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A STATISTICAL EVALUATION OF THE OCCURRENCE OF KERATINOLYTIC FUNGI IN THE SEDIMENTS OF TWO DAM RESERVOIRS

STATYSTYCZNA OCENA WYSTĘPOWANIA GRZYBÓW KERATYNOLITYCZNYCH W OSADACH DENNYCH DWÓCH ZBIORNIKÓW ZAPOROWYCH

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Sediments from two dam reservoirs "Przeczyce" and "Pławniowice" were examined for keratinolytic fungi. The results show the dependence of keratinolytic fungi in sediments on the degree of water contamination with sewage. Ch. keratinophilum *is species associated with sewage inputs to superficial water.*

INTRODUCTION

The results of the surveys of keratinolytic fungi from the sediments of selected dam reservoirs, rivers, ponds, and sewage systems in the Upper Silesia Region of Poland were presented in several previous works [7, 9]. The relations between qualitative and quantitative (q/q) composition of these fungi in the sediments in realation to the degree of sewage contamination in water were generally discussed in another paper [8]. A possible role of keratinolytic fungi as one of the bioindicators of water pollution and degradators of organic compounds in the aqueoues environment was suggested. In this article, we intend to continue this subject showing statistical relationships between q/q compositon of keratinolytic fungi in the sediments of two well documented dam reservoirs with physico-chemical and microbiological data.

MATERIAL AND METHODS

Sediments were sampled in the "Przeczyce" and "Pławniowice" dam reservoirs used for recreational purposes. Althogether, 11 locations were investigated: 2 situated directly in the heavily polluted inflows of rivers into the dam reservoirs and 9 in dam areas (includng the outflows) of different degree of water self-purification. Sediments were sampled twice during the spring season (May-June). At each location, aout 2–3 kg of sediment from shallow places (up to 1 m of depth) and a surgace layer (up to 5 cm of depth) were collected in a clean plastic container. The content of the

plastic container was thoroughly stirred, sedimented, decanted, and placed in a sterilized pot. Water from a superficial layer (up to 0.5 m of depth) was sampled three times. Water and sediment samples were delivered to the laboratory within 4–5 hours.

The hair baiting method [12], using defatted, cut into small pieces, and sterilized human hair as bait, was applied for q/q recognition of keratinolytic fungi in sediments. For each location and sampling, 5–10 hair-supplemented *Petri* dishes were set up. Incubation was carried out at room temperature for 3–4 months. Isolated keratinolytic fungi were identified basing on their micro- and macroscopic characteristics with selected keys and monographs [1, 2, 5, 6]. In detailed examination of fungal population, the following indicators were applied: frequency of isolation of keratinolytic species from sediments (FI), frequency of isolation of individual fungal species (F/species), number of species isolated (NS), number of fungal appearances (NA), L index (L; number of appearances divided by the number of *Petri* dishes set up) compared with the LPP index (number of appearances divided by the number of *Petri* dishes positive for keratinolytic fungi).

Physico-chemical and microbiological water analyses (water temperature [WT], biochemical oxygen demand for 5 days [BOD_5], chemical oxygen demand [COD], dissolved oxygen concentration [DOC], NNH_4, NNO_2, NNO_3, PO_4, pH, alkalinity [ALK], water hardness [WH], Ca, Mg, Fe, Mn, conductivity [CON], chlorides [Cl], sulphates [SO_4], dissolved substances [DS], suspended solids [SS], total and fecal coliforms [TC, FC], total number of aqueous bacteria [TNB_1], mesophilic bacteria [MB] were performed according to the Polish Standards [4].

In analyses of a number of total bacteria [TNB_2] and microscopic fungi [TNF] in sediments, the dilution method with the beef extract peptone (BEA) and *Sabouraud* glucose agars (SGA) were applied [3]. Organic matter content (OM) in sediments was determined by means of the *Tjurin* method.

The CSS "Statistica" program was used for the regression analysis of the above mentioned parameters (means) in relation to the mycological data. Simple linear regressions are mostly presented in this study. Other kinds of correlations (exponencial, multiplicative) are showed when they are better than linear ones.

