

Some correlations between the occurrence frequency of keratinophilic fungi and selected soil properties

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The results of studies on the correlation between the occurrence frequency of keratinophilic fungi, including geophilic dermatophytes and chrysosporium, and soil properties are presented and discussed in the article. The fraction with $\Phi < 0.02$ mm content and the soil pH were the most significant ecological factors that determined the frequency of occurrence and the distribution of these fungi in the soil.

Key words: keratinophilic fungi, arable soils, occurrence, ecological factors, correlations

INTRODUCTION

From the point of view of ecology, soil keratinophilic fungi comprise fungi that decompose native keratin (hair, feathers and other horny structures of animals). They are represented by a group of the so-called geophilic dermatophytes, including some species of *Trichophyton* and *Microsporum* together with perfect stages, as well as the chrysosporium group with the genera *Chrysosporium*, *Ctenomyces* and *Myceliophthora*, the teleomorph of which is usually unknown. As Garrett and Piontelli (1975) claim, keratin matter in the soil is not only a source of nutrients for keratinophilic fungi but also a specific habitat that facilitates their survival and protects them from other competitive microorganisms. The inflow of keratin matter as well as physical and chemical properties of the soil play an important role in the ecology of keratinophilic fungi (Chmel et al. 1972; Chmel and Vlačilicová 1972, 1975; Batelli et al. 1978; Mercantini et al. 1980; Vollenková 1984; Korniłłowicz 1993). The majority of studies on the influence of ecological factors on the occurrence of these fungi in the soil were conducted between 1960 and 1980. The studies in Poland were only fragmentary, and were carried out from the point of view of the occurrence of pathogenic dermatophytes in the soil (Dominik and Majchrowicz 1964, 1965; Prochacki and Biełuńska 1968;

Table 1
Granulometric composition of the soils examined

| № | Soil type - locality | Mechanical formation | Percentage content of mechanical fractions with Ø in mm | | | | | | | | | | | |
|------------|-------------------------|----------------------|---|---------|----------|----------|----------|---------------|----------------|-----------------|--------|---------|----------|-------|
| | | | >1.0 | 1.0-0.5 | 0.5-0.25 | 0.25-0.1 | 0.1-0.05 | 0.05- 0.02 | 0.02- 0.006 | 0.006- 0.002 | <0.002 | 0.1-0.1 | 0.1-0.02 | <0.02 |
| Podzols | | | | | | | | | | | | | | |
| 1. | Wola Lisowska | Weakly loamy sand | n.p | 3 | 25 | 42 | 8 | 12 | 5 | 2 | 3 | 70 | 20 | 10 |
| 2. | Skróbów | Light loamy sand | n.p | 9 | 30 | 26 | 4 | 16 | 4 | 4 | 3 | 65 | 20 | 15 |
| 3. | Firlej | Weakly loamy sand | n.p | 7 | 36 | 33 | 5 | 10 | 8 | 2 | 3 | 76 | 15 | 9 |
| Cambisols | | | | | | | | | | | | | | |
| 4 | Niewęgłosz | Dusty sandy clay | n.p | 4 | 15 | 21 | 12 | 27 | 12 | 5 | 4 | 40 | 39 | 21 |
| 5. | Sobieszyn | Heavy clay | n.p | - | - | - | - | - | - | - | - | - | - | 52 |
| Chernozems | | | | | | | | | | | | | | |
| 6. | Grabowiec | Loess | n.p | - | - | - | 11 | 47 | 23 | 10 | 7 | 2 | - | 40 |
| 7. | Oszczów | Loess | n.p | - | - | - | 11 | 41 | 22 | 7 | 18 | 1 | - | 47 |
| 8. | Werbikowice | Loess | n.p | - | - | - | 9 | 43 | 24 | 9 | 13 | 2 | - | 46 |
| Fluvisols | | | | | | | | | | | | | | |
| 9. | Bezek 1 | Medium clay | n.p | 9 | 20 | 22 | 6 | 7 | 13 | 7 | 16 | 51 | 13 | 36 |
| 10 | Bezek 2 | Medium clay | n.p | 8 | 17 | 16 | 7 | 12 | 16 | 8 | 16 | 41 | 19 | 40 |
| 11 | Dorohucza | Light dusty clay | n.p | 4 | 18 | 16 | 7 | 24 | 13 | 5 | 13 | 38 | 31 | 31 |
| 12 | Świdnik | Light dusty clay | n.p | 3 | 12 | 10 | 6 | 28 | 19 | 10 | 12 | 25 | 34 | 41 |
| Fluvisols | | | | | | | | | | | | | | |
| 13 | Dorohucza | Medium dusty clay | n.p | 0 | 4 | 13 | 9 | 31 | 16 | 8 | 19 | 17 | 40 | 43 |
| 14 | Puławy | Medium dusty clay | n.p | 1 | 3 | 16 | 9 | 21 | 18 | 11 | 21 | 20 | 30 | 50 |
| 15 | Stupcza | Silty dust/loess | n.p | 0 | 0 | 9 | 18 | 36 | 16 | 9 | 12 | 9 | 54 | 37 |
| Renzinas | | | | | | | | | | | | | | |
| 16 | Puławy | Strong loamy sand | n.p | 7 | 27 | 25 | 6 | 15 | 9 | 306 | 5 | 59 | 21 | 20 |
| 17 | Sianice | Light dusty clay | n.p | 2 | 18 | 19 | 10 | 20 | 16 | 5 | 10 | 39 | 30 | 31 |

Explanations: n.p. - not present; (-) - not examined

Nowak 1970). Studies on the ecology of keratinophilic fungi in arable soils in Poland are even more scarce (Ostrowska 1971; Kornilłowicz 1992, 1993).

