ACTA MYCOLOGICA
Vol. 33 (1): 3–23
1988

The effect of contamination of soil by heavy metals on qualitative and quantitative composition of fungi in the rhizosphere of some forest trees

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K o w a l s k i S., S t ę p n i e w s k a H., K r z a n Z., J a n u s z e k K.: *The effect of contamination of soil by heavy metals on qualitative and quantitative composition of fungi in the rhizosphere of some forest trees.* Acta Mycol. 33 (1): 3–23, 1998.

The effect of heavy metals in soils on the formation of fungal communities in the rhizosphere of different forest tree species was studied. Soil samples for heavy metal contamination tests and root samples for the determination of rhizospheric fungi were taken from the same root zone. The reduction of the quantitative as well as qualitative composition of fungi in the rhizosphere of individual tree species clearly correlated with the increase of soil contamination by Pb, Zn, and Cd. The following groups of fungi were distinguished: tolerant, very susceptible, and relatively resistant to contamination of soil by heavy metals. Moreover a great influence of tree species on the qualitative and quantitative composition of fungi in the rhizosphere was demonstrated.

Key words: biodiversity, rhizospheric fungi, heavy metals, industrial areas.

INTRODUCTION

Pollution caused by industry is one of the factors which operates over large areas and may disturb stability in forest environment. According to Z w o l i ń s k i (1995) the contamination of the environment by heavy metals is the main cause of degradation of pine stands growing within a reach of emissions from plants of non-ferrous metallurgy. It affects not only plants but also soil, which in turn leads to disturbances in the qualitative and

quantitative relations in populations of organisms including soil saprotrophic fungi (B å å t h 1989; B a d u r a et al. 1984; G r e s z t a et al. 1979; K o w a l s k i 1996; T y l e r et al. 1989; Z w o l i ń s k i 1995). These changes may lower the natural resistance of microbiological soil environment, not only in relation to root pathogens, but also by disturbing mycotrophy (K o w a l s k i 1987) and populations of rhizospheric fungi (K o w a l s k i 1989). This may lead to overall weakening of pláants, thus lowering their defensive reactions against many weakness pathogens (D o m a ń s k i et al. 1987).

Various species of trees, due to their root secretions, considerably affect microorganisms of the rhizosphere which play an important role in the microbiological processes taking place in the soil (M a c u r a 1971; T i n k e r and S a n d e r s 1975). Moreover they may change the susceptibility of plants to diseases, especially root diseases (J o h a n s s o n and M a r k l u n d 1980; K o w a l s k i et al. 1992; S t ę p n i e w s k a 1992). They also play an important role in proper functioning of mycotrophy of forest trees.

There is no adequate information to what degree industrial pollutants, affect the qualitative and quantitative composition of rhizospheric and mycorrhizal fungi in soil. The changes taking place in this group of fungi may be affected directly by industrial pollutants, especially heavy metals and also indirectly by changes in the metabolism of a plant being under the influence of a long term industrial stress (S c h ü t t et al. 1984).

The objective of this study was to determine the effect of industrial pollutants, especially heavy metals on fungi in the rhizosphere of different forest tree species.

MATERIALS AND METHODS

Investigations were carried out within the range of emissions from zink smelting works „Miasteczko Śląskie" (Upper Silesia Industrial Region) in strongly polluted permanent experimental area No 2 in Brynica (industrial damage zone III) situated in compartments 203 and 204 of the Świerklaniec Forest District (H a w r y ś 1984), and in moderately polluted experimental area in Pniowiec (industrial damage zone II), compartments 77 and 78 of the same district (L a t o c h a 1982). For comparison the investigations were also conducted in area free of excessive pollution (industrial damage zone „0") situated in compartment 49f of the Herby Forest District (K o w a l s k i 1987). Investigated plantations were established on sites of a „fresh coniferous forest" type.

In the study areas situated in the industrial damage zones II and III after removal of a mature *Pinus sylvestris* stand the soil was prepared for planting by full ploughing, and as a result a raw humus horizon strongly contaminated by Pb and Zn was translocated deep into the soil, while horizons Ees and B, mixed to a various degree, were elevated to the surface. The soils in these study areas were described by L a t o c h a (1982) as podzolic and brown podzolic. In zone „0" the soil was prepared for planting by furrow ploughing, and raw humus horizon was mixed with mineral soil. The soil in this area contained more humus in comparison with the soils in zones II and III.

In the industrial damage zones II and III rhizospheric fungi were investigated in 4-are experimental plots. Each plot comprised a 16-year-old pure stand of one of the following tree species: *Betula pendula*, *Quercus robur*, *Larix decidua* and *Pinus sylvestris*.

In zone „0", rhizospheric fungi were investigated in a 21-year-old stand of *P. sylvestris* with group admixture of *B. pendula*, *Q. robur* and *L. decidua*.

Root and soil samples were taken from the same root zone in order to determine the rhizospheric fungi and nutrient content, acidity and level of contamination of soil by heavy metals. The samples were taken from surface sandy mineral horizons, including the horizons Ees and B mixed with each other, and from the initial humus horizon.

The following chemical properties of soil were determined (L i t y ń s k i et al. 1976):

- pH in H_2O and KCl by potentiometric method,
- organic C by oxidometric Tiurin's method modified by the Department of Forest Soil Science of University of Agriculture in Kraków,
- total N by Kjeldahl's method,
- available Mg by Schachtsabel's method,
- exchangable CaO in 1 mole/dcm^3 ammonium acetate extract,
- Pb, Cu, Zn, Cd in 1 mole/dcm^3 HCl extract with an atomic absorption spectrophotometer (ASS 1N).

When samples for rhizospheric fungi identification were taken *P. sylvestris* was just flushing, and the remaining trees had their foliage fully developed.

