CLEAN Soil Air Water
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Quality and Possibilities of Use of Stabilized Sludge Compost Obtained by Inoculation with Geocell-1 Consortium

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Anaerobically stabilized sludge from wastewater treatment is always a challenge from the environmental aspect of management. The agrarian environmental surroundings present a possibility for swift and efficient utilization of compost from anaerobically stabilized sludge in order to increase the quality of the biological product. With intensification of the composting procedure by means of the microbiological consortium Geocell-1 (*Cellvibrio* sp., *Pseudomonas fluorescens* with the addition of micro- and macro-elements), the results show that the compost obtained from stabilized sludge after inoculation is significantly improved in terms of moisture reduction (39–43%), while in the control compost, this value is significantly higher with 61%. The results of the pathogenic effect show a significant reduction in the number of fecal coliform ($<1 \times 10^3$) and *Enterococcus* bacteria ($<1 \times 10^4$) in the inoculated (treated) compost. With a slight decrease in the concentration of limiting factors such as As, Cd, Cu, a quality biological product can be achieved, which can be safely deposited on soil. The phytotoxicological germination test with white mustard (*Sinapis alba*) shows a higher number of sprouting plants with a mixture of treated compost and standard soil for flowers 1:1 and 1:4 compared to the control group.

1. Introduction

Municipal wastewater treatment is an inevitable ecological principle of the European Union. Due to the continuous increase in the amount of wastewater, quantities of waste sludge are expanding as well. In Western Europe, there has been a 50% increase recorded in the last 13 years, and by 2020, this value will reach 13 million tons at the annual level of 12 European Union countries.[1,2] In a global survey in the United States, the amount of waste sewage sludge produced is over 6 million tons per year,[3] while in China this value is significantly higher and amounts to 30 million tons, which has been increased by 13% between 2007 and 2015.[4] Out of the 30 million tons, 80% is inadequately treated.[5]

One of the possibilities is its utilization in agriculture as an organic fertilizer for soil conditioning.[6–8] Due to their chemical properties, communal wastewater, in more than 98% of cases, can be used in fertilization.[9] Final disposal of sludge, and the reuse (recycling) of this sludge (including direct application in agriculture and composting), are the most important options of sludge management in the European Union in the cross section of 15 members (EU-15). In addition, the reuse and sludge incineration will constitute a major practice that will be adopted by the EU-27 (all member states) by the year 2020. Strengthening of these practices in management will lead to the attention and adoption of advanced sludge processing technologies in order to achieve better results for the removal of pathogenic microorganisms and toxic compounds, for controlling unpleasant smells, and for ensuring human health and environmental protection.[10,11] The main ecological guidelines in solving the problems of sludge management depend on a series of multidisciplinary indicators of stabilized sludge quality, and they are determined on the basis of the biological, physical, and chemical characteristics of the sludge. The emphasis is on the results obtained during the examination and proving that it

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DOI: 10.1002/clen.201900023

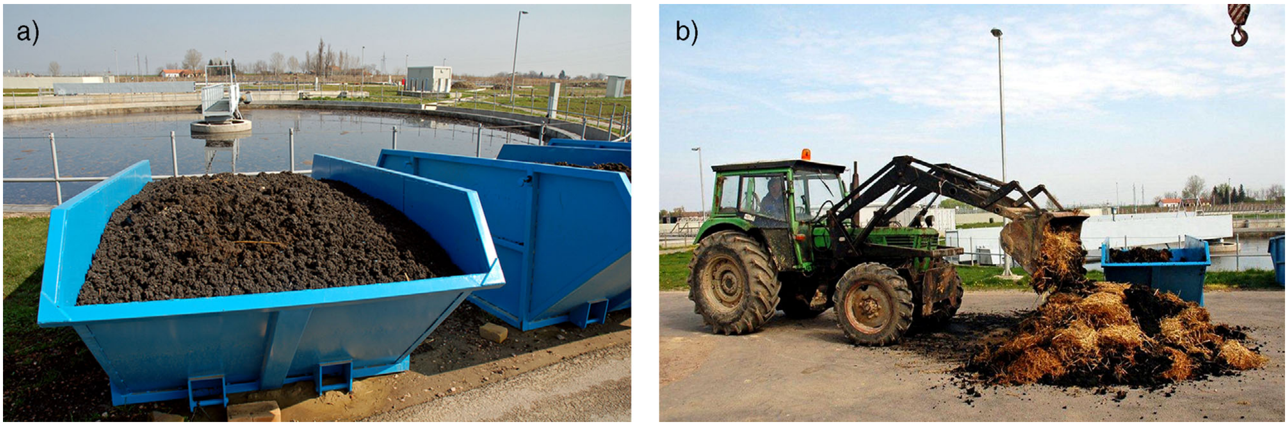

Figure 1. a) Stabilized sludge in containers at the WWTP Subotica and b) mixing of compost material at the WWTP Subotica.

is acceptable and does not cause adverse impacts on the environment. In the European Union, due to the implementation of The Urban Wastewater Treatment Directive 91/271/EEC,[12] the quantity of the sludge and its use for agricultural purposes are further increased.[13] The percentage of sludge in wastewater is between 0.5% and 1%, by its dehydration, this percentage is considerably increased.