The aim of this study was to determine the relationships between the occurrence frequency and distribution of the microorganisms studied and some physical and chemical properties of arable soils. Additionally, an attempt was made to establish the correlation between the occurrence of individual species of keratinophilic fungi.

MATERIAL AND METHODS

The studies comprised arable soils in the area of Lubelszczyzna. Altogether, 17 soils representing 6 types were examined: podzols (3), cambisols (2), chernozems (3), phaeols (4), fluvisols (3) and rendzinas (2). Apart from one instance, samples were collected from arable soils in individual agricultural farms, which use manure for the purposes of fertilisation. Differences in the amount of $\Phi < 0.02$ mm fraction, humus, macro-element content (C, N, P, K, Mg), amount of CaCO_3 and pH were the primary criteria for the selection of the soils.

Table 2
Some chemical properties of the soils examined

| N° | Content in % | | | Content mg in 100g of soil | | | pH | |
|----|--------------|--------|-----------------|----------------------------|----------------------|-------|-------------------------|--------|
| | Humus | N tot. | CaCO_3 | P_2O_5 | K_2O | Mg | in H_2O | in KCL |
| 1 | 1.72 | 0.054 | 0.00 | 21.6 | 13.75 | - | 5.17 | 4.19 |
| 2 | 1.39 | 0.049 | 0.00 | 7.3 | 3.62 | - | 4.52 | 3.36 |
| 3 | 1.05 | 0.041 | 0.08 | 14.0 | 5.15 | - | 4.86 | 4.06 |
| 4 | 1.95 | 0.082 | 0.00 | 11.4 | 16.39 | - | 5.04 | 4.29 |
| 5 | 1.71 | 0.120 | 0.00 | - | - | - | 7.30 | 6.30 |
| 6 | 3.93 | 0.270 | 0.00 | 13.3 | 36.2 | - | 7.89 | 7.15 |
| 7 | 2.28 | 0.200 | 0.00 | 7.8 | 25.7 | - | 6.72 | 6.01 |
| 8 | 2.69 | 0.210 | 0.70 | 16.8 | 69.1 | - | 6.76 | 6.08 |
| 9 | 3.95 | 0.270 | 4.67 | 64.5 | 20.40 | 4.29 | 7.89 | 7.28 |
| 10 | 4.80 | 0.260 | 14.91 | 25.0 | 13.40 | 2.47 | 7.90 | 7.30 |
| 11 | 2.91 | 0.161 | 0.38 | 14.0 | 11.6 | 6.75 | 7.45 | 6.83 |
| 12 | 4.01 | 0.235 | 2.58 | 260.0 | 69.1 | 6.62 | 7.42 | 6.93 |
| 13 | 5.89 | 0.245 | 0.13 | 8.4 | 6.9 | 13.25 | 7.10 | 6.32 |
| 14 | 2.52 | 0.142 | 1.68 | 33.5 | 16.53 | 11.90 | 7.65 | 7.13 |
| 15 | 3.93 | 0.256 | 3.09 | 2.7 | 4.3 | 14.95 | 7.65 | 7.15 |
| 16 | 1.76 | 0.101 | 5.06 | 43.5 | 3.3 | 1.21 | 7.65 | 7.45 |
| 17 | 3.38 | 0.138 | 0.84 | 342.0 | 32.98 | 5.61 | 7.59 | 7.09 |

Explanations: (-) - not examined

Soil samples were collected once in early autumn. Approximately 5 kg of soil was collected in between 20 and 30 places at the humus level Ap (2–20 cm) from each arable soil. The representative sample received was carefully mixed and sieved, mesh diameter 2 mm. The screen analysis of granulometric composition and the determination of chemical properties of soils were conducted in keeping with methods used

in pedologic studies and are given in Table 1 and 2. The correlation between some physical and chemical properties of the soils are given in Table 3.

Table 3
Correlation coefficients of the properties of the soils examined

| | Humus | tot. N | CaCO ₃ | P ₂ O ₅ | Ø < 0.02 mm fraction | pH |
|-------------------------------|----------|----------|-------------------|-------------------------------|----------------------|----|
| Humus | 1 | - | - | - | - | - |
| tot. N | 0.858*** | 1 | - | - | - | - |
| CaCO ₃ | - | - | 1 | - | - | - |
| P ₂ O ₅ | - | - | - | 1 | - | - |
| Ø < 0.02 mm fraction | 0.482* | 0.699** | - | - | 1 | - |
| pH | 0.612** | 0.733*** | - | - | 0.611** | 1 |

Explanations: (-) - no significance of the correlation coefficient; * - significant correlation coefficient on the verge of the level of significance $\alpha = 0,05$; ** - significant correlation coefficient $\alpha = 0,05$; *** - significant correlation coefficient $\alpha = 0,001$

Keratinophilic fungi were isolated in keeping with the methodology presented in Kornilowicz (1993), using chicken feathers as bait. Feathers were prepared in the manner described in Kornilowicz (1992).

