International Journal of Horticultural Science 2019, 25 (3-4): 21-27.
https://doi.org/10.31421/IJHS/25/3-4/3096

Relationship between zinc and cadmium contents and cultivating conditions of gourmet and medicinal mushroom *Agaricus subrufescens*

Jasinska, A.[1,2], Wojciechowska, E.[1], Stoknes, K.[2] , Siwulski, M.[1], Mleczek, M.[3] & Niedzielski P.[4]

[1]Poznan University of Life Sciences, Department of Vegetable Crops, ul. J.H. Dabrowskiego 159, 60-594 Poznan, Poland;
[2]Lindum AS, Lerpeveien 155, 3036 Drammen, Norway;
[3]Poznan University of Life Sciences, Department of Chemistry, ul. Wojska Polskiego 75 60-625, Poznan, Poland;
[4]Adam Mickiewicz University, Faculty of Chemistry, ul. Umultowska 89b,. 61-614, Poznan, Poland
Author for correspondence: agnieszka.jasinska@up.poznan.pl

Summary: Almoust half of the world's population is at risk for inadequate zinc (Zn) intake, a strategic trace element that is necessary for a healthy immune system. A lack of zinc can make a person more susceptible to disease and illness. There is a need of defining additional sources of zinc in diet. Cadmium (Cd), however, and its toxicity in food chain receives considerable public and scientific attention. Cd is primarily toxic to kidney and can cause bone demineralization. In many areas in the EU, intake of Cd is not far from maximum tolerable. Mushrooms are well known for accumulating metal ions such as zinc and cadmium. Objective of this study was to define relationship between cultivation systems and conditions on zinc and cadmium content in fruit bodies of *Agaricus subrufescens* grown on different substrates. Cultivation was performed on mushroom composts based on increasing amount of digestate from anaerobic digestion treatment processes mixed with wheat straw and paper. The Zn and Cd concentration was defined in fruiting bodies, correlated with yield, flush and element concentration in substrates. Results showed percentage of food waste digestate and other components used in experiment had influence on concentration of Zn and Cd in mushroom compost and in *A. subrufescens*. Zn accumulated in collected mushrooms in amounts reaching from 42.8 to 126.9 mg kg^{-1} Cd content ranged 2.6 to 17.9 mg kg^{-1}. Significant correlations for Zn concentration between mushrooms and substrates showed increase of Zn in mushrooms when cultivated on substrates with higher amount of digestate.

Jasinska, A., Wojciechowska, E., Stoknes, K., Siwulski, M., Mleczek, M. & Niedzielski P. (2019): Relationship between zinc and cadmium contents and cultivating conditions of gourmet and medicinal mushroom *Agaricus subrufescens*. International Journal of Horticultural Science 25 (3-4): 21-27. https://doi.org/10.31421/IJHS/25/3-4/3096

Key words: almond mushroom, biofortification, chemical composition, cultivation compost, nutrifoods, edible fungi

Introduction

Zinc (Zn) is one of the most important essential trace metals in human nutrition; not only a vital element in various physiological processes but also a powerful prevention of many diseases. It is needed for the body's defensive (immune) system to properly work. It plays a role in cell division, cell growth, wound healing, and breakdown of carbohydrates. The adult human body contains about 2 to 3 grams of zinc. Zinc is found in cells throughout the human body: in organs, tissues, bones, fluids, and cells. Foods with high protein content, specifically animal protein, are major sources of zinc in human diet (Kaur et al., 2014). Other well known good sources of zinc are nuts, whole grains, legumes, and yeast. Fruits and vegetables are not good sources, the zinc in plant proteins is not as available for use by the human body as zinc from animal proteins. Meat - derived foods have a high amountof Zn, from 0.40 to 6.77 mg per 100 g, grain group - 0.30 to 2.54 mg per 100 g, dairy products - 0.36 to 0.49 mg per 100 g, vegetables - 0.12 to 0.60 mg per 100 g, and fruits 0.02 to 0.26 mg per 100 g (Haeflein & Rasmussen, 1997). Therefore, low-protein diets and vegetarian diets tend to be low in zinc (Osis et al., 1972).

Mushrooms are valuable healthy foods, low in calories, iron, zinc, chitin, fiber, vitamins and minerals (Ouzouni et al., 2009). Mushroom fruiting bodies can be considered as rich in potassium (K), phosphorus (P), copper (Cu) and zinc (Zn). Both Cu and Zn present in the mushrooms are of nutritional value for humans. Content of zinc related in mushrooms ranges from 20 to 350 mg kg^{-1}, sometimes even more in some species (Kalac, 2010; Falandysz & Borovička, 2013; Rzymski et al., 2017).

Due to the fact, that nearly half of the world's population is at risk for inadequate zinc intake, therefore there is a need of defining additional sources of zinc in human diet. Bio-fortification is a recent breeding or genetic engineering strategy that either, increase level of zinc, reduce content of inhibitors (e.g., phytate), or increase expression of compounds that enhance zinc absorption (e.g., amino acids) and have been considered to improve the bio-availability of zinc from plant foods (Roohani, 2013). Higher fungi are known as zinc accumulators. The naturally occuring in mushrooms biosorption of metal ions is viewed as a promising alternative for efficient production of Zn-enriched foods or drugs. The organic form of Zn can produce some new physiological functions as compared to inorganic zinc ions (Fan et al., 1995; Zhou, 2002; Deng et al., 2003).

