Accumulation of Heavy Metals by Fungi in the Cities of Central Black Earth Region

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Abstract. Emissions of heavy metals into the environment lead to their accumulation in living organisms. This article presents the data on the accumulation of heavy metals in the fungi fruit in urban ecotopes of cities in the Western part of the Central Black Earth region. The features of accumulation of heavy metals by macromycetes of different taxonomic affiliation and ecological specialization under multi-level anthropogenic burden are studied. It was noted that MAC of Cadmium (Cd) in edible fungi can be in large excess over 88 times, lead (Pb) – 87 times, copper (Cu) – 6.8 times, zink (Zn) – 6.1 times. The accumulation of heavy metals by macromycetes can be determined by the degree of soil pollution, the bioavailability of heavy metals, as well as environmental specialization of fungi. The highest concentrations of heavy metals were recorded in the fruit bodies of soil and litter saprophytes. Cantharellus cibarius (L.), Murrill, Agaricus melleus (Vahl), Agaricus equestris L. were universal accumulators of Cd, Zn and Cu. Leccinum scabrum (Bull.) Gray, Kuehneromyces mutabilis (Schaeff.) Singer & A.H. Sín., Myxocollybia velutipes (Curtis) Singer are defined as selective Cd accumulators.

1. Introduction
Cities are the main place of living for modern men. Population desity and intensity of economic activity determine the degree and features of transformation of natural ecosystems. The territory of a modern city, as a rule, is presented by anthropogenic and natural-anthropogenic landscapes. Recreational areas, mainly represented by woodlands and forest parks, serve as places of cultural recreation for the population. Environmental wellbeing and the quality of ecosystem components of recreational areas are factors that adequate the health of urban residents.

Heavy metals (HM) are a frequent environmental pollutant in urban areas. Heavy metals pollution is typical for all functional zones [1]. A wide range of anthropogenic HM emissions into the atmosphere of cities leads to their dispersion over the territory and pollution of soil, water and biota. One of the agents of HM accumulation is fungus [2]. Fungi are able to accumulate HM in significant
amounts, and the content of HM in fruit fungi and mycelium often exceeds the concentration of HM in the environment [3, 4]. The HM content can reach up to 10-20% per unit of dry weight of fungi [3]. The accumulating capacity of macrofungi in relation to potentially toxic HM for humans is one of the problems associated with assessing the quality of edible fungi [5], which are collected by residents in forest parks of uptowns and suburbs and are used as food ingredients.

Biosorption of HM by fungi depends on their species and environmental identity, growing conditions (substrate) and bioavailability of HM. Certain types of fungi can be indicators of anthropogenic burden on urban ecosystems and landscapes [3].

Understanding the features of HM accumulation in fungi allows us to solve such important problems of modern ecology as: the indicators of environmental pollution, development of effective methods of bioremediation of soils, prevention of chronic human diseases.

The aim of the work was to identify the factors of accumulation of heavy metals in fruit bodies of micromycetes of various ecological specialization in the conditions of cities of the Western part of the Central Black Earth region.

2. Objects and methods of research

The study was conducted on the territories of cities in the Western part of the Central Chernozem region: Kursk, Zheleznogorsk, Gubkin. Key sites were mostly located in forest areas and wooded lands, which serve as frequent recreation sites for residents and these areas are often places where mushrooms are collected. All the studied sites had a contrasting anthropogenic burden (industrial, residential and recreational zones) and functioned on soils of different Genesis (Table 1).

