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## A PRELIMINARY STUDY ON THE OCCURRENCE OF KERATINOLYTIC FUNGI IN THE STREET SWEEPINGS FROM CHORZÓW

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*The street sweepings from the city of Chorzów were surveyed for keratinolytic fungi. Out of 106 Petri dishes examined, 98 (92,4%) were positive for these micro-organisms. Altogether, 185 fungal appearances belonging to 15 species were observed. Chrysosporium keratinophilum, Malbranchea flava, Ch. europae, Sporothrix schenckii, Ch. anamorphs of Aphanoascus reticulispurus/fulvescens, Ch. an. Arthroderma curreyi, and M. an. Uncinocarpus reessi predominated in the sweepings. The occurrence of Ch. keratinophilum, Ch. europae, Ch. an. A. curreyi, and S. schenckii could depend on the content of heavy metals and the individual fractions in the sweepings. The epidemiological aspect of the presence of pathogenic fungi in the street dust was briefly discussed.*

### INTRODUCTION

Indoor dust within the urban agglomerations has been surveyed for keratinolytic fungi (KF) and related pathogenic species several times [4-6]. It results from available publications, however, that street sweepings have not yet been examined for this group of micro-organisms. In this article, therefore, we present the data of a preliminary study on the occurrence of KF in the sweepings from the city of Chorzów.

### MATERIAL AND METHODS

Street sweepings was sampled during the summer-autumn season in 1993 in the city of Chorzów (Upper Silesia Region, Poland). Altogether, 11 samples from 4 districts of the city were collected. The districts and the numbers of samples were the following: I. the City Centre (samples no. 1, 5, 11); II. the Batory District (samples no. 2, 7, 8); . the Maciejkowice district (samples no. 4, 9); and IV. the Katowicka Street (samples no. 3, 6, 10). These districts differ in the traffic intensity and industrial infrastructure (I - steel metallurgy; II - coke chemistry; III - lead and zinc mining and metallurgy; IV - high traffic street).

A street-sweeper of the Broadway type (made in Sweden) was used for sampling. The street-sweeper was drawn by the truck. Rotating brooms passed the sweepings to the conveyor transporting them to the truck's body. The sweeper machine sprinkled the street simultaneously. Truck's body was filled up after 3-4 km street cleaning. Wet sweep load was ca. 1 t. The content of truck's body was tipped within the municipal landfill site. About 2-3 kg of the wet sweepings were sampled from each tipping. The samples represented material from ca. 10-15 points of the tipping and were dried, sieved with the 1 mm net, thoroughly mixed, and then examined for mycological and physico-chemical features.

The human hair baiting method [13] was applied for qualitative and quantitative (q/q) recognition of KF in the sweepings. For each sample, 7–10 hair-supplemented *Petri* dishes were set up. Incubation was carried out at room temperature for over 6 months. Isolated fungi were identified using selected keys and monographs [1–3, 7, 8]. The following fungal indicators were used: frequency of KF isolation (FI; %), frequency of isolation of predominating species (FIPS; %), number of species isolated (NS), number of appearances (NA), and L index (number of appearances divided by the number of *Petri* dishes set up).

The FIPS abbreviations were the following: CKER, ACUR, SSCH, MFLA, MUR, CHRYS, and CEUR for *Chrysosporium keratinophilum*, *Ch.* anamorph of *Arthroderma curreyi*, *Sporothrix schenckii*, *Malbranchea flava*, *M.* anamorph of *Uninocarpus reessii*, *Chrysosporium* anamorph of *Aphanoascus reticulisporus* + *A. fulvescens*, and *Ch. europae* respectively.

The following physico-chemical parameters were determined for each step sample: size analyses – over 63  $\mu\text{m}$  by sieving method, below 63  $\mu\text{m}$  by sedimentation pipette method; density – by pycnometric method; ignition losses at 600°C (organic matter content) – by gravimetric method; pH in H<sub>2</sub>O – by potentiometric method; conductivity – by potentiometric method; fat content – by naphtha ether extraction method; content of heavy metals (zinc, cadmium, lead, copper, nickel, chromium) – by atomic absorption method. Heavy metals and density were also determined in the sweep fractions. Full physico-chemical characteristics of the sweepings are presented by *Terakowski et al.* [9].

The CSS „Statistica” program was used for statistical analysis of the data obtained.

## RESULTS

Out of 106 *Petri* dishes examined, 98 (92,4%) were positive for KF (Tab. I). Altogether, 185 fungal appearance belonging to 15 species were observed. *Chrysosporium keratinophilum*, *Malbranchea flava*, *Ch. europae*, *Sporothrix schenckii*, *Ch.* anamorphs of *Aphanoascus reticulisporus/fulvescens*, *Ch.* an. *Arthroderma curreyi*, and *M.* an. *Uninocarpus reessii* predominated in the sweep samples. The *Chrysosporium* anamorphs of *A. reticulisporus* and *A. fulvescens* were placed at the same position in Tab. I because they were unrecognisable. FI, NS and L index ranged between 80–100%, 3–7, and 1, 1–2, 7 respectively.