RESULTS

The LPP index was applied in our previous publications [7, 9]. This index shows a very good linear correlation ($L = -1.03 + 0.301 * LPP$; $r = 0.95$, $p < 0.01$) with the L index. While comparing general quantitative indices (FI, NS and L), the best correlations, definitely, were obtained for the L and FI values (Fig. 1).

Linear correlations between mycologival and physico-chemical data are presented in Tab. I and II. Among the general quantitative indices, the best, but negative, correlations were obtained for the L and WT as well as L and pH values ($p < 0.01$). Subsequently, the *F/T. ajelloi* values were positively correlaed with BOD_5, Mn and SS, whereas those of *F/T. terrestre* correlated negatively with many physico-chemical parameters; having the highest correlation coefficient with water alkalinity (ALK). It is to be emphasized, however, that due to the extremely irregular distribution of the points, the correlations of *F/T. ajelloi* with Mn and SS are not reliable. The *F/A. quadrifidum*, *F/Ch. indicum* and *F/Ch. anamorphs* of *A. fulvescens/reticulosporus* values do not show good correlations with physico-chemical parameters. On the contrary, the *F/Ch. keratinophilum* values show very good correlations, e.g. with WT, DOC, NNO_2, pH, and ALK. The *F/Ch. pannicola* values are correlated with DOC and pH, whereas those of *F/A. fulvescens/reticulosporus*, silimilarly to *F/T. ajelloi*, with BOD_5, Mn and SS.

In our previous publications, the anamorph of the fungus *Anixiopsis stercoratia* was erronously named as *Ch. pruinosum*. Later, after getting acquintaned with the revision of the genus *Aphanoascus* by *Cano & Guarro* [1], the revised and correct names of this fungus, *Ch.* an. of *Aphanoascus fulvescens*, were applied. Recently, we have decided to introduce the name *Ch.* anamorphs of *A. fulvescens/reticulosporus* for all old data, because the disitintion between *A. fulvescens* and *A. reticulosporus* based on the reticulation of ascospore wall was previously cery difficult without a good microscopic equipment.

L index (L) is positively correlated with all microbiological parameters, including the OM values (Tab. III). The best correlation, however, was obtained for the L and

Table I. Correlation coefficients for mycological and physicochemical data. Part I

Mycological ndices	WT	BOD_5	COD	DOC	NNH_4	NNO_2	NNO_3	PO_4	pH	ALK
Fl	−0,42	0,52	0,64*	−0,73**	0,48	0,34	0,35	0,09	−0,64*	0,35
NS	−0,59	0,34	0,24	−0,67*	0,36	0,50	0,25	0,54	−0,66*	0,58
L	−0,68*	0,42	0,55	−0,81**	0,57	0,63*	0,17	0,26	−0,85**	0,68*
F/T,aj,	−0,22	0,85**	0,50	−0,36	0,38	0,54	−0,31	0,02	−0,59	0,39
F/T,ter,	0,45	−0,65*	−0,42	0,60*	−0,50	−0,64*	0,07	−0,35	0,71*	−0,74**
F/A,q,	0,62*	−0,30	−0,23	0,16	−0,29	−0,41	0,32	−0,45	0,44	−0,60*
F/Ch,ind,	0,18	0,05	0,29	0,0	−0,06	−0,01	0,17	0,13	−0,10	−0,18
F/Ch,ker,	−0,78**	0,38	0,47	−0,74**	0,43	0,76**	0,03	0,56	−0,90**	0,76**
F/Ch,pan,	−0,60*	0,53	0,43	−0,78**	0,61*	0,49	0,09	0,25	−0,75**	0,70*
F/Ch,A,ful,	−0,07	0,15	−0,08	−0,08	0,10	0,29	−0,46	0,33	−0,04	0,45
F/A,fil,	−0,22	0,79**	0,60*	−0,39	0,45	0,28	−0,22	0,29	−0,63*	0,37

* – $p<0,05$
** – $p<0,01$

Abbreviations:

WT – water temperature (°C)
BOD_5 – biochemical oxygen demand for 5 days (mgO_2/L)
COD – chemical oxygen demand (mgO^2/L)
DOC – dissolved oxygen concentration (mgO_2/L)
NNH_4 – ammonium nitrogen (mg/L)
NNO_3 – nitrite nitrogen (mg/L)
NNO_4 – nitrate nitrogen (mg/L)
PO_4 – phosphates (mg/L)
pH – hydrogen ion potential
ALK – alkalinity (mval/L)
Fl – frequency of isolation of keratinolytic fungi (%)
NS – number of species isolated
L – L index (a number of fungal appearances divided by the number of *Petri* dishes set up)
F/*T. ajelloi* – frequency of *Trichophyton ajelloi* isolation
F/*T. terrestre* – frequency of *T. terrestre* isolation
F/*A. quadrifidum* – frequency of *Arthroderma quadrifidum* isolation
F/Ch. *indicum* – frequency of *Chrysosporium indicum* isolation
F/Ch. *keratinophilum* – frequency of *Ch. keratinophilum* isolation
F/*Ch. pannicola* – frequency of *Ch. pannicola* isolation
F/*Ch. A. fulvescens/reticulosporus* – frequency of *Ch. anamorph of Aphanoascus fulvescens/reticulosporus* isolation
F/*A. fulvescens/reticulosporus* – frequency of *A. fulvescens/reticulosporus* isolation

Table II. Correlation coefficients for mycological and physicochemical data. Part II

Mycological indices	WH	Ca	Mg	Fe	Mn	CON	Cl	SO_4	DS	SS
FI	0,18	0,64*	0,09	0,35	−0,22	0,47	−0,44	0,04	−0,36	−0,15
NS	0,10	0,23	0,0	0,31	0,02	0,71**	0,17	−0,11	−0,11	0,14
L	0,13	0,43	0,08	0,48	−0,12	0,63*	−0,29	−0,27	−0,27	−0,05
F/T,aj,	−0,29	0,0	−0,27	0,35	0,77**	0,70*	0,34	−0,38	0,38	0,77**
F/T,ter,	0,16	−0,20	0,21	−0,59	−0,37	−0,68*	−0,10	0,39	−0,05	−0,42
F/A,q,	−0,17	0,27	−0,25	−0,16	−0,17	−0,46	−0,41	0,44	−0,05	−0,26
F/Ch,ind,	0,02	0,50	−0,15	−0,11	0,15	0,01	−0,17	0,25	−0,03	0,17
F/Ch,ker,	0,09	0,19	0,0	0,30	0,07	0,70*	0,06	−0,37	0,0	0,14
F/Ch,pan,	0,11	0,32	0,10	0,64*	−0,09	0,73*	−0,16	−0,27	−0,28	0,01
F/Ch,A,ful,	−0,29	−0,22	−0,29	0,24	0,40	0,08	0,44	−0,38	0,43	0,35
F/A,ful,	0,0	0,17	0,0	0,32	0,80**	0,63*	0,27	−0,08	0,26	0,86**

* – $p < 0,05$
** – $p < 0,01$

Abbreviations:
WH – water hardness (mval/L)
Ca – calcium concentration (mg/L)
Mg – magnesium concentration (mg/L)
Fe – iron concentration (mg/L)
Mn – maganese concentration (mg/L)
CON – water conductivity (μS)
Cl – chlorides concentration (mg/L)
SO_4 – sulphates concentration (mg/L)
DS – dissolved substances (mg/L)
SS – suspended solids (mg/L)
FI – frequency of isolation of keratinolytic fungi (%)
NS – number of species isolated
L – L index (a number of fungal appearances divided by the number of *Petri* dishes set up)
F/*T. ajelloi* – frequency of *Trichophyton ajelloi* isolation
F/*T. terrestre* – frequency of *T. terrestre* isolation
F/*A. quadrifidum* – frequency of *Arthroderma quadrifidum* isolation
F/*Ch. indicum* – frequency of *Chrysosporium indicum* isolation
F/*keratinophilum* – frequency of *Ch. keratinophilum* isolation
F/*Ch. pannicola* – frequency of *Ch. pannicola* isolation
F/*Ch.* A. fulvescens/*reticulosporus* – frequency of *Ch. anamorph* of *Aphanoascus fulvescens/reticulosporus* isolation
F/A. fulvescens/reticulosporus – frequency of A. *fulvescens/reticulosporus* isolation

