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OCCURRENCE OF BACTERIA *SALMONELLA* SP. IN SEWAGE SLUDGE USED IN AGRICULTURE

Sewage sludge is characterized by good fertilizer properties and contains significant amount of nutrients and organic matter subject to humification in soil. Therefore, sludge has been effectively spread in order to recultivate degraded areas or to replace humus material in green areas of towns, or to lay lawns by the owners of private houses. The sludge used for agricultural purposes has to meet certain chemical and biological criteria. This study examined the occurrence frequency of bacteria *Salmonella* in sewage sludge from eight wastewater treatment plants between 1999 and 2007. The results demonstrated that the occurrence frequency of bacteria *Salmonella* in the sewage sludge strongly depended on the technology used in the treatment plant.

1. INTRODUCTION

Sewage sludge contains large amounts of nutrients and organic matter, which undergoes humification in soil. Therefore, sludge is readily used in recultivation of degraded areas, as a soil replacement in parks and other green areas in towns, and in private gardens for laying lawns [1]. However its agricultural utilization is associated with a considerable risk of introducing into environment chemical residues, primarily heavy metals, and contaminating soil with pathogenic microorganisms. The whole range of such organisms can be found in sludge: both intestinal pathogens and pathogenic bacteria, such as streptococci, staphylococci, *Clostridium*, *Bacillus*, *Listeria*, *Shigella*, *Yersinia*, *Salmonella* and many others. Because of this a sludge utlized in agriculture has to satisfy certain chemical and biological criteria.

Typically, biological criteria are associated with the occurrence of the bacteria belonging to the genus of *Salmonella* and the eggs of such intestinal parasites as *Ascaris lumbricoides, Trichocephalus trichiurus* and *Toxocara* sp. According to current Polish regulations [2], these organisms cannot be present in 100 g of sludge intended

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for agricultural applications. Similar regulations have been applied in other European countries and in the USA, but specific criteria may vary [3].

These organisms were probably selected because of a "raw material" from which the sludge is composed. In this case, a raw material primarily consists of Waste Activated Sludge (WAS) that contains a microbial population, primarily bacteria, forming flocs. These bacteria occur in sewage, i.e. in faeces flowing into the treatment plant. Therefore, it is probable or even certain that both *Salmonella* and eggs of intestinal parasites reach the treatment facility. Sewage treatment technology based on activated sludge process reduces the number of bacteria, including these from the family *Enterobacteriaceae*, by 95–99% [4]. In spite of such a significant reduction in the number of bacteria, microbes of the genus *Salmonella* and the eggs of intestinal parasites are still found in WAS. Therefore, after dewatering, sludge must undergo stabilization in order to eliminate pathogenic organisms and to make it suitable for usage as a biocomponent. The method selected for sludge stabilization (sedimentation, aerobic mesophilic stabilization, anaerobic thermophilic stabilization, composting, or combination of several methods) affects to the greatest extent the total reduction of pathogenic microorganisms [5]. The sedimentation and seasoning processes alone were already found to be entirely insufficient because pathogens were detected in treated sludge even after one year [5], [6]. In contrast, composting may effectively eliminate pathogenic organisms from sludge, provided that the process is carried out at increased temperature maintained throughout a specific period of time [7], [8].

This study examined the occurrence of the bacteria from genus *Salmonella* in sewage sludge stabilized by various methods.

2. MATERIALS AND METHODS

2.1. OBJECT OF SURVEY

Stabilized sewage sludge collected over a period from 1999 to 2007 from eight wastewater treatment plants was subjected to analysis. Sludge was produced in treatment facilities employing different methods of its stabilization and located in towns with different population sizes (the table). A total of 98 sludge samples were analyzed. These samples were collected from several locations (3–5) in the sludge stockpile of a given facility, then they were mixed, and finally ca. 500 g of the mixture obtained were transferred to sterile containers. Sludge samples were transported in insulated containers, in which the temperature did not exceed 7 °C. The analyses were conducted no later than 24 hours after samples delivery to laboratory. Prior to analyses, sludge was refrigerated.

