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INFLUENCE OF CHEMICAL COMPOSITION OF SOIL ON METAL ACCUMULATION IN EDIBLE MUSHROOM SPECIES OF *RUSSULA* GENUS

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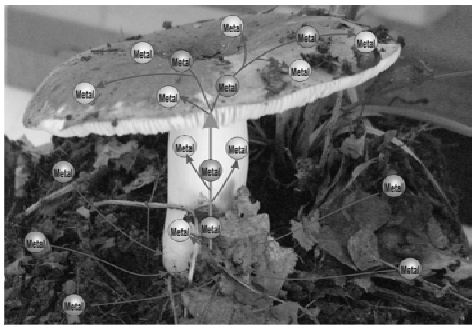
In this study we analysed edible mushrooms species of *Russula* genus, which were harvested from six different woods (in terms of distance to anthropogenic pollution sources) and four types of forestry ecosystems (in terms of dominant species of trees and vegetation). The metal concentration in macromycetes and soil samples was determined by Energy Dispersive X-Ray Fluorescence method. Metal concentrations vary in a wide range between species of *Russula* genus and are different in the morphological parts of fruiting body. The highest concentrations were found in cap of *R. alutacea* for Fe, Zn and Mn, in cap of *R. lutea* for Cu and in stipe of *R. alutacea* for Ni. A pattern of metal bioaccumulation in mushrooms is shown by the correlation loadings of PCA analysis, which explains correlations between chemical composition of soil and metal bioaccumulation in fruiting body, case sensitive for caps and stipes.



INTRODUCTION

The majority of macromycetes species from the *Russula* genus are rated as edible, some are rated as having a low edibility or as non-edible (because of the bad taste or low nutritional properties), but none of these species are rated as toxic or poisoning. The wild growing mushrooms are frequently consumed by humans or some animals. According with this aspect, it is very important to know the level of trace element concentration in mushrooms and factors that influence the metal bioaccumulation in fruiting body. Some of the trace elements are biologically toxic and can affect the health of humans and animals because of accumulation and persistence in the food chain.[1] Trace element concentrations above threshold limit may lead to morphological abnormalities; reduce growth and increase of mutagenic effects in human body or even mortality.[2]

Wild-growing edible mushrooms may also accumulate high concentrations of toxic metals, metalloids or even radio nuclides.[3] Numerous studies concerning the trace elements concentrations in fungal fruiting body were published.[3-8] All of them have showed the capacity of mushrooms to accumulate high concentrations of heavy metals. Some authors have reviewed the literature about the heavy metal concentration in mushrooms and have presented few data about the metal concentration in mushrooms from the *Russula* Genus.[9,10] Only few studies have references about trace element concentration in species from the *Russula* genus. Some of these papers are presenting the influence of municipal sludge application on metal concentration in edible

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mushrooms.[11,12] In a study on 87 species of ectomycorrhizal fungi, Borovička and Řanda[13] have indicated that *Russula atropurpurea* was confirmed as an effective Zn-accumulating species.

Some authors presented the possibility to use macromycetes as bioindicators of soil contamination or even as agents in bioremediation technologies. According with this hypothesis, in the last decades, researchers have studied the capacity of mushrooms to accumulate trace metals and have shaped distribution patterns of these metals in fruiting body, in correlation with the environmental factors such as chemical composition of soil,[14] habitats and location.[15] These studies revealed that an addition of Ca and K in substrate influenced the accumulation and transport (from stipe to cap) of trace elements in the fruiting body. Also they showed that the accumulation pattern is different for the epiphytic mushrooms to those growing on organic matter. Huang et al.[16] studied the cadmium bioaccumulation in *Agaricus* and stated that the accumulation of this metal was positively associated with a phosphorus uptake.

Purpose of this paper is to study the trace element concentration in species of *Russula* genus to reveal the capacity of these species to accumulate trace elements and risk of metal bioaccumulation in the food chain and human health. Trace elements concentration in wild growing edible mushrooms was correlated with the chemical composition of soil (macronutrients, trace elements and pH) to reveal the influence of geochemical factors on trace element bioaccumulation in the fruiting body of *Russula* macromycetes. Part of the analysed species was presented in a previous paper to reveal the risk of metal bioaccumulation for human consumption of wild growing mushrooms.[17] This study brings new data about major and trace element concentration in wild growing mushrooms and a model of environmental geochemistry influence on trace element concentration in wild growing edible mushrooms belonging to a wide spread group of ectomycorrhizal fungi species.

