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MICROBIOLOGICAL EVALUATION OF SEWAGE SLUDGE IN TERMS OF POSSIBILITY OF APPLICATIONS IN SOIL AS A FERTILIZER

Abstract: *The studies were carried out of sewage sludge from wastewater treatment plant for the presence of microorganisms of several main groups, containing pathogenic agents with epizootological significance (Gram-negative aerobic bacteria,* E. coli, Clostridium perfringens*, the genera* Pseudomonas, Staphylococcus, Enterococcus, *fungi, and the total number of microorganisms), to assess the environmental safety of the final product. Alongside similar studies were made of fresh and composted bovine manures. A comparison of the results was made with the bovine compost in order to assess the possibilities for using of sludge for fertilizing. The quantities of microorganisms were given in CFU per 1 g of the investigated material, but also per 1 g of dry substance for each of them. This new approach to reporting of the results per unit dry matter of the investigated materials in our opinion allows their more exact comparison. It was found that the examined sewage sludge from wastewater treatment plant were rich in microorganisms from studied groups and their direct application in soils without prior treatment by aerobic or anaerobic digestion may represent epidemiological danger.*

Introduction

The wastewater treatment is the process of removing contaminants from waste natural waters, domestic, industrial water, those of livestock and other. It includes steps aimed to eliminating the basic physical, chemical and biological contaminating agents. The aim is to obtain environmentally safe wastewater and solid wastes (treated sludge) suitable for disposal or for reuse, usually as a fertilizer in agriculture (Langenkamp and Part, 2001; EPA, 2004).

The treatment of wastewater sludge depends on the quantity of the formed solids, and other specific conditions (Talahassee, 2010). Anaerobic digestion in bioreactors is usually applied in installations with larger scale, and aerobic decomposition is suitable for small stations. It is an

aerobic process, which includes mixing the sludge with carbon sources such as sawdust, straw, waste food. In the presence of oxygen, the bacteria process wastewater solids and by the added carbon source a large amounts of heat is produces, which is an important factor for the decontamination of the final product (Harshman and Barnette, 2000; EPA, 2004; Ivanov, 2004). Concentration of the sludge by dehydrating is also applied in order to reduce the volume for transportation. The removed fluid is usually reintroduced into the cycle of wastewater and the concentrated product in some countries, like the United States, is provided to fertilize the soil. This achieves and reducing the area, which is necessary for the disposal of sludge in landfills (EPA, 2004; Ivanov, 2004).

Applying sewage sludge in agriculture, however, can be a source of biological contamination of soil, water and plants, including with pathogenic microorganisms. It is important to avoid the risks of contamination of the crops' consumers (people and animals) from farmland treated with outputs of wastewater treatment plants. The sure elimination of the risk for groundwater contamination is also important. Therefore, the monitoring and the evaluation of these risks are important to search for environmentally friendly solutions (Harshman and Barnette, 2000; Langenkamp and Part, 2001; EPA, 2004; Wolna-Maruwka, 2009).

The aim of the current work was to perform a microbiological assessment of sludge from the final stages of purification in an urban wastewater treatment plant in view of their epizootiological safety in comparison with mature bovine compost.

Materials and Methods

Samples from different stages of processing in urban wastewater treatment plant near to Sofia were examined. The materials were indicated as follow: • secondary sludge (SS); • mixed sludge (MS), dewatered by belt filter presses; • stayed mixed sludge (SMS); • input into the digester (methane tank) (ID).

Cattle manure. Fresh cattle manure (CM) and composted for 6 weeks (CC) cattle manure of dairy cows were examined, as the latter was used as a comparative control in the microbiological studies.

The data for the dry substances and pH values of the tested materials are presented in Table 1.

Table 1. Dry matter and the pH of the tested materials.

Material	Indicator	
	Dry matter in %	pH
Secondary sludge	0,90	7,52
Mixed sludge, dewatered	41,81	7,56
Mixed sludge, stayed	56,46	6,63
Input into the digester	2,96	7,22
Fresh cattle manure	59,30	7,4
Composted cattle manure	52,20	7,2

Microbiological studies were conducted in accordance with the Ordinance on the terms and conditions for use of sludge from wastewater treatment through its use in agriculture (Decree N339, 2004). The titers of *E. coli* and *Clostridium perfringens* also were established. Additionally were tracked the quantities of bacteria from the genera *Staphylococcus, Enterococcus, Pseudomonas,* Gram-negative aerobic bacteria, fungi, and the total number of microorganisms.

