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Effect of Sewage Sludge Alkalization and Acidification on Keratinolytic and Keratinophilic Fungi

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Abstract

This study aimed to determine the influence of sewage sludge alkalization and acidification on the qualitative and quantitative composition of keratinolytic and keratinophilic fungi in a model experiment. The sludge was alkalized to pH 9 with 0.5N NaOH, 0.5N KOH, 0.5N NH_4OH or with 2% burnt lime mixed with powdered limestone (20g CaO + 5g $CaCO_3$), and acidified to pH 4 with 0.5N HCl, 0.5N HNO_3 or 0.5N H_2SO_4. The sludge with unmodified pH (6.5) served as control. The hair baiting method with four incubation temperatures (23, 29, 33, and 37°C) was used to examine fungi. The sludge alkalization with KOH, NaOH or CaO+$CaCO_3$ increased the number of keratinolytic fungi but decreased the number of keratinophilic fungi. The inhibition of the growth of keratinolytic fungi was observed due to the sludge acidification with H_2SO_4 or HCl. The sludge acidification with HNO_3 or HCl stimulated keratinophilic fungi to grow. Sludge acidification with HNO_3 eliminated keratinolytic fungi, while acidification with H_2SO_4 eliminated keratinophilic fungi. Results are discussed from ecological and epidemiological points of view.

Keywords: keratinolytic and keratinophilic fungi, sewage sludge, alkalization, acidification

Introduction

Sewage sludge contains high quantities of keratinous substrata (mainly hair) of human and animal origin. According to the Majchrowicz and Dominik definition [1], keratinolytic fungi are specialized in decomposition of keratin, being the main component of these substrata. Keratinophilic fungi accompany keratinolytic fungi, utilizing non-protein components of the substrata or the products of keratin decomposition. Favorable environmental conditions imply the abundant growth of keratinolytic and keratinophilic fungi on keratinous substrata in sewage sludge. This allowed studying different relationships between the fungi and environmental factors. Theoretically, all keratinolytic fungi possess potentially pathogenic properties to humans and animals [2]. Indeed, many mycoses with keratinolytic and also keratinophilic fungi as causative agents have been reported [3]. Therefore, studies of the factors influencing the fungi in sewage sludge and sludge-amended soils are also of epidemiological significance.

Keratinolytic and keratinophilic fungi inhabit sewage sludge in extreme abundance [3, 4]. Recent studies [5] have demonstrated that many environmental factors such as pH, temperature, ammonium nitrogen, proteolytic activity, organic carbon and total nitrogen, C: N ratio, total sulfur, C:S ratio, available phosphorus and particle size distribution, affected keratinolytic and keratinophilic fungi in sewage sludge. Among the factors, pH was found to be one of the most important affecting fungal populations. However, statistical relationships found in the quoted studies have to be confirmed under model conditions. Therefore, this study aimed to determine the influence of sewage sludge alkalization and acidification on

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the composition of keratinolytic and keratinophilic fungi in a model experiment.

Material and Methods

Sewage sludge from the Bytom-Miechowice wastewater treatment plant was used for this experiment. It was the excess sludge, after extended aeration (without primary settling tank) and the integrated biological C and N removal process in the Biomix system, dewatered by centrifuging, and piled with plant residues for 1-2 years. The sludge was sampled using a metal spade disinfected with 60% ethyl alcohol. About 200 kg of sludge was collected in plastic barrels disinfected in the same way. The sludge was taken from a pile at different depths (from the surface to the bottom of the pile), cleaned from stones and larger particles, crumbled, and delivered to the laboratory within 2-5 hours. The sludge was then being dried in open air for 4-7 days. The sludge was continuously crumbled and mixed during drying. The dried and mixed sludge was sieved through a 3 mm diameter net and thoroughly mixed again. This sludge was used in model experiments. Physico-chemical characteristics of the sludge were presented elsewhere [5].

The sludge pH in H_2O was 6.5. This pH was modified to 4 and 9, using inorganic acid and alkali solutions. Prior to the final experiment, several preliminary experiments were performed to determine acid and alkali concentrations that did not destroy keratinolytic mycoflora and did not exceed final sludge moisture of 50%. Sludge buffering curves were also determined. Finally, the sludge was alkalized with 0.5N NaOH, 0.5N KOH, 0.5N NH_4OH or with 2% burnt lime mixed with powdered limestone (20g CaO + 5g $CaCO_3$). Subsequently, the sludge was acidified with 0.5N solutions of HCl, HNO_3 or H_2SO_4. Both the sludge acidification and alkalization were performed carefully as follows: acid and alkali solutions were being added to the sludge with small portions and continuous mixing. The entire procedure lasted for 3-4 hours, with continuous pH control. Sludge pH was measured with an electrometric method. The sludge with unmodified pH was used as control.

