Nutritional and Food Safety Aspects Related to the Consumption of Edible Mushrooms from Dambovita County in Correlation with their Levels of Some Essential and Non-essential Metals

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Ten edible mushroom species collected from four sites of Dambovita County, Romania, were analyzed through ICP-MS technique in order to evaluate their content in Fe, Cu, Zn, Pb and Cd, both in cap and stipe. Wild growing species (Russula vesca, Russula alutacea, Macrolepiota procera, Cantharellus cibarius, Boletus edulis, Agaricus campestris and Pleurotus ostreatus) and cultivated species (Pleurotus ostreatus, Agaricus bisporus white and Agaricus bisporus brown) were taken into account. The data related to the elemental composition of the fruiting bodies of mushrooms are important to be known having in view the nutritional and food safety related aspects. Higher concentrations of copper, iron and zinc have been found in Pleurotus ostreatus (cap), Macrolepiota procera (stipe) and Pleurotus ostreatus cultivated (cap) respectively, as follows: $43.90 \pm 0.96 \ \mu g/g \ d.w.$, $715.15 \pm 4.52 \ \mu g/g \ d.w.$ and $379.33 \pm 2.05 \ \mu g/g \ d.w.$ Although significant Health Risk Index were determined for lead in Agaricus campestris (about 0.1 both in cap and stipe) and for cadmium in Pleurotus ostreatus (0.58 in cap), these values are under the regulated PTWI limits for adult person.

Keywords: mushroom species, CP-MS technique, Russula vesca, Russula alutacea, Macrolepiota procera, Cantharellus cibarius, Boletus edulis, Agaricus campestris, Pleurotus ostreatus

Nowadays the food safety represents a major concern for the national and European authorities and the people as well. This is due to the higher incidence of diseases caused by biological hazards and by different chemical substances too. From the last category, extremely heterogeneous, heavy metals represent significant health threats within the food chain, so that at the European level there are a lot of concerns in order to keep under control their maximum level in foodstuffs [1].

By another hand, the nutritious foods represent a vital component of food security, which means that a balanced profile of nutrients and an adequate intake of them are compulsory in order to maintain a healthy lifestyle of the people.

Mushrooms' consumption has become increasingly important in the last years, these ones being considered a good source of proteins, essential amino acids, carbohydrates, fibers, vitamins, minerals and antioxidants [2, 3]. Wild growing mushrooms are very popular in Romania.

Heavy metals are defined as having specific density higher than 5 g/cm³ [4], but the collective term includes now cadmium, chromium, copper, lead, nickel, molybdenum, zinc. They are very harmful because of their non-biodegradable nature and their potential to accumulate in fruiting body [5-8]. For the above mentioned reasons toxicological and environmental studies have prompted interest in the determination of toxic metals in different food [9], including mushrooms. As a result of uptake by edible mushrooms, heavy metals could enter within the food chain and could be the cause for health hazard. Thus, the aim of this research was to investigate the levels of some essential and non-essential metals (Fe, Cu, Zn, Pb and Cd) by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). Finally, the contribution of the mushrooms to the daily intake of the above mentioned trace metals was evaluated.

Experimental part

Materials and methods

Sampling procedure

Mushroom species were collected according to Codex Methods of Sampling [11]. The sampling points were chosen so that to fulfill the following conditions: rural areas without industrial pollution, quite far from roads with car traffic, that do not fall under potential pollution with products used in the intensive agriculture (pesticides, fertilizers and so on).

Sample preparation

The collected fresh fruiting bodies of mushrooms were carefully cleaned by soil and vegetal wastes with deionized water. Then, they were cutted in small pieces with a plastic knife and dried at 60°C for 24 - 30 h, until the constant

Some *Agaricus* and *Macrolepiota* species from genera accumulate high levels of cadmium even in the unpolluted and mildly polluted areas, so that consumption of the accumulating species should be restricted [10].

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weight. Binder drying system was used in the above mentioned scope. The dried material was grinded and a fine powder was obtained. Each sample belonging to a certain species of mushroom was weighed and analyzed by ICP-MS spectrometry.

About 200 mg of each powdered sample (stipe and cap from each mushroom species) were digested in Teflon vessels with 5 mL hydrogen peroxide (31% H₂O₂, Sigma Aldrich) and 3 mL nitric acid (65% HNO₃, Sigma Aldrich). The TOPwave microwave-assisted pressure digestion system (Analytik Jena) was used.

After digestion the vessels were cooled for 30 minutes at room temperature. The solutions obtained were filtered and brought with deionized double-distilled water to 50 mL graduated flasks.

