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DYNAMICS OF CHANGES IN THE NUMBER OF SELECTED MICROORGANISM GROUPS IN SEWAGE SLUDGE AND IN MANURE SUBJECT TO COMPOSTING PROCESS AND IN THE SOIL ENRICHED WITH COMPOSTS

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DYNAMIKA ZMIAN LICZEBNOŚCI WYBRANYCH GRUP DROBNOUSTROJÓW W OSADZIE ŚCIEKOWYM I OBORNIKU PODDANYM PROCESOWI KOMPOSTOWANIA ORAZ W GLEBIE WZBOGACONEJ KOMPOSTAMI

Praca przedstawia charakterystykę mikrobiologiczną osadu ściekowego z mechaniczno-biologicznej oczyszczalni ścieków i obornika oraz wyprodukowanych z nich kompostów. W doświadczeniu sporządzono 4 rodzaje kompostów. Pierwszy składał się z samego osadu ściekowego, drugi z obornika, kolejne komposty powstały w wyniku zmieszania wyżej wymienionych bioodpadów w proporcjach: 75% osadu + 25% obornika oraz 50% osadu + 50% obornika. W kolejnym etapie badań powstałe komposty wprowadzono do gleby. W kompostach oraz w glebie z kompostami oznaczano liczebność: *Salmonella* sp., *E. coli, Clostridium perfringens*, ogólnie bakterii, grzybów oraz promieniowców metodą płytkową na wybiórczych podłożach. Badania wykazały, że proces kompostowania spowodował całkowitą eliminację *Salmonella* sp. oraz zmniejszenie liczebności wszystkich pozostałych grup drobnoustrojów. Zgodnie więc z Rozporządzeniem Ministra Rolnictwa i Rozwoju Wsi z 19 października 2004 r. przekompostowany osad ściekowy nadawał się do wykorzystania na cele rolnicze. Ponadto stwierdzono, że po wprowadzeniu kompostów do gleby, już w sześćdziesiątym dniu zaobserwowano zmniejszenie liczebności w niej większości analizowanych grup drobnoustrojów (z wyjątkiem promieniowców i *E. coli*), w tym bakterii chorobotwórczych z rodzaju *C. perfringens*. Uzyskane wyniki badań wskazują, że wprowadzenie bioodpadów do gleby może zmniejszać przeżywalność niektórych patogenów w środowisku, a więc jest to dobra metoda utylizacji tego rodzaju materii organicznej.

Summary

The paper presents microbiological characteristics of sewage sludge derived from the mechanical-biological sewage treatment plant and farmyard manure as well as composts manufactured from them. In the performed experiment, four types of composts were analyzed. The first of them comprised the sewage sludge alone, the second one – was made up only of farmyard manure, while the remaining composts were prepared by mixing the above-mentioned bio-wastes in the following proportions: 75% sewage sludge + 25% farmyard manure and 50% sewage sludge + 50% farmyard manure. The next stage of experiments involved analyses of the composts incubated with soil. The following assays were carried out in the experimental composts and mixtures of soil and composts: counts of *Salmonella* sp., *E. coli, Clostridium perfringens*, total counts of bacteria, fungi and actinomycetes on selective media employing the plate method. The performed investigations

revealed that the composting process resulted in complete riddance of the *Salmonella* sp. and reduction in the numbers of the remaining groups of microorganisms. Therefore, it can be said that the composted sewage sludge was suitable for the utilization for agricultural purposes in accordance with the Directive of the Minister of Agriculture and Rural Development of October 2004. Moreover, it was found that, as early as 60 days after the introduction of composts into the soil, counts of the majority of the analyzed groups of microorganisms (with the exception of actinomycetes and *E. coli*), including pathogenic bacteria from the *C. perfringens* genus, were found reduced. The obtained research results proved that the introduction of bio-wastes into the soil may decrease survivability in the natural environment of certain pathogens; hence it is a good method of utilization of this organic material.

INTRODUCTION

With the progress in sewage treatment, there increases the amount of the produced sewage sludges, and their storaging causes a threat for the environment. Therefore, it appears essential to work out effective methods of utilization of these materials which would guarantee the recovery of nutrients for plants and microorganisms deposited in sludges and, at the same time, ensure maximum safety for the environment [10]. Diversified chemical composition of sewage sludges causes that they exhibit considerable variability in the content of individual elements and fertilization value [15].

