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Fungi colonizing toxic acid soils in the dumping ground of the "Bełchatów" brown coal mine

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The results of two years' studies on post-mining toxic acid soils of the "Bełchatów" dumping grounds neutralized with chalk, ash, limestone, burnt lime, and ground phosphate rock are presented. The neutralization with ground phosphate rock and ash had the most favourable effect on the development of soil fungi.

Key words: soil fungi, toxic acid soils.

INTRODUCTION

The investigations on post-mining non-toxic raw dumping grounds showed that they exhibited their poor mycological activity. It was found that the activity of fungi increased with the advancing soil-formation processes. Soil fungi played the role of indicators of the development of fertility properties in the reclaimed initial soil (K o w a l i k 1993; K o w a l i k and K o w a l i k 1996).

No studies of this type were conducted in areas of toxic dumping grounds. Hence the aim of the present work was to determine:

– the quantitative and qualitative composition of soil fungi colonizing the dumping ground before and after their neutralization,
– the effect of the applied neutralizers on the species composition and dynamics of soil fungi populations in the toxic acid soils of the top layer of the "Bełchatów" dumping grounds.

MATERIAL AND METHODS

The investigation was carried out in 1995–1996 in a toxic acid (pH in HCl < 3) plot on the top layer of the "Bełchatów" dumping grounds. A detailed description of the grounds and the neutralization methods was given by Krzaklewski et al. (1997). The experimental design included 11 plots differing in the kind and dose of the neutralizer.

Soil samples were taken five times for mycological analyses: prior to soil neutralization (May 5, 1995), before sowing *Lolium perenne* (August 1, 1995), and during its vegetation (October 26, 1995, May 23, 1996, and September 19, 1996). Mycological analyses were carried out according to the methods given by Mańka (1974).

The following determinations were made:
- the dynamics of soil fungi populations,
- the species of dominanting, sub-dominanting, and accessory fungi,
- the density index of the fungi colonies (Kowalik 1993).

RESULTS

During the study 2110 fungi colonies made up of 48 species were isolated from the top layer of the soil from the "Bełchatów" dumping ground. The experimental plots were colonized by the soil fungi to a variable degree: the number of colonies and species ranged from 92–290 and 13–27 respectively in the experimental plots. The smallest fungi populations were isolated from the plots neutralized with a double dose of chalk and burnt lime and from the control plot. The most numerous populations of fungi were isolated in plots neutralized with ground phosphate rock (at the two doses) and with a single dose of chalk (Tab. 1).

The composition of fungi communities isolated from the separate plots differing in the kind and dose of the applied fertilizer, varied considerably.

In the 2-year period studies the toxic acid non-neutralized soils of the control plot were poor in fungi species. They were colonized by scarce soil fungi dominated by species of the genera *Aspergillus*, *Penicillium*, and *Acremonium*. *Aspergillus* spp. constituted 38.92% of the fungi communities; *A. niger* comprised 19.08% and *A. versicolor* 16.03%, whereas *Penicillium* spp. represented by 8 species constituted 35.88%; among which the dominating species were: *P. nigricans*, *P. citrinum*, and *P. funiculosum*.

In plots neutralized with burnt lime, limestone, and chalk a considerable proportion of *Penicillium* spp. and *Aspergillus* spp. was recorded.

In the plot neutralized with a single dose of burnt lime *Aspergillus* spp. constituted 32.51% of the total population of fungi, including *A. versicolor*

