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The Evaluation of Hazards to Man and the Environment during the Composting of Sewage Sludge

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Abstract: Composting is considered an effective treatment option to eliminate or substantially reduce potential hazards relating to the recycling of sewage sludge (SS) on land. The variation of four major types of hazards (heavy metals, instability, pathogenic potential and antibiotic resistance) was studied during laboratory-scale composting of two mixtures of sludge and green waste (1:1 and 1:2 *v/v*). The heavy metal content of the final compost was governed by the initial contamination of SS, with the bulking agent ratio having practically no effect. The composts would meet the heavy metal standards of the United States of America (USA) and the European Union member states, but would fail the most stringent of them. A higher ratio of bulking agent led to a higher stabilisation rate, nitrogen retention and final degree of stability. A good level of sanitisation was achieved for both mixtures, despite the relatively low temperatures attained in the laboratory system. The antibiotic resistance was limited among the *E. coli* strains examined, but its occurrence was more frequent among the *Enterococcus* spp. strains. The type of antibiotics against which resistance was mainly detected indicates that this might not be acquired, thus, not posing a serious epidemiological risk through the land application of the SS derived composts.

Keywords: sewage sludge composting; heavy metals; stability; sanitization; antibiotic resistance

1. Introduction

Composting is particularly regarded as an effective method of treatment for overcoming problems relating to the agricultural use of sludge such as the presence of pathogens, certain pollutants, and the uncontrolled fermentation of unstable organic matter [1–3]. The latter may hamper plant growth and give rise to nuisances, while it may also be a source of methane release to the atmosphere [4–7]. Composting is a controlled aerobic process where complex consortia of microorganisms, whose structure depends on environmental conditions and substrate composition, degrade the readily available organic components or transform them into stable humic-like compounds. A decrease in the content of organic pollutants and a reduction in the bioavailability of heavy metals have also been reported during composting [7–10]. In this context, composting may be considered as a preferred strategy for utilising sewage sludge [11].

However, compost is not always a harmless product. Various organic substrates, including sewage sludge (SS), may contain a number of chemical and biological contaminants that pose health and/or

environmental risks. These contaminants may expose different population groups, ranging from composting plant workers to consumers of compost-treated agricultural products and children playing on compost treated parks to health hazards [12–16].

The most important contaminants, with respect to standards for the protection of public health and the environment, are those relating to inorganic and organic potentially toxic compounds and pathogens [17]. Limits on heavy metal concentration and amounts applied to the soil are set in all national standards regulating compost use because their toxicity to humans and the ecosystem is well established [12,18]. In contrast to metals, some organic contaminants, such as hydrocarbons and many pesticides, may be metabolised by microorganisms during composting [19]. More stable compounds, however, such as PAH, PCBs, chlorinated hydrocarbons, PCDD/F, and some pesticides, may persist in the compost and cause grounds for concern that is reflected in several national compost standards (e.g., France, Germany, Austria) [17]. Pathogens constitute a serious issue of concern, especially for SS-derived composts, as many strains of bacteria, viruses, fungi and parasites are commonly found in the raw material [12,20,21]. Pathogenic microorganisms that may be originally present in the SS constitute a potential threat to public health if they enter the food chain [12,13,22].

Another potential hazard relates to the degree of compost stability, which determines nuisance potential, nitrogen immobilization and leaching, pathogen re-growth potential, and phytotoxicity [5,12,23,24]. Of those three main types of environmental and health hazards, the pathogen presence and stability of the final product depend, particularly on the composting process. The presence of toxic compounds depends more on the initial substrate, although composting may still reduce their chemical and biological availability.

Another issue of increasing concern is the spread of antibiotic-resistant microorganisms in the environment that has been connected to the increase of human clinical use and the application of antibiotics in the animal husbandry as feed additives [25,26]. The presence of antibiotic-resistant bacteria has been reported in the soil, surface water, sewage, sewage sludge, and the food chain [27–29]. Sewage treatment plants have a high concentration of bacteria of faecal origin and may act as a reservoir of antibiotic-resistant genes that can be transferred to pathogens or opportunistic pathogens, such as enterococci and coliforms [27,29–32]. Over the last decade, enterococci have been implicated in nosocomial infections worldwide. Due to their ability to acquire high-level resistance to antimicrobial agents such as vancomycin and erythromycin, they have become a serious threat for public health [33,34].

On this basis, the variation of the main hazards to man and the environment during sewage sludge composting was investigated in two different mixtures of SS and green waste. Especially the factors influencing the stabilisation and sanitisation process of SS were examined in order to optimise the overall process in terms of hazard minimisation and production of a high-quality product. Finally, this work aims to gain an insight on the development of antibiotic resistance of microbial populations in sewage sludge as this may also pose serious risks to public health and soil ecosystems.

