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**Elemental composition of *Russula cyanoxantha* along an
urbanization gradient in Cluj-Napoca (Romania)**

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14 **Abstract**

15

16 How far-reaching is the influence of the urban area over the mineral composition of the
17 *Russula cyanoxantha* mushroom? We studied the metal uptake behavior of this fungus
18 relying on the soil properties. We sampled mushroom and soil from six forests according
19 to an urbanization gradient, and two city parks in Cluj-Napoca (Romania). The elements
20 were quantified using inductively coupled plasma – optical emission spectroscopy (ICP-
21 OES). The concentrations of some elements differed significantly ($p < 0.05$) in the
22 samples from the city ($0.39 \pm 0.35 \text{ mg kg}^{-1}$ for cadmium (Cd), $0.40 \pm 0.19 \text{ mg kg}^{-1}$ for
23 chromium (Cr), $69.1 \pm 29.9 \text{ mg kg}^{-1}$ for iron (Fe), $10.9 \pm 1.3 \text{ mg kg}^{-1}$ for manganese
24 (Mn), $0.76 \pm 0.45 \text{ mg kg}^{-1}$ for titanium (Ti) compared with the samples from the forests
25 ($3.15\text{-}14.1 \text{ mg kg}^{-1}$ Cd, $< 0.18 \text{ mg kg}^{-1}$ for Cr, $22.6\text{-}34.5 \text{ mg kg}^{-1}$ for Fe, $15.9\text{-}19.1 \text{ mg}$
26 kg^{-1} for Mn, $0.19\text{-}0.36 \text{ mg kg}^{-1}$ for Ti. We observed a definite negative trend in the
27 mineral accumulation potential of this fungus along the urbanization gradient. The fungus
28 turned from a cadmium-accumulator to a cadmium-excluder. This highlights a positive
29 environmental influence of the urbanization over the toxic metal uptake of *R.*
30 *cyanoxantha*. The hypothesis, that the urban soil pollution would increase the metal
31 content of the mushroom was disproved. The possible explanation might be the elevated
32 carbonate content of the urban soil, which is known to immobilize the metals in the soil.

33

34 **Keywords:** *Russula cyanoxantha*, elemental composition, urbanization gradient, urban areas,
35 bioaccumulation factor

36

37 1. Introduction

38 The influence of the urban environment on the metal accumulation of macrofungi is mostly
39 unknown. There is a scarcity of papers published on this subject. Schlecht and Säumel (2015),
40 for example, quantified the concentration of Cd and lead (Pb) in several saprotrophic and
41 mycorrhizal mushroom species found in Berlin. Mędyk *et al.* (2017) published the
42 concentrations of some bio- and toxic elements in 10 edible mushrooms from unpolluted areas of
43 a Swedish city. In a recent paper, we highlighted the differences in the elemental composition of
44 the saprotrophic *Agaricus campestris* grown in urban and peri-urban regions. When compared
45 with the peri-urban meadows, the urban environment had a univocal influence on this fungus
46 (Zsigmond *et al.*, 2018). In this paper, we focused on the influence of the urban environment and
47 of the habitat proximity to a major city on the elemental composition of the edible mushroom
48 *Russula cyanoxantha*. We also aimed to contribute to a substantial knowledge about the metal
49 accumulation habit of this species in relation to the soil composition, pH, carbonate and organic
50 matter content. We chose the *R. cyanoxantha* for many reasons: (i) this macrofungus is one of
51 the most commonly spread edible mushroom in the European deciduous forests, (ii) we found it
52 well represented in urban parks, (iii) it is known as sensitive taxa to anthropogenic factors like
53 nitrogen input (Brandrud and Timmermann, 1998; Tarvainen *et al.*, 2003), but less affected by
54 the concentration changes of the heavy metals, base saturation, acidity and organic matter
55 content in the soil (Hansen, 1988), and (iv) according to our knowledge, it is a fungus, which
56 was less studied systematically.

57 The *R. cyanoxantha* is an ectomycorrhizal fungus dependent on the host plant, which in general
58 is a deciduous tree (beech, birch, oak). It is a very tasty and delicious edible fungus, but it is not
59 widely commercialized because of cultivating problems. This fungus is very similar in

60 appearance with other species from the *Russula* genus, like the *R. heterophylla*, *R. alutacea* or *R.*
61 *virescens*, which grow in the same habitat. Nevertheless, the *R. cyanoxantha* has some specific
62 characteristics, like flexible and soft gills, pure white stipes, without brownish or yellow spots
63 and white spore prints (Kuo, 2009).

64 Due to the mycelium, mushrooms have peculiar capabilities of absorbing mineral components
65 from the soil by the process of biomineralization (Gadd, 2013). It is well documented that in
66 contrast with plant rhizomes, fungal hyphae are able to excrete numerous substances as leaching
67 agents for metals and phosphorus from minerals (Rosling, 2009; McMaster, 2012; Smits and
68 Wallander, 2017). That is why wild grown mushrooms contain mineral components in much
69 higher concentrations, than plants. Generally, the mycelium of the *Russula* spp. is small-sized
70 possessing few and short hyphae in contrast with *Suillus* spp. or *Cortinarius* spp. characterized
71 by extensive mycelial systems (Taylor and Alexander, 2005). Probably this is the reason, why
72 these species generally contain mineral components in lower concentrations, than other fungi
73 (Kalač, 2016), and may persist in contaminated soils (Newbound *et al.*, 2010). Since the 1980s, it
74 was demonstrated that the occurrence of the *R. cyanoxantha* seemed not to be greatly affected by
75 the soil and litter characteristics (Hansen 1988). Kutszegi *et al.* (2015) mentioned this fungus as
76 one of the most frequent taxa in the deciduous forests. They also showed that the
77 ectomycorrhizal fungi are the least influenced by the environmental factors, like substratum
78 properties, tree species composition and microclimate. Nevertheless, the *R. cyanoxantha* was the
79 most frequently distributed mushroom in Cluj-Napoca and the surrounding deciduous forest.

80 With respect to the elemental composition of macrofungi, the soil is the most influential driver.
81 Although these organisms occasionally develop macroscopic fruiting bodies, they spend most of
82 their lifetime in the soil in the form of the mycelium. The uptake of metals depends on several

83 factors related to the soil (bedrock geochemistry, organic matter content, pH, moisture
84 availability, and porosity of the soil), as well as the species and lifestyle (saprotrophic or
85 mycorrhizal) of the mushroom (Chatterjee *et al.*, 2017).

