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Evaluation of MSW Compost and Digestate Mixtures for a Circular Economy Application

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Abstract: In order to obtain a product with agronomic characteristics and biological stability consistent with the EU fertilizer decree for the market of EU fertilising products three different mixtures obtained from sludge digestate from municipal wastewater treatment plant, fresh compost and mature compost have been studied and characterized. For the experimental activity, the raw samples and three mixing ones were collected for the analytical characterization. The biological stability was then assessed for all samples using different stability criteria such as Specific Oxygen Uptake Rate, Rottegrad self-heating factor, Residual biogas potential. Specific enzymatic tests provided information about the status of nutrient cycles (C, P and S) and to overall microbial activity. Physical (bulk density, particle density, air capacity and water content), nutritional (C, N, P, K, Mg, and Ca) and toxicological properties (seedling growth tests on *Lepidum sativum* L., *Cucumis sativus* L., *Lolium perenne* L.) were also evaluated in order to assess the feasibility of agronomic use of the digestate-based mixtures. All the digestate-based mixtures responded to the main characteristics of compost quality requirements proposed in national and international regulations. The evidence found in this study highlighted that the strategy of mixing of sludge digestates with the composts allowed to mitigate the environmental risk posed by each starting material and to valorize their nutrient content.

Keywords: digestate; anaerobic digestion; compost; sludge; amendment properties; stability indexes

1. Introduction

Nowadays, the EU Action Plan for the Circular Economy [1] and the Bioeconomy Strategy [2] are the pillars of sustainable development for resource efficiency. The circular economy policy package aims to close the circle through recycling and reuse to reduce the use of virgin resources, while the bioeconomy strategy is an innovative agenda that aims to exploit biomaterials sustainably [3]. The two strategies are connected, in particular regarding the valorization of biowaste in resources [1]. This new approach focuses on the valorization of residues and products from wastewater and municipal solid waste treatment plants [4]. Wastewater sludge is the prevalent residue of wastewater treatment plants and is often treated through anaerobic digestion which converts organic matter into biogas and digestate [5]. The amount of sludge produced in Europe (in 2010), China (in 2006) and the United States (in 2004) is about 9, 3 and 6.5 million tonnes on a dry basis per year, respectively, which is why energy production due to anaerobic fermentation is expected to grow, and with it, the production of digestates from sludge [6]. The use of digestate in agriculture as fertilizer has recently been regulated by the

EU Fertilizer Regulation which promotes the use of fertilizers from recycled biowaste but does not include wastewater sludge digests in the list of sources to which the Regulation applies [7]. Preparatory studies for the EU Fertilizer Regulation [8] claimed that these materials may contain more pronounced traces of organic pollutants than those derived from source separation, leading to a possibly overall higher toxicity; in addition, mention is also made of problems due to heavy metals, pharmaceutical residues, and personal care product residues. Therefore, sludge digestates currently do not find a way to be valorized and are often disposed of in landfills or burned in waste-to-energy plants, and only a remaining part is sent to co-composting plants [9]. The use of sludge digestates as a fertilizer can reduce the need for synthetic inorganic fertilizers [10,11] and can provide some micronutrients that are otherwise not added to the soil [12,13]. Moreover, using sludge digestates as a source of nutrients in agriculture can save non-renewable sources of energy for more sustainable production [10,12].

However, digestate can present a varied chemical and biochemical composition (e.g., high/low total nitrogen content, high/low total phosphorus content, high/low volatile solids, etc.) on the basis of the technology process (e.g., wet/dry process, stripping, etc.) and the input materials (e.g., OFMSW (organic fraction of municipal solid waste), sewage sludges, pig slurry, manure, etc.); the best strategy valorization of digestate as fertilizers or soil amendment can be established on the basis of its typology [14]. The digestate obtained from the anaerobic digestion process of sludge has a high content of organic matter and nutrients, so it is ideal for agronomic use, but to improve its properties it needs further treatment [14] and a new method is proposed in this work.

Therefore, the present work proposes an innovative wastewater digestate management system that foresees the mixing of sludge digestates with the compost produced by the organic fraction of municipal solid waste (OFMSW) in order to obtain a product with agronomic characteristics and biological stability consistent with the EU fertilizer decree for CE marked fertilizers. With this aim, three different mixtures obtained from sludge digestate from municipal wastewater treatment plant, fresh OFMSW compost and mature OFMSW compost have been studied and characterized.

