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DIVERSITY OF FUNGI IN NESTS AND PELLETS OF MONTAGU'S HARRIER (*Circus pygargus*) FROM EASTERN POLAND - IMPORTANCE OF CHEMICAL AND ECOLOGICAL FACTORS

RÓŻNORODNOŚĆ GRZYBÓW W GNIAZDACH I WYPLUWKACH BŁOTNIAKA ŁĄKOWEGO (*Circus pygargus*) WYSTĘPUJĄCEGO WE WSCHODNIEJ POLSCE - WPŁYW CZYNNIKÓW CHEMICZNYCH I EKOLOGICZNYCH

Abstract: General species amount, diversity and frequency of saprophytic and potentially pathogenic for homoiothermic organisms fungal species were studied in 7 nests and pellets of Montagu's Harrier (*Circus pygargus*) from pit bogs of Calcereous Marshes near Chelm (Poland). It was found that examined nests exhibited the environmental properties promoting a great diversity and frequency of *Micromycetes* communities. Mitosporic fungi were the most representative taxonomic groups among these communities. In ecophysiological aspect, the fungal communities found in nests belonged mostly to ubiquitous fungi (polyphags) including cellulolytic species, while keratinolytic species were less abundant. On the other side, both ubiquitous and keratinolytic species were isolated from pellets. Among characterized fungi, the most common were: *Trichoderma viride* in nests and *Doratomyces stemonites* in pellets. The two tested sources were found to be settled by typically saprophytic but also potentially pathogenic fungi, including *Aspergillus fumigatus*, *Scopulariopsis brevicaulis*, *Chrysosporium tropicum* and *Ch. georgii*. Water content, pH and temperature during nestling affected the profile of fungal species in nests and pellets, which was demonstrated by the presence of hydrophilic, alkalitolerant and thermotolerant species isolated from examined material.

Keywords: fungi, nests, pellets, Montagu's Harrier, chemical and ecological factors

Nests of wild birds are the specific niche of microscopic fungi which is rarely studied in ecological and chemical aspects [1]. Relatively precise information concerns keratinolytic species dermatophytes and *Chrysosporium* genus [2]. These fungi colonize

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and gradually degrade keratin-containing material in nests: feather, hair and food leftovers [1-3]. Some of these fungi, eg *Microsporum gypseum*, are opportunistic pathogens responsible for human and animal mycoses. Special danger, also for birds in light of increased mortality among nestling, arises from *Aspergillus fumigatus*, a main factor of lung aspergillosis [1, 4-6]. Pellets are even less studied in mycological aspects [1]. Pellets, produced by some groups of birds, as shrikes (*Lanidae*), corvids (*Corvidae*) and birds of prey, are small bullets containing remains of birds' prey, with lots of indigested leftovers of their quarry. The content of pellets depends of food preferences of particular raptor species; pellets serve also as a medium for fungal growth [7].

Montagu's Harrier nests and pellets, still hardly known as a niche of fungi, are interesting models for studies on diversity of fungal species. This bird is a diurnal raptor, highly specialized at hunting for rodents (*Rodentia*), which supplements his diet with insects (*Insecta*) and small passerine (*Passeriformes*) [8]. Montagu's Harrier nests on ground, which facilitates its access for researchers [9] and its pellet can be collected in nests and under perch sites for adults, localized near nests.

The aim of this work was the presentation of diversity of microscopic fungi found in nests and pellets of Montagu's Harrier (*Circus pygargus*) and analysis of some chemical and ecological factors determining occurrence of particular groups and species of fungi.

Study area and methods

Nests: content and structure

Material for research was isolated from 7 nests of Montagu's Harriers from Blota Serebryckie Marsh, collected in 2004 (nests I and II) and 2005 (nests III, IV, V, VI and VII) year. Blota Serebryckie Marsh is one of pit bogs of Calcareous Marshes near Chelm (Eastern Poland: 51°07'-51°11'N, 23°30'-23°42'E). The Marshes are lowland bogs lying in $CaCO_3$ beds. Bogs are dominated by *Cladietum marisci* community. Water table levels fluctuate in range: 40÷10 cm in the spring and 20÷0 cm in the summer. In studied area, Montagu's Harriers locate their nests exclusively in fields of saw sedge (*Cladium mariscus*) where pH of soil ranges between 7 and 8 [10].

Montagu's Harriers' nests in basal and external parts are constructed with twigs, usually birch's (*Betula* sp.) and willow's *Salix* sp. ones, common reed *Phargmites australis* stems of 10÷50 cm in length and other plants, often including goosefoots (*Chenopodium* sp.) and common broom (*Sarothamnus scoparius*). Nests lining is build from saw sedge leaves. Center of nests, where the birds lay eggs, is stuffed with delicate straw leaves and radicles, mixed with females' feathers. During incubation and early nestling period, the nest is rarely being rebuilt; its extension may happen only in case of water soaking. However, during late nestling period, the nest becomes to be developed and rebuilt. This process lasts until the young leaves the nest. Within this period, the content of organic substances in nest is enriched with new nest material, young's pellets, feces and leftovers of non-consumed food. The nest, at the moment of eggs-lying, is usually oval with 39 cm in diameter (22÷51 cm), with internal lining sphere of 10÷15 cm in diameter (unpublished data). Within the time, the nest, containing feathers, pellets and other materials, becomes pressed by weight of growing young. For the ethic reasons, nests for studies were collected after fledging of chicks, at the end of July 2004 and 2005.

Pellets

Pellets for analysis, containing undigested leftovers of food, were collected from under perch sites (bushes, dead trees, pales or other elements sticking out over the fields of saw sedge, where the birds clean their plumage, rest or look out for a danger. Montagu's Harriers pellets are composed mainly of hair and bones of rodents, usually common voles (*Microtus arvalis*), feathers of passerines and leftovers of insects, mainly great green bush cricket (*Tettigonia viridissima*) and beatles (*Coleoptera*) armours. For the studies, 5 Montagu's Harrier pellets were collected from perches localized near the nests within the nestling period.

Estimation of frequency and identification of fungal species

Nest material was isolated from the lining (inner layer), side part (outer layer) and the part in between (middle layer) [11]. In case of one of the studied nests, with hardly distinguished layers, the material for studies was taken from the whole nest. Samples for experiments (100÷200 g) were collected from randomly chosen fragments of nests, taken together and homogenized. Samples from pellets were collected in analogous mode.

Fungi were isolated by plate dilution method using Martin medium (1951), containing (g in dm^{-3} H_2O): glucose 10.0, pepton 5.0, KH_2PO_4 1.0, $MgSO_4 \cdot 7H_2O$ 0.5, agar 20.0, bengal rose 33.3 mg, streptomycin 0.03%, chlorotetracycline 0.002% and Sabouraud medium [13] containing (g · dm^{-3} H_2O): glucose 40.0, pepton 10.0, agar 20.0, streptomycin 0.03%, chlorotetracycline 0.002%. Choice of isolation media was determined by kind of nestling material, its chemical composition and anticipated ecological status of fungi colonizing the nests. Martin medium is used for isolation of saprophytic fungi colonizing mainly plant leftovers in different environments and was developed for identification of soil fungi [12]. Sabouraud medium is commonly applied for isolation of pathogenic or potentially pathogenic (for people and animals) fungi [13], indirectly transferred by animal substrates. All fungal cultures, in triplicate, were incubated at 26°C.

