Changes in fungal communities in organically fertilized soil

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ABSTRACT

The research project was carried out as a field experiment with application of the following crop rotation system: industrial potato, spring barley for fodder, winter rape and winter wheat, established in the random distribution of blocks in triplicate on gleyic luvisol formed of silty light loam. The aim of the research was to determine the influence of diversified organic fertilization based on composted wastewater sediments and farm manure on the community of soil fungi as compared to fields without fertilization and with NPK fertilization only. The fungi were cultured on the Martin medium and were counted and identified afterwards. As a result of three-year field experiments it was established that organic fertilization had a more determining effect on qualitative composition than numbers of soil fungi. Total number of fungal colony-forming units in the soil fertilized with Biohum at 10 t/ha and 5 t/ha was significantly higher than in soil with mineral NPK fertilization and without fertilization. Most frequently pathogens populated the soil in fields without fertilization and to a lesser extent the soil with mineral NPK fertilization. A positive influence of organic fertilizers on the fungal community structure was recorded. The number of pathogens was limited (to 1.2% in fields fertilized with farm manure) while the population of saprotrophic fungi possessing antagonistic properties increased.

Keywords: soil; organic fertilization; pathogenic fungi; saprotrophic fungi; water extract from compost

Composted wastewater sediments formed as a result of biological treatments can be used for fertilization of soil enriching it with organic matter and at the same time improving its structure. These fertilizers, due to their content of micro- and macroelements, represent a source of nutrients for plants. The increase of the microbiological diversity and activity of soil as a consequence of organic fertilization is widely documented in literature (Shannon et al. 2002, Larkin et al. 2006). It contributes to limiting some soil fungi development, including pathogens from genera *Pythium, Phytophthora* and *Fusarium* (Hoitink and Boehm 1999). At the same time the numbers of positive microflora possessing antagonistic influence in relation to these pathogens increase, which is then a very positive phenomenon. Soil microorganisms, including fungi, represent biological protection of plants against pathogenic factors (Mathur et al. 2006). The above changes in the structure of soil fungi community contribute to improving the health of cultivated crops. Also, the excretions from roots of the cultivated crops can influence the qualitative composition of the soil fungi community (Angus et al. 1994, Grayston et al. 1998).

MATERIAL AND METHODS

The experiment was established by the Chair of Agricultural Chemistry and Environment Protection in 2004 and is located at the Production-Experimental Enterprise in Bałcyny on gleyic lu-

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visol formed of silty light loam (Polish Standard BN-78/9180-11). Prior to the experiment the soil content of P (38.2 mg/kg), K (105.8 mg/kg) and Mg (48.3 mg/kg) and pH (5.04) were determined. The experiment involved rotation system of four crops, namely industrial potato, fodder spring barley, winter rape and winter wheat, as well as an option of organic fertilization based on sewage sediments and composts. The phytopathological studies were carried out during the first three years of the experiment. The studies comprised the following plots: (I) control (no fertilization), (II) mineral NPK fertilization, (III) farm manure 10 t/ha, (IV) farm manure 5 t/ha, (V) Biohum 10 t/ha (municipal sewage sediment with addition of straw, composted, N – 1.07% dry matter, P – 0.75% dm, K – 0.13% dm, Mg – 0.19% dm), (VI) Biohum 5 t/ha, (VII) soil improvement medium 10 t/ha (municipal sewage sediment dried and granulated, N – 1.88% dm, P – 1.28% dm, K – 0.18% dm, Mg – 0.37% dm), (VIII) soil improvement medium 5 t/ha, (IX) municipal sewage sediment from the treatment plant in Tyrowo 10 t/ha (N – 4.66% dm, P – 2.99% dm, K – 0.25% dm, Mg – 0.78% dm), (X) municipal sewage sediment from the treatment plant in Tyrowo 5 t/ha. The farm manure and composted sewage sediments at 10 t/ha were applied once in the crop rotation system, in 2004 before planting potato (Jasia cultivar). Mineral fertilization for potato was 150 kg N (ammonium nitrate 34%), 65 kg P (superphosphate 40%) and 166 kg K/ha (potassium salt 60%). The mineral fertilization on the plots fertilized with NPK was applied in whole before sowing. On the plots with farm manure and sewage sediments the fertilization with N was balanced to 150 kg/ha, depending on their content of total nitrogen, and completed after the main crop with ammonia nitrate. In 2005, only mineral fertilization (90 kg N, 26 kg P and 100 kg K/ha – forms of fertilizers as above) was applied for spring barley (Justyna cultivar); after the harvest and before sowing of winter rape mineral fertilization was applied at 120 kg N, 42 kg P and 134 kg K/ha. Organic fertilization at 5 t/ha was applied only on plots IV, VI, VIII and X. Supplementary fertilization with N was applied on the latter plots to reach 120 kg/h, depending on the content of total nitrogen in composts.

