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The Content of Heavy Metals, Radionuclides and Nitrates in the Fruiting Bodies of Oyster Mushroom Distributed within the Urban Ecosystem

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Abstract. The content of heavy metals, radionuclides, nitrates in edible mushrooms is a topical issue for many researchers in the world. However, such issues on edible mushrooms, in particular, *Pleurotus ostreatus*, which are found naturally within a large city, require more detailed studies because these mushrooms are used in human nutrition. It was found that in some areas, the cadmium content in the studied oyster mushroom specimens is three times higher than the maximum permissible concentration (0.092-0.095 mg / kg). The lead content in the fruiting bodies of oyster mushroom is in the range of maximum permissible concentration (MPC) and is 0.19-0.22 mg / kg. The content of mobile forms of copper exceeded the MPC only in two cases - 5.72 mg / kg and 5.06 mg / kg. The zinc content was 8.87-13.41 mg / kg with MPC being 10 mg / kg. The content of ^{137}Cs was in the range of 0.095-0.1 Bq / kg and that of ^{90}Sr was 0.3-0.4 Bq / kg, which does not exceed the MPC. The content of nitrates in the fruiting bodies of oyster mushrooms did not exceed the maximum permissible concentration of 50 mg / kg. Considering the data obtained on the content of heavy metals, radionuclides and nitrates in *Pleurotus ostreatus*, it should be noted that it is risky to eat such fruiting bodies that have developed in the urban-ecological environment.

Key words: oyster mushroom, heavy metals, radionuclides, nitrates, urboecosystem, city.

Introduction

The oyster mushroom is involved in the destruction of dead wood which is the main habitat of living organisms. (Fontes et al., 2013; Nnorom et al., 2012; Silva et al., 2012). The decomposition of wood creates particularly favorable conditions for the development of ground cover and the restoration of tree species. It is also essential for carbon accumulation and soil protection from erosion (Finley, 2006; Favero et al., 1990).

We studied the content of heavy metals, radionuclides and nitrates in the fruiting bodies of oyster mushroom which develops within the urban ecosystem of the city of Lviv. Lviv (a city with almost one million people located in the west of Ukraine) is the center of a large urban agglomeration. Increasing air pollution and xerophilization of the urban environment is a negative factor for the entire biota of the city's complex green zone. The diversity and species richness of the vegetation and macromycetes of the complex green zone of Lviv is associated with its geographical location, the history of the formation of the terrain relief and climatic changes in previous geological periods (Piskur et al., 2011; Troyan, 1989). The remains of ancient pre-glacial vegetation have almost completely disappeared under the influence of climate cooling in the glacial period, and in its place appeared the northern and northeastern vegetation. The following species were introduced into this region: from the east - steppe species, from the mountains of Central Europe - mountainous, from the west - lowland Atlantic species.

Modern flora of the green zone in Lviv is heterogeneous in composition. It is characterized by the following species (Bellettini et al., 2017; Kycheryavyi, 1999; Szafer 1914): boreal, or taiga species (Norway spruce, Scots pine, spindle tree, wintergreen, etc.), Central European, or of zones of broadleaved forests (European beech, common oak and sessile oak, silver fir, common hornbeam, maple sycamore, perennial liverleaf, etc.), mountain species which include: European mountain species, and among them the Carpathian species, including endemic species (violet (*Viola declinata*)), pontine, or steppe species (*Striated fescue, Feather grass,* etc.).

According to the data of Baysal et al. (2003) and Yang et al. (2013), the oyster mushroom is most common in fresh hazel, fresh hornbeam-oak, and in fresh hornbeam-beech forests, it is very rarely found in pine forest types. It should be noted that in forest conditions, oyster mushroom becomes food for various pests (Munoz et al., 2006), and intensive cultivation of edible mushrooms can often be disturbed by certain bacterial, mold and viral diseases, which often causes significant production losses. These infections are facilitated by special conditions under which mushroom cultivation is usually carried out, such as high temperatures, humidity, carbon dioxide levels (CO_2) and the presence of pests (Kycheryavyi et al., 2018).

