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ORIGINAL ARTICLE

Effects of spring season solar drying process on sanitation indicators in sewage sludge and potential as a method for fertilizer production

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Abstract

The agricultural use of sewage sludge is possible on condition of maintaining microbiological and parasitological standards, and one of the most modern methods improving its sanitary state is solar drying. In the presented study, the effect of this process on the elimination of indicator microorganisms (*Escherichia coli, Salmonella* Senftenberg W_{775}, *Enterococcus* spp.) and eggs of *Ascaris suum* introduced into the biomass of sludge was examined. The experiment was carried out in the spring period with a maximal temperature of 18 °C inside the drying plant. Bacteria and parasite eggs were introduced into special carriers (cylinders filled with sewage sludge) and placed at selected points of the drier. The carriers were removed every 7 days and subject to a research procedure in order to estimate the number of bacteria and percentage of live eggs of *Ascaris suum*. Sanitization of the material was not obtained, since after 28 days of the process the final product contained a large concentration of *Enterococcus* spp. and *S.* Senftenberg W_{775} (10^5-10^9 $MPNg^{-1}$). Only the number of *E. coli* decreased by 6 log. During the process, the fastest decrease in the number of bacteria was observed in *E. coli* (ca 0.2 log/day), slower in enterococci (0.02-0.081 log/day), and the slowest in bacilli of the genus *Salmonella* (0.011-0.061 log/day). Sludge after drying also still contained 57-66% of live eggs of *A. suum*. The study proved that the solar drying of sludge in the spring period results in a product which poses a hazard for public and animal health and environmental sustainability, and should not be used as a fertilizer.

Key words

sewage sludge, solar drying, sanitization, bacteria, *Ascaris suum*

INTRODUCTION

Sewage sludge constitutes a source of valuable biogenic raw materials, but at the same time they can contain many pathogenic bacteria, viruses and parasite eggs [1, 2]. Their presence poses a significant sanitary threat, which should be taken into account when using sludge for agricultural purposes. Infections of people and animals become real during the application of municipal sewage sludge for fertilization, since the period of survival of pathogenic microorganisms in the soil environment is very long. The survival of intestinal bacteria in soil usually does not exceed two months, but under favourable conditions it may be more than two years [3, 4]. Also, viruses introduced into the environment along with sludge not subject to treatment processes are able to maintain their infection ability in soil for several weeks [5, 6].

The eggs of intestinal parasites, particularly *Toxocara* sp. and *Ascaris* sp., also constitute an important group [7]. It has been estimated that parasite eggs can survive for even up to 10 years in soil fertilized with sewage not subjected previously to sanitization [8]. They die very fast under the influence of sunlight, but are resistant to commonly used disinfectants and low temperatures. This may result in serious epidemiological problems, especially in countries where sewage sludge is used on a large scale in agriculture. Therefore, a method is needed which, on the one hand, will allow the elimination of dangerous pathogens, and on the other, will reduce their mass and volume. The only way of reducing the mass and volume of sludge is by thermal drying. However, this requires high energy outlays and, using the conventional fuels that are increasing in price, it is too expensive, especially for middle and small sewage treatment plants. For this reason, using the free sun energy for solar drying seems to be a reasonable alternative [9]. It is assumed that sludge drying lasts 30 days. After this time the product can be used in agriculture, as long as it satisfies microbiological and parasitological standards.

Previous research on the sanitization of sludge subjected to solar drying has been carried out both on a full technical scale in Germany [10, 11] and Australia [12, 13, 14], and on a pilot scale in mini-drying plants in Turkey [15], Greece [16] and Mexico [17]. The studies carried out in Germany by Bux et al. [10] and Hertwig [11] can be regarded as the most comparable to the climatic conditions prevailing in Poland. A small reduction in the number of bacteria was achieved and the drying process was therefore supplemented with liming, keeping sludge in drying plant during a very long drying cycle (as long as 116 days), or storing the dried sludge for at least 18 weeks. Thus, the authors claimed that both high temperature, high pH value and a suitably long time of performing the process are stress factors for bacteria, determining the complete sanitization of sludge.

