Glasnik hemičara i tehnologa Bosne i Hercegovine

Bulletin of the Chemists and Technologists of Bosnia and Herzegovina

2026

66

41-46

Print ISSN: 0367-4444

Online ISSN: 2232-7266

Original Scientific Article

DOI: 10.35666/2232-7266.2026.66.05

UDK: 54[628.19:502.17]

Stabilization of Waste Sludge from Municipal Wastewater Treatment Plant

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Article info
Received: 05/12/2025
Accepted: 17/02/2026

Keywords:
Wastewater treatment
Waste sludge
Sludge treatment
Stabilization
Pyrophyllite schist

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Abstract: Municipal wastewater treatment generates significant amounts of waste sludge that needs to be stabilized before disposal or reuse due to environmental and health risks. This study evaluated the effectiveness of microbiological, biological, and physical sludge treatment methods using physicochemical, microbiological, and ecotoxicological analyses. Microbiological treatment with a mixed microbial culture resulted in odor reduction and partial changes in organic matter content. Biological treatment with *Eisenia fetida* led to gradual structural transformation of the sludge and a reduction in pathogenic indicators, although a longer treatment time was required. Physical stabilization using pyrophyllite schist significantly increased total solids (from 17.75% to 48.32%), reduced volatile solids (to 2.26 %) due to combined stabilization and dilution effects, immobilized selected heavy metals, and eliminated fecal bacteria and other pathogenic microorganisms. Ecotoxicological assessment showed an increase in LC_{50} values from 27% in raw sludge to 58% after physical treatment, indicating reduced toxicity. The results show that pyrophyllite schist is an effective natural material for improving the physicochemical stability and hygienic safety of wastewater sludge and highlight the potential of combining biological agents and natural mineral materials for sustainable sludge management.

INTRODUCTION

Wastewater treatment is a process aimed at reducing pollution to levels at which treated effluent can be safely discharged into receiving water bodies without adverse effects on human health and the environment (Pešević, 2022). An inevitable by-product of municipal wastewater treatment is waste sludge, the amount and composition of which depend on both the characteristics of the influent wastewater and the applied treatment technology. Higher treatment efficiency generally results in increased sludge production and a more complex sludge composition (Vouk et al., 2016). Waste sludge typically represents approximately 1–2% of the total volume of treated wastewater, and retains up to 50–80% of the originally present pollutants. At the same time, sludge is a valuable source of organic matter and nutrients, containing on average about 40% organic carbon, nearly 10% nitrogen, and approximately 3% phosphorus. Due to these characteristics, waste sludge has the potential for beneficial use, particularly in agriculture. However, its application is limited by the presence of heavy metals, pathogenic microorganisms, and various organic contaminants such as polycyclic aromatic hydrocarbons, polychlorinated biphenyls (PCBs), pharmaceuticals, pesticides, and microplastics (Nguyen et al., 2022). Improper treatment or disposal of waste sludge can result in environmental pollution and loss of valuable resources. The importance of effective sludge management has therefore increased significantly, driven by the expansion of sewerage systems, the construction of new wastewater treatment plants, and the modernization of existing infrastructure (Shaddel et al., 2019). In order to reduce environmental risks, sludge stabilization is required to prevent further biodegradation, reduce odors, and improve hygienic safety.

Conventional sludge stabilization methods include biological, physicochemical, and thermal processes (Tezel et al., 2011; Zhen et al., 2017; Zhang et al., 2021). Although these methods can be effective, they are often associated with high operational costs, increased energy consumption, incomplete stabilization, or secondary environmental impacts such as excessive alkalinity or nutrient loss. In addition, many stabilization approaches rely on aggressive chemical agents, which can limit the sustainable reuse of treated sludge. These limitations highlight the need for alternative or complementary stabilization strategies that are environmentally compatible, cost-effective, and based on locally available materials. Natural mineral materials have recently attracted attention for their potential to improve sludge stability through adsorption, immobilization of contaminants, and moisture reduction. In this study, pyrophyllite schist was selected as a physical stabilization agent due to its mineral composition, layered aluminosilicate structure, adsorption capacity, local availability, and low environmental impact. These properties suggest that pyrophyllite schist can contribute to both physicochemical stabilization and hygienic improvement of waste sludge. In addition to physical stabilization, biological approaches play an important role in sludge treatment. In this context, microbiological treatment refers to the application of selected microbial consortia to improve biochemical transformation processes, while biological treatment involves higher organisms, such as earthworms (*Eisenia fetida*), which facilitate organic matter degradation through vermicomposting mechanisms. Although both approaches are biologically based, they differ in their mechanisms, time requirements, and effects on sludge properties.