One hundred Petri dish with substrate were prepared for each soil. The fungal mycelium that appeared after ca. 4 - 6 weeks of incubation in a moist chamber, at the temperature $20^{\circ}\text{C} \pm 2^{\circ}\text{C}$, were transferred onto the glucose Sabouraud agar with acidition and chloramphenicol (Dvořák and Otčenášek 1969). Pure cultures of keratinophilic fungi were isolated and identified up to the level of the species on the basis of macro- and micromorphological observations on plates and in the microcultures, using the following systematic studies: Domsch et al. (1980); van Oorschot (1980); Currah (1985).

The number of soil plates in which the growth of geophilic dermatophytes and fungi of the chrysosporium group, the number of genera, species and strains, as well as the number of species per one plate were considered in the analysis of the occurrence frequency of keratinophilic fungi. It was accepted that one soil plate could be colonized by one strain from a given species only.

The results obtained were analysed using the statistical method of correlation and the analysis of multiple regression. The following linear models of multiple regression were considered: frequency (fungi) = $a + b \text{ humus} + c \text{ Ø} < 0.02 \text{ mm fraction} + d \text{ tot. N} + e \text{ CaCO}_3 + f \text{ P}_2\text{O}_5 + g \text{ pH}$; where: frequency (fungi) - occurrence frequency of keratinophilic fungi (total or individual species) in the soil; a, b, c, d, e, f, g, are constant coefficients, determined using the multiple regression method with the elimination of the least significant components.

Table 4
Indices of the occurrence frequency of keratinophilic fungi in the soils examined

| Growth index | Soils | | | | | | | | | | | | | | | | |
|----------------------------------|---------|------|------|-----------|------|------|------------|------|------|-----------|------|------|-----------|------|------|-----------|------|
| | podzols | | | cambisols | | | chernozems | | | phaeozols | | | fluvisols | | | rendzinas | |
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 |
| Number of occupied plates: | | | | | | | | | | | | | | | | | |
| - total | 100 | 71 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| - geophilic dermatophytes | 100 | 77 | 100 | 100 | 73 | 100 | 95 | 96 | 6 | 10 | 16 | 41 | 96 | 93 | 7 | 3 | 70 |
| - chrysosporium group | 12 | 1 | 4 | 17 | 87 | 76 | 95 | 91 | 96 | 100 | 99 | 100 | 74 | 94 | 92 | 93 | 100 |
| Number of genera | 3 | 2 | 3 | 3 | 3 | 4 | 3 | 4 | 2 | 2 | 2 | 3 | 2 | 4 | 4 | 3 | 3 |
| Number of species: | | | | | | | | | | | | | | | | | |
| - total | 6 | 2 | 5 | 6 | 7 | 7 | 5 | 7 | 2 | 3 | 3 | 6 | 2 | 7 | 7 | 4 | 3 |
| - geophilic dermatophytes | 3 | 1 | 3 | 3 | 3 | 4 | 4 | 4 | 1 | 2 | 2 | 3 | 1 | 3 | 3 | 1 | 1 |
| - chrysosporium group | 3 | 1 | 2 | 3 | 4 | 3 | 1 | 3 | 1 | 1 | 1 | 3 | 1 | 4 | 4 | 3 | 2 |
| Number of strains: | | | | | | | | | | | | | | | | | |
| - total | 137 | 78 | 137 | 129 | 172 | 284 | 178 | 211 | 102 | 110 | 116 | 238 | 170 | 267 | 104 | 171 | 140 |
| - geophilic dermatophytes | 119 | 77 | 122 | 110 | 75 | 204 | 114 | 117 | 6 | 10 | 17 | 70 | 96 | 136 | 7 | 3 | 36 |
| - chrysosporium group | 18 | 1 | 15 | 19 | 97 | 80 | 64 | 94 | 96 | 100 | 99 | 168 | 74 | 131 | 97 | 168 | 104 |
| Number of species per one plates | 1.37 | 0.78 | 1.37 | 1.29 | 1.72 | 2.84 | 1.78 | 2.01 | 1.02 | 1.10 | 1.16 | 2.36 | 1.70 | 2.67 | 1.04 | 1.71 | 1.40 |

RESULTS

General frequency of keratinophilic fungi in arable soils

A mycological analysis of 17 samples of arable soils classified in 6 types has revealed that keratinophilic fungi colonized chernozems and fluvisols most frequently. The number of isolated species and strains of these fungi corroborates this finding (Table 4).

The occurrence frequency of fungi in the group of geophilic dermatophytes, however, was somewhat different from that of the chrysosporium group (Table 4). Geophilic dermatophytes occurred in chernozems and podzols most frequently, and were recorded in phaeols and rendzinas least frequently. The distribution of the representatives of the chrysosporium group differed, and these fungi colonized phaeols and rendzinas most frequently while their weakest growth was recorded in podzols (Table 4).