To determine the composition of species and the quantity of fungi, soil samples were collected at a depth of 10 cm in the first week of August of each year from three points on each plot representing specific combinations. In the laboratory the samples from each plot were combined; test samples of 10 g were weighted to 250 ml flasks and shaken for 20 min in 90 ml of sterile water obtaining the dilution of 10^{-1}. Higher dilutions (10^{-2} to 10^{-4}) were made from that suspension. The fungi was cultured on the Martin's nutrient medium at 22–23°C, and the fungal colonies developed after five days of incubation were converted to grams of dry matter of soil (Dhingra and Sinclair 1995). Next, the fungi were inoculated on agar slopes for later identification of species. At the same time the linear growth of seven potentially pathogenic fungal species, namely *Alternaria alternata, Botrytis cinerea, Colletotrichum coccodes* and genus *Fusarium*, was assessed on the PDA medium supplemented with water extracts from composts analyzed during the experiment. Each compost type in the form of 2 g of dry matter was flooded with 100 ml of sterile water and after 24 h the extracts were filtered through sterile linen. Petri dishes were filled with 2 ml of the filtrate, covered with 10 ml of cooled medium, and mixed by circular movements. On top of the solidified medium, 5 mm agar discs overgrown with 7-day cultures of fungi were placed. The control was provided by dishes with inoculum of pathogens on medium without the filtrates. The experiment was carried out in 4 repetitions (dishes), twice for each species. After 4 and 8 days the diameters of fungal colonies were measured (along two perpendicular lines); on that basis the growth inhibition coefficient, expressed by the formula:

$$I = [(\phi_k - \phi)/\phi_k] \times 100\%$$

where: ϕ_k and ϕ represent the diameter of the control culture and the culture on the medium with the extract from the composts, was computed

The results were processed statistically and subjected to variance analysis. The Duncan's test was used for comparison of the averages.

RESULTS AND DISCUSSION

The results obtained from the three-year experiment indicate that application of organic fertilization diversified the quantitative and qualitative composition of soil fungi communities. The highest total number of fungal colony-forming units was observed in the soil fertilized with Biohum at 10 t/ha (applied during the first year under cultivation of potato) and twice at 5 t/ha (under potato and before sowing of winter rape), and the

lowest total number of fungal colonies was found in the soil without fertilization and with mineral NPK fertilization (the differences were statistically significant – Figure 1). The literature (Sigler and Turco 2002, Spedding et al. 2004, Larkin and al. 2006) reports that mineral and organic fertilization is, next to the type of crop, rotation system and applied plant protection media, a cause of changes in the structure of soil microorganisms' communities.

Among the selected fungal colonies, 52 species and cultures forming no spores as well as yeasts-like fungi were identified (Table 1). Pathogens were represented by *Alternaria alternata, Aureobasidium pullulans, A. bollei, Botrytis cinerea, Colletotrichum coccodes*, *Sclerotinia sclerotiorum* and genus *Fusarium*. The largest number of pathogen colonies was obtained during the second vegetation season of the experiment (9.4%).

