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The effect of different additives on the immobilization of heavy metals, the stability of organic matter, and the ecotoxicity of municipal sewage sludge

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Abstract: There is an ongoing search for the most environmentally friendly use of various materials as additives to improve sludge properties. The aim of this study was to analyze the effect of additive type and dose of biochar (BC), dolomite (DL), bentonite (BN) or diatomite (DT) on heavy metal immobilization, organic matter stability, biochemical activity and ecotoxicity of municipal sewage sludge (SS). Risk Assessment Code (RAC) and Ecological Risk Factor (ERF) values indicated low risk associated with heavy metal (HM) mobility. Higher non-hydrolyzing C contents, compared to SS control, were found in SS to which 1% BC (by 3.6%), 1% DL (by 38.0%), 1% BN (by 25.5%) and 1% DT (by 20.6%) were added. Higher ratio C humic acids/C fulvic acids (Ckh/Ckf) values (more than 30% on average) were obtained when 1% BC, DL or BN were added compared to the 3% addition. Compared to the SS, the Ckh/Ckf values in SS with the addition of BC, DL or BN were on average more than 19% higher. The least varied values of dehydrogenase activity and respiration activity of SS (within dose) were obtained after the application of DT and BC, while the values of both parameters differed the most when BN was added to SS. Based on the results, it is not possible to clearly state which of the applied additives had the most significant effect on reducing the biochemical activity of SS. Sewage sludge and its mixtures generally showed low phytotoxicity to *Sorghum saccharatum* and *Lepidium sativum*, as root growth inhibition was 8–56% and 17–48%, respectively.

Keywords: sludge properties, heavy metals, organic matter, enzymatic activity, ecotoxicity

INTRODUCTION

Sewage sludge (SS) is a valuable but environmentally problematic by-product of human existence. The continuous development of cities and the consequent expansion of the sewerage networks result in the generation of more and more waste water and, consequently, larger quantities of SS. It is predicted that the increasing amount of SS generated will be one of the major waste management problems in many countries (Bolesta et al. 2022, Cárdenas-Talero et al. 2022). According to Molaey et al. (2024), global sludge production will increase by more than 50% by 2050, from the current 45 million dry tons to nearly 68 million dry tons. In recent years, the way in which SS is managed

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in Poland has changed. The amount of SS going to landfills has been decreasing, partly due to changing regulations. As calculated by Bolesta et al. (2022), in 2000 there were 474.5 thousand tons of dry matter stored in Poland, 10 years later (2010) there were 165.9 thousand tons of dry matter, and in 2020 there were only 63.9 thousand tons of dry matter. It should be noted that during this period there was a very large increase in the amount of SS that underwent thermal conversion. Taking into account the reuse of SS, it should be noted that in 2000 as much as 37.77% of all sludge produced was spread on land with agricultural use. 20 years later, the agricultural use of SS had decreased to 21.97% (Bolesta et al. 2022).

The significant content of organic matter rich in plant nutrients predisposes SS to fertilizer use or soil reclamation. On the other hand, SS is a rich source of pathogens, including gastrointestinal parasites, organic and inorganic contaminants (Fijalkowski et al. 2017, Lamastra et al. 2018, Bolesta et al. 2022, Styszko et al. 2022). It is also worth noting that an important barrier to the management of SS is its consistency, as well as its odor (Bień & Bień 2021, Wysocka 2023). The problem of the environmental use of SS is mainly related to the persistence of pollutants accumulated in them. Deposited contaminants from SS in the soil environment can also undergo a number of transformations depending on climatic and soil conditions. This poses a threat to human and animal health and, consequently, to entire ecosystems. Substances deposited in the soil environment may provide a substrate for the formation of intermediates or mixtures of compounds that have toxic, teratogenic or genotoxic effects on living organisms (Gryta et al. 2013, Fijalkowski et al. 2017, Taheran et al. 2018, Bolesta et al. 2022).

The management of SS requires that it undergo prior stabilization processes (Act on waste, 2012). Current EU and national sludge regulations do not consider the latest technologies or alternative options for recovering components from SS (Directive 86/278/EEC, Directive 91/271/EEC, Directive 2008/98/EC, Act on waste 2012, Regulation of Minister of the Environment 2015). In addition to scientific and technological advances and policy changes, activities in the field of the circular economy, bio-economy strategy, farm-to-table strategy or fertilizer product regulations have also changed (Directive 86/278/EEC, European Commission, n.d.). This regulatory and technological mismatch highlights a clear gap in research and implementation, namely the lack of comprehensive, experimentally validated approaches that address environmental safety, material stabilization and resource recovery from SS simultaneously.

Despite the ever-increasing knowledge of the use of various materials as additives to SS to improve their physical, chemical and sanitary properties, the most eco-friendly methods are still being sought. The use of substances, minerals and composites of natural origin is explicitly encouraged. Many scientists still consider the toxicity of SS and the content of heavy metals (HMs) and metalloids to be the most critical issues regarding their use. Research results show that elements accumulated in SS can pose moderate to significant environmental risks, especially when As, Cd, and Pb are present in exchangeable forms that are more bioavailable (Kowalik et al. 2022, Aziz et al. 2023, Islam et al. 2023).

Another important issue is the stabilization of organic matter (OM) found in SS. This is a substantial problem, although it is often disregarded. The large amount of OM accumulated in SS is a potential source of atmospheric emissions of carbon compounds and other volatiles. There are few studies that determine the stability of carbon compounds in SS. Research conducted to date is primarily concerned with determining the odor nuisance of SS in order to identify appropriate techniques for their neutralization (Haider et al. 2022). The biochemical activity of various groups of microorganisms is inextricably linked to OM transformation processes. Indicators of changes in the enzymatic or respiration activity of stabilized SS can provide valuable information that will contribute to the development of more effective methods or additives to reduce the environmental impact of SS as much as possible (Benitez et al. 1999). However, there is a lack of integrated assessments linking OM stability, biochemical activity and ecotoxicological responses under controlled amendment strategies.

