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Original Research

Estimation of Microbiological Status of Sewage Sludge Subject to Composting Process in Controlled Conditions

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Abstract

Microbiological characteristics of sewage sludge from a mechanical-and-biological sewage treatment plant composted in controlled conditions with straw and sawdust are presented. Prepared composts were placed in four bioreactors with airflow of 4 l air·min^{-1}. In bioreactor K1, K2 and K3 the composted mass consisted of 65% sewage sludge (K1–sewage sludge 1, K2 – sludge 2, K3 – sludge 3) + 30% sawdust + 5% straw; while in bioreactor K4 the proportion was: 45% sludge 2 + 50% sawdust + 5% straw. Compost samples were taken from all chambers at the same time, depending on actual temperature.

Microbiological analyses consisted in the determination (by plate method) on selective media of the numbers of mesophilic and thermophilic bacteria, fungi and pathogenic bacteria *Salmonella* sp. *Clostridium perfringens* and *Enterobacteriaceae*. Furthermore, in the experiment, the activity levels of dehydrogenase were determined using 1% triphenyltetrazole chloride as substratum.

Studies have indicated that the composting process caused a decrease of the number of fungi and pathogenic bacteria from *Enterobacteriaceae* family and *Clostridium perfringens* in all composted matters, as well as an increase in the number of thermophilic bacteria. Changes in the number of mesophilic bacteria depended on the compost type. In composts K1 and K2, the composting process caused an increased proliferation of cells, while in the composts K3 and K4 the number of mesophilic bacteria decreased. On the basis of the obtained results, it was also found that in the majority of analysis terms, the lowest activity of dehydrogenases occurred in compost K3, while their level of its activity, in the majority of the studied composts, correlated most intensively with the number of thermophilic bacteria.

Keywords: microorganisms, dehydrogenases activity, sewage sludge, compost

Introduction

Civilizational progress, dynamic increase of population and the necessity to satisfy increasingly growing needs of humanity are the reasons why, from year to year, the amounts of post-production and post-consumption remains keep increasing. Intensive development of industry, urbanization and changes in production technologies contribute in a high degree to such situations. The result of such procedures is the very difficult management of waste materials, which requires a rational solution [1-3].

During the last decade, many new communal sewage sludge treatment plants have been built, existing plants have been modernized. A by-product of sewage treatment

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Table 1. Chemical property values of components used in experiment.

Characteristic		sewage sludge 1	sewage sludge 2	sewage sludge 3	straw	sawdust
Dry mass%		42.43	41.33	41.10	90.00	82.80
pH-H_2O		6.63	6.52	6.63	-	-
$C_{org.}$	g·kg^{-1} d.m.	372.11	388.20	290.41	444.00	500.11
$N_{tot.}$		31.28	31.16	14.75	3.37	4.21
C : N		11.89	12.45	19.68	130.56	118.79

Table 2. The number of microorganisms in sewage sludge, straw and sawdust (beginning of experiment).

Groups of microorganisms	**sewage sludge 1**	**sewage sludge 2**	**sewage sludge 3**	**straw**	**sawdust**
cfu·g^{-1} d.m. of material					
Mesophilic bacteria	$163.00 \cdot 10^5$	$8.01 \cdot 10^5$	$1.50 \cdot 10^5$	$53.00 \cdot 10^5$	$1.00 \cdot 10^5$
Theromphilic bacteria	$2.25 \cdot 10^3$	$2.26 \cdot 10^3$	$1.33 \cdot 10^3$	$1100.00 \cdot 10^3$	$0.01 \cdot 10^3$
Fungi	$9.00 \cdot 10^4$	$16.23 \cdot 10^4$	$2.50 \cdot 10^4$	$35.01 \cdot 10^4$	$47.11 \cdot 10^4$
Salmonella sp.	0	0	0	0	0
Enterobacteriaceae	$309.35 \cdot 10^3$	$405.57 \cdot 10^3$	$441.84 \cdot 10^3$	$2.63 \cdot 10^3$	0
Clostridium perf.	$84.49 \cdot 10^2$	$270.90 \cdot 10^2$	$141.01 \cdot 10^2$	0	0
mg·TPF·kg^{-1} d.m. of material·5h^{-1}					
Dehydrogenase activity	8.33	5.00	2.22	1.16	1.14

plants is a strongly hydrated sludge which constitutes 1-2% of the purified sewage, and the greater the amount of created sludge, the greater the efficiency of the treatment plant [4, 5].

