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Diversity of soil microscopic fungi on abandoned industrial deposits

*Alena KUBÁTOVÁ *, Karel PRÁŠIL & Marie VÁŇOVÁ*

Department of Botany, Charles University, Benátská 2,
128 01 Praha 2, Czech Republic
kubatova@natur.cuni.cz

Abstract – Diversity of saprotrophic soil micromycetes was studied in the period 1993-95 on an abandoned ore-washery settling pit near Chvaletice and on an ash-slag settling pit near Opatovice in the eastern Bohemia (Czech Republic). Deposits on both localities contain higher amounts of metals (esp. Mn and Zn). Despite of a rather toxic character of the studied sites, altogether 108 taxa of soil microfungi were recorded (70 species of *Deuteromycetes*, 25 of *Zygomycota*, and 13 of *Ascomycota*). The most frequent soil microfungi were *Penicillium janthinellum*, *P. simplicissimum*, *Cunninghamella elegans*, *Paecilomyces lilacinus, Trichoderma* spp., *Mucor hiemalis* f. *hiemalis, Coniothyrium fuckelii, Mortierella alpina, Fusarium* spp., *Coemansia aciculifera,* and *Trichoderma virens*. Four fungi, new in the Czech Republic, were discovered: *Gelasinospora calospora*, *Rhopalomyces elegans* var. *apiculatus*, *Syncephalis sphaerica*, and *Westerdykella dispersa*. Some specimens of soil micromycetes are maintained in the Culture Collection of Fungi (CCF), Prague.

soil micromycetes / fungal diversity / toxic substrata / polluted soils / fire / Czech Republic

INTRODUCTION

Many industrial activities lead to artificial redistribution of waste toxic materials in the terrestrial environment and to development of anthropogenic soils. The number of sites with contaminated soils is increasing in the world. The dynamics of succession on these substrata is generally slow because the organisms are stressed by additional unfavourable factors. Fungi are significant component of the soil biota responsible for soil fertility. For this reason, changes of fungal biodiversity during the succession on these polluted soils have been studied (*e.g.* Lebedeva & Lugauskas, 1985; Nordgren *et al.*, 1985; Arnebrant *et al.*, 1987; Fritze & Bååth, 1993; Kowalik, 1995) as well as fungal response to high concentration of individual toxic metals (*e.g.* copper, cadmium, zinc) or organic compounds as phenols (Timonin *et al.*, 1972; Ross, 1975; Marfenina & Lukina, 1989; Gadd, 1993; Morley & Gadd, 1995; López-Llorca & Olivares-Bernabéu, 1997).

On the territory of the Czech Republic many sites influenced considerably by industrial activities can be found. On several localities, microfungal communities were studied, *e.g.* in uranium-mine galleries (Fassatiová, 1970), and in coal-mine dumps (Černý, 1985; Bečvář, 1998). However, no reports on micromycetes in soils polluted with metals are available from this region.

* Correspondence and reprints.

Settling pits as consequence of manganese-pyrite mining near Chvaletice and ash-slag settling pits near coal power station Opatovice in eastern Bohemia can serve as examples of long-term disposal of wastes with higher content of metals. Both localities remained without artificial water input and uncovered. This situation enabled the long-term studies of plant succession in this „natural laboratory“ (Kovář, 1979). The present study is a part of a collaborative programme investigating the biodiversity and biotic interactions during vegetation succession on toxic materials (Kovář & Hroudová, 1996; Kovář, 1999). In the field of mycology, several papers were published. Preliminary results dealing with ecological role of macromycetes on settling pit at Chvaletice were published by Holec (1995). Vosátka et al. (1999) investigated arbuscular mycorrhizal colonization at Chvaletice and Opatovice. Požárová *et al.* (2001) presented results of a study of rhizosphere mycobiota on Chvaletice locality. Results of investigation of coprophilous fungi and parasitic micromycetes are being prepared (Váňová & Kubátová; Marková; resp.). The aims of the present study have been to reveal the overall diversity of soil saprotrophic microfungi, to compare fungal communities on different types of successional series and to find out which fungi are the most stress-tolerant.

MATERIALS AND METHODS

Localities and study sites (Fig. 1)

The locality **Chvaletice** is situated on the left bank of the Elbe river, at an altitude of ca. 200 m, close to the former surface quarry in the northern part of Železné hory hills in Eastern Bohemia (Czech Republic). The mining and processing of Fe-Mn pyrite used for sulphuric acid production lead into the production of waste material containing toxic substances, which were hydraulically transported and accumulated into sedimentation basins. The mining and other activities started in 1952 and ended in mid-seventies. The surface of studied ore-washery settling pit was not reclaimed, the locality was abandoned and the sedimentation pond was spontaneously recolonized.

The dominant mineral is jarosit. In the acidic ore deposits in Chvaletice, sulphides of Fe, Mn, Zn and oxides of Fe and Al were recorded in higher amount (see Table 1). Concentrations of Mn and Zn are by two orders of magnitude higher than average values given for arable soils, contents of Fe and Al are elevated, however the contents of Cu, Cd and Pb are not too high (after Rauch in Kovář & Hroudová, 1996). Compared to natural soils, the content of alkaline elements is higher (see Table 1).