2. Materials and Methods

2.1. Composting Substrates and System

Dewatered sewage sludge was collected from the biological treatment plant of the island of Kos, Greece, which may serve a Population Equivalent (P.E.) of 53,383 inh/day and a daily mass load of 3233 kg BOD₅ per day. The sewage sludge was stabilised through the processes of thickening, aerobic digestion and dewatering [35]. Green waste (tree and bush clippings collected from parks and squares) from the municipality of Kos was used as a bulking agent after shredding with a knife shredder to approximately 20 mm long chips. Two mixtures of sewage sludge and green waste were prepared, at a volumetric ratio of 1:1 (Cy11) and 1:2 (Cy12), respectively. The mixtures were composted for 45 days in two 40-L bioreactors (Columbus Instruments Oxymax system, Columbus, OH, USA) equipped with continuous on-line monitoring of CO₂ production. The CO₂ infrared sensor operated at an absorption

spectrum centred at $4.25 \pm 0.02 \mu\text{m}$ (0–1% operating range). The air-flow was set at a mean value of 0.3 L/min, but fluctuated from 0.1 to 0.4 L/min. Samples were obtained on 0th, 5th, 10th, 17th, 22nd, and 45th days and at each time the bioreactors were reversed along their horizontal axis after sampling.

2.2. Analysis of Abiotic Parameters

Moisture content (MC; % ww), volatile solids (VS; % dw), pH, and electrical conductivity (EC) were determined according to Reference [36]. The volatile solids reduction (VS_{red}; %) was calculated on a constant ash basis. The total carbon and nitrogen amounts were determined using an elemental analyser (ANCA NT) interfaced to a 20-20 stable isotope mass spectrometer (PDZ Europa); inorganic carbon was assumed to be negligible. Heavy metals (Cd, Cu, Ni, Pb, Zn) were determined by an ICP Atomic Emission Spectrometer (Iris Advantage AP/EWR-Duo Option, Thermo Jarrell Ash, SpectraLab Scientific Inc., Markham, ON, Canada) after digesting 0.5 g samples with 65% HNO₃ in a microwave apparatus (MARS 5 CEM, Matthews, NC, USA) and filtering through Whatman 41 (20–25 μm) filters. Two to three sub-samples were analysed, depending on the sensitivity of the parameter.

2.3. Compost Stability

Compost stability was determined on the basis of three respirometric parameters: (a) a modification of Specific Oxygen Uptake Rate test (SOUR test), which measures the maximum rate of oxygen consumption in an aqueous compost suspension of 5 g (ww) compost in 500 mL deionised H₂O at ambient temperature [33]; (b) the Oxygen Demand, (OD₂₀) during the first 20 h of the same experiment, calculated as

$$OD_{20} = \int_{t=0}^{20} (SOUR)_t \cdot dt$$

where (SOUR)_t is the specific oxygen uptake rate, expressed in mg O₂/g VS/h, at time *t* [37,38]; and (c) the commercially available Solvita[®] test, performed and evaluated according to the manufacturer instructions (Woods End Laboratories, Inc., Mt Vernon, ME, USA). Analysis was performed in duplicates.

2.4. Microbiological Analysis

The sanitisation of the composting was assessed using both pathogen indicator microorganisms and specific pathogens. The following microbial populations were measured in each sample: the total mesophilic aerobic microflora (TMM) was quantified by the pour plate method using Plate Count Agar [39]; the total coliforms and *E. coli* were estimated by plating onto the Eosin-methylene-blue (EMB) selective substrate; the faecal streptococci were measured by plating in the Slanetz–Bartley selective agar for enterococci [40]. Staphylococci were enumerated using the same method and the selective substrate Baird-Parker agar. *Clostridium perfringens* population was estimated according to Reference [41]. Salmonella spp. was measured according to USEPs useA (1998). The Api 20E (BIOMERIEUX) system was used for the identification of the isolated strains. The method for the quantification of the *Listeria* spp. population included 3 stages: sample enrichment in Frazer broth, counting and isolation of strains in Palcam agar, and confirming the tests (Gram reaction, oxidase and catalase tests, indole test, the use of citrates as the carbon source, the use of different carbohydrates as the carbon source, Methyl-Red and Voges–Proskauer reactions, hemolysis). The Api-*Listeria* system (BIOMERIEUX) was used for the identification of the isolated strains [42].

