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Microbiological assessment of sewage sludge hygienization in the process of composting with the use of *Salmonella* Enteritidis

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Abstract. The aim of the study is to assess effectiveness of sewage sludge composting efficiency on the basis of survival of *Salmonella* Enteritidis bacteria. Carriers containing a strain of *S.* Enteritidis were added to three layers of a compost pile made of dehydrated sewage sludge from a mechanical-biological wastewater treatment plant and wheat straw. The maximum time of the bacteria survival was established which was found to be the longest in the bottom part of the heap and was 58 days, whereas in the top layer of the pile the survival time of the microorganisms was the shortest, which is 49 days. In the thermophilic stage the highest generated temperatures were from 66 to 73°C for a period of 7 days. It found reflection in the fastest rate of *Salmonella* bacilli inactivation. A thermophilic stage was also found in the middle layer of the pile which lasted for 5 days throughout which period the temperature ranged from 66 to 72°C. Temperatures in the bottom part of the pile were found to be lower, from 30 to 61°C, whereas temperature above 60°C was reported only for 2 days.

1 Introduction

Sewage sludge is characterized by a significant content of organic matter, macro- and micro-components which make it suitable to be used for fertilization of rural and forest areas and recultivation of green areas [1]. However, apart from many beneficial effects making it a good fertilizer, there are also some drawbacks including occurrence of intestinal parasites. Bacteria account for a significant amount of microorganisms settling sewage sludge with prevailing *Enterobacteriaceae*. According to literature *Salmonella* bacillus belong to pathogenic bacteria that most frequently occur in sewage sludge. Stroczyńska-Sikorska [2] says that *Salmonella* bacteria occurs in 100% of raw sludge samples and in 41% of digested sludge. Kłapeć and Cholewa [3] isolated *Salmonella* bacillus in 62% of the samples with digested sludge. The biggest amount of microbiological contamination is found in domestic wastewater where pathogenic microorganisms infiltrate human and animal waste. It is thought that about 0.5–5.0% of human population is infected by sewage sludge. When assessing a sanitary state of

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a sewage sludge, the period of microorganism survival in given environmental conditions as well as the rate of their cell dying, need to be considered. The time of pathogenic microorganism survival outside the host's organism is affected by many factors including: temperature, sun exposure, pH and moist [4]. Long survival of most pathogenic microorganisms is a significant problem connected with biological sewage sludge management, especially its agricultural utilization, where these organisms are able to survive in the soil or plants for months and even years [5, 6]. For this reason, the sewage sludge needs to undergo the process of hygienization before it can ever be used [7]. Composting of sewage sludge is one of the methods of biodegradable waste neutralization. Due to a rich content of organic substances in domestic wastewater, this method should have priority over other forms of waste management such as landfills or incineration [8]. Agricultural management of sewage sludge is most beneficial for small and medium wastewater treatment plants and those which are located in rural areas. Sewage sludge that comes from such facilities is characterized by low content of heavy metals, so they can be composted without any problems and later used for fertilization [9]. Regardless of the sewage sludge source, the process of composting involves processing of organic matter in the conditions of oxygen and interaction of pathogenic microorganisms to obtain a stable, safe and pathogens free fertilizer [10]. Assessment of efficiency of sewage sludge hygienization in the process of composting involves determination of inactivation dynamics for indicator bacteria that is *Salmonella, Escherichia coli* and *Streptococcus faecalis*. The survival time of microorganisms in composted sewage sludge depends, among others, on the input concentration and on the kind of composting technology. Thus, it is important to turn the pile to cause replacement of the compost layers. Temperature is a factor that has a large influence on the process of composting as it boosts activeness and diversity of microorganisms. Monitoring its changes allows to control the process of matter decomposition in the compost piles [11]. According to Marcinkowski [12] alkaline environment is a factor that causes deactivation of *Salmonella* type bacteria. The goal of the research was to assess efficiency of sewage sludge composting on the basis of *Salmonella* Enteritidis survival.

2 Material and methods

An isolated pile was built on a hardened floor of a wastewater treatment plant inside of which perforated pipes were placed to collect the sewage. The compost pile was built from dehydrated sewage from mechanical-biological treatment plant. Wheat straw, cut into 15 cm long sections, was also added to enhance the structure. Particular components were mixed in proportion 1:1. Dimensions of compost pile and distribution of carriers containing bacteria *Salmonella* Enteritidis was presented on the Figure 1.