In the course of the year, some stressing factors for plant and microbial succession have been appeared: a high fluctuation of soil temperature and pH, acidification and secondary salinization. For example, measurements of pH showed strong horizontal and vertical variability within the locality: pH 4.5 - 8.5 (after Rauch in Kovář & Hroudová, 1996). On plots CH3, CH4 and CH5 (see bellow), values pH 5.5 - 6.9 were taken (after Rydlová in Kovář & Hroudová, 1996). A lack of basic nutrients is one of the stressing factors on both localities.

Five successional series have developed on this locality (see bellow). Species richness of vascular plants seems to be low: 35 species of vascular plants were recorded and 4 species of bryophytes (Kovář, 1999).

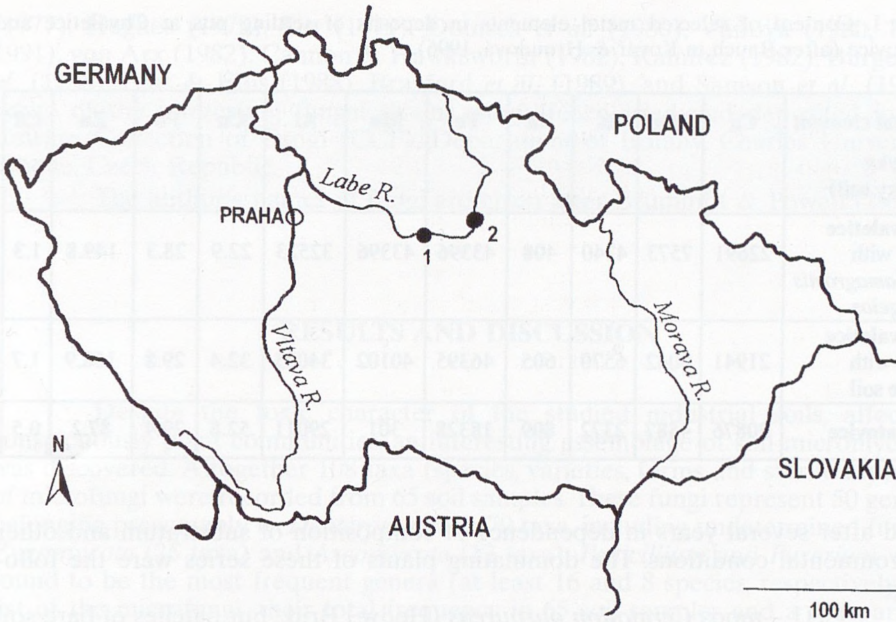

Fig. 1. Map of the Czech Republic showing studied localities (1 – Chvaletice, 2 – Opatovice).

The locality **Opatovice nad Labem** is in the same region, ca 40 km far the locality Chvaletice, at an altitude of ca. 230 m. The coal power station Opatovice produces flying ash which is also hydraulically transported and sedimented in settling pits. One abandoned dry sedimentation pond, which had been revegetated by mixture of grasses, was chosen for the comparison with that in Chvaletice. The accumulation of the ash in this sedimentation pond started at the end of the fifties and ended at the end of the eighties. The deposited material represents a more basic, less toxic type of substratum with lower content of metals than that at Chvaletice (see Table 1). Average values of pH were in the range of 7.9-8.4 (after Rydlová in Kovář & Hroudová, 1996).

A higher species richness of vascular plants was recorded on this locality in comparison with the previous one: 52 species and 2 species of bryophytes (Kovář, 1999).

The mean annual temperature for the studied region is 8.5 °C, with an annual precipitation of 617 mm and 1622 h of sunshine (Czech Hydrometeorological Institute 2000).

Sampling, cultivation and identification

For a study of saprotrophic fungi sixty-five samples of soil were collected from the industrial deposits at Chvaletice and Opatovice during the period of 1993-95. Sampling was made quarterly. The majority of the soil samples were obtained as a mixture from three sites in the depth of five centimetres.

At **Chvaletice**, fifty-two soil samples from five different successional series were collected. The successional series represent vegetation gradient, deve-

Table 1. Content of selected metal elements in deposits of settling pits at Chvaletice and Opatovice (after Rauch in Kovář & Hroudová, 1996).

metal element (mg/kg of dry soil)	**Ca**	**Mg**	**K**	**Na**	**Fe**	**Mn**	**Al**	**Cu**	**Pb**	**Zn**	**Cd**
Chvaletice site with *Calamagrostis epigeios*	22691	7573	4340	408	43396	43396	32523	22.9	28.3	149.8	1.3
Chvaletice site with bare soil	21941	8042	6570	605	46395	40102	34023	32.4	29.8	168.9	1.7
Opatovice	20876	4587	2372	809	18328	301	29011	52.8	29.4	87.2	0.5

loped after several years in dependence of composition of substratum and other environmental conditions. The dominating plants of these series were the following:

CH1 – moos *Ceratodon purpureus* (Hedw.) Brid., but patches of bare soil were prevailing,

CH2 – solitary tufts of *Calamagrostis epigeios* (L.) Roth on bare soil,

CH3 – lichens, mosses and *Calamagrostis epigeios*,

CH4 – lichens, mosses, *Calamagrostis epigeios* and young individuals of *Betula pendula* Roth and *Populus tremula* L.,

CH5 – *Calamagrostis epigeios* and older woody species dominated by *Betula pendula* and *Populus tremula* (trees ca. 3 m high).