2. Materials and Methods

2.1. Materials

The raw samples used in this work were collected at full-scale composting and anaerobic digestion plants operating in central Italy. Each sample consisted of processed biowaste and, according to the input materials and the applied biological treatment, it was classified as follows: Fresh OFMSW Compost (FC), Stable OFMSW Compost (SC) and digestate from municipal digested sludge (DIG). A detailed explanation of the biological treatment plants from which the samples under study were taken is provided:

- Composting: OFMSW and lignocellulosic waste coming from a door-to-door collection system is treated. The biological treatment includes a pre-treatment step consisting of a grinding and a sieving process. After that, the organic fraction is composted in biotunnels (aerated and moistened) for 4 weeks. Biotunnels have a size of $5 \times 5 \times 25$ m and treat about 220 t for two 14-day bioxidation cycles. During the 28 days of treatment, a fan system ensures that the oxygen inside the biotunnels is more than 15% and a wetting system keeps the moisture content of the waste above 35%. Biowaste is kept for at least 3 days at temperatures above 55 °C to ensure hygienization, the temperatures during accelerated oxidation are between 35 and 65 °C. Curing stage takes place in an open compost heaps system. The material is turned over and, if necessary, wet during the 90 days of treatment. FC was sampled after composting at 28 days while SC was sampled at the end of curing after 90 days.
- Anaerobic digestion: Sewage sludge from a municipal wastewater treatment plant are anaerobically digested. The wastewater treatment plant serves only civil effluents; it is an activated sludge plant treating an inlet load of 85,000 population equivalent, with an average flow rate at the biological section of 15,000 m^3/d. Approximately 190 m^3/d of sludge, which feeds the digesters, is

extracted from the sludge line thickeners. The anaerobic digestion compartment consists of two digesters with a volume of 3000 m^3 and 1500 m^3. The material is processed using a wet digestion technology with a Total Solids (TS) concentration of 2% under mesophilic conditions (37 °C) for 21 days. Thanks to dedicated monitoring over a period of 2 years, conducted by the operator in consultation with the control authorities, it has been demonstrated that no pathogens are present in the outgoing digestate (data not shown). After anaerobic digestion, the digested sludge is centrifuged in order to obtain an output material with a total solids content approximately of 20%, hereafter called DIG.

Due to operational reasons, SC and DIG were sampled at the end of the biological process, while FC was collected before the maturation stage. In order to produce commercial mixtures, the raw samples were mixed in different percentages by wet weight. The mixing process was carried out through a manual blade, and the following mixing samples were obtained:

- Mixture 1 (MIX1): mixture composed of 50% of DIG and 50% of SC by wet basis weight;
- Mixture 2 (MIX2): mixture composed of 50% of DIG and 50% of FC by wet basis weight;
- Mixture 3 (MIX3): mixture composed of 33% of DIG, 33% of SC and 33% of FC, by wet basis weight.

For the experimental activity, about 50 kg of the raw samples and the mixing ones were collected for the analytical characterization. For each mix, the samples were prepared by mixing the fractions with a concrete mixer for 30 min. The biological stability was then assessed for all samples using different stability criteria.

2.2. Stability Criteria

2.2.1. Specific Oxygen Uptake Rate

The Specific Oxygen Uptake Rate (SOUR) test estimates the O_2 consumption of organic material suspended in an aqueous solution. This respirometric test is used to assess the stability primarily related to the oxidative stage of a composting process according to Lasaridi and Stentiford (1998) [15]. This methodology has been set up according to Lasaridi and Stentiford (1996) [16], with the modification proposed by Adani et al. (2006) [17]. The approach used in this work is described in Albini et al. (2019) [18]. Briefly, the SOUR test allows the assessment of respiration activity by measuring the dissolved oxygen (DO) concentration consumed by 5 g of fine particles (smaller than 10 mm) of biodegradable matter dissolved in 1-L aqueous solution over an experimental period of 20 h. The flask in which the sample was dissolved was placed on a magnetic stirrer (Velp Scientifica, AREX Digital PRO) and continuously mixed and heated at 30 °C to ensure optimal biological conditions for the degradation process. Besides, 15 mL of phosphate buffer solutions and $FeCl_3$, $CaCl_2$ and $MgSO_4$ solutions, which were developed according to the BOD test procedures (APHA, 2006) [19], were also added to the aqueous solution to avoid process restrictions due to pH or nutrient limitations. ATU (allylthiourea) was also joined into the flask to inhibit nitrogenous oxygen consumption. Since during the biological process oxygen concentration tends to decrease, the suspension was aerated intermittently through a fish-tank air pump following an aeration cycle characterized by 20 min of pump-on (aeration period) and 15 min of pump-off (reading period). During the reading period, a DO probe (Mettler Toledo, InPro6000, Optical O_2 Sensors) provides oxygen concentration measurement. The signals coming from the probe were acquired and processed by an automatic control and data acquisition system (LabView, National Instruments Corporation, Austin, TX, USA) that also provides the aeration cycle.