Note that the natural forest stands in the territory of the city of Lviv were replaced by green plantings of parks, gardens, miniparks, boulevards, and street alleys (Fernandes et al., 2015; Fang et al., 2014), which affects the development of the *Pleurotus ostreatus* populations. Most urban parks are man-made parks. However, there are those that were transformed into parks from forests - Zalizna Voda, Pogulyanka. The forests of Bryukhovychy and Zavadiv, located on the elevated ridges of Roztochya, are being turned into forest parks (Bysko et al., 1982; Bazyuk-Dubey, 2012; Brunets, 2013).

Many well-known scientists conducted their studies to determine the peculiarities of heavy metals entry into plant products and identify the most pollution-resistant agricultural plants. The scientists (Mils & Parker, 1980) studied the effect of high levels of cadmium in soils on various plant species, adding it to the soil in various quantities. The scientist (Garmash, 1982) studied the intake of Pb and Cd from the soil by vegetable crops; for this, these substances were added to the soil in various quantities. The capacity of various wheat varieties to absorb heavy metals was studied. In so doing, wastewater sludge was introduced into the soil (Alekseev, 1987).

The knowledge of the accumulation of heavy metals in mushrooms is limited, as well as the knowledge of their bioavailability in humans. Selenium, arsenic and antimony are not found in high concentrations. Cultivated species, especially *Agaricus bisporus* and *Pleurotus ostreatus*, contain only low levels of trace elements. Very scarce is information on metal losses during the preservation and cooking of mushrooms (Alananbeh et al., 2014).

The distribution of arsenic, cadmium, lead, mercury and selenium was investigated in 1,194 samples of 60 species of common edible mushrooms, collected mainly in the province of Reggio Emilia, Italy. The average amount of lead present in all samples was generally below the maximum permissible concentration. The high Hg content was in the range of 5-10 mg/kg of dry weight. The mushrooms were rich in selenium. The accumulation of heavy metals may be specific to species and, thus, take a taxonomic role, but in these studies it has proved to be unreliable as an ecological index (Demirbas, 2001).

The scientists in (Pavlik, 2005; Pavlik & Pavlik, 2013) present the results of studies on lead content in 238 samples of 28 species of edible mushrooms collected from different sites in the province of Lugo (Spain) during 2005 and 2006. The highest average lead content varied in the range of 2.2–4.1 mg / kg (in the oyster mushroom usually less than 1 mg / kg, which coincides with our data). No statistically significant differences were found between lead levels in hymenophore and in fruiting bodies. The authors proved that the consumption of the mushrooms under study cannot be regarded as a toxicological risk in terms of lead content, and they provide necessary nutritional profile.

Eighteen different types of forest mushrooms (*Agaricus bisporus, Agaricus silvicola, Amanita muscaria, Amanita rubescens, Amanita vaginata, Boletus sp., Hydnum repandum, Hypholoma fasciculare, Laccaria lacceta, Lactarius piperatus, Lactarius sp., Lactarius volemus, Pleurotus ostreatus, Russula cyanoxantha, Russula sp., Russula delica, Russula foetens, Tricholoma terreum*) were analyzed for heavy metal content (Pb, Cd, Hg, Cu, Mn, Zn, Fe, Co, As, Ca, Na, K, Mg, Ba, Ni, Ti, Cr, Al, Bi, Sb, Ag) (Kalac & Svoboda, 2000). The heavy metal content for oyster mushrooms was high and amounted to (mg / kg): Pb – 3.24, Cd – 1.18, Hg – 0.42, Cu – 13.6, Mn – 6.27, Fe – 86.1, Zn – 29.8, which exceeds the figures obtained by the researchers of this work.

The studies (Kalac, 2009) determined the content of heavy metals in 10 species of mushroom. Heavy metal content levels are significantly lower in species such as *P. squamasus, P.ostreatus, B. badius, M. esculenta*, and *M. vulgaris* than in other mushrooms. The levels of Cd, Pb, Zn, and Mn comply with FAO / WHO standards (1976).

