J. Elementol. 2010, 15(1): 39–51

FUNGI COLONIZING SOIL FERTILIZED WITH COMPOSTED SEWAGE SLUDGE AND MUNICIPAL WASTE

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Abstract

A strict field experiment was conducted in 2004-2007 on grey-brown podzolic soil originating from light silty loam, which was 5.04 in pH, highly abundant in P, moderately abundant in K, Zn, Mn and very poor in Mg. The experiment was established in a random block design with four replications. In a four-year crop rotation system, the following sequence of crops was grown: industrial potato, fodder spring barley, winter oilseed rape and winter wheat. The experiment consisted of treatments with farmyard manure and composted sewage sludge (Biohum, Polepszacz, Tyrowo) as well as composted municipal waste (Dano, green waste). The manure and organic fertilizers were applied once (10 t d.m. ha^{-1}) or twice ($2 \cdot 5$ t d.m. ha^{-1}). For comparison, unfertilized plots (control) and NPK fertilized plots were used.

The phytopathological assays were completed in order to determine the effect of natural and organic fertilization on the structure of soil fungal communities. In a laboratory, soil samples were made into suspensions of the dilution from 10^{-2} to 10^{-4}. Fungi were cultured for 5 days on Martin substrate in Petri dishes at 22°C. The grown fungal colonies were calculated into g dry mass and inoculated onto agar slabs for later species identification.

The results suggest positive influence of fertilization with FYM, composted sewage sludge and composted municipal waste on the chemical properties and biotic relationships in soil, i.e. on the growth of fungi which can act antagonistically towards pathogenic species, suppressing at the same time populations of pathogenic fungi. Highest counts of pathogenic fungi were found in unfertilized soil (in the control treatment). The most desirable effect was produced by FYM applied once in a dose of 10 t d.m. ha^{-1}.

Key words: organic fertilization, zinc, manganese, soil, pathogens, saprophytes.

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GRZYBY KOLONIZUJĄCE GLEBĘ NAWOŻONĄ KOMPOSTAMI Z OSADÓW ŚIEKOWYCH I ODPADÓW KOMUNALNYCH

Abstrakt

W latach 2004-2007 przeprowadzono ścisłe doświadczenie polowe na glebie płowej wytworzonej z gliny lekkiej pylastej, pH 5,04 oraz wysokiej zawartości P, średniej K, Zn i Mn oraz niskiej Mg. Eksperyment założono w układzie losowanych bloków, w trzech powtórzeniach. W czteroletnim płodozmianie uprawiano kolejno: ziemniak przemysłowy, jęczmień jary paszowy, rzepak ozimy i pszenicę ozimą. Eksperyment obejmował obiekty z obornikiem i kompostowanymi osadami ściekowymi (Biohum, Polepszacz, Tyrowo) oraz kompostowanymi odpadami komunalnymi (Dano, zieleń miejska). Obornik i nawozy organiczne stosowano jedno- (10 t s.m.$\cdot$ha^{-1}) lub dwukrotnie w zmianowaniu (2$\cdot$5 t s.m.$\cdot$ha^{-1}). Obiekty porównawcze stanowiły poletka bez nawożenia (kontrola) i z nawożeniem mineralnym NPK.

Badania fitopatologiczne wykonano w celu określenia wpływu nawożenia naturalnego i organicznego na strukturę zbiorowiska grzybów glebowych. W laboratorium z pobranych próbek gleby sporządzano zawiesiny wodne o rozcieńczeniu od 10^{-2} do 10^{-4}. Grzyby hodowano w płytkach Petriego, na podłożu Martina, przez 5 dni w temperaturze 22°C. Wyrosłe kolonie grzybów przeliczano na g suchej masy, po czym przeszczepiano na skosy agarowe z zamiarem późniejszej identyfikacji gatunkowej.

Wyniki badań wskazują na pozytywny wpływ zastosowanego nawożenia obornikiem i kompostami z osadów ściekowych i odpadów komunalnych na właściwości chemiczne i stosunki biotyczne w glebie, tj. wzrost liczebności grzybów o uzdolnieniach antagonistycznych względem gatunków patogenicznych, a jednocześnie redukcję populacji tych ostatnich. Najwięcej patogenów zasiedlało glebę nienawożoną (w obiekcie kontrolnym). Najbardziej dobroczynne działanie wykazał obornik zastosowany jednorazowo w dawce 10 t s.m.$\cdot$ha^{-1}.

Słowa kluczowe: nawożenie organiczne, cynk, mangan, gleba, patogeny, saprotrofy.

