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# GENERAL ASSESSMENT OF THE INFLUENCE OF A MUNICIPAL LANDFILL SITE AND ENVIRONMENTAL FACTORS ON THE OCCURRENCE OF KERATINOLYTIC FUNGI IN SOIL

## OGÓLNA OCENA WPŁYWU WYSYPISKA ODPADÓW KOMUNALNYCH I CZYNNIKÓW ŚRODOWISKOWYCH NA WYSTĘPOWANIE GRZYBÓW KERATYNOLITYCZNYCH W GLEBIE

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*The study was to generally determine how a municipal landfill site and environmental factors influenced the distribution of keratinolytic fungi in soil. The landfill site in Sosnowiec with its surrounding area was selected for examination. Keratinolytic fungi occurred abundantly in the soils examined. Some keratinolytic fungal species with pathogenic properties were recorded. Results are discussed from ecological, hygienic and epidemiological points of view.*

## INTRODUCTION

Medically important organisms, which occur in municipal solid waste, can be divided into five groups: helminths, protozoa, viruses, bacteria and fungi. The first two groups are subject to parasitology while the last three are subject to microbiology. In municipal solid waste, chiefly saprophytic microorganisms of water and soil origin are present. However, a considerable amount of the total population of waste microorganisms is of faecal origin. In both groups microbial species pathogenic to plants and animals, including humans are found.

Landfill sites are the places where municipal waste is disposed. These sites are also the sources of microbial contamination and infection. Pathogenic microorganisms spread from landfill sites to their surroundings *via* air, water and animals. Most studies have been concerned with the spread of microorganisms *via* air and have evaluated the public health risk associated with the airborne contamination [10, 11, 15]. The influence of landfill sites on the microbiological quality of soil and the role of waste-contaminated soil in the deterioration of hygienic conditions in the surrounding areas of landfill sites have not yet been extensively studied.

The occurrence of microscopic fungi in municipal waste and the spread of these microorganisms from landfill sites have been the subject of several studies [review in 19]. However, there have been few studies on landfill sites as sources of keratinolytic fungi, microorganisms highly specialised in decomposition of keratin and with potential pathogenic properties to animals including humans [18]. It can be assumed that, due to high humidity and temperature and expected abundance of keratinous debris in municipal waste, keratinolytic fungi find good nutritional conditions for growth in this environment. Fungal pathogenic populations associated with municipal waste and landfill sites require therefore thorough examination. Special attention should be paid to that how different environmental factors affect the distribution of pathogenic fungi in the surrounding areas of landfill sites.

The present study was to generally determine the influence of a municipal landfill site and environmental factors on the occurrence of keratinolytic fungi in soil. The municipal landfill site in Sosnowiec with its surrounding area was selected for examination.

## MATERIAL AND METHODS

Figure 1 shows the municipal landfill site in Sosnowiec with its surrounding area and sampling locations. The description of waste disposal technology together with the land use structure of this area was presented in a previous report [23].

Soil was sampled six times: three times in 1993 (26.04.93; 25.05.93; 12.10.93) and three times in 1994 (4.05.94; 5.07.94; 22.09.94). At each sampling, locations were different and selected at different distances (up to 700 m) from the edge of the landfill site in the downwind and upwind (background) directions. Four locations were on lawns in the residential area of the Kazimierz district (Sosnowiec), at the distance of up to 1000 m from the landfill site to Northeast. Altogether, soil samples from forty-one locations were collected. The locations represented different manmade habitats, i.e., meadows, idle arable soils, sandy wastelands, industrial areas (including a coal ash heap and carbon rock heap) and young self-sown birch and pine forests. Soil was sampled from the superficial layer (up to 5 cm of depth) to plastic bags disinfected with 80% ethyl alcohol solution and UV lamps. At each location and sampling, ca. 3 kg of soil was collected from 50 points within the regular square sampling net (5 x 5 m). This method of sampling covered a wide surface of soil (25 $m^2$) for examination. Samples were delivered to the laboratory within 3–4 hours, first cleaned from municipal waste particles, fresh plant remnants and stones, then crumbled and thoroughly mixed. The mixed samples were examined for microbiological and physico-chemical properties.

The children's hair baiting method was employed for examination of keratinolytic fungi in soil samples. For each sample, 10–20 *Petri* dishes were set up. The method was described in a previous paper [22]. The isolated fungi were identified from their macro- and microscopic characteristics and using selected taxonomic monographs [2–4, 13, 16]. The keratinolytic abilities of the fungi were tested *in vitro* by putting sterilised children's hair onto *Petri* dishes with Sabouraud 1:10 + salts medium, inoculating them with fungal strains and incubating for one month at 25°C.

The occurrence of keratinolytic fungi in soils was characterised by the following indices: frequency of isolation of keratinolytic fungi (FI; the number of *Petri* dishes positive for keratinolytic fungi divided by the total number of *Petri* dishes set up x 100%), number of species isolated (NS), number of fungal strains (NA), frequency of isolation of predominating fungal species (number of strains of a given species divided by the total number of fungal strains x 100%) and L index (number of strains divided by the number of *Petri* dishes set up).

