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THE EFFECT OF SEWAGE SLUDGE SONIFICATION ON SANITARY FACTORS AND PATHOGENIC FUNGI

An increasing amount of sewage sludge produced as a result of water treatment processes still poses a key problem in many communes and the cities in Poland. Therefore new methods of their utilization are indispensable. One of such methods is their natural and agricultural use. However, a drained secondary sludge that has not been subjected to hygenization poses a great environment hazard in terms of sanitary qualities. A sewage sludge contains considerable amount of pathogenic bacteria, parasitic worms and pathogenic fungi, making up for human health potential threats. Therefore sewage sludge used in agriculture ought to be analysed not only for pathogenic bacteria (belonging to *Salmonella* and coliforms) and the helminth eggs, but also for presence of pathogenic fungi. We present the results of investigations on the use of ultrasounds as a method for hygienization of sewage sludge with special regard to the pathogenic fungi survival rate.

1. INTRODUCTION

In Poland, an increasing quantity of sewage sludge coming mainly from communal wastewater treatment plants still poses a basic problem in sewage sludge management [2], [10], [13]. A dynamic development of sewage management in rural communes in Poland, connected with the possibility of using European funds and a growing number of wastewater treatment plants, is responsible for considerable increase in sewage sludge amount [11]. Most biological wastewater treatment plants apply the following processes for sewage sludge treatment: the condensation of sludge, its fermentation in open and closed digestion chambers, and in final stage, the hygienization of sludge (mainly by liming) [3], [4], [11]. The simplest and the cheapest method for utilizing sewage sludge is it natural use in agriculture, forestry, for reclamation of degraded areas; or in the case of large wastewater treatment plants – thermal granulation and use as soil fertilizing and loosening agents [3], [13], [18].

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Directive 86/278/EEC [9] regulates the principles of using sewage sludge in agriculture, and defines the maximal permissible concentrations of heavy metal concentrations in soil and in sewage sludge and the maximal quantities of heavy metals which can be introduced into the soil.

Also the Minister of Environment issued a decree [17] determining the possibilities of the utilizing sewage sludge for non-industrial purposes and defining the conditions, which such a sludge has to meet. The conditions that should be strictly controlled can be itemized as follows: the sanitary quality, chemical composition of the sewage sludge and also chemical composition of top layer of soil. In this decree, however, the minister did not impose the requirements on the content of pathogenic fungi.

In the sewage sludge, which comes from municipal wastewater treatment plants, a huge number of pathogenic bacteria, helmint eggs and pathogenic fungi occur [12], [14]. The occurrence of pathogenic species of fungi should be taken into account if sewage sludge is naturally utilized, especially during fertilization or irrigation the fields and cultivations, as they pose a serious threat for food chain. These microorganisms introduced together with sewage sludge into the soil can infect plant, animals and humans.

Fungi can be responsible for different kinds of allergies and mycoses. Mycoses are mostly skin diseases, which rarely are the cause of internal damages. The diseases caused by pathogenic fungi are usually difficult to diagnose and treat [1], [14], therefore an extensive investigation of fungi and the methods of limiting their content in sewage sludge used for natural purposes are of vital importance.

Because of the above, fungi occurring in the sludge assigned to natural utilization should be examined for their survivability.

2. MATERIALS AND METHOD

2.1. THE SEWAGE SLUDGE SAMPLES

The sewage sludge was collected from two urban wastewater treatment plants, i.e., in Częstochowa and Myszków, Poland. The samples were collected from an open fermentation chamber (Częstochowa) and secondary tank (Myszków) and transported to the laboratory in a cooler. In the laboratory, the samples were stirred and ultrasound disintegration of 10 cm^3 samples randomly selected was carried out with disintegrator UD 20 utilizing a "Sandwich" concentrator (TECHPAN, Warsaw, Poland). The experimental setup included an ultrasonic transducer connected with a low-frequency generator fixed to the bottom of a 1 dm^3 reactor vessel. The ultrasonic fields of 22 kHz and amplitude of 8 μm, 12 μm and 16 μm were used; 10 or 20 min treatment was applied to each group.

2.2. CHEMICAL ANALYSIS

In the samples of sewage sludge, such physicochemical factors as: colour, odour, hydration, total solids, volatile solids, pH were determined.