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In Poland, according to the Order of the Ministry of the Environment of 13 July 2010 [18], municipal sewage sludge can be used if it has suitably small content of heavy metals, and when the sludge is supposed to be used in agriculture, no bacteria of the genus *Salmonella* were isolated from it, and the total number of live eggs of intestinal parasites *Ascaris sp., Trichuris sp., Toxocara sp.* – in 1 kg of dry matter is 0.

To date, apart from a periodical control of the final product, there have been no complex microbiological studies allowing the objective assessment of the sanitary quality of sludge subjected to the process of drying in a solar drying plant. Therefore, the aim of the presented study was to estimate whether sludge is sanitized during the process of drying. Indicator bacteria (*Salmonella* Senftenberg W_{775}, *Enterococcus* spp., *Escherichia coli*) and parasite eggs (*Ascaris suum*) were used, since their presence can indicate the risk of occurring pathogenic microorganisms. It was assumed that introducing the microbiological system of indirect validation will enable calculation of microorganism inactivation rate during the process at different points of the drying hall, which will result in a reduction in transmission of microorganisms and parasite eggs to soil and ground waters.

MATERIAL AND METHODS

The study was carried out in the spring period at a selected solar drying plant for sewage sludge in north-east Poland. The drying plant is a large format facility with polycarbonate roofing which enables filtering of sun rays inside and generation of the greenhouse effect. Mechanically dehydrated sludge was spread with an even layer (0.4 m) on the concrete, warmed floor of the hall. The effectiveness of taking up heat by sludge depends on the water content: more humid sludge gives up its water more easily. The intensity of evaporation was supplemented by mixing and turning the sludge with a turner, and by forced ventilation of the halls.

Preparing the carriers. The carriers used in the study were twelve perforated steel cylinders, each one 90 mm in length and with a diameter of 40 mm. They had special handles allowing fastening at selected observation points: on the turner and in the pile of drier sludge. Sewage sludge inoculated with 1 ml of bacterial mixture of *Escherichia coli, Salmonella* Senftenberg W_{775} and *Enterococcus* spp. were introduced to the carriers – 25 g to each. The concentration of bacteria in the suspension was 10^{8-9} MPN/ml. Perlon sacks containing live eggs of *Ascaris suum* were also placed inside the carrier. The cylinders were filled with sludge and placed at selected points of the drying plant. The control was sewage sludge inoculated with bacteria suspension and stored at room temperature in laboratory conditions. Eight of the 12 carriers used were placed on the appliance for sludge turning (four on the shovels and four on the frame), and the remaining four in the pile formed from sludge. The carriers were removed every seven days and subject to a research procedure in order to estimate the number of bacteria and proportion of live eggs of *Ascaris suum*. The research cycle lasted 28 days and the carriers were therefore analysed at weekly intervals.

Bacteriological investigations. Each bacteria was multiplied in nutrient broth (Merck, 7882) at 37 °C for 24 hours. One ml of bacteria suspension (10^{8-9} MPN/ml) was introduced to 25 g of sewage sludge and placed in the solar drying plant. One gram sludge samples diluted in 9 ml 0.9% NaCl (10^{-2} -10^{-10}) were analyzed. Quantitative analyses were carried out based on calculation of the most probable number of microorganisms [19]. The results were analysed statistically using the Statistica Programme. The theoretical time needed for microorganism inactivation was calculated on the basis of regression lines.

Quantitative determination of *Escherichia coli*. At the first stage, the material samples were added to liquid MacConkey medium (Merck, 5396) and incubated at 43°C for 24 hours. The material was then sieved into agar with tergitol and TTC (Merck, 7680) and incubated at 43 °C for 24 hours. Final identification involved biochemical tests API 20E (Biomerieux, 20100/20160).