Keratinolytic and keratinophilic fungi were examined in the sludge using a modified hair baiting method [6]. Petri dishes were filled with 40 g of sludge and covered with 0.4 g of detergent-defatted, fine cut, and autoclaved children hair each. The dishes were incubated in the dark at 23, 29, 33 and 37°C for four months. Ten dishes responded to each temperature and combination of the experiment. During incubation, stable moisture conditions (ca. 50%) were maintained by adding sterilized redistilled water to the dishes. After 1, 2, 3 and 4 months of incubation, microscopic observations of hair and inoculations of hair attacked by fungi on Sabouraud 1:10 + mineral salts (TK medium) [7], supplemented with chloramphenicol (100 mg/l) and actidione (500 mg/l) were performed. The inoculated TK dishes were incubated at 23 and 37°C for 10 days. The rule was accepted that the growth of a given species on hair, confirmed by its growth on TK medium with antibiotics meant the appearance of the species in a given Petri dish. The fungal growth indices were as follows: number of appearances; isolation frequency (number of Petri dishes positive for fungal growth*100/total number of Petri dishes set up); number of species; and WW index – appearance number increase index.

The WW index reflects the increase of fungal appearance number on hair during a 4-month incubation period. This increase was described with the power function $Y = X^{ww}$, in which X is the incubation time (0, 1, 2, 3 and 4 months)/maximal incubation time (4 months) ratio; Y is the number of appearances after a given incubation month/number of appearances after 4 months ratio (SP_m/SP_4); and WW is the appearance number increase index for a 4-month incubation period. The model is based on the assumption that fungi observed on hair once are present there to the end of a 4-month incubation period. With some exceptions, for instance, in the case of fungal death on hair due to the impact of toxic factors, observations have confirmed the assumption. The WW index values range between 0.02 and 40. The faster increase of fungal appearance number, the lower WW index. High WW index values may testify to inhibition of fungal growth on hair. The WW index can be used in relation to keratinolytic fungi, whose growth on hair is characterized by quantitative succession. Keratinophilic fungi were present on hair from the beginning to the end of the experiment and could not be described with a simple power function.

Pure fungal strains were identified to the species level based on macro- and micromorphological characteristics and using selected taxonomic monographs [8-12]. The *in vitro* hair degradation test was that of Ulfig *et al*. [13].

After the experiment, sludge pH was measured in three Petri dishes for each combination and incubation temperature. Before the pH measurement, sludge from each Petri dish was thoroughly mixed in a plastic container.

Results

Results on the influence of sewage sludge alkalization and acidification on keratinolytic and keratinophilic fungi are presented in Tables 1 and 2, respectively.

Altogether, 464 keratinolytic fungi appearances belonging to at least 12 species were observed in the sludge (Table 1). The *Chrysosporium* anamorph of *Aphanoascus clathratus* (22.2%) with its teleomorph *Aphanoascus clathratus* (2.8%), *Microsporum gypseum* (20.3%), *Chrysosporium keratinophilum* (12.9%) with its teleomorph *Aphanoascus keratinophilus* (1.9%), and *Trichophyton terrestre* (10.1%) with its teleomorph *Arthroderma quadrifidum* (8.2%) prevailed in the sludge. Other keratinolytic species occurred in the sludge with frequencies <10%.

Table 1. The influence of sludge alkalization (to pH 9) and acidification (to pH 4) on keratinolytic fungi.