INTRODUCTION

Beside very small quantities used to reclaim degraded land or re-fill excavation cavities, most sewage sludge, including municipal waste and green waste, is deposited at landfills. However, the high content of organic substance and some mineral components that sewage sludge has makes it suitable for agricultural applications, especially as a substance amending poorly fertile soils. Increased content of organic carbon, owing to the availability of substrates and their improved utilization, enhances the physicochemical properties of soil (Garcia et al. 2000, Hue 1988). Moreover, the value of composts as fertilizer is a consequence of their beneficial influence on the soil's biological life. After all, carbon is known to be a source of nutrition and energy for soil microorganisms. In soil which is fertilized with organic substance, the concentration of macro- and micronutrients rises (Warman, Termeer 2005). There are many references (Ritz et al. 1997, Arti Bhatia Pathak, Joshin 2001) which prove that organic fertilizers help establish proper biotic relations in soils, i.e. rising counts of valuable bacteria and fungi which are antagonistic to plant pathogens, and this supports the biological protection of crops.

Owing to the enhanced microbial activity, the soil enzyme activity increases (de CAIRE et al. 2000). The suitability of composts for biological control of plant pathogens depends on the compost maturity, chemical composition and physical structure (BENDING et al. 2004). The mechanisms which are taken advantage of in this type of plant protection include competition for C and Fe, antibiosis, parasitism and induction of systemic resistance of plants (HORBNY 1990). Many reports (KUTTER et al. 1988, GORODECKI, HADAR 1990) state that natural and organic fertilizers, including sewage sludge and municipal waste-based composts, have a limiting effect on the development of soil pathogens of the genera *Pythium, Phytophthora* and *Fusarium* as well as the species *Rhizoctonia solani*. This study has been carried out to compare the effect of mineral fertilization versus application of FYM or various types of organic fertilizers on the content of zinc and manganese in soil as well as the structure of soil fungal communities.

MATERIAL AND METHODS

In 2004-2007, a strict field experiment was conducted at the Agricultural Experimental Station in Bałcyny. Plots covering 15 m^3 were established on grey-brown podzolic soil formed from light silty loam of class III, complex 4. Before the experiment was established, samples of soil, FYM and composts were taken. Soil was 5.04 in pH, highly abundant in available forms of P, moderately abundant in K, poorly abundant in Mg and moderately abundant in available Zn and Mn.

The experiment was established according to a random block design with three replications. In a four-year crop rotation cycle the following crops were sown: industrial potato, fodder spring barley, winter oilseed rape and winter wheat. The experimental design comprised the following treatments: I – control (no fertilization), II – NPK mineral fertilization, III – farmyard manure 10 t d.m. ha^{-1}, IV – farmyard manure 2·5 t d.m. ha^{-1}, V – Biohum 10 t d.m. ha^{-1} (sewage sludge composted with straw), VI – Biohum 2·5 t d.m. ha^{-1}, VII – Polepszacz 10 t d.m. ha^{-1} (dried and granulated sewage sludge), VIII – Polepszacz 2·5 t d.m. ha^{-1}, IX – Tyrowo 10 t d.m. ha^{-1} (sewage sludge composted alone), X – Tyrowo 2·5 t d.m. ha^{-1}, XI – Dano 10 t d.m. ha^{-1} (compost from unsegregated municipal waste), XII – Dano 2·5 t d.m. ha^{-1}, XII – composted green waste 10 t d.m. ha^{-1}, XIV – composted green waste 2·5 t d.m. ha^{-1}. The composts as well as FYM were applied once during the rotation cycle in a dose of 10 t d.m. ha^{-1} (under potato), or twice, 5 t d.m. ha^{-1} each time (under potato and winter oilseed rape). In the plots fertilized with organic substances and FYM, nitrogen was balanced up to 150 kg ha^{-1} (2004) and to 120 kg ha^{-1} (2005), depending on the concentration of total nitrogen in soil. Spring barley and winter wheat received only mineral fertilization. The concentrations of macro- and micronutrients in the applied organic fertilizers is presented in Table 1.

The phytopathological tests involved isolation of fungi from soil. For this purpose, in the first week of August in each year of the experiment, soil samples were taken from the depth of 10 cm, from three points on each plot. Portions of 10 g were weighted out from aggregated and mixed soil samples and placed in 250 cm^3 flasks. Afterwards, 90 cm^3 of sterile water was added to each flask. The samples were shaken for 20 minutes, and the suspension (concentration 10^{-1}) was used to obtain subsequent dilutions: 10^{-2} and 10^{-4}. A portion of 1 cm^3 of the suspension and 20 cm^3 of Martin nutrient were placed on each Petri plate. After 5-day incubation at 22°C, the grown colonies of fungi were calculated into g dry mass and then inoculated onto agar slabs for species identification according to the keys prepared by BOOTH (1971), ELLIS (1971) and DOMSCH et al. (1980).