Therefore, the aim of this study was to comparatively evaluate microbiological, biological, and physical stabilization methods for municipal waste sludge, with a particular emphasis on the application of pyrophyllite schist as a natural stabilization material. The effectiveness of each treatment was assessed using physicochemical, microbiological and ecotoxicological parameters to determine their potential contribution to sustainable sludge management.

EXPERIMENTAL

Materials

Sludge from the municipal wastewater treatment plant of the city of Živinice was used as the basis for this research. Microbiological treatment of the sludge was carried out using microorganisms from the eMB starter culture (including lactic acid bacteria, photosynthetic bacteria, and PDM 7 group of microorganisms) from the company EM Plus, Doboj, Bosnia and Herzegovina. Biological treatment was carried out using earthworms (*Eisenia fetida*), while physical treatment included the use of pyrophyllite schist purchased from Parsovići-Konjic, Bosnia and Herzegovina, and delivered by AD Harbi d.o.o. Sarajevo. Pyrophyllite schist is a natural aluminosilicate mineral characterized by a layered structure and adsorption capacity, which makes it suitable for binding moisture and inorganic pollutants. Recent mineral science studies have documented its characterization and beneficiation (Bhukte et al., 2024).

Methods

A series of analytical tests were carried out to assess the physicochemical and biological parameters of the sludge. Standard and modified standard methods for wastewater analysis were applied, according to BAS EN ISO, EN ISO, and APHA methodologies. The modifications primarily concerned sample preparation and dilution steps, as standard wastewater treatment methods were adapted to the high solid content and heterogeneous nature of sludge samples.

Physicochemical Analysis

The characterization of the raw material (waste sludge) and the mixtures obtained after treatment was performed by measuring the pH value and determining the total solids (TS) and volatile solids (VS) contents, together with the concentrations of total Kjeldahl nitrogen (TKN) and total phosphorus (TP), expressed on a dry matter basis. The pH value was measured using a pH meter (Mettler Toledo FE20/EL20) with direct electrode immersion into the samples. The total solids (TS) and volatile solids (VS) contents were determined according to Standard Methods 2540 B and 2540 E, respectively (American Public Health Association, 2005). The TKN concentration was determined using Method 4500 (American Public Health Association, American Water Works Association, Water Environment Federation, 2023), while the total phosphorus concentration was analyzed according to the standard BAS EN ISO 6878:2006. Heavy metals were determined by atomic absorption spectrometry in accordance with EN 16174:2012 (European Committee for Standardization, 2012). The concentration of polychlorinated biphenyls (PCBs) was analyzed using the US EPA SW-846 Test Method 3546 (United States Environmental Protection Agency).

Microbiological Analysis

For the purpose of microbiological characterization of the sludge, several key microbiological parameters were analyzed using standard microbiological techniques:

Total aerobic mesophilic bacteria at 22°C and 37°C: Serial dilutions were prepared up to 10^{-6} using a vortex mixer. From each dilution, 1 mL was pipetted into sterile Petri dishes and overlaid with 15 mL of Nutrient Agar. The plates were incubated at 22°C and 37 °C for 72 hours. After incubation, colony-forming units (CFUs) were counted, and results were expressed as CFU/mL.