The sporophore/ substrate ratio for Zn in some species of mushrooms ranges from 1 to 10 mg/kg, which makes them good food with natural ways of bio-fortification in zinc (Bano

et al., 1981; Isiloglu et al., 2001). However, according to Thomet (1999) there is a strong correlation between the distribution of Cd and the chemically related Zn in fruiting bodies of cultivated mushrooms. In general, total Zn concentrations of all the substrates amounted to about 20–30 times those of Cd.

Agaricus subrufescnes (Peck.) (syn. *Agaricus brasiliensis* (Wasser *et al*), *A. blazei* (Murrill ss. Heinem.), discovered and popularised as late as the 20th century, has been known for its exceptional properties in the places of its origin for a long time (Wasser et al., 2002; Lisiecka et al., 2013). This species is considered as a medicinal mushroom containing bioactive polysaccharides and protein complexes (PSPC) which have been shown to function as potent antioxidants, antitumor, and anticancer agents (Endo et al., 2010; Ishii et al., 2011; Oliveira Lima et al., 2011). Metabolic effects and bioactivity after Zn biosorption of *A. subrufescens* studied by Zou (2005) in mushroom fruiting body has a significant effect on the growth, amino acid composition, polysaccharide yields. The experiments have exhibited strong antitumor activity against sarcoma 180 as compared to normal mycelial powder. However this investigation has been studied in submerged cultures, to be used as food additives, whereas there is growing need to supply the market with a large amount of high quality, safe and avirulent foods fortified with zinc ions. However, *A. subrufescens* is also known for high Cd accumulations from the soil into the basidiocarp (Huang et al., 2008; Lin et al., 2010; Sun et al., 2012).

The cadmium content in *A. subrufecens* was recently found to range from 2 to 31 mg kg^{-1} dry matter, respectively (Rzymski et al., 2017). The EU regulation has set a general Cd concentration limit for mushrooms (excluding *A. bisporus*) of 1 mg kg^{-1} fresh matter (Stoknes et al., 2013) which roughly corresponds to 10 mg kg^{-1} dry matter.

Therefore, the aim of the present study was to determine content of zinc, as an essential nutrition element in fruit bodies of *Agaricus subrufescens* cultivated on different substrate, without any additional supplementation in zinc. In addition, to define if association between zinc and cadmium and the growing conditions of mushrooms, will be limiting factor for mushrooms biofortification with zinc.

Materials and methods

Spawn of *Agaricus subrufescens* was purchased from the mushroom spawn laboratory MYCELIA, Belgium. Four mushroom composts (MC) of composed from straw, paper with increasing amount of food waste digestate (FWD) from anaerobic digestion (AD) treatment processes (10%, 20%, 30% and 40% in relation to dry matter of substrate before processing) were compared. The composition was based on dry matter (DM) of substrate before processing, and was evened to obtain 40% of DM of substrate. The digestate from AD of source separated household food waste was separated using a decanter centrifuge.

Compost preparation

Phase I – the composting process

Mushroom growing substrate was composted in rotating composter drums for few days, until volume loss due to heat generation. Later substrate was mixed and moved to insulated containers with controlled air flow through the substrate (to obtain similar conditions as in commercial bulk systems; 70-80°C and 6-9% O_2 v/v). The substrate was turned again twice at an interval of 2 days, giving total of 10 days for phase I.

Phase II – pasteurization

The mushroom compost was moved into a miniature phase II tunnel, where temperature was slowly increased to 60°C over 24 hours and then maintained for 6 hours of pasteurization. During conditioning temperature was decreased to 55°C, for another 6 hours and next to 50°C, until ammonia (NH_3 gas) had dissipated (below 10 ppm). More detailed description of Phase I and Phase II processes are described by Stoknes (2013).

Experiment set-up and measurements

During cultivation experiments, 50 micron polypropylene, autoclavable bags of capacity of 7 L, flat size 38 cm wide × 57 cm high, with four linear ventilation filters were used. Bags were filled with approximately 3 kg of pasteurized compost and left to cooling to room temperature (22°C). The compost was then inoculated with granular spawn on wheat grain, applied at an amount of 3% of fresh substrate weight. The bags were sealed and shaken by hand until the spawn was evenly distributed. The spawning was performed in stable, controlled conditions, at 25°C. After the spawn overgrew the substrate, bags were opened and a 5 cm layer of casing was applied and moved into the growing chamber. The temperature inside the chamber was 25 +/- 2°C for 15 days than decreased by 5°C for next 5 days for initiation of pining. The air humidity for fruit body development was held at 85-95%. The cultivation chambers received LED light with colour temperature 6000 K. The cultivation room was aired so that CO_2 concentration did not exceed 1000 ppm. The first flush was harvested 5 weeks after inoculation. Mushrooms fruiting bodies were collected through the period of 110 days. Five flushes were harvested. Yields were determined as weight of harvested fresh mushrooms from the complete cropping period per fresh weight of substrate at inoculation.

