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HABITAT CONDITIONS AND USABILITY OF THE HOG TRUFFLE (*Choiromyces Meandriformis* Vitt.) FOUND IN BOSNIA AND HERZEGOVINA

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Abstract

Several specimens of the hog truffle fungus (*Choiromyces meandriformis* Vitt.), that belongs to the family *Tuberaceae* Dumort. (1822) were found in the artificially established middle-aged Norway spruce forests (*Picea abies* (L.) Karsten) in the Kneževo area of the Republic of Srpska in Bosnia and Herzegovina. Besides the spruce as a sole species in the tree layer, common hazel, single-seeded hawthorn and old man‘s beard were found in the high shrub layer. Nine species were registered in the low shrub layer and 32 species in the herb layer, with mosses as the most abundant species due to acidification of the upper soil layer by the spruce needles. Unlike the "true truffles", hog truffle partly emerges on the surface, which makes it easier to spot without truffle dogs or other trained animals. Soli under spruce plantation were strongly acid (pH 3.9 - 4.6) and illimerised. Parent materials were flysch, i.e. conglomerates, marls and breccias. Soil texture was silt loam and dominant soil separate was silt (63.59 %). Chemical analysis of the flesh of fungus show that it fulfills criteria related to the edible mushrooms, except concentration of arsenic (As), that is much above (2.1 mg/kg) prescribed limit (0.3 mg/kg). At the same time, chemical analysis of the soil did not show increased concentration of arsenic (found 4,4 mg/kg) in comparison with average value for all soils (5 mg/kg), neither to the allowed interval for uncontaminated soils (0.2 – 40 mg/kg) according to the WHO. This fact leads to the conclusion that hog truffle has particularly emphasized affinity towards arsenic because of its capability to accumulate almost 50 % of arsenic content in the soil, unlike, for example, bolete mushrooms (*Boletus* spp.) which used to accept just around 0.6 % of the available soli arsenic.

Keywords: *hog truffle, usability, arsenic content, habitat conditions*

Introduction

Many fungi species spend the most of their life cycle underground, but majority of them produce large fruit bodies above the earth's surface. Smaller number of the fungi from the classes *Zygomycetes*, *Ascomycetes* and *Basidiomycetes* produce relatively large hypogenous fruit bodies and thus belong to true underground fungi (Hawker, 1954).
Underground fungus hog truffle was for the first time scientifically described by Elias Fries in 1830 under the name of *Mylitta venosa* Fr. At the same time Italian mycologist Carlo Vittadini described the genus *Choiromyces* with the species *C. meandriformis* Vittad. Later on, species *Mylitta venosa* was assigned to this genus as *Choiromyces venosus* (Fr.) Th.Fr. Species *C. meandryformis* Vittad. and *C. venosus* (Fr.) Th.Fr. are considered to be synonyms, while the former name is used more often in southern Europe (Montecchi et Sarasini, 2000). This approach is based on the great similarity of fruit bodies and spore morphology. According to DNA analysis, this fungus is phylogenetically closer to the so called "true" truffles of the genus *Tuber*, than it is with the truffle-like species such as "desert truffles" of the family *Terfeziaceae* (Percudani et al, 1999; Moreno et al., 2011).

Hog truffle lives in mycorrhizal association with many tree species, coniferous as well as deciduous, though Focht (1986) states that false truffle is not obligatory mycorrhizal, but "*it grows on the fields without trees"*. In Sweden it fruits during the summer (June-August), while in USA in October (Castellano et al, 1999). Unlike the "true truffles", it partly emerges on the surface, which makes it easier to spot. But, studies conducted with the truffle dogs have shown that adult fruiting bodies don't always come up on the surface, but rather fully develop underground (Wedén, 2007).
Hog truffle is known from Balkans (Anon., 2010/1; Hrka, 1984), Scandinavian countries (Wedén, 2007), England (Hawker, 1954; Pegler et al., 1993), France, Germany, Hungary (Wedén, 2007), Slovenia (Piltraver et Ratoša, 2006; Poler, 2010), Spain, Italy and Turkey (Anon., 2011), Russia (Wasson et Wasson, 1957), North America (Montecchi et Sarasini, 2000; Castellano et al, 1999) and some other parts of the world.
In Bosnia and Herzegovina hog truffle is considered a vulnerable species (Anon. 2010/1).

