The occurrence of keratinolytic fungi in waste and waste-contaminated habitats

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Summary The data on the occurrence of keratinolytic fungi in sewage, municipal solid waste and waste-contaminated habitats are reviewed. In these habitats, keratinolytic fungi occur with extreme abundance. The factors influencing qualitative and quantitative compositions of the fungi in the habitats are discussed. It is concluded that keratinolytic fungi can be the bioindicators of environmental pollution with waste. The compositions indicate not only the presence of keratin remnants and faecal contaminants in the environment but also respond to the changes in environmental conditions. Fungal indices also indicate the infection risk associated with contamination of the environment with potential fungal pathogens. The needs for further studies are discussed.

Key words Keratinolytic fungi, Sewage, Sewage sludge, Municipal waste, Bioindication

Keratinolytic fungi occur in many natural and man-made habitats. These microorganisms exist in communities together with keratinophilic fungi that have weaker affinity to keratin and utilise chiefly the products of its decomposition [5]. The qualitative and quantitative compositions of the communities depend on a variety of physico-chemical and biological factors. Soil is the main environment of fungal occurrence and activity. In the soil environment, the factors influencing keratinolytic fungi have been relatively well recognised [4,9,14,18,43]. In contrast, little information is available on the occurrence of these fungi in waste and waste-contaminated habitats. Simordová & Hejtmanek [20], de Bertoldi [2], Mangiarotti & Caretta [13], Abdel-Hafez & El-Sharouny [1], Franková [8] and Kornittowicz [11] have studied sewage and freshwater habitats (marine and salty habitats are ecologically different and not included in the present paper). Indoor and outdoor dust within urban agglomerations has been surveyed for keratinolytic fungi and related species several times [7,16,17]. However, no data have been found on the occurrence of these fungi in municipal solid waste, including street sweepings (dust), and soil contaminated with municipal solid waste at urban areas, transfer stations and landfills.

There are two main reasons for the interest in examination of keratinolytic fungi in waste and waste-contaminated habitats. First, abundance of these microorganisms is expected in the habitats because of their richness in keratin remnants of human (mainly) and animal origin and in other substrata needed for fungal growth. Supposedly, keratinolytic fungi play an important role in the decomposition of the substrata in the habitats and could be used in biotechnological processes, for example in bioremediation of waste and waste-contaminated sites. Furthermore, because of different (sometimes-extreme) ecological conditions, the habitats are good objects for examination of relationships between microbial compositions with environmental factors. Second, keratinolytic fungi display potentially pathogenic properties to animals, including human beings. Studies of these fungi in the environment are, therefore, of hygienic and epidemiological importance. The importance increases in highly populated and industrialised areas, because of their high organic and inorganic contamination considerably impacting microbial communities, including those of keratinolytic fungi. An essential element of these studies is evaluation of the effects of waste management and industrial contaminants on the distribution of keratinolytic fungi in the areas.

The majority of the studies presented in this paper have been conducted in the Upper Silesia Region of Poland. Enormous congregation of heavy industry together with a dense human population implicating high sewage and municipal waste production along with high levels of chemical and microbiological contamination of the environment characterises the region. Because of these characteristics, in the last two decades extensive studies have been carried out to explain the factors determining the qualitative and quantitative compositions of keratinolytic fungi in waste and waste-contaminated habitats of the region. The studies have also determined whether keratinolytic fungi might be used as bioindicators of environmental contamination with waste. The present paper is to summarise results of the studies and other data available on the subject.

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Methods

The child or horse hair baiting method [42], with incubation for 4-6 months in the dark at room temperature and without using antibacterial and antifungal agents (actidione and other antibiotics) has been employed in most of the studies presented in this paper. In some studies, the actidione plating method has also been employed [38]. The test for keratinolytic activity of isolated fungal strains has been that of Ulfig *et al*. [38]. The following indices of fungal growth have been used: FI - number of Petri dishes positive for keratinolytic fungi divided by the total number of Petri dishes set up x 100%; NS - number of isolated species; NA - number of fungal strains; FIPS - 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.

The sampling way has evolved during the studies of keratinolytic fungi in waste and waste-contaminated habitats. The evolutionary trend has been that the mixed samples for hair baiting have contained more and more subsamples (up to 50) representing a given environment. Similarly, the number of Petri dishes set up has also increased (up to 100). These changes have allowed using the hair baiting method for quantitative purposes. The influence of the number of subsamples and Petri dishes on the data obtained with the method is to be statistically discussed in a separate paper.

