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Inactivation of *Ascaris suum* Eggs During the Process of Sewage Sludge Composting in Piles

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1. Introduction

The use of sewage sludge as a source of nutrients and organic substance for plants, as part of bioelement circulation in nature, is justified from both the ecological and economic point of view (Christensen et al. 2002, Songin & Hury 2002). Nevertheless, studies by many authors have indicated that sewage sludge contains many pathogenic microorganisms, including parasites (Romdhana et al. 2009, Szala & Paluszak 2008, Szejniuk & Żak 2004). Sewage sludge contains substantial amounts of eggs of helminths, such as: *Ascaris sp.*, *Toxocara sp*. and *Trichuris sp.* (Reimers et al. 1986, Wharton 1980). According to Amin (1988), *Ascaris lumbricoides* and *Strongyloides stercoralis* are the most common species in dried sludge samples. This author claims that a high reproductive potential of *Ascaris* (200,000 eggs/day) probably contributed to the substantial number of eggs found in dried sludge (up to 48 eggs/10 g DM – Dry Matter). Kaniuczak et al. (2009) reported the presence of parasite eggs from the genera *Ascaris lumbricoides* and *Trichuris trichura.* Their numbers in one kilogram of concentrated sludge amounted to 136 and 120 eggs, respectively. Boruszko et al. (2005) observed the presence of live eggs of *Ascaris* and *Toxocara* in two out of three samples of dehydrated sludge after pressing, and their amount stayed on average within the range between 60 and 80/kg DM. Gaspard et al. (1995) reported that eggs of nematodes are highly resistant to most classic sludge treatment methods. The process of sludge prolonged aeration and fermentation was ineffective in elimination of live eggs, since 93% and 66% of them, respectively, were recovered. Similar results were obtained by Black et al. (1982), resulting in a low inactivation rate of live nematode eggs during aerobic and anaerobic sludge stabilization. Gantzer et al. (2001) found on average from 2 to 45 eggs/10g DM in raw sludge, whereas those occurring in the largest numbers were eggs of *Trichuris* (37.7%), *Ascaris* (34.8%) and *Toxocara* (13.7%). *Ascaris* eggs keep their invasiveness for many years in unfavourable biocenotic conditions. The eggs' resistance to adverse environmental conditions is connected, among others, with the multilayer structure of their shields. The hardness of *Ascaris* eggs results from their very tight shell, which has been regarded as one of the most resistant biological structures (Wharton 1980). Composting, that is controlled microbiological changes in the organic substance with structure-forming material, is an advantageous strategy to use for sewage treatment by-products. As a result of this process, sanitary properties of wastes undergo a definite improvement and the sludge structure is changed, as well as its chemical, physical and biological properties (Aubain 2002, Czekała 2008). *Ascaris* eggs are used in monitoring of the effectiveness of hygienization processes. This study was undertaken to estimate the effectiveness of sewage sludge composting in inactivation of *Ascaris suum* eggs.

2. Material and methods

2.1. Preparation of material for composting

Material used in this experiment was sewage sludge derived from mechanical and biological sewage treatment, dehydrated on the belt press, with the composition presented in Table 1.

Sludge was used in addition to straw and hydrated lime in the ratio 1: 0.7: 0.1. Straw cut into fragments with a length of 15-20 cm was used to improve the pile structure and increase the access of air. Preparation of the compost mixture involved mixing sewage sludge with particular components with a dung spreader. A pile was built of the prepared material, with the cross section of a trapezium with the following dimensions: the lower base 2.5 m, the upper base 1.5 m, the height of 1.3 m and the length of 5.0 m. The pile was turned using a dung spreader every day in the 1st and 2nd week, twice a week in the 3rd week, whereas in 4th, 5th and 6th week turning was performed once a week. Then the compost was subjected to maturation for successive 10 weeks.