86 The forest and urban soils present contrasting properties with regard to the acidity, carbonate and
87 metal content. The particular characteristics of urban soils are the elevated pH, alkaline topsoil,
88 less porosity, relatively high concentration of some toxic metals (Lal and Stewart, 2018). The
89 wearing of the buildings and of the street pavements, the emissions from the industrial and
90 construction activities contribute to an alkaline pollutant burden of the local atmosphere in the
91 form of ash and dust (Newbound *et al.*, 2010). Lovett *et al.* (2000) evidenced the deposition of
92 the alkaline ions Ca and Mg to the urban soil surface, which gradually decreased with distance
93 from the city.

94 In the light of these findings, we hypothesized a strong relationship between the pollution level
95 and specific characteristics of the urban soil, and the elemental content of the fruiting body of the
96 *R. cyanoxantha*, when compared with its natural habitat.

97

98 **2. Materials and methods**

99 *2.1. Description of the sampling sites*

100 We carried out our research in Cluj-Napoca (the second largest city in Romania) and its
101 surroundings. It is a dynamically developing city with about 325 000 inhabitants. In order to
102 exclude the varying influence of the air pollution, we chose forests, which lie to the north and to
103 the south from the city center on a trajectory, which is perpendicular to the prevailing wind
104 direction. The seven sampling sites (Fig. 1) were as follows: S1 (a forest near Feleacu, at 7.5 km
105 to south from the city center), S2 (Făget, a forest close to the southern city border, at 5 km from

106 the city center), S3 (a forest near the Mănăştur district, which lies in the southern part of the city,
107 at 2.5 km from the city center), S4 (Iuliu Hațieganu Park and Botanical Garden in the center of
108 the city), S5 (Holia, a forest in the north part of the city, at 2 km from the city center), S6 (a forest
109 near Popești, close to the northern border of the city, at 6 km from the city center) and S7 (a
110 forest near Deușu at 18 km to north from the city center).

111 Fig. 1

112

113 2.2. Sampling and sample preparation

114 We sampled a total number of 95 mushrooms and 95 soil samples in June-August, 2017. All the
115 samples were kept in plastic bags. Each mushroom sample consisted of 7-10 individuals, both
116 small-sized and well developed. The soil samples were taken from the topsoil (0-10 cm) exactly
117 where the mushrooms grew. Before washing the mushrooms, we separated the caps from the
118 stipes using a ceramic knife, and we removed the debris, so that the mass of the anatomic parts
119 could be determined separately. We used these results to determine the mean mass ratio between
120 the cap and the stipe. Next, the mushrooms were washed with tap water, and rinsed with distilled
121 water; they were cut into thin slices and dried at 60 °C, then at 105 °C until constant weight. The
122 samples were grinded and sieved through a stainless steel sieve with pore-size of 315 µm. A
123 mass of about 0.4 g was digested in glass beakers with 5 mL 65% HNO₃ (Merck, Suprapure) and
124 2 mL 30% H₂O₂ (Chempur, pure *p.a.*) at atmospheric pressure. The leaves, stones and living
125 organisms were removed from the soil samples, which were then dried at 20 °C, then at 105 °C
126 until constant weight (Malinowska *et al.*, 2004). About 0.2 g of grinded and sieved (1 mm pore-
127 size) soil samples were digested with 3 mL 65% HNO₃ (Merck, Suprapure) and 9 mL 30% HCl
128 (Merck, Suprapure) in glass beakers. All solutes were diluted to 50 mL with deionized water

129 (Merck, Millipore). The digests were stored in sealed plastic containers. All the glassware was
130 washed after use with tap water, rinsed with distilled water and soaked in 0.1 M HNO₃ for 24
131 hours, then in deionized water for another 24 hours.

132

133 2.3. Instrumental analysis

134 We carried out the elemental composition of the mushroom and soil samples with an ICP-OES
135 (Spectro Genesis, SPECTRO Analytical Instruments GmbH, Germany). The instrument
136 conditions and measurement parameters used throughout this work was described recently
137 (Zsigmond *et al.*, 2018). The method detection limits (MDLs) for each element were calculated
138 using the formula: $(\text{blank} + 3 \times \text{sd}) \times \text{dilution factor}$, where the *blank* is the mean concentration of a
139 certain element quantified in seven blank samples, and the *sd* is the standard deviation of this
140 mean. We used two certified reference materials (CRM): NCS DC 73026 for soil (NACIS,
141 China), SRM 1515 for apple leaves (NIST, USA) for the quality assurance of the instrumental
142 method. For these materials, we followed the same sample pretreatment methods as described
143 earlier. We prepared three samples of each CRM. The MDLs and the recoveries for the studied
144 elements are reported in Table 1. All results were given for the dry weights of the samples.

145

Table 1.

146

147 2.4. The determination of soil properties

148 The pH of the soil samples was determined in two ways: using (1) deionized water and (2) 0.01
149 M CaCl₂ solution as extracting liquids, according to Carter and Gregorich (2008). We added 20
150 mL liquid to a mass of 10 g air-dried soil. We stirred the solution intermittently for 30 minutes,

151 and then we let it stay for one hour. We measured the pH in the supernatant with a combined
152 glass electrode (WTW, Weilheim, Germany).

153 In order to determine the organic matter and the carbonate content of the soil samples, we
154 performed the loss-on-ignition method, according to Heiri *et al.* (2001). Quantities of about 2 g
155 soil samples were placed in ceramic crucibles and dried at 105 °C to a constant weight. We
156 placed the crucibles in an oven and incinerated the samples for 4 hours, followed by a second
157 step at 950 °C for 2 hours. The differences in the weight gave the organic matter content (related
158 to the lost CO₂ and water vapor at 550 °C) and the carbonate content (calculated from the lost
159 CO₂ content at 950 °C).

160

161 *2.5. The bioaccumulation factor*

162 The bioaccumulation factor (BAF) is a dimensionless indicator of the accumulation rate of an
163 element in a living organism (plant, fungus), related to the soil. The BAF for a certain element
164 was calculated by dividing the concentration of the element in the mushroom with the
165 concentration of this element in the soil.