During the reading cycle, the DO probe records DO concentration drops, and, from these measurements, the SOUR value was calculated according to the equation reported in Albini et al. (2019) [18]. The SOUR index represents the maximum oxygen consumption rate during the experimental time. In addition to this, the cumulative oxygen demand in 20 h (OD20), which represents the area

subtended by the SOUR curve, was calculated according to the equation reported in Lasaridi and Stentiford (1998) [15]. The SOUR and OD20 test was performed in quadruplicate for each sample.

2.2.2. Self-Heating Factor

The Rottegrad self-heating factor or Dewar test is an indirect measure of the aerobic biological activity of solid organic materials smaller than 10 mm in size. This method is based on measuring the maximum temperature reached by biomass under standardized conditions placed in a vessel for several days according to Brinton et al. (1992) [20].

The experimental set-up is quite simple and consists of an adiabatic 2-L vessel (Dewar's vessel), two thermocouples and a data acquisition system. Approximately 1–1.5 kg of organic matter is placed into the vessel after sieving (with a 10 mm sieve) and after ensuring optimal moisture conditions (around 35% w/w [21]) for the biological activity, according to the Technical Regulation UNI/EN 16087-2:2012. Two T-type thermocouples continuously record room, and biomass temperatures arranged one in the working environment and the other into the vessel at the half-height from the bottom. All the signals coming from the thermocouples were acquired and processed by a data acquisition system cRIO 9030 controller (National Instruments, Austin, TX, USA).

During the experimental time, the temperature increases due to the exothermic trend of the biological degradation process. The test is considered finished when the reading temperature decreases two days after the maximum value reached. Based on this, the experimental time may vary from 5 to 10 days, depending on the time a sample may require warming up. The degree of decomposition is finally determined by measuring the difference between the highest biomass temperature and room one. The biological stability degree of the tested material can be defined according to the Rottegrad classification proposed by the U.S. Composting Council, 1997. Self-heating factor was measured for each sample in duplicate.

2.2.3. Residual Biogas Potential

Residual Biogas Potential (RBP) is considered another criterion for the assessment of biological stability of an organic matter [7]. The residual biogas potential production can be evaluated by anaerobic biodegradability assays and, considering the composition of the biogas quality, the biochemical residual methane potential (BMP) can also be estimated.

The tests were carried out in triplicate for each sample, and the results were obtained as an indicator of the gas released over an experimental period of 28 days (RBP28 and BMP28) and measured against the Volatile Solids (VS) content of the tested sample.

The test was performed using 1-L stainless steel batch reactors developed at DIEF—University of Florence [22]. The batch reactors were continuously kept in a water bath heated at 37 °C by a thermostat (FA90, FALC Instruments, Treviglio, Italy). Each reactor was filled in with the mass of the sample and mixed with an inoculum (coming from an anaerobic digester treating OFMSW and cattle manure), in a ratio equal to 1.5:1 VS basis. The reactors were closed by a ball valve cap to allow gas sampling, and before starting the experiment, each batch test was purged with nitrogen gas in order to ensure anaerobic conditions [23]. Biogas production was daily checked by measuring the pressure in the reactor headspace through a membrane pressure gauge (Model HD2304.0, Delta Ohm S.r.l., Selvazzano Dentro, Italy). The pressure was then converted into residual biogas potential (RBP$_{28}$) volume, which represents the cumulative biogas production during the overall experimental time [24]. In order to evaluate the methane content of the released gas (BMP$_{28}$), an IR gas analyzer (ECOPROBE 5 – RS Dynamics) was used to determine it based on biogas quality results or a micro-gas chromatograph (INFICON, Bad Ragaz, Switzerland) in case of residual production [25].

2.3. Analytical Methods

The raw samples and the mixture ones were analytically characterized. pH was determined, according to UNI EN 13037. TS, organic carbon and organic nitrogen contents were evaluated by UNI

10780. The protocol UNI 10780 was followed for the determination of heavy metals (Cu, Pb, Cd, Ni, Hg and Cr). The content of total nutrients important for the agronomic productivity (P, K, CaO and MgO) was determined following UNI EN ISO 11885 and EPA 9056A procedures. The methods proposed by Marx et al. (2001) [26] and Vepsäläinen et al. (2001) [27] were followed for the determination of enzymatic activities (butyrate esterase, EC 3.1.1.1; β-glucosidase, EC 3.2.1.21; acid phosphatase, EC 3.1.3.2; and arylsulphatase, EC 3.1.6.1). Briefly, a fresh sample with equivalent dry weight of 3 g, was homogenate by sonicating for 60 s at an output energy of 50 J/s with 75 mL Na-acetate buffer pH 5.5. Equal parts of sample homogenate, and Na-acetate buffer were dispensed into a 96-well microplate. Finally, 100 μL of 1 mM substrate solution were added. Fluorescence (excitation 360 nm; emission 450 nm) was measured, after 0, 30, 60, 120, 180 min of incubation at a temperature of 30 °C, with an a Infinite F200 pro TECAN plate-reader.