Total coliform bacteria (MPN method): The most probable number (MPN) method was used to estimate the number of total coliforms in 100 mL of sample. The method, based on colorimetry, involves inoculation into lactose-based broth (e.g., MacConkey broth) containing Durham tubes. After 48 hours of incubation at 37°C, the presence of acid and gas was used as a positive indicator. The results were calculated using MPN tables and expressed as MPN/1 L.

Yeast/molds: Serial dilutions of the sample were prepared under aseptic conditions. Aliquots from each dilution were transferred to sterile Petri dishes and mixed with Rose Bengal Chloramphenicol Agar (RBCA). After solidification, the plates were incubated at 28°C for 5 days, which is the standard incubation period for the enumeration of yeasts and molds on this medium. After incubation, colonies exhibiting morphology typical of yeasts and molds were counted, and the results were reported as CFU/mL, adjusted for the appropriate dilution factors.

Fecal streptococci (Enterococci): Membrane filtration was used to determine the number of fecal streptococci (enterococci). Salturius nitrocellulose membrane filters with a pore size of 0.45 μm were used. The filters were placed on Slanetz-Bartley agar and incubated at 36°C for 48 hours. Colonies with a red appearance were subcultured onto Bile Esculin agar and incubated for 2 hours at 44.5°C. Colonies that turned dark or formed a dark zone were counted. Results were expressed as CFU/100 mL.

Fecal coliforms: The Eijkman test was used to determine the presence of fecal coliforms. Sample aliquots were inoculated into a lactose-based selective medium and incubated at 44–45°C for 48 hours, the temperature range used to detect thermotolerant coliforms. Tubes showing gas formation were considered presumptively positive.

Proteus species: Serial dilutions were prepared using sterile distilled water. Samples were inoculated into MacConkey broth and incubated for 48 hours at 37°C, followed by streaking on MacConkey agar and incubation for 24 hours at 37°C. Characteristic large, spreading (swarming) colonies indicated the presence of *Proteus* spp.

Sulfite-reducing clostridia (membrane filtration method): After dilution, the samples were filtered through Salturius nitrocellulose membrane filters with a pore size of 0.22 μm, and the membrane filter was carefully placed on the bottom of a sterile Petri dish. Approximately 18 mL of molten complete medium (cooled to about 50°C) was poured over the filter. The plates were incubated under anaerobic conditions at 37±1°C for 20±4 and 44±4 hours. Black colonies were counted and recorded as sulfite-reducing clostridia.

Pseudomonas aeruginosa (MPN method): Five test tubes, each containing 10 mL of Asparagine broth, were inoculated with 10 mL of the diluted sample. After shaking, the tubes were incubated for 48 hours at 35°C. Tubes showing fluorescence or turbidity were considered positive.

Experimental procedure

To complete the sludge treatment process, the following experimental procedures were conducted: microbiological treatment of sludge, biological treatment of sludge and stabilization of sludge with natural material. Raw waste sludge (T0), stored under the same environmental conditions without any treatment, was used as a control sample for comparison with the treated samples.

Microbiological treatment of sludge was carried out using eMB starter cultures containing lactic acid bacteria, photosynthetic bacteria, and PDM (a consortium of seven groups of beneficial microorganisms). In this procedure, the sludge was mixed with a liquid microbial solution by layering. First, a portion of the microbial culture (primarily lactic acid bacteria of dairy origin) was sprayed onto the cleaned concrete surface. Then, a 10 cm layer of sludge was applied, followed by another layer of the microbial solution. This layering process (microorganisms–sludge–microorganisms) was repeated until a pile approximately 80 cm in height was formed. The pile was left to stand for 7 days. A total of 5 liters of microbial solution per 1 m³ of sludge was used. The mixture was aerated by turning the pile every 7 to 10 days. After 45 days, a sample was taken for further analysis.