**Table 1. Key sites of research of micromycetes in the cities of the western part of the Central Black Earth region**

| №  | City, key site, functional zone             | Ecosystem                                      | Soil                          | Geochemical specialization of soil, content Me/kg |
|----|-------------------------------------------|-----------------------------------------------|-------------------------------|-------------------------------------------------|
| 1  | Gubkin, Sergeevka village, recreational   | Oak grove                                      | Voronic Chernozems            | Pb:22.9 Cd:0.2 Zn:60.3 Cu:18,4 Ni:29.8          |
|    |                                           |                                               | Pachic                        |                                                 |
|    |                                           |                                               | Carbic Podzols                |                                                 |
| 2  | Kursk, Gorelii forest, recreational       | Pine forest                                    | Carbic Podzols                | Pb:5,4 Cd:0.1 Zn:6,1 Cu:1,3 Ni:0.8              |
|    |                                           |                                               |                               |                                                 |
| 3  | Kursk, CHP plant-1, industrial            | City waste land with growing birch-trees and ash-leved maples in industrial zone | Technosols                    | Pb:69.5 Cd:2.8 Zn:47.6 Cu:14,5 Ni:32,9          |
| 4  | Kursk, floodplain of Seim river, residential | Mixed forest in dead arm of the river         | Umbric fluvisols oxyaquic     | Pb:32.7 Cd:1.5 Zn:35.2 Cu:7,4 Ni:20,6           |
| 5  | Kursk, Gutorev forest park, recreational  | Pine forest                                    | Carbic Podzols                | Pb:136,8 Cd:1.3 Zn:20,1 Cu:6,3 Ni:37,5          |
| 6  | Kursk, Gremyachenskaya str., residential  | Suburban settlement                            | Greyic Phaeozems Albic        | Pb:20,3 Cd:0.5 Zn:39.2 Cu:13,5 Ni:0.5           |
| 7  | Kursk, forest park Mokva, recreational     | Pine forest                                    | Carbic Podzols                | Pb:9,4 Cd:0.3 Zn:11,6 Cu:2,2 Ni:3,0             |
| 8  | Kursk, Znamenskaya grove, recreational     | Oak grove                                      | Greyic Phaeozems Albic        | Pb:18,7 Cd:0.3 Zn:48,4 Cu:12,2 Ni:32,4          |
| 9  | Zheleznogorsk, train station Mikhailovskii mine, recreational | Oak grove                                      | Greyic Phaeozems Albic        | Pb:7,7 Cd:0.1 Zn:25,1 Cu:9,0 Ni:12,1            |
The choice of key sites was determined by the basic properties (granulometric composition, humus content) of soil cover, as well as the degree of soil pollution of HM. The key areas are represented by the following soil groups: soils of light granulometric composition with a background content of HM, soils of light granulometric composition with HM pollution, soils of heavy granulometric composition with a background content of HM and soils of heavy granulometric composition with HM pollution (Table 1). In low-humus soils of light granulometric composition, the proportion of mobile forms of HM is usually higher than in loamy soils with medium and high humus content, due to the capacity and contrast of sorption and humus geochemical barriers. HM soil pollution modifies this pattern and changes the degree of bioavailability of metals, thus it can affect the accumulation of HM by fungi [6].

At the key sites indicated in table 1, samples of fruit fungi were taken. Sampling was carried out in September – October 2019. 3 average samples (the raw weight of each average sample was 0.5 kg) – fruit bodies of 15 macromycete species were collected on sample plots which size was 50 m × 50 m.

The total HM content in humus-accumulative and humus-eluvial soil horizons (sampling depth 0-20 cm) of the key sites was determined (n=27).

The coefficient of accumulation of HM by fungi was calculated as the ratio of the HM content in the fruit fungi to HM soil content.

Determination of HM concentrations in fungal samples and the total content of HM in soils was carried out by atomic absorption spectrometry in accordance with normative documents – GOST 30692 and Guidelines for the determination of heavy metals in the soils of agricultural land and crop production in 1992.

Statistical data processing, correlation and variance analysis were performed using Microsoft Office Excel package.

3. The content of heavy metals in fruit bodies of macromycetes of different taxonomic affiliation

The results of the study showed that the vast majority of samples of fruit fungi in the key areas was polluted by HM. The maximum lead content in fungi exceeded the MAC 87 times, and zink – 6.1 times. Excess of MAC of these metals was observed in all key areas in 100% of mushroom samples without exception. The excess of MAC of cadmium (max. – 88 times) was observed in 90% of macromycete samples, and the excess of MAC of copper (max. – 6.8 times) was observed in 85% of the samples taken. The maximum concentration limit for nickel in fungi is not established. However, it is worth noting that the anthropogenic burden does not always lead to accumulation of Ni in fungi [7] (Table 2).

