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HEAVY METAL CONTENTS AND BIOACCUMULATION POTENTIAL OF SOME WILD EDIBLE MUSHROOMS

SADRŽAJ TEŠKIH METALA I BIOAKUMULACIJSKI POTENCIJAL NEKIH SAMONIKLIH JESTIVIH GLJIVA

Ivan ŠIRIĆ*, Ante KASAP[1], Ivica KOS[1], Tomislava MARKOTA[2], Draženko TOMIĆ[3], Milan POLJAK[1]

Summary

The concentration of Fe, Zn and Cu in ten edible mushrooms in Medvednica Nature Park was determined. The similarity between the studied species was deterimend by cluster analysis based on concentrations of the aforementioned metals in the fruit bodies. The analyses of heavy metals were carried out by X – ray fluorescence spectormetry. The highest concentration of Fe (153.96 mg kg^{-1}) was determined in *Tricholoma portentosum*, and the highest concentration of Zn (90.60 mg kg^{-1}) was determined in *Tricholoma terreum*. The highest concentration of Cu was determined in *Macrolepiota procera* (78.18 mg kg^{-1}). The concentrations of Zn and Cu significantly differed ($p<0.05$; $p<0.001$) between examined saprophytic and ectomycorrhizal mushrooms. A considerably higher concentration of the analysed elements was found in the cap than in the stipe for all mushroom species. All mushrooms species were bio-exclusors of Fe in relation to the underlying soils. Cluster analysis performed on the basis of the bioaccumulation of the studied metals revealed great similarity of mushroom species belonging to the same genus and partial similarity of species of the same ecological affiliation.

KEY WORDS: heavy metals, edible mushrooms, bioaccumulation potential, ecology

INTRODUCTION
UVOD

Mushrooms are a distinct group of living organisms of considerable nutritive, pharmaceutical and ecological value. They play a vital role in the majority of ecosystems in the biosphere because they are able to biodegrade the substrate on which they grow. Fruit bodies of mushrooms are appreciated for their chemical (Isildak et al., 2004) and nutritional properties (Manzi et al., 1999) and also for texture and flavour. However, it is known that mushrooms can accumulate high concentrations of heavy metals, toxic metalic elements, metalloids and radio nuclids (Kalač 2001; Vetter 2004; Campos and Tejera, 2009). The content of metallic elements in many mushroom species is considerably higher (Kalač, 2010) than in fruits and vegetables (Turkdogan et al., 2003). However, the mechanism of adsorption is still not known (Campos and Tereja, 2011). Mushroom mycelium is able to accumulate considerably higher concentrations of some heavy metals than substrate on which it develops and lives

*1 Dr. sc. Ivan Širić, isiric@agr.hr; Dr. sc. Ante Kasap, akasap@agr.hr; Doc. dr. sc. Ivica Kos, ikos@agr.hr; Prof. dr. sc. Milan Poljak, Agronomski fakultet, Svetošimunska cesta 25, 10000 Zagreb, mpoljak@agr.hr

2 Tomislava Markota, Agronomski fakultet u Zgarebu – studentica

3 Dr. sc. Draženko Tomić, Učiteljski fakultet, Savska cesta 77, 10000 Zagreb, drazenko.tomic@ufzg.hr

Figure 1. Area of the sampling of mushrooms species in Nature Park Medvednica.

Slika 1. Područje prikupljanja uzoraka Park prirode Medvednica

(Campos and Tereja, 2009). The density and depth of the mycelium, which lives in the soil for several months or years, influence the metal content in fruit bodies (Garcia et al., 2009). According to the results of Nikkarinen and Martanen, 2004; Garcia et al., 2009; Aloupi et al., 2012; Petkovšek and Pokorny, 2013., species of mushroom and various environmental factors and soil properties (pH, organic matter, redox potential, type of substrate, geochemistry of substrate, distance from the source of pollution etc.) can affect the metal content in mushrooms. Meanwhile, the relationship between abundance and bioavailability of heavy metals from the substrate is very complex and still not known (Kalač, 2010). However, by calculating the factor of bioconcentration, it is possible to determine the suitability of using mushrooms as bioindicators of environmental pollution (Falandysz et al., 2007).

Mushroom picking is very popular in Central and Southern Europe, as well as in Croatia (Širić et al., 2014). Medvednica Nature Park is located near the largest urban and industrial centre in Croatia, the capital city Zagreb, and there may be increased concentrations of heavy metals in mushrooms. In Medvednica Nature Park, 81 species of mushrooms have been identified to date but there has been no study on their metal contents. The objectives of this study were to (i) determine the iron, zinc and cooper content in wild growing edible mushroom species and the substrate on which they grow, (ii) determine the accumulation capacity (bioconcentration or exclusion) of heavy metals in fruit bodies of mushrooms, (iii) determine the distribution of iron, zinc and cooper in anatomical parts of fruit bodies *(cap and stipe)*, (iv) perform cluster analysis on the mushroom species in relation to their metal content.