Nutrient media. Selective media (Scharlau - Antisel, Bulgaria) were used for isolation and quantitative determination of the microorganisms from the studied groups and types. The following media have been chosen: Mueller Hinton agar for counting the total number of microorganisms in the examined material, 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).

Quantitative determination of the microorganisms was carried out using the conventional method in serial tenfold growing dilutions of the investigated material in a sterile saline solution. Cultures of them were made on the selected 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-colony forming units) in 1 ml or 1 g of starting material were calculated. The corresponding quantities of microorganisms in 1 g dry matter in each of the studied materials also were calculated. For this purpose, 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 performed by the standard method of Student – Fisher, as well as using one-way analysis of variance (ANOVA) followed by Dunnett post-hoc test.

Results and Discussion

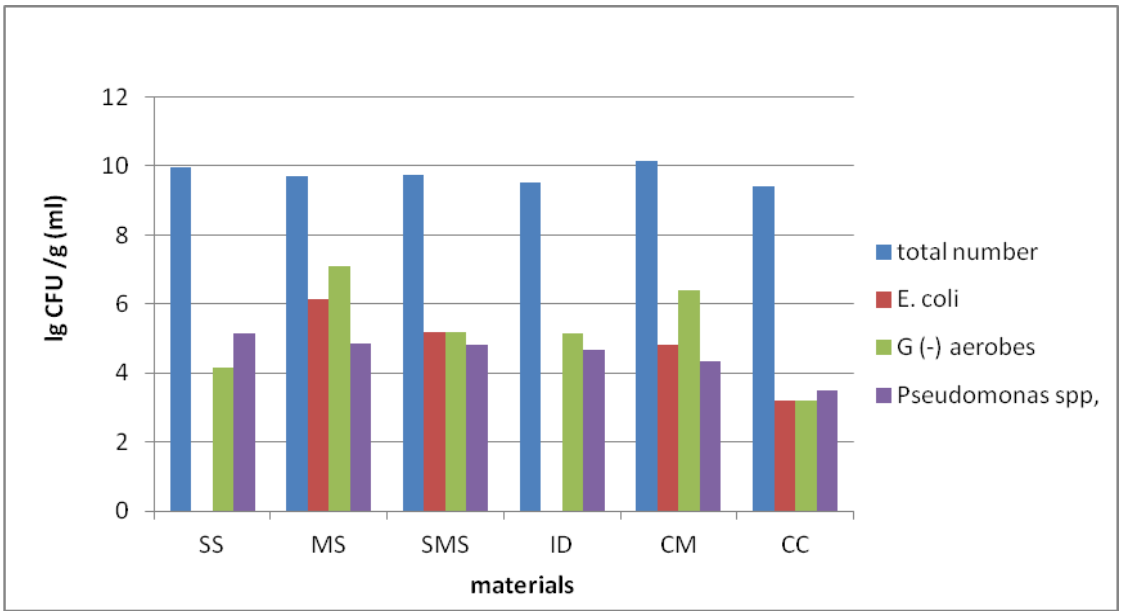

Figure 1. Quantities of microorganisms (total and gram-negative bacteria) in the examined sewage sludge of the various steps of the wastewater treatment plant and cattle manures. SS - secondary sludge; MS - mixed sludge, dewatered; SMS - stayed mixed sludge; ID - input into the digester; CM - fresh cattle manure; (CC) - composted cattle manure.

The results of the quantitative studies of the total number of microorganisms as well as of Gram-negative bacteria in the materials, expressed in CFU in 1 g of the starting materials, are presented in Figure 1. The data for the same materials, presented in CFU in 1 g dry substance, can be seen from Figure 2.

