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Effectiveness of selected stages of wastewater treatment in elimination of eggs of intestinal parasites

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Abstract

The objective of the study was to determine the degree of municipal wastewater contamination with intestinal parasite eggs of the genera *Ascaris, Toxocara*, and *Trichuri*s at individual stages of treatment, and indication of potentially weak points in the hygienisation of sewage sludge. The study was conducted in 17 municipal mechanical-biological wastewater treatment plants which, to a slight degree, differed in the technological process of wastewater treatment and the method of hygienisation of sewage sludge. The selected treatment plants, located in seven regions, included five classified as large agglomerations (population equivalent - PE >100 000), ten as medium-size (PE 15 000-100 000), and two as smaller size with PE 10 000 – 5000. The largest number of viable eggs of *Ascaris* spp., T*oxocar*a spp., and *Trichuris* spp. was found in the sewage sludge collected from the primary settling tank. A slightly lower number of the eggs were found in the samples of excess sludge, which indicates that the sedimentation process in the primary settling tank is not sufficiently long to effectively separate parasites' eggs from the sewage treated. The number of eggs of *Ascaris* spp. and *Toxocara* spp. in the fermented sludge was nearly 3 times lower than that in the raw sludge. The effectiveness of hygienisation of dehydrated sewage sludge by means of quicklime was confirmed in two wastewater treatment plants, with respect to *Ascaris* spp. eggs, in three plants with respect to *Toxocara* spp. eggs, and in one plant with respect to *Trichuris* spp. eggs. The mean reduction of the number of eggs was 65%, 61%, and 100%, respectively. In one wastewater treatment plant, a reduction in the number of viable eggs of *Ascaris* and *Trichuris* species was also noted as a result of composting sludge by 85% and 75%, respectively. In the remaining treatment plants, no effect of hygienisation of sewage sludge was observed on the contents of viable eggs of these nematodes.

Keywords: *Ascaris* spp., *Toxocara* spp., *Trichuris* spp., eggs, sewage sludge.

Introduction

Municipal sewage sludge and its proper management has become an increasingly great problem worldwide. In Poland, a rapid increase in the number of the functioning wastewater treatment plants has been observed, which met the requirements imposed by the Council Directive 91/271/EEC of 21 May 1991 concerning urban wastewater treatment. This resulted in a tremendous increase in the amount of municipal sewage sludge produced. According to Polish Central Statistical Office, its annual production in 2006 exceeded 500 000 tons of dry mass, and it is expected that in 2020 it will reach 950 000 tons of dry mass (10).

A the same time Council Directive No. 1999/31/EEC of 26 April 1999 on the landfill of waste imposed on the member states the obligation to considerably limit the mass of landfilled organic waste, including sewage sludge, and indicated the necessity for their natural use.

The contents of valuable organic substances and mineral components in sewage sludge, or compost produced based of sludge, justifies their use for agricultural purposes and land recultivation (1, 14, 29).

However, the use of sewage sludge as fertilizer poses many hazards related to the possible presence of various pathogens: viruses, rickettsia, bacteria, or fungi, and dispersive forms of parasites in these substances, as

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well as contamination with heavy metals. Both wastewater and sewage sludge enable the transmission of microorganisms to other environments, and may also be the cause of infection in humans and animals (13, 15, 28). Therefore, in the EU, as well as in Polish law, there are regulations which require conducting the studies of sewage sludge in the aspect of its biological and chemical safety. According to these regulations, sewage sludge applied in agriculture cannot contain the eggs of intestinal parasites of *Ascaris*, *Toxocara*, and *Trichuris* species, whereas in sludge used for recultivation of land, cultivation of plants designed for the production of compost, and plants not designed for consumption and production of feed, the total number of the eggs of these parasites cannot exceed 300/kg of dry mass (d.m.) (21).