2.5. Antibiotic Resistance

Escherichia coli strains were isolated in EMB substrate and were identified by morphological (Gram- bacilli) and biochemical characteristics (Api 20E, BIOMERIEUX). These strains were tested for antibiotic susceptibility using the disc diffusion method according to the National Committee for Clinical Laboratory Standards (NCCLS) [43,44]. Fifteen antibiotics were examined: ampicillin (10 μg)-(AM-10), ampicillin (30 μg)-(AN-30), amoxicillin/clavulanic acid (30 μg)-(AMC-30),

cephalothin (30 µg)-(CF-30), chloramphenicol (30 µg)-(C-30), ciprofloxacin (5 µg)-(CIP-5), gentamycin (120 µg)-(CM-120), meropenem (10 µg)-(MEM-10), nalidixic acid (30 µg)-(NA-30), nitrofurantoin (300 µg)-(FM-300), norfloxacin (10 µg)-(NOR-10), ofloxacin (5 µg)-(OFX-5), trimethoprim/sulfamethoxazole (1,25/23,75 µg)-(SXT), tetracycline (30 µg)-(TE-30), and tobramycin (10 µg)-(NN-10). Antibiotic discs (BD, Becton Dickinson, Franklin Lakes, NJ, USA) were placed on the agar plates (Mueller—Hinton Agar CM0337, Hants, UK) and were incubated under aerobic conditions at 37 °C for 24 h. The inhibition zones were measured using a ruler and the results were interpreted according to the manufacturer's standards.

Enterococcus spp. strains were isolated in Slanetz–Bartley and were identified using morphological (Gram+ cocci, catalase-negative) and biochemical tests (ability to grow at 10 and 45 °C in nutrient agar supplemented with 6.5% NaCl and in Bile Aesculin Agar) [39]. These strains were tested for antibiotic susceptibility using the disc diffusion method [43,44]. Twelve antibiotics were examined: gentamycin (120 µg)-(CM-120), streptomycin (300 µg)-(S-300), ciprofloxacin (5 µg)-(CIP-5), ofloxacin (5 µg)-(OFX-5), tetracycline (30 µg)-(TE-30), bacitracin (10 µg)-(B-10), trimethoprim/sulfamethoxazole (1,25/23,75 µg)-(SXT), chloramphenicol (30 µg)-(C-30), kanamycin (30 µg)-(K-30), nalidixic acid (30 µg)-(NA-30), erythromycin (15 µg)-(E-15), and vancomycin (30 µg)-VA(30).

3. Results

3.1. Abiotic Factors

Temperature variation (Figure 1) was typical of lab-scale reactors for materials of moderate energy content, such as the sewage sludge-green waste mixtures used in this trial. The large surface to volume ratio in such reactors leads to larger heat losses and does not allow thermophilic temperatures to evolve to the extent that they would in a larger system. Although constant aeration was provided, temperature peaks were recorded after turning during the first 10 days. Turning improves the structure of the substrate, reduces compaction and short-channelling, increases air-pore space and facilitates aeration, while it also exposes fresh organic matter to microbial attack. As the readily available organic matter is consumed, turning does not affect temperature and denotes the end of the active composting phase. This phase lasted for about four weeks, but higher temperatures were maintained in the 1:2 mixture (Cyl2) probably due to better aeration conditions.

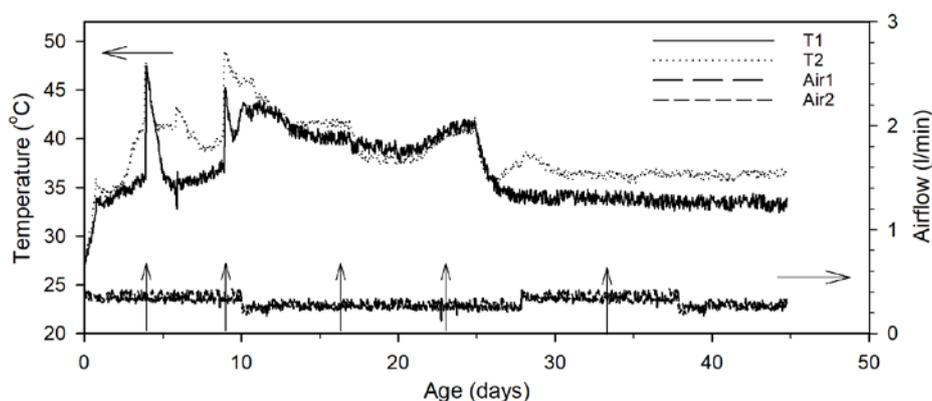


Figure 1. The temperature variation with the composting time. The arrows indicate the bioreactor turning.