166

167 *2.6. Statistical analyses*

168 The statistical analyses were carried out on a data table of 95 rows and 52 columns (containing
169 all the data about the mushroom and soil). We tested the normality of the distributions with
170 Shapiro-Wilk-test. For the homogeneity of the variances, we used the *F*-test for two groups, and
171 the Levene-test for multiple groups. We used the two-sample *t*-test for independent groups
172 (sometimes the Welch-test or the nonparametric Mann-Whitney-Wilcoxon-test) in order to
173 compare the elemental composition of the caps and the stipes. We used the one-way ANOVA

174 (sometimes the Welch-ANOVA or the nonparametric Kruskal-Wallis-test) to compare the mean
175 concentrations of the elements in the seven sampling sites. When the test showed significant
176 differences in the means, we used the proper *post-hoc* test (Tukey-HSD or Games-Howell-test or
177 Dunn's test) to identify the actual differences between pairs. The correlation between the
178 variables was tested with calculation of the Spearman rank-correlation coefficients. These
179 statistical tools are regularly used in the chemometric evaluation of the mineral composition of
180 mushrooms (Malinowska *et al.*, 2004; Brzezicha-Cirocka *et al.*, 2019).

181 In order to test the existence of an urbanization gradient with regard to the elemental
182 composition of the *R. cyanoxantha*, we used simple linear regression and factorial ANOVA. The
183 bioaccumulation factor was taken as response variable, and the habitat distance from the city
184 center, was used as explanatory variable. The normal distribution of the residuals was tested with
185 Shapiro-Wilk test. All the statistics were carried out by R software, version 3.4.4.

186

187 **3. Results and discussion**

188 The mean mass ratio of the fresh anatomic parts (the caps and the stipes) of 228 individuals was
189 of 3.0 g, with a standard deviation of 1.3 g. From about 850 specimen only 228 were measured
190 because we counted for only the healthy, impeccable specimen. The minimum value was of 0.9
191 g, and the maximum value was of 7.2 g. The heaviest cap and stipe weighted 64.2 g and 31.3 g,
192 the lightest ones 2.2 g and 0.6 g, respectively. The average water content of the mushrooms was
193 of 88.5% ($sd = 4.5\%$, $n = 158$). The data table, which contains all results, is provided in the
194 supplementary material as AllData.xlsx (the *m* signifies the mushroom, the *s* signifies the soil).

195

196 *3.1. The elemental composition of soils*

197 According to the decree for soil contaminants released by the Ministry of Environment of
198 Romania (Government Decree No 756/1997), the forest soils presented concentrations lower
199 than the background levels for Cr, Cu and nickel (Ni). In many forest soils the concentrations of
200 silver (Ag), barium (Ba) and Zn were much lower, than the given limits; on the other hand, Cd,
201 Mn, and Pb had concentrations higher than the normal levels (Table 2). The soils from the city
202 park contrasted the forest soils in much higher concentrations of Ag, and considerably lower
203 concentrations of Cd, Mn, and Pb. The Ag exceeded the background level. The Na was detected
204 only in Cluj-Napoca (S4) and in the forest to the north from the city (S5-S7).

205 Table 2.

206 Among the studied sites, the concentration of Cd in the soils exhibited the most heterogeneous
207 distribution. The one-way ANOVA test revealed significantly different concentrations of
208 aluminium (Al), Ba, calcium (Ca), Cu, Cr, Fe, K, magnesium (Mg), Ni, strontium (Sr), Zn in the
209 samples from the sites S1-S4, and the concentrations increased from the southern periphery to
210 the city center (Table S1, Fig. 5-8. in the supplementary material). Meanwhile, to the north from
211 the city, the soils proved to be roughly homogenous in the mineral composition; for example, the
212 S5 and S6 sites resembled the most. In addition, the soils from the city parks were not
213 significantly different in the content of Al, Ca, Cu, Fe, K, Mg, Ni, Sr and Zn from the soils of
214 sites S5 and S6, but significantly richer in these metals, than the soils from site S7. Among the
215 studied sites, the soils of the city parks had significantly the highest concentration of Ag, P and
216 Ti, and the lowest concentration of Mn and Pb.

217

218 *3.2. The elemental composition of mushrooms*

219 We compared the elemental composition of the *R. cyanoxantha* with the literature data for
220 mushrooms (Kalač, 2016). Many of the elements (Ag, Al, Cu, Mn, Na, Ni, S, Ti, Zn) showed
221 concentrations mainly in the lower part of the usual range (Table 2). All the samples had
222 concentrations of Ca, Cr, K, and Mg below the usual range. Furthermore, some studies on the
223 heavy metal content of the *R. cyanoxantha* from the forests of the southern part of Romania
224 showed considerably higher concentrations of Cr, Mn, Fe and Pb; lower concentrations of Cu,
225 Ni, and similar concentrations of Zn (Busuioc *et al.*, 2011; Elekes and Busuioc, 2013). When we
226 compared the results with the data published for *Agaricus campestris* growing in the same urban
227 area, we found lower concentrations in the *R. cyanoxantha*, excepting for Ni and Zn (Zsigmond
228 *et al.*, 2018). It was recently demonstrated, that among the *Russula* spp., the *R. cyanoxantha*
229 could be classified as an average Zn accumulator with concentrations between 50-150 mg kg⁻¹
230 for dry matter, when coming from pristine areas (Leonhardt *et al.*, 2019). We found that this
231 species was less affected by the Zn contamination level of its habitat, since it exhibited very low
232 variations in the Zn content (74.5-108 mg kg⁻¹ average concentrations for dry matter) along the
233 urbanization gradient.

234 A comparison between the samples from the forests with the samples from the city highlighted
235 the ability of the *R. cyanoxantha* to accumulate more Fe and P but less Cd when grown in the
236 city. These low levels of Cd coupled with the undetected Pb levels were somehow unexpected.
237 However, two *Russula* spp. had median concentrations of 2.3-2.5 mg kg⁻¹ for Cd and of 4.8 mg
238 kg⁻¹ for Pb in a recent study from Berlin (Schlecht and Säumel, 2015). Nevertheless, with the
239 exception of S1 and S4, the average Cd concentration of the mushrooms exceeded the usual
240 concentration range.

241 We compared the seven sites using one-way ANOVA test (Fig. 9-11. in the supplementary
242 material). Along the urbanization gradient the alkaline-earth elements (Ba, Ca and Sr) as well as
243 Cu, Na and Zn were evenly distributed in the samples. When compared with the samples from
244 the forests, the influence of the urban environment manifested in significantly higher
245 concentrations of Cr, Fe and Ti, and significantly lower concentrations of Cd and Mn in the
246 samples from the urban parks. Although the highest Ag concentrations were measured in the
247 samples from the city parks, the mean value did not differ significantly from the neighboring
248 forests (S3 and S5). In general, the mushrooms from the S1 site exhibited the lowest mineral
249 concentrations, with the exception of Al, Ba and Ca.