Table 5

Correlation coefficients between the general frequency of keratinophilic fungi and their groups and soil properties

| Fungi | Soil properties | | | | | pH |
|------------------------------|-----------------|--------|-------------------|-------------------------------|----------------------|----------|
| | Content | | | | | |
| | Humus | tot. N | CaCO ₃ | P ₂ O ₅ | Ø < 0.02 mm fraction | |
| keratinophilic fungi – total | - | - | - | - | 0.581* | - |
| geophilic dermatophytes | - | - | - 0.592* | - | - | - |
| chrysosporium | - | 0.474* | - | - | 0.527* | 0.916*** |

Explanations: as for Table 3

It was shown that the general abundance of keratinophilic fungi depended on the level of the Ø < 0.02 mm fraction (Table 5). The greatest number of keratinophilic fungi was recorded in soils rich in this fraction, i.e. formed of loess, as well as heavy and medium clays. A statistical analysis of the occurrence frequency of geophilic dermatophytes and the chrysosporium group showed that the Ø < 0.02 mm fraction content significantly influenced only the distribution of the representatives of the chrysosporium group. No significant correlation between the level of this fraction in the soil and the occurrence frequency of geophilic dermatophytes, however, was recorded. Furthermore, the occurrence frequency of fungi in the chrysosporium group was positively correlated with the nitrogen content and soil pH, properties mutually correlated. The correlation coefficient ($r = 0.916$) was particularly high, most significant ($\alpha = 0.001$) in relation to the soil pH. Thus, as the value of the soil pH increases, the number of the representatives of this group of keratinophilic fungi increases (Table 5). The occurrence frequency of geophilic dermatophytes in the soil was significantly correlated only with the amount of CaCO₃ in the soil. The negative correlation coefficient obtained ($r = -0.592$), at the level of significance $\alpha = 0.05$, proves that the occurrence frequency of geophilic dermatophytes goes up as the content of CaCO₃ in the soil goes down (Table 5). The absence of growth of geophilic dermatophytes was a characteristic feature of the soil with the greatest amount of this component (soil n° 16) (Table 4).

Occurrence frequency of individual species of geophilic dermatophytes and chrysosporium in arable soils

Trichophyton ajelloi (Vanbr.) Ajello in the group of geophilic dermatophytes (Table 6) and *Ctenomyces serratus* Eidam in the chrysosporium group (Table 7) are the most common species in the total of 12 species of keratinophilic fungi isolated from the soils examined. The mean occurrence frequency of these fungi in the soil, calculated on the basis of the occurrence frequency on 1700 soil plates (100 Petri dish per each soil), was 60.2% and 66.4%, respectively (Tables 6 and 7).

The studies conducted revealed that *T. ajelloi* occurred in podzols, cambisols and chernozems most numerously. The weakest growth of this dermatophyte was recorded in phaesols and rendzinas (Table 6). Those soils, on the other hand, were characterised by a high number of *Ct. serratus*, a fungus that appeared frequently also in fluvisols and chernozems. The fungus was not recorded in podzols (Table 7).

Table 6
Occurrence frequency of individual species of geophilic dermatophytes in the soils examined

| Soil type | Number of strains | | | | |
|--------------------|-------------------|---------------------|----------------------|------------------|-------------------|
| | <i>T. ajelloi</i> | <i>T. terrestre</i> | <i>T. georgiense</i> | <i>M. cookei</i> | <i>M. gypseum</i> |
| Podzols | | | | | |
| 1 | 100 (14)* | 18 | 0 | 1 | 0 |
| 2 | 77 (6)* | 0 | 0 | 0 | 0 |
| 3 | 100 (18)* | 18 | 3 | 1 | 0 |
| Cambisols | | | | | |
| 4 | 100 (14)* | 1 | 0 | 0 | 9 |
| 5 | 69 (8)* | 6 | 0 | 0 | 0 |
| Chernozems | | | | | |
| 6 | 85 (4)* | 4 | 0 | 3 | 72 |
| 7 | 95 (8)* | 3 | 0 | 24 | 3 |
| 8 | 96 (7)* | 4 | 0 | 5 | 15 |
| Phaesols | | | | | |
| 9 | 9 | 1 | 0 | 0 | 0 |
| 10 | 6 | 0 | 0 | 0 | 0 |
| 11 | 32 | 0 | 0 | 0 | 0 |
| 12 | 24 | 2 | 29 | | 0 |
| Fluvisols | | | | | |
| 13 | 95 | 0 | 0 | 0 | 0 |
| 14 | 93 | 0 | 0 | 10 | 33 |
| 15 | 5 | 1 | 2 | 0 | 0 |
| Rendzinas | | | | | |
| 16 | 0 | 3 | 0 | 0 | 0 |
| 17 | 36 | 0 | 0 | 0 | 0 |
| Mean frequency (%) | | | | | |
| All soils total | 60.2 (39.7)** | 3.4 (5.1)** | 1.4 (4.6)** | 2.0 (4.1)** | 7.2 (18.6)** |

Explanations: * - number of strains representing perfect stage; ** - standard deviation (%)

Other species of geophilic dermatophytes and chrysosporium, isolated from the soils examined, were characterised by a low occurrence frequency (between ~1% and ~10%). *Microsporium gypseum* (Bodin) Guiart et Grigorakis (Table 6) and *Chrysosporium keratinophilum* (Frey) Carm. (Table 7) were isolated fairly frequently. Populations of *M. gypseum* (also *Microsporium cookei* Ajello) were most numerous in chernozems, while populations of *Ch. keratinophilum* in rendzinas (soil n° 16). As regards the other 7 species, *Trichophyton terrestre* Duric et Frey occurred mainly in podzols, *Trichophyton georgiae* Varsavsky et Ajello, *Chrysosporium tuberculatum* (Kuehn) Dominnik and *Chrysosporium pannicola* (Corda) van Oorschot et Stalpers in phaeols, and *Chrysosporium tropicum* Carm. in cambisol (Tables 6 and 7). *Ch. asperatum* Carm. and *Ch. queeslandicum* Apinis et Rees were isolated only sporadically (individual strains). Twenty-six strains were determined as *Trichophyton* spp.

