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THE INFLUENCE OF SELENIUM ON SELECTED HEAVY METALS CUMULATION IN OYSTER MUSHROOM FRUITING BODIES

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ABSTRACT

Food safety is a very frequent topic. The article deals with the problems of fortification of the most grown mushroom in Slovakia, and the 3rd most grown mushroom in the world, *Pleurotus ostreatus*. Due to the high environmental pollution of soils and air, there is a risk of the production of dangerous fruiting bodies with high heavy metals content. It is known that these substances can promote serious health effects on human body, such as bone weakness or kidney damages (cadmium) and negative process of cognitive developing (lead). The experiment was focused on biofortification with selenium to reduce the accumulation of selected heavy metals (lead, cadmium) in oyster mushroom, grown with intensive cultivation under artificial conditions. This work confirms that the application of sodium selenate to the growing substrate with straw as the main component can reduce the accumulation of cadmium (by 22.45%) and lead (by 64.81%). Research by various authors reported the ability of the oyster mushroom to embed selenium from the substrate into the fruiting bodies. Based on the results of the experiments, we propose to fortify the growing substrate for the production of oyster mushroom by selenium. This way we produce a food with a high antioxidant potential.

Keywords: oyster mushroom; *Pleurotus ostreatus*; heavy metals; food safety; mushrooms; lead; cadmium

INTRODUCTION

Oyster mushroom is popular especially because of its delicious taste. Its composition is also very important. Oyster mushroom contains high amounts of proteins and carbohydrates, minerals such as calcium, phosphorus, iron and other, vitamins like thiamine, riboflavin and niacin as well as low fat **(Sturion and Oetterer, 1995; Justo et al., 1998; Manzi et al., 1999)**.

According to **Silveira et al. (2006)** energy value of *P. ostreatus* is between 139.36 and 213.05 kcal.100 g^{-1} fresh mushrooms. Hyphae are composed of cell wall components such as chitin, other hemicelluloses and β-glucans, which often play a key role in the pharmacological use of mushrooms. For example, in enhancing macrophage function, resisting many bacterial, viral, fungal and parasitic infections, activating non-specific immune stimulation, lowering blood cholesterol levels and blood glucose levels **(Cheung, 2009)**. Although the mushrooms are not plants, they are often included between vegetables in terms of dietary properties. Most species of the genus *Pleurotus* are known for their healing potential.

Selenium (Se) is an essential element with antioxidant effects. It is an important component of several major metabolic reactions, including synthesis of thyroid hormones, antioxidant defence systems and immune functions **(Köhrle and Gärtner, 2009; Gandhi, Nagaraja and Prabhu, 2013)**. The essentiality of selenium was demonstrated in 1957 **(Hegedűs et al., 2006; Hegedűs, Hegedűsová and Šimková, 2007)**. In 1976, the necessity of selenium for humans was proven, despite the fact that previously were highlighted his negative effects **(Hegedűs et al., 2008; Jakabová et al., 2008; Jakabová et al., 2009)**.

It is therefore clear that mushroom enriched with inorganic forms of selenium can produce functional foods with positive effects on human health, anti-inflammatory and anti-tumour effects **(Clark et al., 1996)**.

Cultivation of saprophytic fungi on substrates rich in selenium may be an effective means of producing food with built-in selenium. The content of selenium in mushrooms is generally higher than most vegetables **(Rayman, Infante and Sargent, 2008)**, but this indication is very variable. In contrast with biologically available selenium concentrations in the soil and the related content of selenium in wild mushrooms, in cultivated mushrooms is the content of the embedded selenium variable depending on the species and their maturity. Its content is also dependent on the type and quality of the substrate **(Kalac, 2009)**. The concentration of selenium in the fruiting bodies of the favourite edible mushroom is in the range from <1 – 20 μg $Se.g^{-1}$ dry weight of the fruiting bodies.