Table 1
Fungi isolated from neutralized soils of the "Belchatów" dumping ground

Fungi	Neutralizers – doses										
	Chalk		Ash		Limestone		Burnt lime		Ground ph. rock		O
	1x	2x	1x	2x	1x	2x	1x	2	1x	2x	
1	2	3	4	5	6	7	8	9	10	11	12
Acremonium cerealis W. Gams	1	–	–	–	2	–	–	–	–	–	
A. kiliense Griitr	56	16	–	2	2	4	14	21	23	6	16
A. rutilum W. Gams	2	–	–	–	4	–	1	2	–	–	5
Alternaria alternata (Fr.) Keissler	–	–	4	2	–	–	–	–	–	–	2
Aspergillus fumigatus Fres.	–	–		2	–	–	–	–	–	–	4
A. niger Tiegh.	32	6	2	5	12	39	21	8	5	1	25
A. versicolor (Vuill.) Tiraboschi	4	–	13	85	16	8	30	14	23	10	21
A. wentii Wehmer	–	2	1	–	–	–	2	–	1	–	1
Chaetomium funicola Cooke	–	–	–	2	–	–	–	–	–	–	–
Ch. globosum Kunze ex Steud.	–	–	8	13	–	6	–	–	–	–	–
Ch. olivaceum Cooke et Ellis	–	–	10	5	–	–	–	–	–	–	–
Cladosporium cladosporioides (Fres.) Vries	–	–	–	–	–	–	–	–	4	11	–
C. herbarum (Pers.) Link ex Gray	–	–	–	–	1	1	–	–	–	–	–
C. macrocarpum Preuss	–	–	–	–	–	–	–	–	–	2	–
C. sphaerospermum Penz.	–	–	–	14	5	9	1				
Fusarium sambucinum Fuckel	–	1	–	–	–	6	–	–	–	–	–
Geotrichum candidum Link ex Leman	2	–	–	–	–	8	–	–	–	–	–
Leptosphaeria coniothyrium (Fuckel) Sacc.	–	–	1	4	–	–	1	–	2	–	–
Melanospora acculleata Hans	5	–	–	–	–	–	3	–	–	–	–
Monilia brunnea Gilman et Abbott	–	–	–	–	–	–	2	–	–	–	
M. geophila Oudem.	–	–	–	2	–	–	–	–	–	–	–
Mortierella horticola Linnem.	–	–	–	2	–	–	–	–	12	10	–
M. isabellina Oudem.	–	–	–	–	4	2	–	–	–	–	–

cont. Tab. 1

1	2	3	4	5	6	7	8	9	10	11	12
Nigrospora sphaerica (Sacc.) Mason	–	–	2	–	–	–	–	–	10	–	–
Paecilomyces farinosus (Holm ex Gray) Brown et G. Sm.	2	3	25	–	16	–	2	–	8	20	1
P. fumosoroseus (Wize) Brown et G. Sm.	4	4	41	38	36	11	–	–	14	54	6
Penicillium citrinum Thom	–	–	–	–	–	–	–	–	–	15	8
P. chrysogenum Thom	–	–	–	–	–	–	14	6	–	–	–
P. digitatum (Pers. ex St.- Am.) Sacc.	–	–	3	–	–	–	–	–	4	1	1
P. expansum Link ex Gray	–	–	8	11	18	12	–	–	2	4	2
P. funiculosum Thom	48	18	17	5	8	11	15	33	10	6	9
P. janthinellum Biourge	16	6	3	1	–	5	7	7	15	18	4
P. nigricans Bain. ex Thom	–	–	–	–	–	–	–	2	2	2	18
P. purpurogenum Stoll	15	–	–	–	–	–	–	–	8	–	–
P. restrictum Gilman et Abbott	–	–	–	–	–	–	–	–	16	2	–
P. variabile Sopp	–	–	–	–	–	–	–	–	7	–	2
P. verrucosum var. *cyclopium* (Westl.) Samson, Stolk et Hadlok	8	–	–	3	3	3	2	23	25	18	3
Phialophora cyclaminis Beyma	–	7	–	–	–	2	–	–	–	–	2
Phoma eupyrena Sacc.	–	–	–	–	–	–	1	–	–	–	–
Ph. leveillei Boerema et Bollen	–	–	–	1	–	–	–	–	3	1	–
Ph. medicaginis Malbr. et Roum.	–	–	1	–	20	21	16	5	–	–	–
Pseudeurotium zonatum Beyma	–	–	–	14	–	1	2	–	–	–	
Rhizopus stolonifer (Ehrenb. ex Link) Lind	32	4	14	9	24	30	9	1	5	3	–
Scopulariopsis brumptii Salvanet-Duval	–	–	1	–	18	11	14	10	12	18	–
S. chartarum (G. Sm.) Morton et G. Sm.	2	9	–	–	–	–	–	–	14	11	–
Trichoderma harzianum Rifai	5	–	–	–	–	–	–	–	18	22	–
T. viride Pers ex Gray	16	14	–	–	–	–	–	–	20	33	–
Yeast fungi	7	2	8	11	5	–	10	–	10	13	–
Total number of colonies	257	92	162	231	187	183	163	136	278	290	131
Total number of species	18	13	18	21	15	19	18	14	27	24	19

18.4% and *A. niger* which constituted 12.88%. In the plot where a double dose of this neutralizer was applied the above values were reduced by a half. *Penicillium* spp. constituted 23.31 and 52.2% of the communities among which the dominating species were *P. funiculosum* and *P. verrucosum* v. *cyclopium*. From this plot high numbers of *Ph. medicaginis*, *S. brumptii*, and *R. stolonifer* species were isolated.

