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Microbiological Control in Decontamination of Sludge from Wastewater Treatment Plant

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Abstract: Dynamics of the microbiological indicators of fresh sludge from wastewater treatment plants with a concentration of CaO, 10% and 20%, and ash, −30% and 50%, and treated with quicklime, ash, and microbial fertilizer for a 50-day period of composting were studied. The influence of temperature, water content, and oxygen on the development of microbes was established in laboratory conditions. Microbiological analysis included the determination of non-pathogenic (non-spore-forming bacteria, bacilli, actinomycetes, micromycetes, bacteria digesting mineral nitrogen), and pathogenic (*Salmonella*, *Listeria*, *Escherichia coli*, *Enterococcus*, *Clostridium perfringens*) microorganisms. Of the beneficial microflora in the sludge before treating, the amount of non-spore-forming bacteria was the highest, followed by bacilli and micromycetes. It was found that actinomycetes were absent in the untreated sludge. *Clostridium perfringens* occupied a major share in the composition of the pathogenic microflora, followed by *Escherichia coli*, *Enterococcus*, and *Listeria*. The best results for decontamination of the sludge were obtained by adding 20% quicklime and 50% ash. Alkalization of the sludge after treatment led to the destruction of pathogenic microflora but also reduced the number of beneficial microorganisms. The decrease in pH during the study period determined the redevelopment of pathogenic microflora. Combined variants with lime or ash and microbial fertilizer showed better results for the development of non-pathogenic microflora and the destruction of pathogens.

Keywords: sludge; microflora; liming; ash; microbial fertilizer

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1. Introduction

The management of sludge from wastewater treatment plants (WWTPs) in accordance with environmental principles is an extremely topical issue for the sustainable development of settlements. Its decision is directly related not only to the construction of wastewater treatment plants but also to the use of sludge as an alternative energy source (energy resource) and/or soil improver (raw material resource) [1].

In their use as a soil improver, decontamination and deworming of sludge from wastewater treatment plants is an extremely important pretreatment process. Sludge from wastewater treatment plants deactivated with quicklime can be used for agriculture purposes [2–4]. Pathogenic microorganisms and parasitic worm eggs can be spread by sediment [5,6]. Isolated in the sludge are pathogenic microorganisms such as: *Salmonella* sp., *Listeria* sp., *Escherichia coli*, *Campylobacter* sp., *Clostridium* sp., *Yersinia* sp., and others [4,7–10]. These microorganisms have a strong ability to constantly adapt to changes in the survival environment [11] and can be relatively resistant (especially spore-forming species such as *Clostridium perfringens*) to commonly used sludge stabilization methods [12]. According to a study by Dermendzhieva [3], in sludge from Bulgarian treatment plants, the number of fecal (coliforms, *Escherichia coli*) and intestinal pathogenic (Enterobacteriaceae, Salmonella) microorganisms was above the permissible norms in the Ordinance on the procedure for utilization of sewage sludge

through their use in agriculture [13], which makes them unusable for fertilization in agriculture without applying methods for their decontamination. Co-composting of sewage sludge with lime effectively reduces and even destroys pathogens in the sludge [14–16]. According to Wong and Fang [16], the addition of 0.63% lime before sludge composting slightly improved microbial activity from temperature rise and CO_2 release and did not significantly inhibit bacterial population and β-glucosidase, alkaline phosphatase, and dehydrogenase activity after 100 days of composting. These authors estimated the use of lime at <1.0% (w/w) for co-composting with sewage sludge. On the one hand, this concentration inhibited the development of beneficial groups of microorganisms in sludge to a small degree, but according to other authors [17], the most promising for a short period of time (about 1 month) is the decontamination of sludge by adding a 20% fine and 30% coarser fraction of quicklime. According to them, treatment with quicklime is particularly suitable for this purpose, as lime is not environmentally hazardous and is, at the same time, effective.