The selection of the above-mentioned parasites as indicators has been caused not so much by their pathogencity (sewage sludge may contain many more dangerous pathogens, *e.g.* eggs of *Echinococcus*) but primarily by their common occurrence in faeces, sludge, and fertilizers (24, 25). The selected nematodes are also characterised by a high resistance of their eggs to many physical factors occurring in the environment (temperature, insolation), and chemical substances. The specific structure of eggs of these parasites (*e.g*. the shell of the ascarid egg consists of three layers – an outer protein layer, middle chitinous layer, and inner lipid layer) enables their survival in the external environment for even 10 years. The lack of these pathogens in the sludge examined allows for a conclusion, with a high degree of probability, that the technological processes applied resulted in their removal or inactivation of other, also hazardous, microorganisms and parasites. The process of treatment and disposal of sewage sludge is an integral technological part in any wastewater treatment plant. The technological processes applied in the treatment plants, especially hygienisation, are aimed at the neutralisation of hazardous biological agents creating risk for the health of humans and animals, as well as for the environment.

The objective of this study was to determine which stages of the technological processes of wastewater treatment, and to what degree, contribute to the elimination of parasite eggs.

Material and Methods

The study was conducted in 17 typical municipal mechanical-biological wastewater treatment plants, which, to a slight degree, differed by the technological process of wastewater treatment and the method of hygienisation of sewage sludge. The sewage treatment plants, located in Poland in 7 provinces (Lubelskie, Lubuskie, Małopolskie, Podkarpackie, Łódzkie, Wielkopolskie, and Podlaskie), included five classified into the Group '0' (population equivalent (PE) >100 000), ten into Group 1 (PE 15 000-100 000), and two into Group 2 (PE 10 000 – 5000). In all water treatment plants, polyelectrolytes were used for wastewater dehydration. They were added prior to dehydration of sludge in the press or centrifuges. Differences in technological processes concerned mainly the stage of biological treatment of raw wastewater: the use of a single bioreactor with or without aeration, subsequent application of many bioreactors using anaerobic and aerobic processes. At the stage of biological treatment of sewage sludge, the differences between wastewater treatment plants concerned the type of fermentation chambers (open/closed), chemically supported treatment processes (denitrification and nitrification), and addition of special bacterial cultures (the so-called effective microorganisms, specific to each sewage treatment plant). In four wastewater treatment plants, the denitrification and sedimentation processes were chemically supported, and in three, special bacterial cultures, the so-called effective microorganisms (EM), were used. In nine treatment plants, closed-type fermentation chambers were applied, while in six – the open-type. Multichamber bioreactors were used in technologies applied by 11 treatment plants. The processes of hygienisation of dehydrated sludge were used in 12 treatment plants: in two of them an elevated temperature was applied (periodically), in two – composting, in seven hygienisation with quicklime, and in one – a combination of hygienisation with quicklime and composting.

In each wastewater treatment plant, the samples for the study were collected on completion of the selected stages of the technological process (e.g. preliminary sedimentation, anaerobic fermentation, reactions in oxygen bioreactors, etc.). Fig. 1 presents the points of collecting samples for parasitological examination and general technological scheme of the treatment of municipal wastewater in mechanical-biological wastewater treatment plants.

In each wastewater treatment plant, three to seven samples of sewage sludge were collected. Some installations made it impossible to collect samples at all stages of the technological process. A total number of 92 samples were obtained: raw wastewater (13 samples), sand from grit chamber (9 samples), primary sludge (17 samples), excess sludge (16 samples), fermented sludge (8 samples), dehydrated sludge (17 samples), hygenised sludge (12 samples). Samples collected in various plants at the same stages of treatment differed considerably with respect to the degree of hydration.

The content of dry mass in each sample was determined using a moisture analyser. In the studies of dehydrated and hygenised sludge (containing polyelectrolytes added at the stage of dehydration) our own method was used.

