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THE MICROFUNGAL COMMUNITIES IN THE SOILS TREATED WITH SEWAGE SLUDGE

Sewage sludge rich in organic matter creates a favourable environment for many groups of microorganisms, among other things fungi. Some of them can turn out to be pathogenic for plants, animals and people. The aim of this investigation was to analyse quantitatively and qualitatively fungal communities occurring in the soils treated with sewage sludge. The sewage sludge and soil were mixed in plastic containers at the volume ratio of 1 (sewage sludge) to 3 (sand or clay), and such a substratum was sown with two plants: *Sinapsis alba* and *Dactylis glomerata*. An admixture of sewage sludge increased the number of fungi in soils, independently of the type of sewage sludge, soil and plant. Fungal communities found in the soils enriched with sewage sludge differed from those in clay or sand. Throughout the experiment, the following species known as human pathogens were isolated: dermatophytes (*Sporothrix schenckii, Microsporum* sp.), yeast (*Geotrichum candidum*, *Candida* sp., *Rhodotorula* sp. *Cryptococcus* sp.), and others (*Aspergillus niger*, *Fusarium solani*).

1. INTRODUCTION

High concentration of organic and non-organic compounds (such as nitrogen, phosphorus, potassium) in sewage sludge may contribute to improvement of some chemical and physical properties of soils, particularly of light soils, and to increase in plant crops [1]. Sewage sludge contains also such microelements as cobalt, copper, nickel and zinc, necessary for optimal plant growth. Polish Decree of the Minister of Environment of August 1st, 2002 deals with the possibilities of utilizing municipal sewage sludge for non-industrial targets. Special attention was paid to sanitary conditions, because sewage sludge contains high concentrations of such pathogenic microorganisms as: bacilli of tuberculosis, bacteria from the groups of *Salmonella* and *Shigella*, or eggs of helminthes. The decree does not define many other sanitary

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conditions, e.g. the content of pathogenic fungi which after introducing of sewage sludge to soil can be included into food chain and therefore become dangerous for humans [3]. Sewage sludge is a suitable environment for many species of fungi, e.g.: *Aspergillus* sp., *Fusarium* sp., *Mucor mucedo*, *Penicillium* sp., *Geotrichum candidum*, *Candida* spp., *Epdermophyton* spp., *Microsporum* spp., *Torulopsis glabrata* [4]–[6], *Trichophyton* spp., *Phialophora richardsii*, *Trichosporon* sp., *Rhodotorula rubra*, and *Cryptococcus neoformans*. ULFIG et al. [7] studying the micoflora in sewage sludge from Silesian wastewater treatment plants isolated keratinolytic fungi: *Trichophyton terrestre*, *Trichophyton ajelloli*, *Chrysosporium keratinophilum*, and *Microsporum gypseum*. The objective of this study was to evaluate how sewage sludge admixted with soil affects the structure of soil communities of fungi. Special attention was paid to survival rate of dermatophytes as well as to the influence of soil type and plant on the changes in fungal communities.

2. MATERIALS AND METHODS

The samples of sewage sludge were collected from three wastewater treatment plants (Częstochowa, Częstochowa-Dźbów, Myszków), mixed with soil in plastic containers (15 dm^3) at the volume ratio of 1 (sewage sludge) to 3 (sand or clay) and sown with two plants (*Sinapsis alba* and *Dactylis glomerata*). The soil samples were collected in autumn of 2002 and spring of 2003. Fungi were isolated using the dilution plate method. Then the Sabouraud medium (dextrose agar) containing gentamicin-chloramphenicol was inoculated with fungi. In order to isolate dermatophytes, the DMT medium (Scharlau) was used. Enumeration (Colony Forming Units (CFU)) and identification of species were done according to the methods described in mycological keys [3], [8].

3. RESULTS

The number of fungi in the combinations studied significantly varied, depending on soil and sewage sludge (tables 1 and 2). Sewage sludge in soil increased the number of fungi. This effect was particularly visible in spring 2003. In autumn 2002, the highest number of fungal colonies occurred in sewage sludge from Częstochowa-Dźbów (94.7×10^3 CFU/g) and the lowest (2×10^3 CFU/g) – in sandy soil with *S. alba*. In spring 2003, the highest number of fungal colonies occurred in sewage sludge from Częstochowa-Dźbów (340×10^3 CFU/g) and the lowest (1×10^3 CFU/g) – in soil with *D. glomerata* (table 2).