The removal of pathogenic microorganisms depends on the pH of the sludge, the period of liming, and the water content of the sludge [18]. The main role of liming is to sterilize the sludge from pathogenic microorganisms and parasites. The study by Santos et al. 2021 [19] confirmed that lime mud (LM) and calcined lime mud (CLM) can be used as drying additives and sanitizing agents for sewage sludge. According to these authors, the addition of calcined lime mud using a ratio of between 0.05 and 0.15 g CLM/gwb led to the complete elimination of microbiological contamination in almost all cases. Except for fertilization, the modified sludge with fly ash and loess satisfies the criteria for construction materials [20]. According to Jagaba et al. 2019 [21], incinerated sewage sludge ash (ISSA) can be used as a soil-stabilizing agent. These authors concluded that 7% ISSA additive effectively enhances the strength of soft soils. Some authors [22] have investigated the optimization of conditions for the production of activated carbon (chemical activation ratio, contact time, and activation temperature on the surface area of activated carbon) using sewage sludge as a raw material. According to Gheethi et al. 2018 [4], further treatment using technologies such as solar disinfection, air drying, and lime treatment of sewage effluents and biosolids generated from secondary treatment is necessary to reduce the pathogenic bacteria before reusing for agricultural purposes. Therefore, the usage of sludge in agriculture, construction, activated carbon production, and other purposes implies their decontamination by pathogenic microorganisms. Stabilization of sewage sludge is achieved by raising the pH values to create an alkaline environment that leads to a reduction in nutrient content [23]. In the treatment of sewage sludge with lime, Bina et al. [24] found that when the pH increased to 11–12, *Salmonella* was completely inactivated in the treated sludge after 2 hours, while the removal of up to 99% of fecal coliforms was obtained for two pH ranges. Lime treatment and pasteurization of sludge (50-day sanitation) are very effective methods for disinfection and creating safe sludge in terms of fecal coliform content [25]. In addition to liming and the use of ash, fertilization with microbial fertilizers also supports the development of beneficial microorganisms in substrates [26,27]. For the decontamination of sludge, it would be useful to study the impact of microbial fertilizers containing microorganisms with antimicrobial properties against pathogenic microbes in the sludge, as well as the detection of microorganisms that can accumulate and degrade heavy metals. Similarly, plant species can be studied that, in addition to having antimicrobial properties and accumulating heavy metals, can also be used to deodorize sludge.

The aim of the study was to conduct microbiological control using various methods (treatment with lime, ash, microbial fertilizer, freezing, and thermal effect alone or in combination) to decontaminate sewage sludge. The literature review presented above confirms the effectiveness of liming as a method for the decontamination of sludge, but the proposed methods differ depending on the concentration and size of the quicklime fraction, the treatment period, and the degree of destruction of pathogenic microorganisms. The use of ash [20,21] and microbial fertilizer for the decontamination of sludge are less studied. In

the present research, the analysis of microbiological indicators in dynamics for a period of 50 days after treatment with different ameliorants-alone, in combination, and under different temperature conditions-provides guidelines for creating an effective methodology based on the combined treatment (not only the independent use of ameliorants for a short period) of sewage sludge, compost, and organic waste. This process can reduce and completely destroy pathogenic microorganisms while monitoring the preservation of beneficial microorganisms in the treated substrates. Usually, the microbiological control of sewage sludge is monitored only for their decontamination for safe use; our study compared changes in the amount of non-pathogenic and pathogenic microflora after treatment, which is a prerequisite for the safe and effective use of sludge as a soil improver.

2. Materials and Methods

Sludge from municipal WWTP in Blagoevgrad was used. The wastewater treatment technology included mechanical and biological stages. The sludge underwent thickening, anaerobic stabilization in open digesters, dewatering in belt filter presses, and, finally, deposition to sludge drying beds. Sludge (initial indicators: pH = 6.23; water content 90%, t = 30 °C) obtained from the belt filter presses of the WWTP-Blagoevgrad was studied according to microbiological indicators, in dynamics, composted in greenhouse conditions (air temperature: t-day = 28–30 °C, t-night = 17–18 °C; air humidity: 50–60%), in plastic packages (containers) from 2 L, and treated with quicklime and ash in different concentrations. For some of the variants, fertilization with microbial fertilizer (Baikal EM-1) containing lactic acid and photosynthetic bacteria, nitrogen-fixing bacteria, and Saccharomyces was applied. Each package contained 100 g of dry matter, comprising: 10 g CaO ("Sludge + 10% CaO"), 20 g CaO ("Sludge + 20% CaO"), 30 g ash ("Sludge + 30% ash"), 50 g ash ("Sludge + 50% ash"), and 100 mL of diluted microbial fertilizer solution (for all variants with microbial fertilizer). In laboratory conditions, the sludge samples were placed in different temperatures and aerobic and anaerobic conditions for different periods of time before their analysis. The scheme of variants and the period of microbiological analysis are presented in Table 1a,b, and the composting of the variants in greenhouse conditions is illustrated in Figure 1.

Figure 1. Composted variants in greenhouse conditions.

Table 1. Scheme of variants and period of microbiological analysis.

