Chapter 16
Microbiological Assessment of Sewage Sludge in Terms of Use as a Fertilizer

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Abstract Sewage sludge from wastewater treatment plant was examined for the existence of microorganisms of several major groups such as Gram-negative aerobic bacteria, *E. coli*, *Clostridium perfringens*, the genera *Pseudomonas*, *Staphylococcus*, *Enterococcus*, fungi, and the total number of microorganisms, in order to assess the epizootiological safety of the final product. In parallel similar study was made of composted cattle manure and a comparison of the results was made with the ready for manuring compost in order to assess the possibilities for the use of sludge for fertilizing. It was found that the examined sewage sludge were rich in microorganisms from studied groups and their direct application in soils without prior treatment is not recommended of epizootiological point of view. Methods of processing of sewage sludge should be consistent with epidemiological safety of the final products, but also be aimed at reducing the pollutants' emissions released during the cycle of wastewater sludge, which contribute to global warming.

Keywords Sewage sludge · Cattle manure · Microflora · Epizootological assessment

1 Introduction

In the wastewater treatment plants, waste domestic and industrial waters, those of livestock, natural waters and others for purification enter. The aim is to obtain environmentally safe wastewater and solid wastes (treated sludge) suitable for

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disposal or for reuse in soils as a fertilizer. For treatment of wastewater sludge, anaerobic digestion in bioreactors is usually applied in installations with larger scale, while aerobic decomposition is suitable for small stations [1–3]. In the presence of oxygen, the bacteria process waste solid substances and produce a substantial amount of heat, which is an important factor for the decontamination of the final product. Then the sludge is concentrated through dehydration to reduce the transport volume. The removed fluid is usually brought back into the wastewater process cycle, and the concentrated product most often is applied in soil as fertilizer [2, 4, 5].

According to Department for Environment, Food and Rural Affairs of UK [6] sewage sludge is a by-product of the wastewater treatment process that can be used on farms. Treated sewage sludge (bio solids) contains nutrients and valuable trace elements essential to plants. It is a more efficient and sustainable alternative to inorganic and mineral fertilizers as it provides a source of slow-release nitrogen ideal for use in land restoration and also is a good substitute for peat for land reclamation and thus conserving valuable natural peat land.

In the recent decades more and more wastewater is treated to reduce the outlet of harmful substances and xenobiotics to the environment and this leads to increasing production of sewage sludge. To avoid excessive fertilization and prevent accumulation of contaminants, the use of sewage sludge must be regulated and controlled [7]. Agricultural use of sewage sludge also helps to reduce the use of mineral fertilizers, which are a limited resource, produced under large energy consumption. The ecological consequences of sewage sludge amendment are not fully assess, but the use of sludge as organic fertilizers in agriculture will not have long-term damaging impact on the overall soil quality of arable land. Long-term observations are needed to assess the biological alterations as a result of sewage sludge amendment [7].

In an environmental aspect it must also be borne in mind that the pollutants' emissions released during the whole treatment life cycle of wastewater sludge contribute to global warming. In this respect Houillon and Jolliet [8] had compared six wastewater sludge treatment methods: agricultural spreading, fluidised bed incineration, wet oxidation, pyrolysis, incineration in cement kilns, and landfill. For global warming, incineration in cement kilns has the best balance; landfill and agricultural spreading the worst, while incineration and agricultural spreading have the lowest nonrenewable primary energy consumption.

Organic substances, deposited in soils, contain large amounts of carbon, whose future dynamics can affect climate change and the trend toward global warming. According to Kirschbaum [9] the content of organic carbon in soil may decrease greatly with global warming and thereby providing a positive feedback in the carbon cycle, because cooler soils contain greater amounts of organic carbon. The most often used methods in Japan for sewage sludge treatment are dewatering, composting, drying, incineration, incinerated ash melting, and dewatered sludge melting, each with or without digestion. Other methods used are: landfilling, agricultural application, and building material application. Hong et al. [10] reported that among these approaches sewage sludge digestion can reduce the environmental load and cost through reduced dry matter volume. In China the commonly used sewage sludge treatment techniques are composting, co-combustion in power plant, thermal drying incineration, and cement manufacturing. Dong et al. [11] proved that the anaerobic digestion is an effective pretreatment approach to reduce environmental burden, while the composting poses a positive effect to mitigate global warming, but it introduces high heavy metals to the soil.