Quantitative determination of enterococci. Broth bouillon with glucose and azide was used as an enriching medium for selective growth of *Enterococcus* spp. (Merck, 1590). After 48 hours of incubation in 37 °C the material was streaked to agar with esculine and azide (Merck, 5222). The serological test for confirmation of presumption colony was carried out (Phadabac Strep D Test, Karo Boule Diagnostics AB, Huddinge, Sweden).

Quantitative determination of *Salmonella* Senftenberg W_{775}. Samples were placed in 1% peptonic water (Merck, 7228) and incubated at 37°C for 24 hours. Then, 0.1 ml of the material was transferred to selectively multiplying liquid medium. following Rappaport (Merck, 10236), and incubated at 43°C for 24 and 48 hours. Next, the material was sieved to selective agar medium BPLA, following Kaufmann (Merck, 7236), and incubated at 37°C for 24 hours. Final identification involved serological tests (polyvalent serum HM).

Parasitological researches. Eggs of *Ascaris suum* were isolated from sexually mature female individuals obtained from meat plants. Uteri were dissected and fragments 2 cm in length from the fork were sampled for testing in order to avoid the presence of unfertilized eggs. The eggs were mixed to obtain a suspension with a density of 1-6 thousand eggs/ ml, after which 1 ml was introduced into special carriers, i.e. perlon sacks. Perlon is a perforated material with a mesh of 28 μm, which enables eggs to be affected by physico-chemical factors generated during the processes, while, on the other hand, preventing them from getting into the biomass. At the proper time, the sacks were removed from the apparatuses and their contents transferred to Petri dishes. Incubation of eggs was conducted for 30 days at 28 °C. After that, the percentage of live larvae was calculated under an optical microscope (magnification 1 × 300). The control was a suspension of eggs subject to incubation directly after collecting from the uterus of a sexually mature individual. The results obtained were analysed statistically.

Physico-chemical analyses of sludge. The subject of physico-chemical analyses was mechanically dehydrated municipal sewage sludge and dried sludge, taken from a pile formed at the end of the drying plant hall. Sludge samples collected from this pile of sludge were subject to analyses during the given research cycle at seven-day intervals.

RESULTS

During the research cycle, the ambient temperature, temperature in the pile of dried sludge and that inside the drying plant, insolation, and the relative and absolute humidity outside and inside the drying plant hall were monitored (Fig. 1).

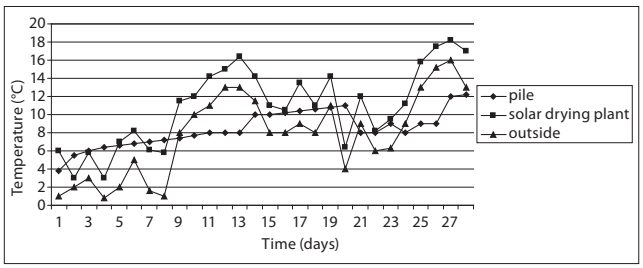

Figure 1. Course of temperatures during the spring cycle

The lowest values of temperatures at all three locations were noted during the first nine days of the cycle. They ranged from 1-8 °C. During the following 10 days, the temperatures increased by about 10 °C. The lowest temperature in the drying plant hall amounted to 3 °C, and the highest – 18.4 °C. During the measurements it was observed that the outside temperature was lower on average by 3 °C than the temperatures inside the drying plant hall.

The distribution of solar radiation was characterized by a large diversity and oscillated within the range 33.2-67.2 W/m^2. Values and course of insolation were shown in Fig. 2.

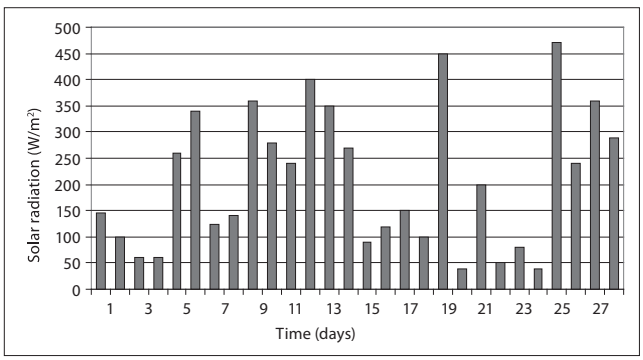

Figure 2. Course of insolation during the spring cycle

The course of the relative air humidity outside and inside the drying plant was very similar and remained within the range 49.1-90.4 % (Fig. 3).

