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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