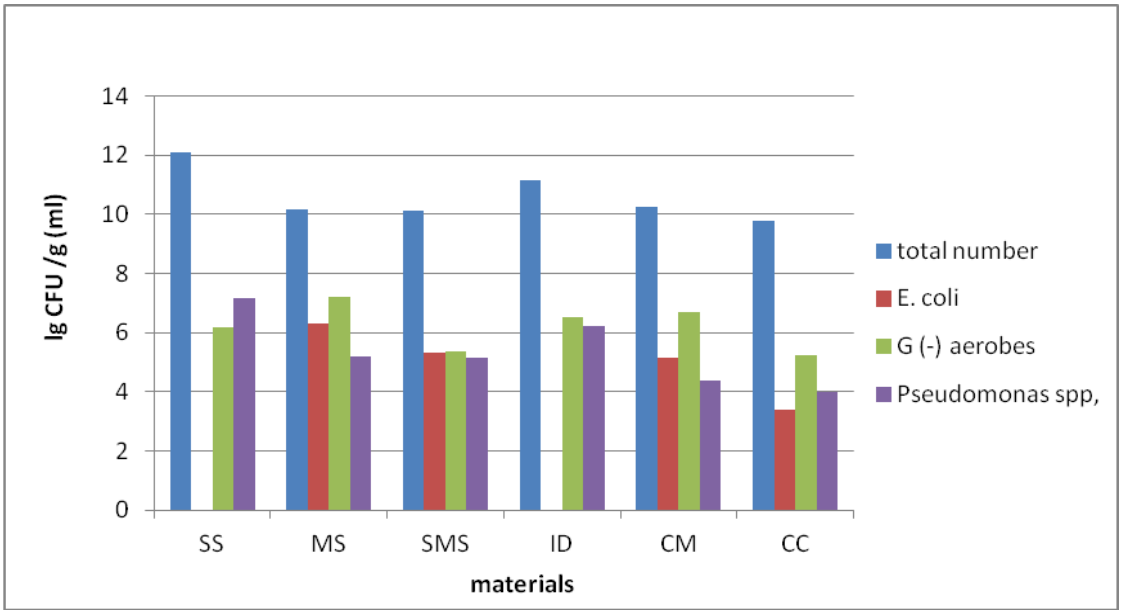

Figure 2. Quantities of microorganisms (total and gram-negative bacteria) presented in a unit of dry matter in the examined materials. SS - secondary sludge; MS - mixed sludge, dewatered; SMS - stayed mixed sludge; ID - input into the digester; CM - fresh cattle manure; (CC) - composted cattle manure.

Figure 3 shows the data from quantitative studies of gram-positive organisms in samples of sludge from the plant in comparison with cattle manure and compost, presented in CFU in 1 g of the starting material. The data for these materials expressed as CFU in 1 g dry substance are given in Figure 4.