RESULTS AND DISCUSSION

Trace elements concentration in mushrooms

Dry matter percentage of mushrooms is species-dependent, but also depends on the age of fruiting body and meteorological conditions. Analysed mushrooms were mature with completed spore forming parts. Dry matter was determined for each individual fruiting body, separately for caps and stipes. The differences between caps and stipes of the same fruiting body were not significant and the mean values for dry matters were: *R. cyanoxantha* – 6.9±0.36%, *R. virescens* – 5.82±0.12%, *R. vesca* – 7.95±0.32%, *R. nigrescens* – 9.42±0.48%, *R. foetens* – 7.38±0.9%, *R. chloroides* – 8.34±1.21%, *R. alutacea* – 15.43±1.34%, *R. lepida* – 18.20±0.16%, *R. aeruginea* – 20.00±3.82% and *R. lutea* – 12.00±0.54%.

Mean concentrations for the analysed trace elements in the fruiting body, with details for each individual species, in cap and stipe, are indicated in Table 1. Each analysed species of mushrooms showed different concentration according to its metabolic activities, but with statistically similarities of bioaccumulation in caps and stipes for each species. Mean trace element concentrations, across all tested species of fungi, were in order: Fe > Zn > Mn > Cu > Ni. The highest concentrations were found for Fe in the cap of *R. alutacea* (689.97 mg/kg), for Cu in the cap of *R. lutea* (34.11 mg/kg), for Zn in the cap of *R. alutacea* (146.75 mg/kg), for Mn in the cap of *R. alutacea* (219.16 mg/kg) and for Ni in the stipe of *R. alutacea* (2.15 mg/kg).

Iron is very abundant in biology and iron-proteins are found in all living organisms, including mushrooms. Despite the important role of iron in living organisms, a high concentration of this metal in daily intake may cause serious abnormalities of gastrointestinal system.[17] For this reason we chose to study this element in edible mushrooms.[18] By analysing species of *Russula* genus we found a range of Fe concentration between 29.41 – 689.97 mg/kg. These results are in agreement with values from previous studies: 31.3 – 1190 mg/kg,[19] 102 – 1580 mg/kg[8] and 30 – 150 mg/kg.[20] For the majority of studied species we observed statistical similarities in metal accumulation comparing the caps and stipes. An exception was *R. lutea* species which showed a statistical difference in accumulation of iron comparing the caps and stipes.

R. lutea species showed the same statistical differences between cap and stipe bioaccumulation of copper. The Cu concentration in the accumulating species of mushrooms are usually 100-300 mg/kg of dry matter, which is not considered a risk for human health[20] and a concentration higher than those in vegetable should

be considered as a nutritional source of this element.[18,21] The results obtained on studied mushrooms showed a copper concentration ranging from 2.86 to 34.11 mg/kg. These values are lower than the normal values for this element, and are similar with values from previous studies: 15.5-73.8 mg/kg,[21] 12-181 mg/kg[22] or 13.4-50.6 mg/kg.[8] These results demonstrated that analyzed species are not good accumulators for Cu.[18]

Zinc is an important trace element for a normal growth of humans and mushrooms are known as good accumulators of this element. The results obtained about zinc concentration in mushrooms range from 19.57 to 146.75 mg/kg. These values are in agreement with the concentrations from literature which have been reported in the range of 28.6 to 179.0 mg/kg,[10] 43.5-205.0 mg/kg[21] or 45-188 mg/kg.[22] Zinc and copper concentrations in most studied species confirmed Kalač's statement[9] that the most elements are distributed in higher concentration in cap when comparing with the stipe of fruiting body.

Manganese is easy absorbed by biosystems and fast transported to the upper parts. A deficiency of this metal was observed at biosystems grown on calcareous soils. Normal values for Mn concentration in mushrooms range between 10 and 60 mg/kg. For some species, like *Boletus edulis* and *Macrolepiota procera*, Mn concentration may exceed 100 mg/kg.[9,21] The results obtained in our study are in agreement with previous studies, the most analysed species indicated a Mn concentration lower that 100 mg/kg. *R. alutacea* and *R. lepida* species indicated a Mn concentration that exceeded this limit and accumulated Mn up to 430 mg/kg.