The Hoekstra et al. (2002) [28] test with *Lepidium sativum* seeds on water extract (1:5, v:v) was followed for the germination test. The heavy metal fractionation was evaluated using the Moćko and Waclawek method (2004) [29]. Physical properties, namely Bulk Density (BD), Particle Density (PD), Total Pore Space (TPS), Air Content (AC), Water-holding capacity (WC), Easily Available Water (EAW), and Water Buffer Capacity (WBC), were determined with the sand-box method (UNI EN 13040). For the seedling growth test, the procedure proposed by Wang et al. (2004) [30] with *Lepidium sativum* L., *Cucumis sativus* L. and *Lolium perenne* L. was used. Ten seeds for each plant species were sown uniformly in each pot filled with the different soil–digestate-based mixtures. After 17 days, the seedling shoots were cut, and the fresh biomass was immediately assessed. The biomass production was reported as a percentage to a no-treated control for each plant.

2.4. Statistical Analysis

All results reported in the text are the means of determinations made on three replicates. Statistical procedures of the Statistica 8.0 software were used. Analysis of variance was used to evaluate the differences ($P < 0.05\%$) between the starting materials (DIG, FC, and SC) and the digestate-based mixtures (MIX1, MIX2 and MIX3), applying as post-hoc test Tukey's method. Principal component analysis (PCA) was applied to identify patterns between selected indicators as variables, and the starting materials (DIG, FC, and SC) and the digestate-based mixtures (MIX1, MIX2 and MIX3) as objects.

3. Results and Discussion

3.1. Biological Stability

In the present work, biological stability tests have been carried out to evaluate the correlation between the indices and the degree of decomposition of the analyzed organic materials (Table 1).

Table 1. Mean RBP_{28}, BMP_{28}, SOUR, OD_{20} and self-heating factor results.

|	RBP_{28} (Nl$_{biogas}$/g VS)	BMP_{28} (NlCH$_4$/g VS)	SOUR (mgO$_2$/g VS*h)	OD_{20} (mgO$_2$/g VS*20 h)	Self-Heating Factor (°C)
DIG	0.14 ± 0.01 c	0.08 ± 0.00 c	18 ± 0 b	221 ± 0 c	11 ± 3 a
FC	0.40 ± 0.01 e	0.21 ± 0.01 e	24 ± 1 c	239 ± 7 c	59 ± 11 b
SC	0.02 ± 0.01 a	0.01 ± 0.00 a	3 ± 2 a	27 ± 3 a	11 ± 1 a
MIX1	0.05 ± 0.00 b	0.02 ± 0.00 b	11 ± 4 b	119 ± 34 b	18 ± 2 a
MIX2	0.23 ± 0.02 d	0.12 ± 0.01 d	17 ± 5 bc	214 ± 63 bc	57 ± 8 b
MIX3	0.16 ± 0.00 c	0.08 ± 0.00 c	10 ± 4 ab	170 ± 61 bc	60 ± 15 b

Note: All the indices are expressed in terms of mean values and standard deviations. Different letters mean values significantly different ($p < 0.05$) between samples.

According to EU Regulation 2019/1009 [31], RBP_{28} values lower than 0.25 Nl$_{biogas}$/g VS and a minimum self-heating factor of Rottegrad III (maximum temperature reachable equal to 30 °C) are

considered limit values for making a fertilizer product available on the EU market. As regards to the Oxygen Uptake Rate, in this study, a different method compared to that reported in the EU Regulation was performed.

Figures 1 and 2 show the cumulative RBP_{28} test curves and the SOUR trends during the corresponding experimental period, which represent the stability indices related to the anaerobic and aerobic biological activity, respectively.

