Saudi Journal of Biological Sciences 25 (2018) 136–143

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

Nests of Marsh harrier (*Circus aeruginosus* L.) as refuges of potentially phytopathogenic and zoopathogenic fungi

Teresa Kornillowicz-Kowalska [a], Ignacy Kitowski [b,*]

[a] Mycological Laboratory, Department of Environmental Microbiology, University of Life Sciences in Lublin, 7 Leszczyńskiego, 20-069 Lublin, Poland
[b] State School of Higher Education in Chełm, Pocztowa 54, 22-100 Chelm, Poland

ARTICLE INFO

Article history:
Received 18 March 2016
Revised 18 February 2017
Accepted 29 March 2017
Available online 31 March 2017

Keywords:
Fungi
Phytopathogenic
Zoopathogenic
Nests
Marsh harrier

ABSTRACT

Birds' nests may be refuges for various species of fungi including that which are potentially phytopathogenic and zoopathogenic. Among the 2449 isolates of fungi obtained from nests of Marsh harriers 96.8% belonged to filamentous fungi. In total, 37 genera were identified from 63 fungi species. Within the mycobiotas of the examined nests populations of fungi which are potentially pathogenic for humans, homoiothermous animals and plants dominated. Among 63 species, 46 (72%) were potentially pathogenic fungi of which 18 species were potentially phytopathogenic and 32 species were pathogenic for homoiothermous animals. Inter alia species of fungi were found in the Marsh harriers nests: *Aspergillus fumigatus*, *Aspergillus flavus*, *Scopulariopsis brevicaulis*, *Chrysosporium keratinophilum* and *Fusarium poae*, *Fusarium sporotrichioides*. In terms of numbers, dominant in Marsh harrier nests were fungi pathogenic to birds, other homoiothermous animals and humans. On that basis it was concluded that Marsh harrier nests are both a source of fungal infections for that species and one of the links in the epidemiological cycle of opportunistic fungi for humans.

1. Introduction

Birds' nests are specific microhabitats which are the place of occurrence of different species of fungi (Hubalek, 2000; Kornillowicz-Kowalska and Kitowski, 2013). Nests constructed on wet soil, on water plants or floating characterized by a high humidity (up 80–90%) with temperature, during breeding increasing up to 35–38 °C are very attractive for hydrophilic fungi (Kornillowicz-Kowalska and Kitowski, 2009, 2013). An attractive ground for saprotrophic fungi are nests of larger wetland birds. They are characterized by diverse composition of organic matter which, with proper humidity allows the development of most fungal saprophytes. Due to the composition of organic matter, temperature and humidity, nests are similar to compost fungal community (Kornillowicz-Kowalska and Bohacz, 2010).

Accumulation of plant biomass dominated by lignocellulose enriched with native feather keratin, promotes the development of cellulolytic, keratinolytic and ubiquitous fungi (Hubalek, 2000; Kornillowicz-Kowalska et al., 2010). The source of fungi can be nest material, soil as well as birds themselves (Kornillowicz-Kowalska et al., 2010, 2011). Within zoopathogenic fungi the most dangerous for bird's nests is *Aspergillus fumigatus* (Hubalek, 2000; Kornillowicz-Kowalska and Kitowski, 2013). The strains of this species cause fungal infections of the lungs and air sacs of birds (aspergillosis) (Barathidasan et al., 2013). Potentially pathogenic fungi isolated from the nests also includes keratinophilic fungi representing geophilic dermatophytes and related species considered as Chrysosporium "group" (Kornillowicz-Kowalska and Bohacz, 2011). Both groups of fungi are able to degrade keratin, using it as the only source of C, N and energy (Kornillowicz-Kowalska, 1997). Among these fungi accidentally pathogenic and pathogenic species are found with wide range of hosts (Hubalek, 2000; Kushwaha, 2000). Among geophilic dermatophytes, human dermatomycosis and animals homoiothermic may be caused most often by *Microsporum gypseum*, while among members of *Chrysosporium* group by *Ch. keratinophilum* (Kornillowicz-Kowalska and Bohacz, 2011). Both species are isolated from nests of wetland birds, but *Ch. keratinophilum* is found more frequently (Kornillowicz-Kowalska et al., 2011).

Perpetrators of dermatomycosis, the most often onychomycosis can be also molds, referred as non-keratinolytic, in particular *Scop-*

Peer review under responsibility of King Saud University.

ELSEVIER Production and hosting by Elsevier

* Corresponding author.
E-mail address: ignacyk@autograf.pl (I. Kitowski).

http://dx.doi.org/10.1016/j.sjbs.2017.03.016
1319-562X/

ulariopsis brevicaulis (Tosti et al., 1996). Some authors consider this species as keratinolytic (Filipello-Marchisio et al., 2000). Dermatomycoses including onychomycosis may be also resulting from the presence of other mold species such as *Fusarium oxysporum*, *F. solani*, *Scopulariopsis acremonium*, *Alternaria* spp. and *Phoma* spp. (Krzysciak et al., 2011). The above mentioned genera and species belong to ubiquitous (polyphagous) and cellulolytic fungi, often isolated from nests (Kornillowicz-Kowalska et al., 2011). Among the phytopathogenic fungi present in the nests the following species deserve attention: *Fusarium* spp., *Verticillium* spp., *Phoma* spp., *Alternaria* spp. and *Pythium* spp. They are causative agents of plant diseases, wild seedling blight or heal blight and necrosis (Domsch et al., 2007; Kwasna et al., 1991). Marsh harrier (*Circus aeruginosus*) is the one of numerous raptor in Europe and Poland (BirdLife International, 2017). This species builds its nests on ground, at reedbeds of lakes, on large fishponds, at small water reservoirs, near recreation reservoirs or near small waterbodies of field. In Poland, water reservoirs on which Marsh harrier nests are frequently used as public beaches or waterholes of cattle. The aim of his paper was to analyze the diversity of mycobiotas in nests of Marsh harriers with special respect to opportunistic potential human, and phytopatogen, animal pathogens.

2. Materials and methods

2.1. Studied nests

Three nests of Marsh harrier were examined. They were collected in middle July of 2006 on small fish ponds located in agricultural landscape of Lublin Region, E Poland: close villages of Nieledew (50°49′47″N 23°47′20″E) - 2 nests, coded: N1 and N3) and Zalesie Kraszeńskie (51°7′16″N, 23°10′11″E) - 1 nest, coded: N2). Nests were collected after definitely left by juveniles. The nests were localized in common reed *Phragmites australis* or common bulrush *Typha latifolia*. Diameter of nests ranged between 50 and 90 cm. c.a. 50 cm of the nests were immersed in water while the above-water dimension was c.a. 70 cm.