The concentration of selenium in the fruiting bodies of mushroom *P. djamor* produced on high selenium substrates

compared to the control substrate without selenium contamination exceeds 800 times the amount, i.e. 141 μg $Se.g^{-1}$ weight of dried mushroom. It has been confirmed that as well as other species of genus *Pleurotus* are able to cumulate selenium **(Wang et al., 2005; Estrada et al., 2009; Da Silva et al., 2012)**.

Scientific hypothesis

Application of sodium selenate into the substrate will reduce the lead and cadmium content in oyster mushroom fruiting bodies.

MATERIAL AND METHODOLOGY

Establishment of experiments to produce oyster mushroom fruiting bodies

In the experiment was used the production strain of oyster mushroom *(Pleurotus ostreatus)* KRYOS B. Mushroom production was based on controlled cultivation conditions, in the premises of The AgroBioTech Research Centre, in two cultivation periods. The production of mushrooms proceeded in the following phases:

- inoculum preparation,
- preparation of the substrate,
- inoculation by inoculum and incubation at 25 °C about 2 weeks,
- initiation of the fruiting bodies at 11 °C for one day,
- fructification (12 hours, 16 °C in dark and 12 hours, 16 °C under light).

The experiment consisted of 4 variants, each variant had 10 repetitions. The whole experiment was carried out in two cultivation periods in the following terms:

- First cultivation period (1. CP) – from 04. April 2016 to 26. May 2016.
- Second cultivation period (2. CP) – from 09. June 2016 to 27. July 2016.

Created variants of experiments:

Preparation of all substrates consisted of wetting of dry straw pellets in water in a weight ratio of 1:2.6.

C – control – without selenium;

X – 0.5 $mg.dm^{-3}$ Se – with addition of 0.5 $mg.dm^{-3}$ Se in the form of sodium selenate aqueous solution;

Y – 1.0 $mg.dm^{-3}$ Se – with addition of 1.0 $mg.dm^{-3}$ Se in the form of sodium selenate aqueous solution;

Z – 2.0 $mg.dm^{-3}$ Se – with addition of 2.0 $mg.dm^{-3}$ Se in the form of sodium selenate aqueous solution.

After the end of the incubation, the containers were evenly bleached, overgrowth with mushroom mycelium, and no visible signs of contamination. In the next step the cultivation containers were covered with transparent plastic layers with a 3 cm hole in the middle of the lid. The microthene foil was cut in the shape of a cross in the place of hole. The initiation of fruiting bodies production was carried out by repeated substrate cooling in two cycles.

The fruiting following after the phase of fruiting bodies germ initiation was similar in 2 cycles from 12 May (1. CP) and from 17 July (2. CP).

Collection and processing of oyster mushroom fruiting bodies

Oyster mushroom fruiting bodies were harvested in the optimal growth phase. Fruiting bodies were lyophilized on a LYOVAC GT2 (Germany) for 24 – 30 hours. The lyophilizate was then homogenized by grinding to a fine powder and further used to determine the selected qualitative parameters.

Analytical methods for determining selected qualitative factors

Quantitative determination of the content of selected risk metals except mercury was carried out in the mineralized samples by AAS (AAS Varian AA Spectr DUO 240 FS/240Z/UltrAA) flame technique. The results were evaluated by the calibration curve method.

Ready-made CertiPur stock calibration solutions (Merck, Germany) was used with well-known concentrations of the heavy metals to be monitored. The final values of the measured parameters were subsequently obtained by software translating the calibration curve with the absorbance of the monitored analyte in the sample.

Statistic analysis

The statistical evaluation of the results was carried out by standard methods using statistical software Statgraphics Centurion XVII (StatPoint, USA) – multifactor analysis of variance (MANOVA), LSD test.