Inductively Coupled Plasma-Mass Spectrometry (ICP-MS)

The elemental content of the mushroom species was determined using a Thermo Scientific iCAP Qc ICP-MS system. The measurements were performed in triplicate in the standard mode (STD), using the Qtegra Intelligent Scientific Data Solution. The relative standard deviation (RSD) values were less than 10%, as follows: 0.5-5.9% for Fe, 0.4-3.6% for Cu, 0.4-4.6% for Zn, 0.6-6.6% for Pb and 1.4-6.3% for Cd respectively. The data were expressed as $\mu g/g$ dried weight (d.w.) material.

Data calculation

The Daily Intake of Metal (*DIM*) was calculated using the above mentioned equation:

$$DIM = CM \times \frac{DFI}{BAW}$$

where:

CM - heavy metal concentrations in mushrooms, $\mu g/g$ d.w.

DFI and *BAW* - daily intake of mushrooms and average body weight, respectively

According [12], 60 kg of body weight was used for intake calculations as the weight of an average consumer. Also, for intake calculations, usually a 300 g portion of fresh mushrooms per meal was assumed, that it means 30 g of dry matter [12]:

$WI = DIM \times 7$

WI - weekly intake (μ g/g body weight)

Provisional tolerable weekly intake (PTWI) is a value established by FAO and WHO [13], being defined as the maximum quantity of contaminant that may a consumer weighing 60 kg intake per week.

Health Risk Index (*HRI*) is defined as the ratio between the daily intake of metals (*DIM*) and the reference oral dose (*RfD*), and can be calculated according with above equation:

$$HRI = \frac{DIM}{RfD}$$

The reference oral doses for Pb and Cd are 1 and 4 μ g. kg¹.day¹ respectively, whereas the estimated exposure was obtained by dividing the daily intake of heavy metals to their safe limits. If the value of HRI is less than *1*, it is assumed that population is not exposed to risk through consumption.

Results and discussions

The essential and non-essential metals content in ten edible mushroom species is shown in figure 1 and figure 2. The results showed that mushrooms accumulate elements in different levels, in caps and stipes, depending on its species. According to [14], the element concentrations are primarily species-dependent and also related to collecting site of the sample, age of fruit bodies and mycelium and distance from a source of pollution.

Copper is known for its ability to adjust the reproductive system, glandular and nervous processes too. Copper is part of metalloenzymes which acting as oxidases to effect the molecular oxygen reduction. It accumulates in the liver first and increases the chances of appearance of free radicals, which in turn damages organs and give rise to the development of diseases and health weakening., levels of Cu ranged from $7.59\pm0.05 \ \mu g/g$ d.w. (cap of *Cantharellus cibarius*) to $48.90\pm0.85 \ \mu g/g$ d.w., the highest level being determined in cap of wild growing *Pleurotus ostreatus*. Excepting *Cantharellus cibarius*, the concentration of copper was higher in cap than in stipe in 9 individual species from 10, higher differences between these anatomic parts being determined for *Russula vesca* and *Macrolepiota Procera* (3 times more).

The reference values known as Dietary Reference Intakes (DRIs) include the Estimated Average Requirement (EAR), Recommended Dietary Allowance (RDA), Adequate Intake (AI), and Tolerable Upper Intake Level (UL) [15].



Fig. 1. The iron, copper and zinc content of mushroom species (C - cap, S - stipe)

The Recommended Dietary Allowance (RDA) for copper for men and women (between 19 and more than 70 years) is by 700 μ g/day [16]. According [17], the recommended intake of copper from food in Romania is by 1.20 mg/day for female and 1.70 mg/day for men, both aged between 31-50 years.

As it can be observed in figure 3, the Daily Intake of copper varied between $4.40 \pm 0.08 \,\mu$ g/kg body weight (cap of *Cantharellus cibarius*) to $31.11 \pm 0.75 \,\mu$ g/kg body weight (cap of *Agaricus bisporus white*), closer to the values mentioned below for a person weighted by 60 kg.

The Tolerable Upper Intake Level (UL) indicates the possibility of overconsumption of a certain nutrient. If an individual's usual nutrient intake remains below the UL, there is little or no risk of adverse effects from excessive intake. The Tolerable Upper Intake Level (UL) of copper for adults is 10 mg/day, a value based on protection from liver damage as the critical adverse effect [16]. Thus, copper may be causing ADHD (attention deficit hyperactivity disorder) symptoms occur to children [18].

The copper levels determined in the present study for *Cantharellus cibarius* were lower than those reported in literature ($32.6 \pm 1.3 \mu g/g$ d.w. found by [19] through analysis of eight different species of wild mushrooms from Greece and $89.5 \mu g/g$ d.w. established by [20] in the fruiting bodies of some macrofungi growing in the East Black Sea region of Turkey, respectively). According [19], mushrooms should be considered a nutritional source of copper, their content in this metal being, in general, higher than in vegetables.