At incomplete decontamination, however, the applying of sewage sludge in agriculture can be a source of biological contamination of soil, water, groundwater, and plants, including with pathogenic microorganisms. It is important to avoid such infection risks of consumers of crops (humans and animals). That is why, the monitoring and the evaluation of these risks are important to the search for environmentally favorable decisions [1, 4, 12]. Sewage sludge is the main source of mould emission into the air at workplaces in wastewater treatment plants and it is a reason for infectious and allergic diseases [13].

The aim of this study was to perform microbiological assessment of sludges of final purification steps in an urban sewage treatment plant in terms of their epizootiological safety in comparison with that of matured bovine compost.

2 Materials and Methods

- **Samples** from different stages of rehash in urban wastewater treatment plant near Sofia were examined. The materials were indicated as follows:—secondary sludge (SS) with 0.90 % dry matter (DM) and pH 7.52;—dewatered by belt filter presses mixed sludge (DMS) with 41.81 % DM and pH 7.56;—stayed mixed sludge (SMS) with 56.46 % DM and pH 6.63;—input into the digester (methane tank) (ID) with 2.96 % DM and pH 7.22.
- **Cattle manure**. Composted for 6 weeks cattle manure (CCM) of dairy cows was examined and used as a comparative control in the microbiological studies. Its dry substance was 52.20 % and pH value—7.20.
- **Microbiological studies** were conducted in accordance with the ordinance on the terms and conditions for use of sludge from wastewater treatment by its use in agriculture in Bulgaria [14]. In addition the titers of *E. coli* and *Clostridium perfringens* were established. Furthermore, quantities of the bacteria of the genera *Enterococcus*, *Staphylococcus*, *Pseudomonas*, Gram-negative aerobic bacteria, fungi, and the total number of microorganisms were tracked.
- **Nutrient media**. Selective media (Scharlau—Antisel, Bulgaria) were used for isolation and quantitative determination of the microorganisms from the studied groups and species, as follow: Mueller Hinton agar for determination of the total number of microorganisms, Eosin Methylene Blue agar for *E. coli* and Gram-negative aerobic bacteria, Cetrimide agar for bacteria of the genus *Pseudomonas*, Chapman Stone agar for those of the genus *Staphylococcus*, Sabouraud agar for fungi, selective medium for enterococci, Salmonella-Shigella agar for Salmonella enterica, and selective agar for *Clostridium perfringens* (Merck-Bio Lab, Bulgaria).

- **The quantification** of the microorganisms was performed in serial tenfold rising dilutions of the investigated material in a sterile saline solution. Cultures of them were made on the selected nutrient media, three for each medium and dilution. After incubation at 37 °C for 24–72 h under aerobic and anaerobic conditions (with Anaerocult® A mini—Merck-Bio Lab, Bulgaria) the mean arithmetical number of developed colonies was determined and the quantities of colonies forming units (CFU) in 1 ml or 1 g of the starting material were calculated. The corresponding quantities of microorganisms in 1 g of dry matter in each of the studied materials were also calculated, as the number of detected CFU was multiplied by the quotient obtained according to the percentage of dry matter in the material.
- **Statistical analysis** of the results was carried out by the classical method of Student–Fisher, as well as using one-way analysis of variance (ANOVA) followed by Dunnett post hoc test.

3 Results

The results of the quantitative examinations of the total number of the microorganisms as well as that of the Gram-negative bacteria in the tested materials, expressed in CFU in 1 g of dry substance, are presented in Fig. 1.