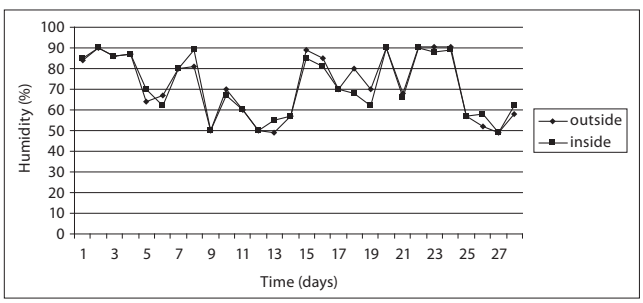

Figure 3. Course of relative humidity during the spring cycle

The results of physico-chemical analyses of sewage sludge are given in Table 1. The contents of fertilizer components – nitrogen, phosphorus, calcium and magnesium, was on the average level characteristic of municipal sewage sludge. Heavy metal content was low, which qualifies sludge for use in agriculture in respect of chemical composition. During the research cycle lasting 28 days, an increase in the dry weight of sludge and a decrease in organic substance was clearly visible. A decrease in total and ammonium nitrogen was observed. The other indexes remained on a constant level.

Table 1. Results of physico-chemical analyses of sewage sludge

Studied parameter	Times of sampling (days)				
	0	7	14	21	28
pH	6.48	6.45	6.55	6.42	6.57
Dry matter (%)	n.s.	34.5	43.6	49.7	52.7
Organic substance (% D.M.)	68.27	68.27	67.63	66.99	67.30
Total nitrogen (% D.M.)	1.67	1.60	1.40	1.29	1.20
Ammonium nitrogen (% D.M.)	0.37	0.37	0.37	0.37	0.12
Total phosphorus (% D.M.)	0.422	0.422	0.420	0.423	0.419
Calcium (% D.M.)	1.379	1.379	1.389	1.378	1.379
Magnesium (% D.M.)	0.383	0.384	0.382	0.381	0.383
Lead (mg/kg D.M.)	31.50	31.49	31.49	31.50	31.50
Cadmium (mg/kg D.M.)	2.56	2.56	2.58	2.57	2.56
Mercury (mg/kg D.M.)	0	0	0	0	0
Nickel (mg/kg D.M.)	22.90	22.89	22.90	22.91	22.91
Zinc (mg/kg D.M.)	1,207.6	1,207.6	1,207.7	1,207.7	1,207.7
Copper (mg/kg D.M.)	203.60	203.60	203.60	203.60	203.60
Chromium (mg/kg D.M.)	79.10	79.10	79.09	79.08	79.10

D.M. – dry matter; n.s. – not studied

Changes in the number of the studied indicator bacteria in the course of sludge drying are presented in Tables 2 and 3. During the entire research period there was a decrease only in the number of *E. coli*. The highest degree of inactivation of the bacilli (0.23 $\log_{10}$/day) occurred in sludge on the frame of the appliance. Their survival was in the range 35-37 days, depending on location of the carriers.