As expected, RBP_{28} and BMP_{28} are directly related to each other since BMP_{28} depends on CH_4 content into the biogas released during the degradation process. At the same time, SOUR and OD_{20} are also correlated, as the former measures the maximum oxygen rate and the latter represents the cumulative oxygen demand.
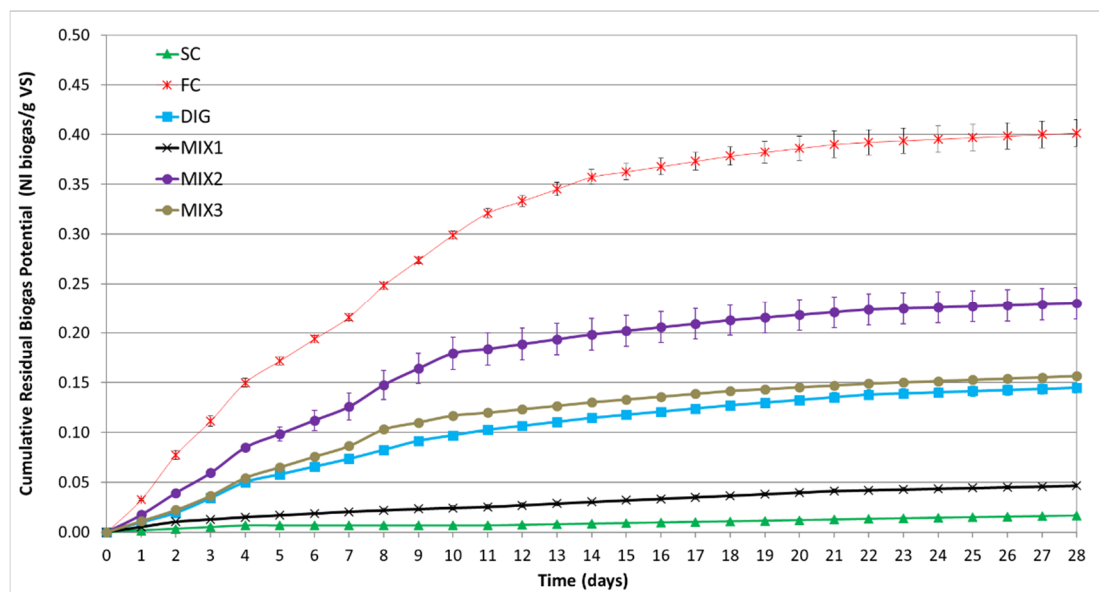

Figure 1. Mean cumulative RBP_{28} test curves expressed in Nl_{biogas}/g VS for 28 days of experimental time. Error bars represent the standard deviations.

Table 1 and Figure 1 show that among the raw samples, only FC has reached RBP_{28} and self-heating factor values that exceed the threshold limit for biological stability. Such condition can be attributed to the sampling point; in fact, FC was collected after only 28 days of the composting process and before the curing stage. The results indicate that the composting process has not been completed yet and the product may be susceptible to further biological degradation. Among the mixture samples, despite the lower RBP_{28} values achieved, the Rottegrad factor for MIX2 and MIX3 overcomes the threshold limit. In particular, the presence of FC into these mixtures led to an increase in the results, unlike MIX1, where only SC and DIG were contained.

Concerning SOUR test, the threshold limit for materials with a medium biological stability degree was proposed by Adani et al. (2003) [17] and Scaglia et al. (2007) [32] equal to 7 mgO_2/g VS*h. The experimental results obtained for all the samples ranged from 3 mgO_2/g VS*h to 22 mgO_2/g VS*h, corresponding respectively to the SOUR value for a processed compost and raw sludge [15].

Figure 2 shows that the maximum SOUR value was obtained for FC (22 mgO_2/g VS*h) which registered no-stability for this sample, as confirmed by the other stability criteria applied. Therefore, from the SOUR curves reported in Figure 2, it can be observed that the trends of MIX2 and MIX3 were affected by the presence of FC. The other samples present SOUR values in accordance with the results obtained for processed organic materials by Schievano et al. (2009) [33], Orzi et al. (2010) [34], Scaglia et al. (2014) [35] and Tambone et al. (2019) [36].
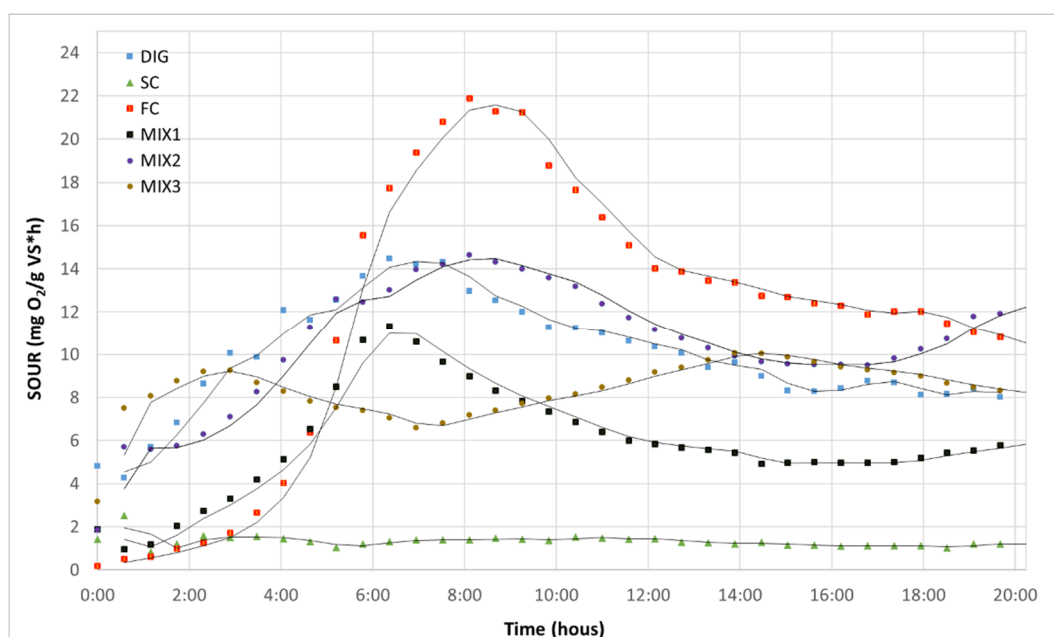
Figure 2. SOUR test trends expressed in mg O_2/g VS*h for 20 h of experimental time.