It was found that the accumulation level of Hg in *P. ostreatus* mushrooms was low and was different in various sites. *P. ostreatus* can be described as a weak Hg collector. Also, such a conclusion was made by the scientists in the work**.** However, if the oyster mushroom is cultivated on substrates polluted with industrial wastewater, this can lead to risks to human health. It is established that high concentrations of Hg greatly reduce the growth of mycelium. The range of the accumulation coefficient was 65-140, that is, very pronounced (Bellettini et al., 2019).

The concentration of cadmium in the fruiting bodies of oyster mushrooms is related to the level of cadmium in the substrate. This metal is present at higher levels in the cap of the mushroom (22-56 mg / kg of dry weight) than in the stipes (13-36 mg / kg of dry weight). The concentration factor (CF) is very low on the control (about 2). The work suggests the presence of a cadmium control mechanism in these types of mushroom. However, the level of cadmium in the fruiting body may pose a risk to consumers of *P. ostreatus* in accordance with the limitations of FAO / WHO (1972) (Favero et al., 1990).

The study investigated the content of heavy metals such as Pb, Cd, As, Hg in rice and edible mushrooms in China to assess the level of pollution and edible safety. Ninety-two rice samples were collected from the main rice cultivation regions in China, and 38 fresh and 21 dried mushroom samples (including oyster mushrooms) were collected at typical markets in Nanjing. It was found that the content of Pb, Cd and As in 4.3%, 3.3% and 2.2% of rice samples, respectively, was higher than the maximum permissible concentration. In fresh edible mushrooms, the content of Pb and Hg in 2.6% of samples was higher than MPC.

However, only the Hg content in 4.8% of dry edible mushroom samples was higher than its MPC. It was concluded that more than 95% of the rice and edible mushroom samples in our testing had high food safety.

The content of phenols, antioxidant activity and the content of metals in *P. ostreatus* grown on coffee chaff enriched with iron (Fe), zinc (Zn) and lithium (Li) were studied. The content of phenolic compounds was different in mushrooms enriched with Fe, Zn or Li. Mineral enrichment contributed to lowering Fe content in three samples. Changes in the content of Zn in the samples did not occur. Li accumulation was observed in a sample enriched with LiCl and in *P. ostreatus* enriched with Fe, Zn, Li. Heavy metals such as Ni, Cr and Cd were not detected, and only low levels of Pb and Al were observed. The study (Fang Y et al., 2014) was the first report to demonstrate an understanding of how the mineral supplements reduce the antioxidant activity in *P. ostreatus* enriched with iron, zinc or lithium.

When growing oyster mushroom on the waste of palm leaves (25%) and the waste of agricultural products (75%), it was found that the content of K was the highest, followed by Na, Mg, Ca, and Zn. This is the first study that reported the success of growing mushrooms on palm leaf waste mixed with other agro-waste (Garcia et al., 2009)**.** The studies of (Gregori et al., 2007), were devoted to the intensive cultivation of oyster mushroom on waste paper, supplemented with peat, chicken droppings and rice husk. It was found that an increase in the rice husk content in the substrate contributed to the acceleration of cultivation, the formation of the cap and fruiting body of mushrooms, which led to an increase in yield, while an increase in peat and chicken manure had a negative effect on the growth. In general, the studies show that the chemical composition of oyster mushrooms grown on paper-derived waste was very satisfactory. The results obtained confirmed the nutritional characteristics of the samples, placing emphasis on effective means for recycling paper.