From the locality **Opatovice** only thirteen samples were examined. They were collected randomly from three types of sites:

– from deposits under *Calamagrostis epigeios, Festuca rubra* L. or *Phleum pratense* L.,

– under mosses *Funaria hygrometrica* Hedw. or *Bryum argenteum* Hedw.,

– from bare soil.

In a laboratory, both soil dilution method and direct inoculation of soil were applied. Different nutrient agar media were used for isolation: soil agar with rose Bengal and glucose, Sabouraud´s agar, corn-meal agar, wort-beer agar, and potato-carrot agar. Streptomycin was added to all media (0.1 g/l). The isolation of thermotolerant micromycetes was made after heat treatment of samples (for one hour at 55 °C). Petri dishes were incubated in the dark at 25 °C and in a refrigerator at 5-10 °C. For the isolation of some other micromycetes, a technique of moist chambers was used: sterile excrements of rabbit and sterile pieces of nails were placed on collected moistened soil samples in Petri dishes. These Petri dishes were incubated on day light at laboratory temperature. After several days visible colonies both from agar plates and moist chambers were transferred to identification agars (Czapek yeast extract agar, malt extract agar, soil extract agar and others previously cited).

Identification of saprotrophic microfungi was made according to Raper & Thom (1949), Ellis (1963), Cejp & Milko (1964), von Arx & Storm (1967), Zycha *et al.* (1969), Gams (1969, 1971, 1976, 1977), Ellis (1971, 1976), Lundquist (1972), Hennebert (1973), Milko (1974), Schipper (1975, 1976, 1978, 1984), de Hoog

(1977), Hughes (1979), Pitt (1979), Domsch *et al.* (1980), Váňová (1980, 1989, 1991), von Arx (1982), Cannon & Hawksworth (1982), Ramírez (1982), Burgess *et al.* (1988), Ellis & Ellis (1988), Brayford *et al.* (1989), and Samson *et al.* (1996). Some of the interesting fungal strains were freeze-dried and deposited in the Culture Collection of Fungi (CCF), Department of Botany, Charles University, Prague, Czech Republic.

The author´s names of fungi are given after Brummitt & Powell (1992).

RESULTS AND DISCUSSION

Despite the toxic character of the studied industrial soils, affecting conspicuously plant communities, an interesting assemblage of soil micromycetes was discovered. Altogether 108 taxa (species, varieties, forms and sterile mycelia) of microfungi were recorded from 65 soil samples. These fungi represent 50 genera belonging presumably to *Deuteromycetes* (70 taxa, including undetermined fungi), *Zygomycota* (25 taxa) and *Ascomycota* (13 taxa). *Penicillium* and *Fusarium* were found to be the most frequent genera (at least 16 and 8 species, respectively). A list of the microfungi, their total frequency in 65 soil samples and a comparison among studied sites are given in the Table 2. The majority of the cited species were rarely isolated. The most frequent species were *Penicillium janthinellum* Biourge (in 49 % of all samples), *P. simplicissimum* (Oudem.) Thom (43 %), *Cunninghamella elegans* Lendn. (43 %), *Paecilomyces lilacinus* (Thom) Samson (38 %), *Mucor hiemalis* Wehmer f. *hiemalis* (28 %), *Trichoderma* spp. (28 %), *Coniothyrium fuckelii* Sacc. (23 %), *Mortierella alpina* Peyronel (23 %), *Coemansia aciculifera* Linder (20 %), and *Trichoderma virens* (J.H. Miller *et al.*) Arx (20 %). On all studied sites the following fungi were found: *Alternaria alternata* (Fr.: Fr.) Keissl., *Cladosporium cladosporioides* (Fresen.) de Vries, *Cunninghamella elegans, Paecilomyces lilacinus,* and *Penicillium simplicissimum.* Due to difficulties with identification, some strains were not determined to the species level (e.g. *Penicillium* spp., *Trichoderma* spp. and *Fusarium* spp.).