Climatic conditions prevailing inside the drying plant did not favour sanitization of sewage sludge. In the carriers on the frame and shovels of the appliance, the number of *Salmonella*

Table 2. Number of studied bacteria (MPN g^{-1})

Location of carrier	Bacteria	Time of drying (days)				
		0	7	14	21	28
Frame	*Salmonella* Senftenberg W_{775}	6.16×10^7	3.83×10^7	2.5×10^8	2.13×10^9	1.96×10^7
	Escherichia coli	5.17×10^8	6.17×10^7	6.17×10^4	6.17×10^3	6.17×10^2
	Enterococcus spp.	1.23×10^5	1.73×10^7	1.32×10^8	1.5×10^6	1.3×10^6
Shovels	*Salmonella* Senftenberg W_{775}	6.16×10^7	2.48×10^8	1.63×10^9	2.8×10^9	1.77×10^9
	Escherichia coli	5.17×10^8	1.97×10^6	0.57×10^3	6.83×10^3	1.23×10^2
	Enterococcus spp.	1.23×10^5	3.83×10^7	1.73×10^7	3.17×10^7	1.67×10^6
Pile	*Salmonella* Senftenberg W_{775}	6.16×10^7	4.17×10^7	2.8×10^8	2.23×10^9	2.83×10^5
	Escherichia coli	5.17×10^8	4.83×10^5	0.47×10^4	5.5×10^3	1.8×10^2
	Enterococcus spp.	1.23×10^5	3.8×10^6	1.07×10^7	1.3×10^7	2.5×10^7

Table 3. Inactivation dynamic of studied bacteria in sewage sludge

Location of carrier	Bacteria	Regression equation	r^2(%)	Survival time (days)
Frame	*Salmonella* Senftenberg W_{775}	y=0.011x+7.89	4.24	717.2
	Escherichia coli	y=-0.231x+8.64	93.82	37.4
	Enterococcus spp.	y=0.020x+6.04	4.46	302.0
Shovels	*Salmonella* Senftenberg W_{775}	y=0.061x+7.81	77.39	128.0
	Escherichia coli	y=-0.228x+7.86	83.07	34.5
	Enterococcus spp.	y=0.034x+6.14	11.24	180.6
Pile	*Salmonella* Senftenberg W_{775}	y=-0.045x+8.28	11.85	184.0
	Escherichia coli	y=-0.215x+7.73	88.40	35.9
	Enterococcus spp.	y=0.081x+5.43	80.67	67.0

Senftenberg W_{775} and *Enterococcus* spp. oscillated, showing a periodical upward tendency, particularly visible, i.e. by as much as 2 log, in the sludge dried on the shovels of the turner (*Salmonella* sp.) and in the sludge pile (enterococci).

The results of parasitological analyses are presented in Fig. 4. In sludge collected from all the carriers a slight inactivation of *Ascaris suum* eggs occurred. A decrease in the percentage of invasive eggs during 28 days was within the range 28-33%, whereas it was the most effective on the shovel of the appliance.

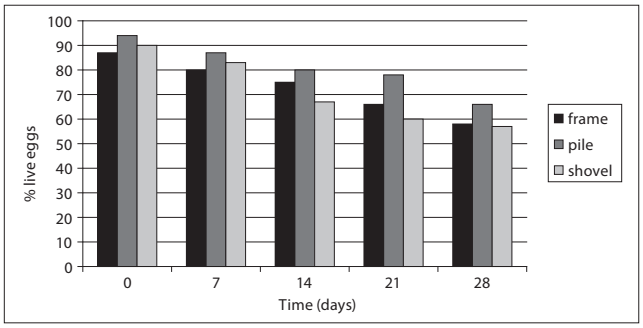

Figure 4. Course of inactivation of *Ascaris suum* eggs in the spring cycle

DISCUSSION

Sewage sludge not subjected to processes of sanitization, or which had been improperly sanitized, and then applied for environmental purposes, becomes the carrier of pathogenic microorganisms, such as bacteria of the genera *Salmonella, Escherichia, Enterococcus* spp. etc., as well as parasite eggs [20, 21, 22]. Therefore, it is essential to stop the process of their spread at as early a stage as possible.

