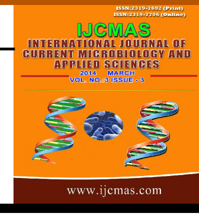


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**Original Research Article**

# Microbiological evaluation of sewage sludge in terms of possibilities of application in soil as a fertilizer

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**A B S T R A C T**

Research was conducted of sewage sludge from wastewater treatment plant for the existence of microorganisms of several major groups, containing pathogenic representatives with epizootological significance (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 environmental safety of the final product. In parallel similar studies were made of fresh and composted cattle manures. 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. The quantities of microorganisms were given in CFU per 1 g of the investigated material, as well as per 1 g of dry substance for each of them. In our opinion this new approach to the reporting of the results per unit of dry matter of the investigated materials, allows for a more accurate 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.

**Keywords**

Sewage sludge; cattle manure; microflora; epidemio-logical assessment.

## 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 represents an aerobic process which comprises mixing sludge with a source of carbon, such as sawdust, straw, or waste food. In the presence of oxygen, the bacteria process waste solid substances and through the added carbon source, produce a substantial amount of heat, which is an important factor for the decontamination of the final product (Harshman and Barnette, 2000; EPA, 2004; Ivanov, 2004). In order to reduce the transport volume, the sludge is concentrated through dehydratation. The removed fluid is usually brought back into the waste water process cycle, and the concentrated product, in some countries such as the United States, is provided for fertilizing the soil. This achieves a reduction in the area necessary for the disposal of the sludge in depots (EPA, 2004; Ivanov *et al*., 2004).

The applying of sewage sludge in agriculture, however, can be a source of biological contamination of soil, water and plants, including contamination from pathogenic microorganisms. It is important to avoid infection risks of consumers of crops (humans and animals) from agricultural areas treated with final products from wastewater treatment plants. The safe elimination of the risk of groundwater pollution is also of importance. That is why, the monitoring and the evaluation of these risks are important to the search for environmentally friendly solutions (Harshman and Barnette, 2000; Langenkamp and Part, 2001; EPA, 2004; Wolna-Maruwka, 2009).

The aim of this work was to carry out the microbiological discretion of sludges to final purification steps in an urban sewage treatment plant in terms of their epizootiological safety, compared to matured bovine compost.

## Materials and Methods

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

Cattle manures. 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.

Microbiological studies were conducted in accordance with the Ordinance on the terms and conditions for use of sludge from waste water treatment by its use in agriculture (Decree N339, 2004). The titers of *E. coli* and *Clostridium perfringens* were also established. Additionally, quantities of the bacteria of the genera *Staphylococcus*, *Enterococcus*, *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. The following media has 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).

The quantification of the microorganisms was performed using the conventional method 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. 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 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.

## Results and Discussion

The results of the quantitative researches 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 the starting material, are presented in Figure 1. The data for the same material shown in CFU in 1 g of dry substance, can be seen on Figure 2.

It was found that the tested sludge and manures does not contain *Salmonella enterica.* The content of the micro-organisms from most studied groups is higher in the sludge in comparison with the cattle compost (CC). 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 titre 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 (MS) and the stayed mixed sludge (SMS) before its disposal, contain the most microorganisms. In these materials, the quantities of *E. coli* significantly exceede those in the researched bovine compost ($P<0,001$ for the values in the materials and in their dry matter).

However, as seen from Figures 1 and 2, *E. coli*, *Enterococcus spp.* and *C. perfringens* in the fresh manure are also in a significantly greater quantities than in the composted ($P<0,001$ for the values in the both manures and for their dry matter). In the secondary sludge the enterococci are in smaller quantities than those in the bovine compost CC ($P<0,001$ for the values in the materials and in their dry matter). Their amount is less also in the fluid, which enters for the anaerobic digestion ($P<0,001$), but by the calculation of the data per unit of dry matter, as seen from Figure 4, the enterococci in this material are in fact a significantly higher amount compared to the bovine compost ($P<0,05$).

They are more also in the fresh cattle manure compared to composted (P<0,001 for the values in both materials and in their dry matter too). As seen from Figures 3 and 4, the enterococci in the mixed dehydrated sludge (MS) are less in comparison with these in the bovine compost, but the differences are unreliable for the test samples, as well as after their qualification for the dry substance (P>0,05). Their quantity in the stayed mixed sludge 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 for the values in the materials and in the relevant dry matters).