TNB, values (Fig. 2). A good multiplicative correlation was also obtained for the L/FC points (Fig. 3). The *F/T. ajelloi, F/A. quadrifidum, F/Ch. indicum, F/Ch.* and *A. fulvescens/reticulosporus* and *F/A. fulvescens/reticulosporus* values are not correlated with any microbiological parameter. Subsequently, the values of *F/T. terrestre* are negatively correlated positively with all parameters under consideration (with the exception of OM). Finally, the values of *F/Ch. pannicola* are strongly correlated with TNB_1.

The best correlations among mycological parameters were obtained for the *F/T. ajelloi-F/T. terrestre* (Fig. 4), *F/Ch. keratinophilum-F/T. terretsre* ($r = -0.763$; $p = 0.006$), and *F/T. ajelloi-F/A. fulvescens/reticulosporus* ($r = 0.861$; $p = 0.001$) pairs.

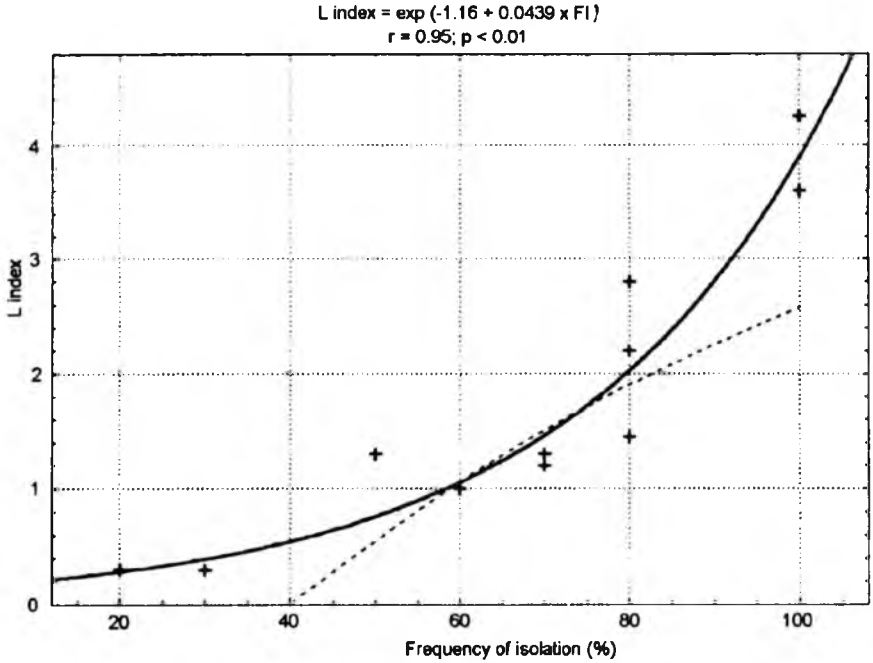

Fig. 1. Correlation between L index (L) and frequency of isolation of keratinolytic fungi (FI)