Table 1

The colony-forming units (CFU) of fungi in the soils treated with sewage sludge (atumn 2002)

Sample studied	CFU × 10^3/1 g of sample	Sample studied	CFU × 10^3/1 g of sample	Sample studied	CFU × 10^3/1 g of sample	Sample studied	CFU × 10^3/1 g of sample	Sample studied	CFU × 10^3/1 g of sample
sand	2.7×10^3	clay	26.8×10^3	sewage 1	32.8×10^3	sewage 2	42.0×10^3	sewage 3	94.7×10^3
sand + *D. glomerata*	15.1×10^3	clay + *D. glomerata*	11.6×10^3	sewage 1 + *D. glomerata*	10.2×10^3	sewage 2 + *D. glomerata*	4.5×10^3	sewage 3 + *D. glomerata*	No data
sand + *S. alba*	2.0×10^3	clay + *S. alba*	45.0×10^3	sewage 1 + *S. alba*	12.4×10^3	sewage 2 + *S. alba*	13.8 ×x 10^3	sewage 3 + *S. alba*	18.3×10^3
				sewage 1 + sand + *D. glomerata*	36.0×10^3	sewage 2 + sand + *D. glomerata*	11.8×10^3	sewage 3 + sand + *D. glomerata*	6.1×10^3
				sewage 1 + sand + *S. alba*	23.0×10^3	sewage 2 + sand + *S. alba*	16.5×10^3	sewage 3 + sand + *S. alba*	42.3×10^3
				sewage 1 + clay + *D. glomerata*	5.0×10^3	sewage 2 + clay + *D. glomerata*	14.8×10^3	sewage 3 + clay + *D. glomerata*	29.5×10^3
				sewage 1 + clay + *S. alba*	26.2×10^3	sewage 2 + clay + *S. alba*	35.1×10^3	sewage 3 + clay + *S. alba*	7.5×10^3

Sewage 1 – the sewage sludge from Częstochowa wastewater treatment plant.
Sewage 2 – the sewage sludge from Myszków wastewater treatment plant.
Sewage 3 – the sewage sludge from Częstochowa-Dźbów wastewater treatment plant.

Table 2

The colony-forming units (CFU) of fungi in the soils treated with sewage sludge (spring 2003)

Sample studied	CFU × 10^3/1 g of sample		Sample studied	CFU × 10^3/1 g of sample		Sample studied	CFU × 10^3/1 g of sample		Sample studied	CFU × 10^3/1 g of sample		Sample studied	CFU × 10^3/1g of sample	
	Sab.	DMT		Sab.	DMT		Sab.	DMT		Sab.	DMT		Sab.	DMT
sand	3.0	0.0	clay	3.0	0.0	sewage 1	102.3	10.0	sewage 2	30.0	3.0	sewage 3	340.0	17.5
sand + *D. glomerata*	3.8	0.0	clay + *D. glomerata*	1.0	0.0	sewage 1 + *D. glomerata*	12.5	0.0	sewage 2 + *D. glomerata*	56.3	1.8	sewage 3 + *D. glomerata*	No data	
sand + *S. alba*	42.4	1.8	clay + *S. alba*	12.9	0.0	sewage 1 + *S. alba*	22.4	0.0	sewage 2 + *S. alba*	29.2	0.0	sewage 3 + *S. alba*	60.0	4.0
						sewage 1+ sand + *D. glomerata*	114.0	1.5	sewage 2 + sand + *D. glomerata*	103.7	1.8	sewage 3 + sand + *D. glomerata*	68.8	0.0
						sewage 1 + sand + *S. alba*	27.3	3.7	sewage 2 + sand + *S. alba*	24.5	1.0	sewage 3 + sand + *S. alba*	188.4	2.8
						sewage 1 + clay + *D. glomerata*	188.9	3.4	sewage 2 + clay + *D. glomerata*	65.4	0.0	sewage 3 + clay + *D. glomerata*	259.4	0.0
						sewage 1 + clay + *S. alba*	125.7	1.0	sewage 2 + clay + *S. alba*	74.9	1.0	sewage 3 + clay + *S. alba*	109.7	64.8

Sewage 1 – the sewage sludge from Częstochowa wastewater treatment plant.
Sewage 2 – the sewage sludge from Myszków wastewater treatment plant.
Sewage 3 – the sewage sludge from Częstochowa-Dźbów wastewater treatment plant.