250 The elemental composition for the caps and the stipes are also given in the supplementary
251 material (Table S1 in the supplementary material). Regardless the habitat, several elements (Cd,
252 Cu, Mg, P, S, Zn) had significantly higher concentrations in the caps, than in the stipes, other
253 elements (Al, Ba, Fe, K, Ni, Sr, Ti) were evenly distributed in the fungi (Tables S2 and S3 in the
254 supplementary material). The Na was the only element, which was present in smaller
255 concentrations in the caps, than in the stipes. This distribution pattern was in accordance with
256 that of the *A. campestris* coming from the same urban and peri-urban area (Zsigmond *et al.*,
257 2018). We identified a habitat-dependent behavior of the *R. cyanoxantha* related to the
258 accumulation of two metals (Ag and Ca). While in the forests the mushroom had significantly
259 higher concentrations of these elements in the caps than in the stipes, there was a considerable
260 shift in the quantity of these elements in the stipes in the urban area (the *t*-statistics have not
261 revealed significant differences of these metals in the caps and the stipes of the mushrooms
262 growing in the city).

263

264 3.3. Soil factors influencing the mineral uptake of the mushroom

265 The *R. cyanoxantha* has manifested a weak accumulating potential for minerals. We found BAF
266 values greater than unity only in the case of the major elements K, P, S and a few minor elements
267 like Cd, Cu and Zn (Table S4 in the supplementary material). The BAF values indicated a strong
268 K, P and S uptake capability of the *R. cyanoxantha* with the highest median BAF values found in
269 the samples from S1 ($BAF_K = 33$, $BAF_P = 21$, $BAF_S = 12$). The BAF values were considerably
270 lower for Cd, Cu and Zn. The highest median values for Cu and Zn ($BAF_{Cu} = 8.7$, $BAF_{Zn} = 3.2$)
271 were also found in the samples from S1; and for Cd ($BAF_{Cd} = 6.2$) in the samples from S7. These
272 results are in agreement with literature data, which revealed that the *Russula* spp. could
273 accumulate cadmium more efficiently than other ectomycorrhizal species (Demirbaş, 2001b;
274 Cejpková *et al.*, 2016; Melgar *et al.*, 2016). For example, both *R. cyanoxantha* and *R. ochroleuca*
275 grown in unpolluted and polluted forests were characterized with bioaccumulation factors of
276 about 10 for Cd. Many studies also revealed the Zn and Cu accumulation property of the *Russula*
277 spp., reporting BAF values of 5.9-7.9 for Cu and 3.4-5.2 for Zn (Demirbaş, 2001a; Alonso *et al.*,
278 2003; Podlasińska *et al.*, 2015; Cejpková *et al.*, 2016).

279 On the other hand, this fungus species proved to be excluder of Pb. Although in some soils the
280 Pb contents were higher than the normal background levels, we couldn't detect this metal in the
281 mushroom. Proskura *et al.* (2017) found similar behavior of the ectomycorrhizal fungus *Boletus*
282 *badius* against Pb. The essential elements like Ca and Mg had maximum median BAF values of
283 0.6 and 0.8 in the samples from site S1, and minimum median values of 0.04 and 0.2 for the
284 samples from both S4 and S5 sites. The other elements had BAF values lower than 0.1; whereas
285 the Fe had the lowest values (< 0.01).

286 The accumulation rates disagreed between the sampling sites suggesting a definite influence of
287 the soil characteristics over the mineral uptake of this fungus (Fig. 2). The city parks proved to
288 be a distinct habitat for this mushroom. Almost in all the cases, we found the weakest
289 accumulation power of the *R. cyanoxantha* coming from the parks. The median BAF values were
290 much lower than in the forests: 0.2 (Cd), 2.1 (Cu), 5.4 (K), 7.8 (P), 10 (S) and 1.3 (Zn). Site S1
291 was also unique from many points of view: it had the lowest concentration of the studied
292 elements in the soil, it had low or average mineral content in the mushrooms with the exception
293 of Al, Ba and Ca, and it exhibited unexpectedly high BAF values for Ca, K, Mg, P and Zn.

294 Fig. 2

295 The one-way ANOVA test revealed a clear trend in the elemental uptake potential of the *R.*
296 *cyanoxantha*. The BAF values significantly decreased toward the city from the southern part in
297 the case of Cu, Mg, P and Zn. On the northern part of the city, the furthestmost forest (S7)
298 exhibited significantly higher BAF values than the S3-S5 sites for Ca, Cu, Mg, K, Zn. It
299 resembled the most with S1 site. We tested the existence of a BAF gradient statistically along the
300 natural-outskirts-city trajectory using two-way factorial ANOVA. The first factor was the habitat
301 and had three levels: the natural habitat (S1 and S7 sites), the outskirts (S2, S3, S5, S6 sites), and
302 the city (S4 site). The second factor was the element, having five levels (Cu, K, Mg, P, Zn). The
303 Fig. 3a. shows the means of the BAFs of each element according to the sampling sites. For each
304 element, the mean concentration decreased from the natural habitat toward the urban area. The
305 Fig. 3b. represents the weighted means of the BAFs of all the tested elements according to the
306 habitat. The differences between the means were significant by ANOVA. This result proves the
307 presence of a negative cumulated BAF gradient from the natural habitat of the mushroom to the

308 city. The urban environment seems to have an inhibitory effect over the mineral accumulation
309 potential of the *R. cyanoxantha*.

310 Fig. 3

311 These results suggested the existence of a tight relationship between the soil properties and the
312 elemental uptake capability of the mushroom. In order to elucidate this, we tested the correlation
313 between the elemental composition of the mushroom and its bioaccumulation properties with the
314 soil properties (Fig. 4). We found few significant correlations between the mineral content of the
315 mushroom and the soil properties. The Spearman rank correlation coefficient values were low ($<$
316 0.45). Other studies also suggested the independence of metal accumulation from the soil
317 properties (Brzostowski *et al.*, 2011; Kojta and Falandysz, 2016; Ivanić *et al.* 2019). However,
318 we found strong correlations between the bioaccumulation factors and the carbonate content of
319 the soil ($r < -0.60$), for almost all the elements with high BAF values, and generally weaker
320 correlation with the organic matter content and the pH of the soil. This pattern shows that the
321 uptake of the elements by the mushrooms from the soil is a complex process. Some factors, as
322 the organic matter (like chelating agents and siderophores) and the acids (like organic acids),
323 originate from at least two sources: both from the mushrooms and the soil. Thus, the
324 mobilization of metals from the soil might be controlled mainly by the fungi and not by the soil
325 properties. However, it seems, that the carbonate content of the soil could hinder the chemical
326 processes initiated by the mushroom in order to solubilize the metals. In the light of these
327 findings, the several extreme BAF values for S1 and S7 in contrast with S4 could be explained
328 by the contrast in the carbonate content and the pH values of the soils (Fig. 5). The rise of the
329 soil pH coupled with higher carbonate content was expected, as the calcium-carbonate (the major
330 carbonate type in the soil) raises the pH of the soil (Essington, 2016). We found Spearman rank

331 correlation coefficients of 0.38 and 0.51 for the carbonate content and the pH of the soil samples
332 (the former value refers to the pH measured when distilled water was added to the soil samples,
333 the last value when CaCl_2 solution was added to the soil samples). In a neutral or slightly
334 alkaline soil, most of the metals are insoluble (Meuser, 2010).