At the present time, a number of methods of sewage sludge utilization are known but most commonly, sewage sludge is composted prior to its introduction into the soil in order to improve its properties, increase nutrient availability and detoxify heavy metals [27, 33, 39]. Following composting processes, wastes containing organic compounds characterized by different sensitivity to decomposition and pathogenic microorganisms are transformed into material which no longer poses hygiene hazards [24]. The incorporation into the soil of composted sewage sludge allows for on the one hand, management of this troublesome waste and, on the other hand, enhances soil fertility due to the increased supply of large quantities of organic matter [13].

The type and quantity of the applied fertilizers affect the content of organic matter in the soil as well as its qualitative composition [21, 31]. All organic matter transformations in the soil are mediated by whole complexes of microorganisms whose activity and diversity depend on the amount and quality of the introduced organic matter [3, 27, 36]. Due to their participation in the decomposition of organic matter introduced into the soil in the form of organic fertilizers, microorganisms play a key role in the formation of humus compounds [14].

The aim of the performed investigations was to determine changes in the microbiological conditions of sewage sludge and farmyard manure subjected to the composting process in laboratory conditions and to assess the dynamics of development of selected groups of microorganisms in the soil following the introduction into it of the above-mentioned composts.

MATERIAL AND METHODS

The experiment was established in laboratory conditions at the Chair of Soil Science at the August Cieszkowski Agricultural University in Poznań. During the first stage of investigations which lasted 90 days, sewage sludge with and without farmyard manure was composted according to the design scheme of experiment presented below (each treatment in three replications):

1. sewage sludge (100%),
2. sewage sludge + farmyard manure (50% : 50% v/v),
3. sewage sludge + farmyard manure (25% : 75% v/v),
4. farmyard manure (100%).

Experiments were carried out in room temperature conditions in plastic containers of 2.5 dm^3 volume [5] and samples for microbiological tests were collected on day 1 and 90 of the trial.

The sewage sludge applied in the experiment originated from communal sewage treatment plant with a small participation of industrial sediments with an advantageous chemical composition and the farmyard manure – from dairy cows. The sewage treatment plant from which the sludge originated is a modern one based on the mechanical-and-biological treatment. The compost mixture was oxygenated by a mechanical mixing of compost every 10–14 days in the first two months of studies and once in the third month.

Selected properties of both components and composts are presented in Table 1 and Table 2.

Table 1. Selected properties of sewage sludge and farmyard manure

Specification	Unit	Sewage sludge	Farmyard manure
Ph-H_2O	–	6.96	8.20
Dry matter	$g \cdot kg^{-1}$	166.5	348.0
Organic C	$g \cdot kg^{-1}$ d.m.	345.5	254.6
Total N		56.3	20.6
C : N	–	6.14	12.36

Table 2. Influence of composting time on changes in organic carbon, nitrogen total and dry mass content in compost samples

Compost	Days of composting							
	1	90	1	90	1	90	1	90
	Carbon		Nitrogen		C:N		d.m.	
	$g \cdot kg^{-1}$ d.m. of compost						%	
Sewage sludge (control)	345.51	230.30	56.31	30.43	6.13	7.57	20.00	44.81
50% sewage sludge + 50% manure	300.20	230.01	39.72	23.62	7.56	9.74	24.81	37.81
75% sewage sludge + 25% manure	312.31	228.01	35.42	27.00	8.82	8.44	25.24	38.80
Manure	254.61	205.00	20.61	18.44	12.35	11.14	53.51	59.69

Following a 90-day composting process, the second stage of experiments was conducted in which the produced composts were incubated in soil. Then, plastic containers were filled with grey-brown podzolic soil in the amount of 975 g d.m. Composts were applied in the amount of 2.5% in relation to soil mass corresponding to 25 g d.m. of composts and the experimental design included the following combinations (each treatment in three replications):

1. soil – control,
2. soil + sewage sludge compost,
3. soil + sewage sludge + farmyard manure compost (50% : 50%),

4. soil + sewage sludge + farmyard manure compost (75% : 25%),
5. soil + farmyard manure compost.

The soil used in the trial was grey-brown podzolic soil containing 6.68 g · kg^{-1} organic carbon, 0.60 g · kg^{-1} total nitrogen and topsoil reaction of pH 5.60.

The incubation process of the soil with composts lasted 60 days, and samples for analyses were collected on the first and last days of the trial.