Table 1

Chemical composition of sewage sludge and composts used for the experiment

Specification	Unit	Sewage sludge			Municipal waste	
		with straw (Biohum)	dried and granulated (Polepszacz)	composted (Tyrowo)	Dano	green waste
Dry matter	%	58.68	85.14	49.31	74.64	78.88
C	%	18.47	38.27	21.39	13.62	8.02
N	$g \cdot kg^{-1}$	10.71	18.82	46.61	8.93	5.33
C/N		17.3	21.3	4.6	15.3	15.1
P	$mg \cdot kg^{-1}$	7.52	2.81	29.93	3.94	4.10
K	$mg \cdot kg^{-1}$	1.35	1.83	2.52	6.31	2.91
Mg	$mg \cdot kg^{-1}$	2.11	3.42	7.83	3.32	2.95
Zn	$mg \cdot kg^{-1}$	109.51	270.40	1310.02	679.50	301.03
Mn	$mg \cdot kg^{-1}$	210.60	228.01	300.51	273.64	326.81

RESULTS AND DISCUSSION

The soil-amending substances tested in our experiment significantly modified the soil content of available forms of zinc, but did not change the soil abundance class (Figure 1). After four years, it was evident that the most beneficial influence on the concentration of this element in soil, as compared with the control (7.3 mg kg^{-1}), was produced by composted sewage sludge from Tyrowo (15.8 mg kg^{-1}). In the soil samples from the other treatments, the available zinc content was on average around 10 mg kg^{-1}.

Microorganisms play an important role in the cycle of oxidation and reduction of manganese. Soil contamination with this element is associated with its form rather than the amount. Excess of easily soluble manganese

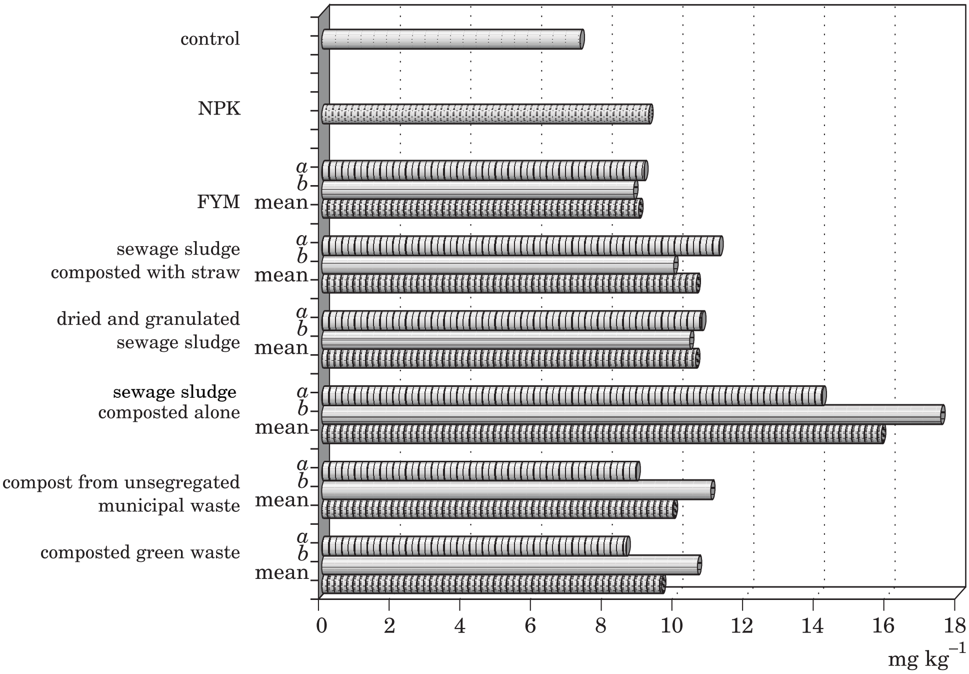


Fig 1. Zink content in the soil after 4-year testing cycles (*a* – 10 t d.m. ha^{-1}; *b* – 2·5 t ha^{-1})

can occur in soils fertilized with municipal waste. Although the amounts of manganese in all the tested fertilizers were approximately the same, their influence on the level of this element in soil was not unambiguous. After four years of the tests, the concentration of manganese in soil ranged from 116.2 (unfertilized treatment) to 156.2 mg kg^{-1} (composted sewage sludge from Tyrowo), but in none of the treatments the level of manganese exceeded the one claimed to be average for this type of soil (Figure 2). The form of the tested fertilizers rather than the way they had been applied had a more profound effect on the content of elements in soil.