The moisture content was above 70% throughout the process in Cyl1, but dropped from an initial value of 65% to 57% in Cyl2 (Figure 2). The variation of VS and pH was typical for the type of materials and the system used [3,45]. The electrical conductivity was low and below 1 dS/m for both mixtures making the compost suitable for plant growth. After 45 days of treatment, VS_{red} was 37.5 and 41.1% for Cyl1 and 2, respectively, with higher differences monitored during the first two weeks

(Figure 2). The CO₂ sensor failed to operate after the first 7 days and caused the loss of important data on the respiration rate in the two reactors. However, large differences in the two mixtures were observed in the brief period of its operation (results not shown), with the cumulative CO₂ production in Cyl2 being twice that in Cyl1 (282 and 147 g CO₂/kg dw, respectively). This was in accordance with the temperature variation and indicates that a higher bulking agent ratio facilitates SS composting probably through the improvement of porosity, moisture content and aeration. However, a complete CO₂ production dataset throughout the composting period would be required in order to assess the effect of the bulking agent ratio on the overall process. A recent investigation, based on gases monitoring during windrow composting of similar mixtures, did not demonstrate any significant difference between the two ratios. The initial higher activity of the higher bulking agent mixture was offset within six weeks of composting. Although there are several references on the effect of the bulking agent ratio on the performance of SS full-scale composting [1,46], further investigation for different composting systems is still needed. This is because the amount of bulking agent is a crucial parameter for the viability of the process in many parts of the world where woody residues and straw are sparse.

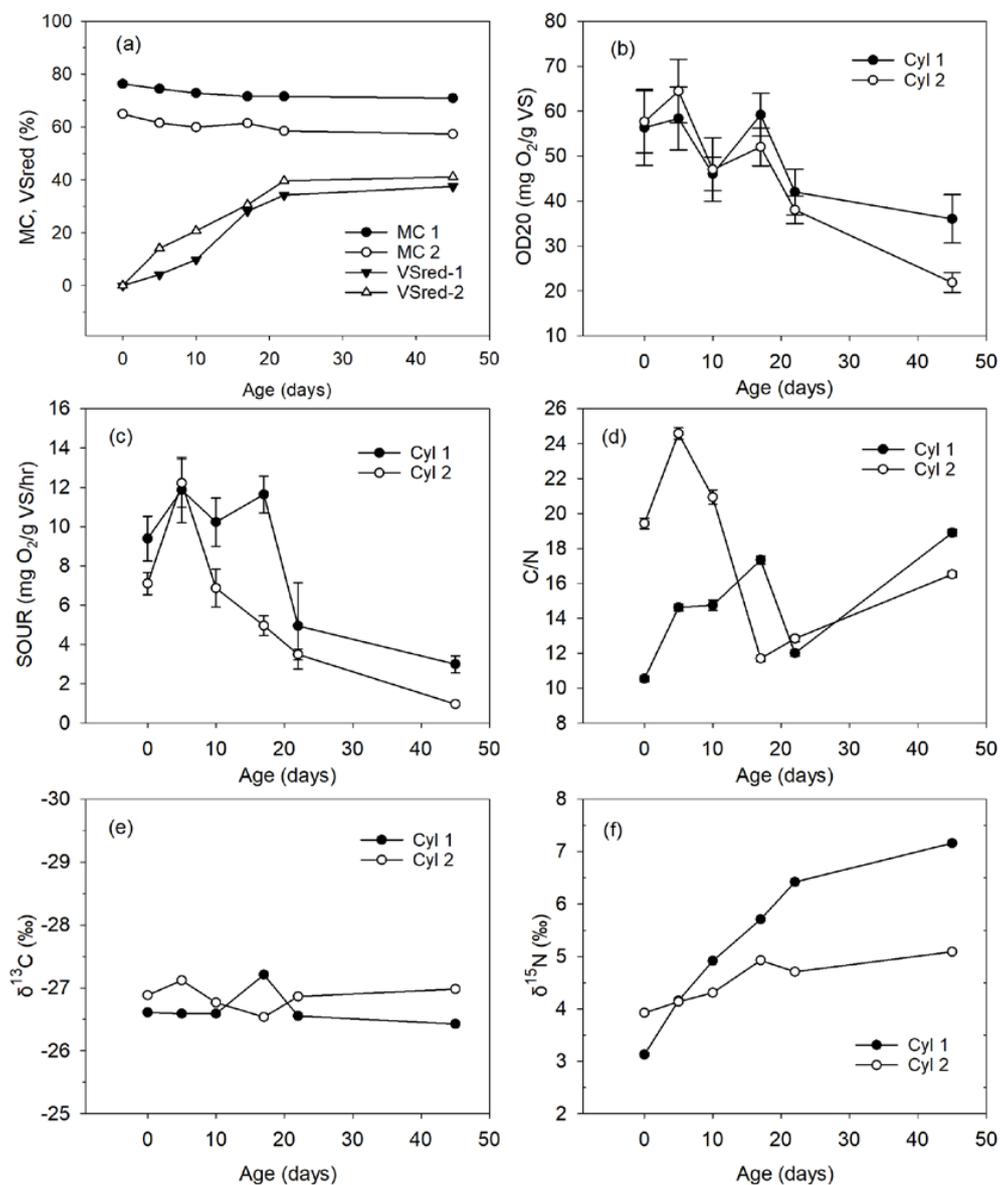


Figure 2. The variation with composting time of: (a) moisture content (%) and volatile solids reduction; (b) OD₂₀; (c) SOUR; (d) C/N; (e) δ¹³C, and (f) δ¹⁵N.