Material and methods

Study area

Hog truffle was found in the artificially established Norway spruce forest (*Picea abies* (L.) Karsten) in the Kneževo area, village Rađići, locality Vrhovine (44°32'42''N, 17°18'48''E), in the second half of the August of 2010 and 2011. Elevation is 840 m, inclination is 5%, eastern exposure, and canopy cover is 60%. Bedrock is carbonate flysch, i.e. conglomerates, marls and breccias. According to available information, the same species was found at southeastern slopes of mountain Vlašić, locality Gluha Bukovica (44°19'50''N, 17°43'44''E), at the straight line distance of 40 km (Anon., 2011/1).

Figure 1: Norway spruce forest where the hog truffle was found
(photo: S. Ljubojevic)

Methods

Age of the plantation was determined by tree-rings counting on the cross-section in the zone of the lower trunk of the representative tree. Floristic composition of the Norway spruce forest was determined using standard phytosociological method (Braun-Blanquet, 1964, according to Stefanović, 1977). Contours of a typical forest association were staking out with GPS „Garmin eTrex Summit". With the same device altitude and exposure were determined, while terrain slope was measured with clinometer "Meridian". Degree of canopy closure was estimated occularly based on conditional scale by Bunuševac (1952).
Plant species were determined using basic and standard floras keys taken from: Javorka et Csapody (1975), Josipović (1970-1977) and Sarić et Josipović (1986).

Physical and chemical analysis of the soil was done using the standard procedures for selected characters. Soil pH in water as active acidity and in 1M of KCl as exchangeable acidity was determined electrochemically using the combination pH electrode on the PHM240 pH/ion meter–radiometer. Humus content in soil was determined using colorimetric method, in the sample with 1N $K_2Cr_2O_7$ and concentrated sulfuric acid. Organic matter was determined by burning at 600 °C. Total nitrogen (% N) was determined by Semimicro-Kjeldahl method, modified after Bremner. Accessible forms of nitrogen in soil (NH_4^+, NO_3^-) were determined using the Bremner method – by extraction of mineral forms of nitrogen using 2N KCl. Amount of the accessible form of phosphorus in the soil was determined using Al-method (Egner-Riehm-Domingo) along with the readings of the optical density on the Jenway Spectrophotometer 6405 UV/Vis. Total phosphorus was determined by “wet burning” method in the solution of acids – with the ammonium vanadate (yellow method). Amount of the accessible form of potassium was determined by Al-method (Egner-Riehm-Domingo) with the direct reading of the concentration on the flame photometer Jenway PFP 7. Total potassium amount was determined by “wet burning” method in the acid solution using the technique of the flame photometry. Total amount of manganese (Mn), zinc (Zn), iron (Fe), copper (Cu), lead (Pb) was determined by destroying the sample with the “aqua regia”, and readings were obtained by the AAS (Atomic Absorbtion Spectometry) using the flame technique. Total amount of mercury (Hg) and arsenic (As) was determined by destroying the sample with the “aqua regia”, and readings were obtained by the AAS and hybrid technique. Mechanical structure of soil was determined by the pipette method using sodium pyrophosphate. Determination of the fungus was done by the macroscopic and anatomical-histological characteristics, according to the standard methodological procedure (Lazarev, 2003). Specimen’s belonging to the genus *Choiromyces* was determined using the key based on the morphology of asci and spores (Trappe et Castellano, 2007). Specimen’s belonging to the species *Choiromyces meandriformis* was determined using the prepared microscope slide of the spore, and comparing it with the referent description and drawing (Hawker, 1954).

Figure 2: Hog truffle
(photo: S. Ljubojevic)

Figure 3: Hog truffle, cross section
(photo: S. Ljubojevic)

Chemical composition of the fungus was determined as follows: protein content was analyzed applying the Kjeldahl method, and lipid content was analyzed following the standard method of Soxhlet. Content of carbohydrates was determined by calculating the difference between 100 and the sum of the contents of proteins, fat, water and ash. Percentages of lipids, carbohydrates and proteins were multiplied by corresponding factors. Thus obtained values were added together, and their sum represents energy value of 100 g of the sample. Arsenic content in the fungus flesh was analyzed following the standard procedure for food products (Anon., 2005). Contents of lead and cadmium were analyzed following the method UMH-243 (method for determination of the metal and metalloid residuals in foodstuff). Nutritive value was assessed by the comparison of the results with the relevant regulations (Anon., 2009).

Results and discussion

Habitat conditions

Several specimens of the hog truffle fungus were found in the vicinity of spruce trees, after their fruit bodies partly emerged on the soil surface.