In the biological treatment experiment, 1 m³ of dehydrated sludge was mixed with 50 kg of a mixture containing California earthworms and compost. The compost included both mature earthworms and newly established colonies of smaller earthworms, mixed with organic residues such as fruit and vegetable scraps, paper, and leaves. The mixture was thoroughly homogenized and left to settle. It was manually stirred every 7 days. After 180 days, a sample was collected and analyzed. In the experimental treatment of sludge with a natural material, dehydrated sludge was mixed with pyrophyllite schist. The ratio used was 1 kg of pyrophyllite schist to 10 kg dehydrated sludge, and the mixture was stored in a dry and dark place. The mixture was stirred every 7 days. By the end of the experiment, the characteristic unpleasant odor of the dehydrated sludge had completely disappeared. After 30 days, the sample was collected and analyzed.

Fig 1. Microbiological treatment of sludge

Fig 2. Biological treatment of sludge

Fig 3. Stabilization of sludge with natural materials

RESULTS AND DISCUSSION

Based on the physicochemical analyses of sludge treated by different methods, the results are presented in Table 1. The addition of pyrophyllite schist to the waste sludge (T3) resulted in a significant increase in total solids (TS) and a change in the ratio of volatile solids (VS), total Kjeldahl nitrogen (TKN), and total phosphorus (TP) in the raw sludge. This effect can be attributed to the high mineral content and adsorption properties of pyrophyllite, which not only increases the solid fraction but also immobilizes certain organic and nutrient components, thereby reducing the volatiles content and changing the availability of nitrogen and phosphorus.

In addition, the physical structure of the schist can improve sludge drainage and compaction, which contributes to the observed changes in the composition of solids and nutrients. The content of metal elements in the treated sludge was lower compared to the untreated sludge, which directly improves the suitability of the material for application. It is particularly important to note that the concentrations of almost all analyzed metals (except Ni) were significantly below the maximum permissible values prescribed by the Regulation on the Determination of Permissible Quantities of Harmful and Hazardous Substances in Soil and Methods of Their Testing (Official Gazette of FBiH, No. 96/22) and the Regulation on Sludge Management from Municipal Wastewater Treatment Plants (Official Gazette of FBiH, No. 28/24).

Table 1: Physicochemical parameters in the analyzed samples.

Parameter	Unit	T_0	T_1	T_2	T_3
pH	-	8.08	7.45	6.82	7.10
TS	%	17.75	22.19	16.17	48.32
VS	%	9.82	12.56	12.89	2.26
TP	g/kg	186.78	156.48	69.5	41.619
TKN	g/kg	9.67	11.07	2.04	11.7
Fe	mg/kg	0.2024	0.217	6.92	0
Mn	mg/kg	0.068	0.08	0.206	0
Cu	mg/kg	118	77.7	141	37.8
Cr	mg/kg	41.7	56.1	88.1	1.14
Pb	mg/kg	27.9	52.7	32.2	18.8
Ni	mg/kg	123	146	96.6	79.8
Ca	g/kg	22.2	26.7	32.3	30.8
PCB	mg/kg	0.01	0.01	0.01	0.01

T_0-raw waste sludge from the Živinice municipal wastewater treatment plant; T_1-microbiologically treated sludge; T_2-biologically processed sludge; T_3-physically stabilized sludge using natural material.

The pH value decreased from 8.08 in the raw sludge to 6.82 in the biologically treated sample (T2), indicating the formation of acidic compounds during the decomposition of organic matter. The slight increase in pH to 7.10 in sample T3 suggests partial buffering and stabilization of the medium after the addition of pyrophyllite schist. The total proportion of solids (TS) increased from 17.75% in the raw sludge to 48.32% in the sample (T3), primarily due to the reduction of moisture and the addition of mineral material. In contrast, samples T1 and T2, showed an increase in volatile solids (VS) content compared to raw sludge, which can be attributed to microbial activity combined with reduced water content, leading to a relative concentration of organic matter in the dry fraction (Arnaiz et al., 2006). A significant drop in VS (to 2.26%) was observed in sample T3, reflecting an increased proportion of inert mineral material and improved stability, thereby reducing the potential for further biological degradation. The total phosphorus content decreased in all treatments, from 186.78 g/kg in the raw sludge to 41.62 g/kg in the sample with the addition of pyrophyllite schist. This decrease indicates the loss of phosphorus compounds during biological processes, where part of the phosphorus is transferred to the liquid phase or consumed in microbial growth. The addition of pyrophyllite schist further reduces the phosphorus content due to the adsorption of phosphate ions on the mineral surface.