Using conventional methods on microbiological selective media, the authors determined: numbers of colony forming units [cfu] of fungi, counts of total bacteria, actinomycetes as well as bacteria from *Salmonella* sp., *E. coli, Clostridium perfringens* genera.

The total number of bacteria was determined on the Merck standard agar medium following 24 hours incubation at the temperature of 35°C [22]. Counts of actinomycetes were determined on chitin medium according to Lingapp and Lockvood [17] following a 10-day incubation of plates at the temperature of 28°C. Fungi were determined on the Martin medium [20] at the temperature of 28°C after 5 days of incubation. Bacteria from the *Salmonella* genus were determined by the plate method on the Merck medium at the temperature of 37°C for 24 hours [29]. In order to make sure that the obtained bacteria were those of the *Salmonella*, procedures recommended by the Polish Standard PN-Z-19000-1 were applied [28]. In order to determine numbers of the *Escherichia coli* bacteria, a selective medium of the Merck Company was used. Plates were incubated at the temperature of 37°C for 24 hours [18]. *Clostridium perfringens* bacteria were determined on the TSC agar medium with tryptose, sulfate and cycloserin incubating the plates in a thermostat with 22% CO_2 atmosphere at the temperature of 44°C for 24 hours [1].

The obtained research results were subjected to statistical analysis using the Statistica 7.1 software.

RESULTS AND DISCUSSION

It is clear from the data presented in Table 3 that on the day of establishment of the experiment (day 1), the highest total bacterial count (1327.39 · 10^5 cfu · g^{-1} d.m. of sewage sludge) occurred in the sewage sludge alone. Bacterial counts in the remaining

Table 3. Total number of bacteria in compost [10^5 cfu · g^{-1} d.m. of material]

Combination	Mean	Standard deviation	Difference (control – combination)
Stage I			
1 day			
Sewage sludge (control)	1327.39	488.87	–
50% sewage sludge + 50% manure	1053.88	497.20	273.51
75% sewage sludge + 25% manure	785.80	601.41	541.59
Manure	205.82	29.45	1121.57**
90 day			
Sewage sludge (control)	269.94	133.23	–
50% sewage sludge + 50% manure	164.90	74.58	105.04
75% sewage sludge + 25% manure	235.43	93.12	34.51
Manure	82.50	42.55	187.44

** – highly significant difference between the number of cells in the control and their numbers in a given combination

combinations were lower by 24% – in the compost with 50% share of sewage sludge and 50% farmyard manure and by 41% in the compost containing 75% sewage sludge and 25% farmyard manure. On the other hand, in the farmyard manure alone, the numbers of bacteria were highly significantly lower by 86% in relation to the number of bacteria in the sewage sludge alone.

Table 4. The number of actinomyces in compost [10^4 cfu · g^{-1} d.m. of material]

Combination	Mean	Standard deviation	Difference (control – combination)
Stage I			
1 day			
Sewage sludge (control)	156.63	43.33	–
50% sewage sludge + 50% manure	185.82	51.12	29.19
75% sewage sludge + 25% manure	300.15	66.25	143.52**
Manure	116.80	18.16	39.83
90 day			
Sewage sludge (control)	106.94	24.55	–
50% sewage sludge + 50% manure	148.73	51.80	41.79
75% sewage sludge + 25% manure	137.93	27.73	30.99
Manure	114.93	36.40	7.99

** – highly significant difference between the number of cells in the control and their numbers in a given combination

The 90-day long period of composting resulted in a several fold decline of bacterial counts in the examined material. The most probable factor which contributed to the qualitative reduction of bacteria was temperature which, in the course of the aerobic (thermophilic) phase of composting of the organic wastes as a rule exceeds 60°C [8, 32]. According to Wieland and Sawicka [35], once the above-mentioned temperature is exceeded, the number of bacterial colonies can decline and only such spore-forming bacteria as *Clostridium* survive.

Apart from proper bacteria, another group of microorganisms whose numbers were analyzed in this experiment were actinomycetes (Tab. 4). According to Bhamidimarii and Pandey [4], besides fungi, these bacteria are the most important group of microorganisms which participate in the process of transformation of the composted material into humus.