Nickel bioabsorption depends on the chemical forms of this element in soil and on the pH of soil.[23] Normally, Ni concentration in mushrooms does not exceed 15 mg/kg[9] as the results showed for the species of *Russula* genus (Table 1), but some authors found a concentration of 58.6 mg/kg in *Coprinus comatus* species.[24] Results of Ni concentration obtained in our study were between the detection limit and 2.15 mg/kg, which confirmed the study of Chen et al.[25] who have indicated that *R. virescens* species had the lowest level of Ni concentration (0.11 mg/kg). Similar results were found for *R. delica* by Isildak et al. – 2.56 µg/g, dry weight[26] and by Semreen and Aboul-Enein – 12.65 µg/g, dry weight.[27]

Results about the trace element concentration in fruiting body of edible mushrooms harvested from an area with any anthropogenic impact showed lower values when comparing with the results of metal concentration in edible mushrooms which grew in a region with the same pedo-climatic characteristics, close to a metal smelter or close to high traffic roads.[28]

Table 1

Metal concentration (mg/kg dry matter) in mushroom species of *Russula* Genus

Species \ Metal		Fe	Cu	Zn	Mn	Ni
R. cyanoxantha	C	308.0±70.2 ^{a}	18.1±18.0 ^{a}	86.0±22.1 ^{a}	35.9±31.2 ^{a}	2.1±2.7 ^{a}
	S	156.1±125.4 ^{a}	8.6±0.8 ^{a}	74.0±21.8 ^{b}	12.9±11.3 ^{a}	1.1±0.7 ^{a}
R.virescens	C	62.6±3.4 ^{a}	6.6±0.8 ^{a}	64.9±31.7 ^{a}	11.1±15.7 ^{a}	0.3±0.4 ^{a}
	S	29.4±41.6 ^{a}	2.9±4.0 ^{a}	34.5±48.8 ^{a}	9.4±13.3 ^{a}	0.3±0.4 ^{a}
R.vesca	C	178.5±113.1 ^{a}	13.3±11.1 ^{a}	90.1±51.1 ^{a}	18.5±32.1 ^{a}	1.1±0.9 ^{a}
	S	114.0±25.6 ^{a}	14.3±8.5 ^{a}	106.1±46.3 ^{a}	19.8±20.8 ^{a}	1.1±0.5 ^{a}
R.nigrescens	C	132.1±86.0 ^{a}	9.0±5.3 ^{a}	30.4±15.1 ^{a}	58.4±37.0	1.1±0.7 ^{a}
	S	56.2±7.7 ^{a}	4.9±0.2 ^{a}	19.6±5.7 ^{a}	ND	0.6±0.05 ^{a}
R.foetens	C	201.0±127.5 ^{a}	17.7±8.1 ^{a}	89.4±52.1 ^{a}	37.7±32.2 ^{a}	1.5±1.1 ^{a}
	S	141.5±129.3 ^{a}	17.8±13.1 ^{a}	77.9±64.0 ^{a}	35.8±46.8 ^{a}	1.3±1.2 ^{a}
R.chloroides	C	81.6±12.1	4.1±1.5	61.1±3.5	7.8±11.1	ND
	S	NA	NA	NA	NA	NA
R. alutacea	C	690.0±725.8 ^{a}	28.0±19.4 ^{a}	146.7±61.5 ^{a}	219.2±309.9 ^{a}	1.6±0.2 ^{a}
	S	79.8±20.4 ^{a}	29.6±19.7 ^{a}	103.6±11.5 ^{a}	183.1±259.0 ^{a}	2.2±0.6 ^{a}
R. lepida	C	508.2±117.1 ^{a}	9.1±0.8 ^{a}	69.0±4.5 ^{a}	209.6±75.5 ^{a}	1.5±0.0 ^{a}
	S	306.2±30.7 ^{a}	10.7±0.7 ^{a}	69.2±5. 6 ^{a}	84.7±14.3 ^{a}	1.7±0.3 ^{a}
R. aeruginea	C	275.4±23.6 ^{a}	20.7±9.1 ^{a}	79.1±35.2 ^{a}	62.9±89.0 ^{a}	1.6±0.4 ^{a}
	S	318.6±24.5 ^{a}	23.7±7.5 ^{a}	92.3±50.3 ^{a}	55.0±77.8 ^{a}	1.8±0.8 ^{a}
R. lutea	C	306.4±13.9 ^{a}	34.1±1.0 ^{a}	51.4±2.2 ^{a}	ND	0.3±0.04 ^{a}
	S	97.3±15.1 ^{b}	29.2±0.9 ^{b}	111.3±4.4 ^{a}	ND	1.4±0.1 ^{a}

C – cap; S – stipe; ND – not detected; NA – not analysed

Differences in elemental contents between caps and stipes of each species were tested with Paired Samples Test: $^{a...b}$ – $p < 0.01$; $^{a...a}$ – $p > 0.01$