MATERIAL AND METHODS
MATERIJAL I METODE

Sampling of mushrooms – *Prikupljanje uzoraka gljiva*

The study was carried out in area of Nature Park Medvednica in the northwestern part of the Zagreb County, Croatia (Fig 1). The study area is covered with a well-preserved deciduous and mixed forest of *Quercus sp*., *Carpinus betulus* L., *Castanea sativa* Mill., *Fagus sylvatica* L., *Picea abies* L., *Acer pseudoplatanus* L. and *Fraxinus excelsior* L.. Macrofungal specimens were collected during the autumn of 2012 (from September till December). Levels of heavy metals (Fe, Zn and Cu) were analysed in 10 edible mushroom species (20 samples per species). Among the sampled species, there were four terrestrial saprophytes (*Agaricus campestris* (L) Fries, *Clitocybe inversa* (Scop. ex Fr.) Pat., *Clitocybe nebularis* Batsch. ex Fr. and *Macrolepiota procera* (Scop. ex Fr.) Sing.), one lignicoluous saprophyte (*Armillaria mellea* (Vahl. ex Fr.) Karst), and five ectomycorrhizal species (*Boletus aestivalis* Paulet ex Fries, *Boletus edulis* Bull. ex Fries, *Lactarius deterrimus* Groger, *Tricholoma portentosum* (Fr.) Quelet, *Tricholoma terreum* (Schff. ex Fr.) Kummer). Completely developed and mature fruit bodies of the investigated mushrooms were collected randomly. At the same time, soil samples of the forest upper soil horizon (0–10 cm) were collected at appropriate sampling places, according to Garcia et al. (2009). All samples were analysed in triplicate.

Analysis of heavy metals – *Analiza teških metala*

Collected specimens and samples of soil substrate were documented, oven dried (24 h; 103°C) milled with laboratory Retch SM2000 and pressed into tablets (r = 16 mm; d = 5 mm) with Chemplex press for further analysis. For analysis x-ray fluorescence spectroscopy was applied (XRF, TwinX, Oxford Instruments). In the first step qualitative analysis was performed. The most frequent pollutants were identified. For those elements calibration curves were prepared and in the second step quantitative analysis was carried out. Most of the measurements were performed on PIN detector (U = 26 kV, I = 115 μA, t = 300 s). The values of bioconcentration factors were calculated as a ratio between the heavy metal contents in the mushroom and the element concentrations in the growing substrate.

pH values and organic matter – *Vrijednost pH i organska tvar*

The pH value of the substrate soil samples was determined potentiometrically in the suspension of the substrate soil and distilled water in the ratio 1: 5. Measurement was carried out acording to methods adapted from „Methods of soil analasys" (Thomas, 1996). The pH value was determined using a pH meter IQ 150 (IQ Scientific Instruments, USA). Organic matter content was determined gravimetrically after combustion of soil (2g air-dried) at 550 °C during 16 h in a furnace horn (Select-Horn. Selecta) (Garcia et al., 2009).

Data analysis – *Analiza podataka*

Statistical analysis and chartings were performed within the R program (R Core Team, 2014) by using two integral and three external statistical packages. Descriptive statistics, calculation of bioconcentration factors and pair-wise comparisons (t-test) of means (concentrations of trace elements) between anatomical parts of the fruit body were obtained within the package „stats", which is an integral package of R. Multiple pair-wise comparisons (TukeyHSD test) of means among species were obtained by the „agricolae" package (de Mendiburu, 2014). Extraction of the information required to create and plot compact letter displays of all pairwise comparisons was performed within the „multcomp" package (Hothorn et al., 2008). Plotting of box-whisker plots was performed with the packages „graphics" (integral) and „lattice" (Sarkar, 2008).

Cluster analysis was performed within the „stats" package. The distance matrix was computed by using the „Euclidian" distance measure, and hierarchical cluster analysis was performed by using a method of complete linkage, which defines the cluster distance between two clusters to be at the maximum distance between their individual components. At every stage of the clustering process, the two nearest clusters were merged into a new cluster, and this process was repeated until the whole data set was agglomerated into a single cluster. The results of cluster analysis were converted into a „phylo" object within the „ape" package (Paradis et al., 2004), and thereafter presented graphically as polar dendrograms.

RESULTS
REZULTATI

Heavy metals in soil substrate – *Teški metali u supstratu tla*

Soil properties (pH value and organic matter content) and average concentrations of iron, zinc and cooper in the area of Medvednica are summarized in Table 1. The mean pH value of the soil substrate at Medvednica was 7.22, within the range of min. 6.30 and max. 8.12. Organic matter content varied from 2.16% to 12.65%, with a mean value of 6.48%. The concentration of hevay metals in the soil substrate indicate that Fe concentration (7569.00 – 8322.00 mg kg^{-1}) was the higest, followed by Zn (42.50 – 94.30 mg kg^{-1}) and Cu (13.21 – 28.33 mg kg^{-1}). The ratio between the highest and the lowest metal concentration (max/min) was highest in Zn (2.21), but only 1.10 in Fe.