The plots neutralized with two doses of chalk were dominated by *A. kiliense*, *A. niger*, *P. funiculosum*, *P. janthinellum*, and *T. viride*. In the plots where the double dose of chalk was applied the number of *Penicillium* spp. declined by 33.85 – 26.09% and thal of *Aspergillus* spp. by 14.1 – 6.25%.

In the plots neutralized with limestone (at two doses) *Aspergillus* spp. constituted 14.97% and 25.7% of the communities whereas *Penicillium* spp. about 16%. In addition a frequent occurrence of *Ph. medicaginis* and *R. stolonifer* was observed.

In the plots neutralized with ground phosphate rock a decrease in the number of fungi of the genus *Aspergillus* and an almost complete elimination of *A. niger* were recorded. Fungi of the genus *Penicillium* constituted 32.03% and 22.76% of the communities among which *P. verrucosum* v. *cyclopium* and *P. janthinellum* dominated. Moreover, the occurrence of such fungi as: *A. kiliense*, *C. cladosporioides*, *C. sphaerospermum*, *M. horticola*, *N. sphaerica*, *P. farinosus*, *P. fumosoroseus*, *Ph. leveillei*, *R. stolonifer*, *S. brumptii*, *S. chartarum*, *T. harzianum* and *T. viride* was recorded. Fungi of *Trichoderma* species constituted 13.66 and 18.97% of total communities. A similar composition of the fungi community (except for *Trichoderma* spp.) was found in plots neutralized with ash. In additions such species as *A. alternata*, *Ch. funicola*, *Ch. globosum*, *Ch. olivaceum*, and *P. zonatum* were noted (Tab. 1).

Among 48 isolated species of fungi 6 were classed as dominants, 16 as sub-dominants and 26 as accessory species.

In the group of dominants such species as *A. versicolor*, *P. fumosoroseus*, *P. funiculatum*, and *A. kiliense* were most numerous. Among the sub-dominants *P. verrucosum* v. *cyclopium*, *S. brumptii*, *T. viride*, *P. janthinellum*, *P. farinosus*, *Ph. medicaginis*, and *P. expansum* were the most frequently occurring species (Tab. 2).

At the different dates of the investigation the variation observed in the number of colonies and species was associated both with the seasonal changes and the effect of neutralizers. In general, it appeared that in the control plot no significant differences occurred in the number of populations during the investigation. Within two months after the neutralization a rapid decrease in the number of fungi (as compared with the control) was noted in the plots neutralized with the two doses of chalk and ash and with the double dose of burnt lime. In the same period the populations of fungi were doubled in plots treated with ground phosphate rock. In the second year of the experiment the

Table 2
Dominant (A) and sub-dominant (B) species of soil fungi isolated from neutralized soils of the "Bełchatów" dumping ground

Fungi	No of fungi colonies	Percentage [%]
A – *Aspergillus versicolor*	224	10.66
Paecilomyces fumosoroseus	208	9.86
Penicillium funiculosum	180	8.53
Acremonium kiliense	160	7.58
Aspergillus niger	156	7.39
Rhizopus stolonifer	131	6.21
B – *Penicillium verrucosum* var. *cyclopium*	88	4.17
Scopulariopsis brumptii	84	3.98
Trichoderma viride	83	3.93
Penicillium janthinellum	82	3.87
Paecilomyces farinosus	77	3.65
Yeast fungi	66	3.13
Phoma medicaginis	63	2.99
Penicillium expansum	57	2.70
Trichoderma harzianum	45	2.13
Scopulariopsis chartarum	.36	1.71
Chaetomium globosum	27	1.28
Penicillium nigricans	24	1.14
Mortierella horticola	24	1.14
Penicillium purpurogenum	23	1.09
Penicillium citrinum	23	1.09
Pseudeurotium zonatum	21	1.00

Table 3
Density index of soil fungi populations in neutralized soils of the "Bełchatów" dumping ground

Combinations	Density index nos/g of soil			
	1.08.95	26.10.95	23.05.96	19.09.96
Chalk 1×	714	8929	13035	23214
Chalk 2×	0	2679	5714	8036
Ash 1×	357	5714	7857	15000
Ash 2×	1250	4038	17142	18750
Limestone 1×	3214	2321	6429	21428
Limestone 2×	7857	4464	9643	10714
Burnt lime 1×	8393	5357	6429	8929
Burnt lime 2×	357	8036	8393	7500
Ground ph. rock 1×	8929	10714	14464	15536
Ground ph. rock 2x×	9821	10536	13928	17500
"O"	4642	5357	· 5357	5714

pattern of succession was more dynamic (particularly in plots treated with chalk, ash, and ground phosphate rock).