Judging by the value of HM accumulation coefficients, the species specificity of the fungi's accumulating ability was determined. Among the species of fungi under analysis, universal HM accumulators were found – fungi that can simultaneously accumulate a significant amount of HM. Thus, Cantharellus cibarius (L.) Murrill, Agaricus melleus (Vahl), Agaricus equestris L. accumulated 5 – 29 times more Cd, 6 – 9.5 times more Zn, 10.3 – 31.9 times more Cu than the content of these HM in the soils of key sites. Macromycetes capable of simultaneous accumulation of significant amounts of Zn and Cu were revealed. The average coefficient of Zn accumulation in edible fungi Lyophyllum decastes (Fr.) Singer and Lepista nuda (Bull.) Cooke was 2.2, Cu – 5.4. Pleurotus ostreatus (Jacq.) P. Kumm. accumulated Zn (the average value of the accumulation coefficient – 3.1) and Cd (the accumulation coefficient – 10.0).

Selective HM accumulators were also identified. The following species showed the ability to selectively accumulate Cd: Leccinum scabrum (Bull.) Gray, Kuehneromyces mutabilis (Schaeff.) Singer & A.H. Sm., Myxocollaabies velutipes (Curtis) Singer. They accumulated in their fruit bodies from 2.2 to 13.7 times more Cd than it contained in the soil. It is worth noting that this effect was observed regardless of soil pollution. Pluteus cervinus (Schaeff.) P. Kumm is a selective Cu accumulator (Cu accumulation coefficient in fruit bodies – 4.7) and an indicator of Pb content in soil (Pb accumulation coefficient in fruit bodies – 1.0).
Table 2. Concentration of HM in fruit fungi, growing in the ecosystems of cities of western part of Central Black Earth region with different anthropogenic burden

| № key site | Fungi * | *Ecological specialization | Content of HM in fruit fungi, mg/kg dry weight |
|------------|---------|-----------------------------|---------------------------------------------|
|            |         |                             | Pb  | Cd  | Zn      | Cu    | Ni    |
| 1          | Leccinum scabrum (Bull.) Gray | SB   | 1.5±0.5                      | 1.5±0.5 | 32.7±6.8 | 12.8±2.7 | 2.7±0.8 |
| 1          | Kuehneromyces mutabilis (Schaeff.) Singer & A.H. Sm. Cantharellus cibarius (L.) Murrill | X    | 1.6±0.5                      | 1.3±0.4 | 54.5±11.4 | 18.3±3.8 | 2.9±0.9 |
| 2          | Agaricus melleus (Vahl) | SB   | 3.1±1.1                      | 0.8±0.2 | 58.2±12.2 | 20.1±4.2 | 0.5±0.1 |
| 2          | Agaricus equestris L. | X    | 2.9±1.0                      | 2.9±0.9 | 55.0±11.5 | 41.5±8.6 | 0.4±0.1 |
| 3          | Myxocollybia velutipes (Curtis) Singer | SB   | 2.7±0.9                      | 0.5±0.2 | 36.6±7.7  | 13.4±2.8 | 0.5±0.1 |
| 3          | Agaricus fulus Bull. | X    | 24.8±8.6                     | 6.1±2.1 | 57.0±11.9 | 27.4±5.5 | 0.9±0.3 |
| 4          | Pluteus cervinus (Schaeff.) P. Kumm. | X    | 4.8±1.7                      | 2.4±0.8 | 27.3±5.7  | 16.5±3.4 | 0.4±0.1 |
| 4          | Lyophyllum decastes (Fr.) Singer | S    | 3.9±1.3                      | 1.6±0.5 | 71.6±15.0 | 34.9±7.3 | 1.1±0.3 |
| 4          | Lepista nuda (Bull.) Cooke | S    | 1.6±0.5                      | 1.0±0.3 | 86.6±18.2 | 45.7±9.6 | 0.4±0.1 |
| 5          | Agaricus melleus (Vahl) | S    | 43.3±15.1                    | 8.8±3.0 | 78.3±16.4 | 67.6±14.2 | 1.4±0.4 |
| 5          | Agaricus fulus Bull. | SB   | 5.3±1.8                      | 0.7±0.2 | 27.9±5.8  | 9.1±2.1  | 2.2±0.7 |
| 6          | Agaricus melleus (Vahl) | X    | 2.3±0.8                      | 0.1±0.0 | 22.7±4.7  | 8.0±1.8  | 0.2±0.07 |
| 7          | Suillus Gray | SB   | 6.1±2.1                      | 0.4±0.1 | 84.9±17.8 | 45.1±9.4 | 0.4±0.1 |
| 7          | Agaricus melleus (Vahl) | X    | 7.1±2.4                      | 3.0±1.1 | 49.6±10.4 | 31.4±6.6 | 0.7±0.2 |
| 8          | Boletus fomentarius L. Clitocybe nebularis (Batsch) P. Kumm. Pluteus cervinus (Schaeff.) P. Kumm. Hypholoma fasciculare (Huds. P. Kumm) | X    | 5.3±1.8                      | 0.2±0.1 | 47.5±9.9  | 10.4±2.2 | 1.8±0.6 |
| 8          | Pleurotus ostreatus (Jacq.) P. Kumm. | X    | 0.6±0.2                      | 1.0±0.3 | 76.3±16.1 | 11.0±2.3 | 0.2±0.1 |
| 9          | **MAC** |                | 0.5 | 0.1 | 20.0     | 10.0     | -     |