335 Fig. 4., Fig 5.

336

337

Journal Pre-proof

338 4. Conclusions

339 This research supported the previous findings related to the *Russula* spp. about the weak mineral
340 accumulation potential of the genus, characterized with lower than the usual levels of all the
341 elements, except for the Zn (generally), and the Cd (sporadically). We did not identify any trend
342 in the mineral content of the *R. cyanoxantha* along the chosen urbanization gradient, but we
343 disclosed a definite fingerprint of the urban environment on the elemental composition of this
344 mushroom. We have also revealed a negative trend in the bioaccumulation potential of the
345 mushroom along the urbanization gradient: the most notable behavior of the fungus was its turn
346 from a cadmium-accumulator to a cadmium-excluder. This is an important finding, which
347 highlights a positive environmental influence of the urbanization over the toxic metal uptake of
348 edible mushrooms growing in Cluj-Napoca. The strongest inhibitory influence over the mineral
349 uptake of the *R. cyanoxantha* turned to be the carbonate content of the soil. We found positive
350 correlations between the carbonate content and the pH of the soil. The weaker correlation
351 between the bioaccumulation potential of the mushroom with the organic matter content and the
352 pH of the soil could show the importance of the peculiar mechanisms exerted by the mycelium
353 for the solubilization of these elements resulting in considerably better uptakes. The hypothesis
354 that the urban soil polluted with heavy metals would increase the content of these elements in the
355 mushroom was thus disproved. The possible explanation might be the elevated carbonate content
356 of the urban soil, which immobilized these metals in the soil.

357

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363

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- 466

467 **Captions to Figures**

468

469 **Fig. 1.** The sampling sites distributed along an urbanisation gradient: S1 – forest (near Feleacu),
470 S2 – Făget forest, S3 –forest (Mănăştur), S4 – the Iuliu Hațieaganu Park and the
471 Botanical Garden inside the city, S5 – Hoia forest, S6 –forest (near Popești), S7 – forest
472 (near Deușu).

473 **Fig. 2.** The variation of the normalized bioaccumulation factors along the sampling sites: S4-S1,
474 growing distance from the city center (S4) to the south; S4-S7, growing distance from the
475 city center to the north. Elements were chosen with BAF values slightly below unity (Ca,
476 Mg) and values above unity (Cd, Cu, K, P, S, Zn).

477 **Fig. 3.** The two-way factorial ANOVA test for the BAFs according to the habitat types and the
478 elements: a) the mean BAFs of some elements according to the habitat type; b) the
479 weighted means for cumulated BAFs according to the habitat types.

480 **Fig. 4.** Spearman rank-correlation coefficient values obtained for the elemental concentrations
481 for the mushroom fruiting body (mg kg^{-1} dry matter) and the soil properties, as well as
482 for the BAF values and the soil properties.

483 **Fig. 5.** Box and whiskers plots for the soil properties (median, IQR, outliers falling below $Q1 -$
484 $1,5\text{IQR}$ or beyond $Q3 + 1,5\text{IQR}$).

485

1 Captions to Figures

2

3 **Fig. 1.** The sampling sites distributed along an urbanisation gradient: S1 – forest (near Feleacu),
4 S2 – Făget forest, S3 –forest (Mănăştur), S4 – the Iuliu Hațieaganu Park and the
5 Botanical Garden inside the city, S5 – Hoia forest, S6 –forest (near Popești), S7 – forest
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15 for the mushroom fruiting body (mg kg^{-1} dry matter) and the soil properties, as well as
16 for the BAF values and the soil properties.

17 **Fig. 5.** Box and whiskers of some physical characteristics of the soil as a function of the
18 sampling sites. (median – solid bold line, 50% – green area, minimum and maximum –
19 whiskers with dotted lines.).

20

21

Table 1

The method detection limits (MDL) of the elements quantified in dry soil and mushroom samples by ICP-OES, and the concentrations and the recoveries found for the reference certified materials (mean \pm sd, n = 3).

Element	MDL (mg kg ⁻¹)		Certified value	Measured value	Recovery (%)	Certified value	Measured value	Recovery (%)
	Soil	Mushroom						
Ag	0.73	0.13	0.068	< MDL		no data	< MDL	
Al	5.4	6.0	54990	41500 \pm 325	75	284	230 \pm 19	81
Ba	0.58	0.51	356	280 \pm 2	79	48.8	48.2 \pm 1.4	99
Ca	37	67	46312	45600 \pm 455	98	15250	15600 \pm 410	102
Cd	1.2	0.30	0.108	< MDL		0.013	< MDL	
Cr	0.66	0.18	43	37 \pm 0.4	86	0.3 ¹	0.2 \pm 0.1	67
Cu	2.4	6.9	28	24 \pm 1	86	5.69	5.2 \pm 0.4	91
Fe	28	4.0	28816	26600 \pm 185	92	82.7	72 \pm 3	87
K	32	4.7	16519	14700 \pm 263	89	16080	14900 \pm 224	93
Mg	4.5	26	17970	19100 \pm 101	106	2710	2830 \pm 62	104
Mn	0.20	0.15	667	637 \pm 5	96	54.1	52.1 \pm 1.1	96
Na	117	23	66693	68600 \pm 722	103	24.4	26.2 \pm 5.8	107
Ni	1.0	0.66	20	20 \pm 1	100	0.94	0.76 \pm 0.11	81
P	297	304	706	810 \pm 11	115	1593	1690 \pm 15	106
Pb	4.0	0.85	3.4	< MDL		0.470	< MDL	
S	87	34	2.70	3.2 \pm 0.3	119	1800 ¹	1870 \pm 32	104
Sr	0.27	0.12	435	520 \pm 16	120	25.1	26.3 \pm 0.7	105
Ti	0.28	0.01	3300	2600 \pm 26	79	no data		
Zn	2.1	1.2	61	58 \pm 2	95	12.45	12.7 \pm 0.4	102

¹Information mass fraction values.