An obligatory condition for the natural use of sewage sludge and other waste materials is to minimize the risks caused by their introduction into the soil environment (Antonkiewicz et al. 2019, Włóka et al. 2020). Plants are an important link in the trophic chain, acting as producers and often as pioneer organisms colonizing different areas (Oleszczuk 2008). In addition, the size and especially the quality of biomass is the key criterion for determining suitability. The identification of potential phytotoxins in agricultural materials and the assessment of their impact on the soil environment are key issues (Oleszczuk 2008, Baran & Antonkiewicz 2017, Włóka et al. 2020). Phytoassays were used to determine the toxicity of various environmental and anthropogenic matrices (Kopeć et al. 2013, Obidowska et al. 2020, Szara et al. 2020, Wieczorek & Baran 2022, Bandarra et al. 2023).

The use of various SS additives can differentially affect heavy metal bioavailability, organic matter stability, biochemical activity, and, ultimately, ecotoxicity. Managing such SS properties through the use of appropriate additives reduces the risk of environmental problems associated with the application of biologically and chemically unstable SS. The balance between SS use and environmental safety remains a key concern in the management of these materials. Despite the growing interest in SS amendment strategies, evaluations that compare and assess the effectiveness of multiple mineral and carbon-based additives within a single, unified experimental framework are still very limited. This hampers the direct comparison of their effectiveness and environmental relevance. The novelty of this study therefore lies in its systematic and comparative assessment of four commonly proposed environmentally benign additives – biochar, dolomite, bentonite, and diatomite – applied at different doses to sewage sludge. The study simultaneously evaluates heavy metal immobilization, organic matter stability, biochemical activity and plant responses (ecotoxicity). This research provides new insights into SS stabilization strategies that is currently lacking in the literature.

The aim of this study was to determine the effects of (i) the type of additive and (ii) the dose of biochar, dolomite, bentonite, and diatomite on heavy metal immobilization, organic matter stability, biochemical activity, and plant responses to the SS stabilization process used.

MATERIAL AND METHODS

Technology for obtaining sewage sludge

One municipal sewage sludge was used in the study. SS came from a wastewater treatment plant located in the Małopolskie Voivodship (Czernichów municipality, 49°97'97.4''N; 19°73'54.4''E).

The selected wastewater treatment plant treats wastewater flowing in through the sewer system as well as that delivered by a septic tanker. The wastewater entering the treatment plant undergoes mechanical treatment on a vertical screen and in a sand trap with aeration, facilitating the separation of fats and easily flotation substances from the raw wastewater stream. The mechanically treated wastewater is subsequently directed to a retention tank from which it is pumped to bioreactors. In the bioreactors, after filling, the phases of dephosphorylation, denitrification, and nitrification alternate. The process is automatically controlled by monitoring the oxygen content of the effluent. At the end of the biological treatment processes, the bioreactor enters the sedimentation phase, during which the activated sludge sinks to the bottom, while the treated wastewater clarifies in the upper part of the bioreactor. After about 2 hours, the treated wastewater is discharged (the first discharge phase is returned to the treatment processes). The treated wastewater is discharged into the Vistula River. After the discharge, part of the excess sludge is pumped out of the bioreactor and goes to the aerobic stabilization chambers. From the aerobic stabilization chambers, the SS is fed to a decanter centrifuge and dewatered. The SS taken for testing contained 20.2 g/kg dry matter (DM), 276 g/kg DM ash and 417.2 g/kg DM total carbon (Ct). It had slightly acidic pH (5.96) and high electrical conductivity (4,580 μS/cm). No live eggs of intestinal parasites were found in the sludge, while the abundance of *Escherichia coli* was 38,232 cfu/g DM, and the abundance of *Salmonella* was less than 10 cfu/g DM. Selected properties of SS are shown in Table 1.

Table 1
Properties of the SS

Determination	Value ± SE[1)]
DM[2)] [g/kg]	20.2 ± 1.0
Ash [g/kg DM]	270 ± 0
pH_{H2O}	5.96 ± 0.05
EC[3)] [µS/cm]	4,580 ± 4
Total carbon [g/kg DM]	417.2 ± 10.0
Zinc [mg/kg DM]	16,877 ± 619
Lead [mg/kg DM]	19.90 ± 0.99
Cadmium [mg/kg DM]	1.39 ± 0.04
Copper [mg/kg DM]	191.0 ± 6.3
Nickel [mg/kg DM]	51.60 ± 3.49
Chromium [mg/kg DM]	663.5 ± 0.1
Coli group [cfu/g DM]	81,072 ± 14,845
Escherichia coli [cfu/g DM]	38,232 ± 9,052
Salmonella [cfu/g DM]	<10

[1)] standard error, [2)] dry matter, [3)] electrolitical conductivity.