* SB – symbiotrophics, X – xylotrophs, S – saprotrophs
** MAC of HM in fruits and vegetables including fungi [8, 9]
Maximum concentrations of Pb (43.3 ppm), Cd (8.8 ppm), and Ni (67.6 ppm) were found in fruit bodies of *Agaricus melleus* (Vahl) at site № 5, represented by polluted Carbic Podzols. Maximum of Zn (121.2 ppm) and Ni (5.9 ppm) were recorded in *Clitocybe nebularis* (Batsch) P. Kumm. on site № 8, represented by Greyic Phaeozems Albic unpolluted by HM. It is worth noting that these are edible fungi that grow in the forested landscapes of Kursk.

4. Accumulating capacity of fungi of various ecological specialization in relation to heavy metals

The chemical composition of the substrate and the feeding type of fungi affect their accumulating capacity in relation to heavy metals [3]. In general, the macromycetes under analysis accumulated zinc and copper more actively (Fig. 1).

![Figure 1](image-url)

**Figure 1.** Influence of ecological specialization on the concentration of heavy metals in the fruit fungi growing on the territories of cities in the Western part of the Central Black Earth region

Active accumulation of Zn and Cu can be explained by the fact that these HM are essential trace elements that participate in the most important metabolic processes and are part of many enzymes [10]. Also, the fungi accumulated quite a lot of Pb (3.91–9.85 ppm). Intensive accumulation of this xenobiotic is due to its high content in the soils of key areas with increased level of anthropogenic burden (in comparison with the background ecosystems of Kursk). To a lesser extent, the fungi under analysis accumulated Cd and Ni. The average content of Cd in the fruit fungi varied from 1.01 to 2.42 ppm, the content of Ni – from 1.11 to 1.92 ppm, which is due to their quantitative content in the substrates.

The results of the research showed that the content of HM in the fruit bodies of macromycetes in the Western part of the Central Black Earth region strongly depended on their ecological specialization (Fig. 1). It was noted that the highest concentrations of Zn and Ni in the fruit fungi were characteristic of soil and litter saprophyles and were 75.04 and 1.92 ppm respectively. Symbiotrophic fungi accumulated significantly less Pb (2.4 and 2.5 times) and Cd (2.3 and 2.5 times) than xylotrophic and saprotrophic fungi. The Cu content in the fruit bodies of symbiotrophs was significantly lower (1.6 times) than in the fruit bodies of saprotrophic fungi.
5. Factors of accumulation of heavy metals in fruit bodies of macromycetes.  