Borovec (1988) cited, that *P. simplicissimum* is able to decompose sulphide minerals. After Illmer *et al.* (1995), *P. simplicissimum* is very effective in solubilizing hardly-soluble $AlPO_4$. Morphologically related species *P. ochrochloron* Biourge is cited as extremely copper-tolerant fungus. According to our preliminary results, *P. simplicissimum* is Cu-tolerant, too. This species was frequently isolated from the dump of copper ore at Špania dolina, Slovakia (Kubátová, unpublished). *P. simplicissimum* was also found with high frequency in substratum of coal-mine dumps in Bohemia (Bečvář, 1998). After many authors (*e.g.* Morley *et al.*, 1996; Sayer *et al.*, 1995; Gallmetzer & Burgstaller, 1998), *P. simplicissimum* can be applied for leaching of metals from industrial waste due its citrate excretion. It seems to be a typical species of industrial soils. Our most frequent species, *P. janthinellum*, is morphologically very close to *P. simplicissimum.* Both species are considered to be conspecific by several authors (Stolk & Samson, 1983; Frisvad & Filtenborg, 1990). However, in this study we have used the narrow species concept according to Raper & Thom (1949) and partly according to Pitt (1979).

Another common species, *Paecilomyces lilacinus*, is also known to be tolerant to high copper content in the soil (Yamamoto *et al.*, 1985). It was found to be tolerant to cadmium (Tatsuyama *et al.*, 1975; Marfenina & Lukina, 1989) and

Table 2. List of soil micromycetes isolated from industrial deposits of settling pits at Chvaletice and Opatovice with frequency of their occurrence.

Locality	Chvaletice - five sites					Opato-vice	Total frequency (%)*
	CH1	CH2	CH3	CH4	CH5		
No. of samples	11	9	11	11	10	13	
Absidia coerulea Bainier **var. coerulea**	-	-	-	27	40	-	**11**
A. coerulea Bainier **var. saccardoi** (Oudem.) Váňová	-	-	9	-	10	8	**5**
A. glauca Hagem **CCF 2842****	-	-	9	9	10	-	**5**
Acremonium murorum (Corda) W. Gams	-	-	-	-	10	-	**2**
Acremonium spp.	-	11	-	-	-	8	**3**
Alternaria alternata (Fr.:Fr.) Keissl.	9	33	9	9	20	8	**14**
Arthrinium arundinis (Corda) Dyko & B.Sutton	9	11	45	-	10	-	**12**
A. phaeospermum (Corda) M.B.Ellis	-	11	-	9	-	8	**5**
Aspergillus fumigatus Fresen.	-	-	-	-	-	23	**5**
A. versicolor (Vuill.) Tirab.	9	-	-	-	-	-	**2**
Cladosporium cladosporioides (Fresen.) de Vries	9	11	18	9	10	31	**15**
C. herbarum (Pers.:Fr.) Link	-	11	27	9	10	15	**14**
C. sphaerospermum Penz.	-	11	-	18	10	8	**8**
Cladosporium sp.	-	-	-	-	-	8	**2**
Coemansia aciculifera Linder	-	-	45	45	30	-	**20**
Coniothyrium fuckelii Sacc. **CCF 3177****	18	56	45	-	10	15	**23**
Cunninghamella echinulata (Thaxter) Thaxter	9	11	18	18	10	-	**11**
C. elegans Lendn.	55	44	64	55	40	8	**43**
Doratomyces stemonitis (Pers.:Fr.) F.J.Morton & G. Sm.	9	-	-	-	10	8	**5**
Drechslera sorokiniana (Sacc.) Subram. & B.L.Jain	-	11	-	-	-	-	**2**
Emericellopsis terricola J.F.H.Beyma **CCF 2853****	-	-	-	-	-	8	**2**
Epicoccum nigrum Link	-	-	9	-	-	8	**3**
Fusarium avenaceum (Fr.) Sacc.	-	-	-	-	-	8	**2**
F. culmorum (W.G. Sm.) Sacc.	-	11	9	-	-	-	**3**
F. graminearum Schwabe	-	11	-	-	-	-	**2**
F. oxysporum Schlecht. **CCF 3182****	-	33	-	-	10	8	**8**
F. solani (Mart.) Sacc. **CCF 3181****	-	-	9	-	10	-	**3**
F. sporotrichioides Sherb.	-	-	-	-	-	8	**2**
F. tricinctum (Corda) Sacc.	-	11	-	-	-	-	**2**
F. verticillioides (Sacc.) Nirenberg	-	11	9	-	-	-	**3**
Fusarium spp.	9	-	-	9	10	15	**8**
Gelasinospora calospora (Mouton) C. & M. Moreau **CCF 3141****	-	-	-	-	10	-	**2**
G. tetrasperma Dowding **CCF 2954****	-	-	-	-	10	-	**2**
Geomyces sp.	9	-	-	-	-	8	**3**
Gliocladium catenulatum J.C.Gilman & E.V.Abbot	-	-	27	27	50	-	**17**
G. roseum Bainier	-	-	-	27	20	-	**8**
Hormoconis resinae (Lindau) Arx & de Vries	9	-	-	-	-	-	**2**
Chaetomium spp.	-	-	-	-	-	15	**3**

* frequency was assessed in % by the number of samples in which a species occurred.
** strain maintained in the Culture Collection of Fungi (CCF), Prague.