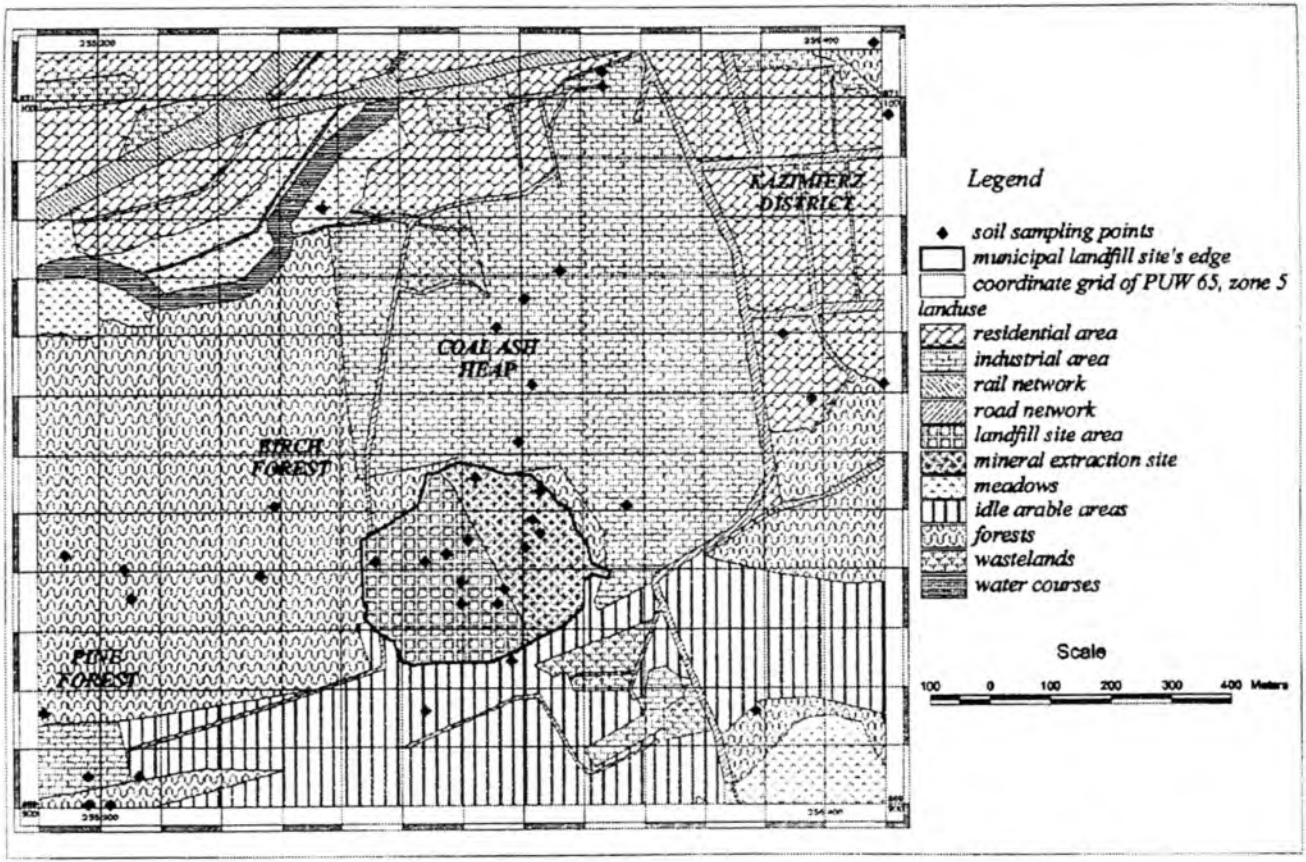



Fig. 1. The municipal landfill site in Sosnowiec with its surrounding area and soil sampling locations

The following physico-chemical parameters were determined for each soil sample: humidity (HUM) by gravimetric method; particle size distribution (PSD) analysis by *Casagrande's* aerometric method in *Prószyński's* modification; pH in $H_2O$ and pH in 1 M KCl by potentiometric method; conductivity (COND) by electrical method; hydrolytic acidity (ACI) by *Kapen's* method; ignition losses at 600 C (IGNL) by gravimetric method; organic carbon ($C_{ORG}$) by *Walklay-Black's* method; total nitrogen ($N_{TOT}$) by *Kjeldahl* method; total sulphur ($S_{TOT}$) by *Eschka* method; sulphate sulphur ($S\text{-}SO_4$) by *Barnsley* and *Lancaster's* method; total phosphorus ($P_2O_{5TOT}$) by colorimetric method; available phosphorus ($P_2O_5$) and available potassium ($K_2O$) by *Egner-Riehm's* method. The C:N ratios were calculated.

The following microbiological parameters were determined in each soil sample: total number of microscopic fungi (TNMF) on malt extract agar (MEA) with chloramphenicol (100 mg/l) at 25 C, number of mesophilic fungi (MF) on YpSs agar with chloramphenicol (100 mg/l) at 37°C, number of thermophilic fungi (TF) on YpSs agar with chloramphenicol (100 mg/l) at 45 C, total number of bacteria (TNB), number of mesophilic bacteria (MB), *Clostridium perfringens* (CP), total coliforms (TC), faecal coliforms (FC), and faecal streptococci (FS). The FC:FS ratios were calculated. The terms "mesophilic" and "thermophilic" are used in the sense of incubation temperatures. Results are presented as the most probable number of bacterial cells in 1 or 100 g of soil dry weight. The methods recommended by the Countryside Hygiene Institute in Lublin, Poland [8] were used to perform the analyses.

In addition to the above-mentioned microbiological and physico-chemical parameters, the $W_W$ index was calculated. This index displays the long-term influence of climatic conditions on soil humidity [19]. It is the value of water deficit (or excess) in the near-surface level of soil at the end of a given period (e.g. a decade), calculated with the following formula:

$$W_W = 1/(1+\Sigma i)*[(P_1-E_1)+\Sigma i(P_i-E_i)]$$

where:

$P_1$, $E_1$ – daily sums of precipitation and potential evaporation respectively on the first day of a given period;

i (1, 2,...., n) – consecutive day in a n-day period.

Negative values of $W_W$ index mean the deficit of water in soil whereas positive values show the overabundance of water in this environment.