There exist numerous methods of sludge utilization, including storage on dumping grounds or incineration. In Poland, the most popular method of sewage sludge utilization is storage. On the other hand, in the legal regulations of the European Union, storing waste materials is regarded as a necessary evil and it may be used only under the condition that the waste material is first segregated, and where possible, reutilized [6]. Therefore, it seems necessary to elaborate efficient methods of making sewage sludge harmless in a more friendly way for the environment. One such method is agricultural utilization [7].

According to Hansson [8], Gambuś, Gorlach [9], Mazur, and Ciećko [10], sludge from communal sewage and from production plants that process animal and plant raw materials contain great amounts of mineral and organic components, and this qualifies them for agricultural utilization. Agricultural use of sludge is not only the shortest and the cheapest method of waste liquidation, but it also permits returning the basic elements and organic substances contained in the sludges into the circulation of matter in nature [11]. The use of sludge in plant cultivation is connected, however, with a potential health risk for humans and animals [12]. Next to a high fertilization value, sewage sludge can be infected to different degrees by pathogenic microorganisms which limit, and sometimes even make impossible, their use as fertilizers [9, 13].

Pathogenic microorganisms and parasites which are present in the sludge used in the form of fertilizers can penetrate into groundwaters and infect plants, and they can threaten human and animal health. Therefore, before agricultural utilization of sludges, they must undergo a process that renders them harmless, and they must be utilized according to environmental protection regulations [14].

In order to decrease or completely eliminate threats caused by the presence of pathogenic microorganisms in sewage sludge, and in order not to permit secondary contamination of the environment, sludge removed from the treatment plant should be stabilized and afterwards subjected to additional detoxication processes. For this purpose, different hygienization treatments can be used [15-17].

There exist many hygienization treatments, such as thermal drying, pasteurization, disinfection with chlorine and calcium, and composting. According to Krzywy et al. [18], composting has become the most popular method of sludge stabilization. It is a traditional method of transformation and detoxication of wastes of communal and agricultural origin. This process consists of the mineralization and humification of the substances contained in sewage sludges with the use of natural decomposition and decaying processes. As a result of this process we obtain a transfor-

mation of wastes containing easily decomposed organic compounds and microorganisms into a safe material from a sanitary point of view [19].

Experimental Procedures

The experiment was established in laboratory conditions in 2006. Material used in the studies consisted of sewage sludge originating from three different sewage treatments plants, and it consisted of wheat and rye straw and sawdust. The microbiological and chemical analyses are presented in Tables 1 and 2. Studies were carried out in four bioreactors of 125,000 m^3 capacity and equipped with electronic sensors for constant recording of some process parameters (temperature, carbon dioxide, methane, ammonia and oxygen). Materials for studies were thoroughly mixed in a container in weight proportion in relation to dry matter: 65% of sewage sludge (bioreactor K1 – sludge 1, K2 – sludge 2, K3 – sludge 3) + 30% sawdust + 5% straw in bioreactors K1, K2 and K3, while in bioreactor K4 the proportion was: 45% of sewage sludge (sludge 2)+ 50% sawdust + 5% straw.

The experiment was conducted with a constant air flow amounting to 4 $L \cdot min^{-1}$ in chambers.

Material in bioreactors was composted for 34 days and 18 hrs (834 hrs), while compost samples were taken from all chambers at the same time, depending on the actual temperature of the composted material.

On microbiological selective medium, using plate method, the numbers of colony-forming units (cfu) of fungi, mesophilic and thermophilic bacteria and pathogenic bacteria (*Salmonella* sp., *Enterobacteriaceae*, *Clostridium perfringens*) were determined.