The mushrooms (*Pleurotus ostreatus*) were cultivated (Černy, 1989) on a basal substrate of rice straw, a basal substrate of wheat straw, a basal substrate of cotton shell, a substrate of mixture of wheat straw and rice straw with different proportions (15%, 30% and 45% in rice straw, 20%, 30% and 40% in wheat straw) to find a cost-effective substrate. It was found that the addition of cotton seeds in rice straw and wheat straw enhanced the launch of mycelium, the initial development and formation of the fruiting body. Compared to the sterilized substrate, the unsterilized substrate had a comparatively higher mycelium growth rate, a shorter period of general colonization and days from the opening of the bag to the formation of primodia. However, the unsterilized substrate did not give a significantly higher yield of mushrooms and biological efficiency than the sterilized substrate; some undesirable characteristics were observed, that is, a smaller diameter of the mushroom cap and a relatively long stipe (Pasternak, 2010; Popovych & Les, 2014).

At the same time, the authors (Huang et al., 2010) show that the fruiting bodies of oyster mushroom, grown on the substrate with bamboo sawdust, contain more free amino acids than those from the control group with coniferous sawdust. It is concluded that bamboo sawdust can be used as the main material for the cultivation of mushrooms. In the future, bamboo sawdust can be used in Malaysia, Thailand and Indonesia with their subtropical climate in order to expand the cultivation of oyster mushrooms.

The cultivation of mushrooms (Gregori et al., 2007)**,** enriched with Se in the substrate of the coffee chaff, was effective, showing increased biological efficiency and Se absorption. Even the lowest concentration of Se, added to the coffee chaff, 3.2 mg / kg, led to the fact that the *P. ostreatus* mushrooms, which contain a sufficient amount of Se, provided the recommended daily intake of Se for adults. These results demonstrate the great potential of coffee chaff in the production of Se-enriched mushrooms and demonstrate the ability of this mushroom to absorb and biomagnetize Se. Selenium (Se) is important for human diet, and it is in a low concentration in the soil, and hence in food. The introduction of Se into fungal proteins shows great potential for improving the nutritional value of the mushroom. Selenium has several physiological functions in protein activity, it increases the function of the immune system, reduces the risk of developing cancer (Isildak et al., 2004).

Mycorhization is the subject of research which found that *Pleurotus ostreatus* can be used as a promising option for the removal of heavy metals from wastewater of the coal industry. The effectiveness of *Pleurotus ostreatus* for absorbing heavy metals was the highest in 50% dilution of wastewater (57.2% Mn, 82.6% Zn, 98.0% Ni, 99.9% Cu, 99.3% Co, 99.1 % Cr, 89.2% Fe and 35.6% Pb) and 25% dilution of effluent (33.0% Mn, 55.1% Zn, 97.8% Ni, 99.7% Cu, 97% Co, 84 , 4% Cr, 87.1% Fe and 73.4% Pb), and raw materials (23.3% Mn, 73.1%)% Zn, 78.7% Ni, 87.5% Cu, 59.3% Co, 64.6% Cr, 34.6% Fe, and 11.3% Pb (Melgar et al., 2009).

Thus, the study of heavy metals, radionuclides, nitrates in edible mushrooms is a topical issue for many researchers in the world. However, the studies on the content of heavy metals in edible mushrooms, in particular, *Pleurotus ostreatus*, which develop naturally within a large city, require detailed investigation since they are used in human nutrition.

Materials and Methods

In the area of green plantings in Lviv, landscape gardening objects (street plantings, miniparks, boulevards, parks) with the presence of trees of different species composition were selected. The distribution and occurrence of the oyster mushroom species was studied using the transect route method (Dudka & Vasser, 1987). The studies were conducted in the spring (April-May) and autumn (October-November) 2014-2018. The fruiting bodies of the oyster mushroom were found in 7 urban environments (Fig. 1). The abundance and growth patterns of oyster mushrooms in urban areas of the city of Lviv are given in Table 1.

The content of heavy metals in the fruiting bodies of mushrooms was determined by the method (Kalac, 2013) in the hydrochloric acid extract (Balyk, 2005). Preparation of mushrooms for laboratory tests included drying of the raw material, grinding and dry ashing in a muffle furnace (t = + 450- + 550 ° C), dissolving the ash in a 10% HCl solution, followed by identification of mobile forms of heavy metals on an atomic absorption spectrophotometer (Baliuk et al., 1999). The chemical analyses of the mushrooms were carried out in a certified laboratory of the Volyn branch of the state institution *Institute of Soil Conservation in Ukraine* (Lutsk, Volyn Region).