The phytopathological tests demonstrated more colony forming units of fungi in the soil from the treatments where organic fertilizers were applied (Biohum in both application variants, Dano in a split dose of 5 t ha^{-1} and green waste in a single dose of 10 t ha^{-1}) than the ones fertilized with FYM, mineral fertilizers or the control treatment (Figure 3).

A study completed by WARMAN and TERMEER (2005) implies that under the influence of composted and fresh sewage sludge, the soil becomes enriched with Zn and Mn, and the mobility of these elements is higher in loamy than in sandy soil (SABIENE et al. 2002). Increasing rates of sewage sludge led to a growth in the population of microorganisms, including fungi, and the enhanced enzymatic activity of soil (AWAD, FAWZY 2004). KUBATOVA et al.

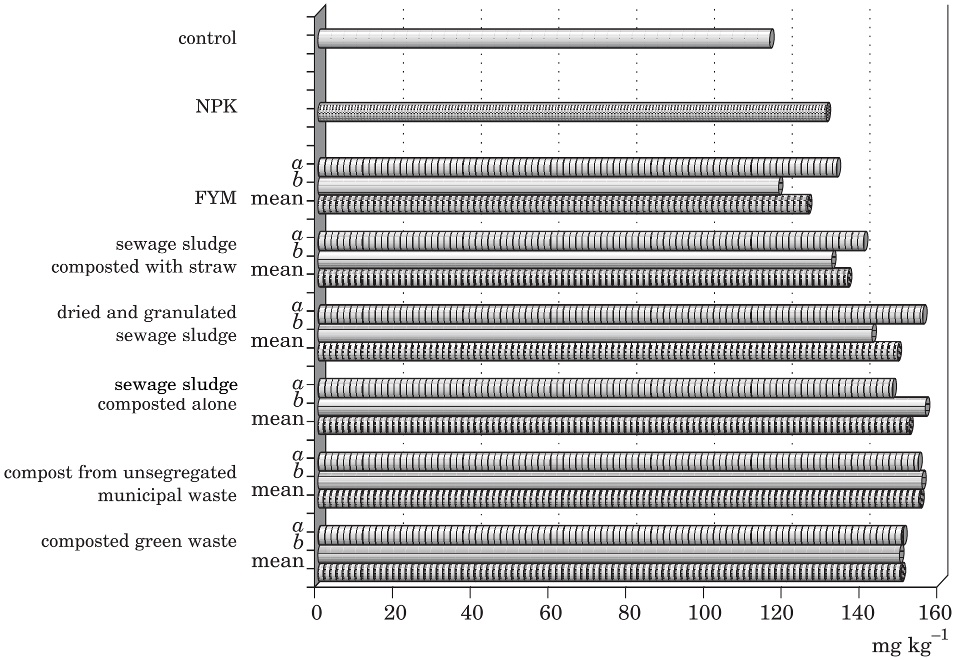


Fig. 2. Manganese content in the soil after 4-year testing cycle (a – 10 t d.m. ha^{-1}; b – 2·5 t ha^{-1})

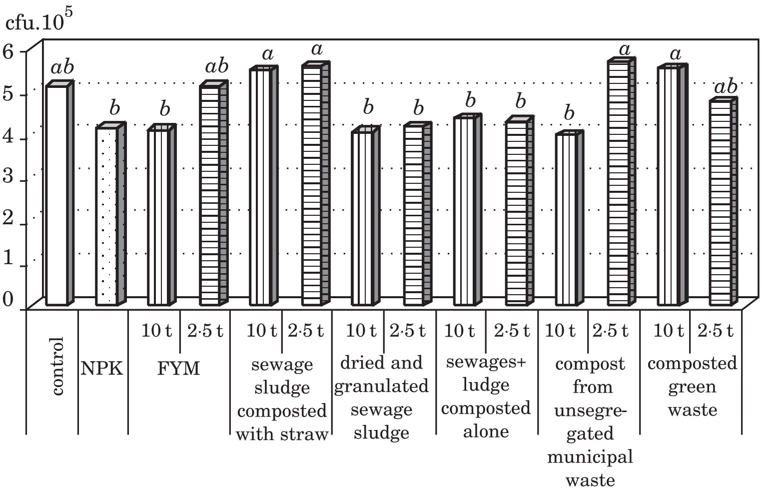


Fig. 3. Number of colony-forming units of fungi in the soil (data marked by the same letters do not differ statistically)