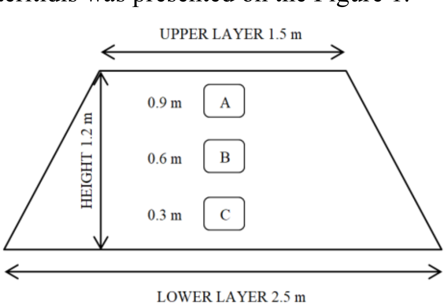

Fig. 1. Scheme of compost pile and distribution of carriers containing bacteria *Salmonella* Enteritidis (A – top; B – middle; C – bottom).

The experiment involved mechanical aeration by turning the composting mixture in time intervals dependent on the stage of composting. In the first and the second week of the experiment the pile was aired every day, whereas later, the biomass was turned 2 times a week. After the sixth week of the experiment the pile was not aired as it entered the process of maturation. In order to determine the impact of sewage sludge composting on elimination of indicator bacteria *Salmonella* Enteritidis ATCC 13076 strain was used. Carriers for tested bacteria were prepared by mixing 1500 g of dehydrated sewage sludge with 150 g of cut straw. In the next stage a suspension containing *Salmonella* Enteritidis bacteria was prepared and its density was established as $9.0 \cdot 10^8$ cells/ml by using an optical densitometer (Vitek Systems ATB 1550). 100 ml of suspension with indicator bacteria was implemented into mixed sewage sludge with straw and placed in 30 perlon bags with dimensions of 7 to 10 cm. The carriers were placed in a pile in the top, middle and bottom layers (Fig. 1). Carries with *Salmonella* Enteritidis were collected from individual layers of a compost pile for a period of 60 days. Measurements of pH were performed for all the three levels of the pile. Temperature and dry matter content were measured throughout the experiment. The pH value of the composted material was determined with the potentiometric method 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 period of the study, using a digital agricultural thermometer with a probe.

Marking the number of indicator bacteria was performed by the MPN method (most probable number). The first stage of *Salmonella* bacilli identification involved proliferation in 1% buffered peptone water (incubation in temperature 37°C for 24 h). Next, motility of a given strain was measured using a semi-liquid selective substrate acc. to Rappaport Vassiliadis Modified Semi-Solid Medium with the use of supplement novobiocin (MSRV). Selective proliferation was performed in a liquid culture, according to Rappaport Vassiliadis with addition of tetrathionate and malachite green (incubation in temperature 41°C for 24 h). The next stage involved replacing the culture onto an agar substrate BPLA with brillantine green, phenol red and lactose and onto agar substrate XLD with xylose, lysine and deoxycholate. Incubation was carried out in temperature 37°C for 24 h. The last stage involved applying serological (reactions with serum HM) and biochemical tests (microtest API 20E).

The results of the tests of *Salmonella* Enteritidis bacteria survival in compost sewage sludge were verified, the results were logarithmized and statistically analyzed on the basis of the analyzed bacteria number change in time, according to formula 1:

$$y = ax + b \tag{1}$$

where: y – logarithm of the number of indicator bacteria, a – elimination of bacteria during one day, b – number of bacteria in the zero stage, x – time counted in days.

The basic statistical analysis was performed by means of Statistica program version 6.0 Microsoft Software and Microsoft Excel 2007.

3 Results and discussion

Changes in the number of *Salmonella* Enteritidis bacteria in particular layers of the compost sewage sludge pile throughout the experiment are shown in Table 1. On the first day of the experiment the amount of *Salmonella* Enteritidis bacteria was found to be similar in the whole pile from $9.0 \cdot 10^8$ cfu/g (lower layer) to $9.5 \cdot 10^8$ (top and middle layers). After 5 days of composting a slight decrease in the number of bacteria was reported. During the next 20 days of the composting process the amount of *Salmonella* Enteritidis bacteria was decreasing, whereas the fastest elimination of the bacteria cells was found for the top layer of the pile, where their number was reported to be at the level of $2.5 \cdot 10^6$ cfu/g. In the same period dead bacteria of the middle part of the pile were isolated in the amount of $1.5 \cdot 10^7$ cfu/g, whereas from the bottom layer $2.5 \cdot 10^6$ cfu/g. El-Housseinim [13] reported a very low amount of *Salmonella* as early as within 4 weeks of the composting process duration. Between 28 and 70 days of the experiment the amount of these bacteria did not exceed the level 10^1 cfu/g. After 8 weeks, the author did not isolate the bacteria any more. In the authors' own experiments the first rapid drop in the number of *Salmonella* Enteritidis of the composted mass was reported on the 30th day of the experiment for the top layer of the pile by 4.58 log cfu/g, in relation to the input concentration. In the middle layer of the pile, bacteria were found to be dying at a slower rate where the drop was 3.32 log cfu/g. However, the lowest elimination level was reported for the bottom layer of the pile where the drop was 2.56 log cfu/g in relation to the initial value. After the next 9 days of the experiment *Salmonella* Enteritidis bacteria were isolated from the carriers in the amount of, respectively: $9.5 \cdot 10^1$ cfu/g (top); $1.5 \cdot 10^2$ cfu/g (middle) and $2.0 \cdot 10^3$ cfu/g (bottom). In the 43rd day of the experiment bacteria were isolated only from the carriers o the bottom layer of the pile, whereas in the 50^{th} and 60^{th} day no bacteria of this kind were not found in the composted sewage sludge.