Municipal solid waste and landfills

A preliminary study of keratinolytic fungi in municipal solid waste was conducted at the Chorzów municipal landfill [21]. It was found that mixed samples of fresh municipal waste (collected immediately after the waste was delivered to the landfill) were rich in keratinolytic fungi. Most of the strains isolated from the samples were *Trichophyton terrestre* with its teleomorph *Arthroderma quadrifidum*. The genus *Chrysosporium* was also represented abundantly in the fresh samples. The storage of the municipal waste at the landfill resulted in the considerable increase of keratinolytic fungi, specifically in the increase of *Trichophyton ajelloi*, *Chrysosporium keratinophilum*, *Aphanoascus reticulisporus* and *A. fulvescens*. Weak keratinolytic strains of *Geomyces pannorum* also occurred in the stored waste. Composting of the municipal waste eliminated all keratinolytic fungi from the fresh samples, probably because of the high temperatures obtained during the process (over 50°C).

Street sweepings are a component of municipal solid waste. However, an opportunity was taken to separately examine street sweepings from the city of Chorzów for keratinolytic fungi [31]. The sweepings were found to be rich in these fungi and the genera *Chrysosporium* and *Malbranchea* predominated among the isolates. Specifically, *Chrysosporium keratinophilum* and *Malbranchea flava* occurred in the sweeping samples with the highest frequency. In contrast to municipal solid waste, the so-called geophilic dermatophytes were poorly represented in the samples. Only some strains of *Trichophyton ajelloi* were recorded. The qualitative and quantitative composition of keratinolytic fungi in the sweepings was associated with pH, the content of heavy metals and particle size fractions.

A long-term study of two municipal landfills (in Sosnowiec and Poczesna near Czêstochowa, Poland) was carried out in 1994-96 [32]. The study was to determine the influence of the landfills on the microbiological quality of the environment. The results have confirmed that landfills are important sources of environmental contamination with potentially pathogenic microorganisms, including keratinolytic fungi. The areas of the landfills with their surroundings as well as the surroundings of waste transfer stations were found to be extremely rich in these microorganisms. The predominating species were *Trichophyton ajelloi, Aphanoascus reticulisporus, A. fulvescens, A. durus, Arthroderma quadrifidum, Chrysosporium* anamorph of *Arthroderma curreyi, Myceliophthora vellerea, C. keratinophilum* with its teleomorph *A. keratinophilus, C. europae, C. tropicum*, and *Microsporum gypseum* with its teleomorph *Arthroderma* sp. The mean frequencies of keratinolytic fungal species for municipal waste-contaminated soils are presented in table 1.

Table 1. The mean frequencies of keratinolytic fungi in habitats contaminated with municipal solid waste.

Fungal species	Frequency (%)
Aphanoascus reticulisporus/fulvescens	14.76
Aphanoascus durus	11.86
Trichophyton ajelloi	10.68
Arthroderma quadrifidum	10.57
Chrysosporium anamorph of *Arthroderma curreyi*	10.38
Myceliophthora vellerea	7.93
Aphanoascus keratinophilus	6.68
Chrysosporium tropicum	5.43
Chrysosporium europae	4.76
Arthroderma spp. (an. *Microsporum gypseum*)	4.20
Chrysosporium pannicola	2.80
Malbranchea an. *Uncinocarpus reesii*	2.60
Aphanoascus terreus	2.08
Microsporum cookei	1.85
Malbranchea flava	1.58
Other species	1.83

Keratinolytic fungi occurred abundantly in the superficial soil layer of landfills and their surroundings. The compositions of keratinolytic fungal communities in the soils differed from that observed for a highly populated and walked area and displayed distinct seasonal variations. The quantitative and qualitative indices of fungal occurrence increased with increasing contamination of the soils with faecal bacteria. The diversities of the communities increased to a certain level of the faecal contamination but decreased in badly contaminated soils. The indices correlated well with physico-chemical factors such as pH, humidity, organic carbon (Tiurin) and nitrogen (Kjeldahl), acidity, alkalinity, conductivity, P_2O_5, K_2O, SO_4, and others. However, the stepwise forward regression analysis showed that, among the factors, soil pH was the most important. The predominating species displayed many different relationships with physico-chemical and microbiological factors. Among the relationships, the positive correlations of *Chrysosporium keratinophilum* and *Malbranchea* anamorph of *Uncinocarpus reesii* with pH, as well as the negative correlations of *Trichophyton ajelloi* and *Arthroderma quadrifidum* with this factor are noteworthy. The positive correlation of *Chrysosporium* anamorph of *Arthroderma curreyi* with humidity, total nitrogen and P_2O_5 content also requires attention.