Changes in the structure of fungal community between individual setups of the experiment and between sampling dates were found (Figure 2a–j). The most numerous populations of pathogens were obtained from the plots without fertilization (14% of the total number of isolates in the setup – Figure 2a). Among them the following species were identified: *A. pullulans, S. sclerotiorum* and genus *Fusarium*. Their numbers increased 2- and 3-fold during the following two years of the experiment, as compared to year one. At the same time the lowest number of saprophytic fungi (23.7%) was obtained from the soil in that combination, as compared to the other combinations. Among them the species of genera *Gliocladium, Paecilomyces, Penicillium, Trichoderma* and order *Mucorales* were identified. They had a particularly low share in the fungal community during the first vegetation season. As a consequence, it can be assumed that the weakened biological barrier in the form of small numbers of antagonistic fungi was the cause of a more intensive development of the above-indicated pathogens.

Less numerous shares of pathogens in soil fungi community during the three-year study was observed on the plots with mineral NPK fertilization – 10.5% (Figure 2b). During the second year of experiment a decrease in their number by a half in comparison to the beginning of the period was observed in the soil under spring barley, while during the third year their presence was not confirmed. At the same time the number of the antagonistic fungi was twofold higher compared to the soil without fertilization.

Organic fertilization had a positive influence on the structure of soil fungi communities'. Its influence was more visible in changes in the qualitative composition of fungi than in their numbers. Hoitink and Boehm (1999) showed that in organically fertilized soil the growth of microorganisms is stimulated; they protect plants against pathogens of genera *Pythium* and *Phytophthora* due to their antibiotic and parasitic influence. In our experiment farm manure showed the best phytosanitary influence among the analyzed organic fertilizers. The share of pathogens in the soil fertilized with farm manure at 10 t/ha was 1.2% (average for three years of studies – Figure 2c). A higher share of these microorganisms (4.1%) was recorded in the soil fertilized by double application of farm manure at 5 t/ha (Figure 2d). In the soil with farm manure