3.2. Heavy Metals

The concentration of all heavy metals, and Cu in particular, in SS, was rather high (Table 1) when considering that there is no heavy industry in a predominantly tourist island. Nevertheless, all metal concentrations were well below the existing EU and Greek limits for agricultural use of sludge (86/278/EEC and Decree 114218, Gr OJ: 1016/B/17-11-97—Table 1). However, the land application of SS is not practised in Greece as the licensing authorities are concerned about potential public health risks and nuisances. It is worth noting that, in most national regulations in the EU, these limit values have been set significantly below the requirements of Directive 86/278/EEC [13]. This variation is also reflected by a great degree of heterogeneity in the compulsory and voluntary compost standards in different countries. It stems from the effort to combine two often contradicting targets: the protection of public health and the environment and the recycling of organic matter. Moreover, the precautionary approach adopted by the EU and the risk assessment approach prevailing in the USA may lead to broad differences in the accepted limit values for a number of critical parameters, such as heavy metals [17]. Even within the EU, there is a wide variation among the limit values adopted by the member countries. The north is usually more stringent than the south and reflects the varying level of progress on environmental protection, but also the different needs in soil organic matter amendment [24].

Table 1. The heavy metals concentration of sewage sludge, initial mixtures, and final compost. Indicative limits are also provided.

		Heavy Metals Concentrations (mg/kg dw)				
		Cd	Cu	Ni	Pb	Zn
Sewage Sludge	Initial	1.2	332	27	151	816
Cylinder 1	Initial	0.9	244	23	114	703
	Final	1.2	292	32	158	896
Cylinder 2	Initial	0.7	180	18	85	519
	Final	1.1	276	27	134	752
Indicative limits	Sludge Directive ¹ & Greek limits for SS ²	20–40	10–1750	300–400	750–1200	2500–4000
	Greek limits for MSW compost ²	10	500	200	500	2000
	Eco-label ³	1	100	50	100	300
	Austrian limits, A+	0.7	70	25	45	200
	A (+50%)	1	150	60	120	500
	B ⁴	3	500	100	200	1800
	French limits ⁵	3	300	60	180	600
	US EPA ⁶	39	1500	420	300	2800
	US Washington State, grade A ⁷	10	750	210	150	1400

¹ Directive 86/278/EEC on the agricultural use of sewage sludge. ² Common Ministerial Decision 114218, Gr OJ: 1016/B/17-11-97: Framework of Specifications and General Programs for Solid Waste Management. ³ Decision 2001/688/EC on the EU eco-label criteria for soil improvers and growing media. ⁴ Compost Ordinance, Quality Class A+ (organic farming), Quality Class A (agriculture, hobby gardening), Quality Class B (landscaping, land reclamation). ⁵ Norme NF U 44-095, May 2002, on Organic soil improvers—Composts containing substances essential to agriculture, spanning from water treatment, ICS: 13.030.20; 65.080. ⁶ USA EPA CFR 40/503 Sludge Rule. ⁷ WA State Department of Ecology, grade A compost (source: [17]).

Regarding the starting mixtures, the heavy metal concentration was 20–25% higher in Cyl1 than Cyl2 and may be explained by the higher green waste content of the latter. As the composting process develops and organic matter decomposes, the mass of the two mixtures is reduced while the quantity of heavy metals remains the same. Therefore, an expected increase in metal concentration [18] was observed in the finished composts, resulting in values similar to the concentration of the SS (Table 1). The reduction in the total heavy metal concentration is only possible through excessive watering and leaching, a process generally avoided due to the costs associated with leachate treatment. On the other hand, composting may reduce heavy metal bioavailability as metals become bound to more

stable humic structures, thus reducing environmental risk [7–9]. In the long term, changes in soil pH and other environmental factors may increase metal mobility and bioavailability. In this respect, regulations worldwide determine the allowed limits on the basis of total heavy metal concentration [18]. In the case that the wastewater sludge exceeds legal standards, mixing it with other organic materials (with properties within limits) is now allowed.