The foundation of the nests was constituted of branches while the edges were made of stalks of Burdock *Arctium* sp. and Canada Thistle *Cirsium arvense*. Padding of the nests was made of common reed, less often common bulrush filled in with edges and grasses. A large part of padding was animal material: pellets containing fur and bones of mammals, as well as small mammals bodies or feathers of avian prey.

Moreover, feathers of females of Marsh harriers who overwent the process of moulting during the incubation and down from fledglings, faeces of nestlings (Marsh harriers are nidicolous birds) were found in the nests. Both components of the nest material: vegetal and non-vegetal were strongly mixed and pressed due to nesting of birds. Apart from water animals, other prey and nest material was delivered to nests by birds from fields surrounding fish ponds.

2.2. Isolation of fungi from nests

Nests (only the above-water part) were collected 7 days after leaving by birds, when they did not fulfil any significant functions. From each nest, from uniformly scattered points 15–20 individual samples were collected of total weight of approx. 200 g. In the case of two nests (Nieledew: N1, N3) with poorly visible marked border between padding and the cortex, because of strong compression, samples were taken exclusively from the central (inner) part of the nest. A single nest (Zalesie Kraszeńskie: N2), with the visibly marked breeding chamber was divided horizontally into 3 parts: inner part (lining material of nest), central part (intermediate part of nest) and outer part (the nest edge), further marked as layers III, II and I, respectively. Samples were collected in a sterile manner into sterile string bags and then cut into 0.5–1.0 cm sections. The contents were mixed for the purpose of averaging and then used for testing.

Mycological analysis of nests included determination of overall number of fungi (mesophilic saprotrophic fungi) isolated on Martin (1950) culture medium in 26 °C. Other fungi were isolated as follows: mesophilic fungi (30 °C); fungi potentially pathogenic for human and animals (Sabouraud medium supplied with antibacterial antibiotics) (Dvorak and Otcenasek, 1969); thermophiles (45 °C, Martin and Sabouraud medium); fungi potentially phytopathogenic from *Fusarium* group (Nash and Snyder medium) (Kwasna et al., 1991) cellulolytic fungi (Waksman cellulose medium on Whatman paper) as the only carbon and energy source (Kornillowicz-Kowalska et al., 2003), chitinophilic fungi using native chitin as a substrate and keratinophilic fungi using native keratin of feathers as a substrate (Kornillowicz, 1993). Fungi, excluding chitin- and keratinophilic, were isolated with usage of plate dilution method. Chitinophilic and keratinophilic fungi were isolated by using baiting technique. From each nest 10–20 plates with crumbled nest material was prepared. Plates were lined with sterile native chitin (insects exoskeletons) and keratin (feathers of chickens) prior to isolation. Plates with nest material were incubated at 26 °C in a humid chamber 4–6 weeks.

Mycelium growing on native chitin were transplanted to the plate with colloidal chitin (g dcm^{-3}): KH_2PO_4 - 1; $MgSO_4 \cdot 7H_2O$ - 0.5; chitin - 5; glucose - 1; yeast extract - 2; microelements - 1 cm^3 ($Fe(NO_3)_3 \cdot 9H_2O$ - 0.7235; $ZnSO_4 \cdot H_2O$ - 0.4398; $MnSO_4 \cdot 4H_2O$ – 0.2030 g dcm^{-3}), agar – 20; streptomycin – 30 mg and chlorocyclin – 2 mg (antibiotics were added after sterilization of the medium). Pure cultures of keratinophilic fungi grown on feathers were prepared on Sabouraud medium with antibiotics, and actidion (Dvorak and Otcenasek, 1969).

To assess the degree of colonization of the nest material by the cellulolytic fungi 0.5 cm fragments of material (totally 100 fragments from each nest) were put onto the Waksman cellulose medium (4/plate). All the fungi cultures isolated with usage of plate dilution method were done in three repetitions. For identification purposes, all colonies grown on plate were isolated. When the number of colonies on the plate was $\leq$10, colonies from 3 plates were isolated.

2.3. Isolation of fungi from samples of water and sediments

The possibility of permeation of the propagules fungal to the surrounding water was assessed by analysing samples of surface water, taken approx. 0.5 m from the nest. The water was collected into a sterile 1000 cm^3 volumetric bottle on the same day as the material of the nest.

Samples of sediments were collected with the ground probe nearby the nest only in the case of N2 nest from Zalesie Kraszeńskie fish ponds. In the case of two other nests terminals (N1, N2) (Nieledew fish ponds) it was impossible due to the location of the nests.

Samples were taken from the upper 5 cm layer, which was transferred to a sterile glass jars. Tests of water samples and sediments included determination of the total number of fungi on a Martin medium (26 °C) and potentially pathogenic fungi for homoieothermic organisms on Sabouraud medium (30 °C).

2.4. Taxonomic identification of fungi

Identification of genera and species of pure cultures of fungi were made on the basis of macro- and micro-morphologic characteristics and on biometric measurements conducted in micro cul-

tures and on plates using standard media. Designation of yeast was carried out considering the morphology of vegetative cells and spores using biochemical tests Fungichrom I from Elitach France SAS.

The final classification of species of the isolated fungi was carried out using reports of Ellis (1971), Kreger-van Rij (1984), Domsch et al. (2007), Nelson et al. (1983), Van Oorschot (1980), Peberdy (1987), Kwasna et al. (1991), Watanabe (2010) and Krzysciak et al. (2011). The criteria for biosafety of fungi potentially pathogenic for humans and animals adopted by the European Confederation of Medical Mycology (ECMM) (Hoog, 1996) were taken. Naming the species was confirmed by visiting Index Fungorum (www.indexfungorum.org).

2.5. Determination of physical and chemical properties of nests

The water content in the nests material was determined by weighing at 105 °C. pH of the nests material, the water and the sediments was determined potentiometrically. C_{tot} and S_{tot} in the nests was determined by elemental analysis by high-temperature combustion in oxygen and determining the thermal conductivity. C_{org} content was determined by Tiurin method; N_{tot}, P_{tot}, K, Ca and Mg contents were determined after wet digestion in a mixture of concentrated H_2SO_4 and hydrogen peroxide by flow spectrophotometry (N_{tot}, P_{tot}) and by the atomic absorption spectrometry (K, Ca, Mg).