The TKN values varied across all treatments. The slight increase in T1 (11.07 g/kg) compared to the raw sludge (9.67 g/kg) can be attributed to the concentration of organic matter and microbial activity. In T2, there was a significant decrease (2.04 g/kg) due to the degradation and loss of nitrogen compounds in the form of ammonia or other soluble forms, while the physically treated sample T3 showed a higher TKN value (11.7 g/kg), which is a result of stabilization and the addition of mineral material, which reduced TKN losses. Regarding heavy metals, the concentrations of all metals were variable. The reported concentrations of iron (Fe) and manganese (Mn), which in certain samples were equal to 0 mg/kg, indicate values below the analytical detection limits of the applied method rather than the complete absence of these elements, resulting in apparently inconsistent trends. In contrast, copper (Cu), lead (Pb), and nickel (Ni) generally decreased only in the sample treated with pyrophyllite schist, suggesting possible immobilization effects due to the addition of the stabilizing material. In the same sample (T3), chromium (Cr) exhibited a significant reduction from 56.1 mg/kg to 1.14 mg/kg, which may indicate its precipitation or adsorption on the mineral surface. The unchanged concentration of polychlorinated biphenyls (PCBs) in all treatments (0.01 mg/kg) indicates the pronounced persistence of these compounds and the limited effectiveness of the applied biological and physical stabilization methods, which is in accordance with literature reports on the long-term persistence of PCBs in the environment (Othman et al., 2022). The results obtained indicate an effective stabilization of organic matter and a partial reduction of potentially hazardous elements. The observed variations in nutrient and metal content reflect the complex biochemical and physicochemical interactions that occur during the biological treatment and stabilization processes. Both biological and physical treatments significantly improved the physicochemical characteristics of the sludge, reducing the organic load and improving its quality. Together with metal concentrations well below the legally permissible limits, these findings confirm that the treated sludge possesses favorable properties for controlled use, provided that continuous monitoring of persistent organic pollutants is ensured. The results of biological parameters after sludge treatment by different methods are presented in Table 2. LC50 values (Lethal Concentration causing 50% mortality after 48 hours) increased after treatment, particularly in sample T3, indicating a reduced acute toxicity of the stabilized sludge. Although a qualitative notation was applied due to methodological constraints, the results suggest improved ecotoxicological safety following physical stabilization. The presence of harmful bacteria, which is considered a key limitation in the application of waste sludge, was significantly reduced after treatment with pyrophyllite schist from the "Parsovići" deposit (AD Harbi, Konjic, Bosnia and Herzegovina). In particular, the use of pyrophyllite schist greatly reduced the presence of pathogenic bacteria. In fact, no bacteria were detected after this treatment.

Table 2: Biological parameters in the analyzed samples.

Parameter	Unit	T_0	T_1	T_2	T_3
LC_{50} (48 h)	%	27	7	20	58
AMB 22°C	CFU/mL	7.2×10^{6}	5.1×10^{6}	2.5×10^{4}	390
AMB 37°C	CFU/mL	2.4×10^{7}	2.2×10^{7}	1.8×10^{4}	17
TCB	MPN/L	2.4×10^{6}	1.9×10^{6}	>3800	9
Yeasts/molds 28°C	CFU/mL	6.7×10^{5}	5.5×10^{5}	1.0×10^{3}	-
Fecal streptococci	/	+	+	+	-
Fecal coliforms (Eijkman test)	/	+	+	+	-
Proteus spp.	/	+	+	-	-
P.aeruginosa	/	+	+	-	-
SR clostridia (10 mL)	/	+	+	-	-

LC_{50}-Lethal Concentration 50 % after 48 hours; AMB-Aerobic Mesophilic Bacteria; TCB-Total Coliform Bacteria; SR clostridia- Sulfite-reducing clostridia; The "+" sign indicates presence, while the "–" sign indicates absence.