Table 1. Results of physicochemical analysis of sewage sludge
Tabela 1. Wyniki analizy fizykochemicznej osadów ściekowych

Specification	% DM	Heavy metal content	mg/kg DM
Organic substance	57.90	Cadmium	2.63
Calcium	3.61	Copper	2.34
Magnesium	0.10	Nickel	63.17
Ammonium nitrogen	0.54	Lead	23.33
Total nitrogen	3.89	Zinc	62.50
Total phosphorus	2.63	Mercury	579.00
Potassium	0.11	Chromium	0.22

2.2. Isolation of eggs

To determine the effect of composting process on the inactivation of *Ascaris suum* eggs, we placed perforated perlon sacks made from material with a pore diameter of 25 μm, containing 1 ml of *Ascaris suum* egg suspension each, at three pile levels (seven at each level) at the following heights: top – 90 cm, middle – 60 cm; bottom – 30 cm. Eggs of *Ascaris suum* were obtained from sexually mature female individuals collected from intestines of pigs from a pig slaughterhouse and 2 cm fragments of mature ascarid matrixes were prepared in order to avoid the presence of unfertilized eggs. Mature eggs of *Ascaris* were squeezed with a glass rod to a Petri dish. The eggs were transferred to a beaker, where they were poured with 200 ml of physiological saline and the solution was shaken together with glass pearls for 10 minutes. The obtained suspension was strained through a fine screen, then 0.2 ml of gentamycin sulfate solution (0.001g/ml) was added and the solution was left for sedimentation for 24 hours. Afterwards, 1 ml of the suspension was introduced with a sterile pipette into each of perlon sacks which were tightly closed using a heat sealer, thanks to which the eggs did not get outside. After such preparation, the samples with *Ascaris suum* eggs were introduced into the composted material and collected from different parts of the pile to evaluate vitality on 11, 28, 37, 42, 51 and 64 days of composting. The sacks were cut in the lab and placed with the inside on sterile Petri plates. Then they were poured with water and incubated for 30 days at 28-30°C. During incubation, the plates were aerated for better access of oxygen. To determine the percentage of invasive eggs, several drops of the suspension were collected from the bottom of plate to the microscopic slide and observed under the microscope 600 x zoom (Zeiss Microbiological Analyzer). Calculations were made for 200 eggs. We looked for eggs in a phase of embryonic development or larvae. Calculations were performed according to the formula (1):

$$E = \frac{E_1}{E_n} * 100\% \tag{1}$$

E – vitality of eggs (%), E_1 – the number of eggs with larvae (pcs.), E_n – total number of observed eggs (pcs.)

The control sample was a suspension of eggs subjected to incubation after collecting them from the matrix of a sexually mature individual. To incubate the eggs, 2 moist chambers were prepared, made of three Petri plates with different diameters. The smallest plates were placed on a scaffold made up of two thin plastic pipes at the bottom of larger vessels, each of which was poured with 10 ml of running water. In this way the proper moisture was provided, necessary to generate the embryonic stage of *Ascaris* eggs. The samples were incubated at 28°C for 14 days, checking the development of invasive eggs with a frequency of 3-4 days. The pH value of the composted material was determined using a potentiometer in the prepared suspension according to the PN-EN 12176. Dry matter was examined according to the recommendations of PN-EN 15934:2013-02. Temperature of the composted biomass was measured at three levels of the pile throughout the study, using a digital agricultural thermometer with a probe. The results of inactivation of *Ascaris suum* eggs in composted sewage sludge were verified and then subjected to the statistical analysis based on changes in the amount of studied eggs in time, according to the formula:

$$y = ax + b \tag{2}$$

where: a – inactivation of eggs during one day, x – time in days, b – percentage of live eggs at the initial phase of the experiment.