2.6. Data analysis

The total number of fungi, number of fungi potentially pathogenic for humans and animals, potentially phytopathogenic *Fusarium* fungi and cellulolytic fungi reported as the number of colony forming units (cfu/1 g dry weight (dw.)) of nest material and sediments (cfu/g), or 1 cm^3 of water (cfu/cm^3). The dry weight of the nest material and sediments was determined after drying to constant weight at 105 °C. The results for the number of fungi are shown as the mean of three replicates for each nest. The degree of occupation of nests by cellulolytic fungi (method of lining of the nest material) was determined on the basis of the number of settled segments/100 taken from each nest. Diversity of fungi communities was calculated on the basis of Shannon-Wiener index: $H = -\sum_{i=1}^{S} Pi\ (\log_2 pi)$ where: S – number of species, pi – participation of i – species in the sample (Krebs, 1994).

3. Results

3.1. Marsh harrier nest material analysis

Characterization of physical and chemical properties of nest material is presented in Table 1; pH around nests marked 1, 2 and 3 were 7.29; 7.43 and 8.30; respectively. The pH in sediments (nest No. 2) was 7.40.

In the micromycetes communities of Marsh harrier nests, mesophilic fungi saprotrophic (Martin medium, 26 °C) and fungi potentially pathogenic for humans and animals (Sabouraud medium, 30 °C) belonged to most frequently occurring. The number of both groups of fungi in all three nests reached or slightly exceeded the concentration of 10^7 cfu g^{-1} d.m. of nest material (Table 2). Numerous were also cellulolytic fungi and fungi isolated on Nash and Snyder medium, including potentially phytopathogenic fungi of the genus *Fusarium* ($10^5 - 2 \cdot 10^7$ cfu g^{-1} d.m. of nest material) (Table 2). The least numerous were thermophilic (45 °C) and saprotrophic fungi as well as potentially pathogenic for homoiothermous ($10^1 - 1.3 \cdot 10^2$ cfu g^{-1} d.m. of nest material; Table 2).

Distribution of the density of the groups of mesophilic micromycetes within a single nest (nest N 2) points to the intensive growth and sporulation of fungi in its peripheral part (nest edge (I) as compared with nest lining (III) (Table 2). The opposite effect has occurred in the case of thermophilic fungi (Table 2).

From all the nests 2449 fungal isolates were separated (Table 3). Only 3.2% of them were represented by yeasts, the remaining 96.8% were filamentous fungi. Altogether 63 species of 37 genera were identified, in 101 isolates only genus was determined (Table 4).

The occurrence frequency of the recorded mesophilic species (saprotrophic and potentially pathogenic homoiothermous organisms) deviated from the assumed and equal amount in all nest layers, both on Martin medium: $\chi^2 = 24.54$; df = 3 < 0.001 as well as on Sabouraud medium: $\chi^2 = 20.63$; df = 3 < 0.001.

The occurrence frequency of the potentially pathogenic fungi (45.6%) was higher as compared with saprotrophic fungi (36.9%).

Table 1
Chemical properties of Marsh harrier nest material.

Nest number	pH		Humidity (in%)	C total	C organic	N total	S total	P total	K	Ca	Mg
	H_20	KCl									
1	6.95	7.44	70.54	45.68	43.95	2.17	0.28	0.15	0.19	0.70	0.043
2-I	7.20	7.44	76.91								
2-II	6.70	6.81	61.42	46.92	42.27	2.27	0.31	0.23	0.40	0.59	0.129
2-III	5.89	6.34	49.58								
3	7.23	6.24	49.56	47.24	42.44	2.17	0.40	0.09	0.10	0.47	0.030

I - outer part (the nest edge); II- central part (intermediate part of nest); III- inner part (lining material of nest).

Table 2
Composition of different fungi (micromycetes) communities in nests of Marsh harrier.

Nest number	A	B	C	D	E	F
	cfu · 10^3					
N1	23082.0	35755.0	14142.0	21837.5	0.0226	0.000
N2-I	249263.0	231564.0	826.0	1165.2	0.0147	0.885
N2-II	82081.0	113185.0	613.4	838.1	0.0345	0.432
N2-III	1223.0	2710.0	185.1	108.0	0.0661	0.152
N3	24650.0	7137.0	403.1	4493.8	1.0441	1.320

A – mesophilic, saprotrophic fungi (Martin medium), B – mesophilic fungi, potentially pathogenic for humans and homoiothermous animals (Sabouraud medium), C – cellulolytic fungi (Martin medium), D – fungi of Nash and Snyder medium; E – thermophilic fungi, (Martin medium), F – thermophilic fungi (Sabouraud medium), cfu –colony forming units, I - outer part (nest edge); II- central part (transition part); III- inner core (lining material, central part of nest).

Table 3
Frequency and diversity of *Micromycetes* in nests of Marsh harriers.

No	Taxa	1	2	3		4		5	6	7
				R	O	M	S			
1.	*Acremonium* spp.	70[1]	32	19	1			27		
2.	*Alternaria* spp.	12			1					4[2]
3.	*Aphanoascus* sp.									6
4.	*Arthroderma* sp.									4
5.	*Aspergillus flavus*	244	117						9	
6.	*A. fumigatus*	46	62	25	19	59	36	38[2]	12	4[2]
7.	*Aspergillus* spp.		2		2		7	4	6	
8.	*Botryotrichum* sp.				8					
9.	*Candida* spp.	1	66							2
10.	*Chaetomium* spp.				21				2	
11.	*Ch. globosum*			100	199					
12.	*Cladosporium* spp.	15	50					52[2]	4	18
13.	*Cunninghamella* sp.								3	
14.	*Doratomyces* sp.		5	14					3	7[2]
15.	*Fusarium* spp.	1	3	54	55			54	4	
16.	*Gliocladium* sp.				5					2[2]
17.	*Mariannea* sp.									2[2]
18.	*Mucor* spp.	6	9							
19.	*Myrothecium* sp.							15[2]		
20.	*Paeciliomyces* sp.						15			2[2]
21.	*Penicillium* spp.	116	32	2	14			111[2]	7	5[2]
22.	*Phoma* sp.						1			
23.	*Rhodotorula* sp.							8[2]		
24.	*Rhizoctonia* sp.			1				7[2]		
25.	*Scopulariopsis* sp.								3	
26.	*S. brevicaulis*	82	79	2				83[2]	22	6[2]
27.	*Thermoascus* sp.					43				
28.	*Torula* sp.									1[2]
29.	*Trichoderma viride*	12	1	9	84				6	
30.	*Trichoderma* spp.	4								
31.	*Trichophyton* sp.									2
32.	*Trichothecium* sp.	10	6	2				2[2]		1[2]
33.	*Verticillium* spp.		4	13			22	5[2]	7	
Total		619	468	241	409	102 183	81	306	88	66
H index		2.74	3.33	2.85	2.62	1.87		3.50	3.74	2.28