Variants	Microbiological Analysis
1a: In Greenhouse Conditions	Before starting the experiment; 10 h after starting the experiment; 10th day after starting the experiment; 25th day after starting the experiment; 50th day after starting the experiment
Sludge without quicklime (CaO)	
Sludge + 10% CaO	
Sludge + 20% CaO	
Sludge + microbial fertilizer + 10% CaO	
Sludge + microbial fertilizer + 20% CaO	
Sludge + 30% ash	
Sludge + 50% ash	
Sludge + microbial fertilizer + 30% ash	
Sludge + microbial fertilizer + 50% ash	
Sludge + microbial fertilizer	
1b: In Laboratory Conditions	Microbiological Analysis
Sludge, +28 °C (aerobic cultivation)	Before starting the experiment, 24 h
Sludge, −4 °C (aerobic cultivation)	24 h
Sludge, −20 °C (aerobic cultivation)	3 h, 6 h, 24 h
Sludge, +70 °C (aerobic cultivation)	6 h, 12 h
Sludge, +28 °C (anaerobic cultivation)	7 days

Microbiological analyses were performed by the method of limiting dilutions, inoculating, and culturing the nutrient media [28]: non-pathogenic microflora: Nutrient Agar for non-spore-forming bacteria and bacilli, MRS Agar for lactobacilli, Actinomycetes Isolation Agar for actinomycetes and bacteria digesting mineral nitrogen, and Czapek Dox Agar for micromycetes; pathogenic microflora: Desoxycholate Citrate Agar for *Salmonella* sp., ChromoBio Listeria Agar for *Listeria* sp., Endo Agar for *Escherichia coli* and coliforms (oxidase confirmatory test), ChromoBio Enterococcus Agar for *Enterococcus*, and Perfringens Agar (TSC and Perfringens Selective Supplement) for *Clostridium perfringens*. A jar with a reagent to generate an anaerobic medium was used to isolate anaerobes. The results are presented as colony-forming units (CFU) recalculated per 1 g of substrate given the amount of inoculation and dilution used [29]. The microbiological analyses were performed in the microbiological laboratory of the University of Forestry, Sofia, Bulgaria, using chemical reagents supplied by Valerus Ltd., Sofia, Bulgaria, nutrient media for microbiological purposes supplied by Optim Co Ltd., Sofia, Bulgaria, and microbial fertilizer Baikal EM-1 supplied by Provision Ltd., Sofia, Bulgaria.

For observing the temperature of the sludge, a temperature probe (model Sv 218) was used for the determination of the water content, a moisture meter for the greenhouse (model Sv 218), and a moisture scale in the laboratory (model DBS 60-3). For the determination of pH (in water), a pH meter (model Portavo 902) was used.

3. Results and Discussion

Liming raised the pH values of the sludge to an alkaline environment: 12.65, "10% CaO"; 12.64, "10% CaO + microbial fertilizer"; 12.72, "20% CaO"; 12.74, "20% CaO + microbial fertilizer." The addition of ash decreased the pH to a smaller degree: 6.97, "30% ash"; 7.01, "30% ash + microbial fertilizer"; 7.85, "50% ash"; 7.56, "50% ash + microbial fertilizer." This trend was characteristic until the 10th day of the study, after which the pH values decreased to a neutral medium at the end of the experiment. The addition of microbial fertilizer lowered the pH of the sludge slightly (pH control = 6.23; pH sludge + microbial fertilizer = 6.12). The water content of the variants decreased from 90% at the beginning of

the experiment to about 40% at the end of the experiment. The temperature of the variants increased by more than 10 °C until the end of the experiment (40–42 °C). These changes in physicochemical parameters affected the microbiocenosis of sludge.

Microbiological analysis of fresh sludge from the treatment plant showed the presence of specific and non-specific (pathogenic) microflora. The number of non-pathogenic groups of microorganisms (in greenhouse conditions) is presented in Table 2.

Table 2. Quantity and qualitative composition of non-pathogenic microflora in raw sludge (cfu/g) in dynamics.