3. Results and discussion

Figures 1 and 2 present the results of the study estimating the effect of composting sewage sludge on the vitality of *Ascaris suum* eggs. At the start of the experiment, the percentage of live eggs in the composted material stayed at a similar level in all parts of the pile, within the range from 87.1 to 89.0%. After 10 days of composting, the presence of numerous invasive *Ascaris* eggs was still recorded, equally in the top (82.6%), middle (85.5%) and bottom (86.6%) parts of the pile. Paluszak et al. (2003), studying the effect of composting on the inactivation of parasite eggs, found that the 16th day of composting brought the complete inactivation of live larvae of *Ascaris suum* in carriers placed in the upper and middle parts of the pile. By contrast, in the outside layer of the pile the percentage of live larvae on the 29th day of composting amounted to 76.3%. In the present study, a marked increase in inactivation of enteric parasite eggs was recorded after 28 days of composting. Then the average percentage of died eggs accounted for 32.8% (Fig. 2). Next 11 days of composting had a destructive effect on the eggs of those parasites. In that period, almost 50.0% elimination of eggs was observed in the middle and top parts of the pile. The eggs placed in carriers in the bottom layer of the pile were characterized with a slower dying rate. A rapid decrease in the number of invasive eggs was recorded after the 42nd day of the experiment, when the inactivation of eggs at a level of 80.2% was observed, on average for the whole pile (Fig. 2). On 51st day of composting, single invasive eggs were observed only in samples collected from the lower part of the pile (2.4%) (Fig. 1). On the 64th day of the experiment, no live parasite eggs were found in composted sewage sludge (Fig. 1,2). According to Koné et al. (2007), the vitality of *Ascaris* eggs during 40 and 60 days of composting was reduced to less than 20.0% and 10.0%, respectively. A high effectiveness of *Ascaris* egg elimination, from 90.0 to 100.0%, was obtained after 80 days of composting due to heat generated during the process.

Proper course of sewage sludge composting depends on many factors, which have direct or indirect effect on the survival rate of eggs of enteric parasites. These include: the type of composted material, the content of nutritive substances, moisture, temperature, pH value and aeration (Aitken et al. 2005, Hassen et al 2001, Taiwo & Oso 2004). Pecson et al. (2005) claim that many factors may affect the degree of inactivation of the eggs of the alimentary tract parasites during biological degradation, both under anaerobic or aerobic conditions, but the temperature is the predominant factor. Similarly, Gantzer et al. (2001) indicated a significant effect of this parameter on obtaining the compost which is safe in respect of sanitary state. According to the authors, a temperature higher than 45°C is able to provide the full hygienization of the composted material. In contrast, Kulikowska and Moszczyńska (2010) claim that only a temperature of 55-60°C can ensure the complete elimination of eggs of the intestinal parasites.

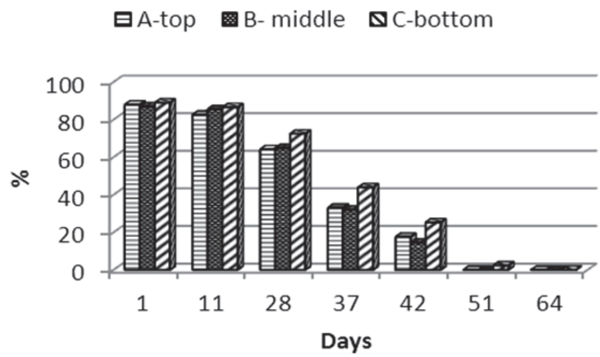

Fig. 1. Percentage (%) of live *Ascaris suum* eggs in individual days of sewage sludge composting in studied parts of the pile
Rys. 1. Odsetek (%) żywych jaj *Ascaris suum* w poszczególnych dniach kompostowania osadów ściekowych badanych częściach pryzmy