Analytical Methods

Sample collection and preparation procedure

Collected samples of substrates and mushroom fruiting bodies (caps and stipes together) were dried at 105±5ºC for 96 h in an electric oven (SLW 53 STD, Pol-Eko, Wodzisław Śląski, Poland) and ground in a laboratory Cutting Boll Mill PM 200 (Retsch GmbH, Haan, Germany). A dry sample (0.50 ± 0.01 g) was digested by concentrated nitric acid (Merck, Germany) in closed Teflon containers in the microwave sample preparation system Mars 5 Xpress (CEM, Matthews, USA). After digestion samples were filtered through paper filters and diluted with water to final volume of 15.0 mL. Each sample was analysed in triplicate using whole sample preparation procedure.

Determination of Zinc (Zn) and Cadmium (Cd)

The technique of inductively coupled plasma optical emission spectrometry (Agilent 5100 ICP-OES (Agilent, USA) was used for Zn and Cd determination. The mode of radial view of plasma was applied. For determination, the following

conditions were used: wave length of 214.439 nm, Radio Frequency (RF) power of 1.2 kW, nebulizer gas flow of 0.7 L min^{-1}, auxiliary gas flow of 1.0 L min^{-1}, plasma gas flow of 12.0 L min^{-1}, viewing height for radial plasma observation at 8 mm, detector CCD (Charge Coupled Device) temperature at -40°C, signal accusation time of 3 replicates. The detection limit has been determined on the level of 0.0026 mg kg^{-1} dry matter (as 3-sigma criteria). The uncertainty for total analytical procedure (including sample preparation) was on the level of 10%. Due to lack of the certified reference material in accordance with samples matrix the traceability was checked using several reference materials CRM S-1 – loess soil; CRM NCSDC (73349) – bush branches and leaves; CRM 2709 – soil; CRM 405 - estuarine sediments and the recovery (80-120%) was acceptable. Every individual sample was analyzed in triplicate. In order to consolidate the number of replications while maintaining randomization, a triple sampling with replacement from the pool of individuals found for each species was conducted. All element contents are given as milligram per kilogram dry matter (d.m.).

Statistical Analysis

Each compost was treated as a separate experiment. Experiments were established in fully randomized design, in 4 replications and 2 cultivation cycles. When comparing the experimental results, the analysis of variance for randomized block with 4 composts treatments was applied (level of significance $\alpha = 0.05$). The results of cultivation experiments were discussed based on mean values from cultivation cycles. The concentration of Zn was measured in medicinal mushroom *A. subrufescens* and correlated with yield, flush and element concentration in substrates. Samples of fruiting bodies and substrate from cultivation in different digestate-based substrates were collected.

Efficiency of Zn species accumulation was expressed in values of the bioconcentration factor (BCF) calculated as the ratio of Zn species concentration in the whole fruiting bodies to the concentration of this element in substrate before cultivation.

Results and discussion

Zinc and cadmium concentration cultivation materials

The results of performed experiment showed that element concentrations were primarily substrate dependent. In this study, at least five factors could affect Zn and Cd concentrations of the edible and medicinal mushroom *Agaricus subrufescens*: substrate characteristic such as amount of digestate from AD, elements levels, pH, organic matter, and yield of the mushrooms fruiting bodies. These factors influence the metal concentrations and the BCFs, as higher fungi are able to bioconcentrate (BCF <1) or exclude (BCF>1) specific metal ions.

Concentration of Zn and Cd in materials and mushroom composts used in experiment are presented in ***Table 1.*** Materials used in experiment characterized with different concentration of analyzed elements. The highest concentration of zinc in components used in mushroom composts was found in wheat straw. This material characterized as well the highest concentration of cadmium. The lowest concentration of Zn was found in paper used in MC. Digestate solids from food waste used for mushroom compost had the lowest concentration of Cd in comparison to other components. Interesting trend was found in mushroom composts used during cultivation – increasing percentage of digestate influenced lower concentration of Zn in mushroom compost and it ranged from 34.78 to 28.75 mg kg^{-1}. Level of Cd in all composts was low and it ranged from 0.19 to 0.36 mg kg^{-1}, the lowest concentration was found in compost with 20% of digestate and the highest in compost with 40% of digestate.

Table 1. Concentration of zinc (Zn) and cadmium (Cd) content of all materials and mushroom growing composts used during experiment (mg kg^{-1} dry matter).