Norway spruce artificial forest (i.e. plantation) in which we found the hog truffle was 41 years old, with 60 % canopy closure. We made two phytocoenological records in the object of the research. First record (Tab. 1) was taken near the plantation fringe, so there we found some species from the surrounding plant communities. On this record we have the layer of high shrubs (thanks to the lower canopy cover), which we don’t have on the other one, taken inside the plantation. Spruce (*Picea abies* (L.) Karsten) was the sole representative of the tree layer, while common hazel (*Corylus avellana* L.), single-seeded hawthorn (*Crataegus monogyna* Jacq.) and old man’s beard (*Clematis vitalba* L.) represented high shrub layer. Lower shrub layer consisted of: blackthorn (*Prunus spinosa* L.), common hazel, dog rose (*Rosa canina* L.), European wild pear (*Pyrus pyraster* Burgsd.), field maple (*Acer campestre* L.), silver birch, spindle (*Evonymus europaeus* L.), single-seeded hawthorn and wild cherry (*Cerasum avium* (L.) Moench). In the herb layer 32 species were recorded, with mosses as the most abundant species due to acidification of the upper soil layer by the spruce needles. The carpet of mosses together with the layer of raw humus are leading to the soil moisture increment, which results in the occurrence of some herbaceous species belonging to the vegetation of wet meadows.

Table 1: Phytocoenological records taken in the study area

No	Species	Record No.			
		1		2	
Tree layer					
1	*Picea abies* (L.) Karsten – Norway spruce	4	5	4	5
High shrub layer					
2	*Clematis vitalba* L. - old man’s beard		+		
3	*Corylus avellana* L. - common hazel		+		
4	*Crataegus monogyna* Jacq. - single-seeded hawthorn		+		
Low shrub layer					
5	*Betula pendula* Roth - silver birch				+
6	*Acer campestre* L. - field maple		+		+
7	*Crataegus monogyna* Jacq. - single-seeded hawthorn		+		+
8	*Cerasum avium* (L.) Moench - wild cherry		r		+
9	*Rosa canina* L. - dog rose		r		+
10	*Prunus spinosa* L. - blackthorn		+		
11	*Evonymus europaeus* L. - spindle		r		
12	*Pyrus pyraster* Burgsd. - European wild pear		r		
13	*Corylus avellana* L. - common hazel				r
Herb layer					
14	*Pteridium aquilinum* (L.) Kuhn	1	1		+
15	*Veronica officinalis* L.		+		+
16	*Hieracium pilosella* L.				+
17	*Dactylis glomerata* L.	1	2	+	1

18	*Poa nemoralis* L.	+	1	1	2
19	*Brachypodium sylvaticum* (Hudson) Beauv.	+	1	+	1
20	*Cruciata glabra* (l.) Ehrend	+	1	+	1
21	*Helleborus odorus* Waldst. et Kit.	+	2		+
22	*Primula vulgaris* Hudson	+	1	+	1
23	*Cirsium erisithales* (Jacq.) Scop.		r		+
24	*Aremonia agrimonoides* (L.) D.C.	1	2		
25	*Bellis perennis* L.		+		
26	*Fragaria moschata* Duchesne		+		
27	*Symphytum tuberosum* L.	+	1		
28	*Sanicula europaea* L.		r		+
29	*Crocus neapolitanus* Merd. et Lois		r		
30	*Platanthera bifolia* (L.) Richard		r		
31	*Veronica chamaedrys* L.				
32	*Taraxacum officinalis* Weber		+	1	2
33	*Galium mollugo* L.		+		+
34	*Potentilla erecta* (L.) Räuschel		+		+
35	*Trifolium repens* L.		+		+
36	*Campanula patula* L.				+
37	*Ranunculus acris* L.		r		
38	*Peucedanum carvifolia* Vill.		+		+
39	*Plantago lanceolata* L.		+		+
40	*Scabiosa leucophylla* Borbas		+		+
41	*Pimpinella saxifraga* L.		r		+
42	*Viola hirta* L.		+		
43	*Prunella vulgaris* L.		r		
44	*Rhodobryum roseum* (Hedw.) Limpr.			+	3
45	*Polytrichum formosum* Hedw.			+	2

Analysis of the physical properties of the soil has shown that according to its texture, soil can be classified as a silt loam. Dominant soil separate is silt (0.06 – 0.002 mm) with 63.59 %. There are also clay (< 0.002 mm) with 23.61 %, and sand (2.0 – 0.06 mm) with 12.80 %. Chemical analysis of the soil is presented in Table 2. Observed ground shown strong acidity; active acidity is 4.6 (pH in H_2O), and exchangeable is 3.9 (pH in KCl). Total arsenic amounted 4.4 mg/kg, while the mercury wasn't detected. According to the World Health Organization (WHO), the content of arsenic in uncontaminated soil ranges between 0.2 and 40.0 mg/kg (Anon., 2010/2), and according to Beyer et Cromartie (1987), average concentration of arsenic in the soil is about 5 mg/kg. With that regard, we can consider arsenic concentration in the observed soil as a normal. Arsenic is characterized by high adsorption and fixation with acid soils, particularly with upper layers, so that it is possible another fungus and mushrooms to have increased content of arsenic on these and similar habitats.