Results of correlation and variance analysis

The results of correlation analysis of the content of HM in soils and the content of HM in fungi showed that a correlation was evident for Pb ($r=0.56$), Cd ($r=0.36$) and Ni ($r=0.44$, with a critical value of $r = 0.32$ at the level of $P_{0.05}$).

Bioavailability of the element in the soil (the ability of HM to move in the soil solution in sandy and loamy soils) and anthropogenic geochemical specialization of the soils (the content of HM in the soil) can indirectly influence the the accumulation of HM in the fruit bodies of xylotrophic. These factors also determine the transportation of HM in woody forms of plants [6]. Two-factor analysis of variance (factorial ANOVA) showed the influence of the studied factors on HM accumulation in the fruit bodies of xylotrophic fungi. The accumulation of Cd significantly depended on these two factors ($R^2 = 0.82$, $P < 0.05$). Differences in the average values of Cd accumulation in fruit bodies of xylotrophs can be explained both by anthropogenic geochemical specialization of soils and by the bioavailability of this metal.

The factor of anthropogenic geochemical specialization of soils had a clear dominant effect and determined 69.9% of the total dispersion, while the bioavailability factor of the element in the soil was only 29.9%. The accumulation of Cu in the fruit fungi in the key areas was determined mainly by the bioavailability factor of this metal. The effect of the Cu bioavailability factor was 76.3% of the total dispersion ($R^2 = 0.62$, $P < 0.05$). Analysis of the accumulating capacity of fungi in relation to Ni showed that 61.7% of the total sample variation of Ni in the fruit bodies of xylotrophs is due to the influence of geochemical specialization of the soil ($R^2 = 0.75$, $P < 0.05$), thus the quantitative content of Ni in the soil determines the accumulation.

A correlation analysis was performed to identify patterns in the relationship of HM accumulation by fungi (Table 3).

Table 3. Correlations of heavy metals in fruit bodies of macromycetes of urban ecosystems in Central Black Earth region

| Element | Pb | Cd | Zn | Cu | Ni |
|---------|----|----|----|----|----|
| Pb | 1.0 | | | | |
| Cd | 0.64 | 1.0 | | | |
| Zn | 0.15 | 0.36 | 1.0 | | |
| Ge | 0.60 | 0.56 | 0.60 | 1.0 | |
| Ni | -0.05 | 0.24 | 0.38 | -0.09 | 1.0 |

Functional relationships between HM accumulated in fungi can be expressed in terms of correlation pleiades [11]. While carrying out the trace element analysis of fruit bodies of macromycetes using correlation analysis, a pleiad was identified that combines the following HM: Pb, Cd, Cu, Zn. The established relationship between HM accumulated in fungi was of high and medium level with varying correlation coefficients of 0.56 – 0.64 with a critical value of $r = 0.32$ at the level of $P_{0.05}$ (Fig. 2).

Figure 2. Correlation pleiades of heavy metals in fruit bodies of macromycetes of urban ecosystems of the Central Black Earth region

6
The closest correlation was observed between cadmium and Pb. These metals are xenobiotics and their active accumulation by biota takes place in case the area is polluted by both Pb and Cd [7]. The correlation of Nickel with other heavy metals is minimal, which is mostly due to the peculiarities of its participation in macromycete metabolism and changes in the bioavailability of Ni affected by its competitive interrelation with other HM.

6. Conclusion
The study found that 85-100 % of samples of fruit fungi taken in urban ecotopes of Kursk exceeded the MPC of HM from 6.1 to 88 times, that surely indicates a high anthropogenic burden in the cities of the Western part of the Central Chernozem region. Among the fungi, universal and selective HM accumulators were identified. Accumulation of HM by macromycetes depends on a number of factors: taxonomic affiliation, ecological specialization, soil pollution level, soil and biotic factors that determine the bioavailability of HM. The highest HM content was observed in soil and litter saprophytes. For heavy metals of technogenic origin (Pb, Cd), the geochemical specialization of soils or the level of pollution plays the determining role in their accumulation in the fruit bodies of xylotrophic fungi, and for essential elements (Zn, Cu) – their bioavailability.

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