The habitat of the coal ash heap badly contaminated by the landfill in Sosnowiec is of special ecological interest [33]. High ignition losses and organic carbon content (derived from partly combusted coal), low concentrations of salts and pH close to neutral characterised the soil of this habitat. Except for Zn, concentrations of other heavy metals were low and the soil was non-toxic to watercress sprout. Under these conditions, lower and higher plants as well as microorganisms were poorly represented. In contrast, keratinolytic fungi occurred rather abundantly in this habitat, with *Aphanoascus durus* and *Chrysosporium europae* as the predominating species. Such composition of keratinolytic fungi has been found for the first time and testifies about the ecological exceptionalness of the habitat.

The air over the landfills was also contaminated with keratinolytic fungi. It was concluded that air transportation of fungal propagules is the main spreading way for these fungi in the vicinity of landfills. The landfills with their exploitation areas were the main sources of microbial contamination of the environment. However, there were many secondary sources of microbial contamination in their surroundings. The most important secondary sources were green filtration belts (forest and shrub areas) situated at the downwind sides on the predominating wind directions. It was found that within a distance of 200 m from the landfills the main reduction process of microbial contaminants in the air and soil took place. However, even at a distance of 500 m from the contamination sources microorganisms originating from the landfills were clearly recognised. Each landfill together with its surroundings created unique conditions for emission, transportation, growth and survival of microorganisms, including keratinolytic fungi.

The results allowed examination of the methods of spatial analysis of the occurrence of keratinolytic fungi and other groups of microorganisms in soil at the landfill in Sosnowiec [3]. It was found that geostatistical models based upon the kriging technique were the best for the description and visualisation of the spatial distribution of the microorganisms at the landfill. The example of such a distribution model for *Aphanoascus keratinophilus* (anamorph *Chrysosporium keratinophilum*) is shown in Figure 1. Since the landfill is remote from populated areas, the background frequencies of this species were zero or close to zero. The errors of estimation (semivariances) that have been calculated for each distribution model allow conclusions to be drawn concerning the completion and verification of the spatial data under examination. The models (generally, the Geographic Information System methods) have appeared to be useful tools in evaluation of the influence of landfills on the microbiological quality of the environment.

Sewage and sewage sludge

Keratinolytic fungi occur rather infrequently in sewage [22]. By contrast, these microorganisms inhabit sewage sludge with extreme abundance. Preliminary reports on the occurrence of keratinolytic fungi in Upper Silesian sludges [25, 26] have indicated that the qualitative and quantitative composition of these fungi in the sludge environment depends on treatment, physico-chemical and microbiological factors. In the above-quoted studies, however, little physico-chemical and microbiological data were available.

Recently, a more detailed study on keratinolytic fungi in Upper Silesian sludges [34] has demonstrated that *Trichophyton terrestre* with its teleomorph *Arthroderma quadrifidum*, *T. ajelloi* with *A. uncinatum*, *Microsporum gypseum* with *Arthroderma* sp., and *Chrysosporium keratinophilum* with *Aphanoascus keratinophilus* are the prevailing species in the sludge environment. The only difference between the previous studies and this study was that *Aphanoascus fulvescens* and *A. reticulisporus* were not isolated from the sludges in the last. However, some isolates of their anamorphs (*Chrysosporium* spp.) were recognised.