The "STATISTICA" for Windows program was used for statistical analyses (descriptive statistics, simple linear correlation, stepwise forward regression, range analysis) of the data obtained. Adding one and logarithming first transformed the microbiological data, due to their original asymmetric distribution.

## RESULTS

Of 495 soil samples (*Petri* dishes) examined, 379 (76.56%) were positive for keratinolytic fungi. The mean L index value was 2.28. Altogether, 1131 strains from 26 species were isolated from the samples. The following species were identified: *Aphanoascus durus* (anamorph + teleomorph; frequency 20.2%), *Arthroderma quadrifidum* (anamorph + teleomorph; 9.5%), *Arthroderma curreyi* (anamorph + teleomorph; 8.6%), *Trichophyton ajelloi* (7.2%), *Chrysosporium* spp. (anamorphs of *Aphanoascus reticulisporus* and *A. fulvescens*; 6.7%), *Myceliophthora vellerea* (6.0%), *Chrysosporium tropicum* (5.9%), *Aphanoascus reticulisporus* (teleomorph; 5.2%), *Arthroderma* sp. (anamorph *Microsporum gypseum* + teleomorph; 5.2%), *Chrysosporium europae* (4.6%), *Malbranchea* anamorph of *Uncinocarpus reessi* (4.3%), *Aphanoascus keratinophilus* (anamorph + teleomorph; 3.8%), *Microsporum cookei* (2.8%), *Malbranchea flava* (2.3%), *Aphanoascus terreus* (anamorph *Chrysosporium indicum* + teleomorph; 2.1%),

Table I. Physico-chemical and microbiological characteristics of soil samples

| Parameter | Unit | Mean | Minimum | Maximum | Standard deviation |
|---|---|---|---|---|---|
| Total coliforms (TC) | MPN/100 g dw | 241274 | 15 | 8630506 | 1348694 |
| Faecal coliforms (FC) | MPN/100 g dw | 215492 | 6 | 8630506 | 1347149 |
| Faecal streptococci (FS) | MPN/100 g dw | 5343 | 15 | 86305 | 18663 |
| FC:FS | – | 5.8 | 0 | 100 | 17 |
| Total number of bacteria (TNB) | CFU/g dw | 2557926 | 1726 | 52701600 | 8862495 |
| Mesophilic bacteria (MB) | CFU/g dw | 153347 | 0 | 4444211 | 737670 |
| *C. perfringens* (CP) | CFU/g dw | 405 | 0 | 6288 | 1201 |
| Total number of microscopic fungi (TNMF) | CFU/g dw | 76354 | 35 | 1405797 | 225614 |
| Mesophilic fungi (MF) | CFU/g dw | 5037 | 0 | 48846 | 9224 |
| Thermophilic fungi (TF) | CFU/g dw | 424 | 0 | 3886 | 762 |
| Humidity (HUM) | % | 8.0 | 0 | 58.4 | 10.8 |
| Ww index | – | –2.27 | –5.5 | –0.2 | 1.81 |
| PSD > 1 mm | % | 3.7 | 0 | 93.0 | 18.6 |
| PSD 1–0.1 mm | % | 83.6 | 42.0 | 97.0 | 15.1 |
| PSD 0.1–0.05 mm | % | 5.0 | 0 | 14.0 | 3.9 |
| PSD 0.05–0.02 mm | % | 3.4 | 0 | 14.0 | 4.3 |
| PSD 0.02–0.005 mm | % | 2.5 | 0 | 12.0 | 2.7 |
| PSD 0.005–0.002 mm | % | 1.7 | 0 | 12.0 | 2.8 |
| PSD < 0.002 mm | % | 3.8 | 0 | 25.0 | 5.6 |
| pH in $H_2O$ | – | 6.8 | 2.8 | 8.0 | 1.3 |
| pH in 1 M KCl | – | 6.2 | 2.6 | 8.8 | 1.4 |
| Conductivity (COND) | µS/cm | 306.2 | 8.3 | 2270.0 | 514.1 |
| Ignition losses (IGNL) | % dw | 14.1 | 0.4 | 49.0 | 15.9 |
| Organic carbom ($C_{ORG}$) | % dw | 7.4 | 0.3 | 29.3 | 8.7 |
| Total nitrogen ($N_{TOT}$) | % dw | 0.3 | 0 | 1.7 | 0.4 |
| C:N | – | 45.2 | 1.5 | 312.1 | 65.3 |

Tabela I cd.

| Parameter | Unit | Mean | Minimum | Maximum | Standard deviation |
|---|---|---|---|---|---|
| Total sulphur ($S_{TOT}$) | % dw | 0.1 | 0 | 0.9 | 0.2 |
| $P_2O_5$ available | mg/100 g dw | 3.4 | 0.1 | 30.5 | 6.5 |
| $K_2O$ available | mg/100 g dw | 10.2 | 0.4 | 79.5 | 19.2 |
| S-$SO_4$ | mg/100 g dw | 11.7 | 0.5 | 96.0 | 21.7 |
| Hydrolytic acidity (ACI) | mval/100 g dw | 12.4 | 0 | 277.0 | 55.2 |
| $P_2O_{5TOT}$ | % dw | 0.1 | 0 | 0.6 | 0.1 |