Material	n	Zn	Cd	Zn/Cd
Food waste digestate solids (FWD)	3	16.25	0.15	108.33
Gypsum added MC	2	22.94	0.26	88.23
Paper, used in MC	2	13.58	0.205	66.24
Wheat straw	1	26.48	0.56	47.28
Chicken manure	2	16.055	0.365	43.98
Garden chalk	2	17.975	0.245	73.36
MC: straw/paper + 10% digestate	3	34.78	0.36	96.61
MC: straw/paper + 20% digestate	3	34.30	0.32	107.25
MC: straw/paper + 30% digestate	1	33.83	0.23	147.08
MC: straw/paper + 40% digestate	2	28.75	0.19	151.36
Casing used in mushroom growing	2	22.7	0.91	24.94

*Percentages (%) given for mushroom composts (MC) are based on dry matter (DM) ; n = number of samples analysed.

Zinc and cadmium concentration in the mushrooms cultivated on different substrates

The chemical composition of mushrooms fruit bodies is principally affected by the chemical composition of the substrate used for cultivation. Commonly used substrates for *Agaricus* sp. cultivation, are mostly composts produced from wheat straw, horse manure, chicken manure and gypsum (Straatsma et al., 2000). In this study we present cultivation of *Agaricus subrufescens* was performed successfully on compost from wheat straw/paper and solid fraction of digestate from anaerobic digestion biogas production as described previously Stoknes, et al., 2013; Jasińska et al., 2016; Stoknes et al., 2016). The concentrations of Zn in the input materials were from 13.58 in paper to 26.48 mg kg^{-1} substrates dry matter in wheat straw. Cadmium concentration was 0.15 in FWD up to 0.56 mg kg^{-1} substrates dry matter in straw. The food waste digestate contained, respectively, 16.25 and 0.15 mg kg^{-1} of substrates dry matter of Zn and Cd, and was much lower than the amounts reported by Govasmark (2011), not exceeding the concentration limits for organic farming (Mattilsynet rapport, 2008). According to Deportes et al. (1995) Zn bioavailability in compost ranges from 1.3% to 60.3%, however there are limited information on the bioavialbility of Zn from AD digestate. The Cd can be high in digestate, and a recent study (Chiew et al., 2015) compared the environmental impact of digestate with mineral fertiliser and concluded the Cd content of food waste digestate can be a challenge with this otherwise sustainable alternative.

In the present study two-factorial statistical analysis **(*Table 2*)** was conducted for all four cultivating substrate and all five harvested flushes, however separately for Zn and Cd. Values which share the same latter do not differ statistically at

significance level α=0.05. Conducted experiment show Zn accumulated in all collected mushrooms in amounts ranging from 43.9 mg kg^{-1} in 4th flush of MC20% to 116.2 mg kg^{-1} in 3rd flush of MC40%. Concentration of Zn in mushroom samples cultivated on selected substrates differed during cultivation and did not show clear trend. Content of Cd ranged from 2.6 mg kg^{-1} in 5th flush of MC10% up to 17.9 mg kg^{-1} in 1st flush of MC40%. Cadmium concentrations in mushroom fruit bodies decreased from flush to flush, showing clear trend.

The levels of zinc in genus *Agaricus* sp. reported in the literature waried; in common button mushroom *A. bisporus* ranges from only 4.17 to 81.4 mg kg^{-1} dry matter; whereas in wild *A. arvensis* it was much higher – from 85.2 – 142 mg kg^{-1} dry matter (Jasińska et al., 2016). There are limited data on zinc levels in fruiting bodies of medicinal mushroom, *Agaricus subrufescens*. Research of Gyorfi et al. (2010), shows significantly higher amounts of zinc than in other species from genus *Agaricus*, from 143 even up to 254 mg kg^{-1}. Recent comprehensive, multielemental analysis of some *Agaricus* species showed there is quite large difference in the range of zinc concentrations within the examined *Agaricus* species: *A. bisporus* (white and brown strain); *A. arvensis* and *A. subrufescens* Rzymski et al. (2017). The study reported mean concentration for *A. bisporus* (white) – 66 mg kg^{-1} (within range 41-95 mg kg^{-1}); *A. bisporus* (brown) 77 mg kg^{-1} (within range 56-168 mg kg^{-1}); *A. arvensis* 129 mg kg^{-1} (within range 10-308 mg kg^{-1}) and *A. subrufescens* 239 mg kg^{-1} (within range 56-681 mg kg^{-1}) Rzymski et al. (2017). *A. bisporus* (white and brown) showed the lowest variability of element contents, whereas the gratest variability of element conetnt was observed in less cultivated species *A. arvensis* and *A. subrufescens*. In our study the mean value of concentration of Zn in *A. subrufescnes*, regardless the substrate was 81.5 mg kg^{-1} (within range 43.9-116.2 mg kg^{-1}, SD 25.20 – data not presented in the tables). The Zn concentrations in *A. subrufescens* in our study presented lower to slightly higher contents of Zn up to the limit established by the Polish legislation (100 mg kg^{-1} DW). The acceptable daily intake was established in 1 mg mg kg^{-1} DW (WHO, 1982), and the recommended dose per day for an adult is 15 mg.

Table 2. Statistical analysis of zink (Zn) and cadmium (Cd) concentration in *Agaricus subrufescens* cultivated on different substrates, during the five flushes of harvests.