The number of mesophilic bacteria was determined on simple nutritive agar by incubation of plates at 26°C for 48 hrs [20]. Thermophilic bacteria were determined on 3% nutritive agar. Plates were incubated for 24 hrs at 55°C [20]. Mould fungi were determined on Martin's nutrient at 24°C for 5 days [21]. *Salmonella* sp. was determined on XLT medium of Merck after 18-24 hours at 36°C [22]. In order to make sure that the bacteria were of *Salmonella* sp. genus, the procedure was conducted according to the Polish Standard PN-Z-19000-1 with an confirming identification [23].

In order to determine the number of bacteria from the *Enterobacteriaceae* family, the selective substratum of Merck was used. Plates were incubated at 37°C for 24 hours [24]. *Clostridium perfringens* was determined on TSC agar substratum with triptose, sulphate and cycloserine by incubation of plates in a thermostat with 22% of CO_2 content at 44°C for 24 hours [25]. Furthermore, in the sampled composted material, the activity of dehydrogenases was identified using the spectrophotometric method. using as substrate 1% TTC (triphenyl-tetrazole chloride), after 24-hour incubation at 30°C, with 485 nm wavelength. Enzymatic activity was expressed in $mg \cdot TPF \cdot kg^{-1}$ d.m. of compost$\cdot 5h^{-1}$ [26].

Statistical analyses applied in the experiment were used on the basis of the Statistica 7.1 program [27].

Discussion of Results

The performed experiment aimed at determining the effect of the composted material type and temperature (its value changes during the composting process), on the dynamics of changes taking place in the number of selected microorganism groups and in enzymatic activity of the composts.

Analyzing the changes in the number of mesophilic bacteria (Table 3) during the composting process, it was found that the proliferation of the bacteria was subject to significant oscillations connected most probably with temperature changes being characteristic of this process. Miyatake and Iwabuchi [28] noted that changes in temperature values during composting have an effect on changes in the activity and diversity of microorganisms, and thereby on composting efficiency.

Comparing between the first two terms the number of mesophilic bacteria in compost K1 (Table 3), it was found that the discussed bacteria more than doubled in term II in relation to term I. The reason was most probably a 12-degree increase of temperature and access to easily decomposing organic matter. Starting with term III (after 31 hrs of composting) together with the temperature increase, the number of mesophilic bacteria significantly decreased. The above status was maintained until term VI. Starting from term VII, together with the drop of temperature in the composted materials, an increase of bacteria proliferation was recorded. Similar tendencies were found by Hassen et al. [29]. Those authors observed, similarly as in our own studies, an increase of the number of bacteria at the beginning of the experiment, followed by a decrease of cell proliferation caused by increased temperature and a successive increase in the number of mesophylls caused by a temperature drop in the composted material.

In the further terms of analyses, the number of the discussed bacteria decreased in spite of a further decrease of temperature in the composts. The reason of this phenomenon was most probably the depletion of the easily decomposable organic matter, or the development of substances poducing actinomycetes with antybiotic properties [30].

Analogically, as in case of compost K1, the number of mesophilic bacteria in the remaining composts was similar, where the proliferation of cells increased several times in term II (after 20 hrs of composting), and then, with the increase of temperature, it was significantly decreased and it increased again after the drop in temperature. Similar tendencies were observed by Czaczyk et al. [31]. Those authors studied the effect of the composting process of plant waste material on a number of selected microorganism groups. It was found that the number of mesophilic bacteria in the initial stage of the composting process gradually increased with the increase in temperature. However, in the mesophilic stage, a decrease of bacteria number was recorded.

Analysis of the number of thermophilic bacteria in the studied composts (Table 3) indicated that in term I of studies,

Table 3. The number of mesophilic, thermophilic bacteria and fugi in composts (cfu·10^5·g^{-1} d.m. of material).