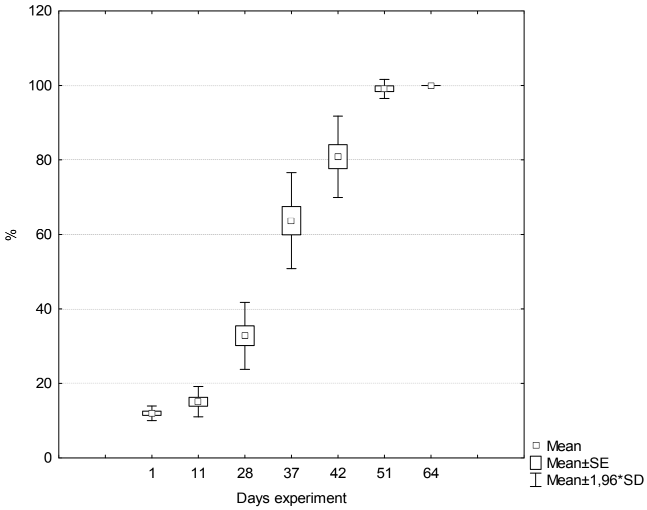

Fig. 2. Inactivation (%) of live *Ascaris suum* eggs in the process of sewage sludge composting
Rys. 2. Inaktywacja (%) żywych jaj w procesie kompostowania osadów ściekowych

In the present study, in the initial period of the experiment the temperature of the composted biomass was low and for the first 2 weeks it did not exceed 20°C. Consequently, the elimination of parasite eggs proceeded very slowly. The effect of a low temperature on long survival time of *A.suum* in sewage sludge was also observed by Berggren et al. (2004). The crucial step in the present study was obtaining the thermophylic phase in the compost pile, which was observed from 35th day of the experiment, when the temperature exceeded 45°C. At the top and middle layers of the compost pile, the temperature stayed at a high level of above 55°C for 10 days. Although in the present study the lowest temperatures were in the bottom part of the pile, since in the thermophilic phase they reached from 48°C to 60°C, they enabled the full inactivation of *Ascaris* eggs, in about two weeks longer than in the top and middle parts. The highest temperatures of the composted material were recorded on the 42nd day of the experiment: 74°C in the top part of the pile, 71°C in the middle part and 61°C in the bottom part (Fig. 3). This resulted in a rapid dying off of *Ascaris suum* eggs. However, Paluszak et al. (2003) report that lack of the thermophilic phase in sewage sludge composting due to insufficient aeration can be an environmental hazard, as *Ascaris* eggs survived 70 days in the outer zone pile.

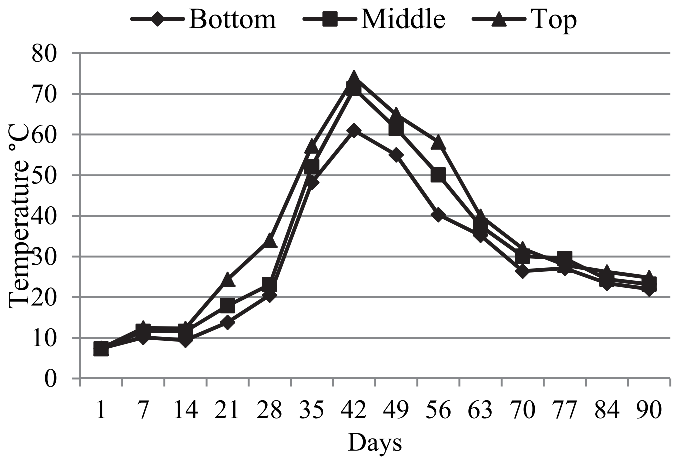

Fig. 3. Temperature values in different parts of the compost pile
Rys. 3. Wartości temperatury w poszczegolnych częściach pryzmy kompostowej

From statistical calculations it follows that the dying rate of eggs was the highest in the middle and top parts of the pile, where the daily elimination rate of live eggs amounted to 1.88 and 1.83%, respectively, whereas the inactivation of *Ascaris* eggs at the bottom layer of the pile was 1.64% (Tab. 2). The theoretical maximal survival time of eggs calculated from regression equations was 54 days in the top and middle parts of the pile and 61 days in the bottom part (Tab. 2).