2.3. BACTERIOLOGICAL ANALYSIS

The bacteriological analysis (estimating the number of bacteria from *Salmonella*) was carried out by means of Koch's method. The dishes containing SS medium were incubated at a temperature of 37 °C for 24 hours. The coliforms were estimated on Eijkman's medium at a temperature of 37 °C for 24 hours.

The fungal communities were isolated using the dilution plate method: the Sabouraud dextrose agar-containing gentamicin and DMT media were inoculated with 4 × 1 cm^3 whose dilution was10^{-2}. The Petri dishes were incubated for 5–10 days at temperatures of 20–28 °C, depending on fungal growth and sporulation. After this time the colonies were enumerated and described as colony forming units (CFU).

The qualitative analysis was conducted subdividing the communities obtained into three main groups belonging to genera: *Mucor, Penicillium* and yeast-like fungi. Fungi that did not belong to any of the above-mentioned three groups [1], [5]–[8], [15], [16] formed the fourth group.

3. RESULTS

It was affirmed that in the case of sewage sludge samples from Częstochowa and Myszków wastewater treatment plants, the coli titre values obtained were independent of amplitude and sonification time and ranged from 10^{-4} to 10^{-5} and from 10^{-5} to 10^{-6}, respectively. The content of *Salmonella* (1200 CFU/1 cm^3) was considerably higher in sewage sludge from Częstochowa wastewater treatment plant than in that from Myszków (30 CFU/1 cm^3) (table 1 and figures 1, 2). The removal of *Salmonella* was close to 100% in the case of sludge sonification for 20 minutes at the amplitude of 16 µm (table 3). The content of pathogenic fungi in samples from both wastewater treatment plants was similar – 1200 CFU/ 1 cm^3 and 1870 CFU/1 cm^3 in the case of samples from Częstochowa and from Myszków, respectively (table 2 and figures 3, 4). In both types of sludge samples, yeast-like fungi dominated – 46% of entire number of fungi isolated from Częstochowa and 93% of entire number of fungi isolated from Myszków (table 3). The sonification of sludge significantly decreased the fungal content in both types of samples; however, in the yeast-like fungi a survival rate was the highest. Sonification at

16 μm and for 20 minutes caused a decrease in the determined colonies from 2700 CFU /1 cm^3 to 100 CFU /1 cm^3 (the samples from Częstochowa) and from 5300 CFU /1 cm^3 to 900 CFU/1 cm^3 (the samples from Myszków). Fungi belonging to other groups, i.e., *Mucor* and *Penicillium* and the other ones, were totally removed.

Table 1

Result of bacterial analyses

Type of sewage sludge	Sewage sludge from Częstochowa		Sewage sludge from Myszków	
	Coli titre*	Number of *Salmonella* colonies in 1 cm^3 of sewage sludge	Coli titre*	Number of *Salmonella* colonies in 1 cm^3 of sewage sludge
Sewage slugde before sonification	10^{-5}	1200	10^{-6}	30
Sewage sludge after 10 minute sonification (amplitude 8 μm)	10^{-4}	660	10^{-5}	30
Sewage sludge after 10 minute sonification (amplitude 12 μm)	10^{-4}	660	10^{-5}	30
Sewage sludge after 10 minute sonification (amplitude 16 μm)	10^{-5}	50	10^{-5}	20
Sewage sludge after 20 minute sonification (amplitude 8 μm)	10^{-5}	550	10^{-5}	30
Sewage sludge after 20 minute sonification (amplitude 12 μm)	10^{-4}	20	10^{-5}	20
Sewage sludge after 20 minute sonification (amplitude 16 μm)	10^{-5}	50	10^{-5}	10

* The smallest dilution (10^{-1}–10^{-8}), in which no coliforms were found.

Table 2

The results of quantitative mycological analysis

Type of sewage sludge	Number of fungi colonies in sewage sludge from Częstochowa					Number of fungi colonies in sewage sludge from Myszków				
	Total	*Mucor*	*Penicil-lium*	Yeast-like fungi	Various	Total	*Mucor*	*Penicil-lium*	Yeast-like fungi	Various
1	2	3	4	5	6	7	8	9	10	11
Sewage slugde before sonification	8000	200	500	2700	4600	5500	0	0	5300	200
Sewage sludge after 10 minute sonification (amplitude 8 μm)	2700	0	100	2600	0	7900	0	0	7900	0