The radionuclides were determined by a scintillation method using the SEG-001 "AKP-S" gamma-ray energy spectrometer and the SEB-01 beta-radiation with digital data calculation software (the average value of the relative measurement error at a confidence level of 95% does not exceed 25%).

The content of nitrates in the fruiting bodies of the mushrooms was determined using the eco-tester of the environment "Soeks".

Table 1. Locations of *Pleurotus ostreatus* L. occurrence within the urban environment of Lviv City.

Sample No.	The name of the street in the city of Lviv where the natural growth of oyster mushroom was found	Abundance (according to O.Drude)	Growth pattern (according to Brown Blanke)
1	Valova Str. (minipark)	sparsae	2
2	29b, Golovaty Str.	sparsae	2
3	180, Shevchenko Str.	copiosae 3	4
4	109, Zelena Str.	solitariae	1
5	Snopkivsky park	sparsae	2
6	Chervona Kalyna ave.	copiosae 3	4
7	Shevchenkivsky grove park	copiosae 3	4


Valova Str. (minipark)


29 b, Golovaty Str.


180, Shevchenko Str.


109, Zelena Str.


Snopkivsky park


5, Chervona Kalyna ave.


Shevchenkivsky grove park

Fig. 1. Identified places of oyster mushroom growth in the city of Lviv. Photos: Mikhail Les. 2014-2018.

Results and Discussion

The content of heavy metals — cadmium, lead, copper, and zinc — in oyster mushroom fruiting bodies was investigated. All the mushroom individuals developed on trees within the city of Lviv, and along the motor roads - samples 2, 3, 4, 6; in gardens and parks - samples 1, 5, 7. It is found that in some areas, the cadmium content in the test specimens of oyster mushroom is three times higher than the maximum permissible concentrations and amounts to 0.03 mg / kg. It is stated that within the city limits, the excess of this heavy metal in the examined oyster mushroom specimens is uneven and is obviously dependent on the contamination of the soil environment and donor wood. Figure 2 shows data on the accumulation of cadmium by oyster mushroom individuals within the city.

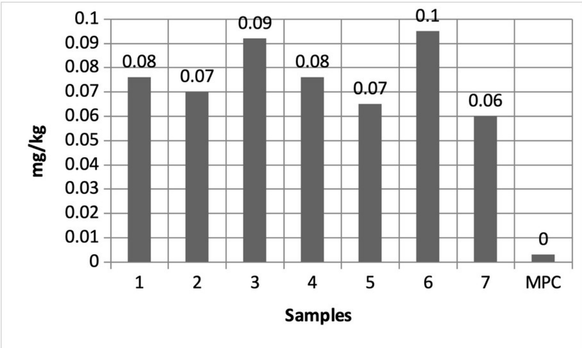


Fig. 2. Cadmium content in the fruiting bodies of oyster mushroom.

The lead content in the fruiting bodies of oyster mushroom is within the maximum permissible concentration (MPC) and is 0.19-0.22 mg / kg. The content of mobile forms of copper exceeded the MPC in only two cases and amounted to 5.72 mg / kg and 5.06 mg / kg. A very different and disappointing situation was with zinc. The studies have shown that the content of this metal in the samples was 8.87-13.41 mg / kg with MPC of 10 mg / kg (Fig.3).

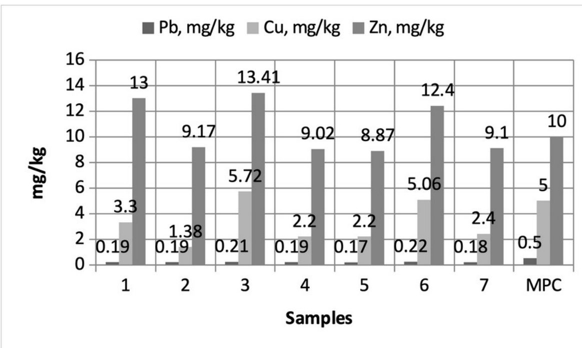


Fig. 3. The content of lead, copper and zinc in the fruiting bodies of oyster mushroom.