One of the methods for improving the sanitary state of sludge is its solar drying. Because pathogenic bacteria are extremely sensitive to loss of moisture, the solar drying of sludge reduces their numbers [23]. This technology works best in regions where warm weather conditions are naturally occurring. The presented study was conducted in spring when the temperatures remained within the range of 3-18 °C; as a result, complete sanitization of the material was not achieved. After 28 days of the process, the dried sludge still contained a high concentration of the indicator microorganisms introduced previously. There was even a periodical increase observed in the number of *Enterococcus* spp. and bacilli of *Salmonella* Senftenberg W_{775} by 1-2 log in the carriers. Through the lack of the thermophylic phase, easy access to nutritious substances and high humidity, pathogenic microorganisms are able to multiply in sludge, which is also confirmed by other authors [20, 21, 22, 23, 24, 25].

The inactivation of thick-shell eggs of digestive tract parasites during the processes of sludge sanitization is very difficult. Literature data proves that the eggs of *Ascaris suum* die during aerobic fermentation at 45 °C after 1 h [26]. From other studies it follows that they should be exposed to the action of a temperature of 42-51 °C for two days [27]. The presented study indicates the lack of effective inactivation of *Ascaris suum* eggs. After 28 days of drying, the percentage of live eggs, depending on the location of sludge, oscillated in the range 57-66%. It seems that the periodical multiplication of pathogenic bacteria and the lack of inactivation of parasite eggs could have resulted from a relatively high value of relative humidity (on average 71.2%) in the hall of the drying plant, as well as from low air temperatures, oscillating within the range 3.2-18.4 °C (on average 11 °C).

Studies of the effectiveness of sludge sanitization in solar drying plants in the spring period have also carried out in other European countries. According to Hertwig [11], also in Germany, satisfying results concerning the elimination of indicator microorganisms were not observed in the spring period. A decrease in count by 1 log of coli bacteria of faecal type was obtained, whereas in the case of enterococci, similar to the present study, their number increased by 3 log. Elimination of *Ascaris suum* eggs was higher than in the presented study and amounted to 43%. It is notable that the author did not isolate bacilli of *Salmonella* in the studied samples, since the sludge collected for the study derived from a dried deposit of sludge previously subjected to the process of oxygen stabilization, which can lead to the partial elimination of pathogenic microorganisms in sludge [28, 29]. Different results were obtained by Nathan and Clarke [13] who carried out the analysis of dried material in respect of its microbiological quality in solar facilities in Australia. The number of pathogens decreased considerably during the drying process. The number of *E. coli* and faecal coliforms at the initial phase of sludge drying was 10^4 cfu/g D.W., whereas at the end of the process it amounted to 3 cfu/g D.W. No bacilli of *Salmonella* or eggs of gastro-intestinal parasites were isolated from any samples.

The physico-chemical properties of sludge were also subject to constant monitoring during the study. In the opinion of many authors, the basic physico-chemical parameter having the impact on the inactivation process of pathogenic microorganisms is the degree of sludge drying [10, 11, 13]. In the presented study, this conclusion is confirmed only in the case of bacilli of *E. coli*, whose number decreased during sludge drying by 6 log.

Sludge solar drying is a technology that does not ensure obtaining a stable material, without pathogenic microorganisms. It is considered that in order to obtain a product safe for use in the environment, sludge should be subject to additional sanitization treatments.

CONCLUSIONS

1. The adopted research model, based on the inactivation of selected pathogenic microorganisms and eggs of intestinal parasites, allows the validation of the method of sewage

sludge solar drying, and predicting the sanitary quality of the product.

2. Indicator microorganisms were characterized by diversified dynamics of inactivation in the course of the process. *Enterococcus* spp. showed a higher resistance, whereas the bacilli of *E.coli* and *Salmonella* Senftenberg W_{775} were more quickly eliminated.
3. No distinct effect was observed by on the elimination rate of the studied indicator microorganisms placing the carrier in the drying plant. Multiplying of the organisms on the shovels of the appliance can be directly related to the possibility of sludge infection during contact with processed material.
4. The study proved the low effectiveness of sludge sanitization during solar drying in the spring period; therefore, the sludge is not fit to be used for fertilization in agriculture.

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