Kind of compost	Temperature of compost (°C)	Mesophilic bacteria		Thermophilic bacteria		Fungi	
		cfu·10^5·g^{-1} d.m. of compost	Standard Deviation	cfu·10^5·g^{-1} d.m. of compost	Standard Deviation	cfu·10^5·g^{-1} d.m. of compost	Standard Deviation
I date – beginning of experiment							
K1	16	**1108.53**	99.51	**5.80**	0.95	**35113.17**	1658.58
K2	18	**1011.71**	18.13	**6.07**	2.51	**33925.69**	3957.17
K3	22	**225.74**	5.50	**1.65**	0.62	**10444.74**	1734.44
K4	19	**109.32**	50.58	**1.94**	0.37	**4919.83**	148.78
II date - after 20 h							
K1	28	**2947.64**	244.00	**59.23**	12.44	**36172.00**	10231.24
K2	40.5	**7729.75**	965.17	**6.74**	0.36	**19470.40**	1146.39
K3	38	**8903.31**	2387.02	**2.98**	0.70	**6349.21**	1734.44
K4	42	**4449.22**	454.80	**2.76**	0.16	**11959.99**	941.75
III date - after 31 h							
K1	40	**274.57**	0.01	**115.10**	70.46	**21416.80**	807.08
K2	52	**186.89**	6.14	**72.53**	11.31	**14506.93**	3061.03
K3	49	**158.48**	7.33	**54.26**	7.33	**11377.25**	1075.62
K4	49	**185.15**	5.69	**2.64**	1.11	**26867.92**	862.71
IV date - after 41.5 h							
K1	49	**26.73**	0.00	**163.99**	5.04	**1383.24**	218.20
K2	72	**8.41**	0.65	**321.84**	35.28	**154.91**	19.17
K3	56	**6.50**	0.25	**277.69**	39.42	**278.73**	44.71
K4	66	**25.13**	14.40	**43.65**	7.85	**84.88**	15.24
V date -after 66.15 h							
K1	67	**0.52**	0.32	**3.35**	2.28	**0.52**	0.21
K2	74	**0.65**	0.11	**6.51**	1.42	**0.30**	0.24
K3	64	**0.22**	0.02	**6.83**	3.04	**0.47**	0.01
K4	74	**1.04**	0.13	**3.03**	1.02	**0.01**	0.00
VI date - after 117 h							
K1	64	**1.15**	0.40	**6.52**	1.23	**0.54**	0.44
K2	68	**0.89**	0.17	**2.34**	0.23	**0.01**	0.00
K3	61	**0.86**	0.36	**15.98**	1.18	**0.01**	0.00
K4	74	**0.61**	0.03	**0.31**	0.26	**0.10**	0.08
VII date - after 498 h							
K1	29	**2515.08**	167.05	**53.38**	1.96	**106.50**	0.01
K2	27	**8350.50**	1909.42	**194.58**	13.62	**2334.16**	1456.49
K3	49	**3038.17**	283.03	**62.85**	4.19	**1137.81**	588.76
K4	43	**3030.30**	140.30	**39.95**	0.59	**806.45**	481.68
VIII date - after 834							
K3	24.5	**6.03**	4.26	**34.80**	31.66	**269.90**	1.23
K4	27	**7.20**	2.94	**24.02**	13.26	**16.22**	1.47

the greatest number of bacteria occurred in compost K2 and it amounted to 6.07 cfu·10^5·g^{-1} d.m.. The composting process, which lasted 20 hours, caused an increase of temperature in the compost chambers by 12-23°C, and at the same time increased the number of bacteria in all composts, which was particularly visible in chamber K1. Starting with term III (after 31 hrs of composting), when the temperature in the chambers increased to 40-52°C, the proliferation of microorganisms increased in the majority of composts. Bacteria increase and develop in conditions of high temperatures not only because of their specific morphological construction, but also due to the physiologico-chemical properties of their cells, the chemical composition of the substrate and adequate pH value [32].

Not before the V term (after 66.15 hrs of composting), when the temperature reached values of 67-74°C, a violent drop in the number of thermophylls was recorded. According to Schlegel [33], in the compost, in the thermophilous phase, there dominate bacteria from Bacillus genus, while the temperature of 70°C is regarded as the critical temperature for the development of thermophylls. In turn, from the studies by Berquist et al. [34], one can infer that at a temperature higher than 70°C, there mainly develops the cellulolytic bacterium *Caldibacillus cellulvoruns.*

A repeated increase of the proliferation of the discussed bacteria was recorded in term VII, when the temperature dropped to 29-49°C.