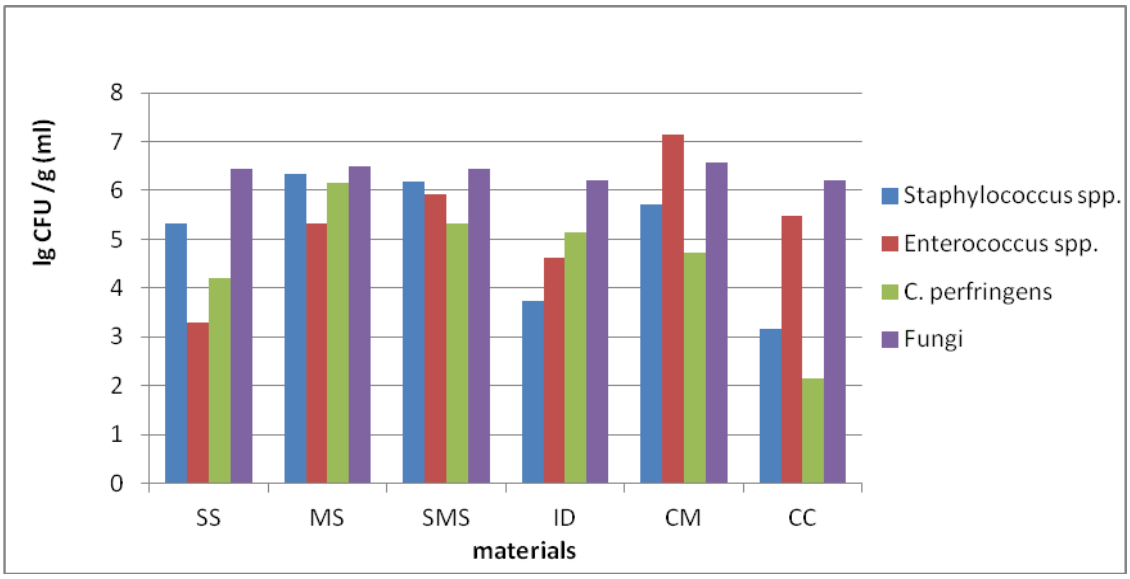

Figure 3. Quantities of Gram-positive organisms in the examined sewage sludge of the various steps of the wastewater treatment plant and cattle manures. SS - secondary sludge; MS - mixed sludge, dewatered; SMS - stayed mixed sludge; ID - input into the digester; CM - fresh cattle manure; (CC) - composted cattle manure.

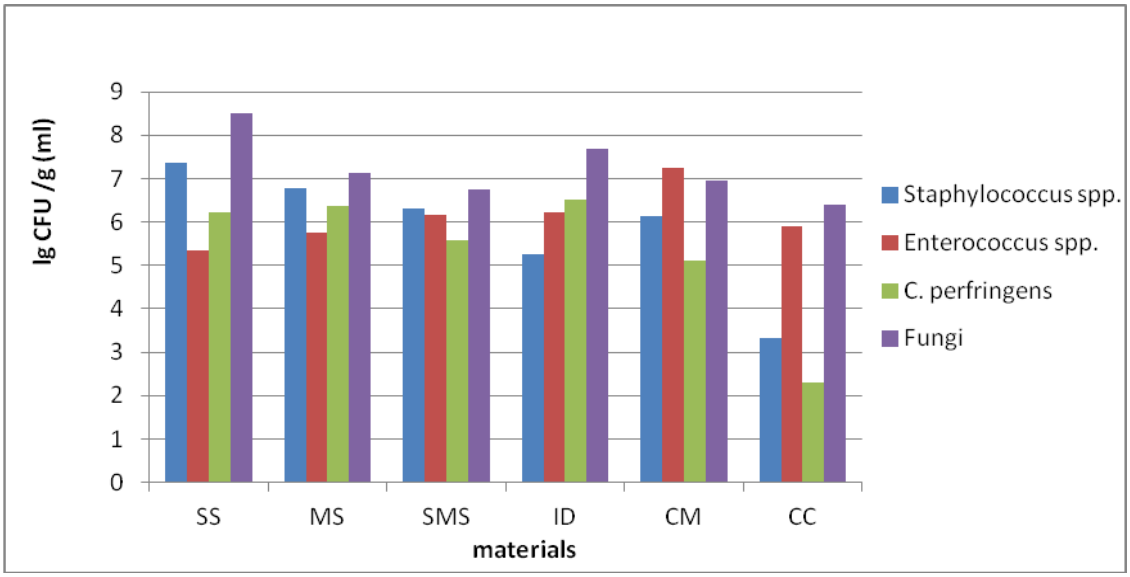

Figure 4. Quantities of Gram-positive organisms presented in a unit of dry matter of the examined materials. SS - secondary sludge; MS - mixed sludge, dewatered; SMS - stayed mixed sludge; ID - input into the digester; CM - fresh cattle manure; (CC) - composted cattle manure.