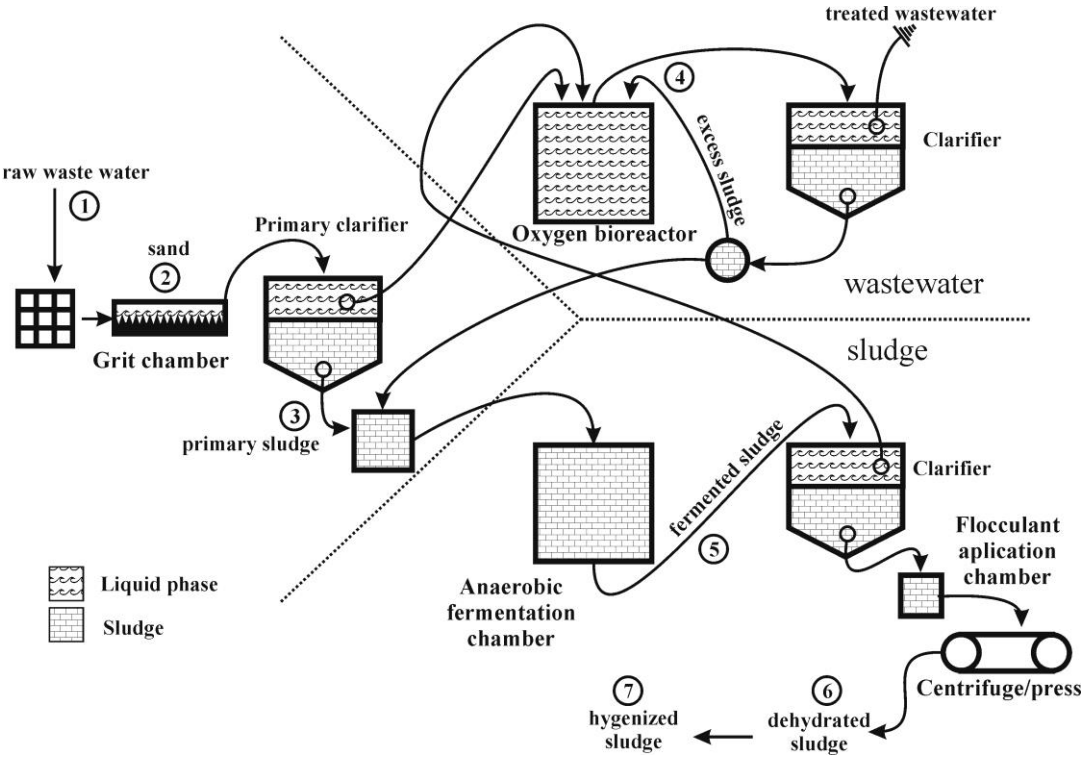


Fig. 1. Points of sampling for parasitological examination presented as a general scheme of municipal wastewater treatment in mechanical-biological treatment plants: 1 - raw sewage; 2 - sand from grit chamber; 3 - primary sludge (from primary clarifier); 4 - excess sludge (from oxygen bioreactor); 5 - fermented/digested sludge (from anaerobic fermentation chamber); 6 - dehydrated sludge; 7 - hygenised sludge

Analytical samples of 50 were subjected to analysis. The samples were initially in a solid consistency, thus a step of thorough mixing was necessary in order to obtain the samples of identical weight. Next, 1600 mL of 0.0025% Tween 20 solution was added to each sample, and subsequently mixed for four hours in a mechanical planetary mixer at high speed. The samples were filtered through a 200 µm mesh, transferred to centrifuge tubes with a volume of 400 mL, and centrifuged for 10 min at 2500 × g. Subsequently, the supernatant was poured out, and the sediment was carefully mixed with floating liquid ($NaNO_3$) at a ratio 1:16; then centrifuged again for 10 min at 2500 × g. After centrifugation, the entire liquid above the sediment was poured into a 5 L beaker, supplemented with water to the volume of 5 L, and left for 1 h. Then, the liquid above the sediment was poured away, leaving approx. 200 mL of sediment at the bottom of the beaker. The sediment was filtered by means of a vacuum filtration device, through polycarbonate filters 4 cm in diameter and pore size of 12 µm. The filters were transferred to a glass slide, and approx. eight drops of glycerol were evenly spread over the whole surface of the filter. The filters were then incubated at 27°C in a humidity chamber. The next day, the filters were observed under a microscope (magnification 100-200×), the location of the eggs found on the filter was registered, and the state of the eggs was determined. Subsequently, the development or degradation of the eggs were periodically (every 7 d) noted and photographically documented. The signs of development of the eggs, or the lack of degenerative changes within four weeks of incubation were considered as confirmation of eggs' viability.