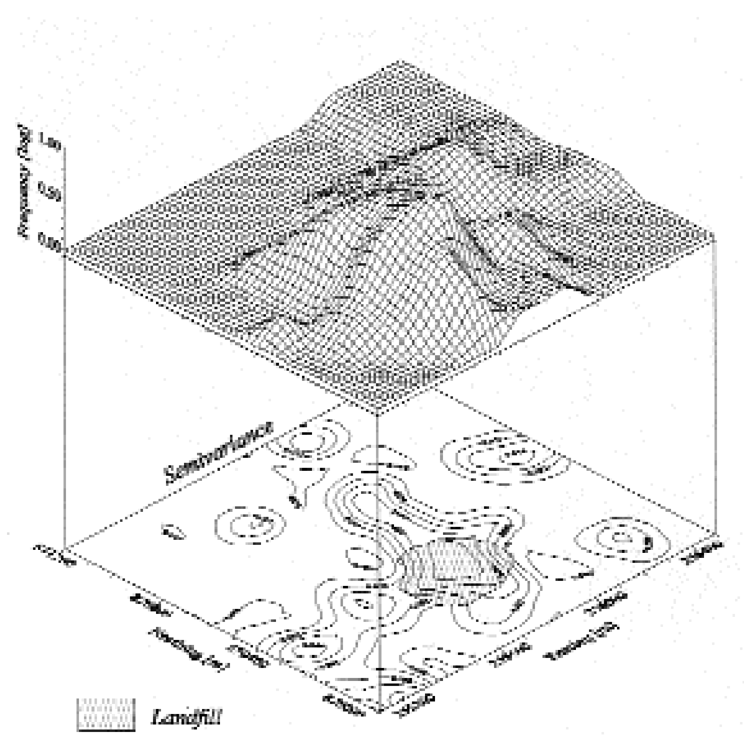

Figure 1. The distribution model of *Aphanoascus keratinophilus* (anamorph *Chrysosporium keratinophilum*) in the surroundings of a landfill site.

It was concluded that this greater understanding of keratinolytic fungi could be a useful tool in the evaluation of sludge treatment processes, including the sludge structure and conditions of humidity. For example, *Chrysosporium keratinophilum* with *Aphanoascus keratinophilus* was associated with the mud sludge structure, high humidity, volatile solids and nitrogen content as well as with the low C:N-ratio in the sludges. These characteristics testify about the low advancement of the sludge treatment processes. The other fungal species, especially geophilic dermatophytes occurred abundantly in the sludges at more advanced stages of the processes. For example, *Trichophyton terrestre* with *Arthroderma quadrifidum* were associated with the structural (soil) and best dewatered (well-aerated) sludges, with the relatively high C:N-ratio. The results have also confirmed that, besides the treatment, structure and humidity conditions, pH is another critical factor determining the distribution of keratinolytic fungi in the sludge environment.

The sludges from Upper Silesia are usually mixed with industrial sludge. This causes higher chemical contamination of the sludges, especially with heavy metals. It can be supposed that low pH along with high Fe, Cr and Ni concentrations eliminated keratinolytic fungi from one sludge sample examined. It was also revealed that *Chrysosporium keratinophilum* was resistant to the 560 ppm Cd concentration in another sludge sample. The influence of Cd on the growth of keratinolytic and non-keratinolytic fungi isolated from waste-contaminated sites has been studied by Plaza *et al*. [19].

Sewage sludge is frequently applied for reclamation or fertilisation of agriculture, forest and devastated areas. It is of ecological and hygienic interest, therefore, to examine the changes in keratinolytic fungal communities in sludge-reclaimed areas. A preliminary field experiment has been performed on this subject [28]. During a 19-month reclamation period, the decreasing incidence of *Aphanoascus fulvescens* and *A. reticulisporus* was observed, concurrent with the enrichment of the sludge-soil mixture with *Arthroderma quadrifidum* and *A. uncinatum*. *Chrysosporium keratinophilum* with weak keratinolytic strains of *Geomyces pannorum* considerably decreased at the beginning of the experiment: after that the partially treated and wet sludge was applied to the soil. The genus *Chrysosporium* and related fungi were completely removed from the mixture after the 17th month of reclamation. Thus, the long reclamation made the fungal quality and quantity in the mixture less diverse but closer to the composition in the control soil. It was concluded that the dewatering, mineralization and structuralization processes together with pH changes in the mixture could explain the changes in fungal composition. This agrees with composition of keratinolytic fungi found in sludge samples collected directly from sewage treatment plants.

Superficial waters and sediments

In superficial waters, keratinolytic fungi occur infrequently or even accidentally [20,22]. It appears that there are no clear relationships between the occurrence of these microorganisms and the degree of sewage contamination in the waters. By contrast, keratinolytic fungi occur abundantly in sediments of the waters. Some earlier studies have already allowed determining general relationships between the qualitative and quantitative composition of these fungi in sediments and the classes of sewage contamination in the waters [27]. The classes were those determined by the Polish standards and included both bacterial (faecal) and chemical contaminants.