Variants	Before Starting	Day 1, 10 h	10th Day	25th Day	50th Day
In Greenhouse Conditions		Non-Spore-Forming Bacteria			
Sludge without quicklime (CaO)	2200	2100	2200	2140	2120
Sludge + 10% CaO		1700	1520	1580	1640
Sludge + 20% CaO		1480	1000	1120	1240
Sludge + microbial fertilizer + 10% CaO		1800	1640	1700	1780
Sludge + microbial fertilizer + 20% CaO		1550	1380	1460	1500
Sludge + 30% ash		1800	1900	1880	1860
Sludge + 50% ash		1600	1740	1700	1680
Sludge + microbial fertilizer + 30% ash		1900	2040	2080	2080
Sludge + microbial fertilizer + 50% ash		1850	2000	2080	2140
Sludge + microbial fertilizer		2240	2300	2320	2340
In Greenhouse Conditions		Bacilli/Lactobacilli			
Sludge without quicklime (CaO)	320	310	400	360	340
Sludge + 10% CaO		170	140	150	160
Sludge + 20% CaO		110	80	100	100
Sludge + microbial fertilizer + 10% CaO		200	160	180	180
Sludge + microbial fertilizer + 20% CaO		170	120	140	160
Sludge + 30% ash		190	260	240	220
Sludge + 50% ash		140	200	180	180
Sludge + microbial fertilizer + 30% ash		220	300	260	280
Sludge + microbial fertilizer + 50% ash		210	280	260	260
Sludge + microbial fertilizer		360	440	460	480
In Greenhouse Conditions		Actinomycetes			
Sludge without quicklime (CaO)	0	0	0	0	0
Sludge + 10% CaO		0	0	0	0
Sludge + 20% CaO		0	0	0	0
Sludge + microbial fertilizer + 10% CaO		0	0	0	0
Sludge + microbial fertilizer + 20% CaO		0	0	0	0
Sludge + 30% ash		0	0	0	0
Sludge + 50% ash		0	0	0	0
Sludge + microbial fertilizer + 30% ash		0	0	0	0
Sludge + microbial fertilizer + 50% ash		0	0	0	0
Sludge + microbial fertilizer		20	20	20	20

Table 2. *Cont.*

Variants	Before Starting	Day 1, 10 h	10th Day	25th Day	50th Day
In Greenhouse Conditions		Micromycetes			
Sludge without quicklime (CaO)	700	600	700	680	660
Sludge + 10% CaO		300	220	240	260
Sludge + 20% CaO		100	40	60	60
Sludge + microbial fertilizer + 10% CaO		400	340	360	380
Sludge + microbial fertilizer + 20% CaO		170	100	140	160
Sludge + 30% ash		400	480	440	440
Sludge + 50% ash		300	360	340	320
Sludge + microbial fertilizer + 30% ash		500	600	580	560
Sludge + microbial fertilizer + 50% ash		440	500	480	480
Sludge + microbial fertilizer		700	800	760	780
In Greenhouse Conditions		Bacteria, Digesting mineral nitrogen			
Sludge without quicklime (CaO)	1980	1920	2140	2120	2080
Sludge + 10% CaO		1780	1620	1680	1720
Sludge + 20% CaO		1500	1320	1360	1360
Sludge + microbial fertilizer + 10% CaO		1850	1740	1780	1800
Sludge + microbial fertilizer + 20% CaO		1580	1440	1480	1480
Sludge + 30% ash		1820	1920	1900	1880
Sludge + 50% ash		1700	1800	1780	1780
Sludge + microbial fertilizer + 30% ash		1900	2000	1960	1960
Sludge + microbial fertilizer + 50% ash		1820	1900	1880	1860
Sludge + microbial fertilizer		2060	2140	2120	2100

The amount of non-spore-forming bacteria and bacteria digesting mineral nitrogen was the highest, micromycetes and bacilli were less represented, and actinomycetes were absent in the studied sludge (except for the sample in which microbiological fertilizer was added). Liming increased the pH values of the sludge (alkaline medium to a pH of about 12), which led to a decrease in the number of non-pathogenic groups of microorganisms studied. This trend was characteristic until the 10th day of the study, after which the number of microbes slowly began to increase, with decreasing pH values over time. The addition of ash led to an increase in the biogenicity of the studied sludge (to a greater extent by the 10th day of the study), which was associated with increased activity of microorganisms that degrade carbon-containing organic compounds. A similar trend was found in the fertilization with microbial fertilizer, as the effect of the preparation increased with the extension of the study period. The trends depended on the added concentrations of quicklime and ash; in the samples with 20% CaO, the development of microorganisms was suppressed to a greater extent than in those with 10% CaO. The variant with 30% ash showed better results than the one with 50% ash, probably because the short-term addition of a high amount of carbon-containing ameliorant inhibited the growth of microorganisms to a greater extent. On the other hand, reducing the water content of the sludge increased the number of microorganisms.