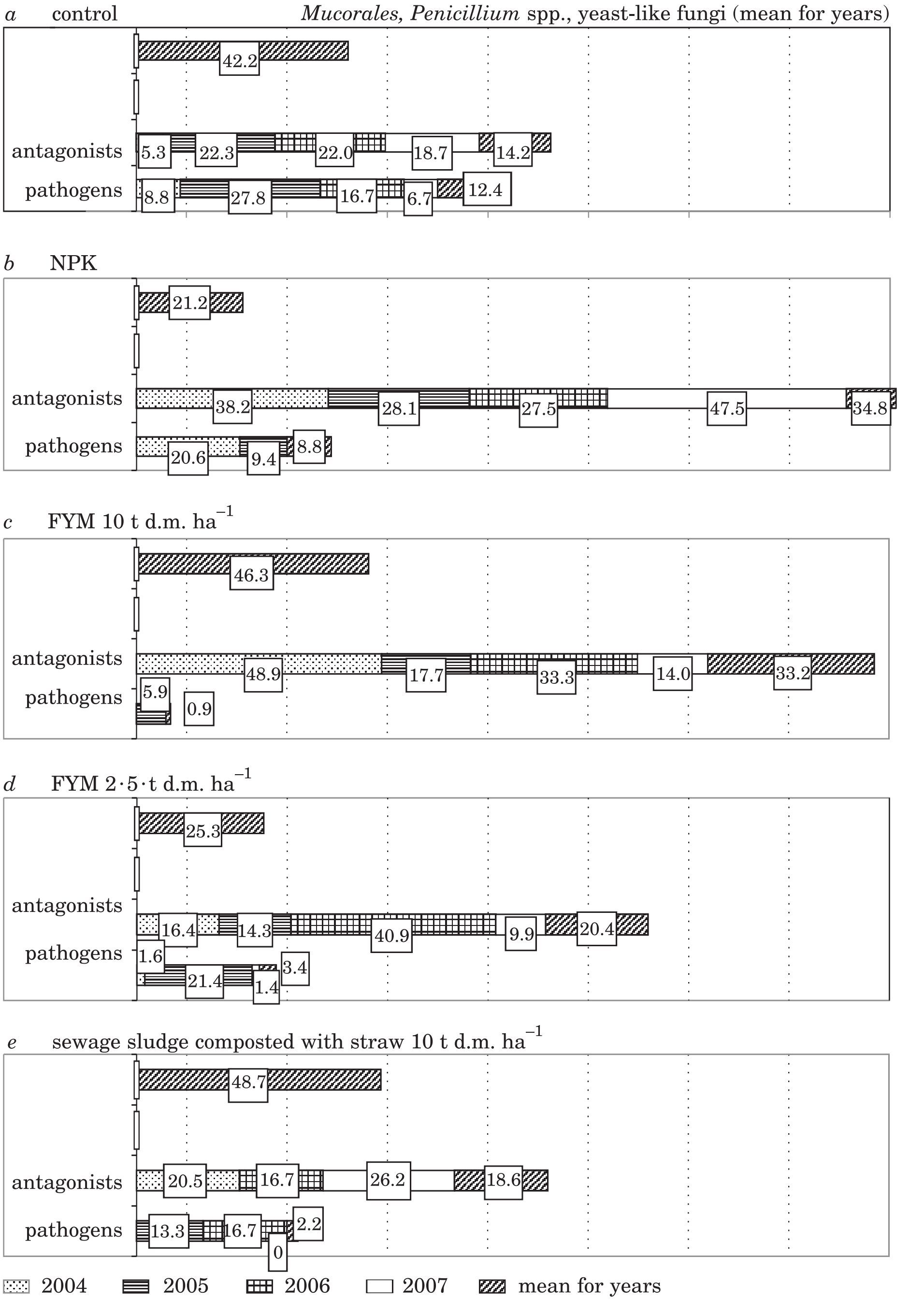
(2002) identified in soil rich in Zn and Mn such fungi as *Paecilomyces lilacinus*, *Mucor hiemalis*, *Mortierella aplina*, *Coniothyrium fuckeli*, species *Trichoderma*, *Penicillium* and *Fusarium*. Pratt (2008) in turn reports that the level of Zn in soil fertilized with waste from hog production tended to be higher than in unfertilized soil, and the level of Mn was rarely or never higher. The above authors also claim that the composition of a fungal assemblage in soil is constant and independent from organic fertilization.