In each experimental plot in zones II and III root samples were taken from 6 trees more or less along the diagonal of each plot, separately for each tree species. In zone „0" the roots of *B. pendula*, *Q. robur* and *L. decidua* were taken from 6 trees representing a group of a given species, while the roots of *P. sylvestris* were taken from 6 trees growing at a certain distance from the remaining tree species. In total 72 samples of roots with adherent soil were taken.

In order to isolate fungi from the rhizosphere of individual trees three 1 g samples of roots up to 1.5 mm in diameter and ($\pm$3.0 cm in length were weighed out, and then rinsed according to the general rules of H a r l e y ' s

and W a i d ' s (1955) method in successive 10 Erlenmeyer flasks, each containing 70 ml of sterile H_2O. Flasks No 9 and No 10 contained in addition 30 g of sterile quartz sand (M a ń k a 1974). After root rinsing, 0.1 ml of washings was taken from the second and ninth washings using sterile pipette, and placed in sterile Petri dishes and then flooded with M a r t i n ' s (1950) medium modified by J o h n s o n (1957).

The isolation of fungi from the rhizosphere of each tree species in individual industrial damage zones was repeated in 60 Petri dishes. In total 720 Petri dishes were used.

RESULTS

Soils from the rhizosphere of trees from the industrial damage zones II and III had weak acid and acid reaction respectively (Tab. 1), while their humus content was very low. In addition they had low or very low nutrient content (Tab. 1). The soils were characterized by relatively high lead, zinc and cadmium contents surpassing the threshold values in the industrial damage zone III (Tab. 2).

Soils from the rhizosphere of individual tree species in zone „0" varied with respect to reaction and humus content. The soil from the rhizosphere of *L. decidua* was strongly acid and had the highest humus content (Tab. 1). The ratio of C : N as well as the morphological appearance of soil indicated a muck-moder type of humus present in these soils.

The soils in the industrial zone III contained 16 times more lead, 4 times more copper, 68 times more zinc, and 36 times more cadmium than the soils in zone „0" (Tab. 2). The soils in zone II contained 3 times more lead, over 4 times more copper, 11 times more zinc, and about 10 times more cadmium than the soils in zone „0" (Tab. 3).

In total 1433, 1136, 1270 and 1083 fungal isolates were obtained from the rhizosphere of *B. pendula*, *Q. robur*, *L. decidua* and *P. sylvestris* respectively (Tab. 3).

The qualitative and quantitative composition of species was more diversified in the communities of fungi isolated from the rhizosphere of broad-leaved trees than in the communities associated with conifers (Tab. 3). The greatest diversity was found in the community of rhizospheric fungi of *B. pendula*. The number of species isolated in zone III amounted to only 52% of the number of species isolated in zone „0", and 66% of the number of species isolated in zone II (Tab. 3). In the rhizosphere of *Q. robur* the number of species in zone III amounted to only 58% of the number of species isolated in zone „0", and as much as 90% of the number of species isolated in zone II (Tab. 3). There was no difference in the number of species isolated from the rhizosphere

Table 1

Some chemical properties of sandy podzolic soil collected from the surface horizon* in zone of small roots of various species of trees in different industrial damage zones

Industrial damage zone	pH		C organic	N total	C : N	Mg	Ca	P	K	Pb	Cu	Zn	Cd
	H_2O	KCl	%			in mg/100 g of soil				in mg/kg of soil			
III	6.0[a] : 6.4[b]	5.1 : 5.3	0.6 : 0.4	0.04 : 0.04	15.0 : 10.0	1.6 : 0.7	26.2 : 15.5	0.7 : 0.5	1.2 : 1.7	150 : 185	3.4 : 4.5	245 : 139	0.05 : 3.2
	6.3[c] : 6.1[d]	5.4 : 4.9	0.8 : 0.7	0.03 : 0.05	26.7 : 14.0	0.0 : 0.4	35.7 : 11.5	0.2 : 0.6	1.3 : 1.8	233 : 149	3.6 : 4.7	305 : 130	0.1 : 2.4
II	5.4 : 6.1	4.4 : 5.1	1.1 : 0.5	0.05 : 0.05	22.0 : 10.0	0.9 : 0.9	22.7 : 10.2	0.9 : 0.5	2.2 : 2.3	30 : 30	2.8 : 5.4	36 : 42	0.05 : 0.9
	5.4 : 5.7	4.6 : 4.8	0.7 : 0.4	0.03 : 0.04	23.3 : 10.0	0.0 : 0.7	16.7 : 3.4	0.5 : 0.9	0.1 : 1.2	30 : 20	2.8 : 5.3	30 : 26	0.05 : 0.5
0	6.7 : 5.0	6.3 : 3.9	5.9 : 3.0	0.29 : 0.19	20.3 : 15.8	4.8 : 4.2	97.2 : 20.5	51.9 : 0.7	39.3 : 3.2	110 : 70	8.7 : 10	13 : 18	0.05 : 0.5
	4.3 : 4.8	3.4 : 3.7	9.2 : 3.8	0.43 : 0.24	21.4 : 15.8	8.4 : 1.0	32.7 : 19.5	1.0 : 2.8	6.3 : 2.8	145 : 61	5.9 : 4.8	65 : 16	0.05 : 0.5

* – in zones II and III soil was prepared for planting by full ploughing. In zone „0" soil was collected from the horizon AEes.
[a] – number in numerator before colon – *Betula pendula* (Brz); [b] – number in numerator after colon – *Quercus robur* (Db); [c] – number in denominator before colon – *Larix decidua* (Md); [d] – number in denominator after colon – *Pinus sylvestris* (So)

Table 2

The content of heavy metals in sandy podzolic soil collected from the surface horizon in zone of small roots of various trees per 1% organic carbon in tested soil, in different industrial damage zones