1 – saprotrophic fungi, Martin medium; 2 – potentially pathogenic for human and animals, Sabouraud medium; 3 – cellulolytic fungi, Waksman medium; 4 – thermophilic fungi, R – plate dilution method, O – method of lining of the nest material, 5 – potentially phytopathogenic included Fusarium genera, Nash and Snyder medium. 6 – chitinophilic fungi, medium with colloidal chitin, 7 – keratinolytic fungi, medium with native keratin of feathers. 1 - numbers of isolates, 2 - accompanying species. In this table are given older names of species. Present and old names of species according to Index Fungorum are given at Table 4

From 7 analyzed ecological communities, the greatest number of species came from cellulolytic fungi (24 species from 13 kinds).

The most frequent fungi of the genus *Aspergillus* were dominated by two species: highly toxic *A. flavus* and *A. fumigatus* potentially pathogenic for homoiothermous organisms (Table 3).

The share of *Aspergillus* spp. within the mesophilic saprophytes (Martin medium, 26 °C) amounted to 46.8% and did not differ significantly ($\chi^2 = 2.457$, df = 1, p = 0.117) from the share of this type in the group of potentially pathogenic mesophilic fungi (Sabouraud medium, 30 °C) ratio of 38.7%.

The occurrence of the most frequent species of *Aspergillus*, *A. flavus* on two above indicated media amounted to 39.4% and 25.0%, respectively (Table 3). Statistical differences in that cases were significant ($\chi^2 = 24.978$; df = 1; p = 0.001). It was found that *A. fumigatus* was characterized by a lower share within the mesophilic fungi than within the thermophilic fungi (Table 3) 7.4% and 57.8%, respectively. Differences between occurrences of thermophilic strains *A. fumigatus* grown on Martin medium versus Sabouraud medium were also statistically relevant ($\chi^2 = 10.076$; df = 1; p = 0.015). This species is ecologically ubiquitous and thermotolerant. This was confirmed by the isolation of this fungi in a wide ranges of temperatures: 26–30–45 °C. *Scopulariopsis brevicaulis* isolated from nests belonged to the fungi potentially pathogenic to humans. It's frequency on mediums of Martin, Sabouraud as well as on Nash and Snyder amounted to 15.4%, 17.5% and 27.0%, respectively (Table 3) Apart from the three indicated species frequently noted were strongly cellulolytic fungi from *Chaetomium* and *Trichoderma* genera (Tables 3 and 4). Both generas represented 75% of cellulolytic fungi colonizing nests' material (the fragments lining method) (Table 3).

The occurrence obtained for *Chaetomium* and *Trichoderm*a, especially for *T. viride* was much lower while isolation was conducted by using dilution method (Table 3).

To the reckoned (23.4%) cellulolytic fungi (isolated by a dilution method) *Fusarium* family can be included. Interestingly a lower occurrence rate was observed (17.6%) for the population of *Fusarium* isolated with using Nash and Snyder medium selective for this species (Table 3). The differences of occurrence of the genus *Fusarium* on both the indicated media were not significant ($\chi^2 = 9.1576$; df = 1; p = 0.282). The decrease in species diversity within the thermophilic saprotrophic fungi and the fungi potentially pathogenic was associated with an increase in the population of *A. fumigatus*. This was confirmed by the negative correlation between the percentages of that species (Table 3) and the Shannon-Wiener coefficient (r = −0.469, n = 8, p = 0.01).

Based on the data presented in Table 4 it was calculated that the ratio of the number of potentially phytopathogenic strains to the number of antagonistic strains is 1:1 (293:265). That relation indicates a state of equilibrium between the populations of those fungi in Marsh harrier nests. The ratio of the populations of fungi poten-

Table 4
List of isolated fungi species and their properties follow (Domsch et al., 2007; Kwasna et al., 1991).