Important organic substances that are part of the sludge composition are fats, proteins, and carbohydrates. In the biochemical process of degradation of organic matter (OM), useful and pathogenic microorganisms are present. According to the literature, the amount of municipal sewage sludge is 30 kg per year per capita on average, but this quantity is significantly increased if there is an industry in a given settlement.[14] On the sludge line wastewater treatment plant (WWTP) Subotica, anaerobic digestion is applied as one of the possible methods of sludge stabilization. The anaerobic digestion process is dominant in the stabilization of the sludge from wastewater treatment and provides a surplus of biogas for the energy supply of the plant. During the digestion, the number of pathogenic microorganisms, unpleasant smells, and volume decrease.[15] The stabilization process requires the continuation of closing the ecological cycle of sludge management and its composting. Composting is the right economic and ecological solution for the stabilization of sludge originating from wastewater.[15] Compost is used as a biological product that increases soil fertility properties, stabilizes soil pH value, replaces artificial fertilizers, and introduces micro- and macronutrients into the soil.[16,17] In the past, there has been numeral attempts for conducting co-composting of waste sludge with various organic wastes (straw, sawdust, grass, etc.)[18] in order to improve the final quality of compost.[19]

The development of nanotechnology enables the global spread of engineered nanoparticles (ENPs). In addition to improving the quality of consumer products, ENPs are also used for the improvement of the environment.[20] Due to nanoparticles (ENPs), the quantity of silver nanoparticles (AgNPs) is significantly increased.[21] Multivariate studies of nitrifying and denitrifying gene relationships of bacteria and physico-chemical parameters in the presence of polyvinylpyrrolidine-coated silver nanoparticles (PVP-AgNPs) proved the possibility of extending the composting strategy and the reduction of nitrogen losses.[22] In the study of the evolution of OM and nitrogen (N) during the process of co-composting of waste sludge and agricultural waste, AgNPs have reduced the total nitrogen (TN) losses, but increased the losses of mineral nitrogen.[23] In addition to reducing the loss (TN) and increasing the concentration of mineralized N at the end of composting, AgNPs caused different effects on the gene structure and functional enzymes of nitrogen biotransformation as well.[24]

Due to its chemical properties, sludge is useful as a resource that contains a high amount of plant nutrients and organic substances that are transformed into humus does not dry the soil and nitrogen compounds are easily accessible to a plant.[25,26] By introducing OM into the soil, the capacity for retaining moisture increases. Organic fertilization reduces bulk density and increases soil porosity.[27] Sewage sludge may also contain potentially toxic elements, as well as toxic metals.[28,29] By the Directive of the European Union Council 86/278,[30] concerning the use of sewage sludge in agriculture, certain limit values (LVs) have been set with the aim of preventing harmful effects on environment. One of the possibilities of improving the quality of compost indicators and increasing the efficiency of the decomposition process is inoculation with microbiological consortia.[31] The success of the inoculation is presented in the stabilization of the toxic element (Pb) in contaminated agricultural waste as well.[32] In addition to the bacterial consortium, a good humic effect has been shown by an inoculum of fungal origin: *Trichoderma viride*, *Aspergillus niger*, and *Aspergillus flavus* during communal solid waste composting.[33]

In the research work, a bacterial consortium (Geocell-1) for the first time was used for the purpose of intensifying the composting process. The microbiological agent is intended for the composting of the sludge from wastewater treatment. During the research period of the composting process, the physico-chemical composition, microbiological quality, as well as the phytotoxicological effect of the accelerated composting biological product were examined and compared with control compost from anaerobically stabilized sludge obtained from the treatment of municipal wastewater at the WWTP Subotica for use in agriculture. In the assessment of anaerobically stabilized sludge and compost quality, the foreign (Hungarian) regulations have been taken into consideration, which are harmonized with the European regulations.[34–37]

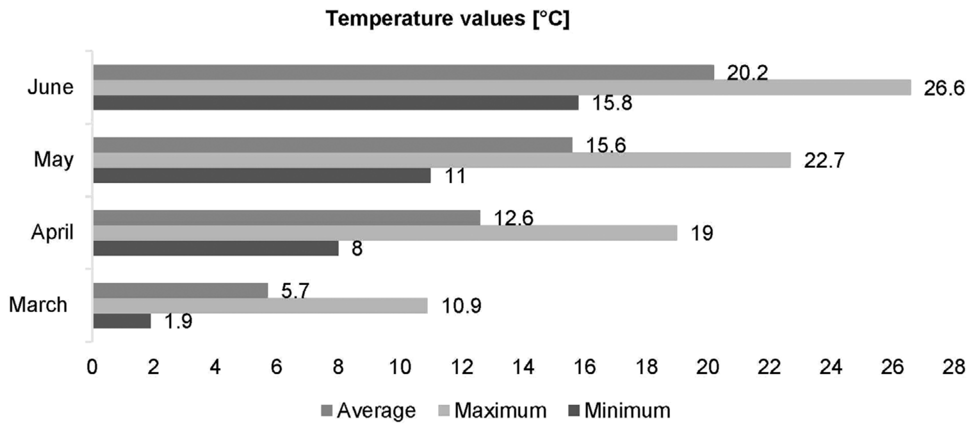

Figure 2. Minimum, maximum, and average air temperature values during composting.