Locality / No. of samples	Chvaletice - five sites CH1 11	CH2 9	CH3 11	CH4 11	CH5 10	Opato-vice 13	Total frequency (%)*
Mammaria sp.	-	-	-	9	10	-	**3**
Mariannaea elegans (Corda) G.Arnaud ex Samson	-	-	-	-	10	-	**2**
Microdochium bolleyi (Sprague) de Hoog & Herm.-Nijh. **CCF 2849****	-	-	-	-	-	23	**5**
Micromucor isabellinus (Oudem.) Arx	-	-	-	-	10	-	**2**
M. ramannianus (Moeller) Arx **var. angulisporus** Naumov ex Váňová	-	-	-	-	10	-	**2**
M. ramannianus (Moeller) Arx **var. ramannianus**	-	-	9	9	10	-	**5**
Mortierella alpina Peyronel **CCF 2861, 2873****	-	-	55	36	20	23	**23**
M. elongata Linnem. **CCF 2862, 2865, 2866****	-	11	45	27	10	-	**15**
M. exigua Linnem.	-	-	9	-	-	-	**2**
M. gemmifera W.Gams	-	-	-	9	-	-	**2**
Mortierella sp.	-	-	27	18	-	-	**8**
Mucor hiemalis Wehmer **f. corticolus** (Hagem) Schipper	-	22	18	18	10	15	**14**
M. hiemalis Wehmer **f. hiemalis**	-	56	36	36	40	8	**28**
M. hiemalis Wehmer **f. luteus** (Linnem.) Schipper	-	-	9	-	-	-	**2**
M. hiemalis Wehmer **f. silvaticus** (Hagem) Schipper	-	11	-	-	-	-	**2**
M. mucedo Fresen. **CCF 2893****	-	-	-	9	-	8	**3**
M. wosnessenskii Schostak.	-	-	-	-	-	8	**2**
Myrothecium roridum Tode: Fr.	-	-	-	-	-	15	**3**
Paecilomyces lilacinus (Thom) Samson	36	33	91	45	10	15	**38**
Paecilomyces sp.	-	-	-	9	-	-	**2**
Penicillium adametzii K.M.Zalessky	-	-	-	18	-	-	**3**
P. brevicompactum Dierckx	9	-	-	-	-	8	**3**
P. canescens Sopp	-	-	-	9	-	-	**2**
P. daleae K.M. Zalessky	-	-	-	-	20	-	**3**
P. funiculosum Thom	-	-	9	-	-	-	**2**
P. glandicola (Oudem.) Seifert & Samson	-	-	9	-	-	-	**2**
P. janthinellum Biourge	91	100	64	45	-	8	**49**
P. manginii Duché & R. Heim	-	-	-	-	10	-	**2**
P. miczynskii K.M.Zalessky	-	-	-	9	-	-	**2**
P. pulvillorum Turfitt	-	-	27	9	-	-	**6**
P. purpurogenum Stoll **var. rubrisclerotium** Thom	9	11	-	9	10	-	**6**
P. restrictum J.C.Gilman & E.V.Abbott	-	-	-	9	20	-	**5**
P. simplicissimum (Oudem.) Thom	55	22	64	73	40	8	**43**
P. thomii Maire	-	-	-	18	30	-	**8**
P. varians G. Smith	-	11	-	-	-	-	**2**
P. verruculosum Peyronel	-	-	-	9	10	-	**3**
Penicillium spp.	18	22	18	-	-	-	**9**

* frequency was assessed in % by the number of samples in which a species occurred.
** strain maintained in the Culture Collection of Fungi (CCF), Prague.