**Abbreviations for units**:
dw – dry weight of soil
CFU – colony forming units
MPN – most probable number

Tabela II. Correlations between the data obtained for keratinolytic fungi with microbiological and physico-chemical parameters

| Indices & frequencies | Correlations with microbiological and physico-chemical parameters (r) at $p < 0.05$ |
|---|---|
| L index | FS (0.74), TC (0.71), FC (0.69), pH in $H_2O$ (0.62), pH in 1 M KCl (0.62), TNB (0.55), CP (0.43), PSD 0.1-0.05 mm (0.41), PSD 0.05-0.02 mm (–0.40) |
| FI (%) | pH in $H_2O$ (0.64), pH in 1 M KCl (0.57), TC (0.46), FC (0.39), FS (0.43), TNB (0.38) |
| NS | TC (0.55), pH in $H_2O$ (0.52), FS (0.46), PSD 1-0.1 mm (0.44), PSD 0.05-0.02 mm (–0.44), FC (0.41) |
| *T. ajelloi* | S-$SO^4$ (–0.41), TF (–0.39) |
| *A. curreyi* | $P_2O_{5TOT}$ (0.44), TC (0.42), FS (0.41), FC (0.36) |
| *C. tropicum* | CP (0.49) |
| *A. reticulisporus/fulvescens* | WW (–0.44) |
| *A. keratinophilus* | $P_2O_5$ available (0.59), pH in 1 M KCl (0.41), CP (0.37), $W_W$ (–0.32) |
| *Malbranchea* an. *U. reessi* | FS (0.48), FC (0.43), CP (0.42), TC (0.41) |
| *Arthroderma* sp. (an. *M. gypseum*) | $W_W$ (0.57), FS (0.49), TC (0.48), FC (0.46), TNB (0.42) |
| *M. vellerea* | pH in 1 M KCl (0.48), TNMF (–0.45), MF (–0.41) |
| *C. europae* | – |
| *A. durus* | – |
| *A. quadrifidum* | FS (0.45), TNB (0.43), FC (0.4), $W_W$ (0.38), TC (0.37), $S_{TOT}$ (0.42) |
| *M. cookei* | $P_2O_5$ available (0.58), TC (0.44), FC (0.38) |
| Other species | PSD 1-0.1 mm (0.41) |

Table III. Mean valuables of fungal indices and frequencies for the ranges of pH in 1 M KCl

| Indices & fungal species | pH in 1 M KCl ranges | | | | |
|---|---|---|---|---|---|
| | < 5 | 5–6 | 6–7 | 7–8 | > 8 |
| L index | 0.23 | 1.11 | 1.71 | 3.56 | 3.24 |
| FI (%) | 25.00 | 57.50 | 71.66 | 99.44 | 87.14 |
| NS | 1.50 | 4.50 | 6.00 | 7.66 | 7.85 |
| Frequencies: | % | | | | |
| *T. alleloi* | 42.85 | 23.07 | 17.06 | 7.97 | 6.94 |
| *A. curreyi* | 0.00 | 6.42 | 14.26 | 11.16 | 3.24 |
| *C. tropicum* | 0.00 | 2.57 | 0.86 | 0.21 | 8.27 |
| *Aphanoascus reticulisporus/fulvescens* | 0.00 | 0.00 | 0.57 | 0.50 | 5.19 |
| *A. keratinophilus* | 0.00 | 0.00 | 1.76 | 1.14 | 4.50 |
| *Malbranchea an. U. reessi* | 0.00 | 0.00 | 1.76 | 1.32 | 7.58 |
| *Arthroderma* sp. (an. *M. gypseum*) | 0.00 | 0.85 | 6.66 | 11.95 | 5.48 |
| *M. vellerea* | 0.00 | 1.60 | 8.43 | 9.38 | 22.61 |
| *C. europae* | 0.00 | 0.85 | 6.66 | 1.70 | 5.64 |
| *A. durus* | 0.00 | 31.62 | 9.63 | 19.67 | 4.87 |
| *A. quadrifidum* | 50.00 | 0.85 | 22.23 | 19.38 | 7.90 |
| *M. cookei* | 7.15 | 1.07 | 1.10 | 5.23 | 2.08 |
| Other species | 0.00 | 6.03 | 7.77 | 9.32 | 5.28 |

*Chrysosporium pannicola* (2.1%), *Aphanoascus fulvescens* (teleomorph; 1.1%), *Botryotrichum piluliferum* (0.4%), *Microsporum racemosum* (0.4%), *Chrysosporium mephiticum* (0.3%), *Myceliophthora* sp. (0.3%), *Malbranchea* anamorph of *Arthroderma tuberculatum* (0.3%), *Arthrographis kalrae* (0.2%), *Amauroascus mutatus* (0.2%), *Scopulariopsis brevicaulis* (0.1%), *Gymnoascus petalosporus* (anamorph + teleomorph; 0.1%) and *Chrysosporium* anamorph of *Arthroderma cuniculi* (0.1%).

The cluster analysis shows that the predominant keratinolytic fungal species can be divided into two groups (Figure 2). The first embraces *A. reticulisporus*, *A. fulvescens* with their anamorphs *Chrysosporium* spp., *Malbranchea* an. *U. reessi*, *A. keratinophilus* and *C. tropicum* while the second comprises the other species. The first group has two subgroups. The first subgroup embraces the *Aphanoascus* species with their anamorphs and the second contains *Malbranchea* an. *U. reessi*, *A. keratinophilus* and *C. tropicum*. The second group has three subgroups. *A. durus* is associated with *C. europae* and the group of "other species", while *M. cookei* is associated with *M. vellerea*. *A. quadrifidum*, *Arthroderma* sp. (anamorph *M. gypseum*), *A. curreyi* and *T. ajelloi* form the third subgroup. The *Chrysosporium* anamorphs of *Aphanoascus reticulisporus* and *A. fulvescens* were morphologically indistinguishable. Some strains of *A. reticulisporus* and *A. fulvescens* with intermediate characteristics of ascospores were also isolated (Figure 3). Because of these similarities and common ecological properties proven by the cluster analysis, both *Aphanoascus* species (teleomorphs + anamorphs) are treated jointly as *Aphanoascus reticulisporus/fulvescens* in further considerations. Keratinolytic fungi occur-