Therefore, it should be noted that the methods used for RBP_{28} and SOUR tests involved the use of a small amount of sample compared to the self-heating factor. This difference in the experimental procedure influenced the results of the tests, especially concerning the mixture samples. In fact, for MIX2 and MIX3, the high self-heating factor does not correspond to the results of the other criteria. This inconsistency is probably due to the heterogeneity of the mixtures analyzed, which indicates the need for a preliminary sieving and grinding process [37,38]. Therefore, it can be affirmed that RBP_{28} and SOUR tests are easily affected by the characteristics of the materials tested due to the low amount of material used.

The biological stability of materials intended for agricultural use is a crucial parameter to be assessed, given that the organic matter degradation should not contribute to the increase of greenhouse gases (e.g., carbon dioxide, methane) [39]. The characterization of organic matter stability in several organic matrices can be performed through the enzymatic activity evaluation. Even though it is not easy to establish universal threshold values to apply enzymes activities as stability indexes [40], the evaluation of the enzymatic activities gives information about the processes of organic matter stabilization. Moreover, enzyme activities (Table 2) give a clear picture of the current state of microbial community metabolism, and about abundance and limitation of substrates/nutrients [41].

Table 2. Enzymatic activities. Butyrate esterase, (BE, mmol MUB/(kg dw h)); β-glucosidase (BG, mmol MUB/(kg dw h)); Phosphatase (PHO, mmol MUB/(kg dw h)); Arylsulphatase (AS, mmol MUB/(kg dw h)).

	BE	**BG**	**PHO**	**AS**
DIG	7651 ± 874 d	1235 ± 87 ab	2835 ± 333 c	2131 ± 62 c
FC	4979 ± 211 b	5256 ± 277 e	1607 ± 127 b	241 ± 1 a
SC	7996 ± 265 d	1388 ± 52 b	1482 ± 7 b	371 ± 2 b
MIX 1	2507 ± 67 a	3455 ± 2 d	1101 ± 247 a	204 ± 85 a
MIX 2	5567 ± 211 c	814 ± 277 a	1719 ± 127 b	1160 ± 1 c
MIX 3	5116 ± 3 b	2220 ± 1 c	1463 ± 18 ab	370 ± 50 b

Note: Different letters mean values significantly different ($p < 0.05$) between samples (N = 3).

The values found for the starting materials and for the mixtures were in line with the values found by Masciandaro et al. (2017) [42] and Peruzzi et al. (2019) [43] for stabilized sludges and stable composts. Moreover, the values of β-glucosidase and butyrate esterase found in MIX1 and MIX3, reflected their stable status, being comparable to values reported for humus soils in a forest [44,45].

DIG and SC presented the highest values for butyrate esterase activity (BE), a non-specific esterase involved in the carbon cycle related to living biomass content [46], thus describing the overall microbial activity; the lowest values were noticed in MIX 1, while all the other samples presented intermediate values.

Esterase activities are usually correlated with phosphatase activity (PHO), an enzyme which hydrolyzes organic and inorganic phosphates so that it is linked to the phosphorus cycle [47]. In this paper, BE and PHO presented an analogous trend between samples, resulting to be positively correlated each other ($r = 0.65$, $p < 0.05$). Moreover, the level of phosphatase and esterase are expected to be the most abundant with respect to the other enzymes, especially in the presence of larger quantities of nutrients and organic matter [48].

On one hand, the level of instability highlighted by the RBP_{28}, BMP_{28}, SOUR, OD_{20} and self-heating factor results for FC sample was also revealed by the level of β-glucosidase activity (BG), an indicator of the presence of labile organic matter easily usable by microorganisms, being a hydrolytic enzyme linked to C cycle. On the other hand, the relative high value found of BG found in MIX1 was mainly related to an increase of C demand by microorganisms, given that the level of overall microbial activity, represented by the butyrate esterase activity, was relatively low.

Arylsulphatase (AS) is an extracellular enzyme that catalyzes the hydrolysis of organic sulfate esters, releasing available sulfates for plant nutrition. It has been suggested to be a very sensitive and useful tool for evaluating compost maturity [49]. In this study, arylsulphatase activity seemed to be related to microbial biomass content being highly correlated to BE ($r = 0.91$, $p < 0.05$).