Frequency of fungi was estimated in average values of cfu g^{-1} of dry mass of nest material. Dry mass was estimated by drying method at 105°C. Pure fungal cultures were transferred to glucose-potato medium (PDA [g · dm^{-3}]: glucose 20.0, agar 20.0, potato extract 1 dm^3 or Sabouraud medium. Identification of isolates was performed on a base of macromorphological features observations (colonies) and micromorphological observations of microcultures, using systematic pictorial dictionaries: Domsch et al [14], Kwaśna et al [15], van Oorschot [16].

Estimation of humidity and chemical composition of nests

Water content in nests was determined using drying method at 105°C. Chemical analysis included estimation of: pH and total carbon and total sulphur contents, using high-temperature firing in oxygen and temperature conductivity methods, organic carbon content by Tiurin method, total nitrogen and total phosphorus contents by flow spectrophotometry method, potassium content by flame emission spectrometry and Ca and Mg content by atomic absorption spectrometry method.

Evaluation of results

Results of fungal groups frequency estimation were analyzed by the statistic method, counting the standard deviations (SD). Analysis of diversity of fungal communities (on a base of fungal isolates frequency representing particular species in analyzed groups) was performed on a base of Simpson factor [17], calculated according to a formula:

$$D = 1 - \sum_{i=1}^{S} p_i^2$$

where p_i is a percentage of isolated species from "i" species in particular fungal community (the nests or one of its layers) and is equal to a ratio of amount of species isolates to amount of isolates of all species on particular isolation medium. Values of Simpson factor ranged between 1 and 1/S, where S is an amount of all species in community [17].

Species domination [18] was determined using a formula:

$$D = 100 \cdot (Sa : S)$$

where Sa is a sum of isolates from species "a" and S is a sum of isolates from tested group. Systemic groups domination on fungal class level was determined analogically, according to the same formula, where Sa is a sum of isolates from systemic unit isolated on particular medium and S is a sum of isolates of all fungi isolated on this medium. For estimation of frequency of fungal species and taxonomic groups, the following scale was accepted:

sporadically	<1%
rarely	1÷10%
often	10÷25%
very often (abundantly)	26÷50%
massively	>50%

Results

1. Chemical content, pH and humidity of Montagu's Harriers nests

Nests of Montagu's Harrier contained high but diverse amounts of basal nutritious components necessary for fungal growth, mainly organic carbon and nitrogen. pH of nests was close to neutral or slightly alkaline values which was probably due to a high Ca content in the soil and also to presence of nitrogen (in a form of NH_4^+ groups, resulting from degradation of keratin from hair and feathers) and uric acid-containing birds' feces.

Table 1

Chemical content of Montagu's Harrier nests

Nest number	Content [% of d.m.]							
	C total	C organic	N total	S total	P	K	Ca	Mg
I	43.15	41.46	2.19	0.25	0.21	0.14	1.27	0.055
II	45.77	42.43	1.44	0.19	0.09	0.11	0.85	0.040
III	45.97	44.14	2.50	0.36	0.19	0.20	0.76	0.046
IV	26.86	25.74	1.31	0.16	0.08	0.22	1.95	0.144
V	44.42	41.60	1.52	0.19	0.14	0.19	1.08	0.059
VI	44.43	41.14	2.72	0.42	0.40	0.25	1.35	0.080

Humidity was high but also diverse and was the highest for nest V and the lowest for nest III. In some nests (IV) humidity of lining was higher than that observed for other layers (Tabs. 1, 2).

Table 2

Water content and pH in particular layers in Montagu's Harrier nests

Nest number	Layer	pH		Humidity [%]
		H_2O	KCl	
III	1	-	-	25.12
	2	-	-	25.17
	3	-	-	23.26
IV	1	-	-	40.15
	2	-	-	63.11
	3	-	-	45.68
V	1	7.60	7.50	70.65
	2	7.36	7.47	70.69
	3	7.33	7.40	70.61
VI	1	7.26	7.35	71.72
	2	7.70	7.60	70.58
	3	7.58	7.60	68.62
VII	-	6.70	7.10	52.50

Explanations: 1, 2, 3 - nest layer; 1 - outside, 2 - middle, 3 - lining; „-" not studied

2. General frequency of fungi in Montagu's Harrier nests

General frequency of fungal isolates in Montagu's Harrier nests was high. In case of saprophytic fungi isolated on Martin medium it ranged between $6.0 \cdot 10^5 \div 1.2 \cdot 10^7$ cfu g^{-1} dry mass (Fig. 1A). Isolation of saprophytic fungi from nests collected in 2004 was impossible, because of the massive overgrowth of isolating plates by *Mucorales*; therefore, in Figure 1A only the data concerning the nests collected in 2005 were presented. Frequency of fungi isolated on Sabouraud medium, favoring the growth of pathogenic species, was lower: $7.0 \cdot 10^4 \div 1.1 \cdot 10^6$ cfu g^{-1} dry mass (Fig. 1B).

Significant diversities in frequency of saprophytic and pathogenic fungal groups for particular nests were found. These parameters were determined by nests humidity. Nests of higher humidity (V, VI and VII) were characterized by higher content of saprophytic species than those of lower humidity (III and also VI) (Tab. 2, Fig. 1A). Fungi were isolated on Sabouraud medium with the highest frequency for nest II and the lowest for nest III (Tab. 2, Fig. 1B).

Frequency of fungal species within the particular nest spaces was also distinct and the most significant in most humid nests: V and VI. In nest V, the most fungal species was observed within the lining in comparison with outer layer. In nest VI the observation was the opposite (Fig. 1A, B). In one case (nest IV) the most numerous fungi were observed for middle layer, when compared with others (Fig. 1B).

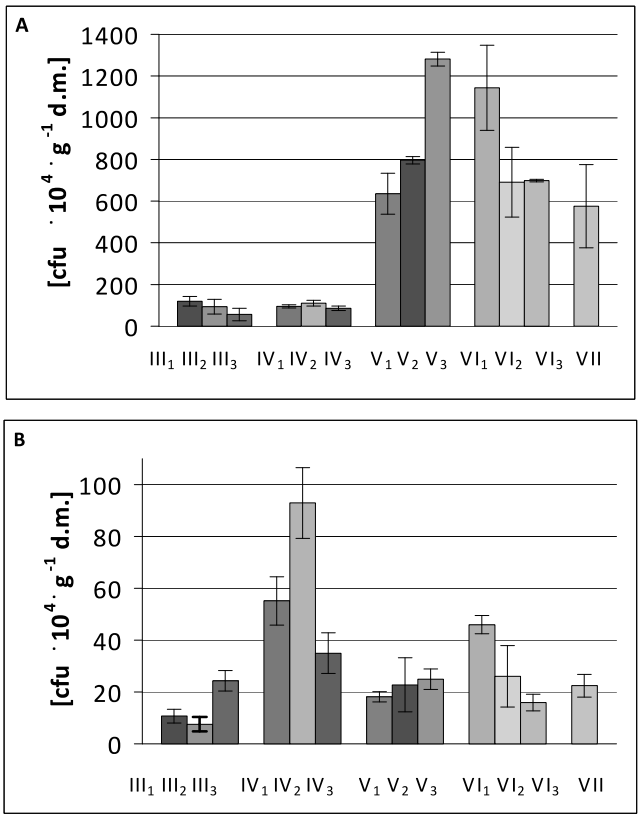

Fig. 1. General frequency of fungi isolated from Montagu's Harrier nests collected in 2005: A - Martin medium, B - Sabouraud medium. Explanation as in Table 2