Both finished composts met the Greek, US, and other lenient standards by a wide margin (Table 1). The French limits (Norme NF U 44-095) stand somewhere in the middle between the usually stricter standards of the European North and the somewhat laxer standards of the South. With the exception of zinc, both composts complied with the French standards. However, they failed to comply with the Austrian class A standards for the application of compost to agriculture and hobby gardening with the exception of nickel. Despite its strict and low limits, Austria allows for a considerable variation of the limit values (up to 50%) in which case Cyl2 would fail only for Cu and Cyl1 for Cu and Zn. National standards should not only be compared according to their limit value, but also their “tolerance band”. Tolerance tends to be greater for countries with strict limits (e.g., Austria, The Netherlands) and zero for countries with higher limits (e.g., Greece, Italy). Both composts would also fail the EU and Austrian standards for organic farming. Comparison with these limits is made only to illustrate the wide gap in requirements as the use of sludge derived-composts is not allowed in organic farming regardless of the compost quality.

3.3. Compost Stability

The variation of several parameters was examined to assess the stabilisation process of the organic matter: OD₂₀, SOUR, C/N, the stable isotopes $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (Figure 2e,f) and the Solvita© test. The VSred (Figure 2a) may also serve as a rudimentary stability indicator. These parameters suggested a good level of SS stabilisation during the 45 days of processing with a slightly faster stabilisation of the mixture in Cyl2. Results for the SOUR correlated well with OD₂₀ (for Cyl1: $r = 0.932$, $p < 0.01$; for Cyl2: $r = 0.908$, $p < 0.05$). The respiration activity decreased for both mixtures from an initial OD₂₀ value of about 55 mg O₂/g VS to 36 and 22 mg O₂/g VS in Cyl 1 and Cyl 2, respectively; the latter value typically corresponds to stabilised composts [5]. The corresponding reduction for SOUR was from the initial values of 9.4 and 7.1 mg O₂/g VS/h to the final values of 3.0 and 1.0 mg O₂/g VS/h for Cyl1 and Cyl2, respectively. The initial increase of respiration activity on day 5 may be attributed to the increased availability of readily assimilable substrates, as the more complex compounds in the sewage sludge are broken down, an effect commonly observed in many composts [5,37,38,47–49].

Similar results were obtained by the Solvita© test which is an indicator of CO₂ and NH₄ production, on the basis of two chromatic scales for the gel indicator paddles (1–8 for CO₂ and 1–5 for NH₄). Both initial mixtures had a CO₂ value of 7 which increased to 8 by day 10 for both cylinders and indicated a low activity throughout the process. However, the NH₄ test indicated pronounced differences between the two reactors evolving from an initial value of 1 for both mixtures to a value of 2 and 5, for Cyl 1 and Cyl 2 respectively, in the final compost. According to the Solvita© scale, these values indicate a low initial C/N ratio for both composts. During composting, ammonia volatilisation took place in both cylinders, although to a different extent. The NH₄ values of the final compost indicated an immature compost in Cyl1 due to high ammonia toxicity and a sufficiently stabilised material in Cyl2. Although the Solvita© test is of a rather qualitative nature, it has been shown to correlate fairly well with other stability tests [47,50,51]. The NH₄ test results result were compatible with the difference in the SS to bulking agent ratio between the two mixtures.

The variation of the C/N ratio (Figure 2) was typical for sewage sludge and indicated a stabilisation of SS during composting, although a mixture richer in the bulking agent (Cyl2) may perform better. The initial C/N ratio of 19.2 in Cyl2 was close to the optimal range of 20–25 for composting [52], while the corresponding value for Cyl1 was very low (10.5). The C/N ratio increase from 10.5 to 17.5 in the first three weeks of composting in Cyl1 was mainly due to the nitrogen loss through ammonia volatilisation. The decrease observed in Cyl2 may be attributed to the higher rate

of carbon mineralization, a hypothesis which is in agreement with the previous results. In the final composts, the differences in the C/N ratio were smothered with recorded values of 18.9 and 16.5 for Cyl 1 and Cyl 2, respectively. In combination with the respiration and Solvita© tests, these results indicate that the excess nitrogen initially present in Cyl1 was volatilised, but the mixture in Cyl2 had retained its N content and provided a better agricultural valorisation for the sludge nitrogen (initial N: 3.5% and 2.3%; final N: 2.0% and 2.4%, for Cyl 1 and Cyl 2 respectively; results not shown).

More information on organic matter transformations was revealed by the stable isotope analysis of nitrogen ($\delta^{15}\text{N}$). The $\delta^{15}\text{N}$ increased sharply in Cyl1, from 3.1‰ to 7.2‰ (Figure 2f), indicating an intense ammonia volatilisation in the lower bulking agent mixture. The corresponding increase in $\delta^{15}\text{N}$ in Cyl2 was smaller, from 3.9‰ to 5.1‰. Where volatilisation of ammonia is a significant process, it will leave the remaining nitrogen enriched in ^{15}N , as the lighter isotope will be preferably volatilised. The loss of ammonia from animal manure results in $\delta^{15}\text{N}$ values above 10‰ or even above 20‰. Hence, it has been suggested that $\delta^{15}\text{N}$ can be used to trace the fate of manure N in the soil-plant system [53] or investigate the bacterial activity in composting systems [54,55]. Indeed, the increase in $\delta^{15}\text{N}$ in Cyl1 was more intense during the first three weeks of composting when most of the ammonia volatilisation occurred. The $\delta^{13}\text{C}$ did not show any significant variation during the composting process, for both mixtures (Figure 2), which is in agreement with Reference [55].