Substrate	Zinc concentration mg·kg^{-1}				
	Flush				
	1	*2*	*3*	*4*	*5*
MC10%	70.9abcde	53.1abc	67.23abcd	60.9abcd	63.9abcd
MC20%	47.1ab	64.3abcd	66.6abcd	43.9a	96.4defg
MC30%	95.3defg	82.7cdefg	113.2fg	103.1efg	112.3fg
MC40%	111.8fg	80.3bcdef	116.2g	89.1defg	92.2defg
Substrate	Cadmium concentration mg·kg^{-1}				
	Flush				
	1	*2*	*3*	*4*	*5*
MC10%	13.7ab	6.0a	6.2a	3.4a	2.6a
MC20%	6.7a	4.4a	3.6a	4.4a	3.7a
MC30%	6.2a	4.9a	4.0a	3.9a	3.6a
MC40%	17.9b	11.8ab	8.7a	7.9a	6.1a

*statistical significance at α=0.05, values which share the same letters do not differ statistically

The amount of Cd taken up in basidiocarps in general is naturally related to Cd concentration in the soil (Melgar et al., 2016). It is also clear that the amount taken up in cultivated *A. subrufescens* is influenced by the total concentration of Cd in the mushroom compost used (Huang et al., 2008; Lin et al., 2010).

As mentioned before increasing percentage of digestate influenced lower concentration of Zn in the mushroom compost and it ranged from 34.78 to 28.75 mg kg^{-1}. The yield of mushrooms expressed in % of substrate (***Table 3***) does not seem to reflect clear trend between concentration of Zn in substrate and yield of mushrooms. The highest concentration of Zn in composts with 10% and 20% of digestate did not affect the highest yield nor the highest concentration of Zn in basidiocarps; which were found in composts with lower Zn concentration and with highest digestate amount. As mentioned before, little is known about the bioavialbility of Zn from AD digestate. Similar relation was found with cadmium concentrations by Stoknes et al. (2019).

Significant correlations for Zn between mushrooms and substrates were found; amount of Zn in mushrooms increased when cultivated on substrates with higher amount of digestate. The bioconcentration factor (BFC), which is concentration in the basidiocarps/concentration in the substrate, is the popular term of describing the ability of fungus to accumulate a particular element.

The results of our investigation showed that Zn is concentrated in mushroom tissue from substrate with transfer factors (substrate/mushroom) of 1.82 - 3.75, and clearly increases with the amount of digestate in the substrate. Accordingly, the amount of straw/paper is decreasing – as shows in C/N ratio most likely making the Zn from digestate more available for mushrooms to absorb.

The fruiting bodies of mushrooms can be considered as rich trace elements such as Cu and Zn. Both Cu and Zn in the flesh of mushrooms collected from background areas are of nutritional value for humans. The situation can be different if the mushrooms emerged at sites polluted with Cu or Zn. This is because in polluted soils other toxic metals (Cd, Pb, Hg) can co-occur with Cu and Zn and can be bio-accumulated to concentrations much above typical contents of edible species and could become toxic to mushrooms (Collin-Hansen et al., 2005).

Mean Zn and Cd concentration in A. subrufescens

The correlation between mean concentration of Zn and Cd in *Agaricus subrufescens* (all flushes) and mean concentration of Zn and Cd in substrate components were analyzed with the significance level of 0.05 (tab. 4). Mean concentration of zinc in *A. subrufescens* was not significantly correlated with cadmium. Similarly, zinc concentration did not affect the mean concentration of cadmium in mushroom samples. However, mean concentration of Zn in *A. subrufescens* was strongly dependent on mean concentration of this element in substrate components. Relations in this analysis were as follows in ***Table 4.***

Strong positive correlation was found between Zn and Cd concentration in *A. subrufescens* and digestate, paper and gypsum used in substrate preparation. Those components affected greater Zn and Cd concentration in mushrooms. Strong negative correlation was found between Zn and Cd concentration in *A. substrate* and wheat straw, chicken manure and garden chalk. Those components affected lower Zn and Cd concentration in mushrooms. There was strong negative

Table 3. Composition and zink (Zn) and cadmium (Cd) content of mushroom composts and basidiocarp mushrooms harvested from all flushes.

Digestate content in compost (by dry weight)	Compost at time of inoculation				Basidiocarps mean all flushes				BCF
	C:N ratio	pH	Zn	+/-SD*	Yield, in % of substrate	+/-SD	Zn	+/-SD	
MC10%	60:1	7.9	34.78	0.644	2.1%	1.439	63.25	12.44	1.82
MC20%	40:1	8.1	34.30	0.41	2.1%	1.373	66.45	26.54	1.93
MC30%	30:1	8.2	33.83	0.63	3.1%	2.14	99.63	18.36	2.94
MC40%	20:1	8.3	28.75	0.73	2.6%	1.817	99.20	16.71	3.75
Digestate content in compost (by dry weight)	Compost at time of inoculation				Basidiocarps mean all flushes				BCF
	C:N ratio	pH	Cd	+/-SD*	Yield, in % of substrate	+/-SD	Cd	+/-SD	
MC10%	60:1	7.9	0.36	0.012	2.1%	1.439	10.5	5.72	29.4
MC20%	40:1	8.1	0.32	0.045	2.1%	1.373	6.6	6.37	20.6
MC30%	30:1	8.2	0.23	0.007	3.1%	2.14	4.7	1.81	20.4
MC40%	20:1	8.3	0.19	0.025	2.6%	1.817	4.6	2.82	22.2

*Yield (=summed total yield through all flushes / weight of the substrate at time of inoculation). C/N = carbon to nitrogen ratio in the composts. Zn values are in mg Zn kg^{-1} dry matter. *SD=standard deviation

correlation between mean concentration of Zn and Cd, and mean concentration of these elements in casing. Chemical composition of casing affected lower concentration of Zn in mushroom samples.