Table 7
Occurrence frequency of individual species of the chrysosporium group
in the soils examined

| Soil type | Number of strains | | | | |
|--------------------|---------------------|-------------------------|---------------------------|----------------------|---------------------|
| | <i>Ct. serratus</i> | <i>Ch. tuberculatum</i> | <i>Ch. keratinophilum</i> | <i>Ch. pannicola</i> | <i>Ch. tropicum</i> |
| Podzols | | | | | |
| 1 | 0 | 0 | 0 | 2 | 0 |
| 2 | 0 | 0 | 0 | 0 | 1 |
| 2 | 0 | 0 | 11 | 4 | 0 |
| Cambisols | | | | | |
| 4 | 0 | 0 | 10 | 2 | 7 |
| 5 | 87 | 0 | 5 | 3 | 2 |
| Chernozems | | | | | |
| 6 | 70 | 1 | 1 | 1 | 3 |
| 7 | 65 | 1 | 1 | 0 | 0 |
| 8 | 91 | 2 | 1 | 0 | 0 |
| Phaeols | | | | | |
| 9 | 100 | 0 | 0 | 0 | 0 |
| 10 | 96 | 0 | 0 | 0 | 0 |
| 11 | 99 | 0 | 1 | 0 | 0 |
| 12 | 99 | 66 | 0 | 0 | 0 |
| Fluvisols | | | | | |
| 13 | 74 | 0 | 0 | 0 | 0 |
| 14 | 83 | 3 | 0 | 45 | 0 |
| 15 | 98 | 0 | 2 | 0 | 1 |
| Rendzinas | | | | | |
| 16 | 68 | 0 | 99 | 0 | 1 |
| 17 | 99 | 0 | 5 | 0 | 0 |
| Mean frequency (%) | | | | | |
| All soils total | 66.4 (39.7)* | 4.3 (15.9)* | 80 (23.7)* | 3.4 (10.8)* | 0.9 (1.8)* |

Explanations: * - standard deviation

Table 8
Correlation coefficients (r) and determination coefficients (R^2) between soil properties and occurrence frequency of species of keratinophilic fungi

| Fungi | Content in the soil | | | | | | | | | | | |
|---------------------------|---------------------|----------|----------|-------|-------------------|-------|-------------------------------|--------|----------------------|-------|----------|----------|
| | Humus | | N tot. | | CaCO ₃ | | P ₂ O ₅ | | ϕ < 0.02 mm fraction | | pH | |
| | r | R^2 | r | R^2 | r | R^2 | r | R^2 | r | R^2 | r | R^2 |
| Geophilic dermatophytes | - | - | - | - | -0.660** | - | - | - | - | - | -0.661** | 0.776*** |
| <i>T. ajellii</i> | - | - | - | - | - | - | - | - | - | - | - | - |
| <i>T. terrestris</i> | -0.527* | - | -0.499* | - | - | - | - | - | - | - | -0.553* | 0.314* |
| <i>T. georgica</i> | - | - | - | - | - | - | 0.523* | 0.273* | - | - | - | - |
| <i>M. cookei</i> | - | 0.737*** | - | - | - | - | - | - | 0.737*** | - | - | - |
| <i>M. gypseum</i> | - | - | - | - | - | - | - | - | - | - | - | - |
| Chrysosporium group | - | - | - | - | - | - | - | - | - | - | - | - |
| <i>C. serratus</i> | 0.629 | - | 0.761*** | - | - | - | - | - | 0.714** | - | 0.925*** | 0.867*** |
| <i>Ck. keratinophilum</i> | - | - | - | - | - | - | - | - | - | - | - | - |
| <i>Ck. panamicola</i> | - | - | - | - | - | - | - | - | - | - | - | - |
| <i>Ck. tropicum</i> | - | - | - | - | - | - | - | - | - | - | - | - |
| <i>Ck. tuberculatum</i> | - | - | - | - | - | - | 0.549* | 0.301* | - | - | - | - |

Explanations: as for Table 3

In the group of the species examined, *Ct. serratus*, *T. ajelloi*, *T. terrestre*, *T. georgiae* and *Ch. tuberculatum* showed a significant correlation with physical and chemical properties of the soil (Table 8).

The most significant ($\alpha = 0.001$) and high correlation coefficient was obtained between the frequency of occurrence of *Ct. serratus* and the soil pH ($r = 0.925$), as well as the N content in the soil ($r = 0.761$). The content of both $\Phi < 0.02$ mm fraction and humus has a significant and positive influence on the growth of *Ct. serratus* in the soil. The four soil properties were mutually significantly correlated (Table 3). The elimination of less significant factors using the regression method showed that the occurrence frequency of *Ct. serratus* was most strongly correlated with the soil pH ($R^2 = 0.867$) – Table 9.

