Stabilized sewage sludge – sanitary aspects and potential for conversion to biosolids

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ABSTRACT

The improvement of wastewater treatment techniques is of crucial importance for effluent quality, but it also results in an increased amount of waste sludge. Dehydrated sludge contains organic matter and nutrients, and therefore it can be used in agriculture and bioremediation, but it is considered a potential source of environmental pollution. As the sludge analyzed in the research does not contain impermissible levels of organic and inorganic pollutants, the aim of the research was to examine microbiological, particularly sanitary, aspects and potential for its further use. Microbial diversity was determined by the standard serial dilution technique and selective media, and sanitary quality indicators (total coliforms, fecal coliforms, *Escherichia coli* and *Salmonella* spp.) were determined by the MPN method. The abundance of fungi, actinomycoetes, and bacteria (ammonifiers, spore-forming bacteria and *Pseudomonas* spp.) indicate possibilities for further use of the sludge. The chemical analysis included the following parameters: total nitrogen (N), phosphorus in the form of P2O5 (available P), organic carbon (C), C/N ratio, pH, and water content. The chemical composition indicates the potential of sewage sludge to be used as a soil fertilizer, but its C/N ratio is not adequate to enable successful conversion to biosolids by the composting process. The obtained results indicate a significant level of microbiological contamination, which was most pronounced in the centre of the stabilized sludge pile. The research showed the necessity to conduct further studies on the microbial diversity and sanitary aspects of sewage sludge for proper waste sludge management.

Keywords: biosolids, *Escherichia coli*, microbial diversity, sanitary quality, sewage sludge.
The quantity of sewage sludge produced in the EU is estimated at more than 10 million tons (dry solids) each year, but the percentage of sludge being recycled and used in agriculture varies across countries (Grobelak and Jaskulak, 2019). Serbia has accepted the Urban Water Treatment Directive (UWWTD 91/271/EEC) and the obligation to solve this problem, practically from the very beginning. Only 12 % of wastewater being discharged to the sewage network (which receives 58% of total municipal wastewater) is being subjected to a purification process (Marko et al., 2020). Consequently, sludge treatment considerations have not received sufficient attention yet.

Sludge separated after wastewater treatment can pose a significant environmental risk due to the potential presence of heavy metals and pathogenic microorganisms, and a high water and organic matter content (Bian et al., 2018). The dry matter of activated sludge consists of 70–90% organic and 10–30% inorganic compounds (Vujović et al., 2016). The high content of organic matter and nutrients (mainly nitrogen and phosphorus) in sewage sludge has indicated its potential application in agriculture or soil bioremediation (Rorat et al., 2019). Despite the significant amount of nutrients, the chemical and microbiological properties of unprocessed sludge are limiting its application, which emphasizes the necessity of further treatment before use or disposal. Aerobic stabilization is a common method because of the high water and organic matter content. After aerobic stabilization and pressing, waste sludge still contains a significant amount of water, 70–75% (Bianchini et al., 2015), which makes it unstable and increases transport costs (Kosowski et al., 2020). The composition of stabilized sludge is difficult to define because wastewater properties are variable and, additionally, sludge is a mixture from different phases of wastewater treatment. As a result, there is a need for comprehensive ecotoxicological, sanitary and environmental considerations before sludge enters the circular economy and is converted to a safe fertilizer.

Sewage sludge makes a perfect environment for different microorganisms, and it is characterized by great biodiversity, which can vary depending on sewage origin, treatment, industrial activity, and other factors (Nascimento et al., 2018). While microflora has the potential for further sludge treatment, harmful microorganisms need to be controlled before sludge application. The most significant pathogens related to sludge are Salmonella representatives, with Salmonella typhi as the most severe, but other enteric bacteria such as E. coli are also commonly detected (Romdhana et al., 2009). According to the EPA waste classification (EPA, 2018), sewage sludge belongs to the group of harmless biodegradable waste (index number 19 08 05).

The levels of total hydrocarbons, polychlorinated biphenyls, polycyclic aromatic hydrocarbons, and heavy metals in the studied sludge are below legislative limits (unpublished data from the facility). Those data indicate possibilities for sludge application after the composting process. The aim of this study was to characterize stabilized sludge from wastewater treatment facility in Šabac (Serbia) and estimate the effect of stabilization on sludge microbiological and chemical characteristics, with particular emphasis on the sanitary aspect.