Materials used as additives to the sewage sludge

Biochar (BC), dolomite (DL), bentonite (BN), and diatomite (DT) were added to SS. The biochar (BC) was produced from coniferous biomass residues by CarbonTeam using pyrolysis at temperatures of up to 500°C. BC was characterized by low ash content (99 g/kg DM), an alkaline pH (7.74), and the highest specific surface area (S_{BET} = 185.6 m^2/g) compared to the other materials used in the study. Dolomite (DL) came from PPUH "Dolomit" Kopalnia Ząbkowice S.A., its pH was alkaline, and the ash content was higher than that of biochar (915 g/kg DM). In addition, DL had the lowest specific surface area (S_{BET} = 3.0 m^2/g) among the materials selected for the study. Commercially available calcium bentonite (BN) (PTH Certech Sp. z o.o., Poland) was used in the study. The ash content of the BN used was 881 g/kg DM, and the S_{BET} value was 36.2 m^2/g. The diatomite (DT) used in the study was obtained from Specjalistyczne Przedsiębiorstwo Górnicze "Górtech" Sp. z o.o. DT was calcined at 750°C for 0.5 h. The ash content of DT was 808 g/kg DM, the pH value was 5.84, and the specific surface area (S_{BET}) was 25.9 m^2/g. The selection of BC, DL, BN, and DT was intentional, based on their diverse physicochemical properties and widespread availability. The additives applied represent four commonly used groups of materials: (i) carbon-rich materials (biochar); (ii) alkaline mineral additives (dolomite); (iii) clay minerals with a high cation exchange capacity (bentonite); and (iv) siliceous materials with a highly porous structure (diatomite). Including them enabled the comparison of materials that differ fundamentally in terms of their surface chemistry, mineral composition, sorption mechanisms and potential to modify pH and soil structure.

Selected properties of BC, DL, BN, and DT are shown in Table 2.

Table 2
The properties of the materials

Determination	BC[1)]	DL[2)]	BN[3)]	DT[4)]
DM[5)] [g/kg]	952 ± 5[6)]	942 ± 5	949 ± 5	855 ± 4
Ash [g/kg DM]	99 ± 0	915 ± 5	881 ± 4	808 ± 4
pH_{H2O}	7.74 ± 0.02	7.87 ± 0.05	9.31 ± 0.01	5.84 ± 0.07
EC[7)] [µS/cm]	330 ± 2	107 ± 2	842 ± 12	392 ± 2
Total carbon [g/kg DM]	711.2 ± 11.1	126.3 ± 2.3	6.9 ± 1.0	16.4 ± 2.7
Zinc [mg/kg DM]	30.0 ± 9.5	380.8 ± 27.2	28.6 ± 3.3	40.7 ± 1.7
Lead [mg/kg DM]	2.3 ± 0.6	110.4 ± 1.0	14.6 ± 0.1	14.1 ± 0.1
Cadmium [mg/kg DM]	4.95 ± 0.01	12.89 ± 2.67	1.06 ± 0.00	0.34 ± 0.09
Copper [mg/kg DM]	14.4 ± 3.3	13.2 ± 1.0	21.6 ± 3.0	45.3 ± 1.2
Nickel [mg/kg DM]	18.77 ± 4.27	1.61 ± 0.21	1.13 ± 0.04	21.67 ± 1.03
Chromium [mg/kg DM]	42.57 ± 0.31	2.21 ± 0.08	4.96 ± 0.08	28.53 ± 0.18
BET surface area [m^2/g]	185.6 ± 6.6	3.0 ± 0.1	36.2 ± 1.3	25.9 ± 0.9
Total pore volume [cm/g]	0.088 ± 0.003	0.009 ± 0.000	0.115 ± 0.004	0.064 ± 0.002

[1] biochar, [2] dolomite, [3)] bentonite, [4)] diatomite, [5)] dry matter, [6)] standard error, [7)] electrolitical conductivity.

Mixture preparation procedure

The procedure for preparing the mixtures involved mechanical homogenization of SS (700-gram sample) with additives at 1% or 3% by dry matter of the materials (Table 3). Prior to mixing, the materials were ground in a laboratory mill to pass through a 0.2 mm. The control was SS without additives.

Table 3
Scheme for mixing SS with mineral and organic additives

Treatment	Proportion
SS[1)] control	700 g
SS+BC[2)]1%	SS (700 g) + BC (1.465 g)
SS+BC3%	SS (700 g) + BC (4.396 g)
SS+DL[3)]1%	SS (700 g) + DL (1.465 g)
SS+DL3%	SS (700 g) + DL (4.396 g)
SS+BN[4)]1%	SS (700 g) + BN (1.465 g)
SS+BN3%	SS (700 g) + BN (4.396 g)
SS+DT[5)]1%	SS (700 g) + DT (1.465 g)
SS+DT3%	SS (700 g) + DT (4.396 g)

[1)] sewage sludge, [2)] biochar, [3)] dolomite, [4)] bentonite, [5)] diatomite.

The mixtures were placed in perforated PVC containers (to allow excess water to drain) and transferred to a 0.8 m × 0.9 m × 0.6 m reactor chamber. The reactor chamber was isolated from ambient conditions. During the experiment, the temperature was maintained at 25°C and the air exchange rate was 0.015 m^3/min, 6 times a day. The SS stabilization process was carried out for 90 days. For better homogenization, the material was removed from the reactor once a week and mechanically mixed. The study was performed in triplicate.

Chemical analyses in sewage sludge with additives

pH and electrical conductivity (EC) ware determined potentiometrically in a suspension of material and water (material : water = 1 : 10) (pH-meter CP – 505, conductivity/oxygen meter CCO – 501). Total carbon content was determined in an Elementar CNS Vario MAX Cube analyzer. The fractional OM composition of the developed materials was determined according to the Schnitzer method (Griffith & Schnitzer 1975, Baran et al. 2019).

Total content of the HMs in the materials was determined after the samples were digested in concentrated acids HCl and HNO_3 (1 : 3 v/v) in a microwave oven (Gondek et al. 2024). The mobilities of HMs after experiment were studied using the standard BCR sequential extraction procedure. In BCR method, four metal fractions were separated: fraction F1 – exchangeable, forms easily soluble in an acidic medium (extracted with 0.11 M CH_3COOH and pH = 2); fraction F2 – forms susceptible to reduction, bound to Fe and Mn oxides (extracted with 0.5 M NH_2OHHCl, pH = 1.5), F3 fraction – oxidation-prone forms bound to organic matter (extracted with hot 30% H_2O_2 and 0.5 M CH_3COONH_4, pH = 2); fraction F4 – residual, mineral-bound forms (hot-digested in concentrated HNO_3 acid). In the extracts obtained, the HM content was determined by inductively coupled plasma atomic emission spectrometry using an ICP-OES instrument, Perkin Elmer Optima 7300 DV.