Table 2

Chemical composition (mg/kg dry matter) and pH of soil in sampling subareas

Metals		Oak	Beech	Durmast	Hornbeam
Macro-elements	Ca	12580±2790	3745±537	10610±2750	6420±1493
	K	22630±3580	281±680	19760±2233	15630±1938
	P	1305±170	1192±139	1291±379	1485±326
	Mg	10970±8735	6743±629	14940±2946	9254±5470
Trace elements	Fe	43850±32120	25765±22310	57430±30590	88750±4968
	Cu	380±235	263±228	241±54	395±95
	Zn	910±1090	81±70	1132±1038	1567±272
	Mn	806±180	719±623	729±81	356±198
	Ni	ND	ND	3.59±4.49	0.41±0.34
pH		5.44±1.26	5.00±0.34	5.89±0.88	5.59±0.49

Chemical composition of soil

In the studied area, substrate of mushrooms contains 2% organic matter,[18] the mean pH_{KCl} was 5.63±0.96 (SD), cation exchange capacity (CEC) was 12.45 g/kg and the mean value of soil moisture was 36.23±2.54 (SD) due to a high ratio of leaf litter. Macronutrient and trace element concentrations in substrate vary across the sampling points, and have a high variability across the forest ecosystem types (Table 2). Higher concentration of calcium in the soil from oak and durmast forest ecosystems led to an increasing value of pH when compare with other two types of studied ecosystems. Potassium and magnesium concentrations were also higher in oak and durmast forests comparing with these metals concentrations in beech and hornbeam forests. The phosphorus concentration was similar across all types of studied forest ecosystems.

Iron concentration increased with increasing altitude and the highest concentrations were in hornbeam forest, at over 400 m altitude (88750±4968 mg/kg). Copper concentration had comparable values for all forest types, but the concentration decreased with increasing altitude and pH. The highest copper concentration was in hornbeam forest (395±95 mg/kg). Zinc concentration increased with increasing altitude and pH. The highest values of Zn concentration were in hornbeam forest, at over 400 m altitude. The mean trace metal content in soil was higher for Zn and Cu when comparing with the normal value for an organic soil – 57-100 mg/kg and 1-115 mg/kg respectively.[29]

Manganese concentration in substrate decreased with increasing altitude and pH. Except hornbeam subarea, in all sampling points, Mn concentration exceeded the threshold limit of normal values, 650 mg/kg.[30] The nickel was found below the detection limit of method, except in the soil from durmast and hornbeam forests. In these samples, the Ni concentration was 3.59±4.49 mg/kg and 0.41±0.34 mg/kg respectively. The results showed lower values comparing with the tolerance limit of an organic soil.[31]

Trace element concentration, such as copper and zinc, were correlated with the geochemical characteristics of studied region, because the anthropogenic impact is minimal.

Models of chemical composition of soil influence on trace metal bioaccumulation

Soil properties, such as pH, redox potential, organic matter content, clay mineralogy, CEC of the soil phase, competition with other metal ions and soil solution influence the absorption and accumulation of metals in mushrooms.[32] Metal concentration and pH are the most important factors which affect the bioavailability of trace elements for mushrooms.

For each trace element, chemical composition of soil has a different influence on bioabsorption and bioaccumulation in wild growing mushrooms. The model of influence of geochemical factors on the trace element accumulation was indicated by the correlation loadings of PCA analysis. This analysis explain the correlations between chemical composition of soil (macronutrients, trace elements and pH) and trace metal accumulation in fruiting body of mushrooms, case sensitive for cap and stipe (Fig. 1). Correlations of geochemical factors and trace elements (Fe, Cu, Zn, Mn and Ni) bioaccumulation were explained 92% by the first principal component (PC) and 5% by the second PC.

Influence of geochemical factors on absorption and accumulation of iron in mushrooms of *Russula* genus was weak to moderate; the highest correlation was between Fe concentration in substrate and accumulation of this metal in the cap of fruiting body (0.5647). The results are statistically significant at a level lower that 0.1% (Table 3). Zinc concentration in substrate showed a moderate positive correlation with the iron bioaccumulation (0.4325 for cap and 0.5433 for stipe). Copper concentration in substrate indicated also a positive weak-moderate correlation with iron bioaccumulation. All these positive correlations indicated that the presence of some trace elements in soil had a synergic influence on the bioaccumulation of Fe in wild growing species of *Russula* genus.