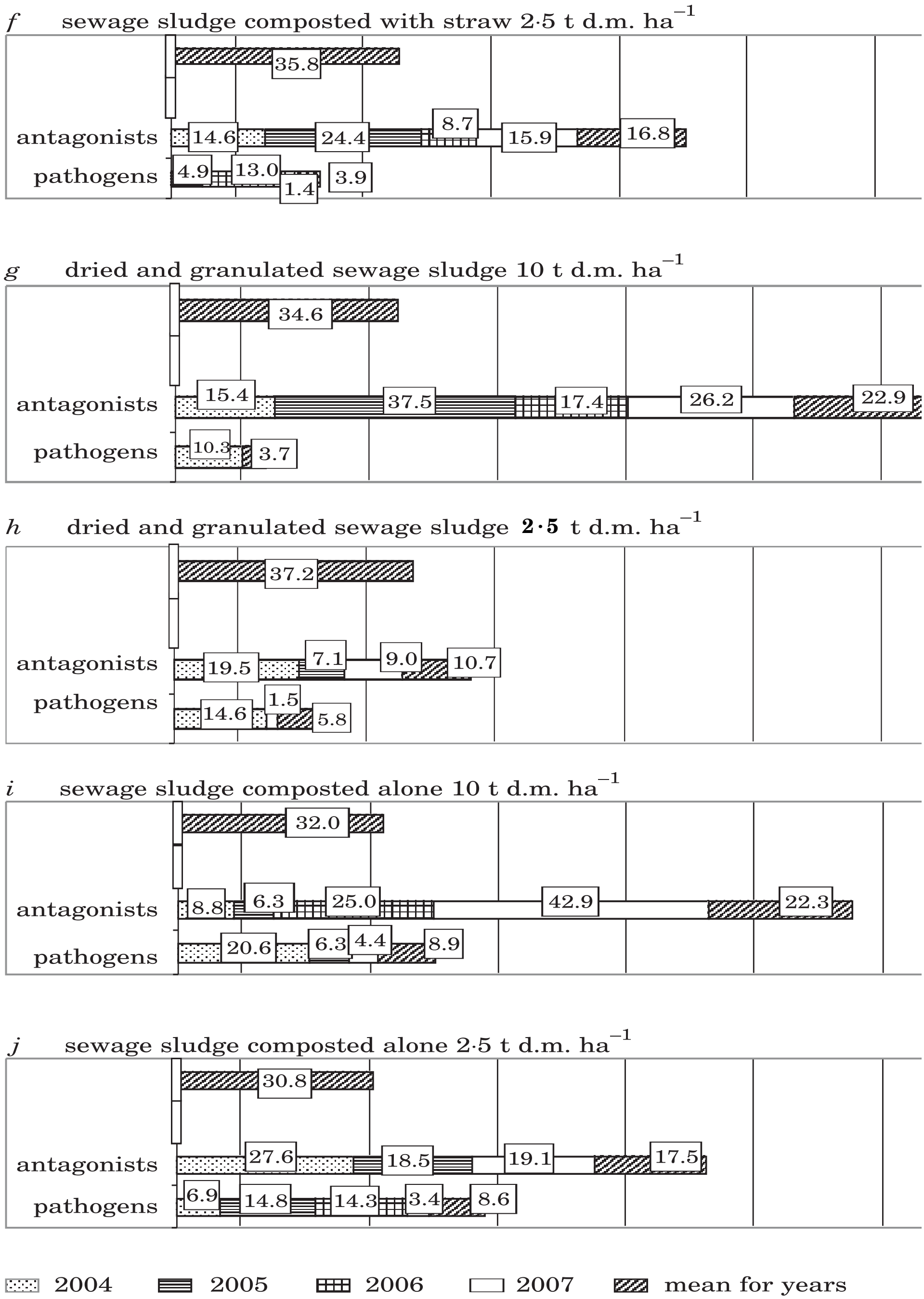
In our own studies, the community of soil fungi was represented by 67 species of fungi as well as non-sporulating cultures and yeast-like fungi. Among the potential pathogens, species of the genus *Fusarium* were identified as well as the species *Alternaria alternata*, *Aureobasidium bollei*, *A. pullulans*, *Botrytis cinerea*, *Colletotrichum coccodes* and *Sclerotina sclerotinum*. Soil from the control treatment had the highest population of fungi (12.4% share in the microbial community; Figure 4a). At the same time, this population contained a low percentage of antagonistic fungi of the genera *Gliocladium*, *Paecilomyces* and *Trichoderma* (14.2%). It was evident that biotic relations in soil fertilized exclusively with NPK mineral fertilizers were superior to those discovered in the control plot (Figure 4b).

Mineral and organic fertilization determines the quantitative and qualitative composition of a community of soil microorganisms, and consequently has influence on the activity of biological life in soil (Shannon et al. 2002, Larkin et al. 2006, Mader et al. 2006, Korniłłowicz-Kowalska et al. 2008).

Our own research has demonstrated that FYM fertilization stimulated the growth of antagonistic fungi while suppressing counts of pathogens (Figures 4c,d). *Fusarium* spp. fungi, in the absence of any other pathogenic fungi, colonized, in the first two years of the experiment only, soil in the treatment where a single application of FYM was performed. In turn, antagonistic fungi appeared in high numbers in all the years of the experiment, with the peak occurrence of 50% in the first year.