Fungal species and growth indices	Acidified sludge			Control	Alkalized sludge			
	HNO_3	H_2SO_4	HCl		KOH	NH_4OH	$CaO+CaCO_3$	NaOH
Chrysosporium anamorph of *Aphanoascus clathratus* Cano & Guarro	-	5	11	17	15	18	24	13
Teleomorph *Aphanoascus clathratus* Cano & Guarro	-	-	-	-	-	7	6	-
Microsporum gypseum (Bodin) Guiart & Grigorakis	-	11	7	18	18	10	16	14
Chrysosporium keratinophilum D.Frey ex Carmichael	-	-	2	6	5	19	8	20
Tel. *Aphanoascus keratinophilus* Punsola & Cano	-	-	-	2	-	1	1	5
Trichophyton terrestre Durie & Frey	-	1	-	8	13	4	10	11
Tel. *Arthroderma quadrifidum* Dawson & Gentles	-	1	-	7	7	4	9	10
Chrysosporium an. *Aphanoascus reticulisporus/ fulvescens*	-	-	-	3	-	15	13	1
Tel. *Aphanoascus reticulisporus* (Routien) Hubálek	-	-	-	-	-	-	1	1
Trichophyton ajelloi (Vanbreuseghem) Ajello	-	1	-	1	11	-	5	5
Chrysosporium zonatum Al-Musallam & Tan	-	-	3	5	-	4	1	-
Arthrographis kalrai (Tewari & Macpher.) Sigler & Carmichael	-	-	-	-	8	-	1	4
Amauroascus mutatus (Quelet) Rammeloo	-	-	-	2	-	-	-	9
Gymnoascus petalosporus (Orr *et al*.) v. Arx	-	-	-	-	1	-	-	2
Chrysosporium indicum (Randhawa & Sandhu) Garg	-	1	1	-	-	-	-	-
Chrysosporium europae Sigler, Guarro & Punsola	-	-	1	-	-	-	-	-
Number of appearances	-	20	25	69	78	82	95	95
Frequency of isolation (%)	-	45	56	85	100	81	100	100
Number of species	-	5	6	8	7	6	8	9
WW index	-	0.80	2.93	0.95	0.40	1.69	0.58	0.75

Altogether, 273 keratinophilic fungi appearances belonging to 16 species were observed in the sludge (Table 2). *Verticillium lecani* (51.6%), *Paecilomyces lilacinus* (16.1%), and *Penicillium janthinellum* (10.3%) prevailed in the sludge. Other keratinophilic species occurred in the sludge with frequencies <10%.

In relation to the control sludge, the sludge alkalization with 0.5N KOH, 0.5N NaOH or 2% $CaO+CaCO_3$ increased the number of keratinolytic fungi appearances and their isolation frequency, and decreased the WW index. Subsequently, the sludge alkalization with 0.5N NH_4OH increased the number of keratinolytic fungi appearances and their WW index but decreased the isolation frequency. The best growth of keratinolytic fungi was observed in the ammonia water-alkalized sludge. The sludge alkalization considerably restricted the growth of keratinophilic fungi as indicated by lower values of growth indices.

In relation to the control sludge, the sludge acidification with 0.5N H_2SO_4 or HCl caused considerable inhibition of the keratinolytic fungi growth. The decrease of fungal growth indices, i.e. the number of appearances, isolation frequency, and the number of species indicated this. The WW index value of the H_2SO_4-acidified sludge was close to that of the control sludge. However, a considerable retardation of the appearance number increase was observed during the HCl-acidified sludge incubation (WW index = 2.93). The sludge acidification with 0.5N H_2NO_3 eliminated keratinolytic fungi. Subsequently, in relation to the control the sludge acidification with 0.5N HCl or HNO_3 caused better growth of keratinophilic fungi. The increase of the number of appearances and the isolation frequency expressed this. However, the number of keratinophilic species was lower in acidified sludges than in the control sludge. The sludge acidification with 0.5N H_2SO_4 eliminated keratinophilic fungi.

The inhibition of the keratinolytic fungi growth due to sludge acidification was observed at all temperatures. Subsequently, the stimulation of fungal growth due to the sludge alkalization was best observed at 23 and 29°C.

The composition of keratinolytic and keratinophilic fungi changed due to the sludge alkalization and acidifi-

Table 2. The influence of sludge alkalization (to pH 9) and acidification (to pH 4) on keratinophilic fungi.