Table 2. Soli chemical analysis

Parameter	Determined value	Parameter	Determined value
pH in H_2O	4.6	Total potassium (K)	0.24 %
pH in KCl	3.9	Accessible potassium (K_2O)	11.0 mg/100g
Organic matter content	16.97 %	Total iron (Fe)	4.85 mg/kg

Humus	7.2 %	Total zinc (Zn)	64.3 mg/kg
Total nitrogen (N)	0.36 %	Total copper (Cu	30.8 mg/kg
Total phosphorus (P)	0.12 %	Total lead (Pb)	45,4 mg/kg
Accessible phosphorus (P_2O_5)	2.0 mg/100g	Total arsenic (As)	4,4 mg/kg

Obtained results are partially agreed (acid soil, wet site, spruce forest) with findings of Rodrigez (2008) who states that in Spain this fungus is found on the acidic, clay soils in the areas with high precipitation, under the English oak (*Quercus robur* L.) and Norway spruce. Habitats that this fungus occurs in Sweden are far more different from observed in Kneževo area. Preliminary results of the Wedén‘s research in Sweden (2007) show that false truffle prefers clay soil, broadleaved symbionts and open habitats with scarce herb layer. Fruit bodies were found in different habitats: garden lawns together with English oak and linden (*Tilia* spp.) or English oak and birch (*Betula* spp.); in the old opened English oak woods; at the fringes of the forests of conifers and birch. These results point to English oak, linden and birch as possible symbionts for the hog truffle, as well as Scots pine (*Pinus sylvestris* L.), fir (*Abies* spp.) and hazel (*Corylus* spp.). Regardless of the habitat variability, the soil was always clayish, and covered with very sparse herb layer, with large share of moss. Researches in the USA have shown that hog truffle is in mycorrhizal relationship with different pine species, with the Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) and the western hemlock (*Tsuga heterophylla* (Raf.) Sarg.), but they did not specified Norway spruce as a possible symbiont (Castellano et al, 1999).

Usability of the hog truffle

Hog truffle’s flash at the intersection is of the white colour, hard and marbled (Fig. 3). It has a distinguish strong odor that is reminiscent of the smell of some inhalational anesthetic, which comes from the present arsenic. It contains 4,99 g of proteins, 0,006 g of fat and 12,36 g of carbohydrates in 100 g of fresh flash (Tab. 3). Related to our most important species of edible fungi, hog truffle is characterized by significantly higher content of carbohydrates and bigger energy value, and much lesser content of fat (Tab. 4). Contents of cadmium and lead are lesser than allowed, while the arsenic content (2.1 mg/kg) is many times higher than allowed (0.3 mg/kg max.), (Anon., 2005). That means this fungus shouldn’t be used in human diet, or sold on the B&H market as a foodstuff.

Table 3. Hog truffle’s flash chemical analysis

Parameter	Measurement unit	Referent value	Determined value
Protein content	g/100g	-	4.99
Fat content	g/100g	-	0.006
Carbohydrates content	g/100g	-	12.36
Arsenic (As) content	mg/kg	< 0.3	2.1
Cadmium (Cd) content	mg/kg	< 1.0	0.26
Lead (Pb) content	mg/kg	0.30	< 0.05
Energy value	kcal/100 g	-	69
	kJ/100 g	-	295

Table 4. Comparative overview of the main nutrients in hog truffle and the most important

mushrooms in Bosnia and Herzegovina (according to Lazarev, 2003)

No	Name of fungi species	Proteins	Fat	Carbohydrates	Energy value kcal/100g
		%			
1	***Choiromyces meandriformis***	**4.99**	**0.006**	**12.36**	**69**
2	*Agaricus campestris*	4.88	0.20	3.57	26
3	*Cantharellus cibarius*	2.64	0.43	3.81	21
4	*Boletus edulis*	5.39	0.40	5.12	33
5	*Lactarius deliciosus*	3.08	0.76	3.09	22
6	*Marasmius oreadus*	6.83	0.67	6.06	41
7	*Suillus luteus*	1.48	0.27	3.95	17
8	*Morchella spp.*	3.28	0.43	4.50	25
9	*Tuber spp.*	7.57	0.51	6.58	44