3. Species and ecological diversity of fungi settling the nests of Montagu's Harrier

Among 7 tested nests of Montagu's Harrier (2 collected in 2004 and 5 in 2005) 2070 fungal species were isolated. They were identified as belonging to 28 genera and 50 species: 45 isolated were classified to genera only while 110 (in majority yeasts and dark pigmented and nonsporing moulds) were not classified (Tabs. 3-5). On average, 8 species of fungi were found per 1 nest. Considering the amount of isolated species, two groups of fungi, isolated either on Martin or Sabouraud medium, were similar and contained 28 or 29 species, relatively; however, they differed in species content. The difference was reflected in, eg, the lack of *Trichoderma* spp. and sporadically appearing *Chaetomium* species within the fungi growing on Sabouraud medium. The differences concerned also the frequency of particular populations. It was found that 81% fungi growing on Martin medium and 66% fungi growing on Sabouraud medium represented mitosporic fungi (*Fungi imperfecti*). 28% and 33% of isolated species, relatively, were classified to *Zygomycetes* (*Absidia, Mucor, Zygorhynchus*). *Ascomycetes* (*Chaetomium*) represented approximately 1% of all fungal isolates (Tabs. 3-5).

Table 3

Fungal species isolated from Montagu's Harrier nests and pellets (2004 and 2005)

No.	Species of fungus	Martin medium	Sabouraud medium
1	*Absidia glauca* Hagem	-	+
2	*Acremonium kiliense* Grütz	+	+
3	*A. strictum* W. Gams	+	-
4	*Actinomucor elegans* (Eidam) C.R. Benjamin & Hesseltine	+	-
5	*Alternaria alternata* (Fr.) Keissler	+	+
6	*Aspergillus flavus** Link ex Gray	+	-
7	*Aspergillus fumigatus* Fres.	+	-
8	*Beauveria bassiana* (Bals.) Vuill.	-	+
9	*Botritis cinerea* Pers. Ex Nocca & Balb.	+	-
10	*Botryotrichum piluliferum* Sacc.& March.	-	+
11	*Candida* sp.	-	+
12	*Ch. elatum* Kunze ex Steud.	+	-
13	*Ch. funicola* Cooke	+	-
14	*Ch. globosum* Kunze ex. Steud.	+	+
15	*Chrysosporium georgii** (Varsavski & Ajello) van Oorschot	+	-
16	*Ch. merdarium** (Link ex Gray) Carm.	+	-
17	*Ch. queenslandicum** Apis & Ress	+	+
18	*Ch. pannicola* (Corda) van Oorschot & Stalpers	-	+
19	*Chrysosporium pannorum* (Link) Hughes	+	+
20	*Ch. tropicum* Carmichael	+	+
21	*Chrysosporium anam. Arthroderma curreyi Berk*	+	+
22	*Cladosporium cladosporioides* (Fres.) de Vries	+	+
23	*Cl. sphaerospermum* Penz.	+	-
24	*Cylindrocarpon destructans* (Zinssm.) Scholten	-	+
25	*Doratomyces microsporus* (Sacc.) Morton & G. Sm.	-	+
26	*D. stemonites* (Pers. Ex Steud) Morton & G. S*m.*	+	+
27	*Emmonsia parva* (Emmons & Ashburn) Cif. & Montemartini		
28	*Fusarium oxysporum* Schlecht. emend. Sny & Hans	-	+
29	*F. sporotrichioides* Sherb.	-	+
30	*Geotrichum candidum* Link ex Leman	-	+
31	*Gliocladium roseum* Bain.	-	+
32	*Mortierella biramosa** van Tiegh.	+	-
33	*Mucor circinelloides* van Tiegh.	+	-
34	*M. hiemalis* Wehmer	+	+
35	*M. mucedo* Mich. Ex St.-Am.	-	+
36	*M. pusillus* Lindt	+	-
37	*M. racemosus* Fres.	+	-
38	*M. spinosus* van Tiegh.	+	+
39	*M. ramonissimus* Samutsevitsch	+	+
40	*Myrothecium roridum* Tode ex Steudel	-	+
41	*Paeciliomyces marquandii* (Massee) Hughes	-	+

42	*P. lilacinus** (Thom) Samson	-	+
43	*Penicillium chrysogenum* Thom	-	+
44	*P. expansum* Link ex Gray	-	+
45	*P. janthinellum** Biourge	+	-
46	*P. nigricans** Bain ex Thom	+	-
47	*P. verrucosum* Dierckx	+	-
48	*Phoma exiqua* Desm.	+	+
49	*P. herbarum* Westend.	-	+
50	*Scedosporium apiospermum** (Sacc.) Sacc.ex Castell. & Chalmes	+	-
51	*Scopulariopsis brevicaulis* (Delacr) Vuill.	-	+
52	*Torula herbarum* Pers. ex Gray	-	+
53	*Trichoderma koningii* Oudem	+	-
54	*T. viride* Pers. ex Gray	+	-
55	*Trichothecium roseum* (Pers.) Link ex Gray	+	+
56	*Trichosporon beigelii* (Kuchenn. Rabenh.) Vuill.	+	+
57	*Verticillium lecanii* (Zimm.) Viegas	+	+
58	*Zygorrhyrchus moelleri* Vuill.	+	-

* - isolated only from pellets

Analysis of diversity of fungal communities isolated from nests, taking into account frequency if fungi representing the species content of studied fungal groups (isolated on Martin and Sabouraud medium) was performed on a base of Simpson's coefficient (D) for 4 nests (III, VI, V, VI), collected in 2005. D values for fungi growing on Martin medium were higher than those for isolates on Sabouraud medium (Tab. 6). This fact was correlated with different quantitative proportions within particular fungal species in both studied groups, which was shown by the lack of fungal dominants on Sabouraud medium in comparison with Martin medium. The most common species within the fungal group isolated on Sabouraud medium reached only 15% of total species amount while on Martin medium - 25% (Tab. 6). Values of this parameter for fungal communities isolated from particular nests layers (lining, middle, outside) were similar, namely, they were lower for these growing on Sabouraud medium than on Martin medium (Tab. 7).

In ecological-chemical aspects of estimation of the species content for fungi colonizing the Montagu's Harrier nests, ubiquitous species (polyphags) growing on relatively accessible organic polymers: polysaccharides including cellulose, proteins (excepting keratin), lipids etc, were the most common. These fungi included *Acremonium, Aspergillus, Doratomyces, Penicillium, Scopulariopsis, Trichoderma, Trichothecium, Verticillum* populations (Tabs. 4 and 5). Among saprophytic fungi isolated on Martin medium, *Trichoderma koningii* dominated (24% of total isolated amount). *T. koningii* and *T. viride* made 34% of all isolates amount (Tab. 4). The most common species isolated on Sabouraud medium belonged to ubiquitous *Doratomyces stemonites* (15%) and *Chrysosporium pannorum* (13%). Sugar fungi, including yeast-like fungi (7%) apart of *Mucorales*, were found with high percentage (35%) on Sabouraud medium (Tab. 5).