The efficacy of additives in the immobilization of HM in SS was analyzed applying: Risk Assessment Code (RAC) (RAC ≤ 1% – no risk, 1% < RAC ≤ 10% – low risk, 10% < RAC ≤ 30% – medium risk, 30% < RAC ≤ 50% – high risk, 50% < RAC – very high risk), Individual Contamination Factor (ICF) (ICF ≤ 1 – low contamination, 1 < ICF ≤ 3 – moderate contamination, 3 < ICF ≤ 6 – considerable contamination, ICF > 6 – very high contamination), and Ecological Risk Factor (ERF) (0 < ERF ≤ 0.4 – low risk, 0.4 < ERF ≤ 1 – medium risk, ERF > 1 – high risk) (Liang et al. 2017, Baran et al. 2024).

Biochemical analyses in sewage sludge with additives

The respiration value (RV) was determined in accordance with the ISO 16072 (International Organization for Standardization 2002) method. The dehydrogenase (DhA) activity was determined by the transformation of colorless, water-soluble 2,3,5-triphenyltetrazolium chloride (TTC) into redwater-insoluble 1,3,5-triphenylformazan (TPF) (Thalmann 1968).

Ecotoxicity analysis of sewage sludge with additives

The phytotoxicity of the mixtures was measured using the Phytotoxkit liquid sample test (Phytotoxkit 2004). This test used: *Lepidium sativum* and

Sorghum saccharatum and two parameters: number of germinated seeds and root length (Phytotoxkit 2004). Three indicators were calculated in the study: percent germination inhibition (IG), percent root growth inhibition (IR), and germination index (GI) (Phytotoxkit 2004, Szara et al. 2020).

Statistics

Data were analyzed using the statistical package Statistica version 13.1 (StatSoft Inc., Tulsa, OK, USA) and Microsoft Excel 2016. Using the Shapiro-Wilk test, the data were checked for normality of distribution. Two-way ANOVA (additives, dose) was used to identify overall significant differences between the treatments. When significant differences were observed, treatment means were separated using the Tukey's (HSD) test with a level of significance of $p < 0.05$. Treatment groups were compared with each other and with SS control. The standard error for the determined properties in the input materials (SS, BC, DL, BN, DT) was calculated using the following formula:

$$Standard\ error = \frac{s}{\sqrt{n}} \qquad (1)$$

where: s – standard deviation, n – number of repetitions.

RESULTS

Selected chemical properties of sewage sludge with additives

After 90 days of stabilization, the dry matter (DM), pH, and electrical conductivity (EC) of the SS analyzed with different additives did not differ significantly (Table 4). Treatments with a 3% addition of BC, DL, BN, or DT generally had slightly higher DM contents and increased pH values by about 0.1 units. An inverse relationship was observed for EC values. There was significant variation in total carbon (Ct) content. The lowest Ct content was found in SS without additives and in plants where SS was mixed with BC (SS+BC1%, SS+BC3%). Ct contents were significantly higher in SS of the other treatments, and the highest Ct content was determined in SS with the addition of 1% DL, BN, or DT.

Table 4
Dry matter content, total carbon, pH, and electrical conductivity values in SS with additives

Treatment	DM[1] [g/kg]	pH H_2O	EC[2] [mS/cm]	Ct[3] [g/kg DM]
SS[4] control	258 a[5]	6.87 a	4.36 a	306 a
SS+BC[6]1%	261 a	6.58 a	4.65 a	306 a
SS+BC3%	264 a	6.72 a	4.11 a	295 a
SS+DL[7]1%	259 a	6.65 a	4.26 a	414 c
SS+DL3%	268 a	6.73 a	3.80 a	366 b
SS+BN[8]1%	278 a	6.67 a	4.07 a	381 c
SS+BN3%	272 a	6.72 a	4.31 a	366 b
SS+DT[9]1%	253 a	6.73 a	4.38 a	372 c
SS+DT3%	277 a	6.81 a	3.91 a	359 b

[1] dry matter, [2] electrolitical conductivity, [3] total carbon, [4] sewage sludge, [5] the same letter in columns means no significant differences between the values at $p < 0.05$, [6] biochar, [7] dolomite, [8] bentonite, [9] diatomite.

Content of HMs in in sewage sludge with additives

The mobility of the tested HMs in SS after 90 days of stabilization in accordance with the RAC indicator varied by the type of element and the additive used. The percentage of Cd content in the F1 fraction of the total content was 5.28% in the SS control (Table 5). In other treatments, the RAC values for Cd were significantly lower, indicating a potentially low risk of release. The DL additive was most effective in reducing the Cd forms accumulated in F1.

Cr, similar to Cu, Ni, and Zn had lower mobility compared to Cd (Table 5). RAC values obtained for SS control were lower compared to RAC values for SS mixed with DL, BN, or DT, regardless of the additive size. However, statistical analysis did not show the significance of the differences and the RAC values obtained for all elements indicated a low potential risk of release.

The percentage of lead content in the F1 fraction of the total content was 1.96% in the SS control. In other treatments, the RAC values for Pb were significantly lower, indicating a potentially low risk of release. The DL additive was most effective in reducing the Pb forms accumulated in F1, as in the case of Cd.

The ICF values were low and indicated low HM contamination (Table 5). The greatest variation in ICF values was observed for Cd and Pb.