Due to its biological significance, zinc is widespread among living organisms. Thus, it is a component of such enzymes, important in the maintenance of the structural integrity of proteins and also in the regulation of the gene expression. It plays a role in immune function. There are three biological functions of zinc: catalytic, structural, and regulatory. During pregnancy, childhood, and adolescence, zinc supports normal growth and development. Amino acids (histidine, lysine and sulfur amino acids) and proteins from food promote the absorption of zinc. The body has no specialized zinc storage system. The excess of zinc in the body can lead to a reduction in HDL cholesterol and inhibiting the absorption of copper in the body. Mushrooms are known as zinc accumulators [21].

In the present study the highest level of Zn was determined in cap of *Pleurotus ostreatus* cultivated $(379.33\pm2.35\,\mu g/g\,d.w.)$, this value being by 55 times higher than in the cap of *Cantharellus cibarius*. The next mushroom species in decreasing order of Zn concentration was *Russula vesca* (53.37±1.05 $\mu g/g\,d.w.$). In 6 from the all 10 species analyzed the concentration of Zn was higher in cap than in stipe, the differences not being obvious as in the case of the copper belonging to the same species.

The Dietary Reference Intakes (DRIs) of zinc for adults is 6.80 ± 0.03 mg/ day for women and 9.40 ± 0.04 mg/day for men. The Tolerable Upper Intake Level (UL) for adults is 40 mg/day [16], a value based on reduction in erythrocyte copper-zinc superoxide dismutase activity. Zinc can become toxic to 150-450 mg/day. It was found out that the Daily Intake of Zinc in the case of *Pleurotus ostreatus* cultivated (240.87±2.75 µg/kg body weight) can be potential dangerous from the safety point of view, the concentration determined being higher in relationship with the DRI of zinc for men.

Iron is the component of a number of proteins, including enzymes and hemoglobin, the latter being an ironcontaining oxygen-transport metalloprotein. It is present in the brain from very early in life, participating in the neural processes. Iron is a component of muscle and blood and it is involved in growth and immune function too. Vitamin C increases the absorption of iron.

In this study the iron concentration ranged between $21.28\pm0.12 \mu g/g d.w.$ (stipe of *Russula vesca*) and $715.15\pm4.52 \mu g/g d.w.$ (stipe of *Macrolepiota procera*). *Boletus edulis* (stipe) and *Cantharellus cibarius* (stipe) were the next mushroom species observed in figure 1, with the iron content by 4 times smaller than those determined for *Macrolepiota procera*.

Fe concentrations mentioned in this paper are in agreement with those reported previously in literature. Thus, the range of Fe concentrations found by [22] in edible mushrooms collected from Yunnan Province, China, was 2.00-826.50 mg/kg, the highest value being determined for *Tuber indicum Cooke et Massee* and the lowest for *Boletus edulis Bull*. [23] reported 11.7-135.8 mg Fe/kg d.w. in mushrooms originated in island of Lesvos, Greece.

According [16] the Dietary Reference Intakes (DRIs) of iron for males (aged between 19 and more than 70 years) is by 6 mg/day and decreases from 8.10 to 5.00 mg/day for females (aged between 19 and more than 70 years). The Tolerable Upper Intake Level (UL) of iron for adults is 45 mg/day, a level based on gastrointestinal distress as an adverse effect.

Excess of iron in body tissues leads to oxidation, to organ damage and disease: large amounts of blood sugar, diabetes, cirrhosis of the liver, irregular heartbeat.

The Daily Intake of metal in the case of iron from *Macrolepiota procera* was $457.69\pm2.55 \ \mu$ g/kg body, value that could means an intake by $27.46\pm0.07 \$ mg/day (almost 61% from the Tolerable Upper Intake Level).

Lead is a toxic and non-essential heavy metal. It accumulates in bones and produces progressive toxicity. Tiredness, weight loss, sleeplessness are health disorders determined by lead. Exposure to lead can occur by contaminated dust, air, water, food, the children being more sensitive. The poisoning with lead affects the nervous system, kidneys, cardiovascular and reproductive systems. Lead is considered a common environmental pollutant, as well.

According to the data from figure 2, the minimum concentration of lead was found as $0.27\pm0.02 \ \mu g/g$ d.w. (cap of *Macrolepiota procera*), while the maximum one was determined both in cap and stipe of *Agaricus campestris* ($2.50\pm0.03 \ \mu g/g$ d.w.). A different situation was registered comparatively with the essential metals concentration, in the sense that it is obvious that lead has accumulated in stipe.

For *Cantharellus cibarius* lead contents in literature were reported to be between 1.00–2.00 µg/g d.w. [12], [24]. Using the AAS method, lowest (undetectable) concentration was determined by [19] for the same species growing in Greece.