2. Materials and methods

2.1. Sewage sludge

The waste material used in the study was sampled in the wastewater treatment facility in Šabac. About 6 million m³ of wastewater are treated each year, and the treatment results in 20–25 t of waste sludge each day. Wastewater treatment in the facility includes primary and secondary treatment. The remaining sludge from the two phases is mixed at a ratio of 1:2–1:3. The mixture is subjected to aerobic stabilization, after which the sludge is pressed and disposed of in the facility surroundings. Two kinds of samples were taken: fresh sludge and sludge subjected to stabilization. For both of them, two layers were sampled: the surface layer and the middle of the pile. Representative samples were stored at 4°C before analyses.

2.2. Chemical characterization

The chemical characterization of sewage sludge included the following parameters: water content (w), total nitrogen content (N), phosphorus in the form of P2O5, total carbon content (C), carbon:nitrogen ratio (C:N), and pH. Water content was determined by drying at 105°C until constant weight, the Kjeldahl method was used for total N determination (Bremner, 1996), pH was measured electrochemically (Eutech pH 700, Eutech Instruments Pte Ltd.). Total P2O5 was determined spectrophotometrically (UV/VIS 1166, Labomed Inc., USA) after acid digestion (AOAC International, 2005), and total C by the dichromate technique combined with selective nutrient media. Fungi were determined on Rose Bengal medium with streptomycin (Peper et al., 1995), total ammonifiers were detected on Nutrient agar (Torlak, Serbia), and Pseudomonas spp. on Citrimide agar (Merck, Germany). Actinomycetes were enumerated on starch ammonia agar consisting of: starch, 10g; (NH4)2SO4, 1g; MgSO4x7H2O, 1g; NaCl, 1g; KNO3, 1g; CaCO3, 3g; agar 20g, and 1L of distilled water. The numbers of sporogenic bacteria were determined on Nutrient agar (Torlak, Serbia) amended with the sample dilution subjected to pasteurization at 80°C for 10 min. Analyses were performed in three replications.

2.3. Microbiological characterization

The microbiological characterization of sewage sludge was performed by the standard serial dilution technique combined with selective nutrient media. Fungi were determined on Rose Bengal medium with streptomycin (Peper et al., 1995), total ammonifiers were detected on Nutrient agar (Torlak, Serbia), and Pseudomonas spp. on Citrimide agar (Merck, Germany). Actinomycetes were enumerated on starch ammonia agar consisting of: starch, 10g; (NH4)2SO4, 1g; MgSO4x7H2O, 1g; NaCl, 1g; KNO3, 1g; CaCO3, 3g; agar 20g, and 1L of distilled water. The numbers of sporogenic bacteria were determined on Nutrient agar (Torlak, Serbia) amended with the sample dilution subjected to pasteurization at 80°C for 10 min. Analyses were performed in three replications.

2.4. Sanitary quality

The MPN method was used for the determination of Salmonella spp., total and fecal coliform bacteria, and E. coli. For the determination of total, fecal coliform bacteria, and E. coli, McConkey broth (Torlak, Serbia) was used for the first test, and Endo agar (TM media, India) for confirmation test, while Selenite Broth (Torlak) and SS agar (Torlak, Serbia) were used for Salmonella determination in the samples. The incubation lasted 48h at 37°C except for fecal coliform bacteria, which were determined after incubation at
44°C. MPN tests were performed in three replications per dilution.

3. Results and discussions

The reuse of sludge originating from wastewater treatment plants in agriculture, forestry, and horticulture is based on its high content of nutrients (Vouk et al., 2011). Organic matter improvement, stimulation of microbiological processes, beneficial soil conditioning properties, and an increase in plant macronutrient availability are the main benefits of sludge use as a biofertilizer (Singh and Agrawal, 2008). At the same time, some negative aspects are also observed, such as an increase in toxic elements and pathogenic microorganisms in the soil (Kulling et al., 2001).

![Figure 1](image)

**Figure 1.** Chemical characteristics of fresh (a), and stabilized (b) sludge from wastewater treatment: total nitrogen (N), available phosphorus in the form of P₂O₅ (available P), and organic carbon (C); ■ surface layer, ■ center.