Table 1. Number of *Salmonella* Enteritidis in particular layers of the pile.

Research days	**Top layer**		**Middle layer**		**Bottom layer**	
	cfu/g	log cfu/g	cfu/g	log cfu/g	cfu/g	log cfu/g
1	$9.5 \cdot 10^8$	8.97	$9.5 \cdot 10^8$	8.97	$9.0 \cdot 10^8$	8.95
5	$1.5 \cdot 10^8$	8.17	$9.5 \cdot 10^8$	8.97	$1.5 \cdot 10^8$	8.17
12	$2.0 \cdot 10^7$	7.30	$7.5 \cdot 10^7$	7.87	$4.5 \cdot 10^8$	8.10
20	$4.5 \cdot 10^6$	6.65	$9.5 \cdot 10^7$	7.97	$2.5 \cdot 10^8$	8.39
25	$2.5 \cdot 10^6$	6.39	$1.5 \cdot 10^7$	7.17	$2.5 \cdot 10^6$	6.39
30	$2.5 \cdot 10^4$	4.39	$4.5 \cdot 10^5$	5.65	$2.5 \cdot 10^6$	6.39
39	$9.5 \cdot 10^1$	1.97	$1.5 \cdot 10^2$	2.17	$2.0 \cdot 10^3$	3.30
43	0	0	0	0	$1.0 \cdot 10^2$	2.00
50	0	0	0	0	0	0

The carried out regression analysis allowed to establish the rate of elimination and theoretical maximal time of the indicator bacteria survival in the composted sewage sludge (Fig. 2–4). Prognostic tests showed that the highest rate of *Salmonella* Enteritidis cells dying were found for the top and middle layers of the compost pile at the level of 0.20 log cfu/day, whereas in the bottom layer of the pile the daily rate of the bacteria inactivation was marked to be 0.18 log cfu. Bauza-Kaszewska and Paluszak [14] found that the rate of *Salmonella* bacteria elimination depends on the layer of the pile and the composting temperature. The highest inactivation rate was reported for the middle layer of the pile where the bacteria cells died before the end of three weeks. A variable rate of *Salmonella senftenberg* W775 bacteria elimination in composted sewage sludge also depends on the applied technology of aeration [14]. According to these authors *Salmonella senftenberg* W775 bacteria, added to an experimental pile in a concentration of $9.15 \cdot 10^7$ cfu/g in an object with mechanical aeration involving turning the mass after 20 days, disappeared from the middle layer, whereas, after 34 days from the top layer and after 41 days from the bottom layer. The authors of the study established, on the basis of regression equations, that the maximal survival time of *Salmonella* Enteritidis bacteria, was found to be the longest in the bottom part of the pile and was 58 days (Fig. 4), whereas the shortest time of survival was found for the top layer 49 days (Fig. 2). The results obtained by Budzińska [15] during composting of sewage sludge oscillated at a similar level. For a material with bacteria carriers whose initial concentration was $9.5 \cdot 10^8$ cfu/g, the theoretical, maximal survival time of *Salmonella senftenberg* in the top layer of the pile was 48.37 days, in the middle part it was 49 days and in the bottom part 55.3 days.

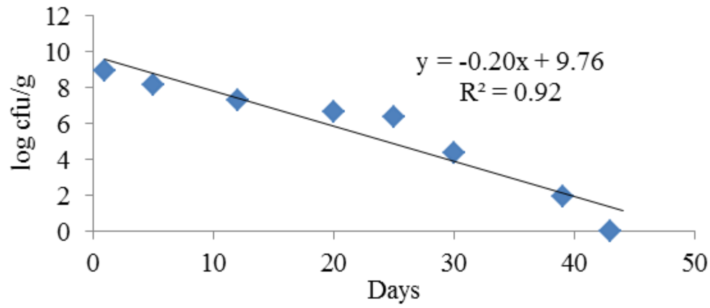

Fig. 2. Regression lines characterizing a drop in the number of *Salmonella* Enteritidis in the top layer of the compost pile.