It was shown that after 90 days of stabilization, the ICF values in SS+DT1% and SS+DT3% treatments were highest for Cd and Pb, although for Pb they were smaller than the ICF values for SS control. For Cr, Cu, Zn, and Ni (except for the SS+BC3% treatment), the ICF values in the BC, DL, BN, and DT treatments were not significantly different from the ICF for SS control.

Lower ERF values indicate an increase in HM content in the less mobile F3 (organic matter-bound) and F4 (residual) fractions (Table 5). The addition of BC, DT and 1% DL to SS was shown to significantly reduce the values of this indicator for Cd. Significant reductions in ERF values for Pb compared to the SS control were obtained in all treatments regardless of the type and amount of additive used. For Cr, ERF values were significantly higher in SS+BC3%, SS+DL3%, and SS+DT3% plants compared to SS control. For Cu, ERF values in SS with BC, DL, BN, and DT additives were comparable to those in SS control, indicating that there was no effect of the additives used on the potential for environmental release of this element.

Table 5
Assessment of HMs mobility in sewage sludge with additives

Treatment	Cd	Cr	Cu	Ni	Pb	Zn
RAC[1] (% in total content metals bound to F1)						
SS[2] control	5.28 b[3]	1.34 a	1.18 a	8.10 a	1.96 b	0.94 a
SS+BC[4]1%	3.41 a	1.46 a	1.25 a	7.56 a	1.60 a	1.14 a
SS+BC3%	3.61 a	1.70 a	1.51 a	9.18 a	1.64 a	1.17 a
SS+DL[5]1%	2.66 a	1.47 a	1.29 a	8.17 a	1.59 a	0.96 a
SS+DL3%	2.72 a	1.78 a	1.49 a	9.06 a	1.29 a	1.11 a
SS+BN[6]1%	2.65 a	1.57 a	1.27 a	8.47 a	1.27 a	1.01 a
SS+BN3%	3.46 a	1.65 a	1.43 a	8.36 a	1.40 a	1.21 a
SS+DT[7]1%	3.93 a	1.63 a	1.43 a	8.49 a	1.39 a	1.22 a
SS+DT3%	3.10 a	1.74 a	1.48 a	9.12 a	1.64 a	1.48 a
ICF[8] (ΣF1–F3)/F4						
SS control	0.128 a	0.077 a	0.025 a	0.194 a	0.047 b	0.061 a
SS+BC1%	0.083 a	0.075 a	0.025 a	0.173 a	0.039 a	0.060 a
SS+BC3%	0.084 a	0.080 a	0.027 a	0.214 b	0.035 a	0.051 a
SS+DL1%	0.074 a	0.082 a	0.027 a	0.189 a	0.040 a	0.053 a
SS+DL3%	0.101 a	0.087 a	0.028 a	0.184 a	0.035 a	0.045 a
SS+BN1%	0.091 a	0.073 a	0.024 a	0.164 a	0.030 a	0.042 a
SS+BN3%	0.111 a	0.078 a	0.025 a	0.149 a	0.033 a	0.045 a
SS+DT1%	0.295 b	0.075 a	0.027 a	0.165 a	0.041 a	0.050 a
SS+DT3%	0.268 b	0.085 a	0.021 a	0.181 a	0.037 a	0.044 a
ERF[9] (F1+F2)/(F3+F4)						
SS control	0.083 b	0.017 a	0.012 a	0.169 b	0.023 b	0.024 b
SS+BC1%	0.051 a	0.019 a	0.013 a	0.153 a	0.018 a	0.025 b
SS+BC3%	0.053 a	0.022 b	0.015 a	0.189 b	0.017 a	0.022 ab
SS+DL1%	0.047 a	0.019 a	0.013 a	0.164 b	0.018 a	0.018 ab
SS+DL3%	0.072 b	0.021 b	0.015 a	0.160 b	0.018 a	0.017 ab
SS+BN1%	0.065 b	0.019 a	0.013 a	0.143 a	0.017 a	0.015 ab
SS+BN3%	0.076 b	0.019 a	0.014 a	0.127 a	0.017 a	0.016 ab
SS+DT1%	0.042 a	0.020 a	0.014 a	0.144 a	0.016 a	0.019 ab
SS+DT3%	0.043 a	0.021 b	0.014 a	0.165 b	0.015 a	0.013 a

[1] Risk Assessment Code, [2] sewage sludge, [3] the same letter in columns means no significant differences between the values at $p < 0.05$, [4] biochar, [5] dolomite, [6] bentonite, [7] diatomite, [8] Individual Contamination Factor, [9] Ecological Risk Factor.

Significantly lower ERF values for Ni compared to SS control were obtained in SS+BC1%, SS+DT1% and after application of BN at both 1% and 3% doses. The highest ERF value for Ni was recorded when SS was mixed with 3% BC additive. ERF values for Zn were not significantly different between SS control and treatments with BC, DL, BN, and DT additives, except for the SS+DT3% treatment where the lowest ERF value was determined. Regardless of the element type and the size and type of SS additive used, the ERF values were low, indicating a low risk of HM release into the environment (Table 5).

Fractional organic matter composition of sewage sludge with additives

In SS to which lower doses (1%) of DL, BN, and DT were added, the humic acid carbon (Ckh) content was significantly higher compared to the addition of 3% of the same materials (Table 6). The opposite was true for the BC addition. The larger addition of BC (3%) resulted in a higher Ckh content in SS after 90 days of stabilization, while the treatment with 1% BC addition had the lowest Ckh content of all the experimental treatments. As with the Ckh content, the lowest fulvic acid carbon (Ckf) content was found in the SS+BC1% treatment (Table 6). Significantly higher Ckf contents compared to the SS control were found in the SS of the other treatments. It should be noted that, inversely to Ckh, higher Ckf levels were found in SS after higher doses of materials were applied.