Content of the arsenic in the plants, including fungi, is usually much lower than in the soil (Živanović, 2010). In the studies that were conducted at 18 localities in Serbia, arsenic content in boletes (*Boletus* spp.) was many times lower than in the soil where the mushrooms grew. In the Norway spruce natural forest at the locality Prijepolje – Jabuka arsenic concentration was 3.53 mg/kg, while in the flash of the boletes were only 0.02 mg/kg or 176 times lesser (Milinović, 2011). In other words, boletes adopt only about 0.57 % of the available soil arsenic. Our study shows significantly higher affinity of the hog truffle towards arsenic accumulation, which is capable do adopt almost half of the available quantities (47.7 %) - 4.4 mg/kg in the soil vs. 2.1 mg/kg in the fungus' flash (Tab.3).

Affinity towards accumulation of arsenic is not exclusive feature of hog truffle. Among others, Byrne et Tusek-Znidaric (1983) registered arsenic in concentrations between 34 and 182 mg/kg of dry matter in caps and stalks of the amethyst deceiver (*Laccaria amethystina* (Bolt. ex Hooker) Murr).

Obtained results are in accordance with opinions of Focht (1986) and Hrka (1984) in Croatia, considered hog truffles as poisonous. However, some authors in Croatia consider it edible (Tomović et Lisjak, 2004; Lukić 2006) and some inedible or with no value (Božac, 1984). Hog truffles are considered poisonous (in fresh condition) in Slovenia (Poler, 2010), as well as in France and Italy (Montecchi et Sarasini, 2000). In Spain it is on the list of fungi that can not be commercially exploited. However, because of the size and colour, it is very often falsely represented as the white truffle (*Tuber magnatum* Picco 1788) (Rodrigez, 2008). At the same time, in the Upper Silesia in Germany it is considered as a mushroom of choice and called "Kaiserpilz". In Turkey it is foraged in significant quantities and exported during the season as a fresh, and during whole year as a dried or frozen (Anon., 2011). Hog truffle is being used in Sweden for the last 100 years. Major foraging areas are around the lakes in the southeastern part of the country. Fruiting bodies are quite large, with the smell that varies from the quite inconspicuous to very pleasant and aromatic, and even more to repellent. These differences reflect the state of fruiting bodies and spores, in the range from immature over mature to state of decaying. Mature fungi are considered a delicacy and used fresh, specially mixed with the butter. In such a way volatile lipophilic compounds are preserved which contribute to complexity of odors. This fungus is also used fried, and for the longer storage it is dried or deep-freeze. Preliminary study of citotoxicity of *Choiromyces venosus* in Sweden shows that the concentration of extracts of this fungus, required for the cell death, is similar to those of common mushroom (*Agaricus bisporus* (J.E.Lange) Emil J. Imbach) and summer truffle (*Tuber aestivum*), (Wedén et al., 2007).

Conclusions

Hog truffle can be found in the artificially established middle-aged Norway spruce forest in the Kneževo area of the Republic of Srpska in Bosnia and Herzegovina, on acid and illimerised solis, at the end of the summer.

Due to the acidification of the upper soil layer by the spruce needles, the most abundant species in the herb layer are mosses. The carpet of mosses together with and the layer of raw humus are leading to the soil moisture increment, which results in the occurrence of some herbaceous species belonging to the vegetation of wet meadows.

Unlike the "true truffles", hog truffle partly emerges on the surface, which makes it easier to spot without truffle dogs or other trained animals.

Hog truffle’s flash at the intersection is of the white color, hard and marbled. Has a distinguish strong odor that is reminiscent of the smell of some inhalational anesthetic, which comes from the present arsenic. Arsenic content is many times higher than allowed, making this fungus inedible or even poisonous. If there is no increased content of arsenic, hog truffles would be considered as a quality edible fungus.

Content of arsenic in the plants, including fungi, usually is much lower than in the soil where they grow, which is not the case with hog truffle in this study. It shows particular affinity to arsenic, and accumulates it in the amount that is equivalent almost to the half of its concentration in the soil.

Arsenic is characterized by high adsorption and fixation with acid soils, particularly with upper layers, so that it is possible another fungus and mushrooms to have increased content of arsenic on these and similar habitats.

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