The samples of sludge from the wastewater facility in Šabac contained significant amounts of N and P (Figure 1), which makes it a good substrate supplement for plant growth. The amounts of these nutrients are within the usual range of concentrations for such waste material, i.e. 2–6% for N, and 1–7% for P (Nakomčić-Smaragdakis et al., 2012). Also, some differences between surface and central layers of the sludge pile were observed, as well as between fresh and stabilized sludge. Stabilized sludge may have a lower content of organic C, since one part of it could be lost during the stabilization process (Yoshida et al., 2015), which was confirmed by our results. The decrease in total C was more pronounced in the central parts of sludge due to increased microbial activity correlated with the water content. The loss of total N is also expected during stabilization processes; in the central part and surface layer, total N content decreased by 21.5% and 23.2%, respectively. Nitrogen loss occurred through the evaporation of ammonia (Smith and Durgam, 2000), and was slightly more pronounced in the surface layers. The amount of available P in surface layers remained unchanged after the stabilization process, but it increased by 4.8% in the central part, probably as a result of microbial activity and aerobic decomposition of organic compounds.

![Table 1](image)

**Table 1.** Carbon/nitrogen ratio (C/N), pH and dry weight percentage (% dw) of residue sludge from wastewater treatment

<table>
<thead>
<tr>
<th>Sample</th>
<th>Parameter</th>
<th>Parameter</th>
<th>Parameter</th>
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<tbody>
<tr>
<td></td>
<td>C/N</td>
<td>pH</td>
<td>% dw</td>
</tr>
<tr>
<td>Stabilized sludge, surface</td>
<td>8.6:1</td>
<td>7.59</td>
<td>80.79</td>
</tr>
<tr>
<td>Stabilized sludge, center</td>
<td>8.2:1</td>
<td>8.31</td>
<td>17.34</td>
</tr>
<tr>
<td>Fresh sludge, surface</td>
<td>7.4:1</td>
<td>7.63</td>
<td>22.71</td>
</tr>
<tr>
<td>Fresh sludge, center</td>
<td>8.0:1</td>
<td>8.17</td>
<td>19.07</td>
</tr>
</tbody>
</table>

Water content in the surface layer of stabilized sludge pile was significantly lower (Tab.1), as the result of longer sludge disposal and dry surface crust formation. The pH of surface layers was lower than that of the central parts, possibly due to a higher abundance of fungi on the surface (Table 2) and their metabolic activity.

The low C/N ratio in sludge samples (Table 1) indicates unfavorable conditions for successful composting. The usual recommendation for the C/N setting is co-composting with some other types of waste containing a high C/N ratio, such as lignocellulose waste from primary agriculture, mushroom production, horticultural waste, sawdust, or other types (Kulikowska, 2016). Previous studies indicate that co-composting with wastes containing a high organic C content can have a positive effect on the composting process and ammonia assimilation by nitrifiers, and can lead to an increase in composting temperature, an increase in humic matter content, and a decrease in pathogenic bacteria abundance (El Kadiri Boutchich et al., 2015; Kulikowska, 2016; Li et al., 2013; Meng et al., 2018). Mixing with lignocellulose waste results in a decrease in the total number of pathogenic
microorganisms in the initial material of the composting pile. At the same time, C/N adjustment will lead to better conditions to achieve higher temperatures, which improve the sanitation of the composting mass (El Kadiri Bouthich et al., 2015).

Table 2.
Microbiological community in sewage sludge (numbers of specific groups of microorganisms as CFU g⁻¹ dw)

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</thead>
<tbody>
<tr>
<td>Stabilized sludge, surface</td>
<td>7.34x10⁷</td>
<td>8.58x10⁴</td>
<td>1.82x10¹⁰</td>
<td>9.28x10⁶</td>
<td>1.41x10⁴</td>
</tr>
<tr>
<td>Stabilized sludge, center</td>
<td>3.46x10⁸</td>
<td>3.34x10⁵</td>
<td>1.91x10⁸</td>
<td>4.99x10⁵</td>
<td>1.65x10⁶</td>
</tr>
<tr>
<td>Fresh sludge, surface</td>
<td>7.93x10⁴</td>
<td>6.43x10⁵</td>
<td>2.28x10¹⁰</td>
<td>1.38x10⁶</td>
<td>1.68x10⁵</td>
</tr>
<tr>
<td>Fresh sludge, center</td>
<td>7.34x10⁴</td>
<td>2.09x10⁵</td>
<td>1.90x10¹⁰</td>
<td>2.10x10⁵</td>
<td>4.19x10³</td>
</tr>
</tbody>
</table>

The abundance of fungi was significantly higher in stabilized sludge than in fresh sludge. The difference between the surface and the inner layer was present in both types of sludge, but it was more pronounced in stabilized sludge (Table 2). Water reduction in the surface layer during sludge stabilization favored fungi over the other microbes. The macromorphological observations of fungal colonies on selective medium indicated that the representatives of Mucor spp. were the most dominant fungal population. The presence of Trichoderma spp. was recorded occasionally. It is known that some Mucor representatives are able to adapt to changes in pH and oxygen content (Reunwai et al., 2010). Mucor and Trichoderma representatives are known as bioremediation agents, and therefore their activity can be involved in the removal of toxic compounds (Khtoon et al., 2021).