Locality	Chvaletice - five sites					Opato-vice	Total frequency (%)*
	CH1	CH2	CH3	CH4	CH5		
No. of samples	11	9	11	11	10	13	
Phoma spp.	9	11	18	9	-	39	**15**
Phomopsis sp.	-	11	9	-	-	-	**3**
Pseudeurotium ovale Stolk	-	-	-	-	-	8	**2**
Pyrenochaeta sp.	-	-	9	-	-	-	**2**
Ramichloridium schulzeri (Sacc). de Hoog var. **schulzeri**	-	-	-	-	-	8	**2**
Rhizopus arrhizus Fisch.	-	-	-	9	-	31	**8**
R. stolonifer (Ehrenb.:Fr.) Vuill.	18	-	9	-	20	-	**8**
Rhopalomyces elegans Corda **var. apiculatus** J.J. Ellis	-	-	9	-	10	-	**3**
Sagenomella sp.	-	-	-	9	10	-	**3**
Sordaria macrospora Auersw.	-	11	-	-	-	-	**2**
Sphaerodes fimicola (E.C.Hansen) P.F.Cannon & D.Hawksw.	9	11	-	-	30	-	**8**
Sporidesmium hyalospermum (Corda) S.Hughes	-	11	-	9	10	-	**5**
Sporormiella intermedia (Auersw.) S.I.Ahmed & Cain	-	-	-	-	-	8	**2**
Stachybotrys atra Corda	-	-	9	-	-	23	**6**
sterile dark mycelium	9	11	27	-	-	31	**14**
sterile light mycelium	9	-	-	-	-	8	**3**
Stilbella sp.	-	-	-	-	10	-	**2**
Syncephalis sphaerica Tiegh.	-	-	-	-	10	-	**2**
Syncephalis sp.	-	-	9	-	-	-	**2**
Talaromyces flavus (Klőcker) Stolk & Samson	-	-	-	18	50		**11**
Talaromyces sp.	-	-	-	-	-	8	**2**
Trichoderma koningii Oudem.	-	-	-	9	-	-	**2**
Trichoderma harzianum Rifai	9	-	27	18	30	8	**15**
Trichoderma virens (J.H.Mill. et al.) Arx **CCF 2848****	-	22	9	45	40	8	**20**
Trichoderma spp.	-	22	27	45	60	15	**28**
Trichophaea abundans (P.Karst.) Boud., anam. **Dichobotrys abundans** Hennebert **CCF 3079****	-	-	-	9	-	8	**3**
Tritirachium sp.	-	-	9	-	-	-	**2**
Ulocladium botrytis Preuss	-	-	-	-	10	-	**2**
undetermined sclerotial fungus	-	-	9	-	-	-	**2**
Verticillium psalliotae Treschew	18	33	36	-	20	8	**19**
Verticillium sp.	-	-	-	9	-	-	**2**
Westerdykella dispersa (Clum) Cejp & Milko **CCF 3088****	9	-	-	-	-	-	**2**
W. multispora (Saito & Minoura) Cejp & Milko **CCF 2870****	-	-	-	-	-	8	**2**
Total No. of species: 108	**25**	**35**	**44**	**45**	**51**	**46**	

* frequency was assessed in % by the number of samples in which a species occurred.
** strain maintained in the Culture Collection of Fungi (CCF), Prague.

was commonly isolated from soils polluted with sulphides, too (Lebedeva & Lugauskas, 1985). On the other hand, Levinskaite (1999) revealed *P. lilacinus* to be sensitive to cobalt, nickel, tin and zinc.

Coniothyrium fuckelii belongs after Timonin *et al.* (1972) among the manganese-oxidizing fungi. *Mortierella alpina* was isolated with high frequency from soil polluted by alkaline deposition (Fritze & Bååth, 1993). Another repeatedly isolated fungus in our study was *Verticillium psalliotae* Treschew. Murphy & Levy (1983) recorded in this species production of calcium oxalate, which can bind copper. Arnebrandt *et al.* (1987) consider this fungus as very tolerant to the effect of copper.

We consider the above cited most frequent micromycetes to be tolerant to the stress factors of the studied habitat, predominantly to the effect of higher amounts of metals in substratum. However, these fungi can not be considered as universal stress tolerant microfungi. This view is supported for instance by Levinskait? (1999) who tested 26 soil micromycetes for heavy metal tolerance (7 metals) and did not found any fungus tolerant to all metals tested.

Many microfungi are not only tolerant to higher content of metals in soil but they are able to effectively bind metals. Especially, chitin and chitosan and melanins located in cell walls possess a high biosorptive capacity for a variety of metal ions (Gadd, 1993; Fogarty & Tobin, 1996). In this study, we isolated besides known melanized fungi (e.g. *Alternaria, Cladosporium*) many dark sterile mycelia, namely at Opatovice and in initial successional series at Chvaletice (CH1 and CH2). Požárová *et al.* (2001) found many dark sterile mycelia associated with roots of *Calamagrostis epigeios* at Chvaletice, too. These dark pigmented microfungi probably play an important role in reducing level of the metal ions in soil and detoxification of soil environment.

The toxicity and availability of the metals for micromycetes and other organisms in soil is dependent on other factors, especially on pH and on solubility of metal salts. For example, fungitoxicity of Cd may increase with increasing pH, while toxicity of Ni may decrease with increasing pH (Gadd, 1993). In our study, no additional experiments were made with the fungi isolated and metals, and therefore it is not possible to evaluate an influence of the individual metals on individual fungal species.

Comparing two localities studied, altogether 91 taxa of soil mycobiota were recorded on five studied sites at locality Chvaletice, and 44 taxa were isolated at locality Opatovice. In this case, a lower quantity of examined samples may play certain role besides the different kind of deposits and earlier stage of succession. Overall data on the structure of soil fungal communities on studied sites at Chvaletice and Opatovice are given in the Table 3. Comparing the observed sites, it has been found that the number of recorded microfungal taxa at Chvaletice are increasing with abundance of vascular plants. On each of the successional series at Chvaletice, one microfungal species was dominating, with increasing quantity of other frequent species and rare taxa, too. On the sites CH4 and CH5 with young trees, *Trichoderma* spp. are playing important role due to the great amount of leaf litter on these plots. It is also the most possible reason for an increased fungal diversity. Vosátka *et al.* (1999) investigated mycorrhizal colonization of *Calamagrostis epigeios* and they found the higher mycorrhizal colonization on the site CH3 then on sites CH4 and CH5.