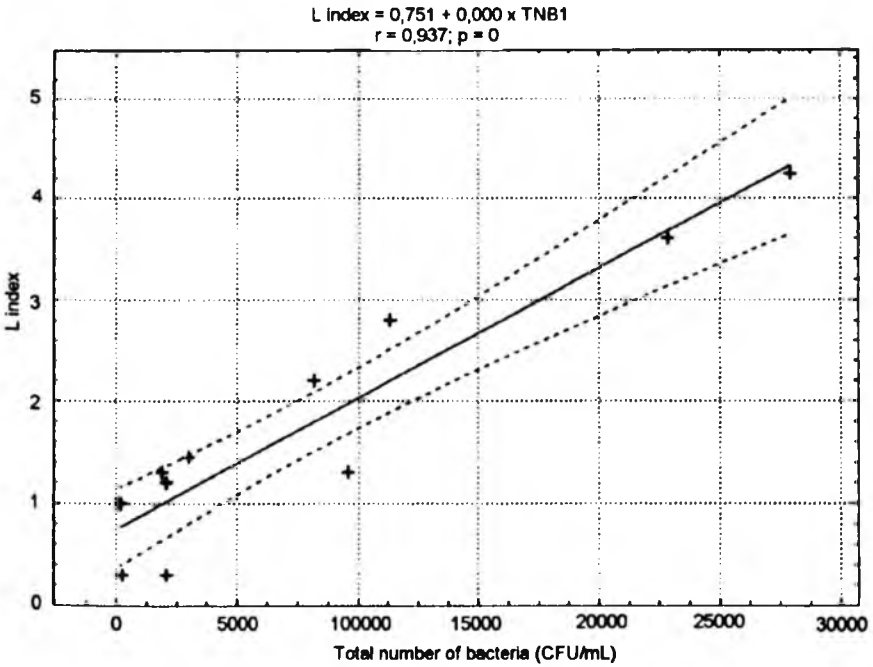

Fig. 2. Correlation between L index (L) and total number of water bacteria (TNB_1)

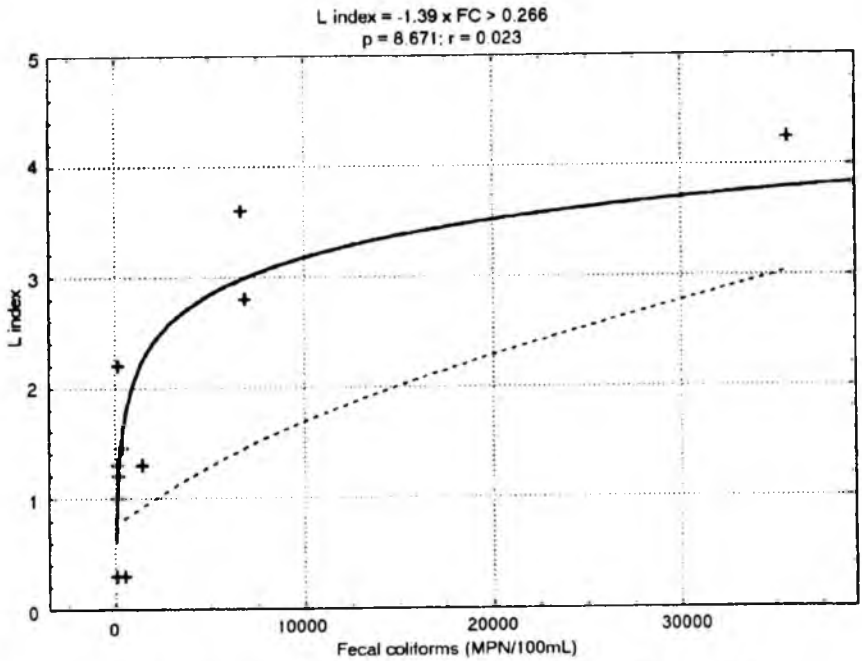

Fig. 3. Correlation between L index (L) and fecal coliforms (FC)