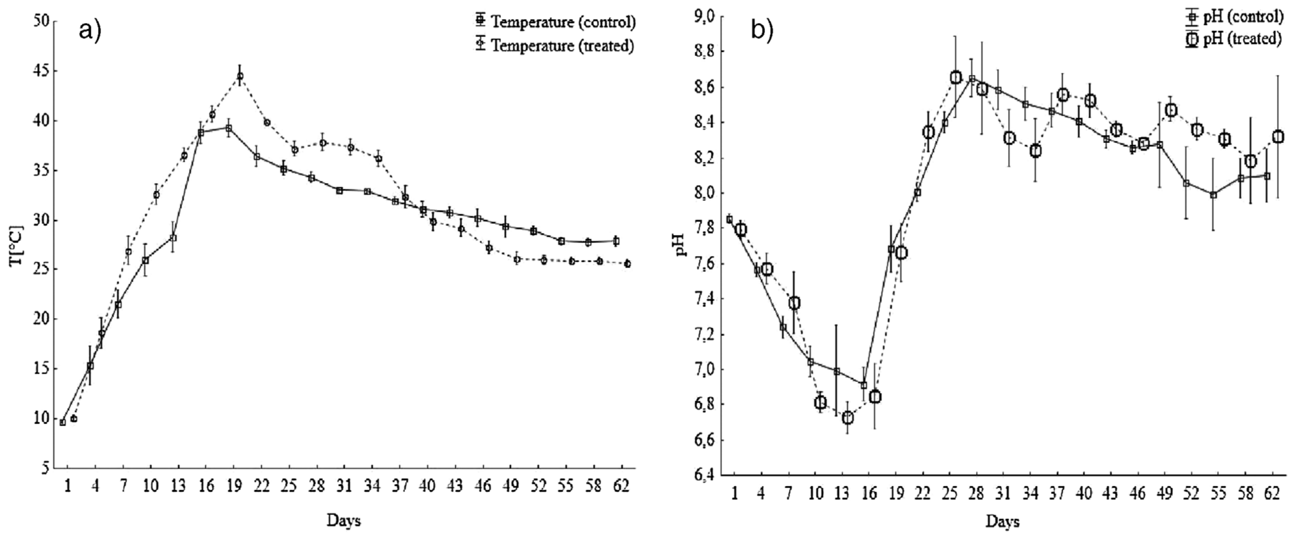

Figure 3. a) Change in temperature and b) pH in special auto-ventilation tank in period (0–62 days).

2. Experimental Section

The material in this research is the digested sludge obtained at WWTP Subotica (which is located at 46°08′ north latitude, 19°69′ east longitude). After detailed physico-chemical and microbiological examinations, digested sludge was subjected to the accelerated composting for three months (March–June) in 2017. The composting was carried out in four open steel containers (A, B, C, and D) of 5 m^3 with auto ventilation (experimental unit with wooden pallets). In the container, digested sludge with 18.6% to 21.6% of dry matter (DM) from wastewater treatment and chopped wheat straw were mixed in the ratio of 1:3 (**Figure 1**a,b). The containers were covered with PVC foils only in case of rain. In the containers with auto ventilation, the material was fully stirred five times during composting in order to achieve better homogenization.[37] For the purpose of achieving a more intensive composting process, a liquid microbiological agent, Geocell-1, (*Cellvibrio* sp., *Pseudomonas fluorescens* with the addition of micro- and macroelements) produced by Geosan Környezetvédelmi, Hungary, was added in the amount of 1 L m^{-3}.

In the first two containers (A and B), composting was carried out without the addition of microbiological strains, while Geocell-1 was added to the other two containers (C and D), in order to improve the composting process. To evaluate the effect of Geocell-1 on physico-chemical characteristics and hygienic parameters, the following schedule was conducted in parallel with a larger sized steel-based container. The compost samples were taken from two controls and two microbiologically treated mixed compost samples at the beginning of the composting process. Five hundred liters of control input compost material and 500 L of inoculated mixed compost were homogenized separately, 250 L of compost was split into six 80-L HDPE containers with auto-ventilation. Three containers were used for control mixed compost, and the other three for inoculated compost composting. Three replications for each treatment confirmed the exact comparative analysis on pH level, core temperature, and relevant hygienic indicators.

Data on the climatic conditions that prevailed during the March–June 2017 were obtained from the Republic Hydrometeorological Service of Serbia (RHMS).[38] The data were read at the Meteorological Station Palić (latitude: 46°06′, longitude: 19°46′).

2.1. Dewar Test

The compost stability was determined by self-heating values. To determine the stability of the compost, determination of the compost maturity degree, self-heating test was used. The test allows the degree of maturity of the examined compost mixture to be determined within 72 h through the achieved maximum temperatures of self-heating.[39] The moisture content of the sludge samples from municipal wastewater was set at 65% by weight under laboratory conditions, and for the purpose of feedback control, a pin-type mobile moisture meter was used both in the control (A, B) and in the mixture treated with the microbiological inoculants (C, D). For the self-heating test, a double-walled cylindrical vacuum chamber of 1.5 L with an inner diameter of 100 mm was used. After filling with a mixture of digested sludge and straw, the temperature was measured for 10 days every 12 h.