Species
Acrostalagmus luteoalbus (Link) Zare W., Gams & Schroers (=*Verticillium tenerum* Ness)[pp,t] [3]
Alternaria alternata (Fr.) Keissl.[pf pp,t] [11]; *A. tenuissima (Kunze) Wiltshne*[pf pp t] [2]
Aphanoascus fulvescens (Cooke) Apinis[k,pp][4]
Arthroderma insingulare A.A. Padhye & I.W. Carmich[k,pp] [9]
Aspergillus flavus Link[pp,t] [370]; *A. fumigatus* Fresesn[pp,t] [301]; *A. niger* Tiegh[pp,t] [15]; *A. parasiticus* Speare[pp,t] [4]; *A. restrictum* G. Sm.[pp,t] [2]
Botryotrichum piluliferum Sacc. & Marchal[c,pf,] [8]
Candida tropicalis (Castell.) Berkhout[pp] [25]
Cephalotrichum microsporum (Sacc.) P.M. Kirk (=*Daratomyces microsporus* (Sacc.) F.J. Morton & G. Sm.)[c,pf] [18]; *C. stemonitis* (Pers.) Morton & G. Sm.[c,pf] [4]
Chaetomium botrychodes Zoph[c,pf] [11]; *Ch. globosum Kunze*[c,pf,pp] [299]; *Ch. indicum Corda*[c,pf] [12]
Chrysosporium keratinophilum D. Frey & J.W. Carmich[k,pp] [8]; *Ch. queenslandicum* Apinis & R.G. Rees[k,pp] [4]; *Ch. tropicum* J.W. Carmich[k,pp] [1]
Circinella muscae (Sorokin) Berl. & De Toni (=*Mucor spinosus* I. Schröt)[ch,s,pp] [6]
Cladosporium cladosporioides (Fresen.) G.A. de Vries[pp,t] [68]; *Cl. herbarum* (Pers.) Link[pp,t] [2]; *Cl. sphaerospermum* Penz[pp,s,t] [47]
Clonostachys rosea f. catenulata J.G. Gilman & E. V. Abbott Schroers (=*Gliocladium catenulatum* J.C. Gilman & E.V. Abbott)[a,s] [7]
Cunninghamella elegans Lendn.[s] [3]
Davidiella macracarpa Crous K. Schub. & U. Braun (=*Cladosporium macrocarpum* Preuss)[s,t] [4]
Fusarium culmorum (W. G. Sm.) Sacc.[pf] [4]; *F. incarnatum* (Desm.) Sacc. (=*F semitectum* Berk. & Ravenel)[pf,t] [48]; *F. oxysporum* Schltdl.[pf,pp] [3]; *F. poae* (Peck) Wellwnw.[pf,t] [48]; *F. sporotrichioides* Sherb.[pf,t] [35]
Gilberella avenacea R.J. Cook (=*Fusarium avenaceum* (Fr.)) Sacc.[pf,t] [3]; *G. sacchari* Summerell & I. F. Leslie (=*Fusarium sacchari* E. J. Butler & Haafiz Khan) W. Gams[pf,t] [20]; *G. tricincta* El-Gholl, McRitchie Shoult & Ridings (=*Fusarium tricinctum* (Corda)) Sacc.[pf,t] [3]
Geomyces pannorum (Link) Singler Carmich. (=*Chrysosporium pannorum* (Link) Hughes)[c,s] [4]
Lecanicillium lecani (Zimm.) Zare & W. Gams (=*Verticillium lecanii* (Zimm.) Viegas[ch,e] [33]; *L. psalliotae* (Treschew) Zare & W. Gams (=*Verticillium psalliotae* (Treschew.)[d.m.,e] [15]
Mariannea elegans (Corda) Samson[c,s] [2]
Microascus brevicaulis S.P. Abbott (=*Scopulariopsis brevicaulis* (Sacc.) Bainier)[e,p,pp] [274]
Mucor racemosus Fresen.[pp] [9]
Myrothecium verrucaria (Alb. & Schwein.) Ditmar[pf,pp,t] [15]
Paeciliomyces variotii Bainier[pp,t] [17]
Penicillium decumbens Thom[s,t] [8]; *P. expansum* Link[pf,t] [39]; *P. glaborum* (Wehmer) Westling (=*Penicillium frequentans* Westling)[a,s] [2]; *P. janczewskii* K.M. Zalessky (=*Penicillium nigricans* Bainier & Thom)[a,c,s,t] [8]; *P. verrucosum* Dierckx[a,s,t] [100]; *P. waksmanii* K.M. Zalessky[a,s] [32]
Phoma herbarum Westend[pf,pp] [1]
Renispora flavissima Sigler et al. (=*Chrysosporium anamorph. Renispora flavissima* Sigler et al.)[k,pp] [33]
Rhodotorula rubra (Schimon) F.C. Harrison[pp] [8]
Saroccladium kiliense (Grütz) Summerb. (=*Acremonium kiliense* Grütz)[pp] [29]; *S. strictum* (W. Gams) Summerb. (=*Acremonium strictum* W. Gams)[pp] [70]
Scopulariopsis brumptii Salv.-Duval[c,ch,s] [3]
Thanatephorus cucumeris (Frahk) Donk (=*Rhizoctonia solani* Kühn)[pf] [8]
Thermoascus aurantiacus Miehe[pp] [43]
Torula herbarum Pers ex Gray[c,s] [1]
Trichoderma koningii Oudm.[a,c,pp,s] [4]; *T. viride* Pers[pp,a,c,s] [112]
Trichophyton terrestre Durie & D. Frey[k,pp] [2]
Trichothecium roseum (Pers) Link[c,s] [21]
Yarrowia lipolytica (Wick., Kurtzman & Herman) Van der Walt & Arx (=*Candida lipolytica* (F.C. Harrison) Diddens & Ladder[s] [44]

a– antagonistic, c – cellulolytic, ch – chitinophilic, dm – mushroom disease, e – insecticide, k – keratinophilic, p – proteolytic, pf – potentially phytopatogenic, pp– potentially pathogenic for homoiothermous, s –saprotrophic, t – toxicogenic, [ ] – number of isolates.

tially pathogenic to humans and homoiothermous animals to the numbers of antagonistic fungi was 7:1 (1801:265). This indicates a vast numerical prevalence of fungi potentially pathogenic to birds, mammals and humans in relation to saprophytic fungi with antagonistic capabilities.

3.2. Analysis of water and sediments from vicinity of nests of Marsh harriers

The number of fungi saprotrophic and potentially pathogenic for homoiothermous organisms in water samples from the water in the vicinity of nests ranged from above 3 to 92 thousand cfu cm^{-3} (Table 5). The largest number of fungi was found in water samples from the vicinity of the nest N 2, especially the potentially pathogenic fungi. The numbers of fungi in samples of sediments (nest N 2) were higher (Table 5). The species composition of filamentous fungi in water samples the surrounding marsh harrier nests were most strongly represented by 4 species: *Cladosporium herbarum*, *Trichoderma koningii*, *Alternaria* and *Aspergillus fumigatus*. The latter two were isolated more frequently from Sabouraud medium (Table 6). Sediments were dominated by fungi from *Trichoderma* genus representing up to 81% of the total saprotrophic fungi isolated on Martin medium (Table 6).

4. Discussion

Our studies showed that so called total number of fungi (=mesophilic saprotrophic fungi) at Marsh harrier nests is relatively high and amounts to $\geq$10 mln cfu/1 g dw of nest material. On a similar level they were as the number of fungi potentially pathogenic for homoiothermous animals. Such the number of microfungi are met periodically in compost (Kornillowicz-Kowalska and Bohacz, 2010). On the other hand this concentrations of fungi are higher than those found in soil, where they usually amount between 10^5 and 10^6 cfu/1 g dw of soil (Posudin, 2014). Populations of potentially phytopathogenic and cellulolytic fungi colonizing studied nests were quantitatively similar to the level of total number of fungi in the soil (100 thous. up to 1 million cfu/1 g dw). Very high number of microfungi in nests of Marsh harrier results from the accumulation of great amount of different food substrates. The variety of carbohydrates of plant origin, mainly cellulose as well as accumulation of N, S and P (Table 1) are crucial for vegetative growth of fungi. Within mycobiotas of the examined nests dominated populations of fungi potentially pathogenic for humans, homoiothermous animals and plants. Among 63 species of fungi up to 46 (72%) were potentially pathogenic, of which 18 species were identified as phytopathogenic, and 32 species as pathogenic for homoiothermous animals (Table 4). Nests of Marsh harriers are not only the refuge of fungi, but also source of infection of phyto- and zoopathogenic fungi. The above is supported by the calculated ratio of frequency of phyto- and zoopathogenic fungi to saprophytic fungi with antagonistic capabilities. In the case of the ratio of phytopathogenic fungi to antagonistic fungi a state of equilibrium was noted. This, however, does not permit to exclude the risk of the environmental threat of phytopathogenic fungi as only a considerable prevalence of antagonistic fungi allows an inhibition of the growth of those micromycetes to be observed. Whereas, the 7-fold numerical prevalence of fungi potentially pathogenic to humans and homoiothermous animals relative to antagonistic fungi shows that the growth of those fungi in nests of the bird species under study is stimulated and not subject to inhibition under the effect of antagonists.