The samples of wastewater and sewage sludge prior to addition of polyelectrolytes were examined using Quinn method (27) with own modification. Samples of liquid consistency were centrifuged at 2500 g for 10 min. Supernatant was poured away, and the sediment examined for the presence of parasite eggs. A total of 400 mL of 0.0025% Tween 20 solution was added to 100 g of the sediment and mixed for 1 min in a magnetic stirrer. The suspension was filtered through a 200 µm mesh. A volume of 100 mL of the filtrate was placed into centrifuge tubes, with the level of liquid marked on each tube with a waterproof pen, and centrifuged for 10 min at 2500 × g. After centrifugation, the supernatant was removed, and 0.0025% Tween 20 solution was added again to the sediment (to the level marked on tubes), stirred with a spatula, and centrifuged for 10 min at 2500 × g. The supernatant was removed and saturated $NaNO_3$ solution was added to the sediment, (to the level marked on the tubes), and stirred with a spatula to obtain a uniform suspension. The suspension was centrifuged for 10 min at 2500 × g. After centrifugation, the upper layer of the supernatant was collected with a pipette (20 mL from each tube) and transferred to a 1 L beaker. The liquid in the beaker was supplemented with water to the volume of 1 L, mixed and left for 1 h. Liquid above the

sediment was carefully poured away, leaving 1/3 of the volume in the beaker. Liquid in the beaker was again supplemented with water to the volume of 1 L, mixed and left for 1 h. The sediment was filtered by means of a vacuum filtration device, through polycarbonate filters with a pore size of 8-15 µm (to facilitate the location of the eggs before filtration, a network was drawn on the filters using a waterproof pen). The filters were transferred to glass slides and approx. Nine drops of glycerol were added to each filter and spread evenly over the whole surface of the filter. The filters were incubated at 27ºC in a humidity chamber. The next day, the filters were observed under a microscope (magnification 100-200×) and the location of the eggs on the filter and their state were registered. Subsequently, every 7 d, the development of the eggs or their degradation was noted and the eggs were photographically documented. The signs of the development of the eggs, or the lack of degenerative changes within four weeks of incubation were considered as the confirmation of the eggs' viability.

Results

The contents of dry mass in raw wastewater ranged from 0.001% to 0.18%, while in sand from the grit chamber – from 39% to 66%, in preliminary sludge from 0.05% to 4.36%, in excess sludge from 0.24% to 6.21%, in fermented sludge from 0.29% to 6.28%, and in dehydrated sludge from 16% to 36%. The differences in the values obtained resulted from the differences in the wastewater treatment installations used, and differences in the location of sites designed for collection of control samples. The results of parasitological examinations were therefore converted to a sludge dry mass unit. The presence of parasite eggs (*Ascaris* spp./*Toxocara* spp./*Trichuris* spp.) is shown in Table 1.

The content of parasite viable eggs (calculated per 1 kg d.m.) found in individual fractions collected at different stages of the technological process is presented in Table 2. Changes in the mean number of parasite eggs in various stages of the technological process in comparison to the preliminary sediment samples (mean number of eggs in the preliminary sludge was considered as 100%) are shown in Fig. 2.

The effectiveness of hygienisation of dehydrated sludge using quicklime was confirmed in one wastewater treatment plant with regard to the eggs of *Ascaris* spp., *Toxocara* spp., and *Trichuris* spp., in one plant with regard to *Ascaris* spp. and *Toxocara* spp. eggs, and in one plant with regard to the *Toxocara* spp. eggs only.