RESULTS AND DISCUSSION

It is generally known that different species of mushrooms tend to accumulate different substances from the substrate and from the environment, with no exception for risk metals. We evaluated the content of selected risk metals (lead and cadmium) during our experiment. For each cultivation period 40 samples of lyophilized fruiting bodies grown on selenium fortified substrates were analysed. Average values for individual variants are given in Table 2. Dehydrated samples of the control substrate without sodium selenium application were also analysed for the presence of metals (Table 1).

In terms of contamination of the oyster mushroom fruiting bodies by risk metals, currently applicable regulations and decrees set the highest limits only for two risk elements - cadmium and lead. According to **Commission Regulation (EU) 2015/1005** of 25 June 2015 amending Regulation (EC) No 1881/2006 as regards maximum levels of lead in certain foodstuffs, the maximum allowed limit for oyster mushroom fruiting bodies intended for consumption is till 0.30 $mg.kg^{-1}$ Pb of fresh matter of the fruiting bodies. As regards **Commission Regulation (EU) No 488/2014** of 12 May 2014 amending Regulation (EC) No 1881/2006, as regards maximum levels of cadmium in foodstuffs, they were determined at levels of up to 0.20 $mg.kg^{-1}$ Cd of fresh fruiting bodies. Since the samples analysed by us were lyophilized, we carried out a conversion to fresh matter of the fruiting bodies. The results are shown in Table 3.

After the calculation we can state, that the critical limits of heavy metals set by the applicable food quality regulations were not exceeded.

Table 1 Average content of metals in samples of dehydrated substrate.

Variant	Pb (mg.kg^{-1})	Cd (mg.kg^{-1})
C	2.20	0.21

Table 1 Average content of metals in lyophilized samples of fruiting bodies*Pleurotus ostreatus* KRYOS B fortified by selenium.

Variant	Pb (mg.kg^{-1} ±SD)	Cd (mg.kg^{-1} ±SD)
C	1.62 ±0.28^{b}	0.25 ±0.05^{b}
X	1.94 ±0.64^{b}	0.26 ±0.03^{b}
Y	1.59 ±1.05^{b}	0.21 ±0.06^{ab}
Z	1.05 ±0.41^{a}	0.19 ±0.04^{a}

Note: C – 0.0 mg.dm^{-3} Se; X – 0.5 mg.dm^{-3} Se; Y – 1.0 mg.dm^{-3} Se; Z – 2.0 mg.dm^{-3} Se; the values in the columns with different letters are significantly different from each other.

Table 3 Lead and cadmium content in the mass of fresh fruiting bodies*Pleurotus ostreatus* KRYOS B and dependence on applied selenium doses.

Variant	Dry matter (%)	average periods
	Pb (mg.kg^{-1})	
C	11.44	0.18
X	10.46	0.19
Y	10.69	0.16
Z	11.69	0.11
	Cd (mg.kg^{-1})	
C	11.44	0.02
X	10.46	0.02
Y	10.69	0.01
Z	11.69	0.02

Note: C – 0.0 mg.dm^{-3} Se; X – 0.5 mg.dm^{-3} Se; Y – 1.0 mg.dm^{-3} Se; Z – 2.0 mg.dm^{-3} Se.

Therefore, these fruiting bodies can be considered as a safe food product for the consumer also after application of selenium. In the case of the interaction of selenium and selected heavy metals, it was statistically proven in both cases that the increased content of selenium in the substrate reduces the accumulation of lead and cadmium. Average for both cultivation periods pointed out that in the variant with 2.0 mg.dm^{-3} Se was the lead accumulation statistically significant reduced by 64.81%. Reduction in accumulation occurred after application of 1.0 mg.dm^{-3} Se (2.47%) too, but it was not statistically significant. In a variant with 0.5 mg.dm^{-3} Se, lead accumulation was increased by 19.75%, but it was not statistically significant (p >0.05).