Table

Technology used to stabilize sewage sludge in treatment plants

Town and number of residents		Technology
Toruń,	>200 000	T UnOx + C
Grudziądz,	50 000–200 000	T UnOx + C
Iława,	50 000–200 000	T UnOx + C
Golub Dobrzyń,	< 50 000	M Ox + C
Lubawa,	< 50 000	M Ox + C
Susz,	< 50 000	M Ox
Unisław,	< 50 000	M Ox + C
Płużnica,	< 50 000	M Ox + C

T – termophilic, M – mesophilic, UnOx – unaerobic, Ox – aerobic, C – composting.

2.2. ISOLATION AND IDENTIFICATION OF BACTERIA FROM SPECIES *SALMONELLA*

The tests for the presence of bacteria *Salmonella* were carried out according to the methodology described in the directive of the Ministry of Environmental Protection from August 1st, 2002 [2]. The analyses were conducted in three replications; each time, a 100 g sample of sludge was analyzed. The test procedure included: non-selective growth in peptone water (composition per 1 dm^3: casein peptone – 10.00 g; K_2HPO_4 – 1.50 g; Na_2HPO_4 – 3.50 g; NaCl – 5.00 g), incubation at 37 °C for 16–24 h. Then 10 cm^3 of the non-selective suspension obtained were transferred to 100 cm^3 of selective medium with acidic sodium selenite (composition per 1 dm^3: casein peptone – 5.00 g; lactose – 4.00 g; sodium selenite – 4.00 g; $NaHPO_4$ – 1.00 g; Na_2HPO_4 – 10.00 g), and 1 cm^3 of non-selective suspension was transferred to 10 cm^3 of MKTTn selective medium (composition per 1 dm^3: beef broth – 4.30 g; casein peptone – 8.60 g; beef bile – 4.78 g; $CaCO_3$ – 38.70 g; sodium thiosulfate – 47.80 g; NaCl – 2.60 g; Novobiocine – 0.04 g; brilliant green – 0.0096 g). The selective multiplication on these media was conducted at 37 °C for 24–48 h. After this time, the solid growth media such as BGA and XLT4 agar were inoculated with the selective colonies of *Salmonella*. The composition of media per 1 dm^3 was as follows: BSA (yeast extract – 3.00 g; peptone – 10.00 g; saccharose – 10.00 g; lactose – 10.00 g; NaCl – 5.00 g; phenol red – 0.08 g; brilliant green – 0.0125 g; agar – 15.00 g) and XLT4 agar (peptone – 1.60 g; yeast extract – 3.00 g; L-lysine – 5.00 g; lactose – 7.50 g; xylose – 3.75 g; saccharose – 7.50 g; ammonium iron(III) citrate – 0.80 g; sodium thiosulfate – 6.80 g; phenol red – 0.08 g; NaCl – 5.00 g; agar – 18.00 g; tergitol 4 – 4.60 cm^3, 37 °C, for 24–48 h). When characteristic colonies were found, polyvalent serological latex tests (Micrgen) were conducted to confirm the presence of bacteria *Salmonella*.

2.3. STATISTICAL ANALYSES

Statistical analyses were carried out based on the program STATISTICA 6.0. The analysis of Variance (ANOVA) was the primary statistical method used in calculations. The occurrence of *Salmonella* was analyzed with regard to the number of people served by a treatment plant, a season, and a technology used in sludge stabilization.