Table 2. Regression line equations of *Ascaris suum* eggs inactivation in the process of sewage sludge composting
Tabela 2. Równania prostych regresji inaktywacji jaj w procesie kompostowania osadów ściekowych

Part of the pile	Regression equation	r^2 (%)	Survival (days)
Top	y = -1.83x+99.35	93.50	54.0
Middle	y = -1.88x+100.84	92.73	54.0
Bottom	y = -1.64x+100.30	91.34	61.0

According to Kosarewicz et al. (1999), for the proper course of the process of sanitization, the temperature in the whole compost biomass should amount to 55°C for 3 weeks. The effect of high temperatures on the loss of *Ascaris suum* invasiveness is connected with damage of the enzymatic system in the eggs of these parasites (Plym-Forshell 1995). This is confirmed by the study by Paluszak et al. (2003), who indicate that failing to obtain high temperatures in the composted material results in improper hygienization of the biomass, and eventually, a possibility of getting considerable amounts of parasite eggs into the environment. Papajová et al. (2005) report that after the process of composting various organic wastes was finished, as much as 60% of them contained live eggs of *Ascaris sp*., *Trichuris sp*., *Toxocara sp*. and *Hymenolepsis sp*. After 150 days of composting, most eggs of *Ascaris suum* retained their vitality (63.47 ± 4.15%). From the observations of Zdybel et al. (2009) it appears that eggs of enteric parasites were found in 38.0% of compost samples. Live eggs were isolated from 8.0% of samples. The samples most often contained eggs of ascarids from the genera *Ascaris* (23.0%), *Trichuris sp.* (15.0%) and *Toxocara* (8.0%).

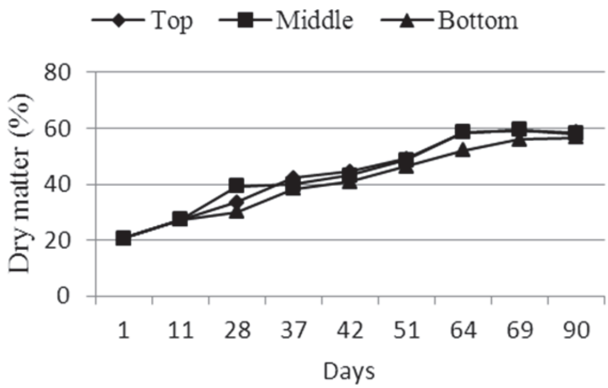

Fig. 4. Changes in dry matter content in composted sewage sludge
Rys. 4. Zmiany zawartości suchej masy w kompostowanych osadach ściekowych

Essential factors affecting the course of the composting process are dry matter content and pH of sewage sludge. On the first day of the experiment, the dry matter content in the composted material amounted to 20.71, at pH value equal to 7.5 (Fig. 4,5). In the experiment there was observed a constant tendency to increase the dry matter content of the pile, which was characterized by the highest content in the 10^{th} week of the experiment, ranging from 55.94 in the bottom part of the pile to 59.39% in the middle part. The pH value during composting increased successively, up to a level of 8.30. The pH value of the composted material began to decrease again from the 51^{st} day of the experiment and at the end of the process this parameter amounted to 6.9 for the whole pile (Fig. 5).

Dach and Zbytek (2005) observed that the dry matter content of composted material ranged from 23.6 to 28.4%, whereas pH was within the range 7.7-8.1. Siuta et al. (2007) indicated that as a result of composting not limed sludge with straw, the dry matter content reached 35.5%, whereas for limed sludge with straw it was 54.0%. The pH values of composts were close to neutral and ranged from 6.3 to 7.3. According to Krzywy et al. (2008), composts made up of sludge, straw and wastes of municipal greenery are characterized by a slightly alkaline pH from 7.25 to 7.36. The values of dry matter content and pH obtained in the present study were typical of the properly proceeding process of composting sewage sludge.