At the locality Opatovice, the samples of deposits were collected from three different types of sites (bare soil, soil under mosses and soil under grasses). However, no distinct differences were observed in species number between these sites, probably due to a low number of samples. No distinct dominant was obser-

Table 3. Structure of soil microfungal communities at Chvaletice (CH1-5) and Opatovice.

Site		The most frequent taxon (100-70 %)	Other frequent taxa (65-30 %)		No. of rare taxa (28-9 %)	Total no. of taxa
CH1		Penicillium janthinellum 91 %	Cunninghamella elegans	55 %	21	25
			Penicillium simplicissimum	55 %		
			Paecilomyces lilacinus	36 %		
CH2		Penicillium janthinellum 100 %	Coniothyrium fuckelii	56 %	27	35
			Mucor hiemalis f. hiemalis	56 %		
			Cunninghamella elegans	44 %		
			Alternaria alternata	33 %		
			Fusarium oxysporum	33 %		
	v		Paecilomyces lilacinus	33 %		
	v		Verticillium psalliotae	33 %		
CH3	v	Paecilomyces lilacinus 91 %	Cunninghamella elegans	64 %	33	44
			Penicillium janthinellum	64 %		
	increasing abundance of plants		Penicillium simplicissimum	64 %		
			Mortierella alpina	55 %		
			Arthrinium arundinis	45 %		
			Coemansia aciculifera	45 %		
			Coniothyrium fuckelii	45 %		
			Mortierella elongata	45 %		
			Mucor hiemalis f. hiemalis	36 %		
			Verticillium psalliotae	36 %		
CH4		Penicillium simplicissimum 73 %	Coemansia aciculifera	55 %	36	45
			Cunninghamella elegans	45 %		
			Trichoderma spp.	45 %		
			Paecilomyces lilacinus	45 %		
			Penicillium janthinellum	45 %		
			Trichoderma virens	45 %		
			Mortierella alpina	36 %		
	v		Mucor hiemalis f. hiemalis	36 %		
CH5	v	–	Trichoderma spp.	60 %	39	51
	v		Gliocladium catenulatum	50 %		
			Talaromyces flavus	50 %		
			Absidia coerulea	40 %		
			Cunninghamella elegans	40 %		
			Mucor hiemalis f. hiemalis	40 %		
			Penicillium simplicissimum	40 %		
			Trichoderma virens	40 %		
			Coemansia aciculifera	30 %		
			Penicillium thomii	30 %		
			Sphaerodes fimicola	30 %		
			Trichoderma harzianum	30 %		
Opatovice		–	Phoma spp.	39 %	42	46
			Cladosporium cladosporioides	31 %		
			Rhizopus arrhizus	31 %		
			sterile dark mycelium	31 %		

* frequency was assessed in % by the number of samples in which a species occured.

ved, either. The assemblage of other frequent fungi isolated from this locality is somewhat different from that one found at Chvaletice (see Table 3). It is obvious that both different soil structure and composition and different plant association affect it.

The effect of an accidental fire was also studied at the locality Chvaletice. The fire occurred on August 1994, approximately in the middle of the studied period. This dramatic disturbance affected the vegetation of successional series CH3, CH4 and CH5. It was found that before the fire altogether 44 species were isolated from 14 soil samples on these three sites. After the fire about 60 species were isolated from 18 soil samples. It is not a too great difference with regard to the highest number of soil samples collected after the fire. Remarkably, a higher occurrence of some species, especially *Cunninghamella elegans, C. echinulata* (Thaxt.) Thaxt. and *Talaromyces flavus* (Klöcker) Stolk & Samson was observed after the fire. Three anthracophilous fungi *Gelasinospora calospora* (Mouton) C.&M. Moreau, *G. tetrasperma* Dowding and *Trichophaea abundans* (P. Karst.) Boud. (anamorph *Dichobotrys abundans* Hennebert) occurred in the post-fire period only. It is in accordance with Widden & Parkinson (1975), who found *Gelasinospora* sp. in soil only after a forest fire. As regards mycorrhizal fungi of *C. epigeios* at Chvaletice, Vosátka *et al.* (1999) observed a strong reduction of sporulation after the fire (mainly *Glomus fistulosum* and *G. intraradices*). Nevertheless, the recovery of populations of mycorrhizal fungi was relatively fast.