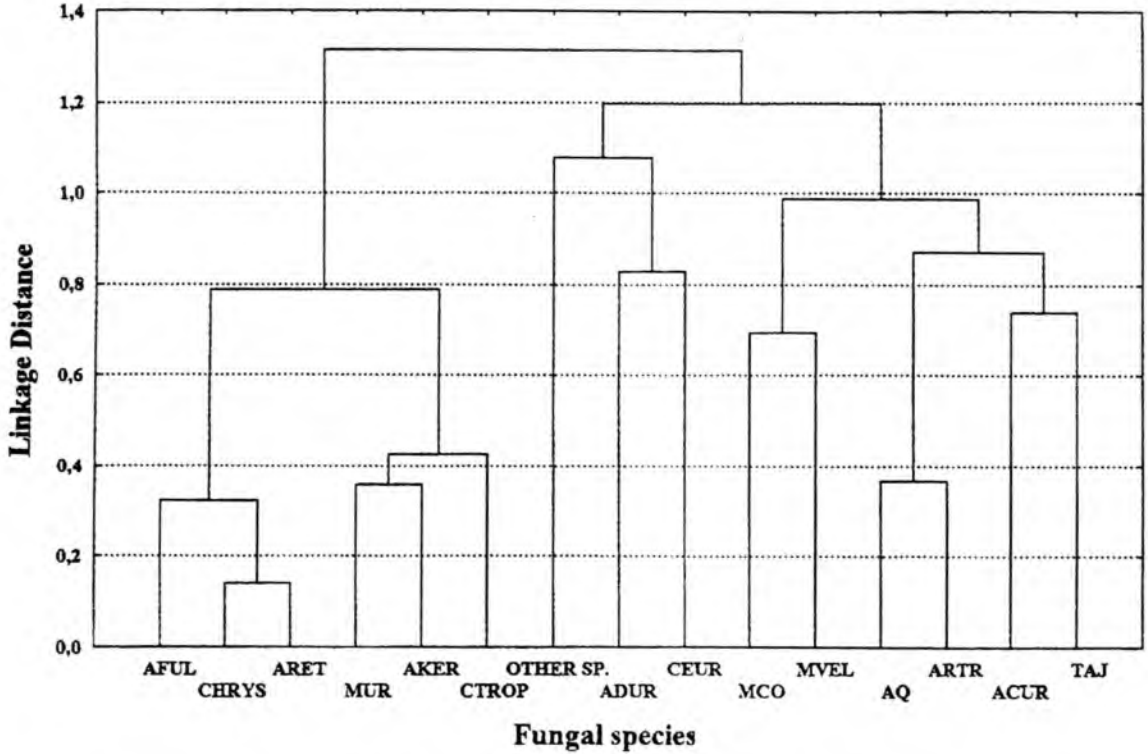
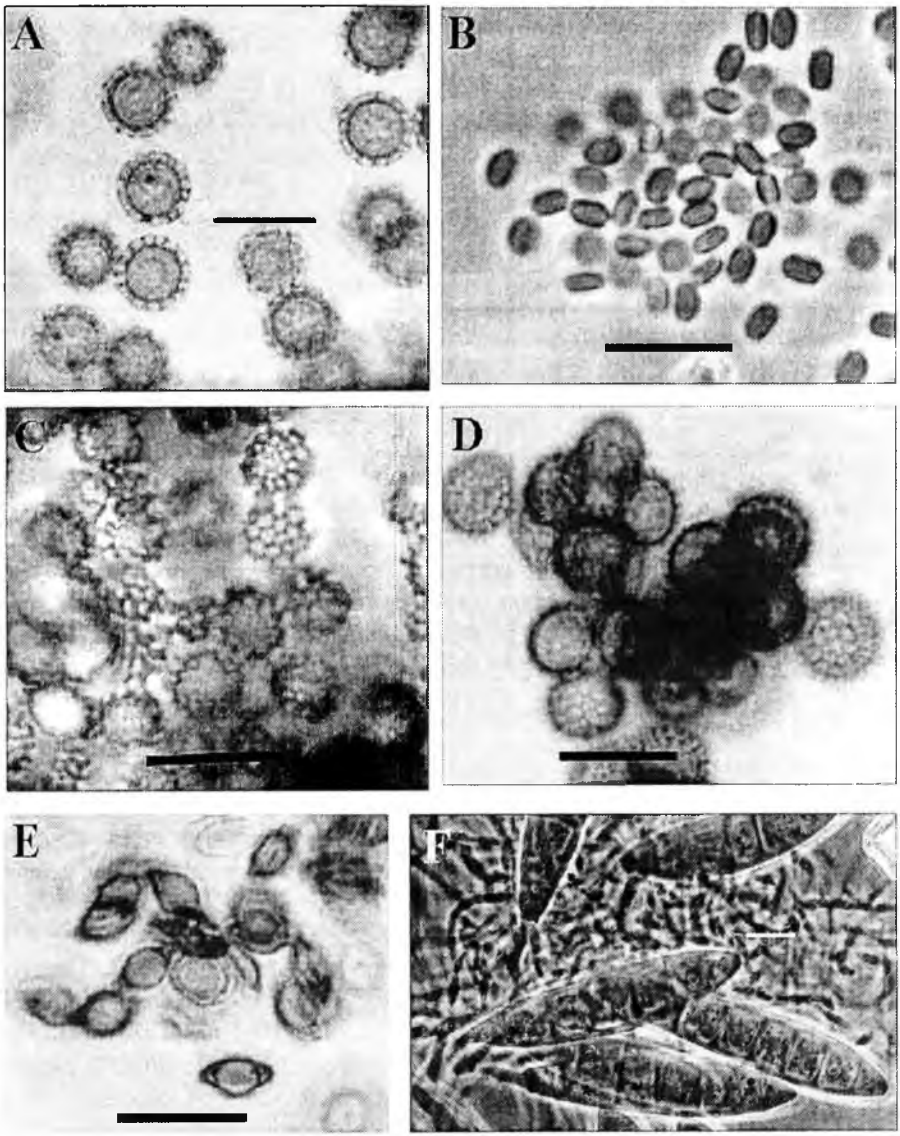