2.2. Physico-Chemical Testing

Physico-chemical testing of digested sludge and produced compost was carried out within the accredited laboratory of SGS Hungaria in Kecskemét. In addition to analytical and calculation determinations, instruments (WTW Multiline P 4; ICP-OES TJA) were used according to Hungarian standards that are equivalent to the following international standardized methods: pH and TN[40]; moisture content and OM[41]; total soluble salts; total phosphorus, phosphorous expressed as P_2O_5 by calculation; potassium, potassium expressed as K_2O by calculation; sodium; density; calcium; magnesium; iron; manganese; boron; zinc; molybdenum; lead; nickel; chromium; cobalt; and cadmium.[42]

2.3. Microbiological Testing

Microbiological analyses were carried out in an accredited laboratory of Food Analytica from Békéscsaba. Detection of pathogenic microorganisms was done according to the method EN ISO Standards.[40]

2.4. Ecotoxicological Testing

The ecotoxicological test was carried out at the water resource recovery facility laboratory within the Public Utility Company Waterworks and Sewerage Subotica and was conducted according to the standard.[43] As a test plant, white mustard (*Sinapis alba*) was used. The test on *S. alba* is suitable for measuring the overall toxic effect of the compost. An analysis of the influence of the compost produced from digested sludge on the germinability of the seed was conducted. A composite (medium) was made from the containers A–D. This composite sample was then divided into three smaller samples and was mixed with standard soil for flowers in the ratio of 1:1 and 1:4. In this way, a test series of 12 sample pots, a volume of 2×10^{-4} m³ was established. In each pot, 25 seeds were sown. Since sowing, the counting of sprouted plants was carried out three times, and the average height of the plants was measured once. The inhibition of germination (X) in relation to the number of grown plants in the control sample is given in percentages partially in each mixture with the following equation:

$$X = ((K - M/K) \times 100 \tag{1}$$

where K is the number of planted seeds and M is the number of sprouted plants.

The inhibition of germination is the mean mathematical value of the parallelly conducted tests.

Table 1. Mean values of the chemical properties of stabilized sludge and compost.

Chemical element (DM)	Stabilized sludge	Compost	LV FVM 36/2006
pH	7.85	8.34	6.5–8.5
Organic matter [g kg^{-1}]	601.0 ± 24.1	643.0 ± 25.7	≥250
Total soluble salts [g kg^{-1}]	17.3 ± 0.7	38.5 ± 1.5	≤40
TN [g kg^{-1}]	54.8 ± 2.2	46.70 ± 1.72	≥10
TP [g kg^{-1}]	20.5 ± 0.8	28.70 ± 0.96	–
P_2O_5 [g kg^{-1}]	48.7 ± 1.8	59.00 ± 2.16	≥50
K [g kg^{-1}]	7.30 ± 0.23	8.50 ± 0.28	
K_2O [g kg^{-1}]	8.70 ± 0.33	10.20 ± 0.36	≥50

Table 2. Comparison of toxic metal concentrations in stabilized sludge and compost with the LVs of international regulations.

Chemical element	EEC 86/278 in soil	EEC 86/278 sludge	FVM 50/2001 sludge	Compost from stabilized sludge
Pb [mg kg^{-1}]	50–300	750	400	90.80 ± 2.98
Cd [mg kg^{-1}]	20–40	20–40	5	3.99 ± 0.58
Cr [mg kg^{-1}]	100–150	1000–1500	350	78.20 ± 2.56
Cu [mg kg^{-1}]	50–140	1000–1750	750	338.00 ± 13.53
Ni [mg kg^{-1}]	30–75	300–400	100	34.60 ± 1.15
Hg [mg kg^{-1}]	1.0–1.5	16–25	5	0.90 ± 0.03
Zn [mg kg^{-1}]	150–450	2500–4000	2000	786.0 ± 26.2

3. Results and Discussion

3.1. Physico-Chemical Properties of Anaerobically Stabilized Sludge and Compost

The sludge from wastewater treatment contains a high percentage of moisture (dehydrated sludge 75–85%). The results show that the percentage of DM ranged between 18% and 21%, and the moisture content was 79% to 82%, in the containers (A–D) at the beginning of the process of intensified composting, and at the end, after 90 days, the moisture content was reduced in the control compost to 61%, while in the containers, due to inoculation, the moisture content ranged between 39% and 43%, close

Table 3. Microbiological parameters in the intersection of the LVs of domestic and international regulations.

Number of microorganisms	Stabilized sludge (control)	Treated stabilized sludge	Decree FVM 36/2006 for compost	Decree FVM 51/2001 for sludge compost
Salmonella sp. (25 g)	–	–	<2 × 10 MPN g^{-1}, or 2 × 10 MPN mL^{-1} negative	2 × 5 g negative
Fecal coliforms (1 g)	4000	3000	<10 MPN g^{-1}, or 10 MPN mL^{-1}	500 MPN g^{-1}
Enterococcus faecalis (1 g)	20 000	15 000	Not defined	Not defined
Streptococcus faecalis (1 g)			<10 MPN g^{-1}, or 10 MPN mL^{-1}	500 MPN g^{-1}
Human intestinal parasites			100 g, or 100 mL	25 g negative

MPN, most probable number.

Table 4. Quantification of fecal coliforms and *Enterocccus* during the composting period (0–60 days).