Referring to Collin-Hansen et al. (2005) it can be stated that in our experiment threat of co-accumulation of toxic elements with Zn and threat of boosting their levels above the established limits does not exist. Moreover, the zinc is an antagonist of other metals (Cd, Pb, Ni), and its presence in some mushrooms reduces the risks associated at high concentrations of other toxic metals. Therefore, the consumption of these mushrooms does not represent a toxicological risk, and they are an important nutritional requirement to the diet Gyorfi et al. 2010).

Table 4. Correlation between zink and cadmium (Zn, Cd) concentration in fruiting bodies of *Agaricus subrufescens* and substrate components.

	Mean of Zn	***Mean of Cd***
Mean of Zn	1	
Mean of Cd	-0.0004*	1
Wheat straw	-0.6028	-0.7256
Paper	0.7469	0.8460
Chicken manure	-0.7469	-0.8460
Lime	-0.7469	-0.8460
Gypsum	0.7469	0.8460
Digestate (food waste)	0.9057	0.9492
Casing	-0.7469	-0.8460

*statistically insignificant, significance level at α =0.05

According to Thomet et al. (1999) there is strong correlation between the distribution of Cd and the chemically related Zn in fruiting bodies of cultivated mushrooms. In general, total Zn concentrations of all the substrates amounted to about 20–30 times those of Cd. Normally, the Zn/Cd ratio of the continental crust is about 100 (Blume et al.,1992).

However, in performed analysis we did not found this correlation. In our experiment we found that Zn/Cd ratio in the substrate were in most of the cases lower than 100 (Table 1). In AD digestate from foodwaste this ratio was 108; and then in the three of four mushroom composts with 10, 20 and 20% of digestate, where Zn concentrations in fruiting bodies were on average level. Surprisingly in the MC with the highest amount of digestate and the highest BCF factor for Zn this ratio was only 79. From the previous study of Thomet et al. (1999) zinc appears to be species-specific, but also mushroom/mycelium age specific in terms of accumulation in the basidiocarps but not as obvious as in Cd. The older mycelium the higher accumulation of zinc but lower of cadmium (Thomet et al., 1999, Kalac et al., 2004). Therefore it is quite safe to say, that mushrooms pick up from the commercial cultivation would be much safer in terms of elements accumulation, due to short lifecycle, than picked up from the wild.

The Cd accumulation in our study was in average of 6.63 mg kg^{-1} (ranges from 1.2 – 21.6 mg kg^{-1}; SD 5.08 data not presented in the tables) which is considerably lower than usually reported for this species; 2.3-35 mg kg^{-1} (Rzymski et al., 2017; Huang et al., 2008; Sun et al., 2012). As stated earlier, there were no statistically significant correlations between accumulations of those two elements found in our study. Thus, Cd and Zn appeared to be transferred from soil to mycelia in different quantities, depending on either species-specific factors. This indicated that the uptake of Cd and Zn could be actually controlled by two separate mechanisms.

Investigation conisdering the zinc levels in *A. subrufescens* were conducted in submerged cultures, to be used as food additives. However our study shows that there is a potential of use the natural ability of mushrooms such as *A. subrufescens* to incerease the concentrations of minerals by simply modifying the substrate. Therefore additional studies are to be performed with use of zinc and other essential for human nutriotion elements as the supplements for cultivation substrate. The mineral composition of the medicinal mushroom *A. subrufescens* can supplement the known positive effects of this mushroom regarding human health care. According to the best knowladge of authors there is limited literature on bioavailibility of zinc from mushroom for human remaining the insufficienlty investigated field waiting for reserchers to be discovered.

Conclusions

Percentage of food waste digestate and other components used in experiment had influence on concentration of zinc and cadmium in the mushroom compost and in sporocarps of *Agaricus subrufescens*. Concentration of zinc in mushrooms increased with the higher amount of food waste digestate in the

substrate. The BCF of 1.82 - 3.75 shows Zn clearly increases with the amount of digestate in the substrate. Accordingly, the amount of straw/ paper is decreasing – as shows in C/N ratio most likely making the Zn from digestate more available for mushrooms to absorb. In the contrary, the content of cadmium decreased in the substrates with more digestate and less straw, which goes in line with results of Stoknes et al. (2019). In the performed analysis we did not found correlation between zinc and cadmium, more over the elements appeared to be transferred from soil to mycelia in different quantities, which shows that the uptake of Cd and Zn could be actually controlled by two separate mechanisms. To answer questions about substrate-mushroom translocation and human health benefits further research on Zn bioavailability from *A. subrufescens* are required.