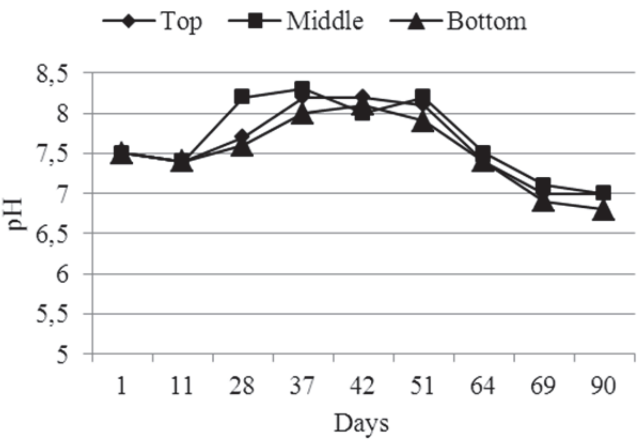

Fig. 5. Changes in pH values during the process of sewage sludge composting
Rys. 5. Zmiany wartości pH w trakcie procesu kompostowania osadów ściekowych

Improper hygienization of sludge biomass results in a large amount of eggs of alimentary tract parasites getting into the environment, posing a serious health threat to people and animals (Capizzi-Banas et al. 2004). From the point of view of environmental biosafety, it is essential to conduct the monitoring of the course of composting process also in the parasitological aspect.

4. Conclusions

1. Sewage sludge composting turned out to be an effective method for elimination of eggs of the parasite *Ascaris*.
2. It was shown that inactivation of nematodes depends, among other things, on the time and temperature of composted material.
3. High temperatures generated during the process (50-70°C) contributed to the complete elimination of parasite eggs at all three layers of the compost piles and allowed for obtaining compost which was safe in respect of sanitary state.
4. Statistical calculations indicate that the time necessary for complete hygienization of sludge ranges from 51 to 64 days.

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Inaktywacja jaj *Ascaris suum* w procesie pryzmowego kompostowania osadów ściekowych

Streszczenie

Przeprowadzone badania miały na celu określenie skuteczności inaktywacji jaj *Ascaris suum* w procesie kompostowania osadów ściekowych. W doświadczeniu wykorzystano osady ściekowe pochodzące z mechaniczno-biologicznego oczyszczania ścieków, odwodnionych na prasie taśmowej Do kompostowania zastosowano oprócz osadów słomę i wapno hydratyzowane w stosunku 1: 0,7: 0,1. Przygotowanie mieszaniny kompostowej polegało na wymieszaniu osadu ściekowego z poszczególnymi komponentami za pomocą rozrzutnika obornika. Z tak przygotowanego materiału usypano pryzmę o przekroju poprzecznym trapezu o wymiarach: podstawa dolna 2,5 m, podstawa górna 1,5 m, wysokość 1,3 m i długość 5 m. Prymę przerzucano w 1 i 2 tygodniu codziennie, w trzecim dwukrotnie, natomiast 4, 5 i 6 przerzucanie odbywało się raz na tydzień. Następnie kompost był poddawany dojrzewaniu przez kolejne 10 tygodni. W celu określenia wpływu procesu kompostowania na inaktywację jaj pasożytów *Ascaris suum* w trzech poziomach pryzmy na wysokości: góra – 90 cm, środek – 60 cm; dół – 30 cm umieszczono perforowane perlonowe woreczki zawierające po 1 ml zawiesiny jaj *Ascaris suum*. Przygotowane w ten sposób próbki z jajami *Ascaris suum* wprowadzano do kompostowanego materiału i pobierano do oceny żywotności w 11, 28, 37, 42, 51 i 64 dniu kompostowania z poszczególnych części pryzmy. Obliczenia prowadzono dla 200 jaj dla każdej próbki poszukiwano jaj w fazach rozwoju embrionalnego lub z larwą. Na początku doświadczenia odsetek żywych jaj w kompostowanym materiale kształtował się na zbliżonym poziomie we wszystkich częściach pryzmy w zakresie od 87 do 89%. Po 10 dniach kompostowania nadal odnotowano występowanie licznych inwazyjnych jaj *Ascaris*, zarówno w górnej (82,6%), środkowej (85,5%), jak i dolnej części pryzmy (86,6%). W badaniach własnych znaczący wzrost inaktywacji jaj pasożytów jelitowych odnotowano po 28 dniach kompostowania, wówczas odsetek jaj zamarłych wynosił średnio 32,8%. Kolejne 11 dni kompostowania wpływało destrukcyjnie na jaja tych pasożytów, w okresie tym stwierdzono prawie 50% eliminację jaj w części środkowej i górnej pryzmy. Wolniejszym tempem zamieralności cechowały się jaja umieszczone w nośnikach w warstwie dolnej pryzmy. Gwałtowny spadek liczby inwazyjnych jaj stwierdzono po 42 dniu doświadczenia, w którym średnio dla całej pryzmy stwierdzono inaktywację jaj na poziomie 80% . W 51 dniu kompostowania pojedyncze inwazyjne jaja obserwowano jedynie w próbkach pobranych z dolnej części pryzmy (2,4%). W 64 dniu doświadczenia w kompostowanych osadach ściekowych nie stwierdzono obecności żywych jaj pasożytów. Wykazano, że inaktywacja nicieni zależy między innymi od czasu i temperatury kompostowanego materiału. Wysoka temperatura generowana w trakcie procesu (50-70°C) przyczyniła się do całkowitej redukcji jaj pasożytów we wszystkich trzech warstwach pryzm kompostowych oraz umożliwiła uzyskanie bezpiecznego pod względem sanitarnym kompostu.