Group	Day	Fecal coliforms	*Enteroccocus*
Control	0	7.8×10^5	6.7×10^5
Treated	0	4.2×10^5	3.7×10^5
Control	30	7.6×10^4	3.5×10^5
Treated	30	3.9×10^4	2.3×10^5
Control	60	7×10^3	3.3×10^4
Treated	60	3.8×10^3	1.8×10^4
Control equation		$y = 2.3 \times 10^3 x^2 – 1.3 \times 10^5 x + 1.8 \times 10^6$	$y = 4.2 \times 10^3 x^2 – 1.9 \times 10^5 x + 2.1 \times 10^6$
Treated equation		$y = 1.1 \times 10^3 x^2 – 6.6 \times 10^4 x + 9.8 \times 10^5$	$y = 1.5 \times 10^3 x^2 – 7.4 \times 10^4 x + 9.7 \times 10^5$
Control R^2		0.95*	0.92*
Treated R^2		0.95*	0.86*

a) Most probable number. *$p < 0.01$.

to the final compost value of 40%, as determined by Haug.[44] The temperature reached maximum values in all four containers between 40 and 44 °C on day 5 (Figure **3**a), but the temperatures are lower compared with previous tests by Mello et al.[16] Three days after prism setting, in the course of their research, the authors recorded values even >60 °C, which corresponds to the manual (European Compost Network) for open prisms as well. In all four containers (A–D) the maximum temperatures ranged between 40 and 44 °C during the initial and thermophilic phases, which caused the reduction and lowering of the risk of pathogenic microorganisms to the environment.[45] However, due to the lack of achieving the optimum temperature value (50–55 °C) in the initial and thermophilic phase according to the authors,[46,47] the hygienization of the compost material was partially achieved. During the composting of anaerobically stabilized sludge, the initial pH ranged between 7.8 and 8.6 (Figure 3b) and the final pH of the compost was between 6.0 and 9.6, which did not affect reaching the optimum temperature value of 55 °C.[47,48]

According to official data on the outdoor air temperature values in Subotica, during the 90-day experiment, the daily temperature values ranged between a minimum of 1.9 °C and a maximum of 26.6 °C (**Figure 2**). Because of this, the mixture of stabilized sludge and straw in the containers in March was exposed to cooling from the environment to a great extent, which was, due to the low-mass effect—the containers large surface area of the temperature discharge, largely preventing the start and progression of the initial and thermophilic phases. This is also indicated by authors,[49] because during the research they found that the weather conditions (temperature of the environment) could have an adverse effect on the compost temperature movement. After 90 days, temperatures at the end of composting, with inoculation of bacterial consortium, ranged between 33 and 24 °C, but with some significant differences. Temperatures were lower in containers to which inoculant (biological consortium) was added. Based on the value of decreasing temperature, we can conclude that the degree of stability[50] of the compost obtained by the treatment with microbiological agent (C and D) is higher than in the controls (A and B). The five categories are split into three major classes according to practitioners and European agencies, the categories are the following: the lowest grade (I) is called "fresh-compost," grades II and III are referred to as "active compost," and grades IV and V are termed "finished compost."[51] These categories are contributed to self-heating temperature allocated ranging between 0 and 10 °C and 40 and 50 °C. The result of the test series conducted according to the value Dewar test belongs to category IV, while to over control test is classified as category III.[47,52,53] The decrease in the volume of the control compost ranged from 18% to 21%, while in the treated compost, this volume decrease was 38–43%. The drop in volume of the treated compost has a favorable effect on storage capacity during management. Mean values of the chemical properties of stabilized sludge and compost, in the absence of domestic regulations related to the widespread use of compost, are compared to the Hungarian Decree FVM 36/2006 (**Table 1**). The following chemical properties were examined: organic matter, pH, total soluble salts, TN, total phosphorus (TP), P_2O_5, K, K_2O, moisture percentage, As, Se, Cu, Pb, Ni, Cr (total), Co, Cd, Hg, Na, Ca, Mg, Fe, Mn, B, Zn, Mo, polycyclic aromatic hydrocarbons (PAH), benzopyrene, total hydrocarbons, and PCB.

Stabilized sludge and compost, during the testing of the pH, the amount of soluble salts and OM, have also shown that these meet and do not exceed the specified LVs in relation to the Decree[34,36] whereas the nutrients, N and phosphorus (P), as well as their compounds, are present in a higher percentage, above the average values for nitrogen (3%) and phosphorus (2%).[54] A higher percentage of nutrients represent increased values of compost quality from the aspect of fertilization.[25,55,56]

Table 5. Correlation between temperature and pathogenic bacteria (fecal coliforms and *Enterococcus*).

	Mean	SD	r (X,Y)	r^2	t	p	N
T (°C) (cont.)	23.5	10.6					
Fecal coliforms (cont.)	290 131.1	381 797.9	−0.934354	0.876**	−6.93725	0.000224	9
T (°C) (cont.)	23.5	10.6					
Enterococcus (cont.)	352 333.3	288 545.9	−0.704842	0.506*	−2.62889	0.033967	9
T (°C) (treat.)	24.4	11.9					
Fecal coliforms (treat.)	154 388.9	206 762.8	−0.840851	0.71**	−4.11015	0.004515	9
T (°C) (treat.)	24.4	11.9					
Enterococcus (treat.)	206 222.2	165 721.7	−0.435825	0.19	−1.28116	0.240945	9

r^2, coefficient of determination; *significant at 0.05; **significant at 0.01.

Table 6. Total number of pathogenic microorganisms in the control and treated composts after 90 days.

Indicator	*Salmonella* in 25 g	Number of fecal coliforms	Number of *Enterococcus*
Compost A (control)	Negative	4×10^3	2×10^4
Compost B (control)	Negative	1×10^3	1×10^4
Compost C with treatment	Negative	$<1 \times 10^3$	$<1 \times 10^4$
Compost D with treatment	Negative	$<1 \times 10^3$	$<1 \times 10^4$

Figure 4. Test plant: White mustard (*Sinapis alba*).