Another factor favouring the development of potentially pathogenic fungi in nests of Marsh harrier was also weaker saprophytic competition among species, conditioned by accumulation of the large amounts of food substrats. Stahl and Christensen (1992) showed that the abundance of substrates may limit competition. The above enables the intensive development of phyto- and zoopathogenic fungi in the nests characterized by a weaker saprophytic competition. The opposite effect is observed in soil, where

Table 5
Number of fungi at water and sediment samples close Marsh harrier nests.

Nests	Number of colony forming units			
	cfu g^{-1} cm^{-3}			cfu g^{-1} 10^3 g^{-1} d.m
	M_W	S_W	M_S	S_S
N1	3.3	8.7	–	–
N2	14.0	92.0	125.4	209.0
N3	5.0	6.3	–	–

M – Martin medium; S – Sabouraud medium; W – water; S – ground; "–" not count; "–" not studied.

Table 6
Species composition of *Micromycetes* at water and sediment samples close Marsh harrier nests. *- number of strains.

Fungi	Medium (dilution 10^3)			
	Martin		Sabouraud	
	Water	Sediments (N 2)	Water	Sediments (N 2)
Acremonium kiliense	2	–	–	–
Acremonium strictum	3	–	–	–
Alternaria alternata	2	–	11	–
Aspergillus flavus	–	4	–	–
Aspergillus fumigatus	8	–	11	–
Cladosporium herbarum	14	6	17	1
Drechslera demiatoidea	–	–	10	–
Doratomyces microsporus	1	–	–	–
Doratomyces stemonites	10	–	–	–
Fusarium pae	–	2	–	–
Gliomastix murorum	–	–	1	–
Penicillium chrysogenum	1	–	–	–
Penicillium verrucosum	1	–	1	–
Trichoderma koningii	18	26	4	–
Trichoderma viride	8	36	7	8
Black pigmenting, non sporing	4	–	–	–
Total	69	77	62	9

N2 –nest number 2.

generally organic matter deficiency is observed and the ensuing heavy competition for carbon and energy sources.

This entails the selection of microbes with strong antagonistic abilities i.e. saprotrophic fungi of the genus *Penicillium* (Frisvad and Filtenborg, 1999).

The largest, in terms of species composition, trophic specialized group at the Marsh harrier nests were cellulolytic fungi (n = 24) (Tables 3 and 4). The level of occupation of the nest material by this species amounted to 100%. The poorest in species (n = 7) were highly specialized trophic keratinolytic fungi associated with native keratin (feathers, pellets). Hubalek et al. (1973) studied 57 nests of 8 passerines. The most frequent cellulolytic fungi genera were: *Fusarium*, *Chaetomium*, *Alternaria*, *Phoma* i *Cladosporium*. Our studies showed that Marsh harriers nests are characterized by a high frequencies mostly of all of *Chaetomium*, mainly *Ch. globosum* (fraction of 50% in the nest colonizing material) as well as *Trichoderma viride* (20.5%, respectively). The third most common were cellulolytic fungi from genera *Fusarium* (fraction of 15%). All 3 of these types represent hydrophilic fungi with a high index of water activity (a_w 0.9–0.95).

They are among the so-called tertiary colonizers with the highest demands on water conditions (Grant et al., 1989). Additionally, *Ch. globosum* i *Trichoderma* spp. belong to fungi which grow and reproduce in flooded areas Domsch et al. (2007). Following ECMM (Hoog, 1996) *Ch. globosum* and *Trichoderma* spp. belong to BSL1 (biosafety level 1), that is saprotrophic or plant pathogens, which rarely cause infections at humans (Krzysciak et al., 2011). The importance of fungi of genus *Fusarium* increases as etiological agents of fungi infections at humans (hyalohyphomycoses). The threat of these fungi relates specifically to people with immunosuppression (Krzysciak et al., 2011). However, *Fusarium* species are plant pathogens and toxicogenic fungi. They made rot of different plant organs, gangrene of seedling and wilting of plants. Mycotoxins produced by *Fusarium*, particularly metabolites such trichothecenes, frequently contaminating grains of cereals are the cause of severe toxicosis in humans and animals (Valcheva and Valchev, 2007; Ma et al., 2013).

A wide variety of species characterized chitinophilic fungi, not yet examined by other authors in the birds' nests. Yet prevalence of these species was low except for *Scopulariopsis brevicaulis*. Prevalence of this opportunistic pathogen amounted to 25% of all chitinophilic fungi. Their frequency was higher in relation to ubiquitous saprophytic and potentially pathogenic fungi (Table 4). That suggests that chitin substrates, insect prey remains and the various stages of insects inhabiting the nest are an appropriate medium for the fungus.

High diversity of invertebrates (included insects) in wetland's birds nests was previously reported (Buczynski et al., 2004). The source of chitin in the nests of birds is also a mycelium. *Scopulariopsis brevicaulis* belonged to the active fungi found in the nests of *Passer montanus* (Hubalek, 1974a, 1974b). From the point of view of biosecurity concerning fungi potentially pathogenic for humans and animals this species is classified as BSL2 (biosafety level 2) (Hoog, 1996). In this group one can find pathogens causing surface and systemic opportunistic fungal infections at patients with immunosuppression. *Scopulariopsis brevicaulis* is responsible for nail infection (onychomycosis) mainly of toenails, rarely internal infections at patients with immunosuppression (Krzysciak et al., 2011). As the cause of human infection originated from presence of this species, the ability for keratin degrading is reported (Filipello-Marchisio et al., 2000). During our studies *S. brevicaulis* was also found on native keratin, though not in the large numbers (Table 3). On the other hand, it was more frequently (22%) isolated from the medium selective for *Fusarium* (Table 3).

Factors facilitating survival of *S. brevicaulis* in nests of Marsh harriers were also thermo-tolerance and predilection for weakly alkaline environments (Domsch et al., 2007).