3.2. Toxicological Evaluation

In order to evaluate the potential fertilizer use of digestate in agriculture, it is essential to check the chemical safety of the material, also considering the effects of the toxic compounds eventually present in the materials onto the flora and fauna of the soil reducing its fertility [50,51].

For what concerns heavy metals, the detected total metal concentrations (Table 3) in DIG were in line with those observed by Fytili and Zabaniotou (2008) [52] for European sewage sludge. It was expected that the mixing of DIG with FC and SC, in particular MIX 2 and MIX 3, mitigate the content of heavy metals, thus resulting in line with threshold value proposed in the Italian regulation (National legislation DL 75/2010) for green and mixed compost.

However, the knowledge of heavy metal bioavailability could be useful to understand their behavior and potential toxicity in the environment.

The procedure for heavy metal fractionation differentiated the heavy metals into four fractions:

1. The exchangeable fraction associated with carbonated phase (Fraction 1);
2. The reducible fraction associated with Fe and Mn oxides (Fraction 2);
3. The oxidizable fraction bound to organic matter (Fraction 3); and
4. The residual fraction (RF) (Figures 3 and 4).

The exchangeable fraction is considered the most toxic fraction, being the most mobile in soil and water media; the Fraction 2 became mobile in acidic and anoxic conditions. The heavy metals in Fraction 3 are not considered bioavailable, given that humic substances retained them. The heavy metals in the residual fraction are in an inert form, bound to minerals and considered to be not extractable.

The origin of the started materials affected the pattern of heavy metal bioavailability, given that different stabilization methods influence the distribution of the metals and the fraction to which they are bound [53]. In general, DIG presented about 47.6% of heavy metals in the most bioavailable fractions (Fraction 1 and Fraction 2), while FC and SC had 33.6% and 30.8%, respectively. The heavy

metals which were present in the mobile fractions, namely Fraction 1 and Fraction 2, were Cu, Pb, and Zn, while Cr and Ni were mainly bound to the residual fraction (83.7% and 88.4%, respectively) (Figure 3). This last result was consistent with findings from other authors for anaerobic sludges [54,55]. Notwithstanding Pb is usually considered not bioavailable in anaerobic sludges [56], DIG presented a considerable quantity of Pb in Fraction 2, the reducible one associated with Fe and Mn oxides [54,57]. It was noteworthy that the mixing strategy of DIG with FC and SC contributed to mitigate the biotoxicity of DIG due to its heavy metal content, given that in the three mixtures, the content of bioavailable fractions decreased until 42.3%, 27.6% and 26.9% in MIX 1, MIX 2 and MIX 3, respectively. These degrees of bioavailability were also reported for sludge stabilized by nature-based solutions [58], for the co-composting process of feedstock mixtures with wood chips [59], and several organic wastes used as a soil amendment [60]. Moreover, these findings were also in line with the germination assay, a test which assesses compost maturity (GI > 80%) suggesting the absence of phytotoxic substances (GI > 50%) (Table 3).

All mixtures did not present phytotoxic effect for seed germination. In fact, all values were higher than 50%, the threshold value expressing phytotoxicity according to Zucconi et al. (1987) [61], notwithstanding the high level of phytotoxicity expressed by DIG (15.4%) (Table 3) [62].

Table 3. Heavy metals content (mg/kg dw) and germination index (GI, %).

	Zn	Ni	Pb	Cr	Cu	GI
DIG	847 ± 130 d	33.7 ± 5.1 a	61.5 ± 9.2	65 ± 10 a	433 ± 64 e	15.4 ± 5.6 a
FC	128 ± 19 a	26.0 ± 3.9 a	23.4 ± 3.5	85 ± 15 a	90 ± 13 a	62.8 ± 19.6 b
SC	129 ± 19 a	56.6 ± 8.5 b	25.7 ± 3.9	146 ± 21 b	100 ± 15 ab	90.4 ± 4.7 c
MIX 1	546 ± 81 c	46.0 ± 6.9 b	43.6 ± 6.5	106 ± 15 b	274 ± 41 d	51.6 ± 10.6 b
MIX 2	272 ± 40 b	24.1 ± 3.6 a	31.1 ± 4.7	88 ± 13 a	154 ± 23 c	50.0 ± 14.1 b
MIX 3	240 ± 36 b	43.5 ± 6.5 b	34.6 ± 5.2	123 ± 18 b	131 ± 19 b	61.5 ± 11.6 b