Analyzing the dynamics of changes in the number of fungi (Table 3) in materials subjected to the composting process, it was found that the increase of temperature in the composted masses was most probably the main factor exerting an influence on the decrease of the proliferation of the discussed microorganism cells. Our studies have shown that the greatest number of fungi during the conducted experiment occurred in compost K1. The above phenomenon was connected most probably with a slower rate of temperature increase in bioreactor K1 during the composting process in relation to the remaining three chambers. The 20-hour composting process of sewage sludges with the additions caused an increase of the number of fungi in bioreactor K1 and K4, and at the same time, a 41-43% decrease of the number of the discussed microorganisms in the K2 and K3 composts. Also in the successive term of analyses (III – after 41.5 hrs), oscillations in the number of fungi were recorded in the composts. In bioreactor K1 and K2, a decrease of the number of those microorganisms was observed, while in composts K3 and K4, an increase of their number was recorded. Starting with term IV, when the temperature in the composted masses reached 49-66°C, the proliferation of fungi cells was significantly decreased. In the two successive terms of analyses, temperature increase was most probably the main factor reducing the number of fungi in the composts. It was found in compost K4 and it was the most perceivable phenomenon in that compost. In K4, compost sewage sludge made 45% (sludge 2), 5% straw and 50% sawdust. According to Hassen et al. [29] (after Beffa et al. 1996, Bollen 1993), in reality it was not the temperature increase during composting which was the main factor limiting the development of fungi. According to

Tabale 4. pH changes during composting.

Kind of compost	Compost temperature (ºC)	pH
I date– beginning of experiment		
K1	16	7.17
K2	18	6.97
K3	22	7.44
K4	19	7.0
II date– after 20 h		
K1	28	7.54
K2	40.5	7.20
K3	38	7.61
K4	42	7.15
III date – after 31 h		
K1	40	7.72
K2	52	7.59
K3	49	7.96
K4	49	7.13
IV date – after 41.5 h		
K1	48	8.07
K2	72	8.39
K3	56	8.41
K4	66	7.42
V date – after 66.15 h		
K1	67	8.58
K2	74	8.33
K3	64	8.58
K4	74	7.37
VI date – after 117 h		
K1	64	8.92
K2	68	8.81
K3	61	8.56
K4	74	8.72
VII date – after 498 h		
K1	29	8.88
K2	27	9.05
K3	49	7.77
K4	43	8.87
VIII- after 834		
K3	24.5	7.42
K4	27	9.07

the opinion of the above-mentioned authors, the microbiological processes of antagonizm, the presence of antibiotics and the change of substrate pH to alkaline reaction were responsible for this phenomenon. The above statement refers to the effect of the pH of substrate on the increase and development of fungi was not reflected in our own studies (Tables 3 and 4).

In the successive term of analyses – VII (after 498 hrs), an increase of the number of fungi was recorded, caused most probably by the drop of temperature in the chambers to 29-43°C. However, after two weeks (term VIII), the proliferation of cells decreased, most probably caused by the depletion of easily accesible organic matter. Similar tendencies were recorded in the studies by Tiquia et al. [35], referring to the composting of manure. In the above experiment. the number of fungi violently decreased in the composted material with an increase of temperature to 50°C, and then, at the drop of temperature below 45°C, a distinct increase of cell proliferation of the discussed microorganisms was recorded.

An analysis of changes in the activity of dehydrogenases (Fig. 1) during the experiment has indicated that the highest mean level of activity of the studied enzymes occurred in compost K1, while the lowest one was in bioreactor K3. The 20-hour composting process caused an increase of temperature in the composting chambers on average by 12-23°C, and at the same time it increased the activity of dehydrogenases in the majority of composts, except for compost K3. Data presented in Fig. 1 indicate that in the mentioned compost, the activity of the discussed enzymes was maintained at a very low level during the whole experiment. The reason for this phenomenon could be the chemical composition of the sewage sludge (sludge 3) being a component of compost 3. That sludge contained the least amount of organic carbon (Table 1). Perhaps it also contained compounds which inhibit the activity of dehydrogenases. Such compounds, as reported by Kucharski and Wyszkowska [36], and Bremner and Tabatabei [37], include nitrates or nitrites and heavy metals occurring in high concentrations.