Aspergillus fumigatus belonged to the deadliest opportunistic pathogens found in nests of Marsh harrier. Mostly it was isolated using selective method of isolation of thermophilic fungi.

His participation in other analyzed species, including mesophilic molds ranged from a few to several percent while *A. fumigatus* in nests of birds were found by Hubalek (1974a, 1974b).

According to the criteria ECMM it is classified as a BSL2 (biosafety level 2). In humans, it causes acute or chronic respiratory infections and at people with immunosuppression also infections of other systems (Latge, 1999). On the other hand, aspergillosis is a severe disease in poultry, pet and free living birds (Nardoni et al., 2006; Tell, 2005; Jung et al., 2009).

Our studies showed that, the number of potentially pathogenic thermophilic (45 °C) strains of *A. fumigatus* in Marsh harrier nests was 360 cfu/g dw. of nest material. In our previous studies (Kornillowicz-Kowalska and Kitowski, 2013) we found that average frequency of thermophilic strains of *A. fumigatus* in nests 7 species of wetland's species was 650 cfu/g dw. of nest material.

In the data presented here frequency of occurrence of *A. fumigatus* among the mesophilic, potentially pathogenic fungi (30 °C) occupying nests of Marsh harrier was $62 \cdot 10^5$ cfu/g dw of nest material. However its rate in species structure of this community was only 13%. Such a high number of *A. fumigatus* is a threat for Marsh harriers. The cause of severe pollution of the examined nests with mycelium spores of *A. fumigatus* and are favorable conditions for the development of the fungus: elevated nest temperature during breeding, weakly alkaline conditions, elevated humidity and abundance of food substrates, large body size of harrier females

incubating eggs and brooding nestlings. *A. fumigatus* grows and sporulates well in habitats of water activities a_w ranging 0.9–0.95, optimal pH = 7.8, heated periodically, rich in carbohydrates and proteins (Kornillowicz-Kowalska and Kitowski, 2013).

During sporulation *A. fumigatus* discharged to atmosphere large quantities of conidia of sizes ø between 2 and 5 μm, vital for many months. Their respiratory inhalation is the direct cause of lung infections (Warris and Verwej, 2005). Water has importance for invasive epidemiology of aspergillosis as disseminations habitat of *A. fumigatus* (Warris and Verwej, 2005; Tell, 2005).

In the samples of surface water collected near Marsh harrier nests the frequency of the propagules of *A. fumigatus* reached 10^4 - cfu cm^{-3}. Water can be the source of *A. fumigatus* infection through aerosolization, or in the process of rinsing the fungi propagules from the nests. We showed that nests of Marsh harrier are also refuge of another danger for birds and humans which was *Aspergillus flavus*. This fungus was frequent in nests of different bird (including raptors) (Hubalek, 1974a). *A. flavus* has habitat needs similar to *A. fumigatus*, but this species was not found among thermophilic fungi communities of studied nests.

Kluczek and Kojder (2000) reported that ca.70% of the *A. flavus* strains are responsible for production of aflatoxins, which are optimally synthesized in the temperatures ranging between 25 and 40 °C and water activity of a substrate between 0.84 and 0.99. Due to this facts Marsh harriers are exposed to inhalation of massive amounts of *A. flavus* conidia containing these mycotoxins. Unfortunately, which can produce adverse effects as birds are extremely sensitive to aflatoxins (Hubalek, 1974a, 1974b; Tell, 2005).

Keratinophilic fungi, in spite of high diversity of keratin substrates in marsh harrier nests were small in number. Probably the main factor limiting their growth was elevated humidity. Most keratinomycetes prefer the environment of low or moderate humidity with the exception of *Chrysosporium keratinophilum* and *Aphanoascus fulvescens* (Hubalek, 2000). These species are hydrophilic and often isolated from wetland birds' nests (Hubalek, 2000; Kornillowicz-Kowalska et al., 2011). They are opportunistic pathogens causing skin infections at human (Hubalek, 2000). In the studied nests the predominance of *A. fumigatus*, *A. flavus*, *Chaetomium globosum*, *Trichoderma viride* and *Scopulariopsis brevicaulis* limited other species. A small number of strains of *Penicillium* and *Mucor* resulted from adverse temperature and humidity, and in the case of some *Mucorales* was a result of a high frequency of the antagonists of genus *Trichoderma*. In studied nests a lack of antibiotic strains such as *Penicillium* genus was observed or their frequency was low. This allowed the development of potentially phytopathogenic fungi (*Fusarium*), as well as already indicated pathogenic homoiothermous organisms.

Acknowledgments

This work was supported by the Ministry of Science and Higher Educations: grant 2PO4GO3330.

References

Barathidasan, R., Singh, S.D., Saini, M., Sharma, A.K., Dhama, K., 2013. The first case of angioinvasive pulmonary aspergillosis in a Himalayan Griffon Vulture (Gyps himalayensis). Avian Biol. Res. 6, 302–306.

BirdLife International, 2017. *Circus aeruginosus*. IUCN Red List of Threatened Species. International Union for Conservation of Nature. <http://www.iucnredlist.org> (accessed: 26.02. 2017).

Buczynski, P., Kitowski, I., Rozwalka, R., 2004. Submerged part of the nests of European bittern *Botaurus stellaris* L. as a substrate for benthic macroinvertebrates. Acta Univ. Biol. Daugavpilensis 4, 77–80.

Domsch, K.H., Gams, W., Anderson, T.H., 2007. Compendium of Soil Fungi. IHW-Verlag Eching.

Dvorak, J., Otcenasek, M., 1969. Mycological diagnosis of animal dermatophytoses. Academia, Prague, Czechoslovakia.

Ellis, M.B., 1971. Dematiaceous Hyphomycetes. Commonwealth Mycological Institute, Kew, Surrey, England.

Filipello-Marchisio, V., Fusconi, A., Querio, F.L., 2000. *Scopulariopsis brevicaulis*, a keratinophilic or a keratinolytic fungus? Mycoses 43, 281–292.

Frisvad, J.C., Filtenborg, O.G., 1999. Revision of *Penicillium* subgenus *Furcatum* based on secondary metabolites and conventional characters. In: Sampson, R.A., Pitt, I. I. (Eds.), Modern concepts in *Penicillium* and *Aspergillus* classification. Plenum Press, New York, USA, pp. 159–172.