Analyzing numbers of actinomycetes on the day of establishment of the experiment (Tab. 4), their highest counts in all compost samples were determined in the compost consisting of 75% of sewage sludge and 25% farmyard manure and the lowest – in the farmyard manure alone. Following 90-day period of composting, the authors observed, as in the case of proper bacteria (Tab. 3), a reduction in the number of actinomycetes in all experimental composts. The most noticeable (in relation to the day of experiment establishment) decline in the cell proliferation reaching 55% was recorded in the compost consisting of 75% sewage sludge and 25% farmyard manure and the lowest (2%) – in the farmyard manure after composting.

Table 5. The number o fungi in compost [10^4 cfu · g^{-1} d.m. of material]

Combination	Mean	Standard deviation	Difference (control – combination)
Stage I			
1 day			
Sewage sludge (control)	188.56	38.96	–
50% sewage sludge + 50% manure	110.57	51.01	77.99
75% sewage sludge + 25% manure	127.46	17.72	61.10
Manure	118.60	63.50	69.96
90 day			
Sewage sludge (control)	53.21	23.15	–
50% sewage sludge + 50% manure	98.85	57.97	45.64
75% sewage sludge + 25% manure	72.55	31.83	19.34
Manure	78.57	40.39	25.36

It is evident from the data in Table 5 that the highest counts of fungi on day 1 of the trial were determined in the sewage sludge (188.56 · 10^4 cfu · g^{-1} d.m. of the material). The composting process reduced numbers of fungi in all the examined compost samples. The strongest reduction of the order of 71%, in comparison with the number of fungi on the day of trial establishment, occurred in the sewage sludge after the termination of the composting process. In the remaining treatments, this decline ranged from 10 to 43%. Initial increase of fungal counts in the composted farmyard manure was also reported by Klamer and Baath [16] and it was followed by a decline of the numbers during the thermophilic phase of the process. Another increase of fungal proliferation was observed when the temperature dropped to below 50°C. Similar research results were reported by Hassen *et al.* [11] who claimed that, following the composting process of solid organic wastes, the numbers of moulds and yeasts declined from 4.5 · 10^6 cfu · g^{-1} d.m. of material to 2.6 · 10^3 cfu · g^{-1} d.m. of material. The authors explain the above phenomenon by the occurrence of environmental factors particularly unfavorable for fungi, i.e. a high temperature dominating in the substrate and the pH reaction within the range of 8–8.5. According to Wieland and Sawicka [35], if temperatures during the composting process exceed 60°C, they can result in the complete reduction of these microorganisms.

Filipkowska *et al.* [9], Hermann [12] as well as Dupray and Derrien [7] maintain that sewage sludge can contain high levels of pathogenic microorganisms, including *Salmonella, E. coli* and *Clostridium perfringens* bacteria. This was also confirmed in our own studies (Tab. 6–8). All pathogenic bacteria present in sewage sludge derive from man – "the producer" of sewage and their species composition depends, among others, on the health condition of the society. It should be stressed that the majority of living bacteria perish in the preliminary sedimentation tanks; nevertheless some of them do survive in the sludge posing a real sanitary hazard. It is evident from experiments that mechanical-biological treatment of sewage frequently does not cause desirable hygiene effects. The above researchers demonstrated it on the basis of three different sewage treatment plants [25]. Sewage sludges derived from these plants were found to contain pathogenic bacteria of: *Salmonella* sp., *Clostridium perfringens* and *Staphylococcus aureus*. This shows that

Table 6. The number of *E. coli* in compost [10^2 cfu · g^{-1} d.m. of material]

Combination	Mean	Standard deviation	Difference (control – combination)
Stage I			
1 day			
Sewage sludge (control)	14.23	10.18	–
50% sewage sludge + 50% manure	6.05	2.48	8.18
75% sewage sludge + 25% manure	4.39	1.54	9.84
Manure	20.77	9.18	6.54
90 day			
Sewage sludge (control)	3.37	1.25	–
50% sewage sludge + 50% manure	10.56	8.37	7.19
75% sewage sludge + 25% manure	13.94	7.37	10.57
Manure	10.39	8.04	7.02

Table 7. The number of *Clostridium perfringens* in compost [10^2 cfu · g^{-1} d.m. of material]

Combination	Mean	Standard deviation	Difference (control – combination)
Stage I			
0 day			
Sewage sludge (control)	103.22	62.36	–
50% sewage sludge + 50% manure	75.98	52.88	27.24
75% sewage sludge + 25% manure	144.19	83.43	40.97
Manure	37.99	38.32	65.23
90 day			
Sewage sludge (control)	18.66	22.27	–
50% sewage sludge + 50% manure	64.60	28.22	45.94
75% sewage sludge + 25% manure	123.79	58.99	105.13*
Manure	18.92	10.60	0.26

* – significant difference between the number of cells in the control and their numbers in a given combination

before their introduction into the soil, various sewage sludges should be subjected to additional hygiene processes in order to eliminate pathogens.