Similar results were also found in the case of cadmium accumulation in fortified oyster mushroom fruiting bodies, on average for both cultivation periods. In variant with 2.0 mg.dm^{-3} Se was statistically proven lower cadmium accumulation by 24%, while in a variant with 0.5 mg.dm^{-3} Se, its accumulation grew by 4%, but statistically not significant. In variant with 1.0 mg.dm^{-3} Se, accumulation of cadmium was statistically not significant decrease by 16%. Based on the above results it can be stated, that the fortification of substrates with selenium not increases the accumulation of selected risk metals statistically significantly in any of the experimental variants. On the other hand, in the variant with 2.0 mg.dm^{-3} Se, was statistically proven decreased accumulation of lead and cadmium in the fruiting bodies of edible oyster mushroom. **Lepšová (2001)** argues that the oyster mushroom cultivated in the wood can accumulate only a small amount of heavy metals from the environment; therefore, there is no need to fear their increased level in the harvested fruiting bodies. The opposite phenomenon can occur in the intensive production of edible mushrooms, where the grain is used as a substrate, which is a by-product of intensive agricultural production.

Stihi et al. (2011) in their study found that wild oyster mushroom in Romania, distant 0.5 km from the source of pollution contained Cr (1.81 mg.kg^{-1}), Mn (12.4 mg.kg^{-1}), Fe (387.00 mg.kg^{-1}), Ni (1.85 mg.kg^{-1}), Cu (12.5 mg.kg^{-1}), Zn (41.30 mg.kg^{-1}), Se (2.64 mg.kg^{-1}), Cd (0.95 mg.kg^{-1}) and Pb (0.64 mg.kg^{-1}) in lyophilized samples. Fruiting bodies in a location 10.5 km distant from the same source of pollution contained only slightly lower concentrations of

the monitored elements, namely Cr (1.08 mg.kg^{-1}), Mn (11.8 mg.kg^{-1}), Fe (284.00 mg.kg^{-1}), Ni (1.29 mg.kg^{-1}), Cu (10.20 mg.kg^{-1}), Zn (37.90 mg.kg^{-1}), Se (2.57 mg.kg^{-1}), Cd (0,87 mg.kg^{-1}) and Pb (undetectable) in lyophilizates. These results confirm the ability of oyster mushroom to accumulate risk metals. In samples of fruiting bodies fortified with selenium, which were analysed in our work, many lower concentrations of the elements were found. The findings can be explained by a better quality of the production substrate.

Siwulski et al. (2017) on the basis of their research indicate that the lowest levels of Al, Fe, Mn, P and Se were observed in the *P. ostreatus* 930 strain. The lowest concentrations of Ca, Cr, K, Nd, Te and Zn were determined in *P. florida*, while in *P. pulmonarius*, were observed trace amounts of Er, Rh, Sc, Tm and Zr. On the other hand, *P. ostreatus* strain 930 contained the highest content of Cu and Lu, whereas *P. florida* of the Rh element. Significant differences were observed in *P. ostreatus* K 22, *P. citrinopileatus* and *P. eryngii*. The highest content of Al, Cr, Er, Fe, Pt, Th, Ti and Tm was determined in *P. ostreatus* K 22, the elements Mg, Mn, P, Re, Se and U were most represented in *P. Citrinopileatus*, and *P. eryngii* accumulated the highest concentration of As, B, Ca, In, Na, Nd and Sr. *P. ostreatus* strain K 22 contained the lowest concentration of In, *P. citrinopileatus* lowest concentration of Na and *P. eryngii* the lowest concentration of Th. From other tested species and strains contained *P. ostreatus* HK 35 lowest content of As and highest level of Te, *P. ostreatus* H 195 lowest content of Cd, Pb and U and highest level of Sc. The authors emphasize that the highest concentration of Pb and Cd was determined in fruiting bodies of *P. ostreatus* 80. The high cumulative potential of the said strain can be specifically used in the soil myco-remediation processes. *P. djamor* was rated as the most efficient K and Zn accumulator. We confirm the fact that the individual strains of edible oyster mushroom are different from each other in the ability to accumulate selected compounds.