In general, as shown by the studies (Pylypets, 2000, pers. comm, Ukraine) on the migration of heavy metals in the soils of Lviv, our data (Cd > Pb > Cu > Zn) basically confirm the author's data (Cd > Pb > Cu > Zn).

The data obtained indicate that the components of forest and other terrestrial ecosystems, which absorb impurities from the atmosphere and accumulate, transport and transfer them, are "sinks" of heavy metals. As is known, the main absorbers of impurities entering park and forest-park biogeocoenoses are soil and plants. Regarding the pollutants such as persistent particles of heavy metals, their storage functions in plants and soil are closely related, since some of the heavy metals such as Cd, Pb, Cu, Zn studied by us, enter the soil through vegetation which supplies litter to the forest floor and litter to park-and-garden plantings.

Scientists estimate that excessive accumulation of Cd, Pb, Cu, and Zn in the organogenic horizon of forest ecosystems slows down the rate of decomposition processes due to the binding of the heavy metals by colloids of organic substances, ions of heavy metals. In their opinion, these processes can have a direct toxic effect on microorganisms-decomposers, or on the enzymes they produce.

In each case, the accumulation of heavy metals is a serious problem from the standpoint of biochemical cycles, and exceeding the MPC, which is found in the samples we studied, is undesirable and harmful from the sanitary and hygienic point of view.

The studies on the radionuclide content in the fruiting bodies of oyster mushroom showed that the contents of ^{137}Cs and ^{90}Sr do not exceed the maximum permissible concentration (GN 6.6.1.1-130-2006). The same conclusions were reached by the researcher in (Cocchi et al., 2006). He found that wild-growing mushrooms accumulated ^{134}Cs and ^{137}Cs, but this did not pose a threat to the human body. The cultivated mushrooms had low radioactivity content caused by natural isotope ^{40}K. The results of our studies are shown in (Fig. 4).

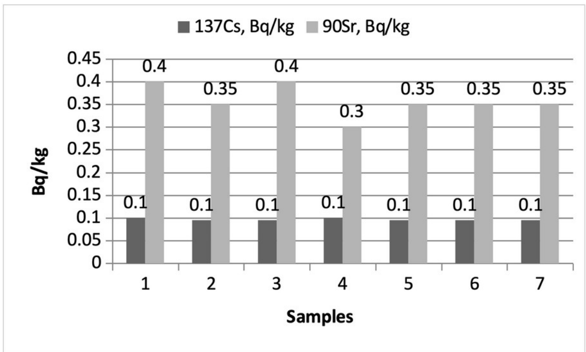


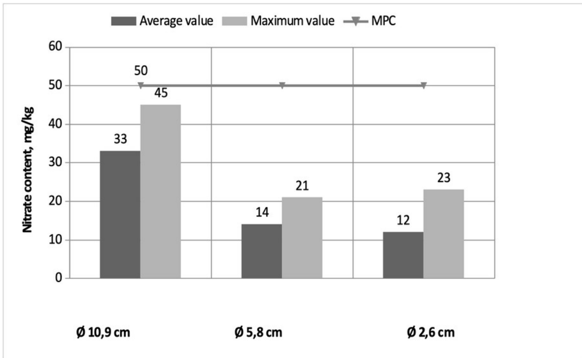
Fig. 4. The radionuclide content in the fruiting bodies of oyster mushrooms.

It should be noted that in plant products, the maximum permissible concentration for ^{137}Cs is 500 Bq / kg, and for ^{90}Sr - 50 Bq / kg.