Fig. 2. Cluster analysis results for keratinolytic fungal species predominating in soils examined
Abbreviations
AFUL – *Aphanoascus fulvescens*, ARET – *Aphanoascus reticulisporus*, CHRYS – *Chrysosporium* anamorph of *Aphanoascus fulvescens/reticulisporus*, MUR – *Malbranchea* anamorph of *Uncinocarpus reessi*, AKER – *Aphanoascus keratinophilus*, CTROP – *Chrysosporium tropicum*, *OTHER SP.* – *other species*, ADUR – *Aphanoascus durus*, *CEUR* – Chrysosporium europae, MCO – *Microsporum cookei*, MVEL – *Myceliophthora vellerea*, AQ – *Arthroderma quadrifidum*, ARTR – *Arthroderma* teleomorph of *Microsporum gypseum*, ACUR – *Arthroderma curreyi*, TAJ – *Trichophyton ajelloi*




Ryc. 3. Some of the keratinolytic fungal species isolated from soils impacted by the municipal landfill site
A. Ascospores of *Amauroascus mutatus*. B. Ascospores of *Aphanoascus durus*. C. Atypical (irregular) ascospores of *Aphanoascus reticulisporus*. D. Typical (regular) ascospores of *Aphanoascus reticulisporus*. E. Ascospores of *Aphanoascus terreus*. F. Macroconidia of *Microsporum racemosum*. G. Bar = 10 μm

Table IV. Mean values of fungal indices and frequencies for the FC ranges

| Indices & fungal species | FC ranges | | | | |
|---|---|---|---|---|---|
| | < 100 | 100 –1000 | 1000– 10000 | 10000– 100000 | > 100000 |
| L index | 1.47 | 2.45 | 2.82 | 4.24 | 5.20 |
| FI (%) | 65.28 | 84.17 | 89.17 | 100.00 | 100.00 |
| NS | 4.44 | 6.25 | 6.33 | 8.50 | 8.00 |
| Frequencies: | % | | | | |
| *T. alleloi* | 0.36 | 0.85 | 0.78 | 0.85 | 0.00 |
| *A. curreyi* | 0.39 | 0.47 | 0.76 | 0.87 | 1.10 |
| *C. tropicum* | 0.23 | 0.39 | 0.54 | 0.54 | 0.00 |
| *Aphanoascus reticulisporus/fulvescens* | 0.94 | 1.26 | 0.61 | 1.29 | 0.00 |
| *A. keratinophilus* | 0.12 | 0.29 | 0.46 | 0.28 | 0.00 |
| *Malbranchea* an. *U. reessi* | 0.04 | 0.32 | 0.24 | 0.30 | 1.03 |
| *Arthroderma* sp. (an. *M. gypseum*) | 0.08 | 0.47 | 0.41 | 0.75 | 1.26 |
| *M. vellerea* | 0.27 | 0.58 | 0.71 | 0.44 | 1.31 |
| *C. europae* | 0.37 | 0.34 | 0.00 | 0.56 | 0.00 |
| *A. durus* | 1.00 | 0.31 | 0.44 | 0.36 | 0.00 |
| *A. quadrifidum* | 0.15 | 0.51 | 0.75 | 1.05 | 1.10 |
| *M. cookei* | 0.04 | 0.27 | 0.24 | 0.22 | 1.31 |
| Other species | 13.37 | 4.90 | 19.06 | 5.61 | 11.54 |

red more abundantly in 1994 than in 1993. In each year, the frequencies of predominating species as well as the values of fungal indices showed high seasonal variability.

Table I shows the descriptive statistics for physico-chemical and microbiological parameters. There were many statistically significant correlations found at $p < 0.05$ between the data for keratinolytic fungi and the above-mentioned parameters (Table II). However, the stepwise forward regression analysis showed that pH in 1 M KCl (exchangeable acidity), FC with FS and TNMF should be considered as the most important factors that influenced the indices of keratinolytic fungi in the soils examined.

In general, the mean L-index, FI and NS values increased with increasing pH in 1 M KCl (Table III). The highest L-index and FI means were calculated for the 7–8 pH range. The means decreased slightly over 8 pH. The NS mean was highest at pH over 8. However, the difference in the NS means between the 7–8 and > 8 pH ranges was small. The mean frequency of *T. ajelloi* was highest at pH below 5. The mean frequencies of this species decreased as pH increased. The mean frequencies of *M. cookei* and *A. quadrifidum* were also highest at pH below 5. However, these species also occurred with high frequencies at higher pH (6–8). *A. curreyi* favoured the 6–8 pH range. Subsequently, *A. durus* favoured the 5–6 pH range, although the species was also isolated with a high frequency from samples with pH between 7–8. *Arthroderma* sp. (an. *M. gypseum*) and the "other species" group occurred with the highest frequency within the 7–8 pH range. *C. europae* occurred with high frequency in the 6–7 and over

8 pH ranges. Five fungal species, namely *C. tropicum*, *A. reticulisporus/fulvescens*, *A. keratinophilus*, *Malbranchea* an. *U. reessi* and *M. vellerea* favoured pH over 8.