Statistical analysis indicated that the low level of dehydrogenase activity in compost K3 correlated positively with the number of mesophilic bacteria and fungi (Table 5). In the case of the three remaining composts, the activity level of the studied enzymes was subject to significant oscillations in the initial terms. However, in term V, when the temperature in the composted materials reached 64-74°C, the activity of dehydrogenases violently decreased. Also, Tiquia et al. [35] noted the decrease of dehydrogenase activity in the thermophilous phase of the composting process. In the successive terms of studies, the biochemical activity in the composts was maintained at a low level with insignificant oscillations, depending on compost type.

On the basis of statistical analysis, it has been shown that the activity of dehydrogenases in composts K1, K2 and K4 was in the highest degree correlated with the number of thermophilous bacteria, while in compost K3 it was correlated with the number of mesophilous bacteria (Table 5).

Table 5. Pearson correlation coefficient between the number of choosen groups of microorgnaisms (cfu·10^5·g^{-1} d.m. of compost) and dehydrogenase activity (mg TPF·kg^{-1} d.m. of material·$5h^{-1}$) in composts.

Dependence type	Compost type			
	K1	K2	K3	K4
Mesophilic bacteria x dehydrogenase	-0.21	-0.22	0.38	0.18
Thermophilic bacteria x dehydrogenase	0.80	0.70	-0.15	0.35
Fungi x dehydrogenase	-0.03	0.21	0.32	0.01

Sanitary studies of sewage sludges detected no presence of bacteria from *Salmonella* genus (Table 2). The absence of *Salmonella* sp. in sewage sludges could be connected with the efficiency of the method of sludge treatments applied in the sewage treatment plants from which the sludges designed for composting originated. Because of the absence of bacteria from *Salmonella* genus, the sludges used in the experiment were suitable for agricultural utilization since, according to the directive of the minister of agriculture and rural development [14], the presence of these microorganisms in sewage sludges completely eliminates them from agricultural use [38]. But sludges used in the experiment contained an excessive disqualifying number of bacteria from the *Enterobacteriaceae* family (Table 2). Therefore, they absolutely required one of the hygienization processes (the composting process).

Analyzing the changes in the number of bacteria from the *Enterobacteriaceae* family in the composted materials (Table 6) during the whole experiment, it was found that the composting process contributed to a significant reduction in the number of the discussed microorganisms. On the day of experiment establishment, the highest number of bacteria was recorded in compost K1 – 432.97 cfu·10^3·g^{-1}, while in compost K4 the number was 247.41 cfu·10^3·g^{-1} d.m. (cfu = colony-forming units).

The 20-hour composting process contributed to a violent proliferation of bacteria from the *Enterobacteriaceae* family, which was most probably caused by an increase by

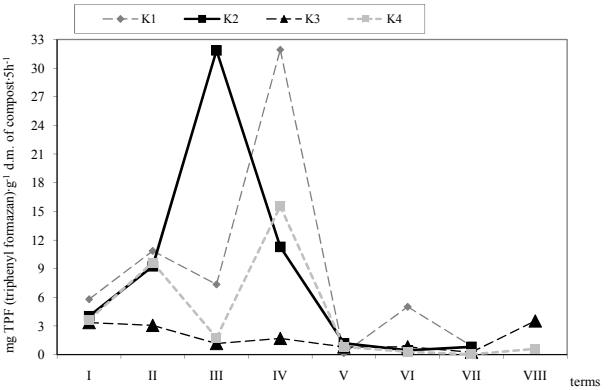

Fig. 1. Changes of dehydrogense activity in composts.

Table 6. The number of pathogenic bacteria in compost.