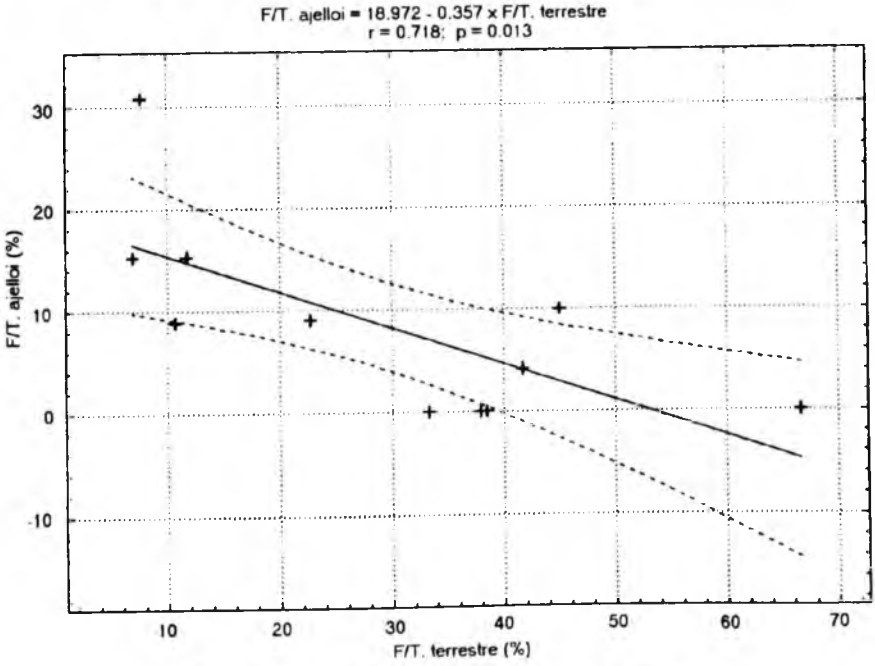

Fig. 4. Correlation between *F/T. ajelloi* and *F/T. terrestre*

Table III. Correlation coefficients for mycological and bacteriological data [organic matter content (OM) values for sediments are also included]

Mycological indices	TC	FC	TNB_1	MB	TNB_2	TNF	OM
FI	0,49	0,52	0,70*	0,65*	0,51	0,69*	0,60
NS	0,24	0,32	0,58	0,47	0,59	0,55	0,45
L	0,73*	0,77**	0,93**	0,89**	0,78c**	0,81**	0,63*
F/T,aj,	0,35	0,31	0,55	0,47	0,30	0,21	0,27
F/T,ter,	−0,43	−0,45	−0,73**	−0,63*	−0,58	−0,54	−0,52
F/A,q,	−0,27	−0,30	−0,41	−0,35	−0,56	−0,42	−0,34
F/Ch,ind,	−0,10	−0,08	−0,01	−0,05	−0,06	−0,26	−0,05
F/Ch,ker,	0,67	0,74**	0,85**	0,81**	0,90**	0,70*	0,47
F/Ch,pan,	0,47	0,51	0,82**	0,70*	0,55	0,65*	0,66*
F/Ch,A,ful,	−0,05	−0,05	0,0	−0,01	0,32	0,18	0,26
F/A,ful,	0,11	0,09	0,44	0,31	0,28	0,23	0,36

* – $p < 0.05$
** – $p < 0.01$

Abbreviations:
TC – total coliforms (MPN/100 mL)
FC – fecal coliforms (MPN/100 mL)
TNB_1 – total number of water bacteria (CFU/mL)
MB – number of mesophilic bacteria (CFU/mL)
TNB^2 – total number of sediment bacteria (CFU/g dry weight)
TNF – total number of fungi (CFU/g dry weight)
OM – organic matter content in sediments (%)
FI – frequency of isolation of keratinolytic fungi (%)
NS – number of species isolated
L – L index (a number of fungal appearances divided by the number of *Petri* dishes set up)
F/*T. ajelloi* – frequency of *Trichophyton ajelloi* isolation
F/*T. terrestre* – frequency of *T. terrestre* isolation
F/*A. quadrifidum* – frequency of *Arthroderma quadrifidum* isolation
F/*Ch. indicum* – frequency of *Chrysosporium indicum* isolation
F/*Ch. keratinophilum* – frequency of *Ch. keratinophilum* isolation
F/*Ch. pannicola* – frequency of *Ch. pannicola* isolation
F/*Ch. A. fulvescens/reticulosporus* – frequency of *Ch. anamorph* of *Aphanoascus fulvescens/reticulosporus* isolation
F/*A. fulvescens/reticulosporus* – frequency of *A. fulvescens/reticulosporus* isolation

DISCUSSION

In a general survey of keratinolytic fungi from superficial waters and sewage systems [8], it was found that the occurrence of these micro-organisms in sediments depends on the degree of sewage contamination in water. Our statistical evaluation of the data obtained for two dam reservoirs clearly supports this observation at both quantitative and qualitative points. It also provides us with some additional findings possibly of cognitive significance. Among the general quantitative indices, L index is applied better than the previously used LPP index. The latter has a narrower range, and it shows the density of fungal appearances in samples positive for keratinolytic fungi but does not characterize all the samples (*Petri* dishes) under examination. It is also clear that FI and

NS indices do not depend on the degree of water pollution to such an extent as the L index does. Thus, the latter has certainly appeared to be the most reliable quantitative index in the assessment of keratinolytic fungal populations in sediments.