Industrial damage zone	Pb	Cu	Zn	Cd
	in mg/kg of soil/1% organic C			
III	250.0[a] : 462.0[b]	5.7 : 11.2	408.0 : 347.5	0.08 : 8.00
	290.6[c] : 212.8[d]	4.5 : 6.7	381.2 : 185.7	0.12 : 3.43
II	27.3 : 61.4	2.5 : 10.8	32.7 : 84.0	0.04 : 1.80
	42.8 : 50.0	3.9 : 13.3	42.1 : 65.0	0.07 : 1.25
0	18.6 : 23.3	1.5 : 3.4	2.1 : 6.0	0.01 : 0.17
	15.8 : 15.9	0.6 : 1.3	7.1 : 4.2	0.00 : 0.13

For explanation see Table 1

Table 3

Fungi isolated from rhizospheres of various trees in different industrial damage zones

Species	Number of isolates in industrial damage zone											
	III				II				„0"			
	Brz	Db	Md	So	Brz	Db	Md	So	Brz	Db	Md	So
1	2	3	4	5	6	7	8	9	10	11	12	13
Absidia coerulea Bain.				1			1			3		
Absidia glauca Hagem					2							
Absidia orchidis (Vuill.) Hagem	2											
Absidia spinosa Lendner									1	7	3	
Acremonium charticola Lindau	6	4							11	2		
Acremonium glaucum W. Gams								5				
Acremonium griseoviride (Onions et Barron) W. Gams					10	12	20	14				
Acremonium diversisporum (van Beyma) W. Gams						28	9	20				
Acremonium kiliense Grütz					3							
Acremonium murorum (Corda) W. Gams									3		9	
Acremonium strictum W. Gams			4				7				4	
Aspergillus parvulus Smith			2			5	17	25			4	
Aspergillus repens de Bary	16								1		3	
Aspergillus versicolor (Vuill.) Tiraboschi	1	13	3	5								8
Aspergillus sp. nr 1360									91		71	
Aureobasidium pullulans (de Bary) Arn. var. *melanigenum* Hermanides-Nijhof	3		4				3		3		2	
Beauveria bassiana (Bals.) Vuill.					1		1					
Beauveria brongniartii (Sacc.) Petch.						3			1			
Botrytis terrestris Jensen									33		15	
Catenularia spp.	1								1	4	2	2
Cenococcum cf. *geophilum* Fr.	21	7		33		13	42	2	3		1	5
Chaetomium cf. *spirale* Zopf											15	
Chalara brevispora Nag Raj et Kendrick								4				
Chalara spp.			2	1								
Chrysosporium pannorum (Link) Hughes					13	3	8		1			3

Cladosporium cladosporioides (Fres.) de Vries		5		1	2		1	1		3		3
Cladosporium elatum (Harz) Nannf.					4							
Cladosporium herbarum (Pers.) Link	2	1										
Cylindrocarpon destructans (Zinssm.) Scholten	1								3		4	
Cylindrophora sp. nr 1006	10											
Exophiala spp.						1					1	13
Fusarium cf. *tumidum* Sherbakoff												2
Geotrichum sp. nr 510				2								2
Heterobasidion annosum (Fr.) Bref.										2		3
Heteroconium sp. nr 20890					5							
Humicola fuscoatra Traaen var. *fuscoatra*					3	3						
Humicola spp.								3	2			2
Hymenula cerealis Ell. et Ev.					12							
Leptodontium beauverioides de Hoog						2						
Leptodontium obscurum de Hoog							1	2				
Monocillium spp.									6		15	
Mortierella alpina Peyronel	8	3	9								1	
Mortierella humilis Linnem.									15		2	
Mortierella isabellina Oudem.						1			39	58	11	3
Mortierella macrocystis W. Gams	2		11		9	13		4	32	4	8	2
Mortierella marburgensis Linnem.					1				1			
Mortierella minutissima van Tiegh.					3			4				
Mortierella nana Linnem.		4	17	12	18	20	11	30	9	5	1	
Mortierella parvispora Linnem.			14						24	8	20	5
Mortierella rammanniana (Moeller) Linnem.				6					2	5		
Mortierella vinacea Dixon-Stewart								1	13		13	4
Mortierella zonata Linnem.									2		13	
Mortierella spp.										5		1
Mucedinaceae sp. nr 21223							29					
Mucor hiemalis Wehmer									4	1	2	
Mucor spp.										11	1	
Mycelium radicis atrovirens Mel.	18	49	29	36	7	31	30	64	22	17	29	60
Oidiodendron cerealis (Thüm.) Barron		8	11	10		5	1			4		
Oidiodendron citrinum Barron	4								52	65	31	2

cont. Tab. 3

1	2	3	4	5	6	7	8	9	10	11	12	13
Oidiodendron echinulatum Barron		7		1			1					2
Oidiodendron flavum Szilvinyi			7	49	6	13	8	5	5	1	4	3
Oidiodendron griseum Robak		17	3	5	8			1	22		19	2
Oidiodendron maius Barron	1						3			20		6
Oidiodendron rhodogenum Robak					6		1		7	6	1	10
Oidiodendron tenuissimum (Peck) Hughes	6	5			1				2	13	25	
Oidiodendron truncatum Barron							2					
Oidiodendron spp.					4				1		1	
Paecilomyces carneus (Duche et Heim) A. H. S. Brown et G. Sm.							6					
Paecilomyces farinosus (Holm ex Gray) A. H. S. Brown et G. Sm.			1						2			
Paecilomyces marquandi (Massee) Hughes					3							
Paecilomyces parvus A.H.S. Brown et G. Sm.	1	5	14	13	1	5	1					
Paecilomyces puntoni (Vuill.) Nannizzi		3										
Paecilomyces varioti Bainier									7			
Paecilomyces spp.	10									7	3	
Penicillium cf. *aculeatum* Raper et Fennel					2	7						
Penicillium adametzi Zaleski										16		4
Penicillium albidum Sopp										5		6
Penicillium aurantio-violaceum Biourge		1	13					1	2	3	1	
Penicillium canescens Sopp				27			10	29				
Penicillium cf. *citreo-viride* Biourge				12	8							
Penicillium citrinum Thom										2		
Penicillium cf. *clavigerum* Demelius			5									
Penicillium decumbens Thom	1		1		12	5	3		1		1	
Penicillium expansum Link		12		1					1		22	8
Penicillium fellutanum Biourge						24	2					
Penicillium cf. *griseofulvum* Dierckx	3								5			
Penicillium implicatum Biourge				2		1				11		6
Penicillium jenseni Zaleski					6			1	45			7
Penicillium cf. *meleagrinum* Biourge									23			
Penicillium melinii Thom					1							1