Quarcoo and Adotey (2013) monitored the accumulation of selected heavy metals by oyster mushroom fruiting bodies in Ghana. They found that the fruiting bodies contained on average 0.04 mg.kg^{-1} Pb, 0.04 mg.kg^{-1} As, 43.77 mg.kg^{-1} Fe, 0.35 mg.kg^{-1} Cd and 0.04 mg.kg^{-1} Hg in a lyophilized mass. Our samples of the fortified fruiting bodies contained lower concentrations of these elements.

Kaya and Bag (2010) analysed 24 species of mushrooms occurring in Turkey. In the case of *Pleurotus ostreatus*, significant levels of heavy metals were found, namely 20.87 mg.kg^{-1} Al, 0.41 mg.kg^{-1} B, 2.11 mg.kg^{-1} Cd, 0.90 mg.kg^{-1} Co, 0.21 mg.kg^{-1} Cr, 39.36 mg.kg^{-1} Cu, 40.57 mg.kg^{-1} Fe, 4.22 mg.kg^{-1} Mn, 1.23 mg.kg^{-1} Ni, 2.14 mg.kg^{-1} Pb and 86.83 mg.kg^{-1} Zn in lyophilizate. Again, we conclude that the concentration of selected elements was lower in our experiment.

Demirbas (2001) found in the dry matter fruiting bodies of the edible oyster mushroom Pb (3.24 mg.kg^{-1}), Cd (1.28 mg.kg^{-1}), Hg (0.42 mg.kg^{-1}), Cu (13.6 mg.kg^{-1}), Mn (6.27 mg.kg^{-1}), Zn (29.8 mg.kg^{-1}) and Fe (81.6 mg.kg^{-1}).

Tuzen, Ozdemir and Demirbas (1998) determined in the dry matter of edible fruiting bodies Pb (0.11 mg.kg^{-1}), Cd (0.55 mg.kg^{-1}), Hg (0.31 mg.kg^{-1}), Fe (48.6 mg.kg^{-1}), Cu (5.0 mg.kg^{-1}), Mn (10.3 mg.kg^{-1}) and Zn (19.3 mg.kg^{-1}).

Lasota, Florezak and Karmanska (1990) prove Cd (11.2 mg.kg^{-1}), Hg (1.2 mg.kg^{-1}), Zn (0.8 mg.kg^{-1}) and Pb (0.0 mg.kg^{-1}) in lyophilized fruiting bodies of oyster mushroom.

All of the above-mentioned findings of a large number of authors point to the fact that edible mushrooms are able to accumulate heavy metals from the environment and the from the substrate into the fruiting bodies, which can greatly worsen the quality of the production. Our argument explains the different concentrations of the rated elements compared to the cited authors, as the content of the selected elements in the concrete fruiting bodies is directly related to the content of the analysed element in the growing substrate. For the intensive production of edible mushroom is important to use only high-quality lignocellulosic material analysed for the content of risk metals. From the point of view of the accumulation of selected metals, we recommended to fortify the cultivation substrate of oyster mushroom with the selenium in order to produce a functional and uncontaminated foodstuff.

CONCLUSION

At work we monitored the accumulation of risk elements – lead and cadmium in the oyster mushroom fruiting bodies. We found that the limit set by the European Commission Regulations, which concern about the content of lead and cadmium in oyster mushroom fruiting bodies has not been exceeded. Fruiting bodies are of satisfactory quality and are suitable for daily consumption.

In the experiment, the reduction of lead and cadmium accumulation was statistically confirmed after application of 2.0 mg.dm^{-3} Se. Cadmium content decreased by 22.45% and lead by 64.81% compared to the control variant. From the results it is clear that by fortification of the substrate (the main component of which is straw) by selenium, we produce not only a high-quality food with a high antioxidant potential, but we can also prevent the accumulation of risk elements of lead and cadmium from the substrate.

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