3. RESULTS AND DISCUSSION

The sludge examined in this study was produced at treatment plants whose operation is based on two different technologies: aerobic mesophilic stabilization and anaerobic thermophilic stabilization (the table). As shown in this study, stabilization carried out under aerobic and mesophilic conditions failed to produce satisfying results. In the sludge treated in such a way, *Salmonella* occurred in 33.33% of the samples analyzed (figure 3). Furthermore, GANZER et al. [8] proved that this method of stabilization was responsible for a common occurrence of these bacteria in treated sludge. It is also evident from the above study that *Salmonella* was found relatively often in sludge stabilized with the aerobic thermophilic method. This finding is quite surprising, which was also pointed out by the authors themselves, because *Salmonella* is characterized by relatively low resistance to high temperature. Typically, during the thermophilic stabilization at 55°C, these bacteria are completely eradicated within 24 hours [9], [11], [12]. However, numerous studies point out that secondary contamination of sludge by bacteria *Salmonella* is possible [9], [12], [13]. This problem is particularly common during composting the sludge amended with plant waste, such as sawdust or straw, which introduce an additional amount of sugars, and thus stimulate the development of intestinal bacilli [6], [12]. The advantages of thermal anaerobic sludge stabilization were also demonstrated in our studies. Both thermal and anaerobic sludge stabilization efficiently reduced the bacteria *Salmonella*, and the number of samples with *Salmonella* were around 7% (figure 3). Similar results were reported by SAHLSTRÖM et al. [13], WATANABE et al. [14] and WAGNER et al. [15] These authors demonstrated that *Salmonella* was efficiently eliminated (< 10% of samples contained these bacteria) during the thermal anaerobic stabilization of sludge. In contrast, the percentage of samples with *Salmonella* increased substantially (> 70%) if the stabilization was carried out under aerobic conditions.

The number of people that produced sewage had no significant impact on the occurrence of *Salmonella* in sewage sludge (figure 1). As shown by SAHLSTRÖM et al. [13], the abundance of these bacteria in fresh sludge always ranges from 10^2 to 10^3 cells per gram, independently of the number of residents [16]. This resulted from the fact that even the smallest treatment plants collect sewage from at least several thousand residents. Hence, some of them have to be unwitting carriers of bacteria, including those of the genus *Salmonella*. Indeed, an increase in the size of population, from which sewage is collected, results in an increased number of carriers, but at the same time, the number of non-carriers also grows. In consequence, this ratio remains relatively stable, and bacteria *Salmonella* are always found in both raw sewage and fresh sludge [17]. In spite of the lack of no statistically significant differences, the results presented in figure 1 showed considerable differences in the occurrence of *Salmonella* between the populations of various sizes. It is most probable that these differences arise from the fact that small plants use exclusively the aerobic mesophilic method of sludge stabilization (the table). In contrast, large treatment facilities universally employ anaerobic thermophilic stabilization methods (methane fermentation and composting of fermentation residue) [18].

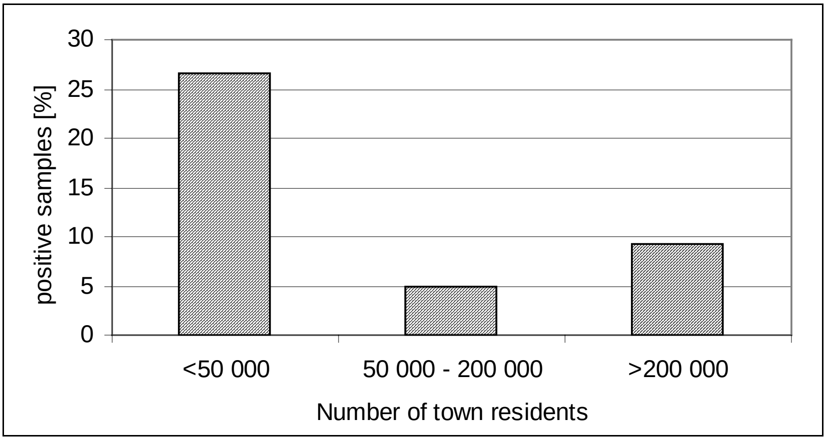

Fig. 1. The impact of the number of residents served by treatment plant on the occurrence of *Salmonella* sp. in sewage sludge. The differences are statistically not significant ($P > 0.05$)