The compost has shown that the concentrations of arsenic and toxic elements such as copper (Cu) and cadmium (Cd) are above the LV of the Decree FVM 36/2006 for composts, while the elements lead (Pb), nickel (Ni), total chromium (Cr), cobalt (Co), and mercury (Hg) are below the permissible LVs (**Table 2**).

The mean values of toxic elements from stabilized sludge and compost have fulfilled the criteria of EEC 86/278 Annex 1B,[30] FVM 50/2001,[36] from the aspect of the LVs, while according to the Decree FVM 36/2006[34] in stabilized sludge and compost, the concentrations of copper (Cu), cadmium (Cd), and arsenic (As) were above the LVs of the Decree FVM 36/2006 for the production of biological product-compost (Table S1, Supporting Information).

The values of organic pollutants in the containers (A–D) were in the range of PAH: 0.51 mg kg^{-1} (<1 mg kg^{-1} (LV)), benzopyrene: 0.01 mg kg^{-1} (<0.1 mg kg^{-1} (LV)), total hydrocarbons: 0.13 mg kg^{-1} (<100 mg kg^{-1} (LV)), and PCB: 0.01 mg kg^{-1} (<0.1 mg kg^{-1} (LV)) which is considerably below the permissible LVs of the Decree 36/2006 for the production of compost for widespread use. The sludge tested in Catalonia,[57] from the aspect of organic pollutants, has shown higher concentrations than stabilized sludge and compost obtained at WWTP Subotica. The comparison of the concentration values of organic pollutants at WWTP Subotica with legislation and standard for compost of some countries of the European Union such as Austria, Luxembourg, Denmark, and Germany, show significantly lower values than the prescribed concentrations.[58] The expressed amounts of toxic metals obtained during the testing are supported by the results[59,60] of safe disposal, since they have proven that the load on the soil with an insignificant or a small amount of toxic metals (below the permissible LV), did not cause accumulation. However, for the purpose of safe management of Subotica's anaerobically stabilized sludge, as well as compost material for a longer period of time, further agrochemical research is required in order to control and prevent possible accumulation of toxic elements at the disposal site.[61,62] By comparing the legislation and standards of the European Union member states, for defining the quality of compost,[58] it can be concluded that arsenic in the composted stabilized sludge, from the aspect of LVs, is above the permissive concentrations of European criteria and it is considered to be the most significant limiting factor. The concentration of 73 mg kg^{-1} is higher than the allowed maximum concentration of 25 mg kg^{-1}. Toxic metals (Cr, Cu, Hg, Ni, Pb, Zn) within this legislation and standards show that the values are below the prescribed minimum concentrations that are defined for sewage sludge.

The recommended minimum concentration in the prescribed range between 3 and 20 mg kg^{-1}, is exceeded only by Cd with 3.9 mg kg^{-1}. Under the name "quality sludge compost" as input material, the European legislation establishes more rigorous criteria for the LVs. The examined compost has, in terms of Cd with 3.9 mg kg^{-1} (up to 2 mg kg^{-1}), Cr with 78.2 mg kg^{-1} (up to 70 mg kg^{-1}), Cu with 338 mg kg^{-1} (up to 300 mg kg^{-1}) exceeded the concentrations, but Cr and Cu only with small differences, whereas Ni with 34.6 mg kg^{-1} (up to 60 mg kg^{-1}), Pb with 90.8 mg kg^{-1} (up to 100 mg kg^{-1}), Zn with 786 mg kg^{-1} (up to 1200 mg kg^{-1}), and Hg with 0.9 mg kg^{-1} (up to 2 mg kg^{-1}) were below the permissive LVs (Table 2). In the comparison named

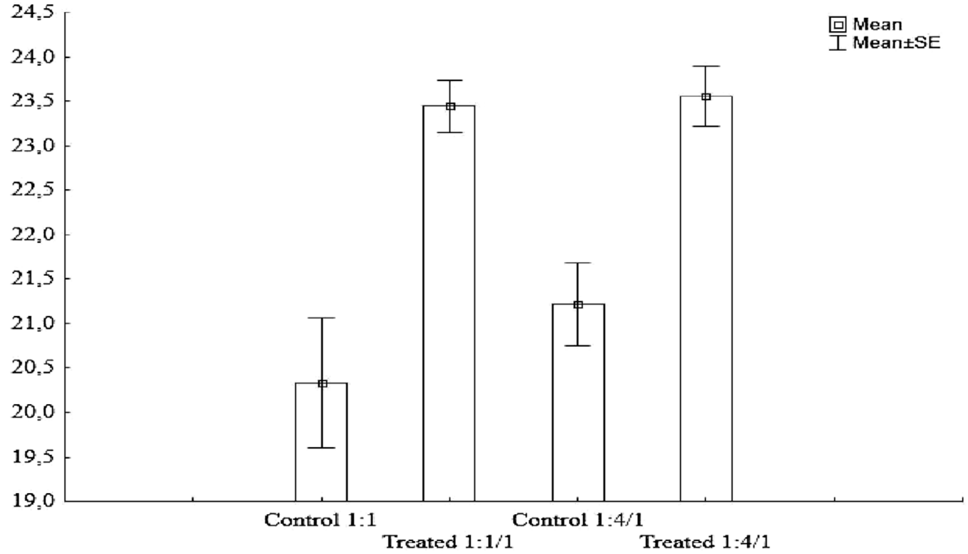

Figure 5. The mean values of the number of sprouted white mustard plants (*Sinapis alba*).