Fungal species and growth indices	Acidified sludge			Control	Alkalized sludge			
	HNO_3	H_2SO_4	HCl		KOH	NH_4OH	$CaO+CaCO_3$	NaOH
Verticillium lecani (Zimm.) Viegas	28	-	22	14	19	18	20	20
Paecilomyces lilacinus (Thom) Samson	13	-	14	8	5	2	-	2
Penicillium janthinellum Biourge	10	-	15	3	-	-	-	-
Fusarium oxysporum Schlecht.	1	-	-	2	7	1	3	1
Pseudallescheria boydii (Shear) McGinnis *et al.*	-	-	1	9	4	-	-	-
Aspergillus fumigatus Fresenius	-	-	5	3	-	-	-	-
Paecilomyces marquandii (Massee) Hughes	2	-	2	1	-	-	-	-
Fusarium solani (Mart.) Saccardo	-	-	3	2	-	-	-	-
Penicillium islandicum Sopp	4	-	-	-	1	-	-	-
Narasimhella marginospora (Kuehn & Orr) v. Arx	-	-	-	-	-	-	-	2
Plectosphaerella cucumerina (Lindf.) W. Gams	-	-	-	1	-	-	-	-
Phialophora melinii (Nannf.) Conant	-	-	-	1	-	-	-	-
Aspergillus versicolor (Vuill.) Tiraboschi	-	-	-	1	-	-	-	-
Aspergillus alutaceus Berk. & Curt.	-	-	1	-	-	-	-	-
Absidia corymbifera (Cohn) Sacc. & Trotter	-	-	1	-	-	-	-	-
Penicillium expansum Link ex Gray	1	-	-	-	-	-	-	-
Number of appearances	59	0	64	45	36	21	23	25
Frequency of isolation (%)	100	0	93	71	63	50	50	53
Number of species	7	0	9	11	5	3	2	4

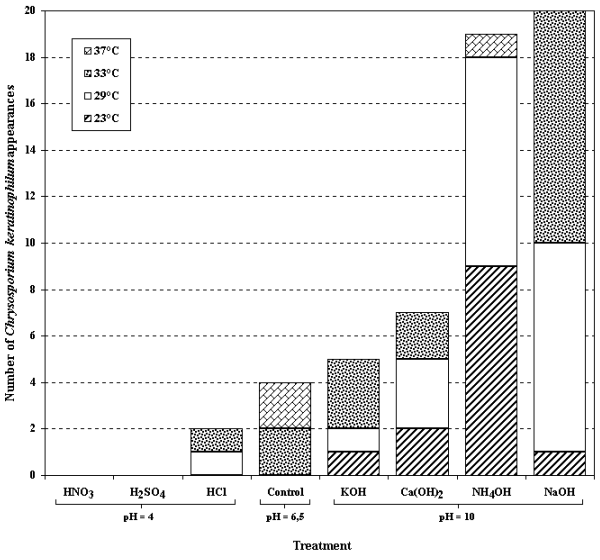

Fig. 1. The number of *Chrysosporium keratinophilum* (teleomorph *Aphanoascus keratinophilus*) appearances in acidified (to pH 4) and alkalized (to pH 9) sludges and in the control sludge at four incubation temperatures.

cation. The most important changes concerned *Chrysosporium keratinophilum* (teleomorph *Aphanoascus keratinophilus*) and the *Chrysosporium* anamorph of *Aphanoascus reticulisporus/fulvescens* (teleomorph *Aphanoascus reticulisporus*) at four incubation temperatures. The number of *Chrysosporium keratinophilum* appearances at four temperatures is illustrated in Figure 1. *Chrysosporium keratinophilum* occurred in the NaOH-alkalized sludge, with a considerable number of appearances, at 29 and 33°C, whereas the fungus prevailed in the ammonia water-alkalized sludge at 23 and 29°C. In the lime-alkalized sludge and the ammonia water-alkalized sludge, considerable numbers of the *Chrysosporium* anamorph of *Aphanoascus reticulisporus/fulvescens* appearances were observed at 23/33°C and 23/29°C, respectively. Subsequently, keratinophilic *Paecilomyces lilacinus* and *Penicillium janthinellum* displayed better growth in acidified sludges.

During a 4-month incubation, the alkalized-sludge pH decreased to 5.3-6.2 and the acidified-sludge pH increased to 5.3-5.8. The control sludge underwent spontaneous acidification, from 6.5 at the beginning to 5.2 at the end of the experiment.

Discussion

Geophilic dermatophytes and most other keratinolytic fungi prefer neutral and alkaline environments [14-18]. In a survey of these fungi in sewage sludge, Ulfig [5] used statistical methods for data interpretation and confirmed these preferences. In comparison with acidic sludges and pH 4-acidified sludges, much higher quantities of fungal appearances in alkaline sludges and pH 9-alkalized sludges are, therefore, understandable and agree with the quoted literature data. It is also known that keratinolytic fungi tolerate wide pH ranges. The experiment also confirmed this phenomenon. However, many keratinophilic fungi prefer acidic pH. For instance, proteolytic enzymes of *Penicillium janthinellum* show the highest activity at acidic pH [19]. The optimal pH for *Aspergillus fumigatus* ranges between 5.9-6.8 [20, 21], although the fungus also produces alkaline proteases [22]. Thus, the good growth of many keratinophilic fungi in acidified sludges also agrees with literature data.