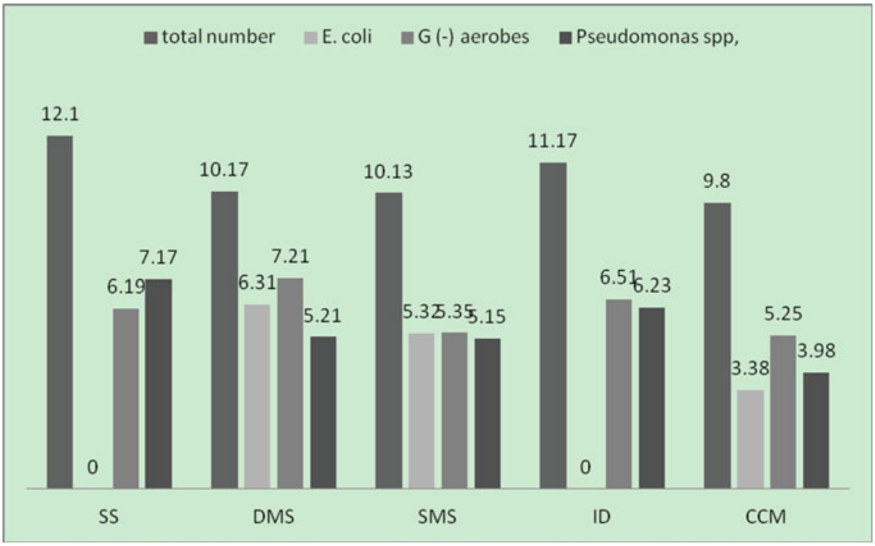

Fig. 1 Quantities of microorganisms (total and Gram-negative bacteria), presented in a unit of dry matter in the examined materials. *SS* secondary sludge, *DMS* mixed sludge, dewatered, *SMS* stayed mixed sludge, *ID* input into the digester, *CCM* composted cattle manure

Figure 2 shows the data of quantitative researches of Gram-positive microorganisms in the samples of the sludge from the treatment plant compared with cattle manure, presented in CFU in 1 g dry matter.

It was found that the tested sludge and manure do not contain *Salmonella enterica*. The content of the microorganisms from most studied groups is higher in the sludge in comparison with the cattle compost. The differences in the total amount of the microorganisms between the separate studied samples are statistically unreliable ($P > 0.05$). From the secondary sludge, as well as from the incoming in the bioreactor liquid, *E. coli* is not isolated in a titer of less than 1 ml. In these materials the enterococci are also less than those in the bovine compost, but *C. perfringens* is settled in them in higher amounts, as well as the fungi. The mixed dehydrated sludge (DMS) and the stayed mixed sludge (SMS) before its disposal, contain the most microorganisms. In these materials, the quantities of *E. coli* significantly exceed those in the researched bovine compost ($P < 0.001$).

However, as seen from Fig. 2, in the secondary sludge the enterococci are in significantly higher amount compared to the bovine compost ($P < 0.05$). The enterococci in the dehydrated mixed sludge (DMS) are less in comparison with these in the bovine compost, but the differences are unreliable ($P > 0.05$). Their quantity in the SMS is superior to that in the bovine compost, but also unreliable ($P > 0.05$). *Clostridium perfringens* is however in considerably higher amounts in each material tested, compared to the matured bovine compost ($P < 0.001$).

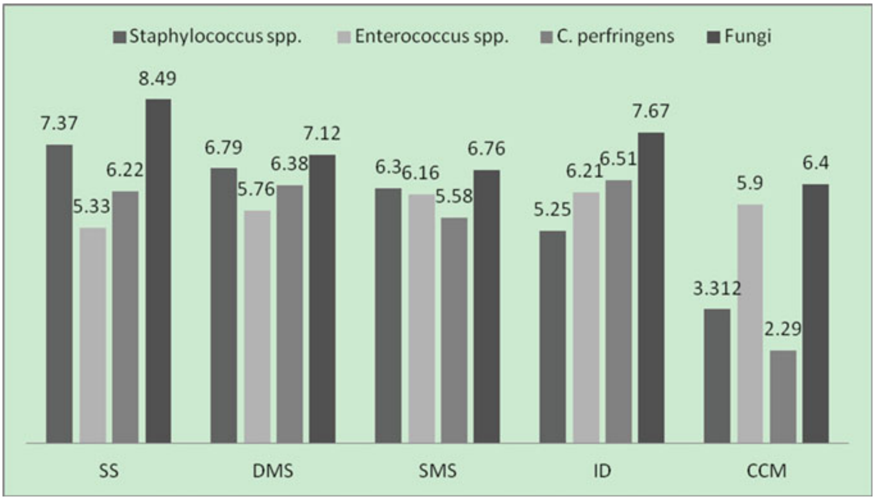

Fig. 2 Quantities of Gram-positive microorganisms, presented in a unit of dry matter of the examined materials. *SS* secondary sludge, *DMS* mixed sludge, dewatered, *SMS* stayed mixed sludge, *ID* input into the digester, *CCM* composted cattle manure