Table 4

Frequency of isolation of particular fungal species from Montagu's Harrier nests (collected in 2005) on Martin medium

No.	Fungal species	III			IV			V			VI			VII	Total
		1	2	3	1	2	3	1	2	3	1	2	3		
1	*Acremonium kiliense*	-	-	-	7	-	-	2	6	-	5	5	2	2	29
2	*A. strictum*	-	-	1	-	-	2	-	-	-	-	3	5	6	17
3	*Acremonium* sp.	-	-	-	-	-	-	3	2	2	3	1	-	-	11
4	*Alternaria alternata*	-	-	-	5	-	5	6	3	4	2	-	-	-	25
5	*Aspergillus fumigatus*	9*	-	11	-	5	16	-	-	-	-	-	-	-	41
6	*Botritis cinerea*	-	-	-	16	8	-	-	-	-	-	-	-	-	24
7	*Chaetomium globosum*	-	-	-	4	-	-	-	-	-	-	-	-	-	4
8	*Chrysosporium pannorum*	-	-	-	-	12	-	-	-	-	-	-	-	-	12
9	*Cladosporium cladosporioides*	1	-	-	-	-	2	-	-	-	-	-	-	-	3
10	*Cladosporium sphaerospermum*	-	-	-	1	-	-	-	-	-	-	-	-	-	1
11	*Doratomyces stemonites*	5	2	-	-	11	3	15	8	9	7	8	-	11	79
12	*Mucor circinelloides*	3	3	-	3	-	-	-	-	-	-	-	-	-	9
13	*Mucor hiemalis*	9	1	2	9	2	5	5	-	-	-	-	-	-	33
14	*Mucor racemosus*	1	5	-	5	-	-	-	-	-	-	-	-	-	11
15	*Mucor spinosus*	1	-	-	7	4	3	-	-	-	-	-	-	-	15
16	*Mucor* sp.	-	-	-	1	-	-	-	2	-	-	-	-	-	3
17	*Penicillium verrucosum*	2	-	-	6	-	-	-	-	-	-	-	-	-	8
18	*Penicillium* sp.	-	-	-	2	-	-	-	-	-	-	-	-	-	2
19	*Phoma* sp.	-	-	-	3	8	-	-	-	22	-	-	-	-	33
20	*Trichoderma koningii*	2	10	-	-	-	-	21	19	17	27	15	21	36	168
21	*Trichoderma viride*	-	-	-	-	-	-	3	6	8	7	15	10	21	70
22	*Verticillium lecanii*	-	-	-	-	-	-	10	14	-	22	8	12	-	66
23	*Zygorrhyrchus moelleri*	11	-	-	-	-	-	-	-	-	-	-	-	-	11
24	nonsporing dark pigmenting	-	-	-	-	-	-	-	-	8	26	-	-	-	34
Total		44	21	14	69	50	36	65	60	70	99	55	50	76	709
		79			155			195			204				

Explanations: III, IV, V, VI, VII - nest number; 1, 2, 3 - nest layer; 1 - outside, 2 - middle, 3 - lining; * isolates amounts

Table 5

Frequency of isolation of particular fungal species from Montagu's Harrier nests (collected in 2005) on Sabouraud medium

No.	Fungal species	I			II			III			IV			V			VI			VII	Total
		1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3		
1	*Absidia glauca*	-	-	29	7	2	8	-	-	-	-	-	-	-	-	-	-	-	-	-	46
2	*Acremonium kiliense*	-	-	-	-	-	1	-	-	-	-	40	26	2	-	-	-	-	-	3	76
3	*Alternaria alternata*	-	1	2	11	4	1	1	2	-	8	1	-	-	-	-	-	-	-	-	31
4	*Beauveria bassiana*	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	2
5	*Botryotrichum piluliferum*	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1
6	*Candida* sp.	23	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	24
7	*Chaetomium elatum*	-	-	3	-	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	11
8	*Chaetomium funicola*	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
9	*Chaetomium globosum*	-	-	-	-	-	-	-	-	-	-	-	2	-	-	5	-	-	-	-	7
10	*Chrysosporium pannicola*	1	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8
11	*Chrysosporium pannorum*	-	28	-	21	-	-	-	-	-	-	85	37	-	-	-	5	-	-	-	176
12	*Cladosporium sphaerospermum*	-	1	-	-	-	-	-	6	-	-	1	-	-	-	-	-	-	-	-	8
13	*Cylindrocarpon* sp.	-	-	-	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4
14	*Doratomyces microsporus*	8	-	3	-	4	-	-	-	-	1	-	-	-	-	6	-	-	-	-	22
15	*Doratomyces stemonites*	21	7	43	18	95	17	-	-	-	-	-	-	-	-	-	-	-	-	-	201
16	*Emmonsia parva*	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	1
17	*Fusarium oxysporum*	-	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5
18	*F. sporotrichioides*	-	-	-	-	4	1	-	-	-	-	-	-	-	-	-	-	-	-	-	5
19	*Geotrichum candidum*	4	1	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3
20	*Gliocladium roseum*	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
21	*Mucor hiemalis*	71	37	20	13	3	1	-	-	-	-	-	-	2	2	-	-	2	2	15	168
22	*M. mucedo*	9	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	17

23	*M. plumbens*	4	40	49	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	93
24	*M. racemosus*	-	-	-	-	-	-	-	-	-	-	-	-	2	2	-	-	2	2	15	24
25	*M. ramonissimus*	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
26	*M. spinosus*	-	-	-	6	4	13	-	-	-	-	-	-	-	-	-	-	-	-	-	23
27	*Mucor* sp.	1	1	1	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	5
28	*Myrothecium roridum*	-	2	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3
29	*Paeciliomyces marguandii*	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
30	*Penicillium expansum*	-	-	-	1	-	-	-	-	-	-	1	1	2	-	-	-	-	-	-	5
31	*P. chrysogenum*	-	-	27	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	27
32	*P. verrucosum*	2	-	-	-	19	11	-	-	-	-	-	-	-	-	-	-	-	-	-	32
33	*Penicillium* sp.	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
34	*Phoma herbarum*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5	5
35	*Scopulariopsis brevicaulis*	-	-	-	-	83	39	-	-	-	-	-	-	-	-	-	-	-	-	-	122
36	*Torula herbarum*	-	-	-	-	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	4
37	*Trichosporon beigelii*	-	2	15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	17
38	*Trichothecium roseum*	-	-	4	14	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	26
39	*Verticillium lecanii*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9	4	2	7	22
40	nonsporing yeasts	-	14	2	-	-	-	5	15	-	19	-	-	-	-	-	-	-	-	-	55
41	nonsporing dark pigmenting	-	-	-	-	-	-	-	6	4	-	-	-	-	-	-	5	3	3	-	21
Total		145	156	204	93	239	97	14	33	26	29	128	66	11	11	13	35	19	12	30	1361
		505			429			73			223			35			66				

Explanations as in Table 4.