Acknowledgements

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 751052 and PULS Young Researchers Subsidy No 507.649.31

References

Anonymous (2006): Commission Regulation (Ec) No. 1881/2006 of 19 December 2006 Setting Maximum Levels for Certain Contaminants in Foodstuffs 5-24.

Bano, Z., Nagaraja, K., Vibhakar, S., Kapur, O. P. (1981): Mineral and the heavy metal contents in the sporophores of *Pleurotus* species. Mushroom Newsletter Tropics : 2: 3–7

Blume, H. B., Brümmer, G. W., Horn, R. (1992): Scheffer/Schachtschabel: Lehrbuch Der Bodenkunde: Enke, Stuttgart, Germany.

Chiew, Y. L., Spangberg, J., Baky, A., Hansson, P. A., Jönsson, H. (2015): Environmental impact of recycling digested food waste as a fertilizer in agriculture - a case study. Resources, Conservation and Recycling: 95:1-14.

Collin-Hansen, C., Andersen, R. A., Steinnes, E. (2005): Damage to DNA and lipids in *Boletus edulis* exposed to heavy metals. Mycological Research; 109:1386–1396

Deng, B. W., Chen, W. Q. (2003): Study on accumulation of zinc in *Polyporus umbellate* in liquid culture. Edible Fungi of China: 22:33–35.

Dèportes, I., Benoit-Guyod, J. L., Zmirou, D. (1995): Hazard to man and the environment posed by the use of urban waste compost: A review. Science of Total Environment.72:197–222.

Endo, M., Beppu, H., Akiyama, H., Wakamatsu, K., Ito, S., Kawamoto, Y., Shimpo, T., Koike, T., Matsui, T. (2010): Agaritine purified from *Agaricus blazei* Murrill exerts antitumor activity against leukemic cells. BBA-General Subjects: 1800(7):669-673.

Falandysz, J., Borovička, J. (2013): Macro and trace mineral constituents and radionuclides in mushrooms: health benefit and risks. Applied Microbiology and Biotechnology: 97:477–501. DOI:10.1007/S00253-012-4552-8

Fan, Q. S., Wei, H., Xie, J. J. (1995) : Effect of *Flamulina velutipes* cultured in zinc enriched medium on the learning ability and immunological function of mice. Acta Nutrimenta Sinica: 17; 89–91.

Govasmark, E., Stäb, J., Holen, B., Hoornstra, D., Nesbakk, T., Salkinoja-Salonen, M. (2011): Chemical and microbiological hazards associated with recycling of anaerobic digested residue intended for agricultural use. Waste Management: 31(12):2577-2583.

Gyorfi, J., Geosel, A., Vetter, J. (2010): Mineral Composition of different strains of edible medicinal mushroom *Agaricus subrufescens* Peck. Journal of Medicinal Food: 13(6):1510–1514.

Haeflein, K. A., Rasmussen, A. I. (1977): Zinc content of selected foods. Journal of American Dietetic Associacion. 70(6): 610–616.

Huang, J. C., Li, K. B., Yu, Y. R., Wu, H. W., Liu, D. L. (2008): Cadmium accumulation in *Agaricus blazei* Murrill. Journal of the Science of Food and Agriculture: 88:1369–1375.

Işıloğlu, M., Yılmaz, F., Merdivan, M. (2001) : Concentrations of trace elements in wild edible mushrooms. Food Chemistry: 73:163–175.

Ishii, P. L., Prado, C. K., Mauro, M. D., Carreira, C. M., Mantovani, M. S., Ribeiro, L. R, Dichi, J. B., Oliviera, R. J. (2011): Evaluation of *Agaricus blazei* in vivo for antigenotoxic, anticarcinogenic, phagocytic and immunomodulatory activities. Regulatory Toxicology and Pharmacology: 59:412-422.

Kalac, P. (2010): Trace element contents in European species of wild growing edible mushrooms: a review for the period 2000–2009. Food Chemistry: 122:2–15

Kalač, P., Svoboda, L., Havlíčková, B. (2004): Contents of cadmium and mercury in edible mushrooms. Journal of Applied Biomedicine: 2:15-20.

Kaur, K., Gupta, R., Saraf, S. A., Saraf, S. K. (2014): Zinc: the metal of life. Comprehensive Reviews. Food Science and Food Safety. 13(4): 358-376.

Jasińska, A. J., Wojciechowska, E., Stoknes, K., Krzesiński, W., Spiżewski, T., Krajewska, K. (2016) : Mushroom cultivation on substrates with addition of anaerobically digested food waste. International Society for Horticultural Science (ISHS), Leuven, Belgium. 199-206.

Lin, C. Y., Guo, H. Y., Chu, C. L., Lee, I. H., Pai, H. L., Shih, H. D. (2010): Factors affecting the amount of cadmium accumulated in culinary-medicinal royal sun agaricus, Agaricus brasiliensis s. Wasser et al. (agaricomycetideae), during cultivation. International Journal of Medicinal Mushrooms, 12(4).