The sweep samples chiefly differed in the FIPS values for *Ch. keratinophilum* and the other species (Fig. 1). The samples formed two groups: The first included the samples no. 1, 3, 8 and the second the samples no. 2, 4–7, 9–11. In these groups, CKER was higher and lower than 40% respectively (Fig. 2).

Selected physico-chemical characteristics of the samples are showed in Tab. II and III. The samples differed mainly in the content of zinc/lead and the fractions

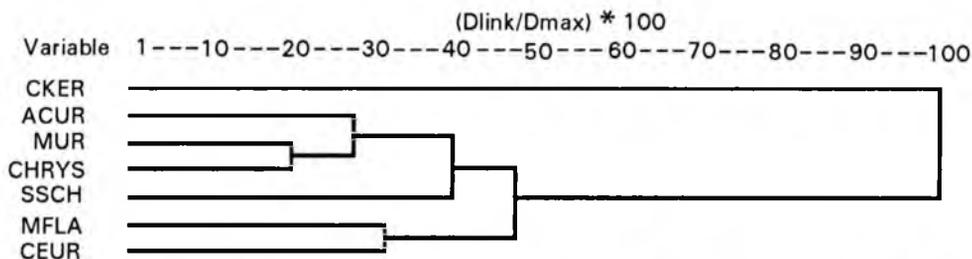


Fig. 1. Cluster analysis of predominating keratinolytic fungi (FIPS) in street sweepings from Chorzów

Table I. The occurrence of keratinolytic fungi in the street sweepings from Chorzów

Keratinolytic species	No. of sweep sample/district											Total
	1	2	3	4	5	6	7	8	9	10	11	
	I	II	IV	III	I	IV	II	II	III	IV	I	
No. of <i>Petri</i> dishes set up	10	10	10	10	10	9	10	10	10	7	10	106
No. of dishes positive for kerat. fungi	9	10	10	10	8	9	8	9	10	7	8	98
FI (%)	90	100	100	100	80	100	80	90	100	100	80	92,4
	No. of dishes positive for:											
<i>Chrysosporium keratinophilum</i>	7	9	8	4	3	7	4	7	7	4	2	62
<i>Malbranchea flava</i>	-	3	-	5	1	3	5	-	4	1	-	22
<i>Chrysosporium europae</i>	-	-	2	6	-	-	2	1	4	3	1	19
<i>Sporothrix schenckii</i>	-	8	-	2	3	-	1	-	1	-	-	15
<i>Chrysosporium</i> an. <i>Aphanoascus reticulisporus/fulvescens</i>	2	1	-	2	-	4	-	-	-	-	4	13
<i>Chrysosporium</i> an. <i>Arthroderma curreyi</i>	2	2	-	-	2	-	1	2	-	1	2	12
<i>Malbranchea</i> an. <i>Uncinocarpus reessii</i>	-	1	-	-	-	5	-	1	2	-	2	11
<i>Aphanoascus reticulisporus</i>	-	1	-	-	-	3	-	-	-	-	4	8
<i>Chrysosporium pannicola</i>	-	1	-	1	-	2	-	-	3	-	-	7
<i>Chrysosporium tropicum</i>	-	-	1	2	-	-	1	-	-	-	1	5
<i>Aphanoascus fulvescens</i>	2	-	-	1	-	1	-	-	-	-	-	4
<i>Trichophyton ajelloi</i>	-	-	-	-	2	-	-	-	-	-	-	2
<i>Botryotrichum piluliferum</i>	-	-	-	-	-	-	1	1	-	-	-	2
<i>Chrysosporium</i> an. <i>Arthroderma multifidum</i>	-	-	-	-	-	-	-	1	-	-	-	1
<i>Myceliophthora vallerea</i>	-	-	-	-	-	-	-	-	-	1	-	1
<i>Myceliophthora</i> sp.	-	-	-	-	-	-	-	-	-	-	1	1
No. of species isolated (NS)	3	7	3	7	5	6	7	6	6	5	7	15
No. of appearances (NA)	13	26	11	23	11	25	15	13	21	10	17	185
L index	1,3	2,6	1,1	2,3	1,1	2,7	1,5	1,3	2,1	1,4	1,7	1,74