Penicillium cf. *nalgiovensis* Laxa			4									
Penicillium nigricans (Bainier) Thom									17	6	57	
Penicillium cf. *purpurogenum* Stoll var. *rubrisclerotium* Thom			11	23								
Penicillium pusillum Smith								10				
Penicillium restrictum Gilman et Abbott										84		1
Penicillium rolfsii Thom					5				8			
Penicillium soppi Zaleski				5						1		4
Penicillium spinulosum Thom	3	12	23	26	10	15	34	31	53	43	61	51
Penicillium spinulosum stirps 2					40			7				5
Penicillium steckii Zaleski								5		10		
Penicillium stoloniferum Thom			8			5	6		19			
Penicillium tardum Thom				18	5					14		
Penicillium terlikowski Zaleski		3				3						
Penicillium thomii Maire										19	4	
Penicillium velutinum van Beyma	2		2			2			34		13	
Penicillium verrucosum Dierckx var. *cyclopium* (Westling) Samson, Stolk et Hadlok	1			1								
Penicillium vinaceum Gilman et Abbott							4	8				
Penicillium waksmani Zaleski				1						1		
Penicillium spp.		9	1	2	14	11	8		65	16	14	3
Phialocephala sp. nr 21133					30							
Phialophora fastigiata (Lagerb. et Melin) Conant	9		11							1	4	
Phialophora richardsiae (Nannf.) Conant	6		8						2	1	14	
Phialophora spp.	6		8		9		11		15		5	
Phoma spp.	5								2			
Ramichloridium subulatum de Hoog				3								
Rhinocladiella pedrosoi (Brupt) Schol-Schwarz				1								5
Scopulariopsis sp. nr 1191									6			
Septonema spp.			1				1		4			
Sphaeropsidales spp.					23							
Thysanophora cf. *canadensis* Stolk et Henneb.		11										
Tolypocladium geodes W. Gams					8						28	2
Tolypocladium spp.											2	3
Torula graminis Desm.			3									

cont. Tab. 3

1	2	3	4	5	6	7	8	9	10	11	12	13
Trichocladium opacum (Corda) Hughes					2						4	
Trichoderma aureoviride Rifai					1		2			2		
Trichoderma hamatum (Bonord.) Bain.	1									7	2	
Trichoderma koningii Oudem.					9		9		13	10		
Trichoderma longibrachiatum Rifai												5
Trichoderma piluliferum Webster et Rifai			3									
Trichoderma polysporum (Link ex Pers.) Rifai									55	25	10	8
Trichoderma pseudokoningii Rifai											5	3
Trichoderma viride Pers. ex Gray				14	2	2			7	21		14
Verticillium cellulosae Daszewska								3				
Verticillium cephalosporum W. Gams					1				1			
Verticillium falcatum (Petch) W. Gams		2										
Verticillium griseum (Petch) W. Gams	25		3	11						1		
Verticillium lecanii (Zimm.) Viegas		3		21		3	1			25	9	29
Verticillium psalliotae Treschow		3		2	8							7
Verticillium spp.	1								2			
Wardomyces pulvinatus (Marchal) Dickinson							9					
Zygorhynchus vuilleminii Namyslowski				4								
Non sporulating*	19	18	27	21	59	32	51	48	55	77	46	59
Total	195	205	267	370	377	268	354	328	856	652	636	374

For explanation of symbols (Brz, Db, Md and So) see Table 1
* – different species of variable numbers
NOTE: Table 3 does not include of fungi sporadically (numbers 1x) occuring in rhizospheres of trees listed below (symbols of industrial damage zones are in parenthesis):

Betula pendula: *Aspergillus* sp. (III), *Acremonium tubakii* (0), *Paecilomyces fumosoroseus* (II), *Sporotrichum* sp. (0), *Trichocladium canadense* (III);
Quercus robur: *Acremonium* sp. (III), *A. terricola* (II), *Alternaria tenuissima* (II), *Aspergillus fumigatus* (III), *Beauveria* sp. (0), *Chaetomium indicum* (II), *Cylindrocarpon* cf. *gracile* (0), *Cytospora* cf. *quercus* (III), *Cytosporella* sp. (0), *Mucor abundans* (0), *Penicillium variabile* (III);
Larix decidua: *Acremonium pteridii* (II), *Candida* sp. (II), *Chalara cylindrosperma* (III), *Chloridium preussii* (II), *Cladosporium sphaerospermum* (0), *Gelasinospora* sp. (II), *Gliocladium* sp. (III), *Mortierella minutissima* var. *dubia* (III), *Mucor plumbeus* (0), *Pestalotia* sp. (III), *Phialophora cinerescens* (II), *Sporothrix* sp. (II), *Zygorhynchus exponens* (III);
Pinus sylvestris: *Arthrinium* sp. (II), *Mucor spinescens* (III), *Paecilomyces elegans* (0), *Paecilomyces* cf. *victoriae* (III), *Penicillium duclauxi* (II), *P.* cf. *granulatum* (0), *P. ochrochloron* (0), *P. rugulosum* (0), *Pseudogymnoascus vinaceus* (0), *Trichoderma harzianum* (0), *Zygorhynchus moelleri* (II).

of *L. decidua* in zones II and III, while the number of species in zone III amounted to 78% of the number of species isolated from the rhizosphere of this tree in zone „0" (Tab. 3). The number of isolates obtained from the rhizosphere of *P. sylvestris* was similar in all the three industrial damage zones investigated, although the number of isolates and species in zone II was the lowest (Tab. 3).