Table IV shows that, in general, the mean values of fungal indices (L index, FI and NS) distinctly increased as the faecal bacterial contamination of soil (FC) increased. The mean frequencies of predominating species also depended on the level of soil contamination with FC. However, the means were usually much lower than those calculated for pH data. Results were similar for FS ranges.

## DISCUSSION

Studies of municipal landfill sites [19] have concluded that the sites are the real sources of environmental contamination with microorganisms potentially pathogenic to plants and animals, including humans. The landfill sites emit numerous bacterial and fungal propagules, including those from actidione-resistant, mesophilic, thermophilic, thermotolerant and keratinolytic species. It is also believed that the landfill sites emit keratinous remnants of human and animal origin. Since microbial propagules chiefly spread from landfill sites *via* the air, and since, besides plant surfaces, the soil surface is the chief receiver of these contaminants, it is of hygienic and ecological interest to study the influence of the sites and environmental (soil and climatic) factors on the compositions of fungal keratinolytic populations in soils of landfill sites' surrounding areas. The present paper displays general data concerning the occurrence of keratinolytic fungi in the surrounding area of the landfill site in Sosnowiec.

In respect of pH requirements, *Hubálek* [7] (after *Garg et al.* [5]) divided the dermatophytes and related keratinolytic fungi into acidophilic, neutrophilic and alkalophilic. Our results have revealed some differences in relation to the above division. In *Hubálek's* division, *T. ajelloi*, *A. curreyi* and *C. tropicum* are acidophilic; *A. quadrifidum*, *C. serratus* (an. *M. vellerea*) and *C. keratinophilum* are alkalophilic; and *M. gypseum* is neutrophilic. In soils contaminated by the landfill site, the acidophilic nature of *T. ajelloi* is unquestionable. However, *A. curreyi,* with the highest frequencies at the 6–8 pH range, should be in fact considered as a neutrophilic species. *M. gypseum* (with its teleomorph *Arthroderma* sp.), with the highest frequencies also at pH between 6–8, can be maintained in *Hubálek's* neutrophilic group. Subsequently, *C. tropicum* is taxonomically and physiologically related to the alkalophilic species *C. keratinophilum* [14]. The ecological relation between these two species has been proved by the cluster analysis. From the data in this study and from river sediments in Catalonia [22], *C. tropicum* should be moved to the alkalophilic group. The study has confirmed the alkalophilic nature of *M. vellerea* and *C. keratinophilum* but, besides *C. tropicum*, the alkalophilic group should also include *A. reticulisporus/fulvescens* and *Malbranchea* an. of *U. reessi*. The alkalophilic nature of *A. quadrifidum* is doubtful, since the species occurred with the highest frequency in soils with pH < 5 and between 6–8. In this study, *A. quadrifidum* together with *C. europae*, *A. durus*, and *M. cookei* had wide ranges of soil pH.

This study has confirmed the importance of pH in the distribution of keratinolytic fungi in soil. However, the stepwise forward analysis has indicated that pH in 1 M KCl (exchangeable acidity) was a more important factor affecting these fungi in soil than pH in $H_2O$ (active acidity) and hydrolytic acidity (Kapen). It has been demonstrated

by *Garg et al.* [5] and confirmed in this study that keratinolytic fungal species occur in wide pH ranges in the soil environment. These wide ranges do not always fit the division of keratinolytic fungi based upon pH optima. This can be explained by the following hypothesis. The main (but not the only) substratum for the growth of keratinolytic fungi is keratinous remnants on the soil surface. However, the fungi are found in active and inactive forms in soil. Both forms are able to attack keratinous substrata but the distinction of inactive forms with the keratin baiting method is more difficult [12]. If keratinolytic fungi exist in a given soil mostly in inactive forms, the method may not reflect the real activity and pH preferences of these microorganisms but only detects the presence of fungal spores in the soil. The wide pH ranges that have been found for some species in the surrounding area of the landfill site in Sosnowiec may support the hypothesis.

There is an additional problem in the interpretation of the data. It appears that keratinous remnants (or baits) on the soil surface provide a highly specific environment in which fungal growth is influenced by both soil and remnant factors. Numerous studies have been published on keratinolytic fungi detectable by keratinous bait [5]. The studies have assumed that soil factors play a crucial role in the distribution of the fungi in soil and that results obtained with the keratin-baiting method fully reflect this role. However, there are little experimental data to determine how far the soil and remnant factors, including pH, influence keratinolytic fungi in the soil-keratinous substratum environment.

Besides pH and the presence of keratinous substrata, other factors affect the distribution of keratinolytic fungi in soil. Among them, more attention should be paid to the contamination of soils with faecal bacteria (FC, FS). The positive correlations between the indices for keratinolytic fungi and the quantities of faecal bacteria in soils are unquestionable. The relationship between the total population of keratinolytic fungi with the contamination of soils with faecal bacteria has been confirmed by the range analysis. Since keratinolytic fungi and faecal bacteria occur abundantly in municipal solid waste, and since both types of contaminants spread from landfill sites over their surrounding areas [18, 19], the association of the above-mentioned groups of microorganisms in soil can be explained. However, the stepwise forward regression, together with the range analysis has also indicated that, generally, pH in 1 M KCl affects keratinolytic fungi more than the faecal contamination of soils.