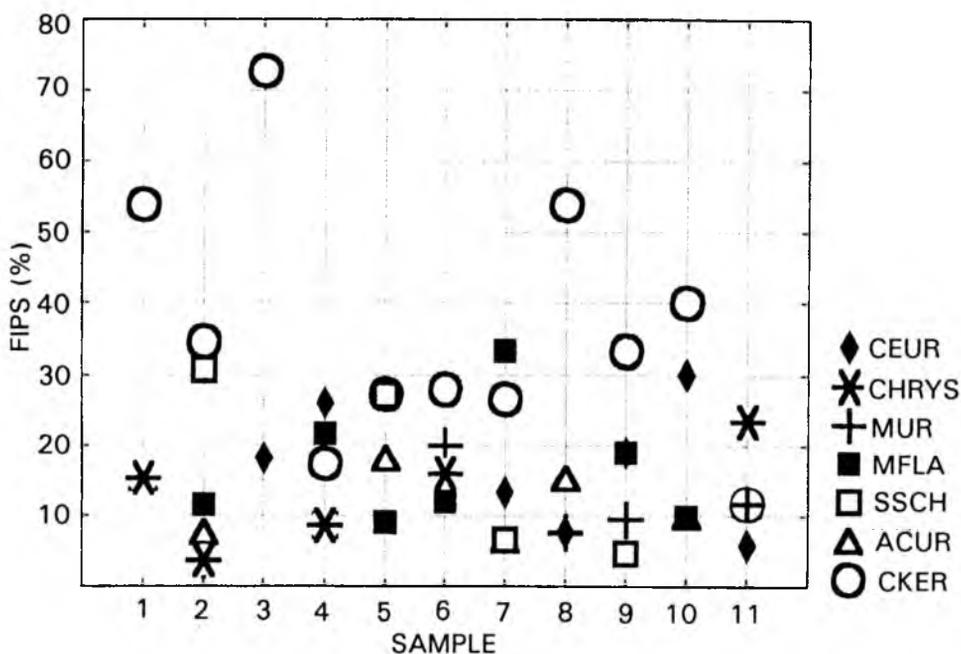


Fig. 2. Occurrence of predominating keratinolytic fungi (FIPS) in street sweepings from Chorzów

1000–500/500 – 250  $\mu\text{m}$ . In addition, the samples from districts I and IV differed from those of districts II and III in the higher content of heavy metals (zinc and lead in particular). The heavy metals were associated with the smaller sweep fractions (<63  $\mu\text{m}$ ).

The mean ACUR and CEUR values were distinctly higher in the districts I/II and IV respectively (15, 1/9, 9 and 0/3, 3; 1, 9/7 and 22, 5/16%). The differences in the mean FIPS values for the other species were not strongly marked.

FI was positively correlated with the lead content ( $p < 0,01$ ;  $r = 0,67$ ). ACUR was negatively correlated with the zinc and cadmium content (-0,66; -0,63) while CEUR positively with the content of these metals and lead (0,68; 0,69; 0,79). In addition, MFLA was negatively correlated with the fat content (-0,66). Finally, CKER was negatively correlated with NS (-0,79).

SSCH was positively correlated with the small sweep fractions (63–32 and <10  $\mu\text{m}$  in particular) whereas CKER negatively with these fractions and positively with the bigger fractions (500–125  $\mu\text{m}$ ).

## DISCUSSION

On the basis of the Fi values, KF must be considered as occurring abundantly in the sweepings examined. However, L index was relatively low. This may indicate that the streets are not the habitat for growth of KF but merely the place of assemblance of fungal propagules. However, our data was obtained with the hair baiting method

Table II. Selected physio-chemical characteristic of the street sweepings from Chorzów

Parameters	No. of sample/district										
	1	2	3	4	5	6	7	8	9	10	11
	I	II	IV	III	I	IV	II	II	III	IV	I
Density (g/cm <sup>3</sup> )	2,58	2,56	2,60	2,47	2,56	2,56	2,57	2,41	2,47	2,57	2,57
Zinc (μg/g)	199,1	388,4	568,2	1400,7	259,8	302,7	199,6	293	1115,1	532	402
Cadmium (μg/g)	3,9	2,85	6,95	8,77	2,74	6,52	5,5	4,36	11,89	7,82	7,86
Lead (μg/g)	93	129,5	322,4	368,5	101,6	265	100,5	126,1	199,3	471,4	149,3
Copper (μg/g)	59,4	52,1	78,7	51,9	43,9	34,5	30,6	48,5	84,3	81,4	43,7
Nickel (μg/g)	43,5	51,8	40,7	39,4	33,4	44,7	39,9	49,7	50,4	50	42,1
Chromium (μg/g)	111,6	93,3	72,6	79,7	99	108,9	132,3	65,4	83,7	174,9	152,9
Ignition losses (%)	6,03	5,86	5,68	21,27	4,52	6,22	4,47	13,38	11,05	6,8	5,36
Fat content (%)	0,31	0,27	0,31	0,23	0,25	0,25	0,26	0,28	0,22	0,29	0,39
pH	7,16	7,33	7,44	7,4	7,51	7,58	7,47	7,09	7,19	7,14	7,05
Conductivity (μS/cm)	300	218	215	248	242	292	241	351	244	302	304