Liming had a more limiting effect on the development of non-pathogenic groups of microorganisms compared with the addition of ash, reduction in water content (from 90% to 40%), and drying of sludge after increasing the temperature by about 10 °C at the end of the experiment. Combined variants with lime and microbial fertilizer, as well as

ash with microbial fertilizer, showed better results for maintaining the development of non-pathogenic microflora compared with the use of lime or ash alone.

Pathogenic microorganisms were found in the studied variants: *Escherichia coli* and coliforms, *Enterococcus*, *Clostridium perfringens*, *Listeria* sp.; *Salmonella* sp. was absent (Table 3).

Table 3. Quantity and qualitative composition of pathogenic microflora in raw sludge (cfu/g) in dynamics.

Variants	Before Starting	Day 1, 10 h	10th Day	25th Day	50th Day
In Greenhouse Conditions		*Escherichia coli* and Coliforms			
Sludge without quicklime (CaO)	2400	2400	2380	2320	2300
Sludge + 10% CaO		1200	620	800	1020
Sludge + 20% CaO		0	0	60	160
Sludge + microbial fertilizer + 10% CaO		1000	540	600	900
Sludge + microbial fertilizer + 20% CaO		0	0	20	40
Sludge + 30% ash		1500	1040	1220	1380
Sludge + 50% ash		800	600	660	680
Sludge + microbial fertilizer + 30% ash		820	700	740	780
Sludge + microbial fertilizer + 50% ash		500	320	380	400
Sludge + microbial fertilizer		1050	920	900	840
In Greenhouse Conditions		*Enterococcus* sp.			
Sludge without quicklime (CaO)	2100	2100	2000	1960	1920
Sludge + 10% CaO		1000	540	680	800
Sludge + 20% CaO		0	0	80	180
Sludge + microbial fertilizer + 10% CaO		880	480	500	560
Sludge + microbial fertilizer + 20% CaO		0	0	40	40
Sludge + 30% ash		1200	960	980	1000
Sludge + 50% ash		600	440	480	500
Sludge + microbial fertilizer + 30% ash		700	540	560	580
Sludge + microbial fertilizer + 50% ash		340	200	240	280
Sludge + microbial fertilizer		800	680	660	660
In Greenhouse Conditions		*Salmonella* sp.			
Sludge without quicklime (CaO)	0	0	0	0	0
Sludge + 10% CaO		0	0	0	0
Sludge + 20% CaO		0	0	0	0
Sludge + microbial fertilizer + 10% CaO		0	0	0	0
Sludge + microbial fertilizer + 20% CaO		0	0	0	0
Sludge + 30% ash		0	0	0	0
Sludge + 50% ash		0	0	0	0
Sludge + microbial fertilizer + 30% ash		0	0	0	0
Sludge + microbial fertilizer + 50% ash		0	0	0	0
Sludge + microbial fertilizer		0	0	0	0

Table 3. *Cont.*

Variants	Before Starting	Day 1, 10 h	10th Day	25th Day	50th Day
In Greenhouse Conditions		*Listeria* sp.			
Sludge without quicklime (CaO)	2000	2000	1960	1940	1920
Sludge + 10% CaO		0	0	40	40
Sludge + 20% CaO		0	0	0	40
Sludge + microbial fertilizer + 10% CaO		0	0	20	20
Sludge + microbial fertilizer + 20% CaO		0	0	0	20
Sludge + 30% ash		0	0	40	60
Sludge + 50% ash		0	0	20	40
Sludge + microbial fertilizer + 30% ash		0	0	20	40
Sludge + microbial fertilizer + 50% ash		0	0	20	20
Sludge + microbial fertilizer		900	800	700	620
In Greenhouse Conditions		*Clostridium perfringens*			
Sludge without quicklime (CaO)	4000	4000	4020	4060	4080
Sludge + 10% CaO		2000	1620	1700	1720
Sludge + 20% CaO		0	0	80	120
Sludge + microbial fertilizer + 10% CaO		400	300	320	360
Sludge + microbial fertilizer + 20% CaO		0	0	60	100
Sludge + 30% ash		3000	2720	2760	2800
Sludge + 50% ash		1800	1600	1640	1680
Sludge + microbial fertilizer + 30% ash		2600	1800	1900	2100
Sludge + microbial fertilizer + 50% ash		200	120	140	160
Sludge + microbial fertilizer		4000	4000	4000	4000