1	2	3	4	5	6	7	8	9	10	11
Sewage sludge after 10 minute sonification (amplitude 12 μm)	500	0	0	500	0	4800	0	100	2100	200
Sewage sludge after 10 minute sonification (amplitude 16 μm.)	200	0	0	200	0	1000	0	0	1000	0
Sewage sludge after 20 minute sonification (amplitude 8 μm)	1900	0	0	1800	100	5700	0	0	5700	0
Sewage sludge after 20 minute sonification (amplitude 12 μm)	100	0	0	100	0	1900	0	0	1800	100
Sewage sludge after 20 minute sonification (amplitude 16 μm)	100	0	0	100	0	900	0	0	900	0

Table 3

The effect of sewage sludge sonification

Type of sewage sludge	Sewage sludge from Częstochowa		Sewage sludge from Myszków	
	Reduction of *Salmonella* [%]	Reduction of fungi [%]	Reduction of *Salmonella* [%]	Reduction of fungi [%]
Sewage sludge after 10 minute sonification (amplitude 8 μm)	57.14	66.25	0.00	0.00
Sewage sludge after 10 minute sonification (amplitude 12 μm)	100.00	93.75	100.00	12.73
Sewage sludge after 10 minute sonification (amplitude 16 μm)	100.00	97.50	100.00	81.82
Sewage sludge after 20 minute sonification (amplitude 8 μm)	71.43	76.25	0.00	0.00

Sewage sludge after 20 minute sonification (amplitude 12 μm)	100.00	98.75	100.00	65.45
Sewage sludge after 20 minute sonification (amplitude 16 μm)	100.00	98.75	100.00	83.64