Studies of keratinolytic fungi in soils with high carbonate content were carried out by Chmel *et al.* [23] and Chmel and Vláčilíková [24]. In recent studies, Korniłłowicz [25] and Korniłłowicz-Kowalska and Bohacz [26] demonstrated that carbonate-rich soils favored the incidence of *Chrysosporium tuberculatum* and some other species from the genus *Chrysosporium*. In contrast, the growth of geophilic dermatophytes, mainly *Trichophyton ajelloi*, was found to be weak in these soils. In the present study, the sludge alkalization with $CaO+CaCO_3$, and also with KOH, NaOH or NH_4OH, considerably increased the number of keratinolytic fungi appearances. In all alkalized sludges, many similarities in fungal qualitative and quantitative compositions were observed. However, considerable differences also were found. *Chrysosporium keratinophilum* prevailed in NH_4OH- and NaOH-alkalized sludges, while the *Chrysosporium* anamorph of *Aphanoascus reticulisporus/fulvescens* occurred with high frequencies in $CaO+CaCO_3$- or NH_4OH-alkalized sludges.

Ammonium was added to the sludge directly with ammonia water or was released, with characteristic odor, during sludge alkalization with the other alkali solutions. Morel and Guckert [27] also observed ammonium release during sludge liming. During the already quoted study, Ulfig [5] found a positive correlation between *Chrysosporium keratinophilum* and ammonium concentration in different sludges. Finally, ammonium toxicity to many organisms, including fungal plant pathogens, was demonstrated [28-30]. On the basis of the experiment and literature data, it can be suggested that pH associated with ammonium action causes essential changes in fungal population in sewage sludge. This important suggestion has to be confirmed in further model experimentation.

The sludge acidification with sulfuric acid eliminated keratinophilic fungi, while the sludge acidification with nitric acid eliminated keratinolytic fungi. Lilly and Barnett [31] already mentioned the antifungal properties of sulfuric acid at pH 4. Subsequently, Kobuz [32] and Bewley and Stotzky [33] demonstrated the inhibition or elimination of many soil microorganisms, including fungi, by sulfur and simulated acid rains. In newer studies, toxic, carcinogenic and teratogenic properties of sulfuric acid were reported [28]. It has also been found that keratin decomposition by keratinolytic fungi leads to the accumulation of sulfates in the medium [34] and that sulfates are the best inorganic sulfur source for these fungi [35]. It can be suggested, therefore, that the survival of keratinolytic fungi in the sulfuric acid-acidified sludge resulted from their resistance to sulfates higher than that of keratinophilic fungi.

Toxic, carcinogenic and teratogenic properties of nitric acid were also demonstrated [28]. No data on the toxicity of nitric acid to keratinolytic and keratinophilic fungi have been found in available literature. However, it has been found that, in contrast to keratinophilic fungi, keratinolytic fungi do not assimilate, or badly assimilate, nitrates [11, 36]. It can be suggested, therefore, that the elimination of keratinolytic fungi from the nitric acid-acidified sludge resulted from their resistance to nitrates lower than that of keratinophilic fungi. Studies on the influence of sulfates and nitrates on keratinolytic and keratinophilic fungi should be continued.

Special attention should be paid to the epidemiological aspect of the incidence of keratinolytic and keratinophilic fungi in sewage sludge and sludge-amended soils. In keratinolytic and keratinophilic fungi, many species with potential pathogenic properties to animals, including humans have been found [2]. Liming is a frequent method used for improvement of soil properties and productivity. Low lime quantities applied in agriculture increase the number of keratinolytic fungi but decrease the number of keratinophilic ones. This could increase the risk of skin mycoses to farmers and other persons when contacted with limed soil. This suggestion agrees with that of Śpiewak [37]. According to the author, mycoses are the most prevalent diseases in farmers (possibly present in over 20% of the farmer population), and soil is one of the fungal infection sources. Liming is also often used for sewage sludge hygienization, with higher lime quantities allowing to reach pH>12 for at least 2 hours. High pH is the major factor of pathogen inactivation and destruction. However, the heat generated and ammonia released in the lime-sludge mixture also contribute to the process [38-40]. The influence of sludge hygienization by liming on keratinolytic and keratinophilic fungi has not yet been studied. Attention should also be paid to potential consequences of sludge and sludge-amended soil acidification. In the present study, sludge acidification with HNO_3 and HCl led to the abundant growth of *Paecilomyces lilacinus* and *Penicillium janthinellum*. The two species are infrequent causative agents of mycoses in humans [2]. However, studies should be conducted to elucidate whether sludge or sludge-soil mixture acidification always results in such a “safe” fungal population.