Before the start of the experiment, regarding the composition of pathogenic species in the sludge, the incidence of Clostridium perfringens (4000 cfu/g) was the highest, followed by *Escherichia coli* and coliforms (2400 cfu/g), *Enterococcus* (2100 cfu/g), and *Listeria* sp. (2000 cfu/g). It was found that *Salmonella* sp. was absent. *Clostridium perfringens* is a spore-forming species that makes it difficult to destroy. Liming led to a reduction in the number of pathogenic microbes (in all variants with liming) and their complete destruction by the 10th day of the experiment in the variants with 20% lime. Marinova et al. [17] also found the best decontamination of sewage sludge is with the addition of 20% and 30% quicklime. According to that study by these authors, *Clostridium perfringens* was destroyed in the indicated concentrations within 31 days after mixing the sludge with fine quicklime. The results of their research confirmed that in order to obtain an epidemiologically safe sludge for a month or less, it must undergo further treatment. The same authors found that the size of quicklime also has a different effect on the development of microorganisms-the larger the fraction is, the more difficult it is for it to homogenize with the sludge, so it is necessary to use a higher rate. According to Santos et al. 2021 [19], the addition of calcined lime mud (CLM) using a ratio of between 0.05 and 0.15 g CLM/gwb led to the complete elimination of microbiological contamination in almost all cases. Conversely, the use of lime mud (LM) did not seem to act effectively as a sanitary agent. Both LM and CLM showed a positive effect on the drying process compared with raw sludge, increasing the drying speed and reducing the drying time. Therefore, the creation of an alkaline environment is a method of purifying the sludge from pathogenic microorganisms. However, lowering the pH values (i.e., less alkaline environment, day 25; neutral environment, day 50) led to the redevelopment of pathogenic microorganisms, to a greater extent for *Clostridium perfringens*. The addition of 20% lime showed better results for the destruction of pathogenic microorganisms compared with the

variants with 10% CaO. In terms of effectiveness, the 50% ash variants followed, which showed results that were almost twice for pathogen eradication compared with the addition of 30% ash. The combined variants—lime and microbial fertilizer and ash with microbial fertilizer—showed better results compared with the independent use of lime and ash. This trend provides grounds to use the combined variants with microbial fertilizer for two reasons: reducing the destruction of pathogens and activating the development of non-pathogenic microflora. Reducing the water content and drying the sludge had a significantly lower effect than liming, which is indicative of the fact that in the non-liming variants, the results remain close for the entire study period. Environmental conditions such as the active reaction of sludge, salt content, heavy metal content, and temperature can lead to changes in the course of microbial activity in the substrate and, consequently, affect the reproductive capacity of microorganisms [7]. The pathogen microorganisms studied have a strong ability to adapt continuously to changes in the survival environment [11] and may be relatively resistant (especially spore-forming species such as *Clostridium perfringens*) to commonly used sludge stabilization methods [19]. Other scientists have also found a positive effect on the decontamination of sewage sludge by applying lime [1,10,12,15,24]. On the one hand, for the complete destruction and prevention of the redevelopment of the pathogenic microflora, additional liming was needed after the 10th day to preserve the alkaline environment, but on the other hand, this reduced the number of beneficial microorganisms. Incinerated sewage sludge ash (ISSA) can be used as a soil-stabilizing agent; 7% ISSA additive can effectively enhance the strength of soft soils [21]. It is, therefore, necessary to combine liming with the addition of ash, microbial fertilizers, suitable plant species with bactericidal and deodorizing action, and other methods in the process of monitoring the development of microflora in sludge in dynamics. According to Gheethi et al. 2018 [4], further treatment using technologies such as solar disinfection, air drying, and lime treatment of sewage effluents and biosolids generated from secondary treatment is necessary to reduce the pathogenic bacteria before reusing for agricultural purposes.

The influence of temperature and oxygen on the development of pathogenic and non-pathogenic microflora was observed in laboratory conditions (Figures 2 and 3).