Table 1. pH, organic matter and heavy metals concentration (mg kg^{-1} d.w.) in soil from study area.
Mean – Mean value; S.D. – Standard deviation; Min. – Minimum value; Max.– Maximum value; C.V. – Coefficient of varioation; O.M. – Organic matter.

Tablica 1. vrijednost pH, organska tvar i koncentracija teških metala u tlu istraživanog područja (mg kg–1)

Mean – srednja vrijednost; S.D. – standardna devijacija; Min – minimalna vrijednost; Max. – maksimalna vrijednost; C.V. – koeficjent varijabilnosti; O.M. – organska tvar.

	Mean	S.D	Min.	Max.	C.V.%
pH H_2O	7.22	0.59	6.30	8.12	0.33
O.M.%	6.48	3.62	2.16	12.65	12.48
Iron	7 953.00	221.79	7 569.00	8 332.00	46.73
Zinc	79.07	13.78	42.50	94.30	37.49
Cooper	22.91	4.42	13.20	28.30	18.54

Metal concentration and bioconcentration factors – *Koncentracija metala i biokoncentracijski faktor u gljivama*

Descriptive statistics on heavy metal concentration and factors of bioconcentration (BCF) are presented in Table 2.

Table 2. Iron, zinc and cooper concentration (mg kg^{-1} d.w.) and bioconcentration factors in the analysed species of mushrooms. Mean – Mean value; S.D. – Standard deviation; BCF – bioconcentration factor

Tablica 2. Koncentracija željeza, cinka, bakra (mg kg^{-1}) i biokoncentracijski faktor u isptivanim vrstama gljiva. Mean – srednja vrijednost; S.D. – standardna devijacija; BCF – biokoncentracijski faktor

Species	Iron			Zinc			Cooper		
	Mean	S.D.	BCF	Mean	S.D.	BCF	Mean	S.D.	BCF
A. campestris	127.94	38.66	0.02	89.53	13.70	1.13	38.09	14.71	1.66
A. mellea	62.08	26.64	0.01	41.99	21.66	0.53	19.39	7.91	0.85
B. aestivalis	84.99	19.48	0.01	81.04	15.61	1.02	19.19	6.47	0.84
B. edulis	69.39	26.01	0.01	82.93	13.70	1.05	22.56	7.26	0.98
C. inversa	54.33	17.88	0.01	62.96	16.31	0.80	19.65	4.83	0.86
C. nebularis	67.73	19.49	0.01	63.48	18.36	0.82	28.48	7.78	1.24
L. detterimus	49.25	14.44	0.01	86.12	13.95	1.09	7.41	2.96	0.32
M. procera	105.99	40.56	0.01	84.55	12.49	1.07	78.18	19.03	3.41
T. portentosum	153.96	35.94	0.02	80.23	16.70	1.01	11.82	5.57	0.52
T. terreum	83.53	30.83	0.01	90.56	18.08	1.15	15.41	5.27	0.67

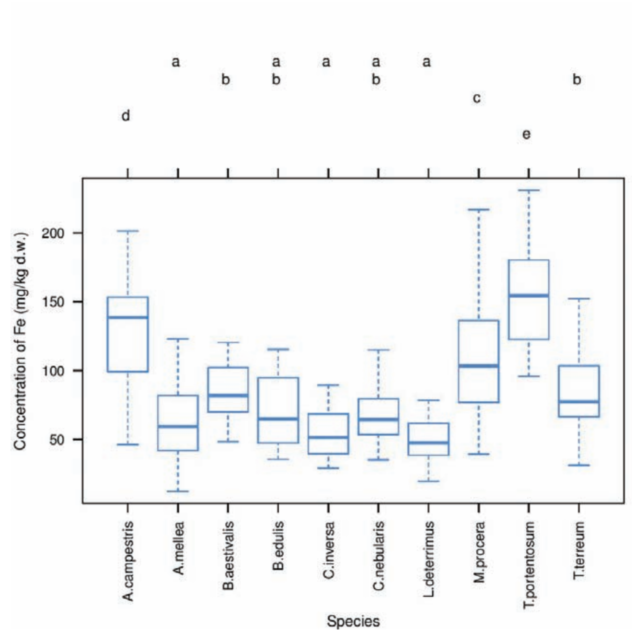

Figure 2. Box and whisker plots representing the distribution of Fe concentration in 10 mushroom species. The box represents the interquartile range (from 1th to 3rd), solid line within the box represents the median (or 2nd quartile), and the whiskers represent the extremes of the distribution. Letters represent the results of Tukey's post-hoc comparisons of mean values among the species.