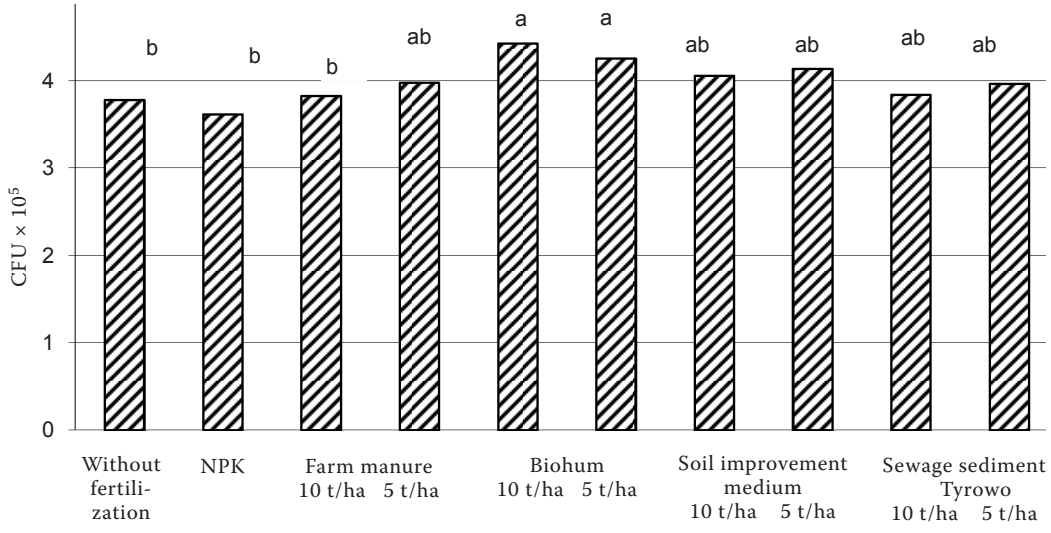

Figure 1. The number of fungal colony-forming units (CFUs) per 1 g soil sample

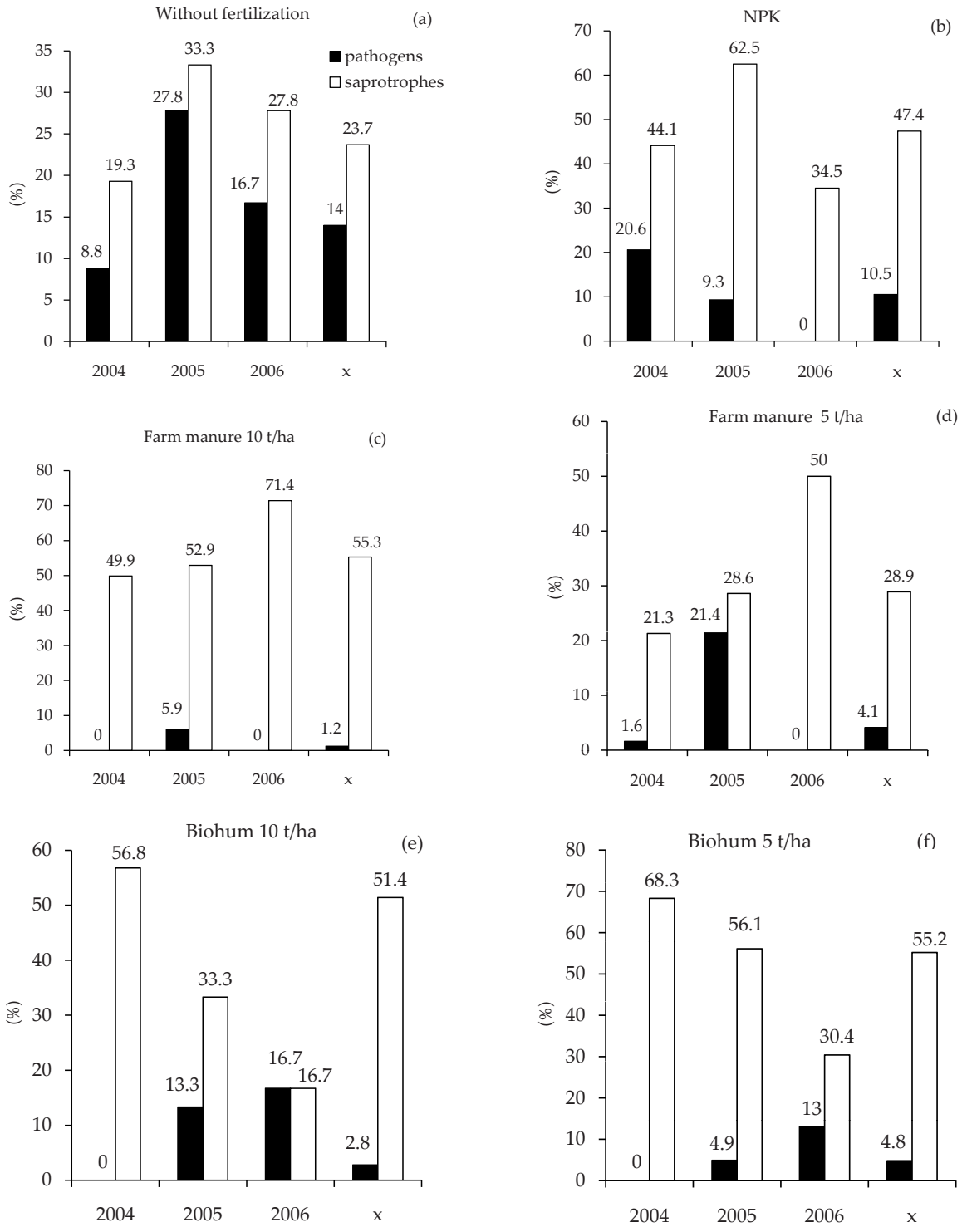

Figure 2a–f. Fungi isolated from soil of different treatments of experiment (years 2004–2006)

applied once or in two doses a total reduction in number of plant pathogens was observed during the third year of cultivation. This was accompanied by an increase in the population of saprophytic fungi, particularly of genus *Trichoderma*, which could explain the inhibited process of pathogen development. The latter fungi are known for their lignolytic and cellulolytic properties (Papavizas 1985).