A comparative qualitative analysis of microscopic fungi occurring in the samples revealed that they occurred in a wide qualitative spectrum, i.e. 115 species were identified. In autumn, the highest number of species (22) was found in sandy soil sown with *D. glomerata* and in sandy soil treated with sewage sludge, also with *D. glomerata*, from Myszków. In spring 2003, the highest number of species (21) was determined in the soil treated with sewage sludge from Częstochowa and sown with *S. alba*. The lowest number of colonies was determined in autumn 2002 in sandy soil sown with *S. alba* (5) and in spring 2003 in sandy soil sown with *D. glomerata*. The representatives of *Fusarium, Penicillium, Mucor, Candida, Cladosporium, Acremonium, Humicola, Alternaria* species predominated in autumn, while in spring, *Candida, Mucor, Mortierella, Saccharomyces, Acremonium* as well as *Fusarium, Trichophyton, Chaetomium* prevailed. Throughout the experiment the following well-known human pathogens were isolated: dermatophytes (*Sporothrix schenckii, Microsporum* sp.), yeast (*Geotrichum candidum, Candida* sp., *Rhodotorula* sp. *Cryptococcus* sp.), and other potential patogens (*Aspergillus niger*, *Fusarium solani*).

4. DISCUSSION

Sewage sludge poses a significant problem, which ought to be resolved quickly, because from year to year its production has increased. The simplest method is their natural use, but is this way safe? Sparse information on the subject is available in literature on mycological analysis of sewage sludge or effects of sewage sludge on the structure of fungal communities in soil, usually a general number of the so-called "mould" is given. Based on the results of different authors it can be concluded that in such an environment mainly yeast (*Candida albicans, C. krusei, Cryptoccocus neoformans, Rhodotorula rubra, Geotrichum candidum*) or filamentous fungi (*Fusarium* spp., *Penicillium* spp., *Mucor* spp., *Absidia* spp., *Mortierella* spp.) [4], [5], [7], [19], [10] have been found. Our results are very similar to those of ULFIG [6] et al. and lead to conclusion that such pathogens as *Candida* spp. (*C. albicans, C. krusei*), *Microsporum* spp., *Trichophyton* spp., *Chrysosporium* spp., *Aspergillus niger*, *Acremonium* spp. occur in sewage sludge. To sum up: fungal population in the soils treated with sewage sludge can be changed and pathogenic fungi, such as *Candida* or *Trichophyton*, can survive, develop and reproduce. For these results, enrichment of soil by sewage sludge should be careful and limited.

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KSZTAŁTOWANIE ZBIOROWISK GRZYBOWYCH W GLEBACH WZBOGACANYCH OSADAMI ŚCIEKOWYMI

Bogate w materię organiczną osady ściekowe są środowiskiem życia wielu grup mikroorganizmów, m.in. grzybów. Niektóre z nich mogą okazać się chorobotwórcze dla roślin, zwierząt i ludzi. Celem naszych badań było poznanie zbiorowisk grzybowych, które zasiedlają gleby wzbogacone osadami ściekowymi z trzech komunalnych oczyszczalni ścieków. W założonym doświadczeniu wazonowym gleby (piasek lub glina) mieszano w stosunku ilościowym 3:1 z osadami i na powstałej mieszaninie wysiewano kupkówkę lub gorczycę. Próby pobierano jesienią 2002 i wiosną 2003 roku. Stwierdzono, że dzięki dodatkowi osadów wzrosła zawartość grzybów w badanych glebach, niezależnie od rodzaju osadów, typu gleby czy wysiewanej rośliny. Wzbogacone w osady podłoża zawierały odmienne zbiorowiska od tych zasiedlających sam piasek czy glinę. Podczas całego doświadczenia izolowano gatunki uznane za chorobotwórcze dla człowieka: dermatofity (*Sporothrix schenckii*, *Microsporum* sp.), drożdżaki (*Geotrichum candidum*, *Candida* sp., *Rhodotorula* sp., *Cryptococcus* sp.) i inne potencjalne patogeny (*Aspergillus niger*, *Fusarium solani*).