On the basis of our own studies, it can be stated that the composting process of the sewage sludge and farmyard manure carried out in laboratory conditions proceeded properly as evidenced by the absence in the obtained composts of bacteria of *Salmonella* genus (Tab. 8). Similar results were reported by Hassen *et al.* [11], who recorded a complete reduction of *Salmonella* sp. already after 25 days of composting. However, different results were reported by Nguyen Thi [24], who found that a 4-month long period of composting of sewage sludge with the addition of straw, sawdust and $CaCO_3$ resulted, in the majority of cases, only in the reduction in the numbers of *Salmonella* sp. and not in their total elimination.

Table 8. The number of *Salmonella* sp. in compost [10^2 cfu · g^{-1} d.m. of material]

Combination	Mean	Standard deviation	Difference (control – combination)
Stage I			
1 day			
Sewage sludge (control)	70.93	36.77	–
50% sewage sludge + 50% manure	89.68	50.40	18.75
75% sewage sludge + 25% manure	68.98	36.83	1.95
Manure	1.24	1.52	69.69**
90 day			
Sewage sludge (control)	0	0	0
50% sewage sludge + 50% manure	0	0	0
75% sewage sludge + 25% manure	0	0	0
Manure	0	0	0

** – highly significant difference between the number of cells in the control and their numbers in a given combination

The data presented in Table 6 indicate that composting did not contribute to the elimination of bacteria from *Escherichia coli* genus. On the day of establishment, the highest numbers of the discussed bacteria were detected in the farmyard manure. This bacterium is a typical intestinal microbe found in humans and animals which explains its higher numbers in the combination consisting of farmyard manure alone. Following a 90-day period of composting, a drop in *E. coli* counts was determined only in the sewage sludge alone and in the farmyard manure in which the composting process was completed (Tab. 6). In the case of the two remaining treatments, counts of the discussed bacteria even increased. The above phenomenon raises serious controversies. However, it is evident from experiments carried out by Dupray and Derrien [7] that some bacteria from the *Escherichia coli* genus subjected to 24- or 72-hour period of incubation in sewage were characterized by a specific modification of cell metabolism or osmoregulatory cell mechanism which allowed them to survive longer in sea water in comparison with non-incubated bacteria. On the other hand, Droffner *et al.* [6] maintain that certain strains of *E. coli* are capable of surviving the temperature of 54°C and appear to be carriers of specific genetic information allowing their thermal resistance. The expression of this information takes place at the temperature of 48°C or higher which makes it possible for the bacteria considered as mesophyllic to survive the thermophilic phase of the composting process.

Also in the case of bacteria from the *Clostridium perfringens* genus, it was found that the composting process failed to eliminate this pathogen completely but only reduced its numbers (Tab. 7). This is a spore-forming bacterium which explains the possibility of its surviving the high temperature characteristic for the thermophilic phase of the composting process. However, the above interpretation is not confirmed by experiments carried out by Nguyen Thi [24] who found that the composting process of sewage sludge with different organic additives eliminated *C. perfringens* completely.

Our own experiments show (Tab. 7) that on day 1 of experiments, the highest cell counts of *C. perfringens* were recorded in the material made up of 75% sewage sludge

and 25% farmyard manure, while the lowest – in the farmyard manure alone. However, the composting process favored reduction in the numbers of these bacteria ranging from 14 to 82%, depending on the experimental object.

In the II stage of studies consisting in the introduction of composted sludges and manure to the soil, it was found that not all microorganisms found there favorable conditions for growth and development. It could have been connected with the sorption properties of the soil causing that pathogenic microorganisms do not penetrate to the deeper layers of groundwater. Another reason could be the competition for food, production of antibiotics by some microorganisms, or the predatory activity of some protozoa.

The composts from incubated experiment introduced into the soil did not cause a statistically significant increase of bacterial counts (Tab. 9). In the case of the soil incubated with the composts made up of 75% sewage sludge and 25% farmyard manure, the determined numbers of bacteria were even lower in comparison with the control soil. A similar phenomenon was observed by Przybulewska *et al.* [27] in the experiment with soil enriched with vermi-compost in comparison with soil alone.