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 (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 in upcoming studies. 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 unaffected under these conditions.

The application in agriculture of sludge from waste water treatment plants is not only the shortest and cheapest way for their utilization, but also it 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 (Siuta and Wasiak, 2001; Nguyen Thi and Obertynska, 2003). The examined by us sewage sludge, however, 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 (Decree N 339 - 14.12.2004). Similar results were obtained also by other authors in their studies of the sewage sludge (Wolna- Maruwka, 2009). In order to achieve effective decontamination it is necessary the studied sludges to go through an analogous process of aerobic decomposition (composting) like animal manures prior to use for fertilizion of the land with purpose reducing the number of potentially dangerous microorganisms and prevent the risk of spread of pathogenic species. 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*. According to Wolna- Maruwka‘s research (2009) by the composting process a reduction is achieved in the quantities of fungi and pathogenic bacteria from the *Enterobacteriaceae* family and *Clostridium perfringens* in the composted matters, as well as an increase in the number of thermophilic bacteria. By examination of the samples from urban sewage and urban sludge Wan Ishak *et al*. (2009) isolated mainly soil bacilli as *Bacillus licheniformis, Bacillus megatherium* and *Bacillus aporrhoeus*. Ivanov *et al*. (2004) also recommend sewage sludge mixed with food waste to undergo a degradation by thermophilic

**Table.1** Dry matter and 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 |

**Figure.1** Quantities of microorganisms (total and gram-negative bacteria) in the studied sewage sludge and bovine fertilizer. SS - secondary sludge; MS - mixed sludge, dewatered; SMS - stayed mixed sludge; ID - input into the digester; CM - fresh cattle manure; (CC) - composted cattle manure

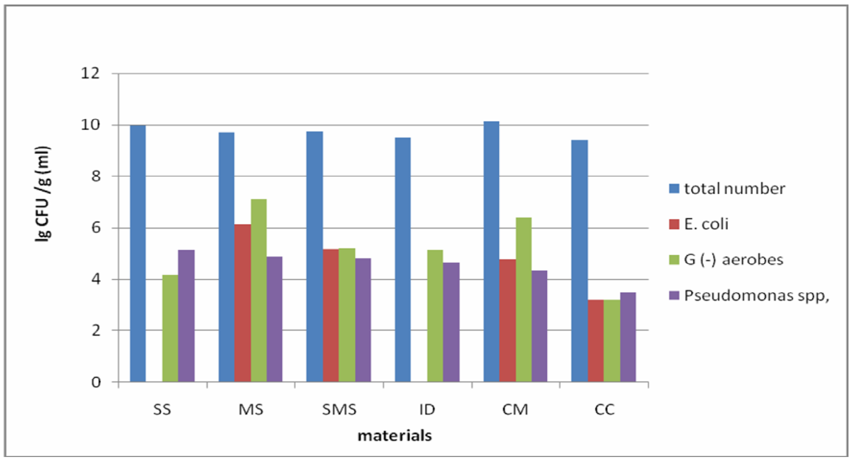



**Figure.2** Quantities of microorganisms (total and gram-negative bacteria), presented in a unit of dry matter in the examined materials. SS - secondary sludge; MS - mixed sludge, dewatered; SMS - stayed mixed sludge; ID - input into the digester; CM - fresh cattle manure; (CC) - composted cattle manure