Among the composted sewage sludge and municipal waste (Figures 4 e-n), the most positive effect on the structure of soil fungal assemblages was produced by composted and dried sewage sludge applied as a single dose under potato (Figures 4g). Pathogenic fungi were isolated from the soil sampled from that treatment only in the first year of the experiment. This was probably due to the fact that antagonistic fungi occurred numerously during the growing seasons in the subsequent years. A comparable influence was produced by Polepszacz (applied twice during the crop rotation) (Figures 4h) and Biohum (in both application variants) – Figures 4e,f. In the soil enriched with these fertilizers, the share of pathogenic fungi would not normally exceed 6%, whereas the percentage of antagonistic fungi ranged from 11 to 19%. The least positive conditions for the health of crops were established in the soil enriched with the compost from Tyrowo (Figures 4i,j) and composted green waste supplied in a split dose (Figures 4n). There, the counts of determined pathogens, including *Fusarium* spp., was the highest. In the same treatments, soil had the highest amount of available forms of zinc.


a control
Mucorales, Penicillium spp., yeast-like fungi (mean for years)
42.2
antagonists
5.3
22.3
22.0
18.7
14.2
pathogens
8.8
27.8
16.7
6.7
12.4
b NPK
21.2
antagonists
38.2
28.1
27.5
47.5
34.8
pathogens
20.6
9.4
8.8
c FYM 10 t d.m. ha⁻¹
46.3
antagonists
48.9
17.7
33.3
14.0
33.2
pathogens
5.9
0.9
d FYM 2·5·t d.m. ha⁻¹
25.3
antagonists
16.4
14.3
40.9
9.9
20.4
pathogens
1.6
21.4
1.4
3.4
e sewage sludge composted with straw 10 t d.m. ha⁻¹
48.7
antagonists
20.5
16.7
26.2
18.6
pathogens
13.3
16.7
0
2.2
2004
2005
2006
2007
mean for years


f sewage sludge composted with straw 2·5 t d.m. ha–1
35.8
antagonists
14.6
24.4
8.7
15.9
16.8
pathogens
4.9
13.0
1.4
3.9
g dried and granulated sewage sludge 10 t d.m. ha–1
34.6
antagonists
15.4
37.5
17.4
26.2
22.9
pathogens
10.3
3.7
h dried and granulated sewage sludge 2·5 t d.m. ha–1
37.2
antagonists
19.5
7.1
9.0
10.7
pathogens
14.6
1.5
5.8
i sewage sludge composted alone 10 t d.m. ha–1
32.0
antagonists
8.8
6.3
25.0
42.9
22.3
pathogens
20.6
6.3
4.4
8.9
j sewage sludge composted alone 2·5 t d.m. ha–1
30.8
antagonists
27.6
18.5
19.1
17.5
pathogens
6.9
14.8
14.3
3.4
8.6
2004
2005
2006
2007
mean for years