Grafikon 2. Kutijasti dijagram (box and whisker plot) distribucije koncentracije Fe u 10 istraživanih vrsta gljiva. Pravokutnik predstavlja interkvartilni raspon (od 1 do 3), crta po pravokutniku označava median, dok gornje i donje horizontalne linije (whiskers) predstavljaju ekstreme distribucije. Slova označavaju statistički značajne razlike prosječnih vrijednosti željeza između vrsta (Tukey post-hoc test).

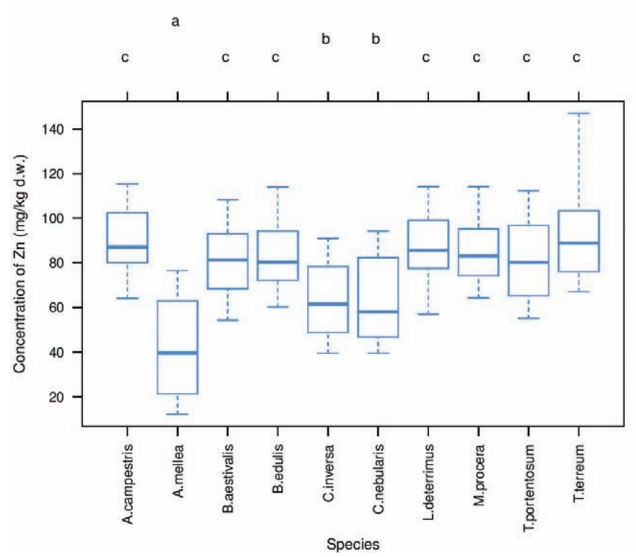

Figure 3. Box and whisker plots representing the distribution of Zn concentration in 10 mushroom species. The box represents the interquartile range (from 1th to 3rd), solid line within the box represents the median (or 2nd quartile), and the whiskers represent the extremes of the distribution. Letters represent the results of Tukey's post-hoc comparisons of mean values among the species.

Grafikon 3. Kutijasti dijagram (box and whisker plot) distribucije koncentracije Zn u 10 istraživanih vrsta gljiva. Pravokutnik predstavlja interkvartilni raspon (od 1 do 3), crta po pravokutniku označava median, dok gornje i donje horizontalne linije (whiskers) predstavljaju ekstreme distribucije. Slova označavaju statistički značajne razlike prosječnih vrijednosti cinka između vrsta (Tukey post-hoc test).

In general, concentrations of Fe varied among the tested species (Table 2). Values varied between 153.96 in the species *T. portentosum* and 49.25 mg kg–1 in *L. deterrimus,* an ectomycorrhizal species that lives only in symbiosis with spruce *(Picea abies* L.*)*. The specified average iron concentration in *T. portentosum* was significantly higher (P<0,05)

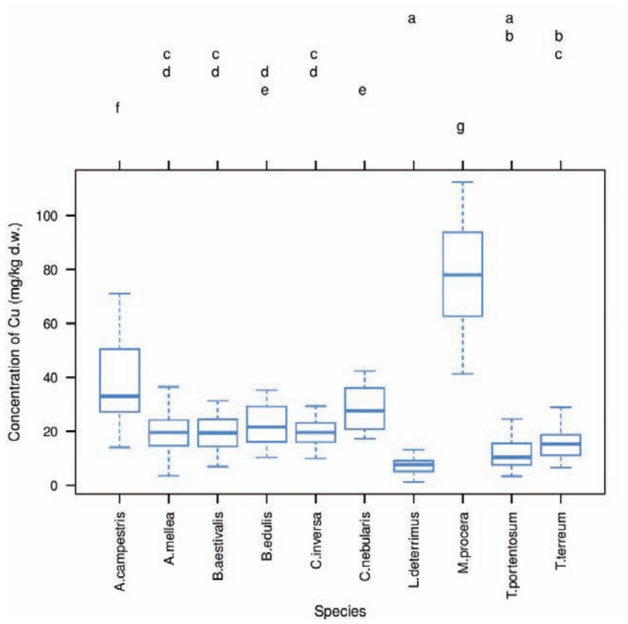

Figure 4. Box and whisker plots representing the distribution of Cu concentration in 10 mushroom species. The box represents the interquartile range (from 1th to 3rd), solid line within the box represents the median (or 2nd quartile), and the whiskers represent the extremes of the distribution. Letters represent the results of Tukey's post-hoc comparisons of mean values among the species.