The quantitative as well as qualitative composition of fungi in the rhizosphere of individual trees was clearly correlated with the contamination of soil by lead, zinc and cadmium (Tabs 2–3). Of 171 species isolated from the rhizosphere of all the investigated trees in zone „0" and zone III only 29% of them were found in both zones, while 46% of species occurred only in zone „0", and only 25% in zone III. The fungi occurring in the aforementioned zones may be considered to be either relatively resistant to contamination by heavy metals or their occurrence in the rhizosphere is affected to a greater extent by root secretions of a given tree species than by the degree of contamination. This was mainly true of *Mycelium radicis atrovirens* and *Penicillium spinulosum*. Although such species as *Aspergillus versicolor*, *Cenococcum geophilum*, *Mortierella alpina*, *M. nana*, *Oidiodendron cerealis*, *O. flavum*, *Phialophora fastigiata* and *Verticillium griseum*, were found in zone III and „0", they were more abundant in zone III. On the other hand *Mortierella macrocystis*, *M. parvispora*, *Oidiodendron citrinum*, *O. griseum*, *O. maius*, *O. tenuissimum*, and *Verticillium lecanii* were more abundant in zone „0" (Tab. 3). Among fungi which occurred only in zone „0" (46%) and formed there relatively numerous populations, the following are noteworthy: *Absidia spinosa*, *Botrytis terrestris*, *Mortierella humilis*, *M. zonata*, *Mucor hiemalis*, *Penicillium adametzi*, *P. albidum*, *P.* cf. *meleagrinum*, *P. nigricans*, *P. restrictum*, *Trichoderma polysporum*, and *T. pseudokoningii*. These fungi may be considered to be very susceptible to industrial pollution. In addition *Mortierella vinacea*, *Tolypocladium geodes*, and *Trichoderma koningii* may be included in this group. They were relatively abundant in zone „0", much less numerous in zone II, and absent in zone III (Tab. 3).

The populations of fungi which seemed to prefer soils contaminated by heavy metals since they were isolated only from zone III were scarce, and only *Paecilomyces parvus* and *Penicillium* cf. *purpurogenum* var. *rubrisclerotium* occurred in relatively large numbers. Moreover *Penicillium canescens* and *P.* cf. *citreo-viride* may be included in this group although they were also isolated in zone II (Tab. 3).

The negative effect of industrial pollutants on the biodiversity of fungi in the rhizosphere of trees may also be demonstrated when analysing quantitative changes in the communities of rhizospheric fungi in zones of high and moderate contamination in relation to the most abundant group of fungi present in the zone free of excessive contamination. In each case the number of species in zones II and III was smaller than in zone „0" (Tab. 4).

Table 4

Changes in the quantitative composition of fungi isolated from rhizospheres of various trees in industrial damage zones II and III in relation to the most abundant group of fungi isolated in zone „0"*

Genus	Industrial damage zone													
	0					II					III			
	Number of species	Percentage of isolates in rhizosphere				Number of species	Percentage of isolates in rhizosphere				Number of species	Percentage of isolates in rhizosphere			
		Brz	Db	Md	So		Brz	Db	Md	So		Brz	Db	Md	So
Penicillium	29	19.0	20.0	13.6	9.0	28	7.1	6.4	5.3	8.7	23	0.5	2.6	5.2	10.7
Mortierella	12	9.5	7.5	5.4	1.4	6	2.1	3.0	0.9	3.6	6	0.7	0.6	4.1	1.7
Oidiodendron	10	6.2	9.6	6.4	2.3	9	1.7	1.6	1.3	0.6	7	0.8	3.3	1.6	6.0
Trichoderma	8	5.2	5.7	1.3	2.7	3	0.8	0.2	0.9	0.0	3	0.07	0.0	0.2	1.3
		39.9	43.0	26.7	15.4		11.7	11.2	8.4	12.9		2.1	6.5	11.1	19.7

For explanation of symbols (Brz, Db, Md and So) see Table 1

* The percentage of isolates was computed in relation to the total number of isolates in rhizospheres of individual tree species from all the industrial damage zones

Much greater differences occurred in the numbers of isolates of these species. The greatest decline in the number of isolates in zones II and III in comparison with zone „0" was found among fungi of the genus *Trichoderma*, which were not isolated from the rhizosphere of *P. sylvestris* in zone II and from the rhizosphere of *Q. robur* in zone III, while their percentage in the rhizosphere of *B. pendula* in zone III was 74 times lower than in zone „0" (Tab. 4). The species of the genus *Penicillium* were also noteworthy. The number of these species was not reduced much, but the percentage of their isolates from the rhizosphere of *B. pendula*, *Q. robur*, and *L. decidua* sharply decreased, and was lower in zone III than in zone „0" by 38, 7.7, and 2.6 times respectively (Tab. 4).

The number and percentage of species which occurred in the rhizosphere of given species of trees in zone „0" and were absent in zone III may be a good indicator of changes taking place in the biodiversity of fungi (Tab. 5). The number of species isolated from the rhizosphere of individual tree species only in zone „0" was 1.6–2 times higher than the number of species isolated only in zone III (Tab. 5).