Abstract

The purpose of the performed study was to determine the effectiveness of *Ascaris suum* eggs inactivation in the composting sewage sludge. Materials used in this experiment was sewage sludge derived from mechanical and biological sewage treatment, dehydrated on the belt press. In addition to sludge, straw and hydrated lime in the ratio 1: 0.7: 0.1 was used. Preparation of the compost mixture involved mixing sewage sludge with particular components with a dung spreader. A pile was built of the prepared material, with the cross section of a trapezium with the following dimensions: the lower base 2.5 m, the upper base 1.5 m, the height of 1.3 m and the length of 5.0 m. The pile was turned using a dung spreader every day in the 1^{st} and 2^{nd} week, twice a week in the 3^{rd} week, whereas in 4^{th}, 5^{th} and 6^{th} week turning was performed once a week. Then the compost was subjected to maturation for successive 10 weeks. To determine the effect of composting process on the inactivation of *Ascaris*

suum eggs, we placed perforated perlon sacks containing 1 ml of *Ascaris suum* egg suspension each, at three pile levels at the following heights: top – 90 cm, middle – 60 cm; bottom – 30 cm. After such preparation, the samples with *Ascaris suum* eggs were introduced into the composted material and collected from different parts of the pile to evaluate vitality on 11, 28, 37, 42, 51 and 64 days of composting. Calculations were made for 200 eggs. Eggs were sought in each sample in a phase of embryonic development or larvae. At the start of the experiment, the percentage of live eggs in the composted material stayed at a similar level in all parts of the pile, within the range from 87.1 to 89.0%. After 10 days of composting, the presence of numerous invasive *Ascaris* eggs was still recorded, equally in the top (82.6%), middle (85.5%) and bottom (86.6%) parts of the pile. In the present study, a marked increase in inactivation of enteric parasite eggs was recorded after 28 days of composting. Then the average percentage of dead eggs were accounted for 32.8%. Next 11 days of composting had a destructive effect on the eggs of those parasites. In that period, almost 50.0% elimination of eggs was observed in the middle and top parts of the pile. The eggs placed in carriers in the bottom layer of the pile were characterized with a slower dying rate. A rapid decrease in the number of invasive eggs was recorded after the 42nd day of the experiment, when the inactivation of eggs at a level of 80.2% was observed, on average for the whole pile. On 51st day of composting, single invasive eggs were observed only in samples collected from the lower part of the pile (2.4%). On the 64th day of the experiment, no live parasite eggs were found in composted sewage sludge. It was shown that inactivation of nematodes depends, among other things, on the time and temperature of composted material. High temperatures generated during the process (50-70°C) contributed to the complete elimination of parasite eggs at all three layers of the compost piles and allowed for obtaining compost which was safe in respect of sanitary state.

Słowa kluczowe:
osady ściekowe, kompostowanie, *Ascaris suum*, temperatura, inaktywacja

Keywords:
sewage sludge, composting, *Ascaris suum*, temperature, inactivation