Grafikon 4. Kutijasti dijagram (box and whisker plot) distribucije koncentracije Cu u 10 istraživanih vrsta gljiva. Pravokutnik predstavlja interkvartilni raspon (od 1 do 3), crta po pravokutniku označava median, dok gornje i donje horizontalne linije (whiskers) predstavljaju ekstreme distribucije. Slova označavaju statistički značajne razlike prosječnih vrijednosti bakra između vrsta (Tukey post-hoc test).

than in other analysed species, with the exception of *A. campestris* (Table 2; Fig. 2). The values of BCF were far below 1 in all analysed mushrooms (Table 2).

The mean Zn concentration in analysed species of wild edible mushroom was 76.34 mg kg^{-1}. The highest mean zinc concentration of 95.56 mg kg^{-1} was found in *T. terreum*, while the lowest concentration was determined in *A. mellea*, a species that lives on wood without contact with mineral particles of soil. Most of analysed species showed values of BCF > 1, while the species *A. mellea, C. inversa* and *C. nebularis* bioexcluded zinc (BFC<1). The best bioindicator potential was determined in *T. terreum* species (1.15).

The highest mean copper concentration of 78.18 mg kg^{-1} was found in *M. procera*, and the lowest concentration was found in *L. deterrimus* (7.41 mg kg^{-1}). The concentration of Cu in *M. procera* was significantly higher (p<0,001) than in other investigated species (Fig. 4). In this work, all ectomycorrhizal and two saprophytic species (*A. mellea* and *C. inversa)* bioexcluded copper (BCF<1). The highest BCFs values of 3.41 and 1.66 were determined in *M. procera* and *A. campestris* (Table 1).

Morphological parts – *Morfološki dijelovi*

Distribution of iron, zinc and cooper between the anatomical parts of fruit bodies *(cap and stipe)* of investigated mushrooms are given in (Fig. 5, 6 and 7). The cap of the

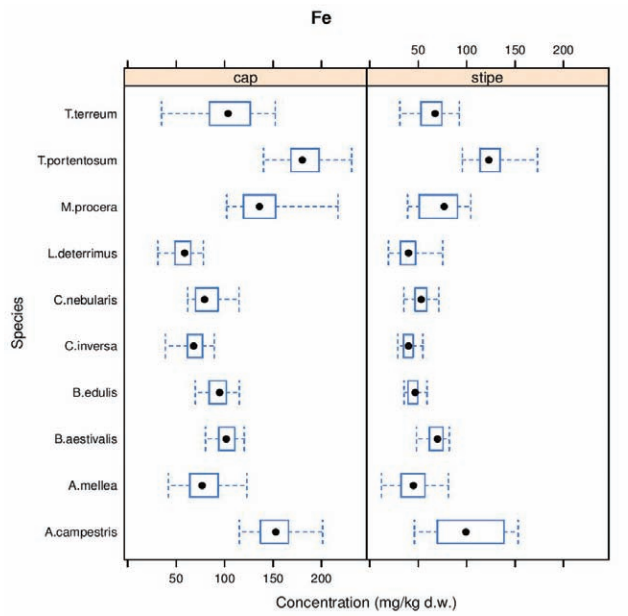

Figure 5. Box and whisker plots representing the distribution of Fe concentration in different anatomical parts of 10 mushroom species. The box represents the interquartile range (from 1th to 3rd), solid line within the box represents the median (or 2nd quartile), and the whiskers represent the extremes of the distribution.

Grafikon 5. Kutijasti dijagram (box and whisker plot) distribucije koncentracije Fe u anatomskim dijelovima plodnog tijela od 10 istraživanih vrsta gljiva. Pravokutnik predstavlja interkvartilni raspon (od 1 do 3), crta po pravokutniku označava median, dok gornje i donje horizontalne linije (whiskers) predstavljaju ekstreme distribucije.

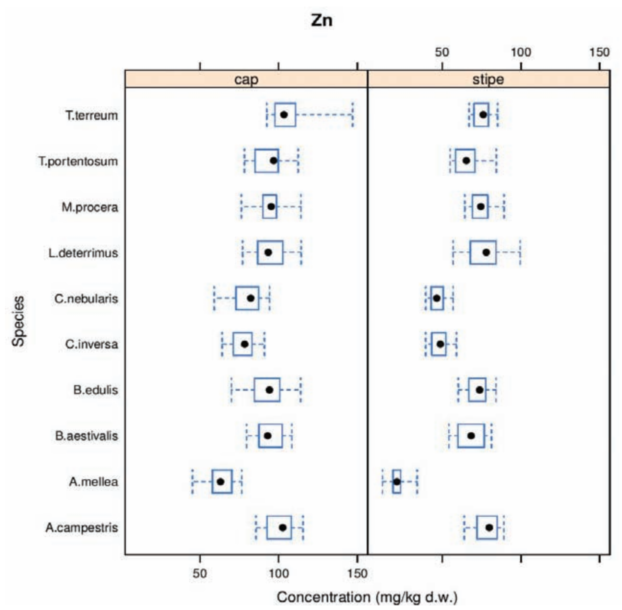

Figure 6. Box and whisker plots representing the distribution of Zn concentration in different anatomical parts of 10 mushroom species. The box represents the interquartile range (from 1th to 3rd), solid line within the box represents the median (or 2nd quartile), and the whiskers represent the extremes of the distribution.