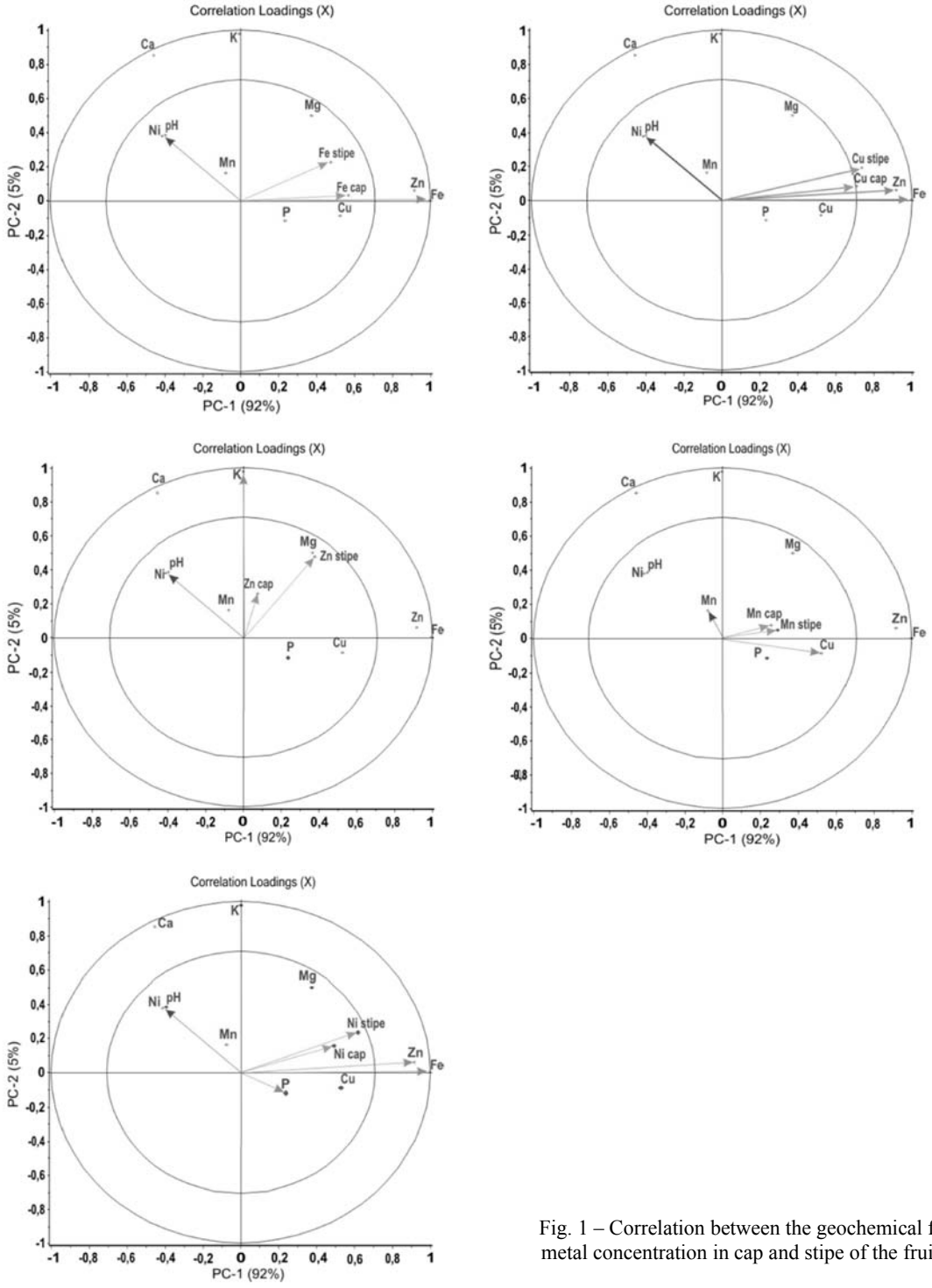

Fig. 1 – Correlation between the geochemical factors and metal concentration in cap and stipe of the fruiting body.

Table 3

Pearson coefficient of correlation between chemical composition of soil and metal concentration in mushrooms from *Russula* genus