The occurrence of the other species dominant, *T. ajelloi*, in the soil depended on the content of CaCO_3 and soil pH (Table 8). The correlation coefficients obtained were negative, thus the number of the populations of this fungus in the soil decreased as CaCO_3 and soil pH increased. The growth of *T. ajelloi*, up to the form of a monoculture, occurred in strongly acidic soils ($\text{pH}_{\text{KCl}} < 4.5$), devoid of CaCO_3 (podzols), to the greatest extent. The fungus was not found in alkaline soils rich in CaCO_3 (rendzina n° 16). The application of the analysis of multiple regression, with the elimination of the least significant components, explained the true variability of the occurrence frequency of *T. ajelloi*. The method used showed that the value of soil pH was the factor that determined the occurrence of *T. ajelloi* populations most strongly (Table 9).

Table 9
Significant correlation coefficients between occurrence frequencies
of the populations of the fungi examined

| Population | Correlation coefficient |
|--|-------------------------|
| <i>T. ajelloi</i> and <i>Ct. serratus</i> | -0.596* |
| <i>T. terrestre</i> and <i>Ct. serratus</i> | -0.560* |
| <i>M. cookei</i> and <i>Ch. pannicola</i> | 0.491* |
| <i>T. georgiae</i> and <i>Ch. tuberculatum</i> | 0.980*** |

Explanations: as for Table 3

The occurrence of *T. terrestre* revealed a significant, although rather weak ($\alpha = 0.05$), correlation with the humus and nitrogen content. The negative correlation coefficients obtained show that *T. terrestre* populations prefer soils with a low nutrient content (Table 8). Moreover, coefficient R^2 shows that the occurrence of this fungus in the soil was inversely proportional to the humus content (Table 9).

In the case of *T. georgiae* and *Ch. tuberculatum*, both the correlation analysis and the multiple regression analysis showed a significant relationship between the occurrence of the fungi and the phosphorus content in the soil. The correlation coefficient and the determination coefficient obtained were, however, low (Tables 8 and 9).

No statistically significant correlation between the occurrence and soil properties was found for the five other species of *Keratinomyces*, (Tables 8 and 9) which rarely occurred in the soils examined.

The presence of other species of keratinophilic fungi in the soil was a factor that significantly influenced the occurrence of some populations of these fungi. The hi-

ghest, most significant ($\alpha = 0.001$) and positive correlation coefficient ($r = 0.980$) was recorded between the populations of *T. georgiae* and *Ch. tuberculatum*. A weaker correlation, although significant ($\alpha = 0.05$), was noticed between the occurrence of *T. ajelloi* and *Ct. serratus*. The negative correlation coefficient shows that the occurrence frequency of *Ct. serratus* decreased as the occurrence frequency of *T. ajelloi* increased, and vice versa. A similar correlation between the growth of *Ct. serratus* and *T. terrestre* in the soil was noticed. A significant, although quite low correlation coefficient was also obtained between the occurrence of *M. cookei* and *Ch. pannicola* in the soil. A positive value of this coefficient shows that the growth of one of these species is accompanied by a growth in number, albeit small, of the other. A positive but not significant correlation coefficient was obtained for the pair of fungi: *T. ajelloi* and *T. terrestre*.

DISCUSSION

The majority of studies (Chmel et al. 1972; Chmel and Vlačilíková 1975; Fattah et al. 1982; Kaul and Sumbali 1992; Kornilłowicz 1993) claim that the occurrence frequency of keratinophilic fungi in the soil is influenced primarily by the content of organic matter. As has been shown in this study, however, there exists no significant correlation between the overall frequency of keratinophilic fungi and the humus content in the soil. On the other hand, a significant correlation between the total occurrence frequency of these fungi and the content of the $\phi < 0.02$ mm fraction in the soil was revealed. These findings indicate that the number of *Keratinomyces* increases as the amount of this granulometric fraction increases. Therefore, keratinophilic fungi prefer soils rich in nutrients, well buffered, with the most favourable air and water relations, as an increase in the $\phi < 0.02$ mm fraction content, consisting chiefly in clay minerals and organic koloids, is accompanied by an enhanced capacity of the soil to store water and nutrients, as well as improved buffer properties. These features are typical of soils formed of medium clays and silt soils, such as chernozems, phaeosols and fluvisols (Table 1). The authors' studies show that these soils are characterised by a higher occurrence frequency of keratinophilic fungi than podzols, low in the $\phi < 0.02$ mm fraction. The conditions in the chernozems studied were particularly favourable for the development of keratinophilic fungi. On the whole, these soils are characterised by base saturation of the sorptive complex, a pH close to neutral, and good oxygenation. As has also been noticed before (Kornilłowicz 1993), a favourable composition of organic matter has come into being in chernozems, characterised, especially in the past, by numerous soil fauna comprising, for instance, small mammals. As Garg et al. (1985) claim, the „animalisation“ of those soils is one of the most important factors influencing the development of keratinophilic fungi in this environment due to the enriched content of keratin proteins. It is therefore not accidental that the greatest number of species and strains of *Keratinomyces*, as well as the greatest number of fungi per one plate were recorded in the chernozem samples. Those soils were occupied particularly numerously by geophilic dermatophytes, including the genus *Microsporum*, often occurring in hairs to be found in this environment, which was reported by Battelli et al. (1978), Vollenková (1984) as well as Garg et al. (1985).