The majority of isolated fungi belong to saprotrophs inhabiting plant debris in the soil. Some of these micromycetes may produce cellulolytic enzymes *(Chaetomium, Fusarium, Gliocladium, Myrothecium, Penicillium, Stachybotrys, Trichoderma)* or chitinolytic ones *(Mortierella, Paecilomyces, Penicillium, Trichoderma, Verticillium).* Several genera belong to phytopathogenic fungi *(Alternaria, Drechslera, Fusarium, Gliocladium, Myrothecium, Phoma, Phomopsis, Trichoderma, Verticillium),* coprophilous microfungi *(Coemansia, Gelasinospora, Sordaria, Sporormiella, Stilbella* and *Syncephalis),* fungicolous micromycetes *(Gliocladium, Sphaerodes, Syncephalis, Trichoderma)* and nematophagous ones *(Rhopalomyces).*

For the isolation of fungi we used several techniques. Some fungi were isolated by one method only. For example, *Gelasinospora* spp., *Mammaria* sp., *Sagenomella* sp., *Talaromyces flavus* and *Trichophaea abundans* were isolated only by heat treatment of samples. Within this group, *Gelasinospora tetrasperma* is known to germinate only after heating (Cain 1950). *Coemansia aciculifera* has grown only on agar plates incubated in a refrigerator or in moist chambers with soil samples and sterile nails. *Sporidesmium hyalospermum* (Corda) S.Hughes, *Stilbella* sp., *Rhopalomyces elegans* Corda var. *apiculatus* J.J. Ellis, and *Syncephalis sphaerica* Tiegh. were recorded only on deposits in the moist chambers. *Verticillium psalliotae* and *Paecilomyces lilacinus* were observed more frequently in the moist chambers than on agar plates.

Most fungi presented in the Table 2 have previously been recorded in the Czech Republic. Four species are considered to be new in the Czech Republic - two ascomycete species: *Westerdykella dispersa* (Clum) Cejp & Milko, *Gelasinospora calospora* and two zygomycete species from order Zoopagales: *Rhopalomyces elegans* var. *apiculatus* and *Syncephalis sphaerica.* Thirteen strains were deposited in the Culture Collection of Fungi (CCF), Prague (see note CCF in the Table 2).

Although different isolation techniques were used, by these methods only a limited part of soil micromycetes can be recorded which are capable of growing under artificial conditions. In addition, the used methods do not allow evaluating the actual enzymatic activity of the isolated fungi in natural environment. Thus, our report gives only preliminary data on fungal diversity of industrial deposits and can only serve as a basis for other more detailed study of effect of metals on micromycetes.

Overall survey of fungal diversity on abandoned sedimentation ponds is reported in a Table 4. In addition to the soil saprotrophic micromycetes, further fungal organisms were detected from this habitat by other specialists.

Kovář (1999) listed 31 species of macrofungi and 29 species of lichens from the locality Chvaletice. Marková (in Kovář & Hroudová, 1996) found 16 parasitic micromycetes. Váňová & Kubátová (still unpublished) found 29 taxa of coprophilous fungi (11 of them were isolated from the soil, too). Vosátka *et al.* (1996) recorded 2 species of arbuscular mycorrhizal microfungi. Požárová *et al.* (2001) isolated 61 taxa of micromycetes from roots of *Calamagrostis epigeios*, 29 species were not isolated in the present study from soil.

Regarding the locality Opatovice, Kovář (1999) listed 11 species of macrofungi and 5 lichens. Marková (in Kovář & Hroudová, 1996) found 8 parasitic micromycetes. Váňová & Kubátová (still unpublished) recorded 5 species of coprophilous fungi (three of them were isolated from soil, too).

Altogether, 219 species of fungi are known from the abandoned sedimentation pond at Chvaletice at present and 72 fungal species from that one at Opatovice. It seems, that a lower number of fungi on Opatovice locality is affected mainly by the lower number of samples investigated.

Table 4. Overall survey of mycobiota diversity on abandoned sedimentation ponds at Chvaletice and Opatovice (figures indicate number of taxa).

Fungal group	**Chvaletice**	**Opatovice**
soil micromycetes (present study)	94	46
root associated microfungi (Požárová *et al.*, 2001)	61 (32 species shared with soil micromycetes)	- (did not studied)
arbuscular mycorrhizal fungi (Vosátka *et al.*, 1999)	2	+ (no detailed data accessible)
coprophilous fungi (Váňová & Kubátová, unpubl.)	29 (11 species shared with soil micromycetes)	5 (3 species shared with soil microfungi)
parasitic micromycetes (Marková in Kovář & Hroudová, 1996))	16	8
macrofungi (Kovář, 1999)	31	11
lichens (Kovář, 1999)	29	5
Total number of fungal taxa	**219**	**72**

The authors concluded that the fungal diversity of studied anthropogenic soils which originated from industrial deposits of abandoned sediment basins is surprisingly high. These industrial soils are very specific especially by their dominant soil micromycetes which differ from those of natural soils. The most frequent microfungi isolated (*Penicillium janthinellum*, *P. simplicissimum*, *Cunninghamella elegans*, *Paecilomyces lilacinus*, *Trichoderma* spp., *Mucor hiemalis* f. *hiemalis*, *Coniothyrium fuckelii*, *Mortierella alpina*, *Fusarium* spp., *Coemansia aciculifera*, and *Trichoderma virens*) are generally considered as tolerant to the effect of stress factors of this habitat (a lack of basic nutrients, high concentration of metals, fluctuation of soil temperature and pH, acidification and secondary salinization) and as an important factor of detoxification and fertilization of these industrial deposits.

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