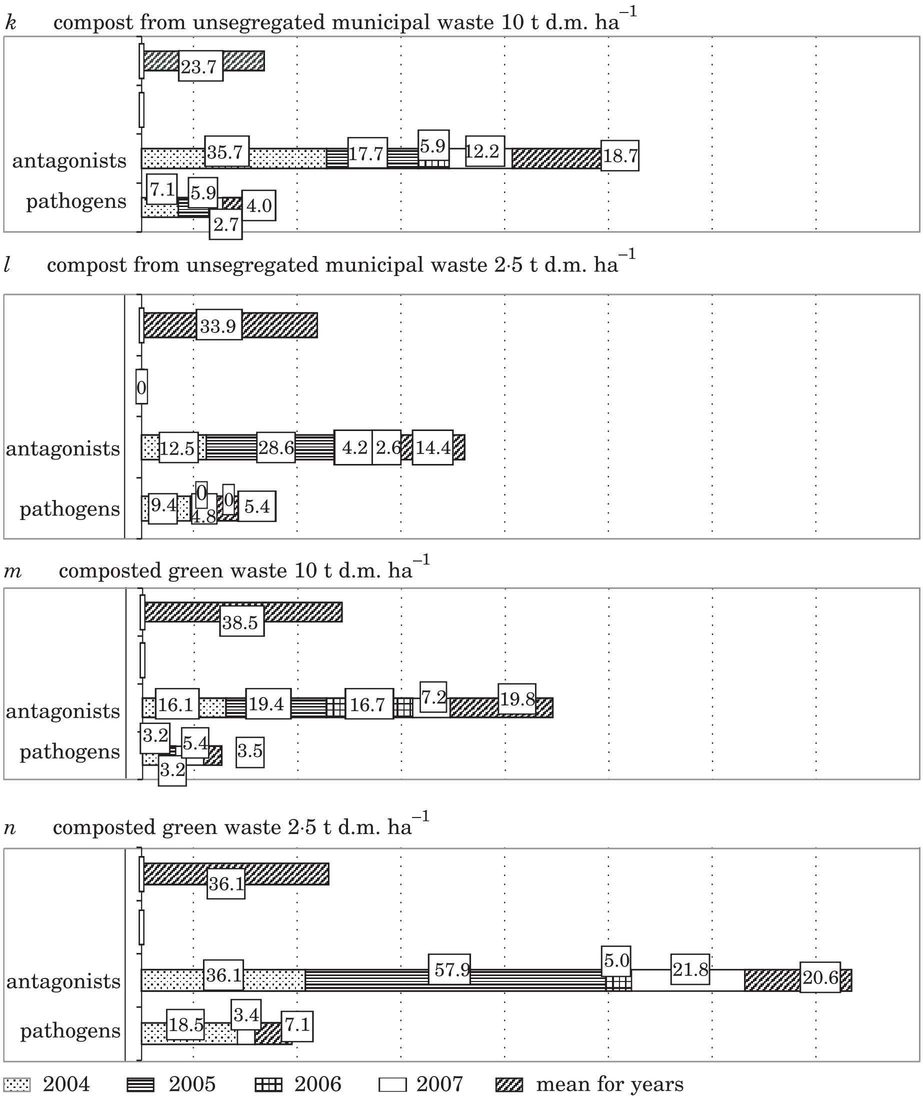


Fig. 4. Structure of fungi isolated from soil

KATO et al. (1981) report that application of composts favoured development of *Fusarium* spp. fungi, and attributed this finding to the fact that the C:N ratio was low and the content of N-NH_4 high. Contrary to that, LEWIS et al. (1992) claim that organic fertilization with composts, including composted sewage sludge, significantly depressed the range of presence of *Pythium*, *Phytophthora*, *R. solani* and *Fusarium* in soil cropped with different plants.

In the present study, the highest counts of fungi of the genus *Trichoderma* were determined in the soil fertilized with sewage sludge-based composts. At the same time, the soil from these treatments was characterized by the highest concentrations of zinc and manganese. Modifications in a soil fungal community leading towards increased counts of beneficial microorganisms are highly desirable, as they condition the biological control of plant pathogens. PANDEY et al. (2006) concluded that organic fertilization of soil favoured the development of fungi which acted as antagonists or parasites of plant pathogens. The presence and number of *T. viride*, as NAAR and BIRO (2006) reported, was positively correlated with the content of Zn in soil. The stimulating activity of the above microelements on the development of species of the genus *Trichoderma* has been verified by *in vitro* tests.

KREDICS et al. (2001) as well as SHANINA KALIM and GANDHI (2003) implied even that Zn and Mn (provided they occur in an optimum rate in soil) can protect some crop species against pathogens which belong to the genus *Rhizoctonia*. However, very high doses of Zn inhibited the enzymatic activity of antagonists which being tolerated by the pathogens of the genus *Pythium* (NAAR 2006), and could even stimulate production of oospores of this fungus-like organisms (ALTAF HUSSAIN BAIG 2003).

On average, the share of species of the order *Mucorales* in the communities of soil fungi assayed during the four years of the study did not exceed 15% in any of the application variants, except the treatments fertilized with Biohum. Good conditions for the growth and development were established for yeast-like fungi and the ones belonging to the genus *Penicillium*. The latter were most often isolated from the soil fertilized twice with Dano, and the lest frequently – from the soil enriched once with FYM or Biohum. At the same time, the smallest and the biggest counts of fungi of the genus *Trichoderma* were isolated from these treatments, respectively. It has been reported that the development of saprophytes of the genus *Penicillium* is suppressed by the above antagonists (DOMSCH et al. 1980).

The results of our experiment seem to suggest that FYM and composts used as fertilizers have positive influence on the chemical and biological properties of soil.

CONCLUSIONS

1. The use of biowaste for soil fertilization increase soil concentration of available forms of Zn and Mn, compared to mineral fertilization or FYM application.

2. Farmyard manure and composts applied in a single dose of 10 t d.m. ha^{-1} rather than a split dose of 2·5 t d.m. ha^{-1} are more efficient at stimulating the development of fungi which act antagonistically towards pathogens; at the same time they are better at suppressing counts of pathogens.

3. Most of potential pathogens colonized unfertilized soil.

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