Table 6
Fractional organic matter composition of SS with additives

Treatment	Ckh[1)]	Ckf[2)]	Cnh[3)]	Ckh/Ckf ratio
	[g/kg DM]			
SS[4)] control	21.59 a[5)]	32.45 a	252.2 a	0.68 ab
SS+BC[6)]1%	18.24 a	26.18 a	261.2 b	0.71 bc
SS+BC3%	24.80 a	36.98 b	232.8 a	0.61 a
SS+DL[7)]1%	31.76 b	34.55 b	348.1 c	0.91 c
SS+DL3%	25.94 a	38.07 b	302.5 bc	0.68 ab
SS+BN[8)]1%	29.98 b	35.89 b	316.4 bc	0.81 b
SS+BN3%	21.99 a	39.84 c	303.7 bc	0.56 a
SS+DT[9)]1%	26.38 b	41.91 c	304.2 bc	0.63 a
SS+DT3%	25.14 a	42.11 c	291.9 abc	0.60 a

[1)] C humic acids, [2)] C fulvic acids, [3)] C nonhydrolizing, [4)] sewage sludge, [5)] the same letter in columns means no significant differences between the values at $p < 0.05$, [6)] biochar, [7)] dolomite, [8)] bentonite, [9)] diatomite.

The content of non-hydrolyzing carbon (Cnh) in SS from each treatment varied not only with the type of additive, but also with the amount added (Table 6). Significantly higher levels of Cnh compared to SS were found in treatments where smaller amounts of BC, DL, BN, and DT were added to SS. Except for the addition of BC, no significant differences in Cnh content (within additive) were found according to the amounts of each material added to SS. Except for the treatments where DT was added to SS, the Ckh/Ckf ratio value was higher after the lower (1%) additions of BC, DL, and BN (Table 6).

Dehydrogenase activity, respiration value, and ecotoxicity of sewage sludge with additives

Except for the addition of BC to SS, DhA and RV showed similar trends (Table 7). Addition of DL to SS at a lower dose (1%) increased DhA and RV compared to the 3% addition. Addition of BN to SS at a lower dose (1%) decreased DhA and RV compared to the 3% addition. 1% and 3% additions of DT to SS did not cause major changes in DhA and RV.

The analysis of GI and IR showed that the applied SS additives increased the phytotoxicity of the tested extracts obtained from each mixture. The doses of the additives had an equivocal effect on the ecotoxicity of samples (Table 7). It is also noteworthy that growth inhibition was observed in all treatments – the GI index was less than 90. For *Sorghum saccharatum*, the greatest growth inhibition was observed in the SS+DT3% treatment and for *Lepidium sativum* in the SS+BN3% treatment. *S. saccharatum* showed a slightly higher sensitivity to the tested SS and its mixtures with different additives than *L. sativium*. The tested parameters were 8–56% (IR) and 37–85% (GI) for *S. saccharatum* and 17–48% (IR) and 54–83% (GI) for *L. sativium*, respectively. Extracts obtained from SS reduced the growth of both plants to the least extent – sludge samples were classified as non-toxic. SS+BC1%, SS+DL3%, and SS+DT1% were non-toxic to *S. saccharatum*. SS+BC3%, SS+DL1%, SS+BN1%, and SS+BN3% showed low toxicity to *S. saccharatum*. All mixtures showed low toxicity to *L. sativum*. Only SS+DT3% was toxic to *S. saccharatum* (Table 7).

Table 7
Ecotoxicological and biochemical indicators in SS with additives

Treatment	Ecotoxicity indicators					
	IG Ss[1)]	IR Ss[2)]	GI Ss[3)]	IG Ls[4)]	IR Ls[5)]	GI Ls[6)]
	PE%					
SS[7)] control	5 a[8)]	8 a	85 b	0 a	17 a	83 a
SS+BC[9)]1%	5 a	15 a	79 ab	0 a	37 a	63 a
SS+BC3%	5 a	26 a	68 ab	5 a	40 a	56 a
SS+DL[10)]1%	0 a	29 a	71 ab	5 a	45 a	50 a
SS+DL3%	5 a	15 a	78 ab	5 a	31 a	64 a
SS+BN[11)]1%	10 a	31 a	59 ab	10 a	44 a	48 a
SS+BN3%	5 a	26 a	69 ab	10 a	48 a	45 a
SS+DT[12)]1%	5 a	13 a	79 ab	5 a	41 a	55 a
SS+DT3%	10 a	56 ab	37 a	5 a	41 a	55 a

Treatment	Biochemical indicators	
	DhA[13)] [mg TPF/g DM/h]	RV[14)] [µg CO_2/g DM/h]
SS control	0.469 c	722.1 b
SS+BC1%	0.317 b	814.8 c
SS+BC3%	0.452 c	807.8 c
SS+DL1%	0.560 d	798.8 c
SS+DL3%	0.270 a	729.7 b
SS+BN1%	0.290 ab	667.4 a
SS+BN3%	0.553 d	1273.4 d
SS+DT1%	0.355 bc	852.0 cd
SS+DT3%	0.358 bc	842.8 cd

[1)] germination inhibition *S. saccharatum*, [2)] root growth inhibition S. *saccharatum*, [3)] germination index *S. saccharatum*, [4)] germination inhibition *L. sativum*, [5)] root growth inhibition *L. sativium*, [6)] germination index *L. sativum*, [7)] sewage sludge, [8)] the same letter means no significant differences between the values at $p < 0.05$, [9)] biochar, [10)] dolomite, [11)] bentonite, [12)] diatomite, [13)] dehydrogenase activity, [14)] respiration value.