Among the composts made of sewage sediments from the treatment plant applied in the experiment, the soil improvement medium had the best influence on the biological life of the soil. Presence of pathogens was found only during the first year

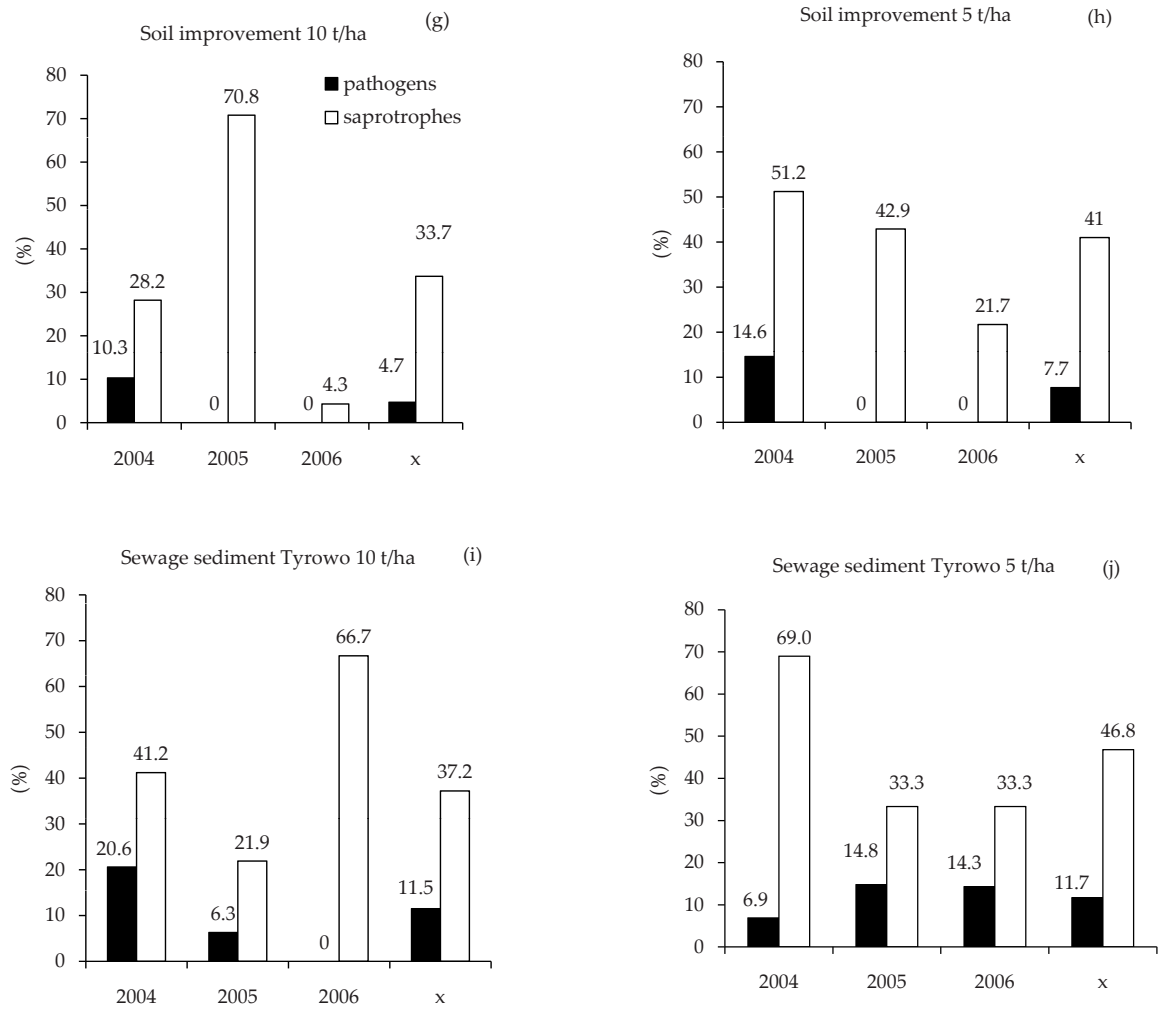

Figure 2g–j. Fungi isolated from soil of different treatments of experiment (years 2004–2006)