Grant, C., Hunter, C.A., Flannigan, B., Bravery, A.F., 1989. The moisture requirements of moulds isolated from domestic dwelling. Int. Biodet. 25, 259–284.

Hoog, G.S., 1996. Risk assessment of fungi reported from humans and animals. Mycoses 39, 407–417.

Hubalek, Z., 1974a. Fungi associated with free-living birds in Czechoslovakia and Yugoslavia. Acta Sci Natur. (Brno) 8, 1–62.

Hubalek, Z., 1974b. The distribution patterns of fungi in free-living birds. Acta Sci. Natur. (Brno) 8, 1–51.

Hubalek, Z., 2000. Keratinophilic fungi associated with free-living mammals and birds. In: Kushwaha, R.K.S., Guarro, J., (Eds.), Biology of Dermatophytes and other Keratinophilic fungi. Rev. Iber. Micol., 17, pp. 93–103.

Hubalek, Z., Balat, F., Touskova, I., Vlk, J., 1973. Mycoflora of birds' nests in nest-boxes. Mycopath. Mycol. Appl. 49, 1–12.

Jung, K., Kim, Y., Lee, H., Kim, J.-J., 2009. *Aspergillus fumigatus* infection in two wild Eurasian black vultures *Aegypius monachus* Linnaeus with carbofuran insecticide poisoning, a case report. Vet. J. 179, 307–312.

Kluczek, J.P., Kojder, A., 2000. Mycotoxins in outline. University of Sciences and Technology in Bydgoszcz, Bydgoszcz, Poland (in Polish).

Kornillowicz, T., 1993. The frequency of occurrence and distribution of keratinophilic fungi in some arable soils. Acta Mycol. 28, 3–17.

Kornillowicz-Kowalska, T., Kitowski, I., 2013. *Aspergillus fumigatus* and other thermophilic fungi in nests of wetland birds. Mycopathologia 175, 43–56.

Kornillowicz-Kowalska, T., Kitowski, I., 2009. Diversity of fungi in nest and pellets of Montagu's harrier *Circus pygargus* from east Poland – importance chemical and ecological factors. Ecol. Chem. Eng. S 16, 453–471.

Kornillowicz-Kowalska, T., Bohacz, J., 2010. Dynamic of growth and succession of bacterial and fungal communities during composting of feather waste. Biores. Technol. 101, 1268–1276.

Kornillowicz-Kowalska, T., Kitowski, I., Iglik, H., 2010. The occurrence of cellulolytic fungi and *Fusarium* in nests of *Circus pygargus*. Acta Mycol. 45, 97–114.

Kornillowicz-Kowalska, T., Kitowski, I., Iglik, H., 2011. Geophilic dermatophytes and other keratinophilic fungi in the nests of wetland birds. Acta Mycol. 46, 83–107.

Kornillowicz-Kowalska, T., 1997. Studies on the decomposition of keratin wastes by saprotrophic microfungi. I. Criteria for evaluating keratinolytic activity. Acta Mycol. 32, 51–79.

Kornillowicz-Kowalska, T., Iglik, H., Wojdyło, B., 2003. Correlation between the abundance of cellulolytic fungi in selected soil properties. Acta Mycol. 38, 161–172.

Kornillowicz-Kowalska, T., Bohacz, J., 2011. Biodegradation of keratin waste. Theory and practical aspects. Waste Manage. 31, 1689–1701.

Krebs, C.J., 1994. Ecology. Harper Collins, New York, USA.

Kreger-van Rij, N.J.W., 1984. The yeasts a taxonomic study. Elsevier Sci. Publ, Amsterdam, Netherlands.

Krzysciak, P., Skora, M., Macura, A.B., 2011. Atlas of Human Pathogenic Fungi. Wroclaw, MedPharm, Poland (in Polish).

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

Kwasna, H., Chełkowski, J., Zajkowski, P., 1991. Polish flora. Fungi Mycota XXII, Deuteromycetes, Hyphomycetales, Fusarium. Polish Academy of Sciences, Warsaw –Krakow, Poland. (in Polish).

Latge, J.P., 1999. Aspergillus fumigatus and aspergillosis. Clin. Microbiol. Rev. 12, 310–350.

Ma, L.J., Geiser, D.M., Proctor, R.H., Rooney, A.P., O'Donnell, K., Trial, F., Gardiner, D. M., Manners, J.M., Kazan, K., 2013. Fusarium pathogenomics. Annu. Rev. Microbiol. 67, 399–416.

Martin, J.P., 1950. Use of rose bengal and streptomycin in plate method of estimating soil fungi. Soil Sci. 19, 215–233.

Nardoni, S., Ceccherelli, R., Rossi, G., Mamcianti, F., 2006. Aspergillosis in *Larus cachinnans micaellis*, Survey of eight cases. Mycopathologia 161, 317–321.

Nelson, P.E., Toussoun, T.A., Marasas, W.F.O., 1983. *Fusarium* species, an Illustrated Manual for Identification. Pennsylvania State University Press, USA, Philadelphia.

Peberdy, J.F., 1987. *Penicillium* and *Acremonium*. Biotechnology Handbooks, vol. 1. Plenum Press, New York, USA.

Posudin, Y., 2014. Methods of Measuring Environmental Parameters. John Wiley & Sons Inc., Hoboken, USA.

Stahl, P.D., Christensen, M., 1992. In vitro mycelial interactions among members of a soil microfungal community. Soil. Biol. Biochem. 5, 765–771.

Tell, L.A., 2005. Aspergillosis in mammals and birds, impact on veterinary medicine. Med. Mycol. Suppl. 43, 571–573.

Tosti, A., Piraccini, B.M., Stinchi, C., Lorenzi, S., 1996. Onychomycosis due to *Scopulariopsis brevicaulis*, clinical features and response to systemic antifungals. Br. J. Dermatol. 135, 799–802.

Valcheva, A., Valchev, G., 2007. The fusariotoxins zearalenon and deoxinivalenol as natural contaminators of some cereal components in the production of combined feed. Bulg. J. Agric. Sci. 13, 99–104.

Van Oorschot, C.A.N., 1980. A revision of *Chrysosporium* and allied genera. Stud. Mycol. 20, 1–89.
Warris, A., Verwej, P.E., 2005. Clinical implications of environmental sources for *Aspergillus*. Med. Mycol. Suppl. 43, 559–565.
Watanabe, T., 2010. Pictorial atlas of soil and seed fungi. Morphologies of cultured fungi and key to species. CRS Press Taylor & Francis Group, Boca Raton-London-New York.