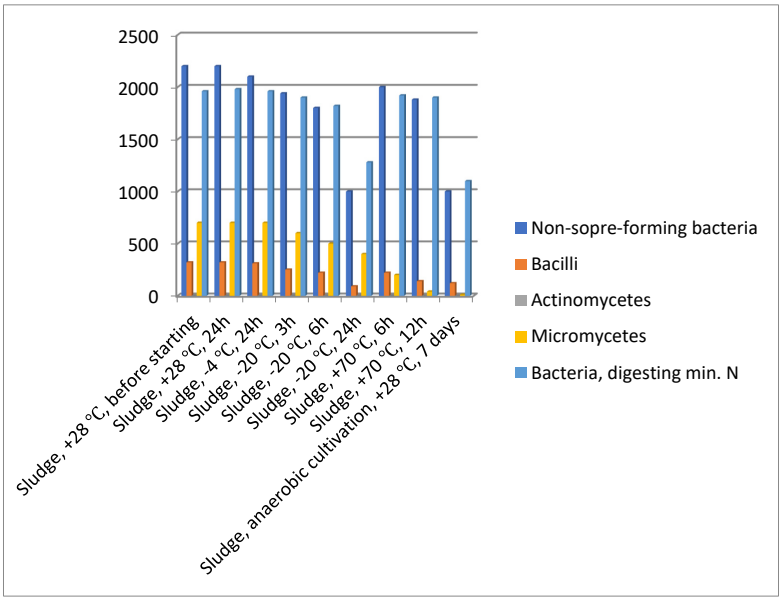

Figure 2. Non-pathogenic microflora (cfu/g).

At negative and higher positive (70 °C) temperatures, mesophilic microorganisms stopped their development and activity, and psychrophiles and thermophiles (which are less than mesophiles) actively developed in samples cultured at 28 °C. Thermal treatment (100 and 130 °C) without adjuvants reduced microbiological contamination in the sewage sludge [19]. By lowering the temperature to −20 °C and prolonging the cultivation time, the

number of microbes decreased. An exception to this trend was found for *Clostridium;* their amount was lower at −20 °C for 6 h compared with the same temperature for 24 h, which is explained by their mechanisms for adaptation to environmental conditions. Anaerobic microorganisms were fewer than aerobic ones, with the exception of *Clostridium perfringens*, which are anaerobes, and lactobacilli, which are microaerophiles. Placing the samples at extreme temperatures and in an oxygen-free environment before analyzing reduces the development of pathogens but does not destroy them. The amount of *Clostridium perfringens* depends least on the influence of extreme temperature conditions, as they are spore-forming and can better adapt and survive changes.

Pictures of some isolated microorganisms in the sludge are presented in Figure 4.

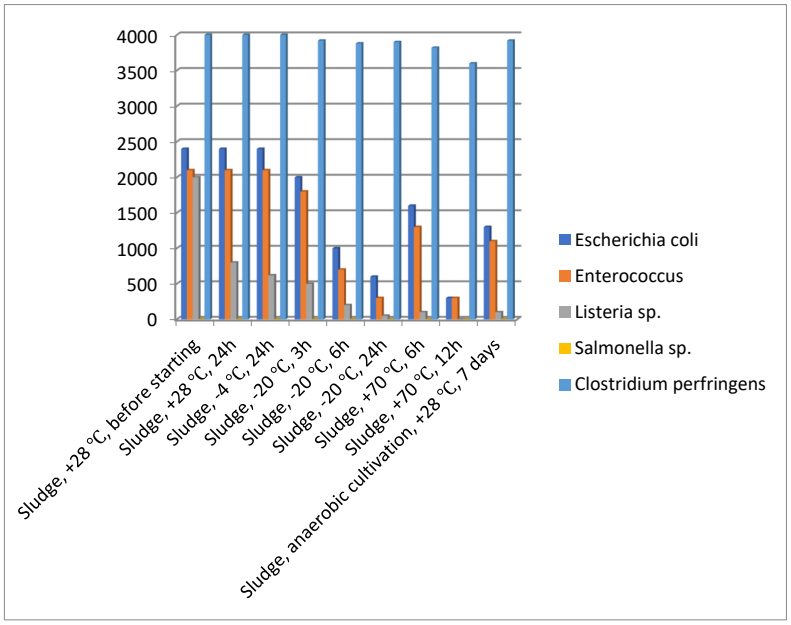

Figure 3. Pathogenic microflora (cfu/g).