Integrated summary of results

After 90 days of stabilization, the basic physicochemical properties of SS amended with BC, DL, BN, or DT remained largely unchanged, with only slight increases in dry matter and pH in treatments with 3% additives. More substantial differences were observed in carbon fractions. DL, BN, and DT at 1% increased total carbon and favored higher Ckh and Cnh carbon contents. Heavy metal mobility indicators (RAC, ICF, ERF) consistently confirmed a low environmental risk across all treatments. DL was the most effective in reducing the proportion of mobile Cd and Pb forms, while Cr, Cu, Ni, and Zn showed generally low mobility regardless of additive type. Several treatments promoted a shift of metals to less mobile fractions, further reducing potential release. Biochemical responses indicated moderate changes in microbial activity depending on additive type, and ecotoxicity tests showed that most mixtures increased seedling growth inhibition relative to untreated SS, though toxicity remained low in nearly all cases. Only SS+DT3% reached the toxic category for *S. saccharatum*. Overall, the additives did not substantially alter the general properties of the stabilized sludge, but they affected carbon stabilization pathways and metal speciation. Lower doses of DL, BN, and DT improved carbon stabilization and metal immobilization, while BC effects were dose-dependent. The observed improvements in carbon stabilization and metal immobilization at lower doses of DL, BN and DT can be explained by their distinct physicochemical mechanisms, which act primarily through pH regulation, cation exchange capacity and surface sorption. Lower additions of dolomite increased pH, promoting heavy metal precipitation and organo-mineral complex formation. However, excessive alkalization may have limited the positive effects of this process. Bentonite and diatomite, characterized by a high specific surface area and sorption capacity, effectively immobilized heavy metals and stabilized organic matter at lower doses. However, higher amendment rates likely led to the saturation of the active sites involved in heavy metal sorption. In contrast, the effect of BC was dose-dependent due to its heterogeneous nature. At a lower dose, biochar may have stimulated microbial activity, temporarily increasing organic matter mineralization and heavy metal mobility. At a higher BC dose, sorption processes likely predominated, resulting in enhanced heavy metal immobilization and improved stabilization of organic carbon. These findings emphasize the importance of optimizing the dose when selecting amendments for sewage sludge stabilization. Despite these differences, all of the amended sludge variants maintained a low ecological risk.

DISCUSSION

For many years, alternative ways to stabilize SS have been sought (Awasthi et al. 2016, Zhang et al. 2017). Various technologies and additives are applied to corrected the physical properties of SS to increase the efficiency of HM mobility reduction and SS hygienization, while reducing adverse environmental effects.

HM content, in addition to sanitary properties, is the primary indicator of the potential for natural, including agricultural, use of SS (Molaey et al. 2024). The addition of BC, DL, BN, or DT to SS undoubtedly caused changes in HM immobilization, as confirmed by the three indicators RAC, ICF, and ERF. The largest changes were observed in the indicator values for cadmium and lead. In general, the RAC, ICF, and ERF values obtained for both elements were lower than those obtained for the SS control (except for the ICF value for Cd after DT addition). A detailed review of the scientific literature by Tytła and Widzewicz-Rzońca (2021) shows that there are not many publications on ecological risk assessment of HM in SS. Ren et al. (2021), using attapulgite as an additive to SS, effectively reduced the bioavailability of Cu, Cr, Ni, and Zn as a result of the conversion of soluble, reducible, and oxidizable forms to the residual fraction. The HM stabilization mechanism was through the fixation of heavy metals in the attapulgite matrix. Yang (2003) evaluated the effectiveness of clinoptilolite modified with ultrasound in binding HM to SS. According to the cited author, the main parameters affecting the ion exchange capacity between clinoptilolite and trace elements from sewage sludge are the value of the metal's ionic radius, the charge density of the cations, and the electric charge of the ions. The present study showed that the values of RAC, ICF, and ERF for Pb and Cd exhibit the most significant alterations. According to Cheng et al. (2021), the use of modified bentonite as a conditioning agent is effective in promoting the stabilization of HM in SS. This can be explained by the fact that the dominant component in BN is montmorillonite. Montmorillonite can adsorb heavy metals through interlayer cation exchange reactions caused by the electrostatic interaction between the positively charged metals and the negatively charged mineral surfaces and the formation of inner-sphere complexes induced by Si–O− and Al–O− functional groups at the mineral edges (Kraepiel et al. 1999). The study did not confirm a significantly better effect expressed by the RAC, ICF, and ERF indicators in relation to the addition of BN to SS. One possible explanation may be the relatively low dose of BN (1% and 3%) applied in the experiment. At such concentrations, the amount of active sorption sites introduced with BN may have been insufficient in relation to the total heavy metal content present in the sewage sludge. Consequently, the adsorption capacity of montmorillonite may have been quickly saturated, limiting its ability to significantly alter metal fractionation and mobility. Huang et al. (2017) refer to the combination of different methods of HM stabilization in SS. In the cited study, the authors chose two types of stabilizers, i.e. fulvic acids and phosphogypsum, which were added to SS before its carbonization (at 350°C, for 1 h). The results revealed that adding fulvic acids and phosphogypsum to SS increased the number of functional groups, such as carboxyl, phenolic, hydroxyl and amino groups, in the resulting materials. This undoubtedly contributed to reducing the mobility of HMs in SS after carbonization. In the present study, although no carbonization process was carried out after the addition of BC to SS, it is expected that the mechanism by which HMs were immobilized may have been similar. Immobilizing HMs in SS is not straightforward due to the variation in the chemical composition and physical properties of the SS matrix. Additionally, important parameters that determine the success of the process include the properties of the elements themselves, such as metal ion radius value, cation charge density, and ion electric charge. Analyzing the value of the specific surface area (S_{BET}) of the additives used, it should be noted that the main immobilization mechanisms of the studied HMs were adsorption processes for the BC additive and probably the formation of hydroxide precipitates and ion exchange for the DL additive. In the case of BN and DT additives, possible mechanisms of HM immobilization could have been adsorption, ion exchange, and hydroxide precipitation. The use of different SS additives for HM stabilization is certainly determined by their physical and chemical properties, although the additive size can also play a major role. According to Wang et al. (2018), as the amount of fly ash added increases, changes in HM mobility may be accompanied by alterations in the activity of processes involved in OM humification in SS.