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References

1. MAJCHROWICZ I., DOMINIK T. Further contribution to the knowledge of keratinolytic and keratinophilic soil fungi of the region of Szczecin – keratinolytic and keratinophilic fungi in the immediate surroundings of cattle. Ekol. Pol. (Seria A) **17**, 87, **1969**.
2. DE HOOG G. S., GUARRO J., GENE J. AND FIGUERAS M. J. Atlas of Clinical Fungi; Centraalbureau voor Schimmelcultures & Universitat Rovira i Virgili: Utrecht, Reus, **2000**.
3. ABDEL-HAFEZ A. I. I., EL-SHAROUNY H. M. M. The occurrence of keratinophilic fungi in sewage sludge from Egypt. J. Basic Microbiol. **30**, 73, **1990**.
4. ULFIG K., TERAKOWSKI M., PŁAZA G., KOSAREWICZ O. Keratinolytic fungi in sewage sludge. Mycopathologia **136**, 41, **1996**.
5. ULFIG K. Factors influencing the occurrence of keratinolytic and keratinophilic fungi in sewage sludge. Mycotechnology: Current research and Future Prospectives, S. Deshmukh Ed., Oxford IBH (in press), **2004**.
6. ULFIG K. Studies of keratinolytic and keratinophilic fungi in sewage sludge by means of a multi-temperature hair baiting method. Polish J. Environ. Studies **12**, 461, **2003**.
7. TAKASHIO M. Etudes des phénoménes de reproduction liés au vieillissement et au rajeunissement des cultures de champignons. Ann. Soc. Belge Méd. Trop. **53**, 427, **1973**.
8. SIGLER L., CARMICHAEL J. W. Taxonomy of *Malbranchea* and some other Hyphomycetes with arthroconidia. Mycotaxon **4**, 349, **1976**.
9. RAPER K. B., FENNEL K. D. The genus *Aspergillus*. R. E. Krieger Publishing Company: Huntington, New York, **1977**.
10. VAN OORSCHOT C. A. N. A revision of *Chrysosporium* and allied genera. Studies in Mycology **20**, 1, **1980**.
11. DOMSCH K. H., GAMS W., TRAUTE-HEIDI A. Compendium of Soil Fungi. Academic press: London-San Francisco, **1980**.
12. CANO J. F., GUARRO J. The genus *Aphanoascus*. Mycol. Res. **94**, 355, **1990**.
13. ULFIG K., PŁAZA G., TERAKOWSKI M., STASZEWSKI T. A study of keratinolytic fungi in mountain sediments. I. Hair-baiting data. Roczn. PZH **49**, 469, **1998**.
14. GARG A. P., GANDOTRA S., MUKERJI K. G., PUGH G. J. F. Ecology of keratinophilic fungi. Proc. Indian Acad. Sci. (Plant Sci.) **94**, 149, **1985**.
15. SIMPANYA M. F. Dermatophytes: their taxonomy, ecology and pathogenicity. Biology of Dermatophytes and other Keratinophilic Fungi; Revista Iberoamericana de Micología, pp 1-12, **2000**.
16. ULFIG K. The occurrence of keratinolytic fungi in waste and waste contaminated habitats. Biology of Dermatophytes and other Keratinophilic Fungi; Revista Iberoamericana de Micología, pp 44-50, **2000**.
17. KUSHWAHA R. K. S. The genus *Chrysosporium*, its physiology and biotechnological potential. Biology of Dermatophytes and other Keratinophilic Fungi; Revista Iberoamericana de Micología, pp 66-76, **2000**.
18. BÖHME H., ZIEGLER H. The distribution of geophilic dermatophytes and other keratinophilic fungi in relation to the pH of the soil. Mycopathol. Mycol. Appl. **38**, 247, **1969**.
19. NORTH M. J. Comparative biochemistry of the proteinases of eucaryotic microorganisms. Microbiological Reviews **46**, 308, **1982**.
20. ROSENBERG S. L. Temperature and pH optima for 21 species of thermophilic and thermotolerant fungi. Can. J. Microbiol. **21**, 1535, **1975**.
21. UTZ R., COLE G. T., RÜCHEL R., MONOD M. Molecular cloning and targeted deletion of PEP2 which encodes a novel aspartic proteinase from *Aspergillus fumigatus*. Int. J. Med. Microbiol. **290**, 85, **2000**.
22. GOW N. A. R., GADD G. M. The Growing Fungus. Chapman & Hall: London, **1995**.
23. CHMEL L., HASILÍKOVÁ A., HRAŠKO J., VLÁČILÍKOVÁ A. The influence of some ecological factors on keratinophilic fungi in the soil. Sabouraudia **10**, 26, **1972**.
24. CHMEL L., VLÁČILÍKOVÁ A. The ecology of keratinophilic fungi at different depths of soil. Sabouraudia **13**, 185, **1975**.
25. KORNIŁŁOWICZ T. The occurrence and distribution frequency of keratinophilic fungi in selected arable soils (in Polish). Acta Mycologica **28**, 3, **1993**.
26. KORNIŁŁOWICZ-KOWALSKA T., BOHACZ J. Some correlations between the occurrence frequency of keratinophilic fungi and selected soil properties. Acta Mycologica **37**, 101, **2002**.
27. MOREL J. L., GUCKERT A. Influence of limed sludge on soil organic matter and soil physical properties. The influence of sewage sludge application on physical and biological properties of soils, Proceedings of a Seminar organized jointly by the Commission of the European Communities, Directorate-General for Science, Research and Development and the Bayerische Landesanstalt für Bodenkultur and Pflanzenbau, Munich, Federal Republic of Germany, Munich, D. Reidel Publishing Company, Dordrecht-Boston-London, pp 25-42, **1981**.
28. RICHARDSON M. L., GANGOLLI S. The Dictionary of Substances and their Effects. The Royal Society of Chemistry, Clays Ltd.: London, **1994**.
29. LOCKWOOD J. L., FILONOW A. B. Responses of fungi to nutrient-limiting conditions and to inhibitory substances in natural habitats. Advances in Microbial Ecology vol. 5, Plenum Publishing Corporation: pp 1-61, **1981**.
30. SCHIPPERS B., MEIJER J. W., LIEM J. I. Effect of ammonia and other soil volatiles on germination and growth of soil fungi. Trans. Br. Mycol. Soc. **79**, 253, **1982**.
31. LILLY V. G., BARNETT H. L. The Physiology of Fungi (Polish edition). PWRiL, Warszawa, **1959**.