Microsporum gypseum and other species from the genus as well as *Myceliophthora vellerea* mainly occurred in sediments of very badly polluted (classless) waters. The incidence of *Trichophyton ajelloi, Chrysosporium keratinophilum, Geomyces pannorum* and some other species increased with increasing water pollution. The incidence of *Aphanoascus fulvescens, A. reticulisporus* and *Chrysosporium pannicola* was the highest in sediments from 3rd class waters. It was also found that *Chrysosporium europae* was associated with slightly polluted waters. The incidence of *Aphanoascus terreus* (anamorph *Chrysosporium indicum*) and *Chrysosporium* anamorph of *Arthroderma curreyi* in sediments bore little relationship to levels of sewage contamination in the waters. Finally, the incidence of *Trichophyton terrestre* with its teleomorph *Arthroderma quadrifidum* were the highest in sediments from unpolluted and only slightly polluted waters (1st and 2nd classes).

The relationships have been confirmed in two natural lakes with different trophic levels outside the Upper Silesia Region [11]. It is of special ecological interest, however, to compare the Silesian results with those obtained for river mouths in Catalonia (Spain) [36,37]. The incidence of *Arthroderma quadrifidum* and *A. uncinatum* (with their anamorphs) were much higher in Silesian sediments while a higher incidence of *Aphanoascus fulvescens*, *A. reticulisporus*, *Chrysosporium* anamorph of *Arthroderma curreyi* and *C. tropicum* was observed in Catalonian sediments. Another difference between the sediments is that among the *Aphanoascus* species *A. reticulisporus* predominated in Silesian sediments whereas *A. fulvescens* prevailed in Catalonian sediments. In both studies, however, strains (teleomorphs) with intermediary characteristics were found. The conclusion is that the differences in fungal compositions resulted from the differences in climatic conditions (mainly temperature) between both countries.

The data from two Silesian dam reservoirs and Catalonia were statistically analysed [35,40]. Using the stepwise forward regression method, good relationships between fungal indices with the quantity of aqueous bacteria, alkalinity, NO_3, Mg, pH and dissolved oxygen concentration (DOC) were obtained for Silesian dam reservoirs. In the Catalonian study, the statistical relationships were weaker and the most important factors were the total number of microscopic fungi, temperature, cyanides, DOC, faecal coliforms, chemical oxygen demand, NH_4, dissolved substances, pH, salinity, phenols, and detergents. The differences could have resulted from different conditions under which the studies were carried out. In the Silesian short-term study, the results concerned dam reservoirs with water self-purification areas clearly observed. In the long-term Catalonian study, polluted river mouths with different ecological conditions and origins of water contamination (municipal, agricultural, and industrial) were investigated. It appears that further development of statistical methods is necessary for interpretation of the data.

The studies have demonstrated that both water contaminants and natural factors such as temperature, solar radiation and salinity influence the compositions of keratinolytic fungi in sediments. The diversities of keratinolytic fungal communities increased to a certain level of water contamination but decreased in badly contaminated waters. The results have displayed the impact of climatic conditions on these fungi and the essential role of pH in determining keratinolytic fungal communities in an aqueous environment. It was also observed that cyanides, detergents, and phenols associated with petroleum contamination considerably altered the communities in some Catalonian rivers (toxic effects).

It is also noteworthy that the keratinolytic mycoflora determined by the hair baiting method differed from that obtained by the actidione plating method [38]. It was explained chiefly by that the hair baiting method is generally more selective in relation to fungal strains with strong keratinolytic properties and environmental conditions influence the selectiveness of the methods. In both methods, the association of fungal species with water pollution factors were demonstrated in particular. In the hair baiting method, *Chrysosporium keratinophilum*, *Aphanoascus reticulisporus*, *A. fulvescens*, *Trichophyton ajelloi* and some other species correlated well with the factors. In the actidione plating method, the association of *Narasimhella marginospora* with badly polluted Catalonian waters is unquestionable.

Recent studies have concerned the occurrence of keratinolytic fungi in mountain sediments. In sediments from mountain streams in Brenna (Beskid Slaski Mountains), the influence of water contaminated by sewage on a keratinolytic fungal community was observed [41]. The changes in the diversity of the community depended on the organic matter content, the concentrations of salts and the particle size distribution. The impact of local climatic conditions on the composition of keratinolytic fungi in the sediments was also demonstrated. Apart from the mesophilic *Microsporum gypseum*, a rare psychrophilic dermatophyte, *Keratinophyton ceretanicus*, occurred abundantly in mountain sediments. The fungus specializes in the decomposition of keratin remnants under severe mountain conditions. In contrast to the polluted mountain sediments from Brenna, no keratinolytic fungi were isolated from sediments of unpolluted oligotrophic lakes in the Tatry Mountains.