4 Discussion

The application in agriculture of sludge from wastewater treatment plants is the shortest and cheapest way for their utilization. It also allows the return in the natural cycle of the basic elements and organic substances, containing in them. This, however, may be associated with potential health risks for animals and humans [15, 16]. The examined by us sewage sludge do not fully meet the requirements of the Bulgarian Ordinance on the terms and conditions for use of sludge from wastewater treatment through its use in agriculture [14]. Similar results were obtained also by other authors in their studies of the sewage sludge [12].

The data from current research indicate that the smallest amounts of the microorganism were contained in the bovine compost, intended for manuring. Results from our previous studies indicate, that the decontamination of composted cattle manure in regard to imported therein pathogenic test bacteria from different groups occurs in a period of at least 3 weeks [17]. This fact gave us reason to use the ordinary 6-week cow compost as a control for comparison of the results in upcoming studies. In order to achieve effective decontamination, it is necessary for the studied sludges to go through an analogous process of aerobic decomposition (composting) like animal manures prior to use for fertilization to reduce the number of potentially dangerous microorganisms.

According to Wolna-Maruwka [12] by the composting process a reduction is achieved in the quantities of fungi and pathogenic bacteria from the Enterobacteriaceae family and *C. perfringens* in the composted matters, as well as an increase in the number of thermophilic bacteria. Ivanov et al. [5] also recommend sewage sludge mixed with food waste to undergo degradation by thermophilic aerobic bacteria using starter cultures from *Bacillus thermoamylovorans*. In our opinion according to the obtained results the disposal of final dehydrated sludge for a time sufficient for the inactivation of the pathogens is important and right from an environmental point of view, as its microbial content is high, including bacteria with sanitary significance as *E. coli*, *Enterococcus* spp. and *C. perfringens*.

Sewage sludge can be raw or treated and there are different sorts of treatment [6]. Use of different acids as acetic, propionic, and others is proposed as an ecological and fast way for decontamination of such waste materials [18, 19]. This is not so appropriate for ordinary use, because it is not recommended to spread sludge on a soil with a pH below five [6]. Scheinemann et al. [20] proposed ecological method by mesophilic lactic acid fermentation of cow manure or sewage sludge during which Gram-negative bacteria, enterococci, and yeasts are inactivated within several days. Ammonia also is used in this direction for the sanitization of fecal sludge [21] and other organic wastes [22], as well as formaldehyde [23] and calcium oxide [24]. To test the inactivation of pathogenic bacteria in sewage sludge and cattle manure, they were used suspensions *E. coli*, *Salmonella Anatum*, *Listeria monocytogenes*, *Clostridium sporogenes, or C. perfringens.* For the same purpose Popova et al. [17] used test strains of *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*, multiresistant to amphenicols and tetracyclines, in comparative investigations of the efficacy of different methods for decontamination of manures (anaerobic digestion, ordinary composts, and after treatment with CaO).