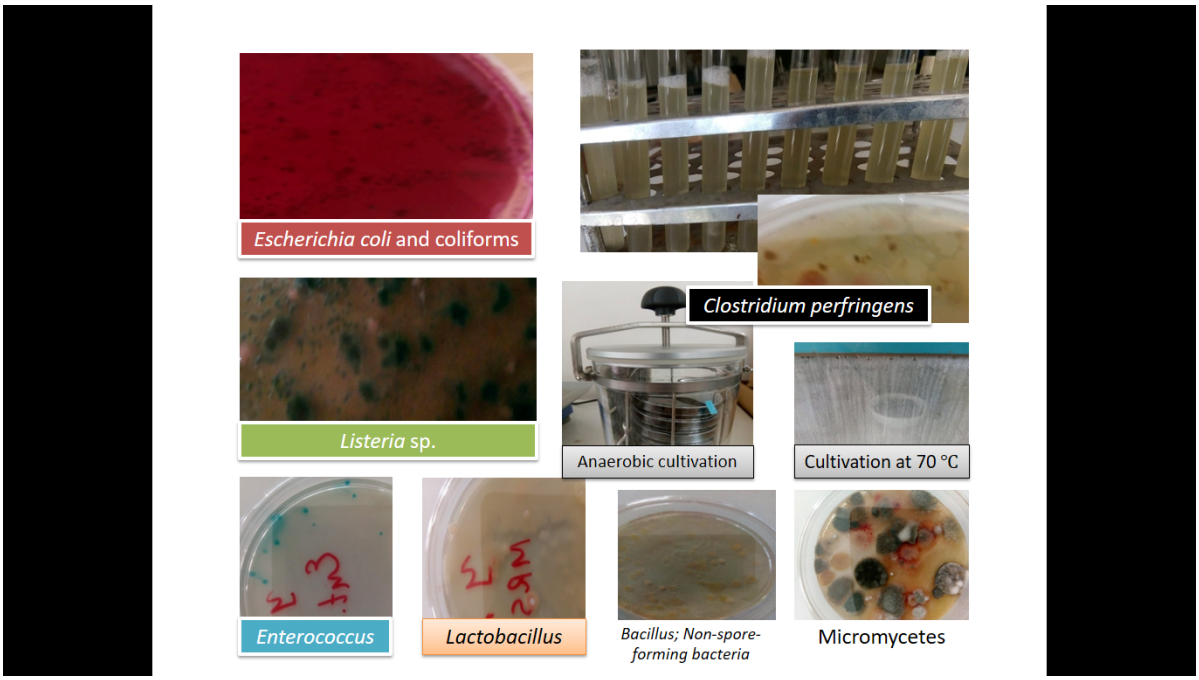

Figure 4. Some isolated microorganisms in the composted variants.

4. Conclusions

The dynamics of non-pathogenic and pathogenic microorganisms were studied using sludge from the treatment plant after decontamination with quicklime and ash, in different concentrations and for a period of 50 days. Of the beneficial microflora in the sludge, the highest was the number of non-spore-forming bacteria and bacteria digesting mineral nitrogen, followed by bacilli and micromycetes; actinomycetes were absent. *Clostridium perfringens* had a major share in the composition of the pathogenic microflora, followed by *Escherichia coli* and coliforms, *Enterococcus*, and *Listeria* sp. It was found that *Salmonella* sp. was absent in the studied sludge.

The best results for the decontamination of sludge were given by the addition of 20% quicklime (10th day): complete destruction. At the same time, however, the creation of an alkaline environment led to a reduction in the number of beneficial microorganisms. On the one hand, additional liming was needed after the 10th day to completely destroy and prevent the regrowth of pathogenic microbes, and on the other hand, this probably led to a greater reduction in beneficial microorganisms. The decrease in water content and drying of the sludge during the study period showed a weaker effect on the dynamics of both pathogenic and non-pathogenic microorganisms compared with the effect of increasing pH.

The addition of ash reduced the pathogenic microflora but not their complete destruction. Better results in terms of pathogens destruction were obtained using 50% ash compared with 30%. In terms of non-pathogenic microflora, the 30% ash variant gave better results than the one with 50% ash; probably the short-term addition of a high amount of carbon-containing ameliorant initially inhibited the development of microorganisms to some extent. The combined variants showed better results for the development of non-pathogenic microflora and the destruction of pathogens compared with the use of only one ameliorant.

Cultivation at sub-zero and higher (70 °C) temperatures inactivated the development of mesophilic microorganisms, determined the formation of cultures of psychrophiles and thermophiles, which are less than mesophiles, and actively developed in samples cultured at 28 °C. By lowering the temperature to −20 °C and prolonging the cultivation time, the number of microbes decreased to a greater extent. An exception to this trend was found for *Clostridium*, which is spore-forming and survives better in extreme conditions.

The results show that the addition of ameliorants such as lime, ash, and microbial fertilizers, the creation of acidic aerobic and anaerobic environments, and the use of different cultivation temperatures are factors that can be used to reduce or destroy pathogenic microorganisms in sewage sludge, compost, and organic waste. Long-term research and a multifactorial approach are needed to identify suitable combined variants for both the complete decontamination of sludge and the preservation of beneficial microflora in order for sludge to be used safely as organic fertilizer in agriculture.

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