Grafikon 6. Kutijasti dijagram (box and whisker plot) distribucije koncentracije Zn u anatomskim dijelovima plodnog tijela od 10 istraživanih vrsta gljiva. Pravokutnik predstavlja interkvartilni raspon (od 1 do 3), crta po pravokutniku označava median, dok gornje i donje horizontalne linije (whiskers) predstavljaju ekstreme distribucije.

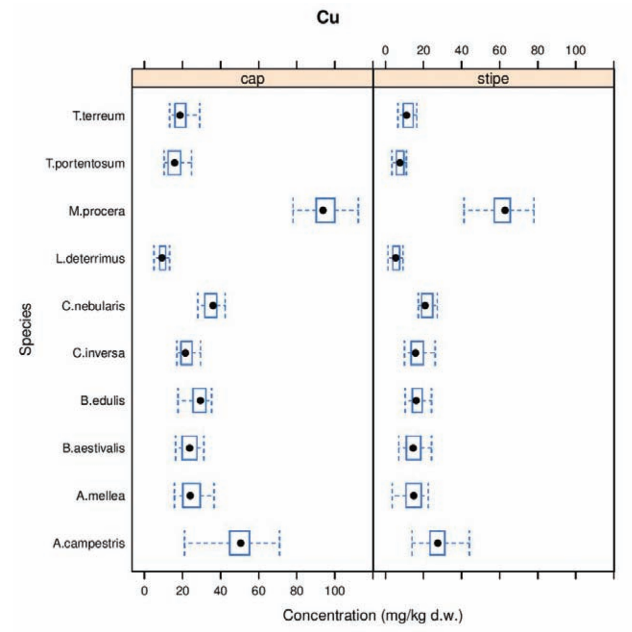

Figure 7. Box and whisker plots representing the distribution of Cu concentration in different anatomical parts of 10 mushroom species. The box represents the interquartile range (from 1th to 3rd), solid line within the box represents the median (or 2nd quartile), and the whiskers represent the extremes of the distribution.

Grafikon 7. Kutijasti dijagram (box and whisker plot) distribucije koncentracije Cu u anatomskim dijelovima plodnog tijela od 10 istraživanih vrsta gljiva. Pravokutnik predstavlja interkvartilni raspon (od 1 do 3), crta po pravokutniku označava median, dok gornje i donje horizontalne linije (whiskers) predstavljaju ekstreme distribucije.

mushrooms showed that mean levels of investigated metals were considerably higher than in the stipe in all tested mushrooms.

Comparison of heavy metal concentrations between saprophytic and ectomycorrhizal mushroom species – *Usporedba koncentracije teških metala između saprofitskih i ektomikoriznih vrsta gljiva*

Our data on heavy metals concentrations for different life styles of mushrooms (saprophyte and ectomycorrhizal) are shown in (Table 3). A higher heavy meatl content in saprophytic mushrooms in comparison with ectomycorrhizal species was confirmed for copper (p<0.001). Otherwise specified, higher concentrations of zinc have been observed in the ectomycorrhizal species than in saprophytic ones (p<0,001), while there were no significant differences in iron concentrations among saprophytic and ectomycorrhizal mushroom species (Table 3).

Table 3. Iron, zinc and copper concentrations (mg kg^{-1} d.w.) in mushrooms of diferent ecological type.
Mean – Mean value; S.D. – Standard deviation

Tablica 3. Koncentracija željeza, cinka i bakra (mg kg^{-1}) u gljivama različitog ekološkog tipa
Mean – Srednja vrijednost; S.D. standardna devijacija

	Fe	Zn	Cu
	Mean ± SD	Mean ± SD	Mean ± SD
Saprophytic	83,62 ± 41,27	68,50 ± 23,89	36,75 ± 24,91
Mycorrhizal	88,23 ± 44,04	84,17 ± 15,09	15,28 ± 7,77
t-statistic	–1,08	–7,71	11,63
p- value	0,28	0,00	0,00

Cluster analysis – *Klaster analiza*

A dendrogram of hierarchical cluster analysis is shown on (Fig. 8). Cluster analysis based on the accumulation of heavy metals revealed similarity among species belonging to the same genus. In addition to these within-genera similarities, great similarity based on accumulated heavy metals was found between *A. campestris* and *T. portentosum,* and between *L. deterrimus* and *T. terreum*. However, the former were found to be more diverse to species of the genus *Macrolepiota (M. procera).*