The data have also revealed the influence of climatic factors on the distribution of keratinolytic fungi in soil. Among these factors, the $W_W$ index requires special attention. Although no correlations between fungal indices (L-index, FI and NS) and the frequencies of predominating species with soil humidity were observed, the correlations between these frequencies and the $W_W$ index were clear. This means that the water deficit in the near-surface soil layer affected individual species more than it did the total fungal population. On the one hand, *A. reticulisporus/fulvescens* and *A. keratinophilus* were found to occur more abundantly in the periods with higher water deficit in soil. On the other hand, *Arthroderma* sp. (an. *M. gypseum*) with *A. quadrifidum* favoured the periods with lower water deficit. The relationships concerning the *Aphanoascus* species disagree with the observations that have been made in sewage sludges [21]. In the sludge environment, all *Aphanoascus* species were characteristic for the mud sludge

structure and high humidity (water excess). The explanation for this disagreement may be that mostly inactive *Aphanoascus* propagules of municipal waste origin occurred in dry sandy soils around the landfill site examined. Fungal keratinolytic activity was initiated by adding water to the soil samples supplemented with hair bait.

The statistical analysis has indicated that the occurrence of keratinolytic fungi in soils may depend on other environmental factors, particularly on the size distribution of soil particles (PSD). These dependencies must be proved in laboratory studies. Some workers [9, 17] have also suggested the importance of biological interactions within fungal populations and individual preferences of fungal species to different keratinous substrata in the distribution of keratinolytic fungi in soil. However, there is little evidence to support these suggestions for soil conditions. This problem also requires further attention.

Keratinolytic fungi occurred abundantly in the exploited area of the landfill site and its surroundings. The influence of environmental factors on the qualitative and quantitative composition of keratinolytic fungi in soils was complex. Since many habitats with different ecological conditions were found in the surrounding area of the landfill site examined, and since the keratin (hair) baiting method provides some interpretation problems, it is more difficult to explain our data. Each of the habitats mentioned showed unique conditions for the growth and survival of keratinolytic species originated from the landfill site. For example, the occurrence of keratinolytic fungi in the ash heap soil impacted by the site has been demonstrated previously [20]. The distribution of keratinolytic fungi in the habitats examined apparently requires a separate paper. In particular, the spatial distribution of the fungi requires special illustration methods. The thesis for using microorganisms, including keratinolytic fungi, as bioindicators of environmental contamination with municipal waste should be best shown with these methods.

According to *Ajello's* classification [1], all the dermatophytes and other keratinolytic fungi isolated in this study should be considered as geophilic. This means that these fungi display saprophytic activity in soil and are not specialised in pathogenic life. However, among the dermatophytes the pathogenic properties of *M. racemosum* have been clearly demonstrated [review in 20]. Also, *M. gypseum* and *M. cookei* have both been reported to cause infections in humans, cats, dogs and horses [review in 17]. Subsequently, among the non-dermatophytes *A. fulvescens* and *S. brevicaulis* have been reported as human and animal pathogens [review in 6]. Thus, our results have proven that municipal solid waste and landfill sites are the sources of potentially pathogenic fungi with keratinolytic properties. This finding cannot be ignored from the hygienic and epidemiological points of view.

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## GENERAL ASSESSMENT OF THE INFLUENCE OF A MUNICIPAL LANDFILL SITE AND ENVIRONMENTAL FACTORS ON THE OCCURRENCE OF KERATINOLYTIC FUNGI IN SOIL

Summary

The study was to generally determine the influence of a municipal landfill site and environmental factors on the distribution of keratinolytic fungi in soil. The landfill site in Sosnowiec was selected for examination. Keratinolytic fungi occurred abudantly in soils of the landfill site examined and its surrounding area. Of 495 soil samples (*Petri* dishes) examined, 379 (76.56%) were found to be positive for keratinolytic fungi. Altogether, 1131 strains from 26 species were isolated from the samples. Among the fungi, some species with pathogenic properties (*Microsporum racemosum*, *M. cookei*, *M. gypseum*, *Aphanoascus fulvescens* and *Scopulariopsis brevicaulis*) were recorded. The influence of environmental factors on the qualitative and quantitative composition of keratinolytic fungi in the soils was complex. Among these factors, exchangeable acidity (pH in 1 M KCl, in particular), faecal bacterial contamination and the level of water deficit in soil were the most important. The conclusion has been drawn that municipal landfill sites are the sources of potentially pathogenic fungi with keratinolytic properties.

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## OGÓLNA OCENA WPŁYWU WYSYPISKA ODPADÓW KOMUNALNYCH I CZYNNIKÓW ŚRODOWISKOWYCH NA WYSTĘPOWANIE GRZYBÓW KERATYNOLITYCZNYCH W GLEBIE

Streszczenie

Celem badań była ogólna ocena wpływu wysypiska odpadów komunalnych i czynników środowiskowych na występowanie grzybów keratynolitycznych w glebie. Do badań wybrano wysypisko odpadów komunalnych w Sosnowcu. Grzyby keratynolityczne obficie występowały w badanych glebach. Na całkowitą liczbę 495 próbek gleby (szalek *Petriego*) wzrost tych grzybów stwierdzono w 379 próbkach (76,56%). Łącznie wyizolowano 1131 szczepów grzybowych należących do 26 gatunków. Wśród wyizolowanych grzybów kilka gatunków (*Microsporum racemosum*, *M. cookei*, *M. gypseum*, *Aphanoascus fulvescens* and *Scopulariopsis brevicaulis*) posiada właściwości chorobotwórcze wobec ludzi i zwierząt. Wpływ czynników środowiskowych na skład jakościowy i ilościowy grzybów keratynolitycznych w glebie był złożony. Wśród tych czynników do najważniejszych należy zaliczyć: kwasowość wymienną (pH w 1 M KCl), zanieczyszczenie bakteriami kałowymi oraz deficyt wody w glebie. Wyciągnięto wniosek, że wysypiska odpadów komunalnych są źródłem skażenia środowiska grzybami chorobotwórczymi o właściwościach keratynolitycznych.

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