Table 6

Simpson coefficients (D) for 4 whole nests of Montagu's Harrier (*Circus pygargus*)

Nest number	Sabouraud medium	Martin medium
III	0.485	0.850
IV	0.535	0.909
V	0.787	0.830
VI	0.594	0.772
Total for III-VI	0.760	0.908
Pellets	0.575	0.639

Table 7

Simpson coefficients (D) for particular layers of Montagu's Harrier (*Circus pygargus*) nests

Nest layer	Sabouraud medium	Martin medium
outside	0.740*	0.909*
middle	0.661*	0.878*
lining	0.754*	0.865*

* average values for nests: III, IV, V, VI

Among fungal species isolated on both media, native cellulolytic (*Chaetomium* sp.) and native keratin-degrading species (*Chrysosporium pannicola*, *Ch. tropicum*) (Tabs. 4 and 5) were found, although with a low frequency. These highly nutritially specialized fungi are less abundant in their natural communities than ubiquitous species and are less efficient as saprophytic competitors. Therefore, isolation of these species requires more selective media and methods, as eg baiting method.

Mycobiota of Montagu's Harrier nests, aside of typical saprophytic strains as *Trichoderma* spp., *Gliocladium* spp., *Chaetomium* spp., included many potentially pathogenic species, classified to opportunistic pathogens, causing dermatomycoses or organ mycoses: *Aspergillus fumigatus, Acremonium kiliense, Candida* spp., *Chrysosporium* spp., *Emmonsia parva, Geotrichum candidum, Mucor* spp., *Scopulariopsis brevicaulis, Trichosporon beigelii*. Considering general frequency, the species mentioned above belonged to rarely or sporadically appearing ones; however, in some nests they were isolated very often. It concerned specially: *A. kiliense*, appearing in lining and middle layer of nest IV with frequencies 30% and 40%, respectively, *S. brevicaulis*, found in analogous layers in nest II with frequenies 35% and 40%, respectively, and especially *A. fumigatus*, with frequencies of 44% and 79% of all strains isolated from nests IV and III (Sabouraud medium) (Tab. 5). Mycotoxins-producing strains were represented by *Penicilium* spp., specially *P. verrucosum*, *A. fumigatus*, *Alternaria alternata*, *Fusarium* spp. (Tabs. 4 and 5). The last two species: *Alternaria* and *Fusarium*, but also *Botrytis cinerea*, *Cladosporium* spp., *Cylindrocarpon* spp., *Phoma* spp. represented fungi of potentially pathogenic properties (Tabs. 4 and 5). Montagu's Harrier nests were also colonized by fungi known as insecticidal and nematodicidal: *Beauveria bassiana*, *Paeciliomyces marquandii*, *P. lilacinus*, *Verticillium lecani* (Tabs. 4 and 5) which, as well as chitin leftovers in pellets, reflected probably the presence of many insects and other invertebrates in nests.

4. Species content and diversity of fungi colonizing Montagu's Harrier pellets

751 fungal strains were isolated from 5 examined pellets (395 on Martin and 356 on Sabouraud medium). Collected material was classified to 22 species, although 32 isolated

strains, mainly dark pigmenting and nonsporing ones, remained not classified (Tab. 8). Fungi growing on Martin medium belonged to 18 species and these on Sabouraud medium - to 8, which corresponded, in calculation per one pellet, to 3.6 and 1.6 species, respectively. Considering all isolated species (on both media), fungal growth coefficient was equal to 4.4 species per one pellet. All isolated species (22) belonged to: anamorphic (mitosporic) fungi (77%; 17 species), *Ascomycetes* (represented by *Chaetomium*) (14%; 3 species) and *Zygomycetes* (9%; 2 species). *Doratomyces stemonites* was a dominating species - over 50% isolates on Martin medium and 60% on Sabouraud medium; codominanting species were *Chrysosporium*, with frequencies of 38% and 26%, respectively. The most frequently isolated representatives of *Chrysosporium* were: *Ch. tropicum* (24% on Martin and 17% on Sabouraud medium) and *Ch. georgii* (17%, exclusively on Martin medium).

Table 8

Fungal species isolated from Montagu's Harrier (*Circus pygargus*) pellets

No.	Fungal species	Martin medium	Sabouraud medium	Total
1	*Actinomucor elegans*	6*	-	6
2	*Aspergillus flavus*	3	-	3
3	*Chaetomium elatum*	5	-	5
4	*Chaetomium funicola*	1	-	1
5	*Chaetomium globosum*	1	-	1
6	*Chrysosporium georgii*	50	-	50
7	*Ch.merdarium var. roseum*	1	-	1
8	*Ch. pannicola*	-	13	13
9	*Ch. pannorum*	1	-	1
10	*Ch. queenslaudicum*	6	-	6
11	*Ch. tropicum*	92	61	153
12	*Chrysosporium* anam. *Arthroderma curreyi*	1	18	19
13	*Doratomyces stemonites*	199	213	412
14	*Mortierella biramosa*	4	-	4
15	*Paeciliomyces marquandii*	-	15	15
16	*Penicillium lilacinus*	-	22	22
17	*Penicillium janthinellum*	1	-	1
18	*P. nigricans*	1	-	1
19	*Penicillium* sp.	1	1	2
20	*Scedosporium apiospermum*	1	-	1
21	*Trichosporon beigelii*	1	-	1
22	*Trichothecium roseum*	-	1	1
	not identified			
23	yeasts	19	12	31
24	nonsporing dark pigmenting	1	-	1
	Total	395	356	751

* - isolates amount

In ecologically-physiological aspect, species colonizing the pellets were represented mainly by typically keratinolytic (*Chrysosporium georgii, Ch. merdarium, Ch. pannicola, Ch. queenslandicum, Chrysosporium anam., Arthroderma curreyi*) and ubiquitous (including *Chaetomium* spp. and *Trichothecium roseum*, highly specialized at cellulose degradation) fungi (Tab. 6). These two fungal groups appeared in following proportions: 38% or 54% (Martin medium) and 26% or 70% (Sabouraud medium).

Isolation of fungi from pellets was facilitated by very low frequency of *Mucorales*. The representatives of the last group belong to very quickly growing fungi, hindering or even making impossible the growth of slowly growing species (including keratinolytic ones) on isolating media. Sugar fungi (non-identified yeasts, *Candida* spp., *Trichosporon beigelii* and *Actinomucor elegans*), found in Montagu's Harrier pellets, were isolated using Martin and Sabouraud media with frequencies of 7% and 4%, respectively (Tab. 8). Among them, *Chrysosporium* spp., *Candida* spp., *Emmonsia parva, Trichosporon beigelii* and *Scedosporium apiospermum* belonged to opportunistic pathogens and *Aspergillus flavus*, *Paeciliomyces* spp. and *Penicillium* spp. - to potentially toxinogenic ones.

Discussion

The results obtained during the course of performed studies lead to the conclusion that Montagu's Harrier nests are the microhabitat characterized by high frequency and species diversity of *Micromycetes*. Generally, amount of propagules found on Martin medium, reaching even up to 12 million cfu g^{-1} of dry mass of nest material, corresponded to general amount of fungi in composts prepared of feather and plant leftovers [19]. However, these values exceeded average amounts of fungal species in soils, reaching $1 \div 10 \cdot 10^5$ cfu g^{-1} [20, 21]. High frequency of microscopic fungi in Montagu's Harrier nests resulted from accessibility and broad spectrum of organic carbon sources in nest material, continuous inflow of nitrogen-rich substrates (feather, prey's leftovers and feces), high content of other macroelements (P, K, S, Mg, Ca) and humidity close to optimal for fungal growth (50÷60%) [20, 22]. Even in nest of lower (23÷25%) humidity, frequency of fungal species exceeded $1 \cdot 10^6$ cfu g^{-1} which may be explained by high tolerance of fungi to drought, reflected in their low water-activity coefficient (a_w): 0.6÷0.91 [22, 23]. Hydrological parameters of peatbogs, characterized by high water table fluctuations, promoted such an adaptation of fungi.