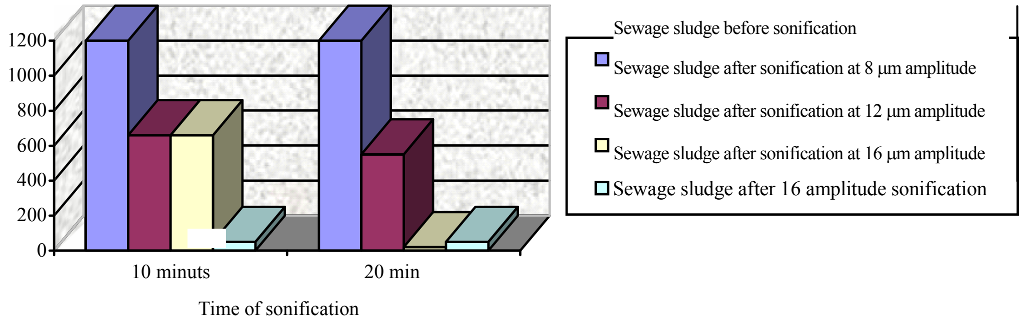

Fig. 1. Reduction of *Salmonella* in sewage sludge from Częstochowa

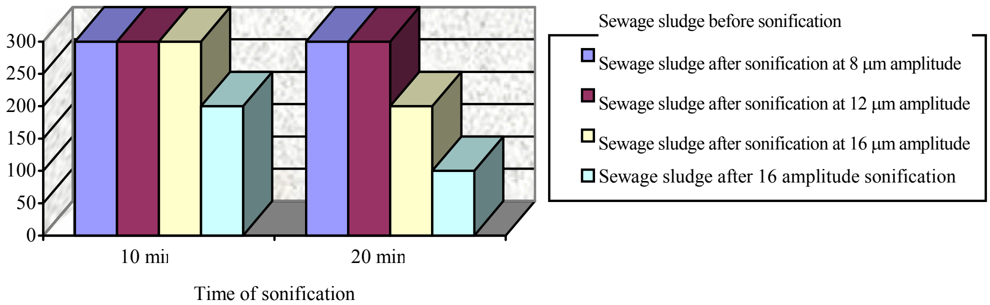

Fig. 2. Reduction of *Salmonella* in sewage sludge from Myszków

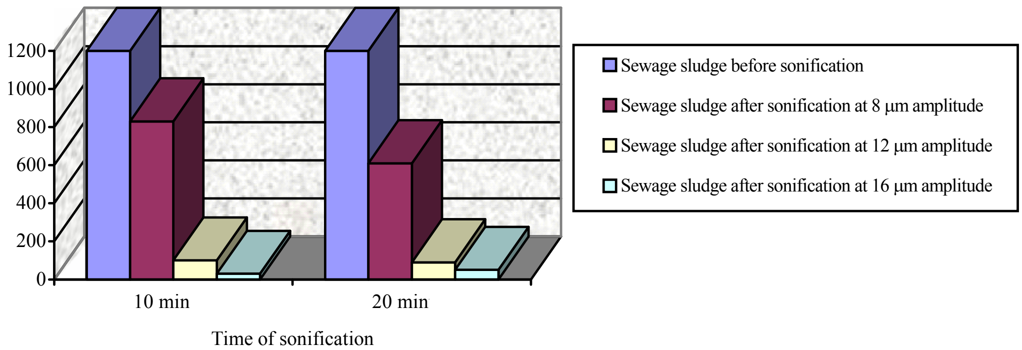

Fig. 3. Reduction of fungi in sewage sludge from Częstochowa

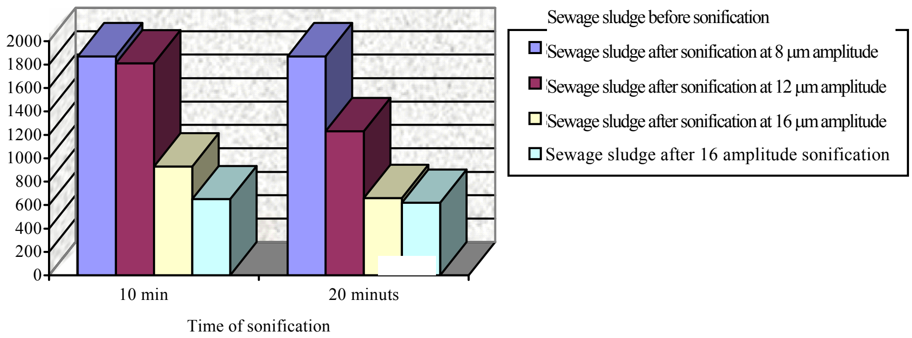

Fig. 4. Reduction of fungi in sewage sludge from Myszków

4. DISCUSSION

The results presented indicate that sonification of sewage sludge is a remarkably effective method of their hygienization in terms of removing *Salmonella* and pathogenic fungi. The sonification of sewage sludge (especially for 20 minutes at the amplitude of 16 μm) had a significant influence on the reduction of pathogenic fungi content, mainly yeast-like fungi which predominated in the sewage sludge tested. From sonificated sewage sludge there were also isolated such pathogenic species as: *Candida, Trichophyton, Gliocladium*, *Fusarium, Geotrichum* and *Rhodotorulla* (in appearance order). There was no a clear correlation between the content of both *Salmonella* and pathogenic fungi and the coli titre. A ultrasonic field did not influence significantly reduction in content of coliforms.

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WPŁYW NADŹWIĘKAWIANIA OSADÓW ŚCIEKOWYCH NA WARTOŚCI WSKAŹNIKÓW SANITARNYCH ORAZ GRZYBÓW CHOROBOTWÓRCZYCH WYSTĘPUJĄCYCH W OSADACH

Wzrastająca ilość osadów ściekowych powstających w oczyszczalniach ścieków wciąż stanowi nierozwiązany problem wielu gmin i miast w Polsce. Dlatego też nieustannie poszukuje się nowych metod ich utylizacji. Jedną z metod zagospodarowania osadów ściekowych jest ich przyrodnicze i rolnicze wykorzystanie. Należy pamiętać jednak, że odwodniony osad wtórny, nie poddany procesom higienizacji stanowi duże zagrożenie pod względem sanitarnym. Zawiera nie tylko znaczne ilości bakterii chorobotwórczych i robaków pasożytniczych, ale także grzybów patogennych, stanowiących potencjalne zagrożenia dla zdrowia i życia ludzi. Dlatego też tak ważne jest, aby osad przeznaczony do przyrodniczego
i rolniczego wykorzystania był badany (w części mikrobiologicznej) także pod względem zawartości grzybów chorobotwórczych dla człowieka, a nie tylko ze względu na zawartość bakterii chorobotwórczych (z rodzaju *Salmonella* i grupy coli) i jaj robaków pasożytujących w jelicie. W niniejszym artykule przedstawiono wyniki badań nad nadźwiękawianiem jako metodą higienizacji osadów ściekowych ze szczególnym uwzględnieniem przeżywalności grzybów chorobotwórczych.