Due to population growth, the quantity of sewage sludge produced in the world continuously grows. Today the predominant method for the disposal of this sludge is its storage and agricultural application. However, the legislation will limit these avenues of sewage sludge disposal. Therefore, new effective methods of thermal sewage sludge utilization such as pyrolysis, gasification, combustion, and co-combustion are developed [25]. A new type of sewage sludge incineration providing 50 % energy savings and 40 % reduction of CO_2 emissions in comparison with a conventional plant was proposed by Murakami et al. [26]. This advanced method can realize energy recovery and savings as well as a low environmental impact. Hong and Li [27] found that the use of sewage sludge as secondary raw material in cement production have little or no effect on changing the overall environmental potential impact generated from general cement production. Using this technology could reduce the pressure on the environment from dramatically increased sludge disposal. Wet oxidation [28] also is an alternative solution to incineration for recovering energy in sewage sludge while converting it to mostly inorganic residues. This technology, although requiring a certain increase of technical complexity at the wastewater treatment plants, may contribute to environmental and economic advantages. Kim and Kim [29] evaluated feed manufacturing, composting, and landfilling for food waste disposal options from the perspective of global warming and resource recovery. These common treatment methods currently employed as environmentally friendlier than other methods, can negatively affect the environment if their by-products are not appropriately utilized as intended. Smith [30] highlighted, that soil carbon sinks and sources are included in attempts to meet emission reduction targets according to the Kyoto Protocol. Croplands are the largest biospheric source of carbon lost to the atmosphere and serious reductions in carbon emissions will be required over the next decades. There are options available in this direction including more efficient use of organic amendments (animal manure, sewage sludge, compost). Zhao et al. [31] compared co-composting and utilization of sludge and woodchips to reveal potential impacts to environment and human health. They point out the impacts of co-composting to acidification and terrestrial eutrophication due to ammonia emission, and trace gaseous compounds have marginal impacts to global warming and toxicity categories.

Obviously, processing methods for sewage sludge are many, but of paramount importance is to be environmentally safe and end products should not contain pathogenic microorganisms.

5 Conclusions

The comparative study of the sewage sludge and matured bovine compost for content not only of *E. coli*, *Enterococcus* spp., *C. perfringens*, but also for the main groups of Gram-negative and Gram-positive microorganisms, allows for a more certain assessment of their decontamination.

The examined sewage sludge contains microorganisms of the species *E. coli*, *C. perfringens*, *Enterococcus* spp., etc., in amounts, exceeding those in the matured bovine compost, and should not be deposited in the soil without prior aerobic or anaerobic treatment.

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References

1. Langenkamp H, Part P (2001) Organic contaminants in sewage sludge for agricultural use. European Commission Joint Research Centre, Institute for Environment and Sustainability, Soil and Waste Unit. Brussels, Belgium, pp 1–73
2. EPA (2004) United States Environmental Protection Agency. Office of Water. Office of Wastewater Management. Washington, DC 20460 (2004). Primer for Municipal Waste Water Treatment Systems. Document no EPA 832-R-04-001
3. Talahassee FL (2010) Florida Department of Environmental Protection. Ultraviolet disinfection for domestic waste water, 17 Mar 2010
4. Harshman V, Barnette T (2000) Wastewater odor control: an evaluation of technologies. Water Engineering and Management, 28 Dec 2000. ISSN:0273-2238
5. Ivanov VN, Wang JY, Stabnikova OV, Tay STL, Tay JH (2004) Microbiological monitoring in the biodegradation of sewage sludge and food waste. J Appl Microbiol 96(4):641–647
6. Department for Environment, Food and Rural Affairs (2013) Guidance. Managing sewage sludge, slurry and silage. Farming and the environment. Gov. UK
7. Jensen J (2004) Ecotoxicological effect assessment and risk characterisation of selected contaminants in sewage sludge. PhD thesis. National Environmental Research Institute, Department of Terrestrial Ecology, Denmark, p 70. ISBN:87-7834-611-8
8. Houillon G, Jolliet O (2005) Life cycle assessment of processes for the treatment of wastewater urban sludge: energy and global warming analysis. J Clean Prod 13(3):287–299
9. Kirschbaum MUF (1995) The temperature dependence of soil organic matter decomposition, and the effect of global warming on soil organic C storage. Soil Biol Biochem 27(6):753–760
10. Hong J, Hong J, Otaki M, Jolliet O (2009) Environmental and economic life cycle assessment for sewage sludge treatment processes in Japan. Waste Manag 29(2):696–703
11. Dong J, Chi Y, Tang Y, Wang F, Huang Q (2014) Combined life cycle environmental and exergetic assessment of four typical sewage sludge treatment techniques in China. Energy Fuels 28(3):2114–2122
12. Wolna-Maruwka A (2009) Estimation of microbiological status of sewage sludge subject to composting process in controlled conditions. Pol Environ Stud 18(2):279–288