of cultivation (Figure 2g and h). The numbers of saprotrophic organisms in soil with double fertilization showed a decreasing trend over the study period while in the soil with single application of the fertilizer under potato, the largest number of these fungi was obtained during the second year of the study. Abbo-Sedera (2006) observed that under the influence of organic fertilization the number of microorganisms in the potato root zone increased. In the soil fertilized with composts of sewage sediments from the Tyrowo treatment plant the share of pathogens exceeding 11% was found (Figure 2i and j). The dynamics of changes in the fungal community of the soil during the period of three years of the study was more favorable in the case of a single application at 10 t/ha of the fertilizer; a decrease in number of pathogens from 20% during the first year of study to 6% during the following year and to the total reduction of population during the third vegetation season was observed coupled with simultaneous increase of saprotrophes to ca. 70% in the total population of fungi. Double application at 5 t/ha resulted in a two-fold increase in the number of pathogens during the later two years of the experiment as compared to the first year, coupled with a decrease in the population of saprotrophes.

In the case of experimental setup with Biohum fertilization, the average share of pathogens of 2.8% and 4.8% during the three study years was found for the dose of 10 t/ha and twice 5 t/ha, respectively (Figure 2e and f); however, the changes occurring in the structure of fungal community were not satisfactory. During the two later vegetation seasons, in particular the third one when winter rape was cultivated, a decrease in saprophytes is recorded, which might be caused by the simultaneous increase in the number of pathogens. The modifying influence of plant roots excretions on soil microorganisms may be suggested; it would be in line with the literature (Funck-Jensen and Hockenhull 1984).

Table 1. Fungi isolated from organically fertilized soil during investigation period 2004–2006 (%)

Fungi	Treatments									
	I	II	III	IV	V	VI	VII	VIII	IX	X
Acremoniella spp.	9.7									
Acremonium strictum W. Gams	2.2	2.2	1.2	21.1	19.3	4.7		2.6	3.8	3.8
Alternaria alternata (Fr.) Keissler									2.6	1.3
Arthrinium sphaeospermum Fuckel	1.1		1.2	1.0		1.9	1.2		3.8	1.3
Aspergillus spp.	1.1	1.1	4.7							1.3
Aureobasidium bolleyi								3.8	1.3	1.3
Aureobasidium pullulans (de Bary) Arnaud	6.5	7.4		3.2	0.9	3.8	2.4			3.9
Botrytis cinerea Pers.										1.3
