bodies of Amanita vaginata

The content of total and extractable forms of all tested heavy metals in the soils was not significantly correlated with their content in the fruiting bodies of the tested samples of Armillariella mellea.
A significant correlation was obtained only between the total content of Cd in the soils and its contents in the samples of the Boletus aestivalis.
The content of the total forms of heavy metals Ni, Cu, Zn, Fe, Cd, and Pb in soils was not correlated with their content in the fruit bodies of Cantharellus cibarius. On the other hand, a significant correlation was found between the total content of Mn and its content in the samples of C. cibarius. The content of the extractable forms of heavy metals in the soils was not correlated with their content in the tested fungi samples. These results have particular significance because C. cibarius is an edible and economically very important fungus.
A significant correlation was found between the extractable forms of Fe in the soils and its content in the fruiting bodies of G. dryophilus. The total content of Cu in the soils was correlated with its content in the fruiting bodies of Gymnopus dryophilus.
A significant correlation was obtained between the content of the total forms of Zn and Cd and their content in the fruiting bodies of Hypholoma fasciculare. Regarding the extractable forms of heavy metals in the soils, a significant correlation was obtained between the content of the extractable forms of Fe in the soils and its content in the samples of H. fasciculare.The relationship between the concentration of Cu in the fruiting bodies of Hypholoma fasciculare with the same substrate is 0.47 (Radulescu et al. 2010) [10]. In the case of Elekes et al. (2010)[2], for Bucegi Mountain in Romania, it was ascertained that they amounted to 0.22 mg·kg-1 in the fruiting bodies, or 0.086 mg·kg-1 in the substrate. The relationship between the concentration of Mn in the fruiting bodies and the content of the same metal in the soil was 0.49 (Radulescu et al. 2010) [10].
A significant correlation was obtained between the total Fe and Pb content of the soils and their content in the fruiting bodies of Laccaria laccata.
A significant correlation between the total content of Zn and Cd in soils from the examined region and their content in the fruiting bodies of Moreades was recorded.
The total Cd content in the soils was in positive correlation with its content in the fruiting bodies of Russula cyanoxahtha. Regarding the extractable forms of heavy metals, a significant correlation was established between the content of Zn in the soils and its content in the tested samples.
A significant correlation was obtained between the extractable content of Cu in the soil and the content of this heavy metal in the fruiting bodies of Suillus granulatus. Compared to the correlation shown in the data in certain Swedish sites for Cu, $p < 1.93$ are lower. (Vinichuk 2012) [14]. However, the dependence on the content of Cu in the soil and the fruiting bodies of this type of edible fungus does not constitute a potential hazard in its consumption, since it is known that Cu is an essential element [3],[5].
A significant correlation was found between the total content of Cu, Fe, and Cd in the soils from the examined region and their content in the fruiting bodies of Suillus luteus. Also, the extractable Mn content in the soils was significantly correlated with its content in the examined samples of S. luteus.
The obtained results showed no significant correlation between the content of the total and extractable forms of the tested heavy metals in the soils and their content in the fruit bodies of Armillariella mellea. This species is lignified and lives very briefly, and the fruiting bodies of this fungus decompose for several days which is probably the reason why the content of the total forms of heavy metals Ni, Cu, Zn, Fe, Mn, and Pb in soils was not correlated with their content in the fruiting bodies of B. aestivalis.

4. Conclusion

Many mushroom species from the Kichevo valley (Republic of North Macedonia) are consumed by the human population regardless of the heavy metal content and uptake in the human body.

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