Seventeen months after the neutralization the greatest mycological activity expressed by the density index of fungi colonies was recorded in the plots neutralized with single doses of chalk and limestone or with ash and ground phosphate rock at the two rates. The density index of fungi colonies was four times higher in the case of chalk and limestone than in the control plot, and 3 times higher in the case of ground phosphate rock and ash neutralization (Tab. 3).

DISCUSSION

The quantitative and qualitative composition of fungi communities isolated from toxic acid soils neutralized with chalk, limestone, and burnt lime was decidedly different from that of the communities isolated from developed agricultural and forest soils and also from reclaimed untoxic dumping grounds sown with grasses (Kowalik 1993, 1995a). The present results indicated that most species of soil fungi were sensitive to very low pH values of the environment (Franz 1973; Lehto 1994). The only spectrum approximating to fungi communities from soils sown with grass on reclaimed non-toxic grounds, were fungi isolated from plots neutralized with ground phosphate rock and ash (Kowalik and Kowalik 1996). Fungi of the genera *Cladosporium*, *Mortierella*, *Nigrospora*, *Paecilomyces*, *Phoma*, *Rhizopus*, *Scopulariopsis*, and *Trichoderma* which occurred in the soil neutralized with ground phosphate rock belonged to microbiocenoses which were fairly common in developed soils (Kowalik and Kowalik 1996). Here the numerous occurrence of structure-forming fungi *T. harzianum* and *T. viride* played a particularly important role. In plots neutralized with ash this observation was supported by the occurrence of *Chaetomium* spp. These fungi and numerous *Penicillium* spp. contributed to the formation of soil aggregates (Harris et al. 1966), thus constituting the structure-forming factor.

The colonization of toxic acid soils of the control plot and of some plots not neutralized to neutral pH by numerous *Penicillium* and *Aspergillus* species, such as *A. fumigatus*, *A. niger*, *A. wentii*, *P. funiculosum*, *P. purpurogenum*, *P. variabile*, and *P. verrucosum* v. *cyclopium* confirmed their acidiphilic properties reported by Trique (1970) and Franz (1973).

A decrease in the number of fungi colonies within 2 months after neutralization with chalk and burnt lime may be explained by the radically changed pH of the grounds (from toxic acid to neutral or slightly alkaline) and thereby the change of habitat conditions of the fungi. The more dynamic pattern of succession in the second year after the neutralization suggested the stabilization of the community of "primary colonizers" and the colonization of great parts of the area by new species of fungi (K o w a l i k 1995b). The introduction of perennial ryegrass, development of its root system, and mineral fertilization (K r z a k l e w s k i et al. 1997) unquestionably affected a more dynamic development of populations in the second year of the investigation.

CONCLUSION

The mycological activity was poorly marked in the toxic acid non-neutralized soils of the top layer of the "Bełchatów" dumping ground which were only colonized by scarce soil fungi with *Penicillium nigricans*, *P. funiculosum*, *P. citrinum* and *Aspergillus niger*, *A. versicolor* as the dominating species.

For the toxic acid soils of the top layer of the "Bełchatów" dumping ground the most beneficial neutralizers in the mycological aspect were ground phosphate rock and ash, since they stimulated the development of fungi which produced microbiocenoses similar to these of non-toxic soils of the dumping grounds under agricultural reclamation conditions and sown with grasses.

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Grzyby zasiedlające toksycznie kwaśne grunty zwałowiska Kopalni Węgla Brunatnego "Bełchatów"

Streszczenie

Badania prowadzono w latach 1995–1996 na rekultywowanych, toksycznie kwaśnych gruntach zwałowiska „Bełchatów", neutralizowanych kredą, popiołem, wapniakiem, wapnem palonym i mączką fosforytową. Celem badań było poznanie grzybów glebowych zasiedlających grunty wierzchowiny zwałowiska przed i po ich neutralizacji, a także określenie wpływu zastosowanych neutralizatorów na skład gatunkowy i dynamikę rozwoju ich populacji. Określono dynamikę liczebności populacji, gatunki grzybów dominujących i influentnych oraz wskaźnik zagęszczenia kolonii.

Stwierdzono, że toksycznie kwaśne grunty zwałowiskowe są ubogie pod względem mikologicznym; zasiedlają je tylko nieliczne grzyby glebowe, głównie z rodzaju *Penicillium* i *Aspergillus*. Wykonanie neutralizacji spowodowało rozwój zbiorowisk grzybów glebowych zróżnicowany w zależności od zastosowanego neutralizatora.

Przeprowadzono ocenę zastosowanych w doświadczeniu neutralizatorów w aspekcie ich wpływu na aktywność mikologiczną, tworzonej w procesie rekultywacji, gleby inicjalnej.