Table 1. Number of samples containing parasite eggs collected from different stages of treatment technological process

Type of nematode	Number of samples containing parasite eggs from stages of treatment					
	raw wastewater	sand from grit chamber	preliminary sludge	excess sludge	fermented sludge	dehydrated sludge
Ascaris spp.	2	0	1	4	1	3
Toxocara spp.	0	0	2	0	1	0
Trichuris spp	1	0	0	0	0	1
Ascaris spp./*Toxocara* spp.	1	0	4	1	3	4
Ascaris spp./*Trichuris* spp.	0	1	3	0	0	0
Toxocara spp./*Trichuris* spp.	0	0	1	0	0	1
Ascaris spp./*Toxocara* spp./*Trichuris* spp.	2	0	2	2	3	5
Positive samples in total (%)	46%	11%	76%	44%	100%	82%

Table 2. Mean number and range of values (min-max) of viable eggs isolated from raw wastewater, sand from grit chamber, and sewage sludge (calculated per 1 kg d.m.)

Type of nematode	Raw wastewater n = 13	Sand from grit chamber n = 9	Preliminary sludge n = 17	Excess sludge n = 16	Fermented sludge n = 8	Dehydrated sludge n = 17
Ascaris spp.	1000 0-8450	20 0-190	15 670 0-233 940	10 320 0-141 040	6100 0-42 190	3550 0-51 530
Toxocara spp.	540 0-4320	0 0	2670 0-29 240	1570 0-7080	980 0-3490	250 0-1460
Trichuris spp.	420 0-1710	40 0-380	270 0-2090	280 0-1920	210 0-1140	120 0-620

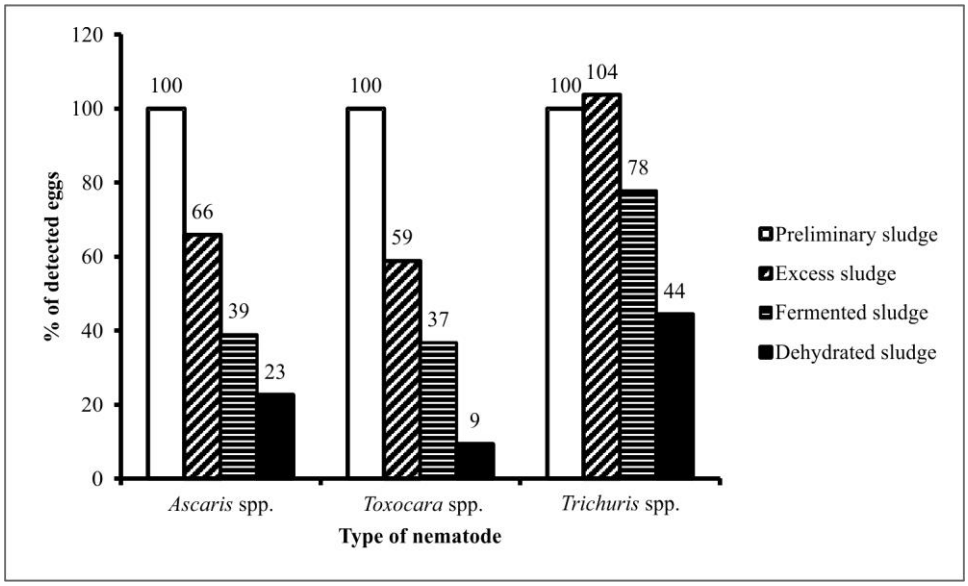


Fig. 2. Changes in the mean number of parasite eggs in various stages of the technological process in comparison to the preliminary sediment samples (mean number of eggs in the preliminary sludge was considered as 100%)

The mean reduction in the number of eggs (in relation to the content of eggs in dehydrated sludge) was 65%, 61%, and 100%, for *Ascaris* spp., *Toxocara* spp. and *Trichuris* spp. respectively. In one wastewater treatment plant the reduction in the number of viable eggs of *Ascaris* spp. and *Trichuris* spp., by 85% and 75% respectively, resulted from the process of sludge composting. In the remaining wastewater treatment plants, where hygienisation of sewage sludge was applied, no effect of these procedures on the contents of viable nematodes eggs was noted.

Discussion

During the process of wastewater treatment, especially as a result of sedimentation in clarifiers and flow through the remaining units, the number of parasite eggs may be reduced by 78% to 100% (5). However, eggs may be partly present in the purified wastewater, and considerable numbers cumulate in sewage sludge (23, 30). The presence of parasites was also confirmed in sludge subjected to fermentation and aerobic stabilisation (5). The results of our study show that the number of parasite eggs in sewage sludge (preliminary, secondary and fermented) is relatively large.