The frequency of occurrence of chrysosporium representatives in chernozems was much lower than that of dermatophytes, which may be influenced by, generally speaking, their lower keratinolytic activity resulting from, among other things, simpler structures for destruction of native keratin (English 1965, 1969). It may lead to a decrease in the substrate range of these fungi which, as a result, may recede from habitats richer in less readily available forms of keratin, such as hair α -keratin, and come to occupy habitats containing more easily available feather keratin. This suggestion is supported by observations which reveal an accumulation of these fungi on feathers in the soil and bird plumage (Pugh and Evans 1970; Dixit and Kushwaha 1991; Pinowski et al. 1999).

The statistical analysis of the frequency of occurrence of geophilic dermatophytes and the chrysosporium group carried out in this study corroborates the earlier observation (Kornilłowicz 1993) that these fungi colonize soil environments with different properties. In the case of geophilic dermatophytes Chmel and Vlačilíková 1975, the level of CaCO_3 was a factor, which significantly influenced the frequency of occurrence. The number of these fungi increased as the content of this constituent decreased, and decreased as the amount of CaCO_3 rose. The results of studies conducted by (1977), as well as Kornilłowicz (1992, 1993) also reveal a weaker distribution of geophilic dermatophytes in soils rich in CaCO_3 than that of chrysosporium. Given that an increase in CaCO_3 in the soil is accompanied by an increase in soil pH, the effect observed should be laid down to the selective impact of pH on the enzymatic activity of these fungi rather than to the influence of calcium ions. The most favourable pH values for the keratinolytic activity of dermatophytes are usually lower than those for chrysosporium representatives (Garg et al. 1985). The author cited classifies most geophilic dermatophytes as acidophilic or neutrophilic, and fungi in the chrysosporium group as nitrophilic and alkalophilic. In the studies conducted in this work, the absence of geophilic dermatophytes and an accumulation of chrysosporium representatives in alkaline soils exemplified this correlation.

The studies conducted in this work show that *Trichophyton ajelloi* and *Ctenomyces serratus* belong to the species of keratinophilic fungi that are most common in the soil. The occurrence frequency of both species was strongly, but inversely, correlated with the soil pH. As the soil pH increased, the number of *Ct. serratus* went up, while the number of *T. ajelloi* went down. The inverse effect took place when the soil pH decreased: the occurrence of *Ct. serratus* went down, and that of *T. ajelloi* went up.

The stimulation of the development of *T. ajelloi* in acidic and strongly acidic soils, corroborated by the results of the statistical analysis, complies with earlier observations made by various authors who claim that the species belongs to acidic environments (Böhme and Ziegler 1969; Pugh and Evans 1970; Chmel and Vlačilíková 1977; Vollenková 1984; Garg et al. 1985; Kornilłowicz 1993). The domination of *Ct. serratus* in neutral and weakly basic soils, demonstrated in this work, confirms earlier findings (Pugh and Evans 1970; Garg et al. 1985; Kornilłowicz 1993), which identify it as a neutrophilic species. In the authors' earlier works (Kornilłowicz 1993), it was noticed that the preference of *T. ajelloi* and *Ct. serratus* for soil environments whose pH differed could be attributed to the anta-

gonism between these fungi. The negative correlation between the occurrence frequency of both populations obtained in this work supports this hypothesis.

The studies conducted also revealed a negative correlation between the distribution of *Ct. serratus* and *T. terrestre* populations. It turned out that *T. terrestre* colonized mainly podzols, low in organic matter and nitrogen, and characterised by acidic pH, not tolerated by *Ct. serratus*. A negative, although fairly weak, correlation with those soil properties would suggest that there exists a relationship between the occurrence of *T. terrestre* and the soil type rather than that between its occurrence and individual soil properties. A three chain analysis: soil properties > soil type > keratinophilic fungi, may provide further data and will be carried out in the future. At the moment, it may only be suspected that *T. terrestre*, considered by some authors (Garg et al. 1985) to be a xerophile, occurs mainly in light soils characterised by a negative water balance. Otčenašek et al. (1969) and Chmel et al. (1972) also reported a frequent occurrence of *T. terrestre* in soils low in humus content and moisture.

The dermatophytes *Microsporum gypseum*, *M. cookei* and *Trichophyton georgiae*, as well as *Ch. keratinophilum* and *Ch. tuberculatum* in the chrysosporium group, are those fungi among the 7 isolated species of keratinophilic fungi in the soils studied that also deserve special attention.

Even though Chmel et al. (1972) report that *M. gypseum* occurs mainly in soils with high humus content, no significant correlation between the frequency occurrence of this fungus and the soil properties studied was found in this work. However, the occurrence of the *M. gypseum* population, which was limited mainly to chernozems, may suggest, similarly to *T. terrestre*, a relationship with the soil type. A similar assumption may be made in connection with the distribution of the *Microsporum cookei* population. As Chmel and Vlačilíková (1977) and Kornilowicz (1992, 1993) observed, these fungi were found chiefly in chernozems. The „animalisation“ of chernozems is most probably of great importance in this respect. *Microsporum gypseum* and *M. cookei* usually do not occur in environments that do not contain hairs (Otčenašek et al. 1969). Furthermore, as the authors cited claim, both species occupy the same soils, which was also confirmed in this work. Böhme and Ziegler (1968) claim otherwise. In their opinion, *M. gypseum* and *M. cookei* are mutually exclusive. In the light of the data given by those authors, as well as on the basis of the authors' research, which reveals a positive, but fairly low, correlation between the occurrence frequency of these species, both theses seem accurate. They prove their similar habitat requirements, which bring about nutritive competition and consequently a limitation of the growth of one of the partners. Between those two species, *M. cookei* is the weaker partner, which is indicated by a lower frequency of occurrence of this fungus than that of the *M. gypseum* population, as observed in the authors' studies.