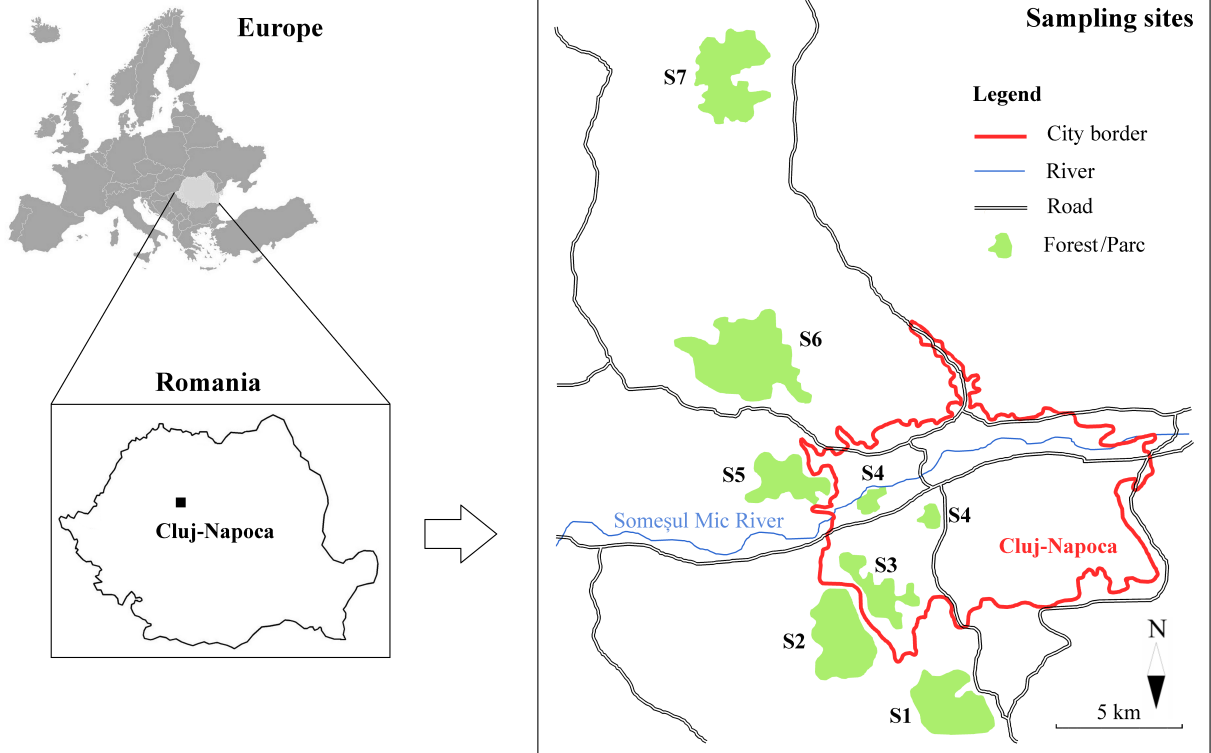
Table 2

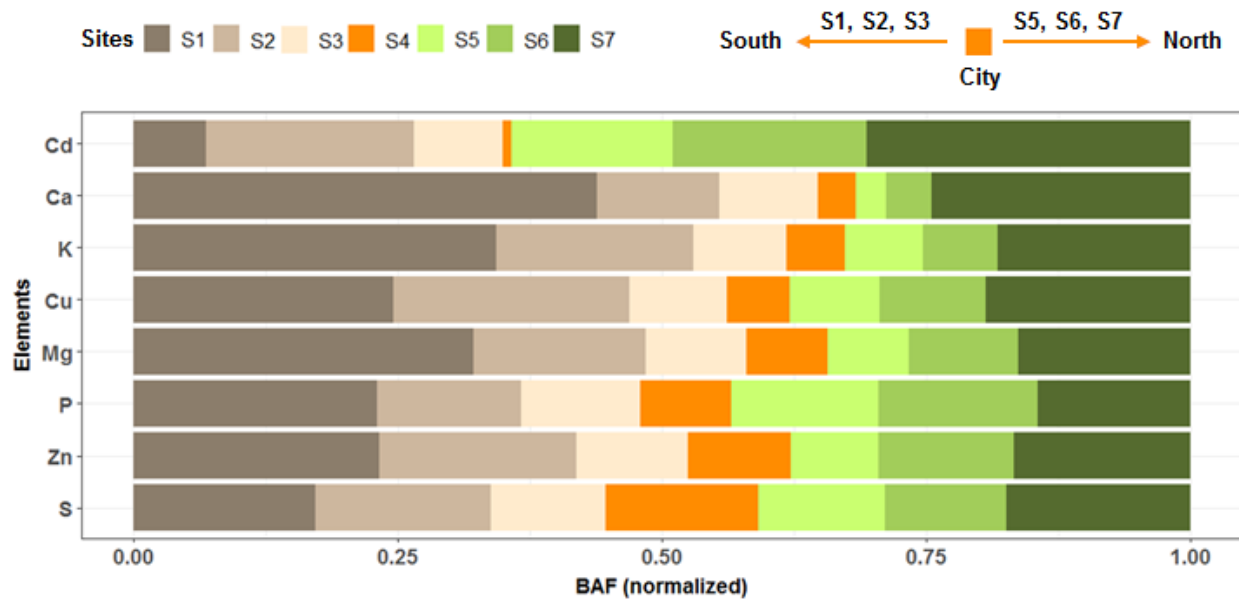
The elemental composition (mean \pm sd) of the *R. cyanoxantha* fruiting body (FB) and of the soil in the six forests and in the city parks given in mg kg⁻¹ (dry matter). The statistics were calculated after removing the outliers.