Important ecological factor, affecting the frequency and fungal species affiliation, is a chemical content of nest material. More abundant populations of fungi isolated on Martin medium, in comparison with those isolated on Sabouraud medium, pointed on a fact that saprophytic fungi, dependent on plant organic material dominating in nests over animal-derived (feather, prey's leftovers, feces) organic substance, were a major group among all fungi isolated from nest material. Saprophytic fungi appearing in natural habitats, in contrast to bacteria [20], are basically adapted to biodegradation of plant biomaterial with a high C:N rate, as eg straw (C:N is 180:1; non-published data). Fungi specialized at degradation of animal-derived leftovers (of lower C:N rate; eg feather's C:N rate is 3:1) are less common [24]. This feature arises from fungal high requirement for organic carbon because 50% of carbon substrate in growth medium of these fungi is being built into their mycelium [20]. During the degradation of substrates of low C:N rate, the majority of organic carbon is being immobilized in the mycelium while the excess of nitrogen is being released to surrounding environment in a form of NH_3 [24]. Preferential conditions of growth for this fungal group, specially the fungi potentially pathogenic for people and animals, are provided by Sabouraud medium, containing less nitrogen than Martin medium.

Apart of general frequency, intensity of fungal growth in Montagu's Harrier nests was measured by amount of the species and frequency of their appearance. Material taken from 7 nests allowed for isolation of 50 *Micromycetes* species, representing mainly mitosporic fungi, with overall score of approx. 7 species isolated per one nest. These numbers suggest that the nests were colonized by numerous fungal species.

Simpson coefficients confirmed the diversity of fungal species in nests or their layers. The higher these values were, the lower was the quantitative diversification of populations representing particular organisms communities and vice versa [17]. It was found that, in contrast to general fungal species frequency, the species diversity was higher in case of fungi growing on Sabouraud medium (lower values of D coefficient) than on Martin medium. This is an indirect proof for higher quantitative diversification of *Micromycetes* populations colonizing the animal components of studied nests in comparison with the plant ones. It was therefore clear that Sabouraud medium did not allow to isolate any particular dominant between fungal species: only 2 often but not common species (*Doratomyces stemontes* and *Chrysosporium pannorum*) were found on this medium. However, the group growing on Martin medium showed the presence of highly dominating population, represented by *Trichoderma* spp., specially by *T. koningii*. *Trichoderma* was selected from studied materials due to its high affinity to cellulose-rich stems of Saw Sedge, main component of Montagu's Harrier nests. *Trichoderma* species were reported to be specialized at cellulose degradation [25]. Their presence in nests was also related to lignin presence [7]. *Trichoderma* is also highly tolerant to varying environmental conditions, especially to humidity [6, 26]. *Trichoderma* is a fungus which appears and grows intensively in humid soil and on wood [27]; moreover, it shows highly antagonistic properties [26, 28] facilitating quick colonization of growth medium.

Nutritious specialization at cellulose degradation concerned also the nests-colonizing fungi other than *Trichoderma koningii* and *T. viride*, namely *Doratomyces stemonites*, *D. microsporus*, *Chrysosporium pannorum*, *Trichothecium roseum* [6, 14, 29]. These fungi (except of *T. roseum*) were also classified by some authors [30-32] to keratinolytic species. Isolation media used in this work, however, do not favor the growth of *Cheatomium* - the most efficient, except of *Trichoderma*, cellulose-degrading *Micromycetes* species. Such a phenomenon resulted from weak abilities of this taxon representatives to saprophytic competition with fast-growing on Martin medium representatives of sugar and ubiquitous fungi. This species, however, was detected with a high frequency on cellulose-containing medium (data not published).

Relatively numerous colonizers of Montagu's Harrier nests belonged also to simple organic carbon-utilizing sources: *Mucor*, *Absidia* yeasts and ubiquitous fungi, preferring significantly humid environments: *Acremonium*, *Fusarium*. Also Hubalek [7] frequently isolated the species mentioned above from water birds nests.

Apart of substrate affinity and humidity, pH and temperature belong to important ecological factors determining species content of fungi colonizing Montagu's Harrier nests. Alkaline pH, significant environment humidity and high temperature of nest during the nestling period undoubtedly favored the growth of majority of *Penicillium* species. This genus colonizes mainly niches of low and moderate temperature and humidity, and its representatives are classified to psychrophilic and xerophilic fungi [23]. High temperatures during nestling period, however, favored the growth of thermophilic and thermotolerant species represented by *Aspergillus* (*A. fumigatus*). This species is

classified as a thermotolerant mesophile growing in a broad range of temperatures: 12÷52°C [23] with an optimum at 37°C [33]. Its appearance is specially abundant in environments of raised (also temporarily) temperature, such as composts [34] and nests [7].

Absidia glauca and keratinolytic (as *Chrysosporium pannicola*) and proteolytic (as *Scopulariopsis brevicaulis*) species belonged to specially alkaliphilic and alkalitolerant colonizers of Montagu's Harrier nests [2, 14, 20, 35]. *S. brevicaulis* was one of species the most frequently isolated from starlings (*Sturnus vulgaris*) feather. This fungus was isolated from feather of 10 from 110 studied birds which made approx. 9% of total amount of tested starlings [36]. Appearance of these fungi was analyzed also in soil with high content of feather under the alkalization-accompanied degradation process [37, 38] and it was showed that protein ammonification coupled with fungal keratinolysis of feather resulted in release of high amounts of ammonium and followed by alkalization of environment to values ≥8.0.

Specially high diversity of keratinolytic, proteolytic and alkaliphylic fungi was observed in Montagu's Harrier pellets found near the nests and containing non-digested prey's leftovers. High frequency of *Chrysosporium* spp., mainly *Ch. georgii* and *Ch. tropicum*, should be explained by high abundancy of rodents (*Rodentia*) and passerines (*Passiformes*) (including *Passer* spp. and *Sturnus vulgaris*) in tested birds' diet. These both keratinolytic fungal species often appears in fur of small mammalian species and in feather of mentioned birds [1, 36, 39]. Among the fungi of strong proteolytic properties or weakly keratinolytic and alkalitolerant together ones, *Paeciliomyces* spp., specially *P. lilacinus*, were found. *P. lilacinus* belongs to common feather decomposers [26, 37]. Korniłowicz-Kowalska [24] showed that when this species was cultured on medium containing feathers as a sole carbon and nitrogen source, it degraded this substrate in 33%. Very high frequency of appearance of *Doratomyces stemonites*, a species colonizing feathers of numerous wild birds, in the pellets may indicate that it reveals high potency for keratinolysis, which was previously reported by Dominik [31] and Dominik & Majchrowicz [32]. Hubalek [7] defined this species as often appearing in water birds nests. Appearance of *D. stemonites, S. brevicaulis*, *Chrysosporium* spp., *Chaetomium* spp., *Paeciliomyces* spp. in both nests and pellets suggested high similarity between these two microenvironments, with mainly quantitative differences.