In a recent study, different incubation temperatures (room temperature 20±2°C, 33 and 37°C) for badly polluted sediment samples supplemented with hair were used [39]. At these temperatures, three different qualitative and quantitative compositions of keratinolytic fungi were obtained. At room temperature, geophilic dermatophytes, i.e., *Trichophyton ajelloi*, *T. terrestre* and *Microsporum gypseum* (with their teleomorphs) prevailed in the samples. At higher temperatures, the dermatophytes were observed sporadically and *Chrysosporium keratinophilum* together with *Aphanoascus reticulisporus* predominated on hair bait.

A separate problem is an explanation of the ecological role of keratinolytic fungi in superficial waters. Based on the available data, especially on those obtained during *in situ* and *ex situ* experiments with hair-fulfilled metal teaballs [30], it can be presumed that aqueous proteolytic bacteria play a crucial role in the decomposition of keratin debris in the waters. Aquatic fungi and, to a lesser extent, soil fungi with keratinolytic properties grow on hair in the superficial layer of sediments and in the overlying water under good aeration and nutritional conditions. Hence, the abundance of keratinolytic soil fungi on hair bait can result not only from the assemblance of fungal propagules in sediments but also from the certain behaviour of these fungi in the aqueous environment (in shallow near-shore habitats in particular).

Keratinolytic fungi as bioindicators of environmental waste pollution

In ecological and sanitary studies, a number of bioindicators of environmental pollution are employed. For example, bacteriological indicators such as faecal coliforms and streptococci are commonly used in sanitary practice. These indicators display the actual level of environmental contamination with human and/or animal faeces and allow evaluating the risk of infectious diseases resulting from the contamination [12]. It appears that keratinolytic fungi could also be indicative microorganisms. However, their indicative values rely on different relations.

The highest physiological and ecological specialisation characterises keratinolytic fungi. These microorganisms exist in the environment in anthropogenic and zoogenic communities "separated" on keratin remnants from other microbial populations. The qualitative and quantitative compositions of the communities reflect the complex influence of many environmental factors. The most important factors are as follows:

- delivery and congregation of keratin remnants and fungal propagules together with the decomposition of the remnants in a given environment;
- level of environmental pollution with human and/or animal faeces;
- physico-chemical factors, including climatic conditions;
- presence of toxic compounds and elements of industrial and natural origin in the environment.

Keratin is considered as the main natural substratum for keratinolytic fungi [7] but no study measuring the quantity of keratin reached a given environment has been found in the available literature. Since analytic separation of keratin from other soil compounds seems to be difficult, this lack of information is understandable. However, some studies have compared populated and unpopulated areas, as well as polluted and unpolluted environments [5,15,27,29,32]. Based on their results, it can be hypothesised with high probability that the delivery of keratin remnants together with fungal propagules is the most critical factor implicating the abundance of keratinolytic fungi in the environment. Human beings and animals, organic fertilisers, wind, waters and waste deliver the remnants and propagules to the environment. To prove the above hypothesis, the adequate method of analysis for the quantification of keratin in soil and other media has to be designed. This is a challenge for soil chemists.

Keratinolytic fungi are associated with human and/or animal activities. In particular, these microorganisms commonly occur within highly populated areas to which keratin remnants are continuously delivered. In unpopulated areas, the abundance of keratinolytic fungi is restricted mostly to fields treated with organic fertilisers (e.g., manure) and to the places constantly or temporarily penetrated by animals [9,24]. However, in sewage and municipal waste extremely high amounts of keratin remnants and other compounds needed for fungal growth are expected. In fact, large keratin particles (hairs and nails) were clearly observed on sieve nets during the sieving of sludge samples. The large particles are only a part of the total keratin amount present in the samples. Smaller keratin particles (for instance, shaved hair and epidermal elements) are difficult to separate and observe. Because of the high keratin amounts and good nutritional conditions, the extreme abundance of keratinolytic fungi characterises sewage sludge, municipal waste and waste-contaminated environments. In these environments, keratin remnants always occur together with faecal bacteria and fungal propagules. Presumably, the relationship between the abundance of keratinolytic fungi and faecal bacteria of human or/and animal origin is characteristic for most or even all keratin-rich environments. The quantity of keratin remnants along with their slow decomposition fixes the fungal composition for a long time. It can be assumed that bacterial indicators (except for sporing bacteria) reflect the short-term or even transitory contamination of the environment while the fungal composition displays the long-term effects of the contamination.