Although compost stability is strongly related to the environmental and health risk associated with compost use, there is no stability test universally accepted and many national standards do not include stability limits. The few that do are mainly based on respirometric tests, e.g., the self-heating potential and AT4 in Germany, the RD4 in the UK, and the Dynamic Respiration Index in Italy [56–59]. However, limit values are not directly comparable with the parameters tested herein.

3.4. Compost Sanitisation

The variation of the different microbial populations is presented in Table 2. The total mesophilic population remained practically unchanged during composting at about 5×10^7 cfu/g dw for both mixtures. Fungi increased by one and two orders of magnitude in Cyl1 and Cyl2 respectively, as the material became drier. As pathogen indicators, the total coliforms and faecal streptococci were only reduced in Cyl1 from 8.4×10^5 cfu/g dw to 9.4×10^4 cfu/g dw and from 1.4×10^6 cfu/g dw to 2×10^5 cfu/g dw, respectively. Sanitisation appeared more efficient when judged on the basis of specific pathogens. The *E. coli* population decreased from about 2.5×10^5 cfu/g dw to below detection limits in both mixtures and indicated a good sanitisation of the compost. Similarly, *Salmonella* spp. was detected in the SS and the raw mixtures (60 cells/g dw) but was absent from both composts. *Staphylococcus aureus* and *Listeria monocytogenes* were absent in both the raw material and the composts. Both are human pathogens transmitted with food and associated with serious epidemiological problems [60–62]. *Clostridium perfringens* was reduced from 2.4×10^4 and 3.1×10^4 cells/g dw in the raw mixtures to 8×10^2 and 6×10^2 cells/g dw in the final compost, in Cyl 1 and Cyl 2, respectively. This bacterium is a common member of human enteric flora but also an opportunistic human pathogen. It can survive in high temperatures because it transforms into endospores and its reduction by two orders of magnitude is considered satisfactory.

Table 2. The effect of the composting process on microbial populations (CFU/g dw). Results are expressed as mean \pm SD ($n = 3$).

Microbial Population	Sewage Sludge	Cylinder 1		Cylinder 2	
		Initial	Final	Initial	Final
Total mesophilic ($\times 10^7$)	5.3 \pm 2.5	4.2 \pm 1.8	4.6 \pm 3.6	6.1 \pm 3.8	3.3 \pm 0.3
Fungi ($\times 10^5$)	0.3 \pm 0.1	0.5 \pm 0.2	1.5 \pm 0.7	1.1 \pm 0.7	28 \pm 12
Total coliforms ($\times 10^5$)	9.3 \pm 0.9	8.4 \pm 0.6	0.9 \pm 0.4	9.2 \pm 0.9	13 \pm 7
Faecal streptococci ($\times 10^6$)	1.7 \pm 0.4	1.4 \pm 0.3	0.2 \pm 0.1	1.8 \pm 0.6	1.1 \pm 0.4
<i>E. coli</i> ($\times 10^5$)	2.5 \pm 0.7	2.1 \pm 0.7	ND †	2.8 \pm 0.8	ND
<i>Clostridium perfringens</i> ($\times 10^2$)	320 \pm 110	240 \pm 110	8 \pm 2	310 \pm 100	6 \pm 2

† Not detected.

The French standards on sludge compost (Norme NF U 44-095), which are among the most explicit regarding sanitisation, require that *E. coli*, *Clostridium perfringens*, and Enterococci are below 10^4 , 10^3 and 10^5 CFU/g dw, respectively, while Salmonella and *Listeria monocytogenes* are absent. For all parameters examined, both composts complied with the French regulation. Greek standards for Municipal Solid Waste (MSW) and sewage sludge compost (Common Ministerial Decision 114218, 1016/B/17-11-97) require the absence of Salmonella and Enterobacteria, but the latter are seldom absent even in commercial composts [12,24]. The Directive 86/278/EEC does not include specific requirements for pathogens in SS used in agriculture, but takes the approach of allowing a long safety period between sludge application and crop picking to ensure pathogen destruction. For compost utilisation there are no such compulsory time lags, therefore, most national regulations have provisions to restrict pathogen content in order to reduce possible health risks. Sanitisation is determined either indirectly, based on a required minimum temperature: exposure time profile, or directly, based on product testing. Despite the wide differences among regulations, it seems that the composting process in this study achieved a good level of sludge sanitisation for both bulking agent ratios.