It should be emphasised that the results of parasitological examinations of raw wastewater, due to the very low contents of dry mass, are burdened with a significant error. In some cases, it was not possible to obtain a mass of sludge sample sufficient for examination (100 g) after centrifugation of wastewater (even 5 or 10 L). In addition, in the majority of wastewater treatment plants there are sites where sewage transported by asenisation cars (*e.g.* sewage from local small slaughter houses or processing plants) is added to the influent from the sewage system. Due to this situation, the composition of inflowing raw wastewater may undergo many changes.

The data presented show that the grit chamber does not play any role in the reduction of the number of parasite eggs. The flow of liquid through the grit chamber leads to the collection of heavy and relatively large particles (e.g. larger grains of sand) (6). However, it is possible that the speed of this flow is too high for parasite eggs to settle. Nevertheless, the detection of parasite eggs in these samples was accidental.

The first sample fully suitable for examination due to its good homogenisation was that of preliminary sludge. The flow of wastewater through preliminary settling tanks is slow, resulting in a division of two phases: water and particles suspended in it. Sand, grit, etc., being heavier than water, settle at the bottom of the unit as a result of the sedimentation phenomenon (6, 22). Due to the slowed flow, suspension particles from various sources (i.e. influent from the sewage system, discharge from asenisation cars) are collected in the preliminary settling tank within a longer period. The data presented show that the largest number of viable eggs of all three species of parasites was found in sludge from preliminary settling tanks. A slightly lower number of viable eggs was observed in the samples of excess sludge, which shows that the process of sedimentation in the preliminary settling tank is not sufficiently long-lasting to effectively separate all parasite eggs from the treated wastewater, and the processes of bacterial denitrification and dephosphatation of wastewater in bioreactors do not significantly affect the viability of parasite eggs. Similar results were obtained by other authors (8, 9, 17, 27). Nevertheless, the effectiveness of the processes of

anaerobic digestion in the reduction of the number of viable parasite eggs was not fully confirmed. In the fermented sludge, nearly three times fewer eggs of *Ascaris* spp. and *Toxocara* spp. were found, compared to the preliminary sludge. However, at this stage of technological cycle, no decrease in the content of viable eggs of *Trichuris* spp. was noted. Similar results were obtained by Gaspard (12) and other authors (4, 16). Different results were obtained by Bartkowska *et al*. (3). They showed better effectiveness of aerobic fermentation in the treatment (liquation) of parasite eggs (3). However, the number of eggs observed in this study was too low to draw such definitive conclusions.

The most popular method of hygienisation is the use of quicklime. However, in our study incomplete effectiveness of liming was observed. Liming may be effective against parasite eggs only when the highest doses of this agent are applied. The use of such high doses (20%) is, however, relatively expensive; hence, the addition of lower doses of quicklime, most frequently used in treatment plants, is not sufficiently effective in egg inactivation. The lack of effectiveness of hygienisation might have resulted from, among other things, irregularities in the procedures applied (e.g. too low dose of CaO, application of the agent only on the top surface of the prism formed from collected dehydrated sludge, excessively low humidity of sludge, and the lack of sufficiently high temperature produced) (11, 18).

The second widely used method of hygienisation is composting. However, our study revealed this procedure also did not show full efficacy against parasite eggs either. Composting of sludge, during which the required temperature in the entire prism (>60ºC) is not sufficiently obtained, does not lead to the complete inactivation of parasite eggs present in sludge. The effect of temperature within the range of 35-45ºC, which is obtained in the surface layers of compost prisms, may be too short-lasting under aerobic conditions to have the full inactivation effect on the eggs (2, 7, 19, 26).

In conclusion, the results of our study show that live parasite eggs were present in sludges at all stages of treatment. Hygienisation processes usually used in wastewater treatment plant have limited efficacy in elimination of parasite eggs. This points out the need to introduce more effective treatment technologies, especially because of the risk for human health concerning the use of dehydrated sludges as organic fertilizers in agriculture.

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