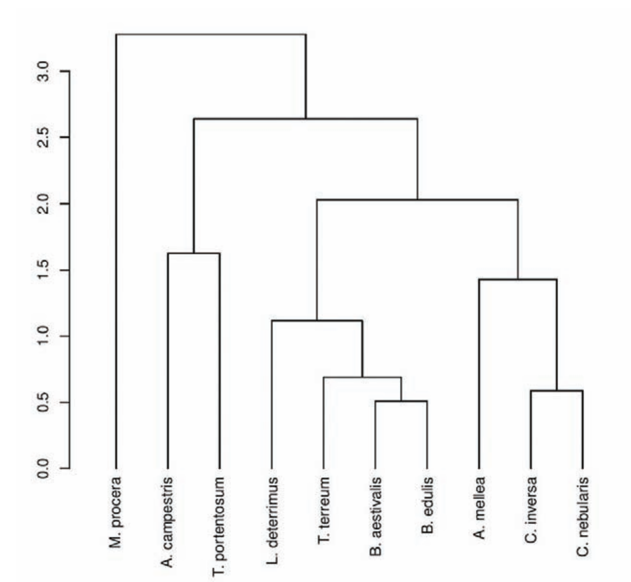

Figure 8. The dendrogram of cluster analysis on the examined species of fungi in relation to their content of heavy metals.

Grafikon 8. Dendrogram klaster analize na ispitivanim vrstama gljiva u odnosu na njihov sadržaj teških metala.

DISCUSSION
RASPRAVA

The concentrations of iron in this study are in agreement with results reported by (Rudawska and Leski 2005 a, b; Borovička and Randa 2007; Isildak et al. 2007; Kalač 2010; Kojta et al. 2011). The highest mean concentration of iron was found in ectomycorrhizal species *T. portentosum*, which is in accordance with the results of Isildak et al. (2007). However, the ectomycorrhizal species *L. deterrimus* accumulated the lowest mean concentration of iron (Table 2). The established values are in accordance with the reports of Rudawska and Leski (2005a, b). Extraordinarily high concentration of iron (1304–2075 mg kg $^{-1}$) was found in *Suillus variegatus* (Borovička and Randa, 2007). This species is well known as an accumulator of Fe (Valiulis et al. 1995; Falandysz et al. 2001, Kalač, 2010). Moreover, iron is distributed unevenly within a fruit body, with the highest contents in the cap, and lower in the stipe, which is in accordancet with previously reported results (Kalač 2010; Falandysz et al. 2011; Kojta et al. 2011; Jarzynska and Falandysz 2012). Iron content of macrofungi is generally lower than that in soil (Table 1). Consequently, the BCF of all mushrooms species for iron was lower < 1, which implies that all the investigated mushrooms species are bioexclusors of iron. These results are in major agreement with the results of Malinowska et al. (2004).Accumulation of zinc in the investigated species of fungi is close to the accumulation of iron, although the concentration of zinc in the analysed samples of soil was considerably lower than the concentration of iron (Table 1). According to different authors, concentrations of zinc in mushrooms ranges from 21 to 110 mg kg^{-1} (Cayir et al., 2010), 30 to 150 mg kg^{-1} (Kalač,2010), 35 to 136 mg kg^{-1} (Radulescu et al., 2010) and 29 to 146 mg kg^{-1} (Sarikurkcu et al., 2011). The values of zinc in this study were in the previously specified range. The lowest concentration of zinc determined in the species *A. mellea* can be explained by lifestyle of mushrooms, because it is a lignocellulous species that lives on wood without contact with mineral particles of soil. This is in accordance with studies of Campos (2011), which have confirmed the lowest content of zinc in *A.mellea*. The highest mean value in *T. terreum*, can be explained that zinc adapted to ectomycorrizal fungi can be used as biological barriers to the accumulation of metals in symbiotic trees Adriansen et al. (2006), thereby increasing the concentration of zinc in the fruit bodies of mushrooms. The lower accumulation of zinc in the saprophytic species can be caused due to microbial immobilization of zinc, but also because of antagonism of zinc with phosphor, calcium, manganese, iron and cooper. A considerably higher concentration of zinc in the cap than in the stipe determined for all species examined in this study is in major agreement with the results of Rudawska and Leski (2005a, b) and Alonso et al. (2003). Bioconcentration factor was mostly < 10 Kalač (2010), which is in an agreement with our results, although some species of mushrooms were found to be bioexclusor for zinc (BCF<1).