Table 9. Total number of bacteria in soil [10^5 cfu · g^{-1} d.m. of soil]

Combination	Mean	Standard deviation	Difference (control – combination)
Stage II			
1 day			
Soil (control)	60.70	20.18	–
Soil + sewage sludge	84.27	57.36	23.57
Soil + 50% sewage sludge + 50% manure	89.56	1.23	28.86
Soil + 75% sewage sludge + 25% manure	37.48	26.78	23.22
Soil + manure	69.39	39.64	8.69
60 day			
Soil (control)	33.14	5.90	–
Soil + sewage sludge	27.29	10.12	5.85
Soil + 50% sewage sludge + 50% manure	35.36	14.23	2.22
Soil + 75% sewage sludge + 25% manure	18.31	5.75	14.83
Soil + manure	62.48	4.18	59.34

After 60 days of incubation, there followed a decrease of cell proliferation dynamics both in the control combination (soil) and in that enriched with composts. The causes of this drop should be attributed to the microflora of the sewage sludge or farmyard manure which is of intestinal or faecal origin [11] and even if it survived the composting process, it did not find suitable living conditions in the soil [36].

A different phenomenon was observed in the case of actinomycetes (Tab. 10). Following the introduction of composts into the soil, the highest counts of actinomycetes were recorded in the soil with the compost made up of 75% sewage sludge and 25% farmyard manure, whereas their smallest numbers were found in the control soil. Elevated levels of actinomycetes in the treatments enriched with organic matter were caused most probably by the introduction into the soil of composts rich in cells of these microorganisms. Their counts ranged from 106.94 to 148.73 · 10^4 cfu · g^{-1} d.m. of material (Tab. 10).

Table 10. The number of actinomyces in soil [10^4 cfu · g^{-1} d.m. of soil]

Combination	Mean	Standard deviation	Difference (control – combination)
Stage II			
1 day			
Soil (control)	2.39	1.85	–
Soil + sewage sludge	5.53	2.20	3.14
Soil + 50% sewage sludge + 50% manure	3.21	0.71	0.82
Soil + 75% sewage sludge + 25% manure	5.53	2.37	3.14
Soil + manure	4.24	2.15	1.85
60 day			
Soil (control)	82.67	2.83	–
Soil + sewage sludge	77.94	16.07	4.73
Soil + 50% sewage sludge + 50% manure	90.02	2.84	7.35
Soil + 75% sewage sludge + 25% manure	83.90	9.51	1.23
Soil + manure	105.63	11.94	22.96**

** – highly significant difference between the number of cells in the control and their numbers in a given combination

After 60 days of incubation, counts of actinomycetes in all analyzed soil objects increased several dozen times. The highest increase of this group of microorganisms was observed in the combination with farmyard manure and it was statistically significantly higher in comparison with the control soil. Also Nowak *et al.* [26] and Przybulewska *et al.* [27], in their experiments with farmyard manure and vermi-compost, found a significant increase of actinomycete counts in the soil. Moreover, the development of these microorganisms might have been enhanced by temperature whose optimum for actinomycetes, according to Marcinkowska [19] is 27°C.

The mycological analysis of soil samples (Tab. 11) carried out on the day of establishment of the experiment showed that the smallest number of fungi occurred in the control soil ($1.9 \cdot 10^4$ cfu · g^{-1} d.m. of material). The number of fungi in the remaining soil samples was several times higher. The observed higher numbers of the discussed microorganisms in the soil fortified with composts was caused most probably by the fact that in these combinations, apart from typical soil microflora, fungi derived from composts were also isolated. The extension of the incubation time of the soil with composts to 60 days did not exert a significant impact on changes in the numbers of fungi (Tab. 11).

Soil is not a suitable environment for the growth and development of pathogenic microorganisms whose natural habitat is human or animal organism. That is why, already on the day when composts were introduced into the soil, *E. coli* counts were found to be reduced to the level of $0.21–3.75 \cdot 10^2$ cfu · g^{-1} d.m. of material (Tab. 12).