Lisiecka, J., Sobieralski, K., Siwulski, M., Jasinska, A. (2013): Almond mushroom *Agaricus brasiliensis* (Wasser et al.) –properties and culture conditions. Acta Scientiarum Polonorum-Hortorum Cultus: 12(1):27-40.

Melgar, M. J., Alonso, J., García, M. A. (2016): Cadmium in edible mushrooms from NW Spain: bioconcentration factors and consumer health implications. Food and Chemical Toxicology: 88, 13-20.

Mattilsynet M., Rapport J. (2008): Rester av plantevernmidler i vegetabilske næringsmidler (English Summary). Available: <http://www.mattilsynet.no/mattilsynet/multimedia/archive/00040/Rapport__Rester_av_p_40466a.pdf>.

Oliveira Lima, C. U. J., de Almeida Cordova, C. O., de Toledo, N. O., Funghetto, S. S., de Oliveira, G., Karnikowski, M. (2011): Does the *Agaricus blazei* Murill mushroom have properties that affect the immune system? An Integrative Review. Journal of Medicinal Food: 14:2–8

Osis, D., Kramer, L., Wiatrowski, E., Spencer, H. (1972): Dietary zinc intake in man. American Journal of Clinical Nutrition. 25(6): 582-588.

Ouzouni, P. K., Petridis, D., Koller, W. D., Riganakos, K. A. (2009): Nutritional value and metal content of wild edible mushrooms collected from west Macedonia and Epirus, Greece. Food Chemistry. 115:1575–1580

Roohani, N., Hurrell, R., Kelishadi, R., Schulin, R. (2013) : Zinc and its importance for human health: an integrative review. Journal of Research Medical Sciences: The Official Journal of Isfahan University Of Medical Sciences : 18(2):144.

Rzymski, P., Mleczek, M., Siwulski, M., Jasińska, A., Budka, A., Niedzielski, P., Kalac, P., Gąsecka, M., Budzyńska, S. (2017) : Multielemental analysis of fruit bodies of three cultivated commercial *Agaricus* species. Journal of Composition and Analysis : 59:170-178.

Stoknes, K., Beyer, D.M., Norgaard, E. (2013): Anaerobically Digested food waste in compost for *Agaricus bisporus* and *Agaricus subrufescens* and its effect on mushroom productivity. Journal of the Science and Food Agriculture: 93:2188–2200.

Stoknes, K., Scholwin, F., Krzesiński, W., Wojciechowska, E., Jasińska, A. (2016): Efficiency of a novel ‘‘Food to Waste to Food” system including anaerobic digestion of food waste and cultivation of vegetables on digestate in a bubble-insulated greenhouse. Waste Management: 56:466-476, Doi: 10.1016/J.Wasman.2016.06.027

Stoknes, K., Scholwin, F., Jasińska, A., Wojciechowska, E., Mleczek, M., Hanc, A., Niedzielski, P. (2019): Cadmium mobility in a circular food-to-waste-to-food system and the use of a cultivated mushroom (*Agaricus subrufescens*) as a remediation agent. Journal of Environmental Management: 245: 48-54.

Straatsma, G., Gerrits, J. P. G., Thissen, J. T. N. M., Amsing, J. G. M., Loeffen, H., Van Griensven, L. J. L. D (2000): Adjustment of the composting process for mushroom cultivation based on initial substrate composition. Bioresource Technology: 72:67–74.

Sun, L., Liu, G., Yang, M., Zhuang, Y. (2012): Bioaccessibility of cadmium in fresh and cooked *Agaricus blazei* Murill assessed by in vitro biomimetic digestion system. Food and Chemical Toxicology: 50(5):1729–1733, Http://Dx.Doi.Org/10.1016/J.Fct.2012.02.044.

Thomet, U., Vogel, E., Krähenbühl, U. (1999): The uptake of cadmium and zinc by mycelia and their accumulation in mycelia and fruiting bodies of edible mushrooms. European Food Research and Technology: 209(5):317-324.

Wasser, S. P., Didukh, M. Y., Amazonas, M. A. L, Nevo, E., Stamets, P., Eira, A. F. (2002): Is a widely cultivated culinary-medicinal royal sun *Agaricus* (The Himematsutake Mushroom) indeed *Agaricus blazei* Murrill? International Journal of Medicinal Mushrooms: 4:267–290.

World Health Organization (1982): Evaluation of certain food additives and contaminants. 26th report of the Joint of FAO/WHO Expert Committee of Food Additives. WHO Technical Report Series. 683. Geneva.

Zhou, J. L. (2002): Zn biosorption by *Rhizopus arrhizus* and other fungi. Applied Microbiology and Biotechnology: 51:686–693.

Zou, X. (2005): Effects of Zn supplementation on the growth, amino acid composition, polysaccharide yields and anti-tumour activity of *Agaricus brasiliensis*. World Journal of Microbiology and Biotechnology: 21(3):261-264.