Compost type	Compost temperature (ºC)	*Enterobacteriaceae*		*Clostridium perfringens*	
		cfu·10^3·g^{-1} d.m. of compost	Standard Deviation	cfu·10^2·g^{-1} d.m. of compost	Standard Deviation
I date – beginning of experiment					
K1	16	**432.97**	205.07	**11.60**	13.40
K2	18	**411.95**	48.86	**456.38**	9.32
K3	22	**269.54**	48.90	**35.37**	40.85
K4	19	**247.41**	72.44	**91.10**	92.57
II date- after 20 h					
K1	28	**19262.07**	313.27	**282.14**	181.69
K2	40.5	**18496.88**	1124.12	**590.53**	162.38
K3	38	**39249.64**	5331.95	**643.57**	39.99
K4	42	**15249.37**	2693.06	**310.37**	10.35
III date - after 31 h					
K1	40	**4288.85**	133.16	**2169.13**	348.75
K2	52	**2575.39**	329.38	**937.24**	376.43
K3	49	**3341.32**	373.38	**568.86**	138.28
K4	49	**1411.32**	313.73	**132.07**	21.78
IV date - after 41.5 h					
K1	49	**2491.98**	395.19	**2379.67**	1327.59
K2	72	**422.68**	25.44	**901.44**	393.22
K3	56	**1307.12**	131.01	**1414.36**	53.84
K4	66	**196.44**	37.80	**200.07**	147.01
V date - after 66.15 h					
K1	67	**0.61**	0.19	**5.16**	2.08
K2	74	**1.33**	0.12	**0.99**	0.45
K3	64	**0.16**	0.02	**3.34**	0.44
K4	74	**1.27**	0.12	**8.30**	6.06
VI date - after 117 h					
K1	64	**0.05**	0.03	**4.48**	5.24
K2	68	**0.01**	0.01	**0.09**	0.10
K3	61	**0.01**	0.01	**16.68**	20.81
K4	74	**0.08**	0.05	**0.19**	0.15
VII date - after 498 h					
K1	29	**3.39**	0.46	**36.91**	27.23
K2	27	**1.36**	0.12	**11.46**	8.28
K3	49	**3.26**	1.27	**35.66**	1.74
K4	43	**0.34**	0.24	**7.39**	1.25
VIII date - after 834 h					
K3	24.5	**0.18**	0.08	**30.04**	15.05
K4	27	**0.01**	0.01	**1.39**	0.52

several (or even by more than ten) degrees of temperature in the composted materials. The highest number of the discussed bacteria was recorded at that time in compost K3 39,249.64 cfu·10^3·g^{-1} d.m., with an increase of temperature to 38°C and pH 7.61 (Table 4). Such violent proliferation of *Enterobacteriaceae* bacteria was most probably caused by the access of easily degraded organic substances in the initial period of composting and by the optimal temperature for the growth of the discussed bacteria and by pH. According to Truszczyński [39], the optimal temperature for the growth of *Escherichia coli* bacteria belonging to the *Enterobacteriaceae* family is 37°C, although their growth is possible within the interval of 10 to 45°C and the most adequate pH value is 7.2-7.4 .

On the basis of data presented in Table 6, we can infer that further growth of temperature in the composted masses (term III – after 31 hrs of composting) contributed to a violent decrease of the number of the discussed bacteria. The highest decrease of the proliferation of *Enterobacteriaceae* bacteria was recorded in compost K3 – 3341.32 cfu·10^3·g^{-1} d.m. at 49°C and pH 7.96. Also, Shaban [40] and Vinneras et al. [41] noted in their studies that the violent increase of temperature to 50-70°C in the composted materials caused the inactivation of pathogenic microorganisms.

Cekmecelioglu et al. [42] confirmed that the reduction of the number of *Enterobacteriaceae* took place even in a temperature increase to 54.7-56.6°C.

According to Mckinley and Vestal [43], the thermophilous phase of composting has a significant effect on the change and succession of microorganism population. On the one hand, such high temperature causes an inactivation or reduction of mesophilous (not spore-forming) microorganisms (e.g. *Salmonella, E. coli*). On the other hand, it exerts an effect on the growth and activity of thermophilous microorganisms. In the successive three terms of the composting process (Terms III-V), it was found that the thermophilous conditions dominating all composted materials caused a further decrease of the number of discussed bacteria.

From the studies of Smith et al. [44], it follows that *Escherichia coli* and *Salmonella* are not destroyed in mesophilic temperatures and their inactivation takes place only in thermophilous conditions. Droffner et al. [45] wrote about the possibile survival of some mesophylls, for example *Escherichia coli* in thermophilous conditions, even at more than 54°C. Some strains of *E. coli* are carriers of specific genetic information that conditions their thermal resistance. The expression of this information takes place at 48°C or higher, and it permits bacteria regarded as thermophiloous to survive the thermophilous phase of the composting process. According to Szala and Paluszak [46], the depth, at which pathogens develop in the composted mass also exerts an effect on the rate of pathogenic bacteria inactivation. In the presented studies, it was found that *Salmonella* died faster in the surface layers of compost than in the deeper ones.