The transformation of OM in SS during its stabilization is contingent upon various factors, including the conditions (e.g., air availability, temperature) and the additives introduced into SS (Rigobello et al. 2017, Klučáková 2018, Michalska et al. 2022). The content of OM and the ratio between the content of humic acids and fulvic acids may be important for the stability of the HMs contained in SS (Zhang et al. 2017). He et al. (2009) suggested that heavy metal speciation and phytotoxicity of SS depended on factors such as pH and OM content and properties. In the present study, the total carbon content of the stabilized SS was at similar levels, with the lower additions of BC, DT, BN, and DT favoring higher Ct content in SS. A similar trend (except for the BC additive) was observed for the Ckh content. OM transformations are inextricably linked to the activity of biochemical processes. The results showed that the addition of BC, DL, BN, or DT to SS had an ambiguous effect on the activity of microorganisms, which was reflected in the DhA and RV values. Higher (3%) addition of BC, BN, and DT showed higher DhA. Only in the BN treatment, the elevated DhA at higher additions was associated with a heightened RV value.

Based on the available literature, it can be concluded that SS is highly phytotoxic (Baran & Antonkiewicz 2017). This toxicity can be caused by various substances contained in SS, such as HM, polycyclic aromatic hydrocarbons (PAHs), antibiotic or pesticide residues (Baran & Antonkiewicz 2017, Włóka et al. 2020). Baran and Antonkiewicz (2017) found that SS contained high total content of HMs and their readily available forms. In the present study, low phytotoxicity was generally observed in the samples analyzed. The lower phytotoxicity may be due to three factors. First, the extracts of the mixtures were examined, rather than the solid phase. Second, both SS and their mixtures were characterized by a slightly acidic or neutral pH, which greatly reduced the HM mobility. Third, the low phytotoxicity may have resulted from the reduced availability of HMs due to their immobilization by OM contained in SS. Other authors also indicated that the above factors could have an important impact on the ecotoxicity of SS to organisms (Obidowska et al. 2020, Szara et al. 2020). Root growth inhibition showed higher sensitivity than seed germination. Germination capacity is an indicator that is relatively unaffected by the presence of toxic substances in the waste (Szara et al. 2020). Plants germinate in a polluted environment, but later growth is inhibited, so the parameter for assessing root growth is more useful in this type of study. The highest phytotoxicity was shown for *S. saccharatum* in the SS+DT3% treatment. The high toxicity of diatomite to *Heterocypris incongruens* was also reported in previous studies (Gondek et al. 2023). The toxic effects of diatomite were attributed to its acidic pH and the shape of its grains. Sharp, porous, small diatomite fragments can damage the digestive tract of *H. incongruens* (Gondek et al. 2023). The negative effects of diatomite on plant organisms were also observed in a study by Borroso et al. (2021), which showed increased inhibition of plant root growth in a 3-day Phytotoxkit assay where diatomite (DT) was added to the soil.

The results of the study provide important information on HMs in SS and have both scientific and practical potential. It was shown that the addition of BC, DL, BN, and DT significantly affects HM immobilization, with the greatest effects observed for cadmium and lead. The values of the RAC, ICF, and ERF indicators indicate that the additives reduce the mobility of the most toxic HM forms, which is crucial for minimizing environmental risk and SS phytotoxicity. The results demonstrate which additives and at what doses are most effective in HM stabilization, which can directly contribute to the safer use of SS in agriculture or land reclamation. Observed changes in the content of OM, particularly in the carbon content of humic and fulvic acids, indicate that chemical and microbiological processes in SS are key for long-term metal immobilization. The findings suggest the potential use of additive combinations (e.g., biochar + aluminosilicates) to enhance HM stabilization efficiency while simultaneously reducing phytotoxicity. The study highlights the importance of considering microbial activity and OM transformations as integral components of SS stabilization strategies, which may lead to more comprehensive and sustainable management approaches.

CONCLUSION

The stabilization of SS is a key treatment to improve its biological, chemical, and physical properties. Regardless of the element, additive and dosage applied to the sewage sludge, the RAC and ERF values corresponded to low release risk, and the ICF values corresponded to low HM contamination. Despite the low values of the calculated indicators, the study indicates that cadmium and lead may be the most significant problems in the environmental application of sewage sludge. Significantly higher levels of non-hydrolyzing C compared to SS were found in treatments where smaller amounts of BC, DL, BN, and DT were added to SS. In addition to the treatments where DT was added to SS, the Ckh/Ckf quotient values were on average more than 30% higher after the smaller (1%) addition of BC, DL, and BN compared to the 3% addition of these materials. This indicates a more efficient humification of OM. The values of SS dehydrogenase activity and respiration activity, which varied the least as a function of dose, were obtained after the application of DT and BC, and the most after the addition of BN. The tested sewage sludge and its mixtures generally showed low phytotoxicity to the test organisms, *Sorghum saccharatum* and *Lepidium sativum*. Considering the different effects of the analyzed parameters, further research should focus on the development of mixtures of the materials used and their functionalization, e.g. thermal functionalization, to achieve the better stabilization of municipal sewage sludge.

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