Table 5

Quantitative and qualitative comparison of fungi differentiating the community of rhizospheric fungi of trees in zones „0" and III

Species	Percentage of species isolated only in zone „0" or only in zone III (numbers with symbol*) in the rhizospheres of the investigated trees			
	Brz	Db	Md	So
1	2	3	4	5
Absidia coerulea		0.4		0.1
Absidia orchidis	0.2*			
Absidia spinosa	0.1	0.8	0.3	
Acremonium murorum	0.3		1.0	
Acremonium tubakii	0.1			
Acremonium sp. nr 575		0.1*		
Aspergillus fumigatus		0.1*		
Aspergillus repens			0.3	
Aspergillus versicolor	0.1*	1.5*	0.4*	
Aspergillus sp. nr 1360	8.6		7.8	
Beauveria brongniartii	0.1			
Botrytis terrestris	3.2		1.7	
Catenularia spp.		0.5	0.2	0.3
Chaetomium cf. *spirale*			1.7	
Chalara cylindrosperma			0.1*	
Chalara spp.			0.2*	0.1*
Chrysosporium pannorum				0.4
Cladosporium herbarum	0.2*	0.1*		
Cladosporium sphaerospermum			0.1	

cont. Tab. 5

1	2	3	4	5
Cylindrocarpon destructans			0.4	
Cylindrocarpon cf. *gracile*		0.1		
Cylindrophora sp. nr 1006	1.0*			
Cytospora cf. *quercus*		0.1*		
Cytosporella sp. nr 277		0.1		
Exophiala spp.			0.1	1.7
Fusarium cf. *tumidum*				0.3
Gliocladium sp. nr 1121			0.1*	
Heterobasidion annosum		0.2		0.4
Humicola sp. nr 1222	0.2			
Monocillium spp.	0.6		1.7	
Mortierella alpina	0.7*	0.4*		
Mortierella humilis	1.4		0.2	
Mortierella isabellina	3.7		1.2	0.4
Mortierella minutissima var. *dubia*			0.1*	
Mortierella parvispora	2.3	1.0		0.7
Mortierella rammanniana	0.2	0.6		0.8*
Mortierella vinacea	1.2		1.4	
Mortierella zonata	0.2		1.4	
Mortierella spp.		0.6		0.1
Mucor abundans		0.1		
Mucor hiemalis	0.4	0.1	0.2	
Mucor plumbeus			0.1	
Mucor spinescens				0.1*
Mucor spp.		1.3	0.1	
Oidiodendron cerealis				1.3*
Oidiodendron citrinum		7.6	3.4	0.3
Oidiodendron echinulatum		0.8*		
Oidiodendron griseum		2.0*		
Oidiodendron maius	0.1*	2.3		0.8
Oidiodendron rhodogenum		0.7		1.3
Oidiodendron tenuissimum			2.8	
Paecilomyces elegans				0.1
Paecilomyces farinosus	0.2		0.1*	
Paecilomyces parvus				1.7*
Paecilomyces puntoni		0.4*		
Paecilomyces varioti	0.7			
Paecilomyces cf. *victoriae*				0.1*
Paecilomyces spp.	1.0*	0.8	0.3	
Penicillium adametzi		1.9		0.5
Penicillium albidum		0.6		0.8
Penicillium canescens				3.3*
Penicillium cf. *citreo-viride*				1.6*
Penicillium citrinum		0.2		
Penicillium cf. *clavigerum*			0.6*	
Penicillium expansum	0.1	1.4*	2.4	
Penicillium granulatum				0.1
Penicillium cf. *meleagrinum*	2.2			

cont. Tab. 5

1	2	3	4	5
Penicillium melinii				0.1
Penicillium cf. *nalgiovensis*			0.5*	
Penicillium nigricans	1.6	0.7	6.3	
Penicillium cf. *purpurogenum* var. *rubrisclerotium*			1.3*	3.0*
Penicillium restrictum		9.7		0.1
Penicillium rugulosum				0.1
Penicillium soppi		0.1		
Penicillium steckii		1.2		
Penicillium stoloniferum	1.8			
Penicillium tardum		1.6		2.4*
Penicillium thomii		2.2	0.4	
Penicillium variabile		0.1*		
Penicillium verrucosum var. *cyclopium*	0.1*			0.1*
Penicillium waksmanii		0.1		0.1*
Pestalotia sp. nr 1092			0.1*	
Phialophora fastigiata	0.8*	0.1		
Phialophora richardsiae		0.1		
Pseudogymnoascus vinaceus				0.1
Ramichloridium subulatum				0.4*
Scopulariopsis sp. nr 1191	0.6			
Septonema spp.	0.4		0.1*	
Sporotrichum sp. nr 1204	0.1			
Thysanophora cf. *canadensis*		1.3*		
Tolypocladium geodes			3.1	0.3
Tolypocladium spp.			0.2	0.4
Torula graminis			0.4*	
Trichocladium canadense	0.1*			
Trichocladium opacum			0.4	
Trichoderma aureoviride		0.2		
Trichoderma hamatum	0.1*	0.8	0.2	
Trichoderma harzianum				0.1
Trichoderma koningii		1.2		
Trichoderma longibrachiatum				0.7
Trichoderma piluliferum			0.4*	
Trichoderma polysporum	5.3	2.9	1.1	1.1
Trichoderma pseudokoningii			0.6	0.4
Verticillium falcatum		0.2*		
Verticillium griseum	2.4*	0.1	0.4*	1.4*
Verticillium psalliotae		0.4*		
Zygorhynchus exponens			0.1*	
Zygorhynchus vuillemini				0.5*
Total	35.6	40.9	41.1	11.7
	6.8*	8.9*	4.8*	16.9*

Explanation: the percentage of isolates was computed in relatin to the total number of isolates in zone „0" and III;
For explanation of symbols (Brz, Db, Md and So) see Table 1