Presence of the same *Micromycetes* species in Montagu's Harrier pellets and nests suggested the phenomenon of fungal species transmission between different birds of prey's species and also between birds and their nests. This phenomenon may suggest the transmission of saprophytic and potentially pathogenic fungi by some birds of prey and their pellets. Such a fungal-transmission aspect of birds behavior must be taken into account. Montagu's Harrier is an Eurasian migratory species, having its wintering area in Africa and India. It may therefore play a role of a migratory factor of opportunistic mycoses-causing species (including *Chrysosporium* spp.) and toxinogenic ones (as *Aspergillus flavus, A. fumigatus, A. brevicaulis*). The phenomenon of transmission of fungi of different pathogenic spectrum against people and animals by water-mud birds was previously highlighted by Kisicka-Madziar [40].

A. fumigatus, frequently isolated from birds' pellets [40] lungs, air sacks [41], feathers [7] and birds' (also water birds') nests [5, 7] is one of the most important fungal

species, from epidemiological and epizootiological points of view. This species is a main pathogenic factor causing bird aspergillosis (mainly of the lungs) [42]. Thermotolerance and presence of protein (also keratin)-containing substrates in Montagu's Harrier nests were main factors favoring the appearance of this zoopathogens. *A. fumigatus* presents high activities of extracellular proteases (including elastase, collagenase, keratinase) [43-45] - enzymes participating in fungal pathogenesis. Its abundant presence in some studied nests causes a real danger for young of wetland birds, considering the conditions in nests (temperature, humidity, pH), favoring *A. fumigatus* growth and proteolytic activity of this fungus [45]. The highest amounts of this species were found in internal nests layer, where the young live and concentration of fungal aerosol is usually the highest. Kisicka-Madziar [40] showed that the young of Wader birds (*Charadridae*) were more susceptible to fungal infections than the adults. *A. fumigatus* is also considered as the most dangerous fungal species causing human lung mycosis [46].

Other common species of opportunistic, often isolated from Montagu's Harrier nests pathogen is *Scopulariopsis brevicaulis*, a thermotolerant fungus presenting proteolytic [14] and keratinolytic properties [47], causing human nail mycosis.

Apart of high frequency of mentioned opportunistic pathogens, Montagu's Harrier nests were characterized by presence of other potentially dangerous fungal species: *Acremoniu kiliense, Aspergillus flavus, Geotrichum candidum, Candida* spp. *Chrysosporium* spp., *Fusarium* spp., *Aspergillus*, *Candida* and *Geotrichum* spp. were also often isolated from litter of goshawk *Accipiter gentilis* nests [48]. In contest of these facts, nests of Montagu's Harrier may be considered as one of mycosis and mycotoxycosis reservoirs in the environment common for birds of prey and people.

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References

[1] Hubalek Z.: *Keratinophilic fungi associated with free living mammals and birds*. [In:] Biology of dermatophytes and other keratinophilic fungi. R.K.S. Kushwaha and J. Guarro (eds.). Rev. Iber. Micol., 2000, **17**, 104-108.

[2] Kushwaha R.K.S.: *The genus Chrysosporium, its physiology and biotechnological potential*. [In:] Biology of dermatophytes and other keratinophilic fungi. R.K.S. Kushwaha and J. Guarro (eds.). Rev. Iber. Micol., 2000, **17**, 66-76.

[3] Hubalek Z.: *Pathogenic microorganisms associated with free living birds (a review)*. Acta Sci. Natur., Brno, 1994, **28**, 1-74.

[4] Joseph V.: *Aspergillosis in Raptors.* Seminars in Avian and Exotic Pet Medicine, 2000, **9**, 66-74.

[5] Kruszewicz A.G., Pinowski J., Kruszewicz A.H., Mazurkiewicz M., Pawiak R. and Małyszko E.: *Occurrence of fungi in House Sparrow (Passer domesticus) and Tree Sparrow (Passer montanus) nestlings*. [In:] Nestling mortality due to microorganisms and toxic substances: syntesis. J. Pinowski, B.P. Kavanagh, B. Pinowska (eds.). WN PWN, Warszawa 1995, 283-290.

[6] Pinowski J., Pinowska B. and Haman A.: *Fungi in birds plumage and nests*. Int. Stud. Sparrow., 1999, **26**, 3-28.

[7] Hubalek Z.: *Fungi associated with free-living birds in Czechoslovakia and Yugoslavia*. Acta Sci. Natur., Brno, 1974, **8**, 1-62.