Metal		Fe	Cu	Zn	Mn	Ni
Ca	C	-0.2285 [c]	-0.2404 [c]	0.2329 [c]	-0.0882 [c]	-0.0203 [c]
	S	-0.0047 [c]	-0.1996 [c]	0.2890 [c]	-0.1027 [c]	-0.0659 [c]
K	C	0.0506 [c]	0.1146 [c]	0.2841 [c]	0.0783 [c]	0.2443 [c]
	S	0.2895 [c]	0.1761 [c]	0.5042 [c]	0.0699 [c]	0.2934 [c]
P	C	0.0172 [b]	0.3984 [b]	-0.0626 [b]	-0.0861 [b]	0.7166 [b]
	S	0.3278 [b]	-0.0879 [b]	-0.0499 [b]	-0.0654 [b]	0.2261 [b]
Mg	C	0.1303 [c]	0.1953 [c]	0.0005 [c]	0.1359 [c]	-0.0319 [c]
	S	0.0745 [c]	0.3988 [c]	0.1938 [c]	0.0491 [c]	0.1421 [c]
Fe	C	0.5647 [c]	0.7044 [c]	0.0745 [c]	0.2566 [c]	0.4884 [c]
	S	0.4730 [c]	0.7305 [c]	0.3781 [c]	0.2886 [c]	0.6134 [c]
Cu	C	0.3067	0.3021 [c]	0.2906 [c]	0.2297 [c]	0.2485 [c]
	S	0.3671 [c]	0.3099 [c]	0.0548 [c]	0.3779 [c]	0.4027 [c]
Zn	C	0.4325 [c]	0.6561 [c]	-0.0659 [c]	0.1981 [c]	0.3889 [c]
	S	0.5433 [c]	0.6729 [c]	0.3688 [c]	0.1976 [c]	0.6103 [c]
Mn	C	-0.2992 [c]	-0.1866 [c]	-0.0605 [c]	-0.1733 [c]	-0.1719 [c]
	S	0.0452 [c]	-0.1667 [c]	0.1023 [c]	-0.2479 [c]	-0.1634 [c]
Ni	C	-0.2628 [c]	-0.3359 [c]	-0.1098 [c]	-0.0136 [b]	-0.1537
	S	-0.3069 [c]	-0.3831 [c]	-0.2253 [c]	-0.1652 [a]	-0.3464
pH	C	-0.1043 [c]	-0.3988 [c]	0.0002 [c]	0.0297 [b]	-0.2131 [c]
	S	-0.4803 [c]	-0.3350 [c]	-0.1945 [c]	0.0044 [a]	-0.2761 [c]

C – cap; S – stipe

[a] - $p < 0.05$; [b] - $p < 0.01$; [c] - $p < 0.001$

Nickel and calcium concentration in substrate showed a negative weak-moderate influence on iron bioaccumulation, which means that Ni and Ca had an antagonistic influence on iron bioabsorption and accumulation. Macronutrients in soil indicated a weak influence on Fe bioaccumulation, and correlations were positive for K, P and Mg. The pH had a negative correlation with the Fe bioaccumulation. The strongest correlation was showed by pH and iron accumulation in the stipe of fruiting body. This led to the idea that in a soil condition with higher pH, the mobility of Fe increased and this metal was bioaccumulated in the upper part of fruiting body.

Copper accumulation in studied mushrooms was positively influenced by the metal concentration in substrate, but with weak-moderate level of correlation (<0.5). The accumulation of this metal was negatively influenced by the pH, with a weak-moderate correlation (-0.3988 for cap and 0.3350 for stipe), without statistically significant differences at level lower than 0.1%. Iron and zinc concentration in substrate showed a positive strong correlation (0.7 and 0.6 respectively) with the copper bioaccumulation. This may indicate a synergic influence of Fe and Zn on the absorption and bioaccumulation of Cu in wild fungi of *Russula* genus. These correlations had similar values for Cu accumulation in both cap and the stipe. A competition between Cu, Mn and Ni ions in soil was indicated by a negative weak-moderate correlation. Macronutrients in substrate showed a positive weak-moderate influence on Cu bioaccumulation in mushrooms, except calcium which showed a negative correlation.

Zinc accumulation in mushrooms of *Russula* genus was species dependent. Correlation between the chemical composition of soil and metal bioaccumulation was weak for the most analysed macronutrients and trace elements in soil, with statistically significant differences at level lower than 0.1%. Iron and zinc concentration in substrate had a positive weak-moderate influence on Zn accumulation in the stipe of mushrooms (0.3781 and 0.3688 respectively). The strongest influence on Zn bioaccumulation was associated with the K concentration in soil, which was indicated by a positive moderate correlation (0.5042).

Accumulation of manganese in fruiting body of studied mushrooms was differently influenced, for

cap and stipe, by geochemical factors. Manganese concentration in substrate influenced negatively the accumulation of Mn in both cap and stipe (-0.1733 and -0.2479 respectively). Differences were statistically significant at level lower than 5%. Except nickel, presence of trace elements in the substrate had a synergic influence (positive weak correlations) on the accumulation of Mn in *Russula* mushrooms. Both macronutrients in soil and the pH showed a very weak influence on Mn bioaccumulation.