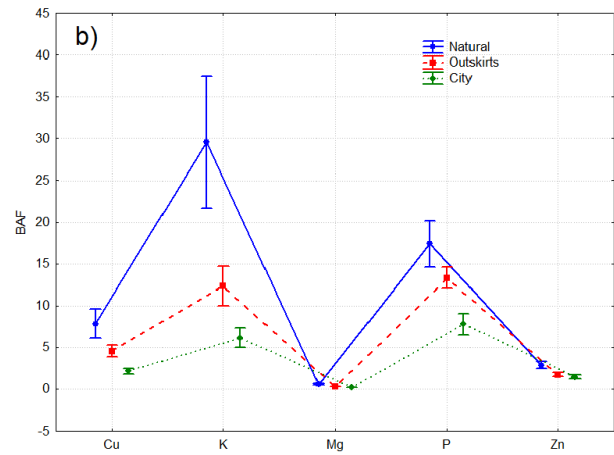
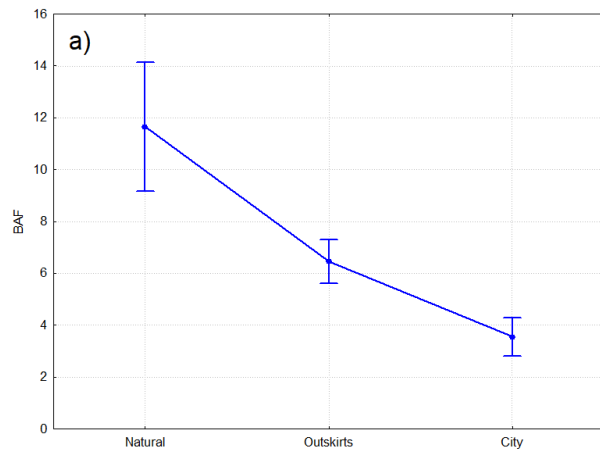
Element	Samples	Feleacu (S1) n = 13	Făget (S2) n = 15	Mănăştur (S3) n = 13	City (S4) n = 16	Hoia (S5) n = 14	Popeşti (S6) n = 12	Deuşu (S7) n = 12	Usual content ^a Background level ^b
Ag	FB	0.29 \pm 0.28	< MDL	0.28 \pm 0.19	0.76 \pm 0.50	0.48 \pm 0.47	0.18 \pm 0.14	0.21 \pm 0.20	0.2–3
	soil	< MDL	< MDL	< MDL	4.57 \pm 4.00	< MDL	< MDL	< MDL	< 2
Al	FB	170 \pm 8.5	51.2 \pm 10.4	153 \pm 7.1	49.7 \pm 26.2	68.6 \pm 14.4	40.4 \pm 14.1	25.4 \pm 8.7	50–200
	soil	5460 \pm 1130	12000 \pm 3790	14500 \pm 4700	18700 \pm 3650	16600 \pm 5600	18000 \pm 6360	13000 \pm 1710	–
Ba	FB	1.39 \pm 0.66	< MDL	0.67 \pm 0.28	0.65 \pm 0.32	0.63 \pm 0.47	1.27 \pm 0.79	< MDL	1–5
	soil	45.9 \pm 23.7	72.2 \pm 24.6	88.5 \pm 19.2	104 \pm 28	120 \pm 33	171 \pm 69.4	59.9 \pm 8.24	< 200
Ca	FB	236 \pm 90	107 \pm 17	167 \pm 32	167 \pm 98	218 \pm 141	221 \pm 102	173 \pm 67	200–1000
	soil	415 \pm 192	875 \pm 377	1220 \pm 628	3490 \pm 805	4270 \pm 2050	3920 \pm 2290	480 \pm 108	–
Cd	FB	3.15 \pm 3.70	8.22 \pm 4.90	6.13 \pm 4.18	0.39 \pm 0.35	9.54 \pm 5.46	12.2 \pm 8.8	14.1 \pm 8.9	0.5–5
	soil	< MDL	2.08 \pm 0.26	2.54 \pm 0.74	1.23 \pm 0.45	3.13 \pm 0.40	3.39 \pm 0.90	2.32 \pm 0.37	< 1
Cr	FB	< MDL	< MDL	< MDL	0.40 \pm 0.19	< MDL	< MDL	0.25 \pm 0.14	0.5–5
	soil	5.44 \pm 1.66	16.4 \pm 5.89	18.9 \pm 6.13	27.2 \pm 6.25	27.1 \pm 9.77	25.9 \pm 7.31	22.7 \pm 3.30	< 30
Cu	FB	37.1 \pm 35.5	48.4 \pm 45.5	37.6 \pm 38.7	37.6 \pm 33.8	47.1 \pm 48.4	55.0 \pm 48.4	47.8 \pm 46.8	20–70
	soil	5.99 \pm 3.67	7.84 \pm 3.28	11.6 \pm 4.05	18.6 \pm 2.76	14.3 \pm 4.25	21.6 \pm 9.19	7.08 \pm 1.43	< 20
Fe	FB	23.7 \pm 8.54	23.4 \pm 10.4	22.6 \pm 6.76	69.1 \pm 29.9	23.9 \pm 5.29	34.5 \pm 14.1	27.4 \pm 4.28	30–150
	soil	5040 \pm 600	12000 \pm 3090	14500 \pm 3720	18900 \pm 4140	17400 \pm 2660	18000 \pm 5000	13000 \pm 1420	–
K	FB	16400 \pm 3580	16300 \pm 2540	15800 \pm 2770	20000 \pm 1780	18800 \pm 3020	19700 \pm 3510	21400 \pm 2350	20000–40000
	soil	479 \pm 149	1090 \pm 505	1740 \pm 858	3540 \pm 938	2660 \pm 979	2610 \pm 770	1230 \pm 337	–
Mg	FB	564 \pm 79	602 \pm 53	543 \pm 76	723 \pm 87	634 \pm 99	672 \pm 112	787 \pm 137	800–1800
	soil	800 \pm 218	1900 \pm 730	2450 \pm 770	4370 \pm 1630	3500 \pm 495	3440 \pm 1020	2270 \pm 430	–
Mn	FB	17.2 \pm 3.5	15.9 \pm 3.2	18.3 \pm 3.4	10.9 \pm 1.3	17.7 \pm 3.6	18.8 \pm 5.1	19.1 \pm 3.0	10–60
	soil	514 \pm 229	1140 \pm 447	1380 \pm 540	478 \pm 49.9	1030 \pm 303	1320 \pm 662	960 \pm 187	< 900
Na	FB	150 \pm 90	116 \pm 78	258 \pm 149	252 \pm 112	136 \pm 110	115 \pm 78	337 \pm 222	100–400
	soil	< MDL	< MDL	< MDL	145 \pm 78	< MDL	< MDL	< MDL	–
Ni	FB	1.22 \pm 0.52	1.50 \pm 0.49	1.35 \pm 0.44	2.49 \pm 1.52	1.07 \pm 0.57	1.42 \pm 0.55	1.74 \pm 0.76	0.5–5
	soil	5.13 \pm 1.49	14.6 \pm 6.88	18.0 \pm 7.10	23.9 \pm 4.38	29.1 \pm 6.57	29.9 \pm 11.3	14.9 \pm 3.58	< 20
P	FB	3610 \pm 480	4420 \pm 310	3580 \pm 290	5190 \pm 400	4020 \pm 680	4500 \pm 740	5480 \pm 610	5000–10000
	soil	188 \pm 64.7	367 \pm 39.7	327 \pm 64.2	707 \pm 197	338 \pm 110	322 \pm 109	428 \pm 32.2	–
Pb	soil	16.2 \pm 2.68	22.5 \pm 3.22	22.4 \pm 4.07	14.7 \pm 1.97	31.1 \pm 2.93	28.4 \pm 9.39	23.6 \pm 2.00	< 20
	FB	1730 \pm 150	2020 \pm 150	1850 \pm 140	2550 \pm 330	2170 \pm 400	2200 \pm 330	2220 \pm 400	1000–3000
S	soil	136 \pm 30.4	178 \pm 26.5	244 \pm 72.9	224 \pm 73.5	315 \pm 151	317 \pm 118	189 \pm 55.8	–
	FB	0.59 \pm 0.18	< MDL	0.52 \pm 0.08	0.82 \pm 0.45	0.74 \pm 0.60	0.81 \pm 0.34	0.43 \pm 0.10	< 2
Sr	soil	3.61 \pm 1.27	8.91 \pm 3.13	12.8 \pm 5.37	24.2 \pm 3.95	27.4 \pm 9.55	24.9 \pm 13.0	6.58 \pm 0.90	–
	FB	0.19 \pm 0.12	0.20 \pm 0.12	0.22 \pm 0.09	0.76 \pm 0.45	0.21 \pm 0.15	0.28 \pm 0.14	0.36 \pm 0.14	< 10
Ti	soil	110 \pm 13.6	210 \pm 80.3	202 \pm 79.0	429 \pm 123	225 \pm 94.5	282 \pm 96.7	364 \pm 95.5	–
	FB	85.5 \pm 19.1	102 \pm 26	74.5 \pm 15.8	104 \pm 29	86.3 \pm 18.8	108 \pm 35	101 \pm 21	30–150
Zn	soil	27.2 \pm 5.49	43.7 \pm 12.6	51.7 \pm 13.5	72.8 \pm 16.5	84.6 \pm 10.2	81.4 \pm 31.4	44.5 \pm 6.41	< 100