The concentration of cooper was significantly different (p<0,001) between life style of investigated fungi. This is in accordance with the results of Alonso et al. (2003), who found significantly higher (p<0,001) cooper concentration in the saprophytic compared to ectomycorrhizal species of fungi. According to data of Kalač (2010), cooper contents in the most species of mushrooms from unpolluted areas may vary between 20 and 100 mg kg^{-1}, with a few exceptions in species with high bioaccumulative potential, such as *Agaricus macrosporus, Agaricus silvaticus, Macrolepiota procera* (Alonso et al. 2003; Svoboda and Chrastny, 2008). Accordingly, the present study demonstrates the highest mean values of cooper in *M. procera* (p<0.001). The opposite was found for ectomycorrhizal species *L. deterrimus,* where the lowest concentration of cooper was determined (Table 2), which is equivalent to the results of Aloupi et al. (2012). This could be due to symbiotic lifestyle of the ectomycorrhizal species *L. deterrimus* (Aloupi et al., 2012). Higher deposition of copper in the cap versus the stipe determined in our study is in agreement with the results of Kojta et al. (2011) and Jarzynska and Falandysz (2012). Also, Kalač (2010) determined shows higher content of cooper in the cap than in the stipe in several mushroom species from the family *Boletaceae.* Available scientific literature does not mention the reason for the higher accumulation of nickel in the cap than in the stipe. A possible explanation for this is not just higher biological activity in spores, which are part of the cap (Chang and Chan, 1973), but also the possibility of the influence of atmospheric deposition, depending on the area of mushroom sampling. In this study, all metals bioconcentration factors were below 1 in the ectomycorrhizal species of mushrooms, which suggests that ectomycorrhizal species are bioexclusors of cooper. We consider that higher bioconcentrations of cooper in saprophytic species *M. procera*, that develop mycelium in the upper horizon of the soil. Our results are in agreement with values reported by Alonso et al. (2003).

Grouping of the species based on the concentration of heavy metals in their fruit body (hierarchical cluster analysis) revealed that species of the same genus have a similar ability to accumulate metals from their growing environment. The similar nutritional habits of species belonging to the same genus were probably the major reason for such consistent clustering of species on the basic level. This clustering was mainly in accordance with our expectations, bearing in mind that species within the same genus share some common physiological characteristics. Grouping of the species in separate clusters on the next level was more or less in accordance with our expectations, since the majority of analysed species (or even whole genera) were separated in clusters in accordance with their ecological affiliation (ectomycorrhizal and saprophytic species). However, this clustering was not completely consistent, since species of the genus *Tricholoma* were found to be more similar to the terrestrial saprophytes than to members of its own ecological affiliation. In view of the ecological habits of the examined species, the positioning of *M. procera* within a separate cluster can also be considered to be a partially unexpected result. This species is a terrestrial saprophyte, and we therefore expected its grouping in a cluster with other species of the same ecological affiliation. The results of cluster analysis were finally presented graphically as dendrograms.

CONCLUSIONS
ZAKLJUČCI

Iron, zinc and cooper concentration of 10 mushrooms species collected from Nature Park Medvednica, Croatia were determined. The heavy metal concentrations in the mushrooms are mainly affected by species and their lifestyle. All mushrooms species were bioexclusors of iron. On the other hand, bio-accumulation features in some of the investigated mushroom species for the metals zinc and copper were determined. The average concentrations of the investigated metals between the anatomical parts of the fruit body (cap and stipe) were considerably different. The determined values of analysed elements in mushrooms correspond to levels in unpolluted areas. Based on the determined concentration of metals in mushrooms and soil substrate, it can be concluded that the environment of the investigated area is not contaminated with the analysed elements. The heavy metal levels of wild edible mushrooms and area on which they grow should be analysed more often in order to evaluate the possible danger to human health.

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Sažetak

Predmetnim istraživanjem utvrđivana je koncentracija Fe, Zn i Cu u deset samoniklih jestivih vrsta gljiva Parka prirode Medvednica. Sličnost između ispitivnih vrsta gljiva ustanovljena je klaster analizom na temelju koncentracije navedenih metala u plodnom tijelu gljiva. Analiza teških metala provedena je metodom XRF – rentgenske fluoroscentne spektrometrije. Najveća koncentracija Fe od 153.96 mg kg^{-1} utvrđena je u *Tricholoma portentosum*, dok je najveća koncentracija Zn od 90.60 mg kg^{-1} ustanovljena u vrsti *Tricholoma terreum*. Najveća koncentracija Cu utvrđena je u vrsti *Macrolepiota procera* (78.8 mg kg^{-1}). Analizom teških metala u gljivama ustanovljene su značajne razlike ($p<0.05$; $p<0.001$) u koncentraciji Zn i Cu između saprofitskih i ektomikoriznih vrsta gljiva. Utvrđena je znatno veća koncentracija ispitivanih metala u klobuku u odnosu na stručak. Sve istraživane vrste gljiva isključene su kao mogući bioindikatori onečišćenja okoliša željezom. Klaster analiza provedena na temlju koncentracije teških metala u gljivama otkrila je veliku sličnost vrsta gljiva koje pripadaju istom rodu i djelomične sličnosti vrsta iste ekološke pripadnosti.

KLJUČNE RIJEČI: teški metali, jestive gljive, bioakumulacijski potencijal, ekologija