Nickel accumulation in fruiting body of *Russula* species was negatively influenced by the pH and Ni concentration in substrate. An increasing of Ni concentration in substrate decreased the bioavailability of this element for mushrooms and the mobility within the fruiting body. Iron, copper and zinc concentration in substrate had a positive moderate influence on Ni bioaccumulation in analysed mushrooms, and correlations were stronger for the stipe of fruiting body (0.6134 for Fe, 0.4027 for Cu and 0.6103 for Zn). Potassium and phosphorus concentration in substrate showed a positive influence on Ni bioaccumulation, and the strongest influence was indicated by a positive correlation between phosphorus concentration in substrate and nickel accumulation in cap of fruiting body (0.7166), with statistically significant differences at level lower than 1%. Mobility of nickel within the fruiting body was positively associated with the phosphorus concentration in substrate.

EXPERIMENTAL

Sampling and preparation

The biological samples used in this study are represented by ten species of wild growing mushrooms of *Russula* Genus, edible or non-edible, harvested from a forest ecosystem, located at 15-20 km from Târgovişte City, Roumania, between 44°53′ - 44°57′ N, and 25°19′ - 25°24′ E (Fig. 2). Studied area is located at 10-15 km NW from a metal smelter, 3.5 km SW from an oil extraction platform and 2 km NE from a high traffic road. In terms of relatively high distance from the metallurgical pollution source, there is a weak influence of the sedimentable dusts. Oil extraction activities and traffic have also a weak influence on soil pollution because studied area is at least 200 meters inside the forest. We chose to study an area with minimal influence of any pollution source, because we intended to evaluate only the heavy metal accumulation induced by the geochemical composition of soil.

We studied two forest ecosystems of 75 and 25 km^2, with four subareas according with the dominant species of trees: Durmast Oak (*Quercus petraea*), Oak (*Quercus robur*), Beech (*Fagus sylvatica*) and Horbeam (*Carpinus betulus*). The mushroom sampling was conducted during summer (June and July) and early autumn (September) between 2008 and 2010. Daily temperatures during sampling periods were about 25-30 °C, at an altitude of 240-430 m. In each studied subarea, we established five sampling points of 0.25 km^2 each, 1 km apart, distributed in the four corners of each subarea and one in the middle.

The harvested mushrooms were mature, with sporophores and the whole fruiting body, caps and stipes were collected separately (Table 4). Each species was sampled in 6-9 replicates from every sampling point and the replicates were grouped in 3 mean samples. Substrate underneath the fruiting body was also collected, down to a 5 cm depth. After harvesting, the fresh mushrooms were washed with deionized water to remove soil particles, dried at 60 ºC and then ground to a fine powder. The substrate samples were completely dried at 40 ºC, ground to a fine powder and sieved at 250 μm (according to SR ISO 11464).

Table 4

Analyzed species of wild growing mushrooms, habitat and edibility

Sample	Species	Dominant species of tree	Edibility
1	*Russula cyanoxantha*	Durmast, Hornbeam	edible
2	*Russula virescens*	Durmast, Beech	edible
3	*Russula vesca*	Durmast, Oak	edible
4	*Russula nigrescens*	Durmast	edible
5	*Russula foetens*	Durmast, Oak	non edible
6	*Russula chloroides*	Beech	edible
7	*Russula alutacea*	Hornbeam	edible
8	*Russula lepida*	Durmast	edible
9	*Russula aeruginea*	Durmast, Hornbeam	edible
10	*Russula lutea*	Durmast	edible