Table 7. Data (two-sample *t*-test assuming unequal variances) of the control and treated compost.

	Control (1:1:1)	Treated with microbiological agent (1:1:1)
Mean	20.33333333	23.44444444
Variance	4.75	0.777777778
Observations	9	9
Hypothesized mean difference	0	
df	11	
t Stat	−3.969734748	
p (T< = t) one-tail	0.001098548	
t Critical one-tail	1.795884814	
p (T< = t) two-tail	0.002197096	
t Critical two-tail	2.200985159	
	Control (1:4:1)	Treated with microbiological agent (1:4:1)
Mean	21.22222222	23.55555556
Variance	1.944444444	1.027777778
Observations	9	9
Hypothesized mean difference	0	
df	15	
t Stat	−4.060293254	
p (T< = t) one-tail	0.000512818	
t Critical one-tail	1.753050325	
p (T< = t) two-tail	0.001025636	
t Critical two-tail	2.131449536	

df, degrees of freedom.

"compost" as input material, only Cd is above the allowed limit (up to 3 mg kg^{-1}) with its 3.9 mg kg^{-1}.[58] According to the European Union Directive EEC 86/271 – Annex 1 C, the possibilities of disposal of stabilized sludge, as well as the compost on the soil were also examined. According to the Nitrates Directive (EEC 91/676)[63] the soil load, with stabilized sludge or compost, was calculated taking into account the concentrations of toxic elements that can burden 1 ha in 1 year. In order to determine the maximum dose, the soil load was tendentiously modeled in quantities of 1, 10, and 20 t (Table S2, Supporting Information).

Based on the results of the studies, it can be concluded that in case of a dose of 20 t ha^{-1}, the content of nitrogen in sludge does not exceed the total concentration of 170 kg ha^{-1} per year, as permitted for nitrate-sensitive soils,[63] and the toxic elements is very favorably and their excepted values are under the limit line regulated by the European Union.

Toxic elements at a load up to a maximum of 20 t ha^{-1} per year are significantly lower in quantities in relation to the permissible LV of EEC 86/278 Annex 1 C.[30] Both the total Cu and Cr concentrations were lower compared to the permissible dose when disposed on land. The total copper concentration was 8.33 times lower, while the total chromium concentration was 35 times lower than the regulation limits. By comparing the results of the LVs of the 27 European Union countries for the purpose of disposal on agricultural land, the concentration of Cd did not meet the LVs of the regulations of Slovenia, Finland, Denmark, and the Netherlands. In terms of the concentration of Cr, only the Netherlands, while in terms of Cu of the 27 countries, only nine have prescribed more rigorous limits. The chemical elements mercury (Hg) and nickel (Ni) cannot comply with only the more stringent regulations of Denmark and the Netherlands (only), while lead (Pb) is below the permissible concentrations and corresponds with each EU member state regulation. From the aspect of the chemical element arsenic (As), out of 27 countries, only eight have defined the concentration limits for agricultural disposal, and the examined sludge has shown that the concentration of arsenic was only below the permissible limit of Hungary and Belgium (Flanders).[64]

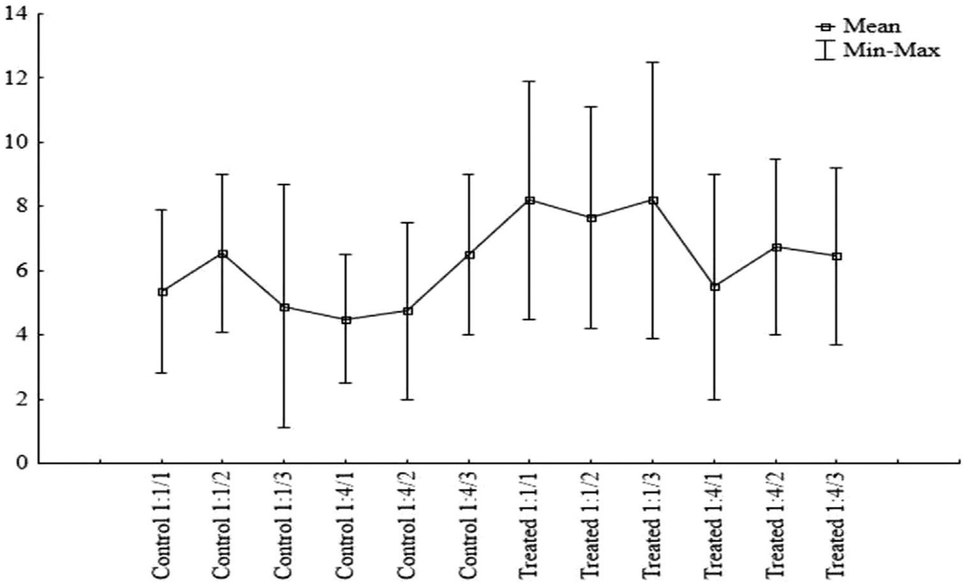

Figure 6. The height of sprouted plants (*Sinapis alba*).

3.2. Microbiological Analysis of Stabilized Sludge and Compost

Due to the technological possibility of exploiting the thermal effect, in the mesophilic range (37–42 °C), of the temperature of anaerobic digestion of stabilized sludge at the WWTP Subotica, before starting the degradation process, the pathogenic microorganism, *Salmonella*, was not detected, which confirms the allegations of Mello et al.[16] about the favorable technological impact of anaerobic digestion in the elimination of this pathogen.