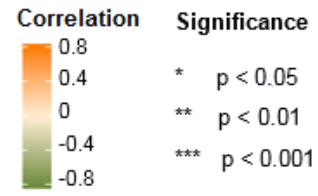
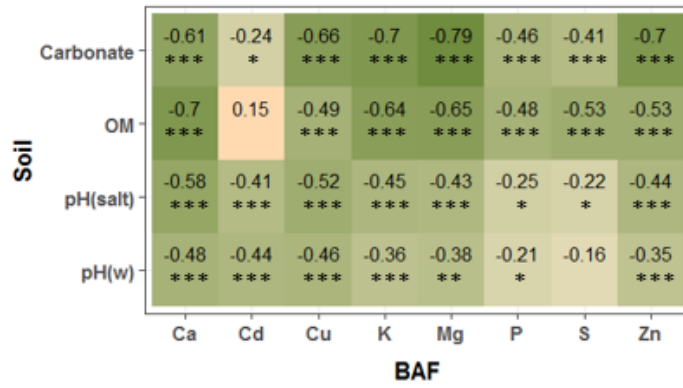
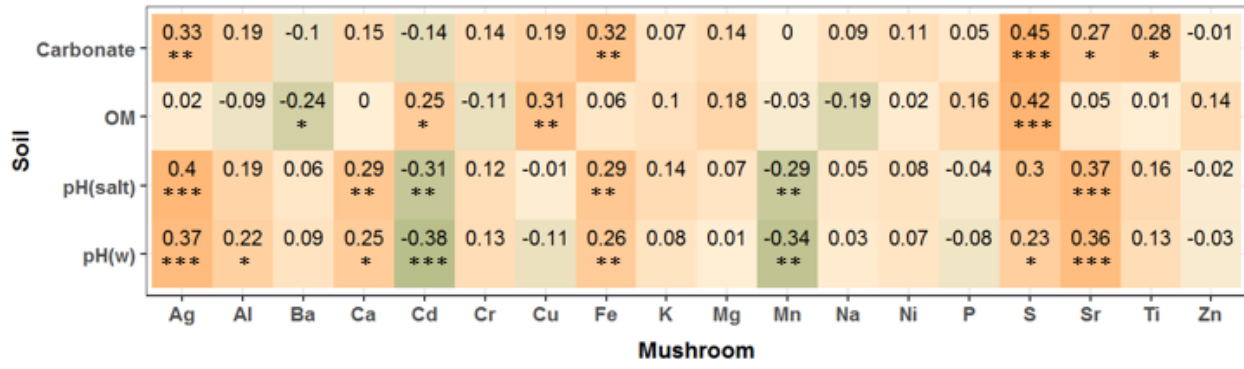
^aUsual content of elements in mushrooms from unpolluted areas according to Kalač (2016).

^bBackground levels for soils given by the Ministry of Environment of Romania (Government Decree 756/1997, November 3, 1997).

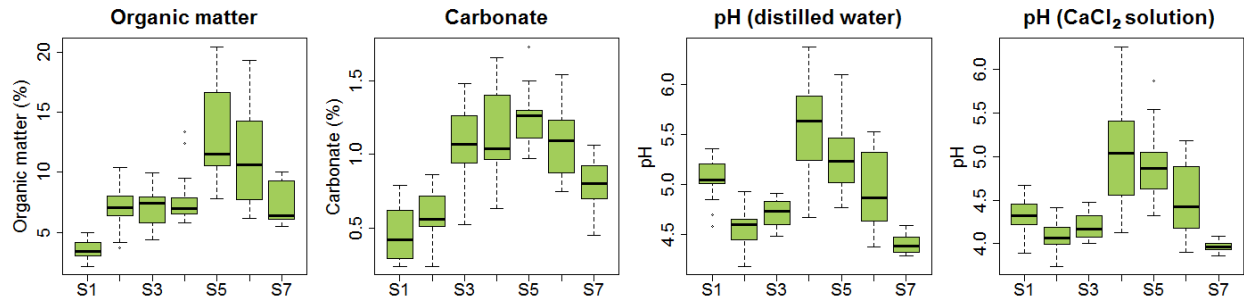








Journal Pre-proof



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The city had local influence over the mineral composition of this mushroom.

The soil properties strongly affected the elemental uptake capability of the fungus.

The mineral accumulation potential of the fungus gradually decreased toward the city.

The carbonate content of the soil impeded the uptake of many metals by the mushrooms.

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