Little has been known so far about the distribution of the *Trichophyton georgiae* populations in the soil. Garg et al. (1985) report that the species is connected with soils containing a high level of humus and thus characterised by high fertility. The studies conducted in this work demonstrate that it is a rare species in the soil, correlated positively with the phosphorus level. A positive correlation between the occurrence frequency of *Chrysosporium tuberculatum* and the phosphorus content, as well as the occurrence frequency of *T. georgiae* in the soil also show that the occurrence of *Chrysosporium tuberculatum* in the soil may be conditioned by similar factors. Both spe-

cies, however, did not occur in the soil with the highest phosphorus content (soil n° 17) in the group of the 17 soils studied. It was found, however, that both species colonized first of all phaeosols rich in CaCO_3 . Chmel and Vlačiliková (1975), as well as Kornilowicz (1993) also made similar observations. It may thus be suspected that the type of soil was more significant than its individual properties also in the case of these two fungi.

Ch. keratinophilum and *Ch. pannicola* were also the species, which accumulated in individual soil samples. As the studies conducted by Chmel and Vlačiliková (1977) show, they are *Chrysosporium* species most frequently encountered in the soil. The domination of *Ch. keratinophilum* in a strongly alkaline soil (carbonate rendzinas) observed in this study corroborates a close affinity of this fungus with alkaline soils, recorded by Garg et al. (1985). Alkaliphilous preferences of *Ch. keratinophilum* are connected with the production of alkaline keratinolytic proteinase (optimum pH=9), which was demonstrated by Dozie et al. (1994). A growth stimulation of *Ch. pannicola* occurred in one of the three fen soils examined (soil n° 14), and was related to the occurrence of many other *Keratinomycetes* species. Probably the composition of all ecological factors significant for the development of *Keratinomycetes* was particularly favourable in this soil. Chernozem n° 6 was the other soil in which the biocenosis of keratinophilic fungi was most numerous and most diverse in terms of species. Typical features of both soils were a high content in $\phi < 0.02$ mm fraction (40-50%) and the pH close to neutral (7.13-7.15). In the light of the studies conducted in this work, those two properties exert the greatest influence on the occurrence and the distribution of keratinophilic fungi in the soil. The level of $\phi < 0.02$ mm fraction has a greater impact on the abundance of the *Keratinomycetes*, while the soil pH has a greater impact on their species composition.

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Korelacje pomiędzy częstością występowania grzybów keratynofilnych, a niektórymi właściwościami gleby

Streszczenie

Celem pracy było poszukiwanie korelacji między częstością występowania grzybów keratynofilnych i właściwościami gleby. Badaniami objęto 17 gleb uprawnych reprezentujących: gleby bielcowe, brunatne, czarnoziemy, czarne ziemie, mady i rędziny z terenu środkowo-wschodniej Polski.

Przeprowadzone badania wykazały, że ogólna frekwencja grzybów keratynofilnych w glebie jest istotnie dodatnio skorelowana z poziomem części spławialnych ($r = 0,581$ przy $\alpha = 0,05$). Częstość występowania dermatofitów geofilnych była ujemnie skorelowana z poziomem CaCO_3 ($r = -0,592$ przy $\alpha = 0,05$), natomiast grupy *chrysosporium* skorelowana dodatnio z wartością pH gleby ($r = 0,916$, $\alpha = 0,001$). W przypadku poszczególnych populacji *Keratinomyces* istotne korelacje z właściwościami gleby wykazywały *Trichophyton ajelloi*, *T. terrestre*, *T. georgiæ*, *Ctenomyces serratus* i *Chrysosporium tuberculum*. Najbardziej istotną i dodatnią korelację otrzymano między występowaniem *Ct. serratus* i poziomem pH gleby ($r = 0,925$, $\alpha = 0,001$). Odczyn gleby w największym stopniu warunkował również rozmieszczenie populacji *T. ajelloi* w glebie. Obserwowana relacja miała odwrotny charakter ($r = -0,661$ przy $\alpha = 0,01$; $R^2 = 0,776$ przy $\alpha = 0,001$), niż w przypadku populacji *Ct. serratus*. Zasiedlanie odmiennych,

pod względem odczynu, środowisk glebowych oraz ujemny współczynnik korelacji między częstością występowania populacji *Ct. semmatis* i *T. ajelloi*, wskazywały na antagonizm tych gatunków w glebie.

Wpływ czynników edaficznych, na występowanie pozostałych w/w populacji grzybów keratynofilnych, był mniej jednoznaczny. Skłaniał on raczej ku tezie wskazującej na silniejsze powiązania z typem gleby niż jej określonymi właściwościami.