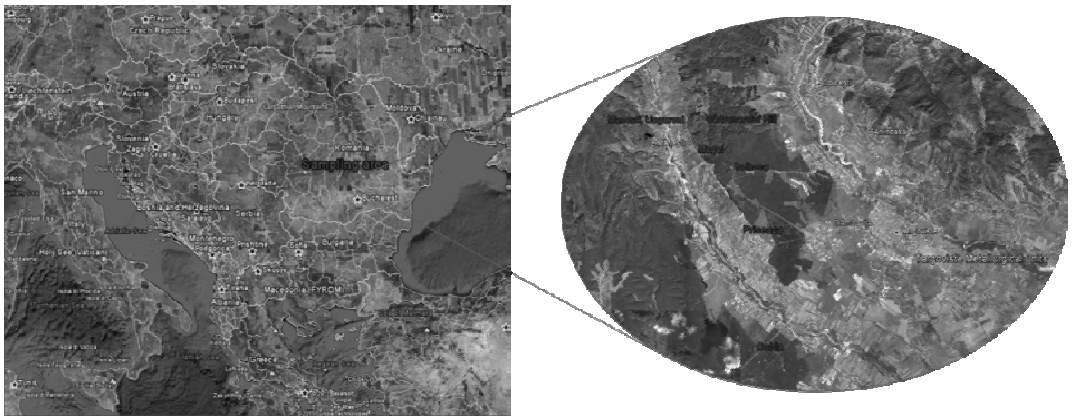

Fig. 2 – Location of studied area, in South Romania, 240-430 m altitude, Oak, Durmast Oak, Beech and Hornbeam forest – red marks; metallurgical unit – yellow mark (Google Earth Map).

Table 5

Observed and Certified values of macronutrients and trace metals in Orchard leaves and Montana soil (n=5)

Element			Certified value	EDXRF value	Recovery (%)
SRM 2710 – Montana soil	(%)	Ca	1.25±0.03	1.21±0.12	97
		K	2.11±0.11	2.23±0.10	106
		P	0.106±0.015	0.12±0.02	113
		Mg	0.853±0.042	0.87±0.07	102
		Fe	3.38±0.10	3.25±0.15	96
		Mn	1.01±0.04	1.11±0.08	110
	(mg/kg DM)	Cu	2950.00±130.0	2973.00±152.0	101
		Zn	6952.00±91.0	6895.00±138.0	99
		Ni	14.30±1.0	14.54±1.3	102
NIST-SRM 1571	(mg/kg)	Fe	300.00±20.0	307.22±18.3	102
		Cu	12.00±1.0	11.75±1.6	98
		Zn	25.00±3.0	23.96±2.2	96
		Mn	91.00±4.0	89.72±6.7	99
		Ni	1.30±0.2	1.41±0.6	108

Analytical procedure

The elemental content of mushrooms and substrate was established by Energy Dispersive X-Ray Fluorescence method (EDXRF),[33] using ElvaX Spectrometer having an X-ray tube with an Rh anode operated at 50 kV and 100 μA. Two grams of each sample were manually pressed, without any chemical treatment, in a plastic vial with Mylar on the bottom. The samples were excited for 300 s, and the characteristic X-rays were detected by a multichannel spectrometer based on a solid state Si-pin-diode X-ray detector with a 140 μm Be window and energy resolution of 200eV at 5.9 keV.

The accuracy and precision of the results were evaluated by measuring a certified reference sample NIST SRM 1571 – Orchard leaves for mushrooms samples and SRM 2710 – Montana soil (Table 5), for substrate samples. All elements which were in a concentration higher than 0.3 mg/kg were then recorded. Every result is the average of five determinations. The final results were reported to dry substances and calculated in mg of metal per kg of dry matter (mushrooms or soil) – mg/kg DM.

For environmental sample analysis, the EDXRF method has the advantage of a rapid and inexpensive method, with a simple preparation of sample and no reagent consumption. Quantitative and qualitative analysis by EDXRF techniques are performed without chemical digestion and a great number of elements can be determined simultaneously, in a short time.[1,34]

Data analysis

The heavy metal concentrations in both mushrooms and the soil underneath were expressed as means and standard deviation of the samples for each studied species. To establish the Pearson coefficient of correlation between chemical compositions of soil and trace metal accumulation in mushrooms we conducted a *t*-test for means. Pattern of these correlations was established by Principal Component Analysis, using UnscramblerX CAMO software, 10.1 version (trial).

CONCLUSIONS

Metal concentration was found different across studied species of mushrooms, case sensitive for

the morphological part of fruiting body, cap or stipe. Metal concentration was higher in mushrooms harvested from hornbeam and oak forests and was correlated with a higher trace element content in substrate.

Metal concentration in substrate had a high variability according to the dominant species of tree in the forest ecosystems and showed different levels of correlation with the trace element bioaccumulation. Calcium concentration in substrate was negatively associated with trace element accumulation (except Zn bioaccumulation) in mushrooms. Manganese and nickel presence in substrate was also negatively associated with trace element bioaccumulation. Potassium, magnesium, iron, copper and zinc concentration in substrate was positively associated with the accumulation of trace elements in mushrooms from the *Russula* genus. The strongest influence of chemical composition of soil was indicated by the presence of iron and zinc in substrate, which was positively associated with copper bioaccumulation in studied mushrooms.

Model of influence of soil chemical composition on trace element bioaccumulation is useful in the evaluation of risk in case of human consumption, and for using these species as a bioindicator.

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