3.5. Antibiotic Resistance

Eleven *E. coli* strains were tested for their susceptibility to 15 antimicrobial agents. Only one strain (EC4) was found resistant to ampicillin (9%) among the five antibiotics inhibiting the cell wall synthesis (AN-30, AMC-30, AM-10, MEM-10, CF-30). Two strains (EC10, EC16) demonstrated resistance to norfloxacin (18%), a nucleic acid inhibitor, among the five antibiotics of this type examined (CIP-5, OFX-5, NA-30, NOR-10, FM-300). All the strains were susceptible to the group of protein inhibiting antibiotics (TE-10, K-30, GM-120, C-30, NN-10), while one strain (EC4) was resistant to the SXT (9%), an inhibitor of the folic acid metabolism. As the number of the *E. coli* strains tested was limited, the results should be treated with caution. Nevertheless, the results reported from other studies that examined strains originating from sewage, effluents from different stages of wastewater treatment and sewage sludge were also variable. Reinthaler et al. [63] measured up to 18% resistance to ampicillin in activated sludge samples, while Niemi et al. [64] observed an ampicillin resistance up to 42% in faecal coliforms originating from untreated domestic sewage in Finland.

Among the 28 *Enterococcus* spp. strains tested for antibiotic susceptibility, none were found to be resistant to vancomycin. Vancomycin and teicoplanin are glycopeptides used as antimicrobial agents in the treatment of hospital infections caused by multi-resistant Gram-positive bacteria [28] and the spread in the environment of vancomycin-resistant enterococci (VRE) is considered a health risk of great concern [28]. One strain (3.6%) was found resistant to bacitracin, an inhibitor of the cell wall synthesis. The greatest resistance was observed against nalidixic acid (89%), while resistances of 18% and 14% were obtained for the other quinolones, ciprofloxacin, and ofloxacin, respectively. The majority of the strains (75%) were found to be resistant to SXT which is an inhibitor of the folic acid metabolism. Another important group of antibiotics consists of aminoglycosides, whose use in clinical practice has been attenuated because of the persistence of resistant bacterial strains. Regarding this group of

antibiotics, a significant number of strains were found resistant to kanamycin (57%), one isolate was resistant to streptomycin (3.6%), and none to gentamycin. Resistance (18%) was also found against tetracycline, erythromycin, and chloramphenicol.

Resistant enterococci to vancomycin and erythromycin have been found in a study of four different sewage treatment plants in Spain [32]. It is noteworthy that resistance against these antibiotics has been connected with transferable genetic elements [34,65]. The appearance of resistant enterococci that might carry such elements (plasmids, transposons) gives rise to a very large discussion about the health risks posed by the dissemination of these genetic elements in the environment through the reuse of treated waste (manures and sewage sludge). The absence of vancomycin resistance and the low erythromycin resistance found in this study indicates that land application of the Kos sewage sludge should not be restricted due to concerns for antibiotic resistance. However, further investigation is required for determining the type of the resistance identified (intrinsic or acquired). Finally, multi-resistant enterococcal strains (more than two antibiotics) were also isolated: 2 strains were resistant to 6 antibiotics, 1 strain to 5 antibiotics, 6 strains to 4 antibiotics, and 11 strains to 3 antibiotics.

4. Conclusions

The heavy metal content of sludge from the island of Kos allows its agricultural application and the beneficial use of derived composts according to Greek, US, and several European standards. The heavy metal concentration of the composts is mainly governed by the contamination of the raw sludge and is not affected by the bulking agent concentration. Overall, the $\delta^{15}\text{N}$ signature seems to offer a good indication of the progress of the stabilization process during composting. On the contrary, the $\delta^{13}\text{C}$ did not show any significant variation during the composting process.

A higher concentration of green waste, at a 1:2 (*v/v*) ratio, facilitated the composting process evolution and control and lead to a higher stabilization rate, nitrogen retention, and final degree of stability. Although the large surface to volume ratio in these laboratory-scale reactors led to larger heat losses and did not allow thermophilic temperatures to evolve to the extent that they would in a larger system, a satisfactory level of sanitisation was achieved for both mixtures. As the total mesophilic population remained practically unchanged during composting, sanitisation appeared more efficient when judged on the basis of specific pathogens

The antibiotic resistance was limited among the *E. coli* strains examined. However, its occurrence was more frequent among the isolated *Enterococcus* spp. strains. The type of antibiotics against which resistance was mainly detected indicates that this might not be acquired, thus, not posing a serious epidemiological risk through the land application of sewage sludge. Further investigations are required to verify this hypothesis.

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