A very high prevalence of ammonifiers was noted in analyzed samples (Table 2). This was expected, considering the high organic matter content of the sludge, and its tendency to be unstable and easily decomposed. This part of microbiota plays an important role in the composting process. Aerobic conditions in the surface layer increased the presence of spore-forming bacteria, which made up a significant portion of the ammonifier group. Spore-forming ammonifiers belonging to Bacillus genus are the main bacterial component in organic carbon degrading micrflora, especially in the thermophilic phase of the composting process (He et al., 2013).

Table 3.
Sanitary quality of sewage sludge MPN/10g dw

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total coliforms</th>
<th>Fecal coliforms</th>
<th><em>Escherichia coli</em></th>
<th><em>Salmonella</em> spp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stabilized sludge, surface</td>
<td>&gt; 13,580</td>
<td>&gt; 13,580</td>
<td>247</td>
<td>247</td>
</tr>
<tr>
<td>Stabilized sludge, center</td>
<td>&gt; 64,706</td>
<td>&gt; 64,706</td>
<td>212</td>
<td>64,706</td>
</tr>
<tr>
<td>Fresh sludge, surface</td>
<td>&gt; 50,000</td>
<td>&gt; 50,000</td>
<td>&gt; 50,000</td>
<td>414</td>
</tr>
<tr>
<td>Fresh sludge, center</td>
<td>&gt; 57,895</td>
<td>&gt; 57,895</td>
<td>&gt; 57,895</td>
<td>8421</td>
</tr>
</tbody>
</table>

The surface layer of stabilized sludge was characterized by the lowest level of all examined microbial quality indicators (Tab. 3). The other samples contained more than 50000 MPN/10g of total and fecal coliform bacteria, as well as of *E. coli* in the surface and center of fresh sludge. The center of stabilized sludge had a specifically pronounced abundance of *Salmonella* spp., which are among the most important microbial contaminants of sludge (Romdhana et al., 2009). According to the current legislative regulations in the Republic of Serbia, the limit for *Salmonella* spp. in compost used in agriculture is 10 MPN/10g dw (Official Gazette RS, No. 67/2011, 48/2012, and 1/2016). According to the USA legislation (US EPA, 1993),
composts originating from biosolids can be classified as A class (without detected pathogens) and B class (with certain levels of pathogenic microorganisms), but the standard sets restrictions on their use in agriculture. The restrictions are related to crop rotation and restricted periods for use of agricultural products after compost application. E. coli was also a common contaminant of both fresh and stabilized sludge (Wery et al., 2010).

The presence of pathogenic bacteria in sludge can put food production at risk if sludge is used on fields. E. coli and Salmonella spp. are capable of surviving and multiplying inside compost particles for up to a few months after compost application (Eamens et al., 2006). Given their propagation potential in soil and risk for human health, their populations in waste materials should be of particular interest. The results indicate that sludge should be further treated before use and that these treatments should include sanitation. One solution could be to use composting as a strategy for stabilized sludge treatment. The proper maintenance of elevated temperature during composting leads to a significant improvement in microbiological quality (Epstein, 2002; Đokić et al., 2014).

4. Conclusions

The stabilization of sewage sludge results in moderate changes in its water content and chemical and microbiological characteristics. Further treatments are needed to enable sludge involvement in the circular economy. Composting of stabilized sewage sludge is a cost-effective process that has many benefits such as reducing the total volume of waste, enhancing soil fertility, improving sludge structure and water holding capacity, reducing the abundance of pathogenic bacteria and xenobiotics, recycling other lignocellulose waste at the same time. Variability in biological communities and their diversity indicate the need for further research focused on the sanitary characterization of sewage sludge.

Acknowledgment

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Declaration of competing interest

The authors have no conflict of interest to declare.

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