32. KOBUS J. The influence on plants. The influence of microorganisms (in Polish). Sulfur in the biosphere, PWRiL: Warszawa, pp 210-212, **1980**.
33. BEWLEY R. J. F., STOTZKY G. Simulated acid rain (H2SO4) and microbial activity in soil. Soil Biol. Biochem. **15**, 425, **1983**.
34. KUNERT J. Physiology of keratinophilic fungi. Biology of Dermatophytes and other Keratinophilic Fungi; Revista Iberoamericana de Micología, pp 77-85, **2000**.
35. KUNERT J. Inorganic sulphur sources for the growth of the dermatophyte Microsporum gypseum. Folia Microbiol. **26**, 196, **1981**.
36. SINGH K. V., AGRAWAL S. C. Nutritional requirements of keratinophilic fungi and dermatophytes for conidial germination. Mycopathologia **80**, 27, **1982**.
37. ŚPIEWAK R. Zoophilic and geophilic fungi as a cause of skin disease in farmers. Ann. Agric. Environm. Med. **5**, 97, **1998**.
38. STRAUB T. M., PEPPER I. L., GERBA C. P. Hazards from pathogenic microorganisms in land-disposed sewage sludge. Rev. Environm. Contam. Toxicol. **132**, 55, **1993**.
39. DUMONTET S., DINEL H., BALODA S. B. Pathogen reduction in sewage sludge by composting and other biological treatments: a review. Biol. Agricul. Horticul. **16**, 409, **1999**.
40. DUMONTET S., SCOPA A., KERJE S., KROVACEK K. The importance of pathogenic organisms in sewage and sewage sludge. J. Air & Waste Management Assoc. **51**, 840, **2001**.