13. Cyprowski M, Sowiak M, Soroka PM, Buczyrńska A, Kozajda A, Szadkowska-Stańczyk I (2008) Assessment of occupational exposure to fungal aerosols in wastewater treatment plants. Med Pr 59(5):365–371
14. Ordinance (2004) On the manner of utilization of sludge from wastewater treatment through its use in agriculture. Decree N339, 14 Dec 2004, SG 112, 23 Dec 2004 (in Bulgarian)
15. Siuta J, Wasiak G (2001) Principles of sewage sludges using on unindustrial goals (natural). Inż Ekol 3:13
16. Nguyen Thi BL, Obertynska E (2003) Effect of sewage sludge on some soil microorganisms under mazie cultivation. Zes. Probl Post Nauk Roln 494:305
17. Popova TP, Petkov YP, Baykov BD (2009) Following of the surviving of pathogenic microorganisms in poultry litter in sequens is bath process of methane fermentation and comparative assessment in relation to the decontamination in other methods of bioprocessing. In: Proceedings of the 14th ISAH congress, International Society for Animal Hygiene, vol 2, 19–23 July 2009, Vechta, Germany, pp 1029–1032
18. Lind O (1937) Untersuchungen über die bakteriziden Eigenschaften der Milchsäure. Buch-, Verlags- und Akzidenzdruckerei Eduart Seibert, Essigsäure und Salzsäure und ihre Verwendung als Konservierungsmittel für Fleisch Giessen
19. Goepfert JM, Hicks R (1969) Effect of volatile fatty acids on Salmonella typhimurium. J Bacteriol 97:956–958
20. Scheinemann HA, Dittmar K, Stöckel FS, Müller H, Krüger ME (2015) Hygienisation and nutrient conservation of sewage sludge or cattle manure by lactic acid fermentation. PLoS ONE 10(3):e0118230. doi:10.1371/journal.pone.0118230
21. Fidjeland J (2015) Sanitisation of faecal sludge by ammonia. Treatment technology for safe reuse in agriculture. Doctoral thesis, Swedish University of Agricultural Sciences, Faculty of Natural Resources and Agricultural Sciences, Uppsala, p 79
22. Popova TP, Baykov BD, Shindarska Z (2013) Comparative testing of effect of ammonium phosphates for decontamination of cattle manure. Archiva Zootechnica 16(1):89–97
23. Popova TP, Baykov BD (2014) Decontamination of sewage sludge by treatment with formaldehyde in vitro. Int J Current Microbiol Appl Sci 3(10):982–989
24. Popova TP, Marinova-Garvanska SM, Kaleva MD, Zaharinov BS, Gencheva AB, Baykov BD (2014) Decontamination of sewage sludge by treatment with calcium oxide. Int J Current Microbiol Appl Sci 3(9):184–192
25. Werle S, Wilk RK (2010) A review of methods for the thermal utilization of sewage sludge: the polish perspective. Renew Energy 35(9):1914–1919
26. Murakami T, Suzuki Y, Nagasawa H, Yamamoto T, Koseki T, Hirose H, Okamoto S (2009) Combustion characteristics of sewage sludge in an incineration plant for energy recovery. Fuel Process Technol 90(6):778–783
27. Hong J, Li X (2011) Environmental assessment of sewage sludge as secondary raw material in cement production—a case study in China. Waste Manag 31(6):1364–1371
28. Bertanza G, Canato M, Heimersson S, Laera G, Salvetti R, Slavik E, Svanström M (2015) Techno-economic and environmental assessment of sewage sludge wet oxidation. Environ Sci Pollut Res Int 22(10):7327–7338. doi:10.1007/s11356-014-3378-6
29. Kim MH, Kim JW (2010) Comparison through a LCA evaluation analysis of food waste disposal options from the perspective of global warming and resource recovery. Sci Total Environ 408(19):3998–4006
30. Smith P (2004) Carbon sequestration in croplands: the potential in Europe and the global context. Eur J Agron 20(3):229–236
31. Zhao Y, Lu W, Damgaard A, Zhang Y, Wang H (2015) Assessment of co-composting of sludge and woodchips in the perspective of environmental impacts (EASETECH). Waste Manag 42:55–60