In the successive term of analyses (VII – after 498 hrs of composting) an increase of the bacteria number was recorded. It was probably connected with the drop in temperature. Also, Wieland and Sawicka [30] reported that the cold phase of composting represents a stabilization period of compost in which an activity of mesophilous microflora is observed again.

Different than in the case of *Enterobacteriaceae* was the course of changes in the number of *Clostridium perfringens* bacteria in the studied composts (Table 6).

On the day of experiment establishment, in compost K2, the highest number of bacteria was detected – 450.381 cfu·10^2·g^{-1} d.m. in comparison with the remaining composts. In turn, in compost K1 the least number of bacteria was found – 11.608 cfu·10^2·g^{-1} d.m.. According to Truszczyński [39], Clostridium perfringens develops best within a temperature interval of 18 to 38°C, while the optimal growth and development temperature is 37°C, and optimal pH is 7.0-7.5.

In the successive terms of analyses (II–IV), a gradual increase of *Clostridium perfringens* in all composted masses was recorded. The increase in the number of discussed types of bacteria was, however, more visible in compost K3, where it reached the maximal value – 1414.36 cfu·10^2·g^{-1} d.m. in term IV. After 66.15 hours of composting (term V), a further temperature increase was recorded and, at the same time, the number of *Clostridium perfringens* decreased.

Holmquist and Stenstrom [47] reported that next to temperature being one of the most important factors exerting an effect on the reduction of pathogenic microorganisms, a significant role is also played by pH and the biological properties of sludge. Sidhu et al. [48] ascribe the reduction of the number of pathogenic microorganisms in compost to the presence of the indigenous antagonistic microflora. Also, Szember [49] stated that the factor that causes the dying of pathogenic microorganisms in composts is not only the periodical effect of high temperature, but probably a sterilizing effect is also exerted by a certain number of antibiotics produced primarily by numerous actinomycetes occurring in composts.

Further maintenance of the thermophilic phase (term VI) caused a decrease of the bacteria numbers in composts K1, K2 and K4, and a several-fold increase in the proliferation of bacilli in compost K3. The increased number of the discussed bacteria in compost K3 could have been connected with the production of endospores by *Clostridium perfringens*. From the studies by Hassen et al. [29], one can see that soon after the temperature in the composted material reached 60°C, there followed a significant increase of the number of dormant spores.

In the successive terms of analyses, in our own studies, when the temperature dropped to mesophilous conditions we recorded an increase of bacteria number in all analyzed composts. Analyzing the changes in the number of *Clostridium perfringens* bacteria in the composted materials, one can state that the composting process carried out in controlled conditions did not contribute, similar to the case of bacteria from the *Enterobacteriaceae* family, to a complete hygienization of the compost, but it only caused a reduction in the number of pathogens.

Conclusions

1. The reduction dynamics of the studied groups of microorganisms (with the exception of thermophilic microorganisms) in composts was shifted in time, in the majority of cases, according to the temperature increase.
2. Dehydrogenase activity correlated most intensively with the number of thermophilous bacteria in the majority of the studied composts.
3. The studied sewage sludges applied in the experiment did not meet the sanitary standards required by the directive of the minister of agriculture and rural development (2004) since, in spite of the absence of bacteria from *Salmonella* genus, the number of bacteria from the *Enterobacteriaceae* family was higher than 1000 $cfu \cdot g^{-1}$ of compost.
4. The composting process of sewage sludges' significant reduction of their number of *Enterobacteriaceae* and *Clostridium perfringens*.
5. On the basis of the presented studies, it was found that the developed composts K3 and K4 met the standards foreseen for sewage sludges designed for agricultural purposes, because the bacteria of *Salmonella* genus have not been isolated from them and the number of *Enterobacteriaceae*, in the final terms of studies, was significantly smaller than 1000 $cfu \cdot g^{-1}$ of compost.
6. Furthermore, it has been stated that composts K1 and K2 did not meet the required standards regarding the content of *Enterobactreiaceae* in natural fertilizer dosing for agricultural use.

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