On the other hand, in the control treatment (soil), this bacterium was not found either on the day of establishment of the experiment or after 60 days of incubation. In the case of the soil fortified with sewage sludge alone after composting, it was found that a 60-day period of incubation eliminated completely the discussed bacteria. In the case of the three remaining soil combinations to which compost with farmyard manure was introduced,

Table 11. The number of fungi in soil [10^4 cfu · g^{-1} d.m. of soil]

Combination	Mean	Standard deviation	Difference (control – combination)
Stage II			
1 day			
Soil (control)	1.99	1.23	–
Soil + sewage sludge	3.96	1.62	1.97
Soil + 50% sewage sludge + 50% manure	8.72	1.42	6.73*
Soil + 75% sewage sludge + 25% manure	3.83	2.28	1.84
Soil + manure	7.71	3.91	5.72*
60 day			
Soil (control)	8.78	1.23	–
Soil + sewage sludge	5.14	3.24	3.64
Soil + 50% sewage sludge + 50% manure	6.88	4.44	1.90
Soil + 75% sewage sludge + 25% manure	4.68	1.74	4.10
Soil + manure	4.24	3.16	4.54

* – significant difference between the number of cells in the control and their numbers in a given combination

Table 12. The number of *E. coli* in soil [10^2 cfu · g^{-1} d.m. of soil]

Combination	Mean	Standard deviation	Difference (control – combination)
Stage II			
1 day			
Soil (control)	0	0	–
Soil + sewage sludge	3.75	2.64	3.75
Soil + 50% sewage sludge + 50% manure	0.68	0.75	0.68
Soil + 75% sewage sludge + 25% manure	0.21	0.52	0.21
Soil + manure	0.38	0.59	0.38
60 day			
Soil (control)	0	0	–
Soil + sewage sludge	0	0	0
Soil + 50% sewage sludge + 50% manure	1.37	1.74	1.37
Soil + 75% sewage sludge + 25% manure	0.63	0.69	0.63
Soil + manure	0.77	0.59	0.77

an increase of *E. coli* counts was recorded during the final period of the experiment. This could have been associated with the occurrence in the farmyard manure of some specific strains of bacteria more resistant to unfavorable environmental conditions and development of *E. coli* strains of prolonged period of survivability [23].

The period of survivability of this bacterium in the soil varies and may range from 38 days to 8 weeks, depending on the type of soil or the presence of plants [2, 34].

Following the introduction into the soil of composts, on the day of the initiation of the experiment, the highest number of *C. perfringens* bacteria was recorded in the soil with the addition of the compost made up of 75% sewage sludge and 25% farmyard manure and the lowest – in the control soil, without compost (Tab. 13).

Table 13. The number of *Clostridium perfringens* in soil [10^2 cfu · g^{-1} d.m. of soil]

Combination	Mean	Standard deviation	Difference (control – combination)
Stage II			
1 day			
Soil (control)	0.39	0.61	–
Soil + sewage sludge	8.70	5.23	8.31
Soil + 50% sewage sludge + 50% manure	13.77	5.37	13.38
Soil + 75% sewage sludge + 25% manure	35.35	9.51	34.96**
Soil + manure	42.02	26.42	41.63**
60 day			
Soil (control)	0.59	0.65	–
Soil + sewage sludge	4.15	0.65	3.56
Soil + 50% sewage sludge + 50% manure	6.88	4.77	6.29
Soil + 75% sewage sludge + 25% manure	9.58	4.25	8.99
Soil + manure	2.31	1.26	1.72

** – highly significant difference between the number of cells in the control and their numbers in a given combination

The presence of pathogenic bacteria in the control soil without the addition of composts may cause controversy. However, it is not possible to exclude cases of accidental soil contamination prior to its collection for analyses by faces animals, of primarily of birds, living on the field from which the soil was collected (Tab. 13). Soil incubation with composts during the period of 60 days reduced numbers of *C. perfringens* bacteria but failed to eliminate them completely, irrespective of the experimental objective.

CONCLUSIONS

1. The composting of sewage sludge resulted in the reduction of counts of all the examined groups of microorganisms and total elimination of bacteria from the *Salmonella* sp. genus.
2. In conditions of the incubation of composts with the soil, there followed a decrease of the number of the studied microorganism groups with the exception of actinomycetes and *E. coli*
3. The results of the performed investigations revealed that the incorporation into the soil of composted sewage sludge can be treated as one of the ways of reducing the risk of occurrence of bacteriological-epidemiological hazards caused by the presence in them of *Clostridium perfringens*.

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