In the rhizosphere of *B. pendula* the following species are worthy of notice: *Aspergillus* sp. No 1360, *Botrytis terrestris*, *Mortierella isabellina*, *Penicillium meleagrinum*, *P. nigricans*, and *Trichoderma polysporum*. Their numerous populations were found in zone „0", while in zone III these fungi were absent not only in the rhizosphere of this tree, but were not isolated from the rhizosphere of any of the investigated tree species. The same situation was observed in the case of *Penicillium adametzi*, *P. restrictum*, *P. thomii*, *Trichoderma koningii*, and *T. polysporum* in the rhizosphere of *Q. robur*, *Aspergillus* sp. No 1360, *Botrytis terrestris*, *Monocillium* sp., *Penicillium nigricans*, *Tolypocladium geodes*, and *Trichoderma polysporum* in the rhizosphere of *L. decidua*, and *Exophiala* sp., *Oidiodendron rhodogenum* and *Trichoderma* spp., in the rhizosphere of *P. sylvestris* (Tab. 5).

The following species were numerous in zone III and absent in zone „0": *Cylindrophora* sp. No 1006 in the rhizosphere of *B. pendula*, *Thysanophora* cf. *canadensis* in the rhizosphere of *Q. robur*, *Penicillium purpurogenum* var. *rubrisclerotium* in the rhizosphere of *L. decidua*, and *Paecilomyces parvus*, *Penicillium canescens*, *P.* cf. *citreo-viride*, and *P. purpurogenum* var. *rubrisclerotium* in the rhizosphere of *P. sylvestris* (Tab. 5).

DISCUSSION

The soils in the zone of high damage caused by industrial emissions (zone III) were characterized by high lead and zinc contents exceeding the threshold values (according to Kloke after Greszta and Panek 1989), which was also confirmed by other authors (Zwoliński 1995; Olszowska 1997). The content of cadmium increased in these soils while that of copper did not exceed the threshold value, which points to the lack of contamination of these soils by this chemical element. Thus zinc, lead, and cadmium could have cause a considerable quantitative and qualitative differences in the communities of the investigated rhizospheric fungi, which confirms the results obtained by Olszowska (1977) indicating a clearly negative effect of these heavy metals on the activity of soil enzymes: urease, asparaginase, and dehydrogenase. The reactions of soil microorganisms are determined, among others, by a global concentration of all heavy metals present in the soil (Zwoliński 1995). Therefore, the differences found in the communities of rhizospheric fungi cannot relate to the levels of individual heavy metals. They result from interactions between the contaminated (not only by heavy metals) environment and individual forest tree species.

Mycorrhizal fungi should also be taken into account in the communities of rhizospheric fungi. However the isolation of these fungi requires the use of special methods. Generally they are very susceptible to higher levels of heavy

metals. However, their role in limiting the transfer of toxic metals to plants may be considerable (Colpeart and Van Assche 1992). Changes in the qualitative and quantitative composition of these fungi may be determined indirectly by their fructification in the polluted environment. Many studies indicated that the number of fructifications as well as the number of species of these fungi were considerably reduced by increasing the contamination of soil by heavy metals (Kowalski et al. 1989; Rühling and Söderström 1990).

Different reaction of various species of soil fungi as well as their strains to the contents of heavy metals indicate a necessity to identify the fungi to the species in order to evaluate the effect of soil contamination on their population. This fact was emphasized by Bååth (1989) and Tyler et al. (1989). These authors cited the results of different studies indicating that species from the genera *Mortierella*, *Oidiodendron*, and *Penicillium* were susceptible to contamination of soil by heavy metals. Among species of *Zygomycotina*, *Mortierella* spp. were the most susceptible to copper (Arnebrant et al. 1987). Badura et al. (1984) found that high contamination of soil by lead might drastically limit the qualitative composition of fungi in soil all the way down to monocultures of *Aspergillus flavus*, *A. fumigatus*, and *Penicillium funiculosum*. In our study, only *A. fumigatus* was found exclusively in the rhizosphere of *Q. robur* in the zone of the greatest contamination.

Our studies confirmed the high susceptibility of fungi of the genera *Mortierella*, *Oidiodendron*, and *Penicillium* to industrial pollution. These groups of fungi isolated from the soil beyond the limits of the rhizosphere in the same study areas showed similar susceptibility (Kowalski 1996). This was mainly true of *M. isabellina*, *M. vinacea*, and *P. nigricans*, which were abundant in the rhizospheric soil as well as outside the rhizosphere in zone „0", while they were not isolated in zone III at all. However, species from the genus *Trichoderma* seemed to be most susceptible to contamination of soil by heavy metals. They were quite abundant in the rhizospheric soil of all the tree species investigated in zone „0", while they were absent or sporadic in zone III. Similar results were obtained in studies concerning fungi in the soil outside the rhizosphere (Kowalski 1996). The number of species in the rhizospheric soils was reduced from 8 in zone „0" to 3 in zone III. High susceptibility of fungi of the genus *Trichoderma* to pollution was also pointed out by Černý and Cudlin (1989). The susceptibility of other rhizospheric fungi indicated by these authors is in agreement with our results. However, among fungi of the genera *Mortierella* and *Oidiodendron* there were also species, e.g. *M. nana* and *O. flavum* whose number increased considerably in zone III.

When discussing the effect of industrial pollution on the quantitative and qualitative composition of rhizospheric fungi in different contamination zones

the effect of root secretions of individual tree species on fungal communities in their rhizosphere should also be taken into account. The studies showed that the differences in the number of isolates and fungal species isolated from the rhizosphere of different trees in a single contamination zone were larger than the differences in the communities of fungi isolated from the rhizosphere of a single tree species but in different zones situated not very far away from one another.