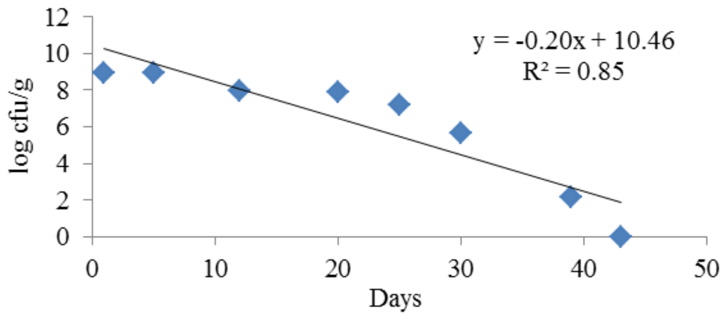

Fig. 3. Regression lines characterizing a drop in the number of *Salmonella* Enteritidis in the middle layer of the compost pile.

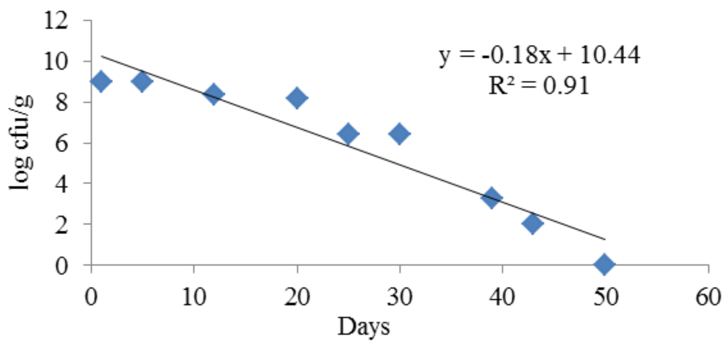

Fig. 4. Regression lines characterizing a drop in the number of *Salmonella* Enteritidis in the bottom layer of the compost pile.

The main factors determining elimination of pathogenic bacteria in the composted material include: the pile temperature which exerts a significant influence on the sanitary-hygienic state of the obtained compost [16, 17]. According to the results of the authors' own research, in the first period of composting, the temperature was low in all layers of the pile.

From 1 to 15 day of the experiment the temperature of the top layer increased from 11 up to 22°C. In this composting stage, temperatures in the middle and the bottom parts of the pile were slightly different, respectively, 9–18°C and 10–18°C. Such an arrangement of temperatures in this period of experiment can be explained by a slight decrease in the number of *Salmonella* Enteritidis in the carriers introduced to particular layers of the pile. A significant growth of temperature was found in the top layer from 16 to 38 day of the experiment, where temperatures between 40 and 73°C were reported. It needs to be emphasized that, in the thermophilic stage of composting, the highest temperatures in the pile were from 66 to 73°C, for the period of 7 days. This found reflection in the highest inactivation rate of *Salmonella* bacilli. A thermophilic stage was also reported for the middle part of the pile, which lasted for the period of 5 days, where the temperature ranged at the level of 66–72°C. In the bottom part of the pile there were lower temperatures ranging from 30 to 61°C, whereas the temperature above 60°C was reported only for two days. In the 90th day of composting, Wolna-Maruwka and Czekała [18] found no *Salmonella* bacilli. Such an output was possible for the biological material due to its reaching the thermophilic stage (60°C). Lack of a thermophilic stage has a direct impact on prolongation of the studied microorganism inactivation time whose survival increases under such conditions. In order to obtain consistent and safe fertilizer to be used in agriculture the temperature in the pile needs to increase from 45 to 70°C, which determines elimination of pathogenic microorganisms from the composted material [19]. It needs to be stressed that in the authors' own research, despite lower temperatures occurring in the bottom layer of the compost pile, the achieved temperatures enabled total elimination of indicator bacteria from the sewage sludge, though after a period longer by 9 days as compared to the top layer and by 6 days as compared to the middle layer (Fig. 5). Literature data indicates that, achievement of the sewage sludge full hygienization effect is possible after reaching temperature over 55°C, which should maintain for the period of app. 3 weeks [10].