The total number of coliform bacteria, which is a fecal pollution indicator, ranged from 3×10^3 to 4×10^3, while *Enterococcus faecalis* was between 1.5×10^4 and 2×10^4 (**Table 3**, **Table 4**).

The correlation rate of 0.87 (control) and 0.71 (treated) shows that the correlation between fecal coliforms and temperature is positive and statistically significant at the level of 0.01 (**Table 5**). In compost in all four containers with and without treatment, *Salmonella* sp. was negative, while the number of fecal coliforms and *Enterococcus* bacteria (coliforms and *E. faecalis*) was significantly reduced in the containers where a microbiological (inoculative) agent for the intensification of composting was used (**Table 6**).

In addition to reducing the number of pathogenic bacteria using the Geocell-1 bacterial consortium, composting time has been reduced as well, and the compost quality was improved. A similar result has been achieved during inoculation with a microbiological consortium *Bacillus subtilis* B1U/1, *B. subtilis* D3L/1, or *Pseudomonas* sp. RAT/5 at a ratio of 1:1:1 by Pan et al.[65] In order to reduce the total number of pathogenic microorganisms below the LV according to Hungarian harmonized European regulation[34,36] in stabilized sludge and compost, a complete implementation of the controlled composting process is necessary in order to achieve the temperature required for the elimination of pathogenic bacteria.[66]

3.3. Ecotoxicological Results of Compost Testing

The period of germination during the ecotoxicological research was between days 3 and 7 after sowing as it is predicted in the soil without toxic load, during their ecotoxicological studies with the test plant white mustard (**Figure 4**). [67] During the germination test (the number of sprouted seeds), the heights of the sprouted plants, however, have also shown differences between the inoculated and the control compost. After the third week since sowing, the test plant, white mustard (*S. alba*), showed a greater number of sprouted plants in the pots with a mixture of treated compost and standard soil for flowers with ratios of 1:1 and 1:4 compared to the control group (**Figure 5**).

Statistical analysis data (*t*-test) was done for the performance of germination tests where compost has shown enhanced properties from the ecotoxicological aspect of testing. The completed statistical data analysis (*t*-test) after the ecotoxicological germination test with *S. alba* shows that there are statistically significant differences in the number of sprouted seeds between the control and the treated sample. In particular, for 1:1: *t*-statistics is –3.97, with (p-value < 0.001), therefore, it can be concluded that there are statistically significant differences in the number of spiked seeds between the control and the treated sample. Similarly, for 1:4: *t*-statistics is −4.06, with p-value <0.0005 (**Table 7**). Based on statistical analysis, we can conclude that the *X* sludge compost treated with the microbiological agent (Geocell-1) for accelerated composting gives better results of germination, and thus makes the compost ecotoxicologically safer.

On the fourth week after sowing, the average height (cm) of the sprouted test plant, white mustard (*S. alba*), was measured as well (**Figure 6**). The best result from the aspect of height was achieved in pots with a mixture of treated compost and standard soil for flowers with ratios of 1:1:1 and

1:1:3, where the measured heights of the plant were 11.9 and 12.5 cm.

A mixture of the compost treated with the preparation and the standard soil for flowers have provided a significant difference in the average height of the plants compared to the entire control group. The lowest values of plant height were measured in control groups (pots). The ecotoxicological test with an indicator plant showed generally good results from the aspect of toxicological effect.[68] In both the control group and the treated group, the number of germinated seeds ranged between 82.4% and 94.4%. There were no visible phytotoxicity effects regarding anaerobic sludge and compost on the indicator plant, in contrary to some ecotoxicological examinations of dehydrated and anaerobically stabilized sludges using white mustard (*S. alba*).[69]

4. Conclusions

Composting of digested-stabilized sludge is one of the modern forms of rationally closing the ecological cycle of sludge management in agriculture. The results show that the compost obtained from stabilized sludge in the process of intensified composting with the bacterial consortium Geocell-1 at WWTP Subotica has been significantly improved compared to the control compost. Testing for *Salmonella* in the treated compost was negative, and the number of fecal coliforms ($<1 \times 10^3$) and *Enterococcus* bacteria ($<1 \times 10^4$) has improved in terms of volume and pathogenicity reduction. The compost therefore can be safely used in disposal on soil. A quality biological product, compost, could be achieved by means of a controlled industrial composting process by reducing the concentration of limiting factors such as As, Cd, and Cu. In Subotica it is predicted that by 2038 the amount of stabilized sludge obtained from wastewater treatment will be ≈314 000 m³. This volume would have a significant utilization value, from the aspect of nutrition (nutritional value) and could reduce artificial fertilizers usage. The disposal of an enormous amount of stabilized sludge is and will be a burden on the community and the sewage sludge producer.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

Acknowledgements

The results of this experimental work were accomplished by the implementation of semi-industrial composting within the framework of the cooperation between Waterworks and Sewerage PUC Subotica, Serbia and Agrogeo Kft., from Kecskemét, Hungary. We thank Branislav Miščević, Ph.D., for useful discussions.

Conflict of Interest

The authors declare no conflict of interest.

Keywords

compost, ecotoxicology, inoculation, microbiological consortium, stabilized sludge

Received: January 16, 2019
Revised: April 6, 2019
Published online: October 1, 2019

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