This study provided evidence of a distinctive effect of high contamination of soil by heavy metals on fungi in the rhizosphere of forest trees. It was found that contamination considerably reduced the qualitative composition of fungi as well as the population size of many species. However, it also indicated a diversity of fungal communities occurring in the rhizosphere of individual tree species in the contaminated zone. This diversity was also evident in relation to saprotrophic fungi occurring in the soil outside the rhizosphere (K o w a l s k i 1996), macrofungi (K o w a l s k i et al. 1989), and mycorrhizal associations (K o w a l s k i 1987). Therefore it is advisable, to introduce various species of trees during convertion of degraded stands. Such silvicultural treatment may speed up the revitalization of degraded soil by adding to it a more diversified rhizospheric mycoflora, thus improving the nutrition and health conditions of trees in recultivated areas previously damaged by industry.

CONCLUSIONS

1. Contamination of soil by lead, zinc, and cadmium considerably reduces the qualitative composition of fungi as well as the population size of many species in the rhizosphere of forest trees. However trees may affect the formation of fungal populations in the rhizosphere.

2. Introduction of various species of trees during the convertion of degraded stands is advisable from the microbiological point of view as well. Such silvicultural treatment adds to a degraded soil a more diversified rhizospheric mycoflora which may improve the nutrition and health conditions of trees in recultivated areas.

3. When taking into consideration the qualitative composition of fungi in the rhizosphere of trees it is advisable to plant on sites prepared by full ploughing *B. pendula* in mixture with *L. decidua*, and *P. sylvestris* in mixture with *Q. robur* during stand convertion.

The study was in part supported by the Polish Committee for Scientific Research (KBN) – Grant No 6P04F04208.

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Wpływ skażenia gleby metalami ciężkimi na skład jakościowy i ilościowy grzybów w ryzosferze wybranych gatunków drzew leśnych

Streszczenie

1. W strefie wolnej od nadmiernego skażenia imisjami z ryzosfery badanych gatunków drzew otrzymano 2531 izolatów grzybów, które należały do 123 gatunków i 55 kultur grzybów niezarodnikujących, zaś w strefie II – 1345 izolatów grzybów, należących do 102 gatunków i 30 kultur grzybów niezarodnikujących, natomiast w III strefie skażenia imisjami jedynie 1046 izolatów, które należały do 92 gatunków grzybów i 38 kultur niezarodnikujących.

2. Prowadząc analizę składu jakościowego oraz ilościowego grzybów wyizolowanych z ryzosfery poszczególnych drzew w różnych strefach skażenia imisjami przemysłowymi stwierdzono w zbiorowiskach grzybów z ryzosfery drzew iglastych i liściastych duże różnice; największe były w ryzosferze brzozy brodawkowatej. Liczba izolatów grzybów stwierdzona w ryzosferze tego gatunku drzewa w III strefie skażenia imisjami była ponad 4 razy niższa niż w strefie „0" i 2-krotnie niższa niż w II strefie skażenia. Liczba izolatów grzybów w ryzosferze dębu i modrzewia w III strefie skażenia była mniejsza odpowiednio o 3 i 2,4 razy w stosunku do strefy „0" i jedynie 1,3 razy w stosunku do II strefy skażenia imisjami. Natomiast liczba izolatów grzybów z ryzosfery sosny pospolitej w trzech badanych strefach była podobna. Również większą redukcję w III strefie skażenia w stosunku do strefy „0" stwierdzono w składzie gatunkowym grzybów w ryzosferze zarówno brzozy i dębu jak też modrzewia i sosny.

3. Redukcja składu jakościowego i ilościowego grzybów w ryzosferze poszczególnych gatunków drzew wyraźnie korelowała ze wzrostem skażenia gleby takimi metalami ciężkimi jak Pb, Zn, Cd.

4. Z ogólnej liczby gatunków grzybów wyizolowanych z ryzosfery wszystkich badanych gatunków drzew w strefach „0" i III, w obu strefach skażenia stwierdzono jedynie 29%, natomist aż 46% gatunków stwierdzono tylko w strefie „0" i jedynie 25% gatunków tylko w III strefie.

5. Wydzielono grupy grzybów bardzo wrażliwych na zanieczyszczenia przemysłowe, tolerancyjnych w stosunku do imisji przemysłowych oraz stosunkowo odpornych na te zanieczyszczenia. Z grzybów, które występowały licznie w badanych środowiskach, do grupy pierwszej należały: *Absidia spinosa*, *Botrytis terrestris*, *Mortierella humilis*, *M. zonata*, *M. vinacea*, *Mucor hiemalis*, *Penicillium adametzi*, *P. album*, *P.* cf. *meleagrinum*, *P. nigricans*, *P. restrictum*, *Tolypocladium geodes*, *Trichoderma polysporum*, *T. pseudokoningii* i w mniejszym stopniu *T. koningii*. Do grupy drugiej można zaliczyć: *Mycelium radicis atrovirens*, *Penicillium spinulosum* oraz takie, których liczebność wyraźnie wzrastała w III strefie skażenia imisjami: *Aspergillus versicolor*, *Mortierella alpina*, *M. nana*, *Oidiodendron cerealis*, *O. flavum*, *Phialophora fastigiata* i *Verticillium griseum*, natomiast w grupie trzeciej znalazły się: *Paecilomyces parvus*, *P. purpurogenum* var. *rubrisclerotium*, *P. canescens* i *P. citreo-viride*.

6. Wpływ poszczególnych gatunków drzew na kształtowanie się zbiorowiska grzybów w ich ryzosferze niekiedy był tak znaczny, że różnice w liczbie izolatów i gatunków grzybów w zbiorowiskach grzybów wyizolowanych z ryzosfery w jednej strefie skażenia były większe pomiędzy poszczególnymi gatunkami drzew, aniżeli różnice w zbiorowiskach grzybów wyizolowanych z ryzosfery jednego gatunku drzewa ale w niezbyt odległych od siebie strefach skażenia imisjami.