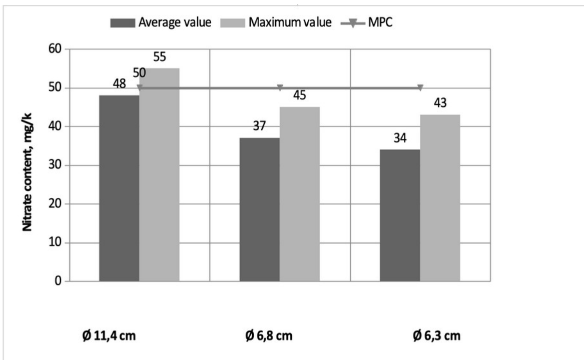
In the process of the nitrogen cycle, a gradual decomposition of organic compounds occurs, as a result of which nitrogen passes into the nitrate form. The nitrates formed in the soil are quickly assimilated by tree roots and are accumulated by both xylophytes and saprophytes (Bressa et al., 1988). Thus, natural nitrates are formed which are included in the subsequent cycle of nitrogen (Vaseem et al., 2017). However, owing to the growing burning of fuels (factories, internal combustion engines), nitrogen oxides are formed in the atmosphere, and they enter the soil with rain in large quantities and are converted there with the help of nitrogen-fixing bacteria into nitrates. Excessive concentration of nitrates in plant products is harmful to people, especially in childhood (Kycheryavyi, 2010). Therefore, when collecting mushrooms and passing them to the consumer, we must ensure that the maximum permissible concentrations are not exceeded.

Our observations, conducted in urban parks, have found that in the same urban environment, as the oyster mushroom, the honey mushroom (*Armillariella mellea*) develops. These species fructify at the same period of time. Therefore, it was decided to collect samples of the fruiting bodies of individuals of both species and measure the content of nitrates in them (Kycheryavyi et al., 2016).

It was found that high levels of nitrate content are characteristic of those fruiting bodies of *Pleurotus ostreatus* and *Armillariella mellea* specimens which have the largest cap diameters. In oyster mushroom with different cap diameters, the nitrate content did not exceed the maximum concentration which is 50 mg / kg (fig. 5a). As can be seen from (fig. 5b), in all three variants of the experiments (the size of the caps was different), the average and maximum rates of nitrate accumulation were higher in honey mushrooms. At the same time, the honey mushroom sample with the largest cap diameter (11.4 cm) was found to exceed the maximum permissible concentration by 5 mg / kg.



a)



b)

Fig. 5. The nitrate content in the fruiting bodies of the oyster mushroom *Pleurotus ostreatus* (a) and the honey mushroom *Armillariella mellea* (b).

The obtained results are in favor of the oyster mushroom which assimilates less nitrates and is safer for the consumer.

Conclusions

As a result of studying the content of heavy metals in the fruiting bodies of oyster mushroom, which develops within the urban ecosystem of Lviv, it has been found: in some areas, the content of cadmium in the investigated individuals is three times higher than the maximum permissible concentration and amounted to 0.092-0.095 mg/kg; the lead content in the fruiting bodies is within the maximum permissible concentration and is 0.19-0.22 mg/kg; the copper content exceeds the MPC in two cases and amounted to 5.72 mg/kg and 5.06 mg/kg.

The studies on the radionuclide content in oyster mushroom fruiting bodies have established that the contents of ^{137}Cs and ^{90}Sr do not exceed the maximum permissible concentrations. The ^{137}Cs content was in the range of 0.095-0.1 Bq/kg, and 90Sr - 0.3-0.4 Bq/kg.

The highest nitrate levels are found in those fruiting bodies of *Pleurotus ostreatus* and *Armillariella mellea* which have the largest cap diameters. In oyster mushroom with different cap diameters, the nitrate content did not exceed the maximum concentration which is 50 mg/ kg.

Taking into account the data obtained on the contents of heavy metals, raiodnuclides and nitrates in *Pleurotus ostreatus*, it should be noted that to eat such fruiting bodies that have developed in the urban-ecological environment of Lviv is risky.

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