Physico-chemical factors (including climatic conditions) exert a considerable impact on the compositions of keratinolytic fungal communities in keratin-rich environments. Among the factors, pH, temperature, organic carbon and nitrogen content, C:N-ratio, salts, humidity, faecal bacteria and oxygen availability appear to be the most important. Except for oxygen availability, the influence of the other factors on many keratinolytic fungal species has been relatively well-recognised [9]. However, some waste-contaminated environments also contain toxic compounds and/or elements of industrial and/or natural origin such as detergents, cyanides, phenols and other BTEXs, PAHs, petroleum hydrocarbons, heavy metals, salts, etc. These contaminants can markedly impact keratinolytic fungi, in extreme cases causing their total or near total elimination. Some fungal species, i.e., *Chrysosporium keratinophilum* and *Trichophyton ajelloi*, display high resistance to the toxic factors under field conditions. This finding requires confirmation in laboratory studies.

The impact of temperature on the occurrence of keratinolytic fungi in the environment (as an element of climatic conditions) and on their growth on some artificial media has been demonstrated [6,14,40]. However, incubation temperature of hair-supplemented samples is also an important factor determining the results obtained with the hair-baiting method. The data obtained by Ulfig & Plaza [39] indicate that at room temperature (20±2°C) the fungi with the lowest temperature optimum remove or mask mesophilic species on hair bait. The last species cover the hair bait at higher temperatures. Thus, the differences in keratinolytic mycofloras between countries with different climatic conditions can result, to a high degree, from different temperatures used during the so-called room incubation. This finding indicates the absolute need to use several temperatures to fully characterise communities of keratinolytic fungi.

In general, the qualitative and quantitative composition of keratinolytic fungi can be a multifunctional bioindicator of environmental pollution with waste. It means that the composition indicates not only the presence of keratin remnants and faecal contaminants in the environment but also respond to the changes in environmental conditions. Additionally, the fungal growth indices inform us about the infection risk associated with the contamination of the environment with potential fungal pathogens. Each indicator of environmental pollution should have certain universal characteristics to be used under different ecological, including climatic conditions. The composition of keratinolytic fungi shows at least two such characteristics, i.e., the restricted number and similar for different habitats set of predominating species and the possibility of use of fungal growth indices under any conditions. The results have confirmed the usefulness of the composition as a bioindicator of environmental waste pollution in long-term and monitoring studies. The analytic procedures are too long to be used in expedited site characterization. However, implementation of the composition to the ecological and sanitary practice implies the need for further studies to thoroughly examine the most important factors influencing the occurrence of these fungi in the environment. These studies should be complex and one of their essential elements is elaboration of the proper statistical method for data interpretation. Another problem is further modification of the hair-baiting method for quantitative purposes.

A separate problem is determination of the exposition of human beings and animals to epidermal mycoses in the areas rich in keratinolytic fungi. It is assumed that active fungal elements (inoculum) lodging in a skin abrasion can cause infection [44]. Hence, the richer community of fungal potential pathogens implies the higher probability of fungal infection in a given area. Most of the keratinolytic fungal species isolated from sewage, municipal waste and waste-contaminated environments are saprophytic, rarely causing epidermal mycoses in animals, including human beings. Among the saprophytic species, however, *Microsporum gypseum*, *Aphanoascus fulvescens* and *Chrysosporium keratinophilum* and some others are frequently recorded from the contaminated environments and encountered more often than the other species in medical laboratories [10]. In addition, three obligatorily pathogenic fungi, i.e., *Microsporum canis*, *M. persicolor* and *Trichophyton mentagrophytes* have also been isolated from polluted sediments. It has also been proved that pathogenic dermatophytes are able to survive in aqueous and sludge environments for a long time [23]. Finally, a rare saprophytic but pathogenic dermatophyte, *Microsporum racemosum*, was found to occur in the coal ash heap soil, markedly influenced by the landfill in Sosnowiec [33]. Thus, the risk of fungal infection increases in the environments contaminated with sewage sludge and municipal waste. Epidemiologists, hygienists and waste managers can not ignore these findings.

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