Colletotrichum coccodes Hughes (Wallr.)		1,1			0.9					
Chaetomium globosum Kunze ex Fr.	1.1	11.6	1.2	13.4	0.9	1.0	2.4	7.7		2.6
Cladosporium cladosporioides de (Fres) Vries		1.1	1.2			3.8	1.2	3.8	6.4	1.3
Coniothyrium minitans Sacc.		1.1	3.5	1.0					2.6	
Endothia spp.						1.0				1.3
Epicoccum spp.										1.3
Fusarium culmorum (W.G. Sm.) Sacc.	1.1								3.9	
Fusarium equiseti (Corda) Sacc.			1.2			1.0		3.8	1.3	1.3
Fusarium oxysporum Schlecht.	3.2				0.9					
Fusarium poae										2.6
Gilmaniella humicola Barron	5.3							5.1		3.9
Gliomastix murorum (Corda) Quequen	1.1	3.3	1.2	6.2	1.8	1.9	2.4	7.7	1.3	3.9
Gliocladium catenulatum Gilman and Abbott	1.1		3.6			1.0			3.8	
Gliocladium penicillioides Corda					0.9					
Humicola brevis Gilman and Abbott	1.1		1.2			4.7		1.3		1.3
Humicola fuscoatra Traaen							1.2			2.6
Humicola grisea Traaen	1.1	1.1					2.4	1.3	1.3	1.3
Monodictis glauca (Cooke et Harkn.) Hughes							1.2			
Monodictis laevis (Wilttshire) Hughes					1.8	1.0				
Mortierella acuminata Linn.			8.2		3.6					
Mortierella alpina Peyronel				1.0	6.4	1.0			2.6	3.9
Mortierella isabelina Qudemans	5.3	1.1	1.2		10.1		1.2	2.6	1.3	2.6
Mortierella zonata Linn.					9.7	6.7	1.2		6.4	
Mucor hiemalis Wehmer		2.2	1.2	1.0	0.9	1.0		3.8	1.3	
Paecilomyces lilacinus (Thom.) Samson				4.1						
Paecilomyces nivalens (Thom.) Samson	1.1	4.2				7.6	4.7	1.3		2.6
Paecilomyces roseum (Thom.) Samson		3.3	1.2	3.2		1.0				1.3
Papulaspora sepedonioides Preuss								2.6	1.3	
Penicillium spp.	6.5	11.6	4.7	4.1	2.7	11.4	7.0	10.3	20.5	16.9
Periconia macrospinosa Lefebvre et Johnson							1.2	2.6		
Phoma eupyrena Sacc.	1.1	6.4			0.9	4.7	9.4	1.3	1.3	3.9
Rhizopus nigricans Ehrenberg		2.2	1.2	1.0	0.9	18.1	7.0	7.7		10.4
Sclerotinia sclerotiorum (W.G. Sm.) Sacc.	3.2	2.2		1.0			2.4		2.6	
Sporormia spp.									1.3	
Sporotrichum olivaceum Fries	1.1	4.2	1.2	2.1	0.9		1.2	2.6	1.3	2.6
Taeniolella stilbospora Corda (Hughes)		3.3								
Trichocladium canadense Hughes				1.0						
Trichoderma aureoviride Rifai			3.5	3.2		4.7		3.8		
Trichoderma hamatum (Bon.) Bain	5.3	11.5	9.4			1.9	8.2	2.6	3.8	6.5
Trichoderma harzianum Rifai	3.2		3.5		4.6	1.0	5.8			
Trichoderma koningii Qudemans		5.4	14.1	1.0	1.8		1.2		1.3	1.3
Trichoderma polysporum (Link: Pers) Rifai	1.1	3.3	2.4	9.3	4.6					
Trichoderma viride Pers. ex S.F. Gray		5.4	1.2	1.0	5.5		2.4	3.8	1.3	5.2
Zygorhynchus spp.										1.3
Nosporulatig fungi	5.3	1.1		1.0	2.7	14.3	16.3	2.6	11.3	1.3
Yeast-like fungi	31.2	2.4	26.8	20.2	17.4	1.0	16.3	15.4	10.3	2.6
Total	186*	190	170	194	218	210	172	156	156	154