L index shows the best correlations with DOC and pH. Since the values of pH and DOC considerably increased, and since the L index values decreased during the self-purification processes in the water of two dam reservoirs under examination, the relationships found are quite understandable.

It was demonstrated in a previous study [8] that on hair laid in samples from unpolluted and only slightly polluted waters, fungal populations were small, with monoculture of *Trichophyton terrestre* and its teleomorph *Arthroderma quadrifidum* frequently occurring. Rarely were other fungi isolated from these samples. Intensive growth of *T. ajelloi* and *Chrysosporium*, in particular *Ch. keratinophilum*, was found to be characteristic for polluted sediments. *Ch. pannicola*, *Aphanoascus fulvescens/reticulosporus* (anam + teleom.), *Microsporum gypseum* and *M. cookei* were also isolated more frequently from polluted samples. Our statistical evaluation has confirmed the association of *T. terrestre* with unpolluted and only slightly polluted waters, as well as *T. ajelloi* and *Chrysosporium* with polluted aqueous environments. In addition, it has revealed that *T. ajelloi* and *A. fulvescens/reticulosporus* depend on the total input of readily degradable organic matter (BOD_5) into superficial waters, when *Ch. keratinophilum* and, though to a smaller degree, *Ch. pannicola* are better correlated with other physico-chemical parameters (WT, DOC, NNO_2, pH, ALK). Interestingly, the species dependent on BOD_5 are not correlated with microbiological data, whereas those associated with many physico-chemical parameters also show very good correlations with microscopic fungi and bacteria, including those of fecal origin. Since the factors influencing fungal survival, transport and growth in aqueous environments are practically unknown, the relationships showed are difficult to explain. However, *Ch. keratinophilum* together with *Ch.* and *A. fulvescens/reticulosporus* were found to be characteristic for municipal-industrial sludges in our region. Rarely did geophilic dermatophytes prevail in the sludges [10, 11]. This may indicate that *Ch. keratinophilum* is the species particularly associated with sewage inoputs to superficial waters. *T. ajelloi*, *T. terrestre* and *A. fulvescens/reticulosporus* behave differently in the waters, displaying the dependence chiefly on organic matter content and, supposedly, soil or runoff origin. More comprehensive ecological data ought to be obtained to thoroughly confirm and explain these statistical relationships. Our results can be useful in application of keratinolytic fungi as one of the bioindicators of water pollution.

K. Ulfig

STATYSTYCZNA OCENA WYSTĘPOWANIA GRZYBÓW KERATYNOLITYCZNYCH W OSADACH DENNYCH DWÓCH ZBIORNIKÓW ZAPOROWYCH

Summary

Osady pochodzące z dwóch zbiorników zaporowych, "Przeczyce" i "Pławniowice", badane były pod względem występowania grzybów keratynolitycznych. Otrzymane wyniki wraz z danymi fizyko-chemicznymi i mikrobiologicznymi dla wód oraz osadów dennych poddane zostały analizie statys-

tycznej. Analiza ta wyraźnie potwierdziła nasze wcześniejsze obserwacje dotyczące zależności występowania grzybów keratynolitycznych w osadach dennych od stopnia zanieczyszczenia wód ściekami. Wskazała również, że *Ch. keratinophilum* jest gatunkiem w sposób szczególny związanym ze zrzutem ścieków do wód powierzchniowych. *T. ajelloi, T. terrestre* i *A. fulvescens/reticulosporus* odmiennie "zachowują się" w tych wodach, wykazując zależność od łatwo przyswajalnej materii organicznej (BZT_5) i przypuszczalnie glebowe pochodzenie. Istnieje potrzeba dalszych laboratoryjnych i terenowych badań, aby potwierdzić i wyjaśnić otrzymane zależności.

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