It was found that the tested sludge and manures was not contained *Salmonella enterica*. The content of microorganisms from the most studied groups was higher in the sewage sludge, versus in the bovine compost (CC). The differences of the total quantities of microorganisms between the examined samples were statistically implausible (P>0,05). From the secondary sludge, as well as from the inlet fluid into the digester, *E. coli* was not isolated in a titer less than 1 ml. In these materials the enterococci were also less than those in the bovine compost, but *C. perfringens* was recovered in higher amounts into these materials, as well as fungi. Most microorganisms were found in the mixed dewatered sludge (MS) and in stayed mixed sludge (SMS) before its depositing. In these materials the quantities of *E. coli* significantly exceeded those in the studied bovine compost (P <0,001 for the values in the materials and per their dry matter). However, as seen from Figures 1 and 2, *E. coli*, *Enterococcus* spp. and *C. perfringens* into the fresh manure were also in a significantly greater quantities than in the composted one (P<0,001 for the values in the both manures and for their dry matter). In the secondary sewage sludge, the enterococci were in smaller quantities than those in the bovine compost CC (P<0,001 for the values in the materials and per their dry matter). Their number was less and in the fluid, which entered for anaerobic digestion (P<0,001), but in calculating of the data per unit of dry matter, as seen from Figure 4, the enterococci in this material were in fact in significantly higher quantity, compared with the bovine compost (P<0,05). These were more also in the fresh cattle manure than in the composted (P<0,001 for the values in both materials and per their dry matter). As seen from Figures 3 and 4, the enterococci in the mixed dewatered sludge (MS) were less in comparison with these in the bovine compost, but the differences were implausible for the tested samples and at their determination per the dry matter (P>0,05). Their quantity in stayed mixed sludge was superior to that in the bovine compost, but also implausible (P>0,05). However, *Clostridium perfringens* was in significantly higher levels in all tested materials in comparison with the bovine compost (P<0,001 for the values in the material and per the relevant dry matters).

Discussion

The data from these studies indicate that the smallest amounts of the microorganism were contained in the bovine compost, intended for application as fertiliser. Results from our previous studies were shown that decontamination of composted cattle manure in respect of imported therein pathogenic test bacteria from different groups occurs in a period of at least 3 weeks

(Popova et al., 2009). This fact gave us reason to use the ordinary 6-week cow compost as a control for comparison of the results of this study. The established relatively high levels of enterococci in the fresh cow dung were probably due to its higher pH, to which these bacteria are tolerant. The spores of fungi are also stable under such conditions.

Application in agriculture of sludge from wastewater treatment plants is not only the shortest and cheapest way for their utilization, but also allows the return in the cycle of nature of the basic elements and organic substances, contained in them. This, however, may be associated with potential health risk for animals and humans (Siuta and Wasiakq 2001; Nguyen Thi and Obertynska, 2003). Studied by us sewage sludge, however, 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 (Decree N 339 - 14.12.2004). Similar results were obtained also by other authors in studies of sewage sludge (Wolna- Maruwka, 2009). In order to achieve effectively decontamination it is necessary the studied sludges to undergo a similar process of aerobic decomposition (composting) like animal manures before use to fertilize the soil, for reducing the number of potentially dangerous microorganisms and prevent the risk of spread of pathogenic species. The landfill of final dewatered sludge for a time sufficient for the inactivation of the pathogens is important and right from an environmental perspective, as its microbial content is high, including bacteria with sanitary significance as *E. coli, Enterococcus* spp. and *C. perfringens*. According Wolna- Maruwka (2009) the composting process causes a decrease of the number of fungi and pathogenic bacteria from *Enterobacteriaceae* family and *Clostridium perfringens* in the composted matters, as well as an increase in the number of thermophilic bacteria. When examining the samples from municipal sewage and municipal sludge Wan Ishak et al. (2009) isolated mainly soil bacilli as *Bacillus licheniformis, Bacillus megatherium* and *Bacillus aporrhoeus*. Ivanov et al. (2009) also recommend sewage sludge mixed with food waste to undergo degradation by thermophilic aerobic bacteria using starter cultures from.*Bacillus thermoamylovorans*.

Conclusions

The comparative study of the sewage sludge and mature bovine compost for content of *E. coli, Enterococcus spp., C. perfringens*, but also for the main groups of Gram-negative and Gram-positive microorganisms, allows more certain estimation of their decontamination.

Reporting of results per unit dry matter of the sludges and the examined manures allows more accurate comparison of the content of microorganisms therein.

The studied sewage sludge and fresh bovine manure contain microorganisms of the species *E. coli, C. perfringens, Enterococcus* spp. and etc. in amounts, higher than those in mature bovine compost, and should not be deposited in the soil without prior aerobic or anaerobic processing.

Acknowledgement

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