I – control (no fertilization); II – mineral NPK fertilization; III – farm manure 10 t/ha; IV – farm manure 5 t/ha; V – Biohum 10 t/ha; VI – Biohum 5 t/ha; VII – soil improvement medium 10 t/ha; VIII – soil improvement medium 5 t/ha; IX – sewage sediments (treatment plant in Tyrowo) 10 t/ha; X – sewage sediments (treatment plant in Tyrowo) 5 t/ha; *number of isolates

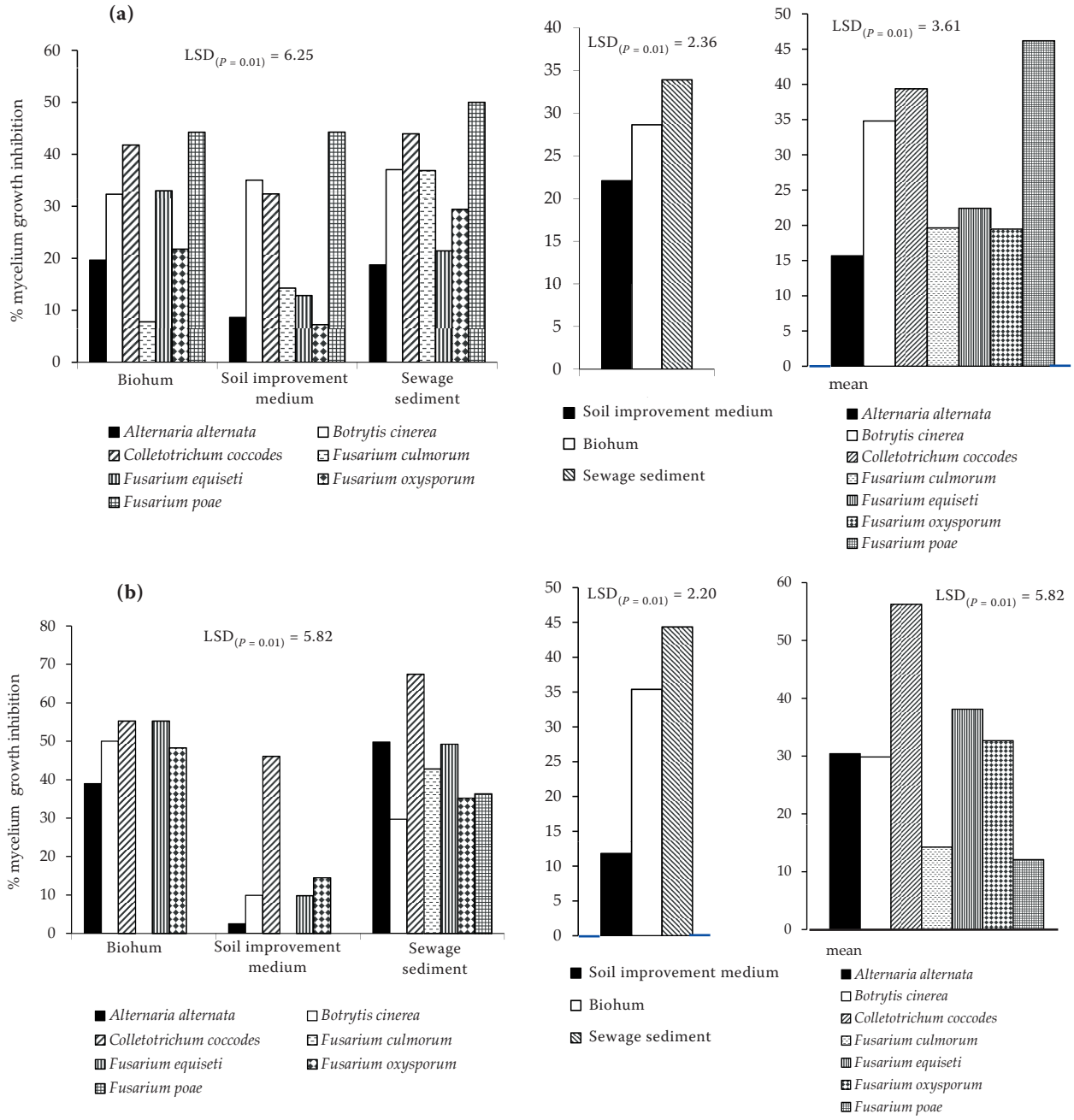

Figure 3. Percentage of pathogen mycelium growth inhibition on PDA with water extracts from composts (a) after 4 days, (b) after 8 days

The authors show that the species of *Brassicaceae* family that produce substances of glucosinolates and isocyanates can inhibit the development of saprophytic species. However, Smith and Kirkegaard (2002) found out that species of *Trichoderma* genus are tolerant to the above compounds. Curl (1982) even stated that plant excretions can improve the phytosanitary condition of soils through a better development of fungi inhibiting the growth of pathogens. As a conclusion, farm manure and soil improving medium were characterized by the best agricultural suitability.

The aquatic extract from composted sewage sediments tested *in vitro* inhibited the growth of mycelia of the studied pathogenic fungi at both times of measurement. The stronger fungistatic influence was found after 8 days than after 4 days of culture. The strongest inhibition of the fungal growth was recorded in the case of Tyrowo compost; fungal mycelium growth inhibition coefficient as compared to the controls was 33.9% and 44.4% during consecutive measurements, respectively (Figure 3). The lowest biological activity was presented by compounds and microorganisms con-

tained in the extract of soil improving medium (22.1% and 11.8 % inhibition of mycelium growth). The *Fusarium poae* and *Colletotrichum coccodes* species were the most susceptible after 4 days of culture and *C. coccodes* and *F. equiseti* after 8 days of culture. *In vitro* studies confirmed the inhibitory influence of composts of sewage sediments on development of selected plant pathogens in soil although differences in effectiveness of individual fertilizers were found, which is understandable because of the differences in conditions. Many authors indicated in their studies the protective influence of extracts from various fertilizers against plant pathogens from genera *Phytophthora, Fusarium* and *Rhizoctonia solani* (Aryantha and Guest 2006).

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