During the biological treatment of the sludge (T1–T3), the decrease in odor intensity and changes in sludge appearance qualitatively supported the microbiological and ecotoxicological results. A significant decrease in aerobic mesophilic bacteria (AMB) and fecal indicators (total coliform bacteria and sulfite-reducing clostridia) was observed, indicating effective pathogen reduction. After six months of treatment, the unpleasant odor was no longer present, and the sludge showed a heterogeneous coloration, which is consistent with partially biologically stabilized fractions. LC_{50} values increased after treatment, particularly in sample T3, suggesting reduced acute toxicity and improved ecotoxicological safety. Taken together, these observations demonstrate the effectiveness of the applied treatments in improving sludge stability and reducing biological hazards.

CONCLUSION

Waste sludge can be stabilized by microbiological, biological, or physical treatment methods, primarily by altering its microbiological and physicochemical characteristics. The use of a mixed culture of microorganisms significantly reduced unpleasant odors and contributed to the reduction of sludge volume, while at the same time stimulating the development of new microbial communities. Biological treatment using earthworms (*Eisenia fetida*) in combination with compost material is a relatively long process, resulting in gradual structural changes in the sludge and a noticeable reduction in odor intensity, consistent with partial biological stabilization. The use of a natural clay material, pyrophyllite schist, provided several additional advantages. In addition to odor reduction, this treatment resulted in a final product with a significantly lower moisture content and the complete removal of harmful microorganisms, particularly fecal bacteria. Compared to the commonly used alkaline stabilizing agent $Ca(OH)_2$, which is quite aggressive and strongly alkaline, pyrophyllite schist proved to be a milder but effective alternative for sludge stabilization. These results indicate that combining biological agents and natural materials in waste sludge treatment can produce more stable and environmentally acceptable by-products with reduced odor emissions, highlighting the potential of this approach to improve the sustainability of sludge management practices.

AKNOWNLEDGEMENT

The authors would like to sincerely thank AD Harbi d.o.o. Konjic for kindly providing the pyrophyllite schist used in this research. Their support played an important role in making this study possible.

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Summary/Sažetak

Tretman komunalnih otpadnih voda proizvodi značajne količine kanalizacionog mulja koji zahtijeva stabilizaciju prije odlaganja ili ponovne upotrebe zbog potencijalnih rizika po okolinu i zdravlje ljudi. U ovom radu procijenjena je efikasnost mikrobioloških, bioloških i fizičkih metoda tretmana mulja primjenom fizičko-hemijskih, mikrobioloških i ekotoksikoloških analiza. Mikrobiološki tretman mješovitom kulturom mikroorganizama doveo je do smanjenja neugodnih mirisa i djelimičnih promjena sadržaja organske materije. Biološki tretman primjenom vrste *Eisenia fetida* rezultirao je postepenom strukturnom transformacijom mulja i smanjenjem patogenih indikatora, ali je zahtijevao produženo vrijeme tretmana. Fizička stabilizacija primjenom pirofilitnog škriljca značajno je povećala ukupni sadržaj suhe materije (sa 17,75% na 48,32%), smanjila sadržaj isparljivih materija (na 2,26%) usljed kombinovanih efekata stabilizacije i razrjeđenja, imobilizirala odabrane teške metale te dovela do eliminacije fekalnih bakterija i drugih patogenih mikroorganizama. Ekotoksikološka procjena pokazala je povećanje vrijednosti LC_{50} sa 27% u sirovom mulju na 58% nakon fizičkog tretmana, što ukazuje na smanjenu toksičnost. Dobijeni rezultati potvrđuju da pirofilitni škriljac predstavlja efikasan prirodni materijal za unapređenje fizičko-hemijske stabilnosti i higijenske sigurnosti kanalizacionog mulja te ukazuju na potencijal kombinovanja bioloških agenasa i prirodnih mineralnih materijala kao održivog pristupa upravljanju muljem.