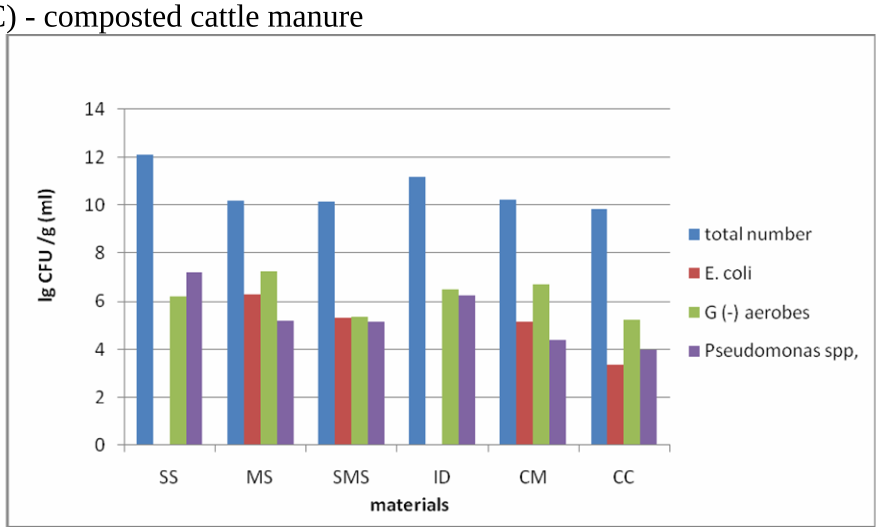


**Figure.3** Quantities of Gram-positive organisms in the examined sewage sludge from the treatment plant and beef fertilizers. SS - secondary sludge; MS - mixed sludge, dewatered; SMS - stayed mixed sludge; ID - input into the digester; CM - fresh cattle manure; (CC) - composted cattle manure.

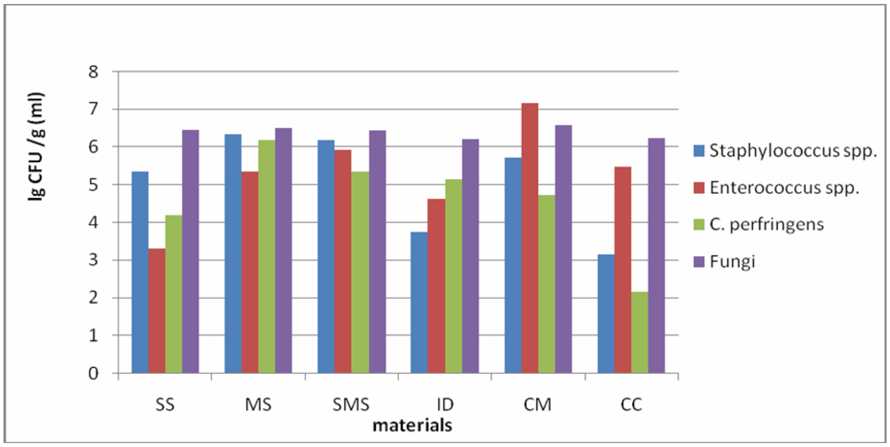
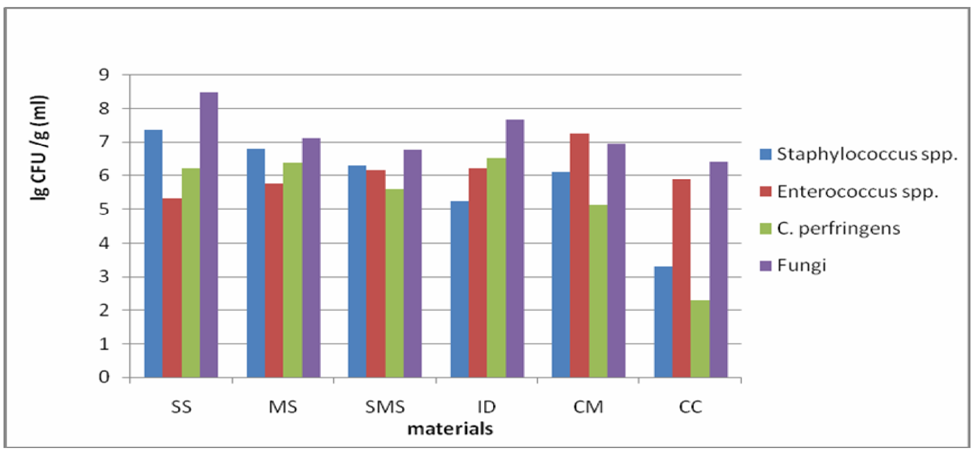



**Figure.4** Quantities of Gram-positive organisms, presented in a unit of dry matter of the examined materials. SS - secondary sludge; MS - mixed sludge, dewatered; SMS - stayed mixed sludge; ID - input into the digester; CM - fresh cattle manure; (CC) - composted cattle manure.




aerobic bacteria using starter cultures from *Bacillus thermoamylovorans*.

The comparative study of the sewage sludge and matured 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 for a more certain assessment of their decontamination. The reporting of results per unit dry matter of the sludges and the examined manures allows a more accurate comparison of the content of the microorganisms inside them. The studied sewage sludge and fresh bovine manure contain microorganisms of the species *E. coli, C. perfringens, Enterococcus* spp. and etc. in amounts, exceeding those in the matured bovine compost, and should not be deposited in the soil without prior aerobic or anaerobic processing.

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