Cadmium is characterized by a serious toxicity and it accumulates mainly in spleen, liver and kidneys, inhibiting many life processes. The overexposure may occur even if trace quantities of cadmium are found, due to its low permissible exposure limit. It can be taken into account that mushrooms can be very rich in cadmium, which is a byproduct of the production of zinc and lead [19]. According with data reported in scientific literature [12], the cadmium level in blood serum increases considerably with mushroom consumption. For this reason cadmium seems to be the most deleterious among heavy metals in the mushrooms.

The cadmium content in the mushroom species analyzed was, with the exception of *Pleurotus ostreatus* (cap) smaller that the lead concentration in the same





Fig. 3. Daily Intake of Metal Values for mushroom species (C - cap, S - stipe)

samples. It ranged between $0.05\pm0.01 \ \mu\text{g/g}$ d.w. (cap of *Cantharellus cibarius*) and $0.91\pm0.01 \ \mu\text{g/g}$ d.w. (cap of wild growing *Pleurotus ostreatus*).

8-times and even 39-times higher concentrations of cadmium than in the present study were reported by [19] and [20] respectively, for *Cantharellus cibarius*.

The Daily Intake of Metal Values for all the mushroom species analyzed are shown in figure 3, being obvious that for essential metals can be taken into account *Macrolepiota procera* (Fe), *Pleurotus ostreatus* cultivated (Zn) and *Agaricus bisporus white* (Cu). Health risk assessment was made having in view the Health Risk Index as indicator (Figure 4).

Closer values for *Cantharellus cibarius* were reported by [19] for the daily metal intakes by a normal, 60 kg consumer, expressed in mg/serving, as follows: for Fe 3.57 ± 0.19 , for Cu 0.978 ± 0.04 and for Zn 1.62 ± 0.08 respectively.

The PTWI for lead is by $25\mu g/kg$ b.w. and for cadmium is by 7 $\mu g/kg$ b.w. [12]. In this study the Weekly Intake values for lead and cadmium were lower than the above mentioned indicators. Thus, the PTWI for lead were 10.00 $\mu g/g$ b.w. and 10.75 $\mu g/g$ b.w. for stipe and cap of *Agaricus campestris* respectively, while the Health Risk Index for this mushroom species was by 0.1 (10 times lower than the unsafe value). The Weekly Intake for cadmium was 4.09 $\mu g/g$ b.w. for the mushroom species mentioned the last one, while its Health Risk Index was by 0.58 (i.e. 5.8 times lower than the unsafe value). Similarly results related to the Pb and Cd concentrations, below their detection limits, were found by [25] in two oyster mushroom (*Pleurotus ostreatus* and *Pleurotus florida*). For *Cantharellus cibarius* growing in Greece value by 0.012 ± 0.00 daily Cd intakes by a normal, 60 kg consumer in mg/serving, was determined [19].

On the contrary, in mushroom samples collected from polluted geographical area, different authors reported Health Risk Indexes greater than "1" for Pb and Cd. Thus, [26] found out that $PTWI_{Cd}$ and that $PTWI_{Pb}$ values were exceeded in several samples originated from surroundigs of the historical mining and metal processing area (4.11 fold and 1.35 fold respectively for *Suillus grevillei*). Also, in Yunnan Province, China, Pb and Cd in most of the edible mushrooms analyzed exceeded the limit levels, concrete policies to protect the wild edible mushroom resources being recommended [22]. Having in view that HRI_{Cd} values were greater than 1 in species including *Boletus edulis* and *Russula vinosa*, while HRI_{Pb} of all the samples were less than 1, it was concluded that Cd posed a potential human risk, while Pb did not.

Conclusions

The levels of some essential and non-essential metals (Cu, Fe, Zn, Pb and Cd) of ten edible mushroom species (cultivated and wild growing) in cap and stipe too were determined using ICP-MS technique [27-34]. In the present study was observed that the essential metals have been accumulated in cap rather in stipe, while the toxic metals vice versa.

Although differences quite significant were established between species concerning to their metals contents, it



Fig. 4. The Health Risk Index (HRI) of the analyzed mushroom species (C cap, S - stipe)

can be stated that the sampling areas originated from Dambovita County can provide mushrooms as valuable food resource for well-balanced diets, having in view their content of functional minerals. According the data presented, the areas were not polluted, the levels of the heavy metals Pb and Cd in all studied species being sufficiently low and didn't pose health risks. However, an increased attention should to be paid to wild growing species *Agaricus campestris* and *Pleurotus ostreatus*, characterized by a higher capacity of accumulation of the non-essential metals Pb and Cd. Further studies related to the potential synergistic effects between these metals will be taken into account by authors.

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