[8] Kitowski I.: *Trends on parental care in Montagu's Harrier during nestling period in south east Poland.* Berkut, 2003, **12**, 112-118.
[9] Kitowski I.: *Breeding ecology of Montagu's harrier (Circus pygargus) in marshes of eastern Poland: importance of aggregated nesting.* Acta Zoolog. Lituan., 2008, **18**, 83-89.
[10] Buczek A.: *Habitat conditions, ecology, resources and protection of Saw Sedge Cladium mariscus (L.) Pohl. in Lublin macroregion*. Acta Agrophys. 2005, **129**, 1-126 (in Polish).
[11] Pugh G.J.F.: *Associations between bird's nests, their pH and keratinophilic fungi*. Sabouraudia, 1966, **5**, 49-53.
[12] Martin J.P.: *Use of acid rose bengal and streptomycin in the plate method of estimating soil fungi*. Soil Sci., 1950, **19**, 215.
[13] Dvořak J. and Otčenašek M.: Mycological diagnosis of animal dermatophytoses. Academia, Prague 1969.
[14] Domsch K.H., Gams W. and Anderson T.H.*:* Compendium of Soil Fungi. 1. Acad. Press., London 1980.
[15] Kwaśna H., Chełkowski J. and Zajkowski P.: Flora polska. Grzyby (Mycota), 22: (Deuteromycetes), (Hyphomycetales), (Fusarium). WN PWN, Warszawa 1991.
[16] Oorschot van C.A.N.: *A revision of Chrysosporium and allied genera*. Stud. Mycol., 1989, **20**, 1-89.
[17] Krebs C.J.: Ecology. The Experimental Analysis of Distribution and Abundance. Harper Collins, New York 1994.
[18] Trojan P.: General Ecology. PWN, Warsaw 1981 (in Polish).
[19] Korniłowicz-Kowalska T. and Bohacz J.: *An attempt to compost feather waste with the application of fungi inoculum.* Acta Agrophys., 2002, **73**, 189-197 (in Polish).
[20] Griffin D.H.: Ecology of Soil Fungi. Chapman and Hall, London 1972.
[21] Kjőller A. and Struwe S.: *Microfungi in ecosystems: fungal occurrence and activity in litter and soil*. Oikos, 1982, **39**, 391-422.
[22] Cooke R.C. and Rayner A.D.M.: Ecology of saprotrophic fungi. Longman, London 1984.
[23] Griffin D.H.: Fungal Physiology. Wiley-Liss, New York 1993.
[24] Korniłowicz-Kowalska T.: *Studies on the decomposition of keratin wastes by saprotrophic microfungi. I. Criteria for evaluating keratinolytic activity*. Acta Mycol., 1997, **32**, 51-79.
[25] Korniłowicz-Kowalska T., Iglik H. and Wojdyło B.: *Correlations between the abundance of cellulolytic fungi and selected soil properties.* Acta Mycol., 2003, **38** (1/2), 157-168.
[26] Korniłowicz-Kowalska T.: *Effects of soil fungi (Micromycetes) on plant pathogens and pests and their practical aspect*. Fragm. Agronom., 2000, **17**, 135-155 (in Polish).
[27] Nielsen K.F.: Moulds growth on building markers. Ph.D. thesis, By og Byg. Statens Byggefarskningsinstitut, Danish Building and Urban Research, 2002.
[28] Pietr S.: *The mode of action of Trichoderma: short summary*. [In:] Trichoderma spp., other microorganisms and plant extracts in plant diseases control. VIII Conference of the Section for Biological Control of Plant Diseases of the Polish Phytopathological Society. Skierniewice, Poland, 1997, 7-15.
[29] Hubalek Z., Balat F. and Touskova J.: *Mycoflora of birds nests in nest-boxes.* Mycopathol. Mycol. Appl., 1973, **49**, 1-12.
[30] Carmichael J.W.: *Chrysosporium and some other aleuriosporic hyphomycetes*. Can. J. Bot., 1962, **40**, 1137-1173.
[31] Dominik T.: *Chrysosporium Corda 1833*. Zesz. Nauk WSR w Szczecinie, 1967, **24**, 37-66 (in Polish).
[32] Dominik T. and Majchrowicz J.: *Second contribution to the knowledge of keratinolytic and keratinophilic soil fungi in the region of Szczecin*. Ecol. Pol., 1965, A.**13**, 415-447.
[33] Kozakiewicz Z. and Smith D.: *Physiology of Aspergillus*. [In:] T. Atkins, R.F Sherwood (ed.): Biotechnology Handbooks 7. Aspergillus. Plenum Press, New York 1994.
[34] Fischer J.L., Beffa T., Lyon P-F. and Aragno M.: *Aspergillus fumigatus in windrow composting: effect of turning frequency.* Waste Manage. Res., 1998, **16**, 320-329.
[35] Hubalek Z.: *Influence of pH on the occurrence of fungi in birds nests*. Z. Allg. Mikrobial., 1976, **16**, 65-72.
[36] Camin A.M. and Chabasse D.: *Keratinophilic fungi associated with starlings* (*Sturnus vulgaris*) *in Britany, France*. Mycopathology, 1998, **143**, 9-12.
[37] Korniłowicz T.: *Studies on mycoflora colonizing raw keratin wastes in arable soil.* Acta Mycol., 1991/92, **27**, 231-241 (in Polish).
[38] Korniłowicz-Kowalska T.: *Studies on the mycoflora colonizing keratin-bark-urea manure*. Acta Mycol., 1993, **28**, 19-30 (in Polish).

[39] Cabanasse D.: *Taxonomic study of keratinophilic fungi isolated from soil and some mammals in France*. Mycopathologia, 1988, **101**, 133-140.
[40] Kisicka-Madziar J.: *Transmission of potentially pathogenic fungi for human by shorebirds.* Unpublished Ph.D. thesis. University of Warmia and Mazury, Olsztyn 2006 (in Polish).
[41] Shin T.K., Lee H.J., Lee D.S., Kwon O.D., Yang K.C., Kim O.N. and Kim W.T.: *Aspergillus fumigatus infection in wild goose*. Korean J. Vet. Clin. Med., 1996, **13**, 195-197.
[42] Mikaelian J., Gauthier F., Fitzgerald G., Higgins R., Clavean R. and Martinean D.: *Primary causes of death in wild birds of Quebec*. Med. Vet. Quebec., 1997, **27**, 94-102.
[43] Frosco M.C. and Mcmillan J.D.: *Purification and properties of the elastase from Aspergillus fumigatus*. Infect. Immun., 1992, **60**, 728-734.
[44] Monod M., Paris S., Sanglard D., Jaton-Ogay K., Bille J. and Latge J.P.: *Isolation and characterization of a secreted metalloprotease of Aspergillus fumigatus*. Infect. Immun., 1993, **61**, 4099-4104.
[45] Santos R.M.D.B., Firmino A.P., de Sa C.M. and Felix C.R.: *Keratinolytic activity of Aspergillus fumigatus Fresenius.* Curr. Microbiol., 1996, **33**, 364-370.
[46] Dynowska M.: *Clinical aspects of infestation by mould fungi.* [In:] Grajewski J. (ed.): Mycotoxins and Mould Fungi., 2006, 70-80 (in Polish).
[47] Filipello Marchisio V., Fusconi A. and Querio F.L.: *Scopulariopsis brevicaulis: a keratinophilic or a keratinolytic fungus.* Mycoses, 2000, **43**, 281-292.
[48] Wieliczko A., Piasecki T., Dorrestein G.M., Adamski A. and Mazurkiewicz M.: *Evaluation of the health status of goshawk chicks (Accipiter gentilis) nestling in Wrocław vicinity.* Bull. Vet. Inst. Pulawy, 2003, **47**, 247-257.

RÓŻNORODNOŚĆ GRZYBÓW W GNIAZDACH I WYPLUWKACH BŁOTNIAKA ŁĄKOWEGO (*Circus pygargus*) WYSTĘPUJĄCEGO WE WSCHODNIEJ POLSCE - WPŁYW CZYNNIKÓW CHEMICZNYCH I EKOLOGICZNYCH

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Abstrakt: Zbadano ogólną liczebność, skład i frekwencję gatunków grzybów saprotroficznych i potencjalnie chorobotwórczych dla organizmów stałocieplnych w 7 gniazdach oraz wypluwkach błotniaka łąkowego (*Circus pygargus*) występującego na torfowiskach węglanowych koło Chełma (Polska). Badane gniazda okazały się mikrosiedliskiem kształtującym dużą różnorodność oraz wysoką liczebność zbiorowisk *Micromycetes*. Do najliczniejszych grup taksonomicznych należały grzyby mitosporowe. Pod względem ekofizjologicznym badane zbiorowiska grzybów gniazdowych zdominowane były przez grzyby ubikwistyczne (polifagi), w tym celulolityczne. Grzyby keratynofilne były mniej liczne. Wypluwki obok grzybów ubikwistycznych cechowały się wysokim udziałem grzybów keratynofilnych. W obrębie wyodrębnionych gatunków najwyższą frekwencją odznaczały się *Trichoderma viride* w gniazdach oraz *Doratomyces stemonites* na wypluwkach. Obok gatunków typowo saprotroficznych gniazda oraz wypluwki zasiedlały grzyby potencjalnie chorobotwórcze, w tym *Aspergillus fumigatus* i *Scopulariopsis brevicaulis* oraz *Chrysosporium tropicum* i *Ch. georgii*. W obrębie wyodrębnionych gatunków spotykano gatunki hydrofilne, alkalotolerancyjne oraz termotolerancyjne, co miało związek z zawartością wody, pH oraz temperaturą podczas lęgów.

Słowa kluczowe: grzyby, gniazda, wypluwki, błotniak łąkowy, czynniki chemiczne i ekologiczne