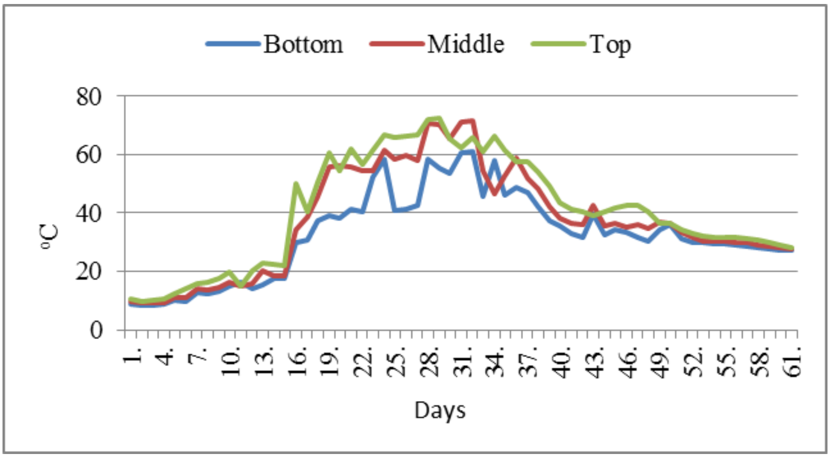

Fig. 5. Changes in temperature in the studied material during the composting process.

The content of dry matter and pH of the sewage sludge are factors that determine the process of composting. Dach and Zbytek [20] found out that the dry matter content ranged from 23.6 to 28.4%, whereas pH was between 7.7 and 8.1. In turn, Siuta et al. [21] proved that for unlimited sewage sludge composting with straw addition the content of dry matter reached 35.5%, whereas, for a limed sludge with straw addition it was 54%. In the authors' own research, the dry matter content on the day of the experiment start, was 20.20% for the whole pile. The experiment was characterized by constant tendency of the pile dry matter increase which on the 12th day of the experiment was the highest in the top layer of the pile at the level from 23.08%, whereas, the lowest was 21.90% in the bottom layer. The content of dry matter was growing gradually while on the 43rd day of the sewage sludge composting, its significant growth was reported reaching 49.68% in the top part of the pile, whereas in the middle and bottom parts, it was respectively: 46.28 and 43.94%. The highest contents of dry matter was found in the stage of the compost maturation, on the 60th day and for the top, middle and bottom layers they were, respectively: 56.34%; 55.36%; 50.15% (Table 2).

Table 2. Changes in dry matter share (%) in the studied material during the composting process.

Research days	Top layer	Middle layer	Bottom layer
1	20.20	20.20	20.20
5	21.12	21.89	20.97
12	23.08	22.10	21.90
20	26.92	26.49	24.39
25	27.23	27.33	26.77
30	31.39	35.42	27.13
39	36.41	40.98	40.61
43	49.68	46.28	43.94
50	55.36	54.00	49.94
60	56.34	55.36	50.15

The composting process is often accompanied with the pH changes which result, among others, from intensity of decomposition processes that occur inside the pile as well as from acids that are produced. The changes are small and usually, after a short, slight decrease, pH turns into the alkaline, which is the effect of ammonium release alkalinizing the environment. Additionally, composting of a sewage sludge involves presence of mineral and organic compounds which can cause periodic changes of the compost pH [22]. Table 3 shows pH changes in the composted biomass which at the beginning of the experiment ranged at a similar level in all layers of the pile and was similar to slightly alkaline pH.

Table 3. Changes in the pH value in the studied material during the composting process.

Research days	Top layer	Middle layer	Bottom layer
1	7.16	7.22	7.13
5	7.76	7.98	7.69
12	7.88	7.99	7.83
20	8.42	8.24	8.23
25	8.30	8.36	8.11
30	8.25	8.39	8.00
39	8.18	8.20	7.90
43	7.49	7.50	7.40
50	7.06	7.11	6.90
60	7.07	7.10	6.99

In the first three weeks of composting the experiments revealed pH increase up to 8.42 in the upper part of the pile, up to 8.23 in the bottom part, after which a slow drop in pH value was reported for all the pile layers. In the 60th day of the experiment pH of the composted sewage sludge was 6.99 to 7.10. Wolna-Maruwka and Pilarski [11] obtained results similar to those achieved by the authors. The contents of dry matter and pH obtained in the authors' own experiment were typical for a normal process of sewage sludge composting [23]. In conclusion, it needs to be said that the process of composting has a destructive impact on pathogenic microorganisms inhabiting in sewage sludge. Due to the impact of various environmental factors, pathogenic microorganisms are being slowly eliminated during the composting process, whereas the dynamics of changes can be characterized by high diversification [24, 25].

4 Conclusions

The experiments have shown effectiveness of the process of sewage sludge composting for elimination of *Salmonella* Enteritidis bacteria. The experiments have proved that the most important parameter providing achievement of the sanitary safest compost is temperature which in the thermophilic stage depends on the pile layer. The composting process is affected by the content of dry matter and pH of the sewage sludge. The obtained results have proved that the total elimination of *Salmonella* Enteritidis bacteria from the composted biomass was possible after 58 days of the process duration.

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