Our results prove that the occurrence of bacteria *Salmonella* in sewage sludge is also affected by the season of collecting the samples (figure 2). Also GANZER et al. [9] confirm that the season in which samples are collected affects the occurrence of *Salmonella*. According to their study, *Salmonella* is most common in the samples collected in late winter and spring. GANZER et al. [8] explain this result by lower temperature of stabilization, since the system is obviously cooled by cold atmospheric air. However, statistically significant results of this study show that bacteria *Salmonella* were most frequently found (25%) in sludge samples collected in autumn (figure 2). During spring and summer, the frequency of the occurrence of *Salmonella* was similar (7–10%). However, in the samples collected in winter, these bacteria were not found at all in the entire period of the study (1999–2007). This result is quite surprising because in summer, higher temperature should have accelerated eradication of *Salmo-*

nella regardless of the technology used. A detailed data analysis revealed, however, that the vast majority of the samples collected in "summer" were obtained in last days of June. For this reason, the stabilization temperature may not have reached its maximum and possibly that is why a large part of the sludge samples subjected to analysis contained bacteria *Salmonella*. Still, the results of the tests for the presence of *Salmonella* from the seasonal perspective seem puzzling and require a further detailed study.

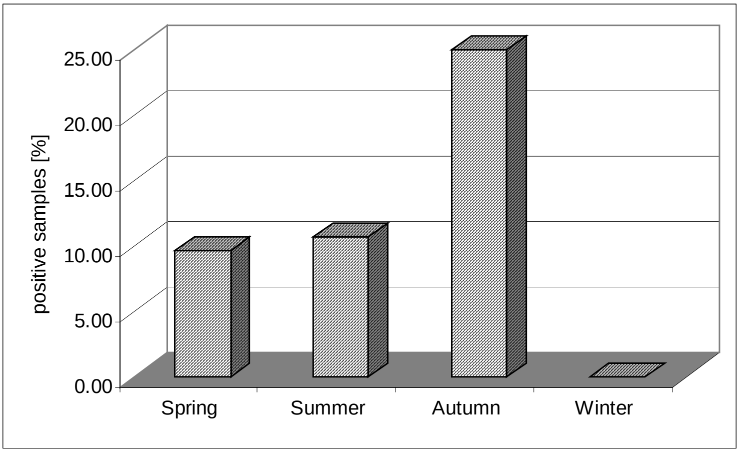

Fig. 2. Relations between season and the occurrence of *Salmonella* sp. in sewage sludge. The difference between autumn and other seasons is statistically significant ($P < 0.05$)

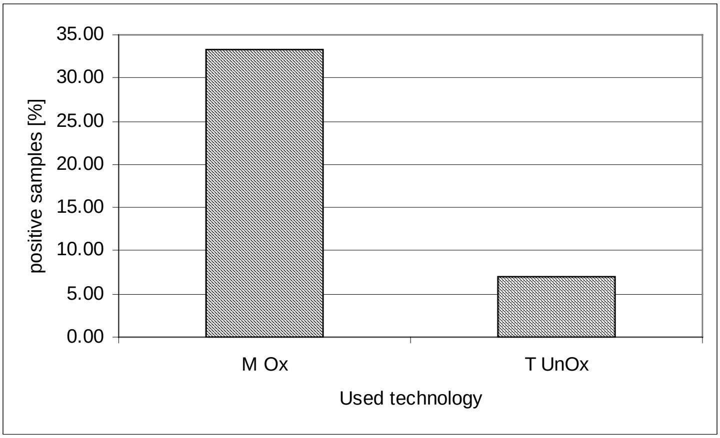

Fig. 3. Relation between the technology used to stabilize sewage sludge and the occurrence of *Salmonella* sp. The differences are statistically significant ($P < 0.05$).
M – mesophilic, T – termophilic, Ox – aerobic conditions, UnOx – unaerobic conditions

To sum up, it can be concluded, based on the results presented, that the technology used, and particularly temperature, affects most seriously the occurrence of *Salmonella* in sewage sludge.

4. CONCLUSIONS

Sewage sludge is characterized by good fertilizer properties as it contains large amounts of nutrients and organic matter; the latter undergoes humification in soil. Therefore, sludge is readily used in recultivation of degraded areas, as a soil replacement in parks and other green areas in towns, and in private gardens for laying lawns. The sludge applied for agriculture has, however, to meet certain chemical and biological criteria.

This study examined the occurrence frequency of the bacteria of the genus *Salmonella* in sewage sludge from eight wastewater treatment plants between 1999 and 2007. The results obtained showed that the occurrence frequency of *Salmonella* in sewage sludge was largely affected by a treatment technology used by a given plant.

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