Table III. Percentage participation of the following fractions in the street sweepings from Chorzów

Fraction	No. of sample/district										
	1	2	3	4	5	6	7	8	9	10	11
	I	II	IV	III	I	IV	II	II	III	IV	I
1000-500	41,35	24,39	34,1	58,45	44,47	46,9	36,43	42,89	33,45	33,49	41,16
500-250	43,87	50,37	47,87	30,5	35,48	42,7	45,61	42,07	40,82	46,53	42,44
250-125	11,23	19,37	15,93	8,19	13,84	8,5	13,48	11,27	18,42	16,55	11,58
125-63	2,41	3,67	1,7	1,88	4,14	1,3	2,39	2,61	5,45	2,38	3,22
63-32	0,8	1,46	0,33	0,65	1,59	0,47	1,37	0,83	1,32	0,59	1,01
32-20	0,195	0,367	0,043	0,162	0,258	0,06	0,404	0,166	0,359	0,332	0,283
20-10	0,094	0,255	0,016	0,12	0,14	0,054	0,284	0,112	0,153	0,117	0,254
<10 μm	0,056	0,094	0,007	0,036	0,08	0,009	0,034	0,052	0,034	0,013	0,053

that does not ever reflect the real KF activity in the environment [12]. Therefore, the above hypothesis should be confirmed with other methods.

In general, the differences between the sweep samples in the q/q composition of KF were found to be rather small. This could result from the small differences in the physico-chemical characteristics of the samples (except for the content of heavy metals). In the samples, pH ranged between 7,05–7,58. This range could favour *Ch. keratinophilum* in this habitat. In addition, high CKER was associated with low NS. This indicates that *Ch. keratinophilum*, one of the species with the highest keratinolytic and biological activity [2], could eliminate other species from the hair bait. This also concerns pathogenic species including zoo- and anthropophilic dermatophytes.

The occurrence of KF could also depend on the content of heavy metals in the sweepings. The content of these metals, lead and zinc in particular, was distinctly higher in the districts with high traffic intensity as well as lead and zinc industry. In a study of KF in badly polluted sediments [11], *Ch. keratinophilum* was found to be the species most resistant to pollutants, including heavy metals. Our data confirmed this finding and indicates that this species is particularly associated with human activity (wastes). Other fungi that might be dependent on heavy metals are *A. curreyi* and *Ch. europae*. In the quoted study, however, the last fungus was found to be most sensitive to pollutants. The influence of heavy metals and other toxic factors on KF must be, therefore, carefully examined in laboratory studies.

Our data also indicate that some KF are probably associated with the sweep fractions. For example, *Ch. keratinophilum* could be associated with the bigger fractions in which bigger keratin remains of human and animal origin are possible present. Subsequently, *S. schenckii* would be associated much smaller fractions. This hypothesis must be confirmed in future studies of the occurrence of KF within the individual sweep fractions.

*S. schenckii* was the only pathogenic species isolated in our study. It must be emphasized, however, that some fungal pathogens are not able to compete on hair with the highly specialized geophilic KF [10]. In the future study, therefore, we intend to use the dilution method combined with several selective media and temperatures [6].

The association of KF with the street sweepings and their individual fractions is possible important from an epidemiological point of view. Street dust are lifted from the street and pavement surfaces to the air and may cause allergic diseases. Besides, we believe that the sweepings contain many more pathogenic fungi of human and animal origin and they are the source of fungal infections.

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#### WSTĘPNE BADANIA WYSTĘPOWANIA GRZYBÓW KERATYNOLITYCZNYCH W ZMIOTKACH ULICZNYCH MIASTA CHORZOWA.

Zmiotki uliczne z miasta Chorzowa przebadano pod względem występowania grzybów keratynolitycznych. Na ogólną liczbę 106 szalek *Petriego* w 98 (92,4%) stwierdzono obecność tych mikroorganizmów. Łącznie zaobserwowano 185 pojawów grzybów należących do 15 gatunków. *Chrysosporium keratinophilum*, *Malbranchea flava*, *Ch. europae*, *Sporothrix schenckii*, *Ch. anamorfi*, *Aphanoascus reticulisporus* i *A. fulvenscens*, *Ch. an. Arthroderma curreyi* i *M. an. Uncinocarpus*

*reessii* dominowały w badanych zmiotkach. Występowanie *Ch. keratinophilum*, *Ch. europae*, *Ch. an. A. curreyi* i *S. schenckii* mogło być uzależnione od zawartości metali ciężkich i składu ziarnowego zmiotków. Przedyskutowano aspekt epidemiologiczny związany z obecnością grzybów chorobotwórczych w pyłe ulicznym.

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