Note: Different letters mean values significantly different ($p < 0.05$) between samples (N = 3).
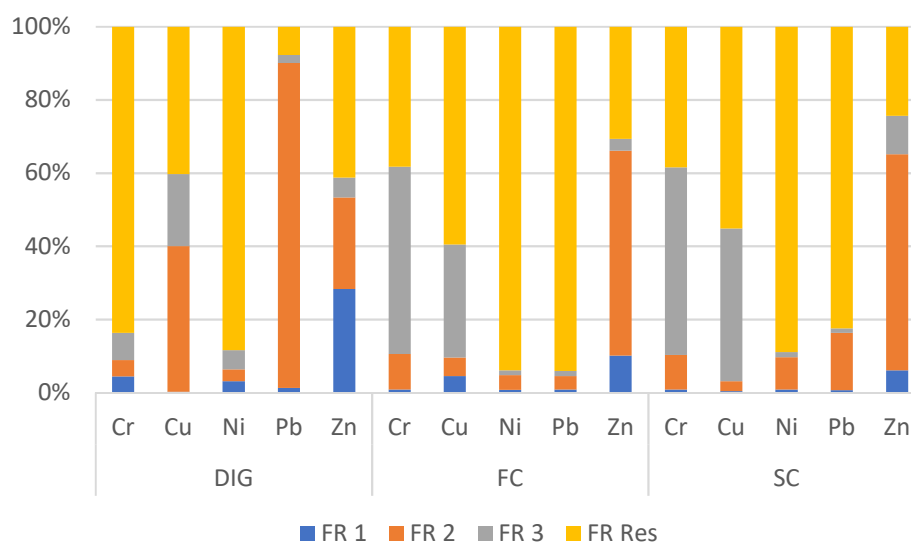
Figure 3. Heavy metal fractionation (%). Percentage distributions of the different chemical fractions of heavy metals in the starting materials (DIG, FC and SC).
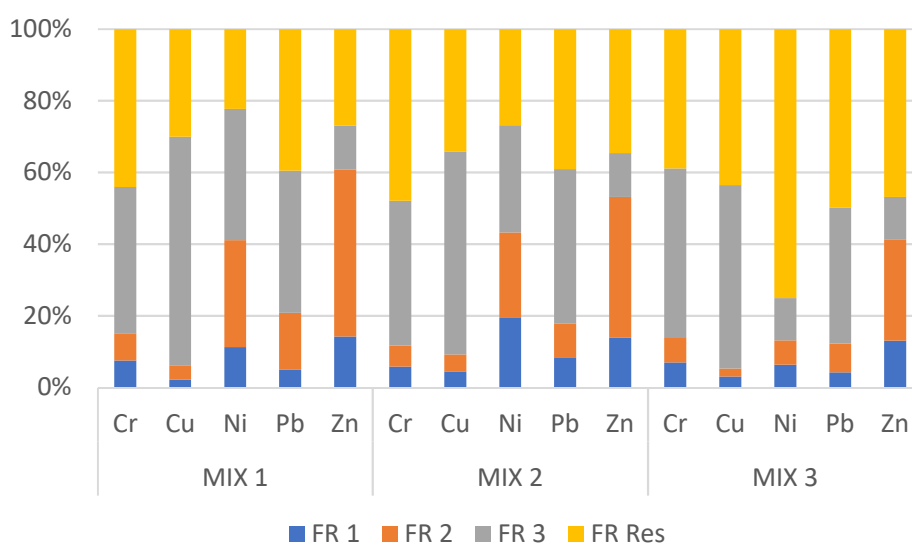
Figure 4. Heavy metal fractionation (%). Percentage distributions of the different chemical fractions of heavy metals in the digestate-based mixtures (MIX1, MIX2 and MIX3).

3.3. Principal Component Analysis

Given that BMP_{28}, RBP_{28}, and Self-Heating factor were highly correlated ($r > 0.99$ and $r > 0.73$), and that SOUR and OD_{20} present a positive correlation ($r > 0.93$), BMP_{28} and OD_{20} were selected for principal component analysis.

The PCA of the data set indicated 78.3% of the data variance as being contained in the first three components. PC1, PC2 and PC3 accounted for 31.6%, 30.5% and 16.2% of the total variance, respectively. PC1 was linked to enzymatic activities, while PC2 was tightly associated with biological stability of the samples, with butyrate esterase, BMP_{28} and OD_{20} and heavy metals bound to organic matter (Fr 3). On the other hand, PC3 explained the toxicological degree of the samples, being associated with germination index, and with heavy metal fractionation (Fr 2 and Residual Fraction) (Table 4).

The bubble-plot of scores provided a graphical representation of the starting materials and of the mixtures, pinpointing the variables that appear to be more associated with each sample (Figure 5). As expected, the starting materials were discriminated in the plot. While DIG resulted associated with parameters that highlighted its biological instability, SC presented a high level of biological stability, being located at different extremes of PC2. FC revealed its high degree of instability (PC2) and it is also associated with enzymatic activity linked to the C cycle (PC1). However, FC and SC were also correlated with indicators of the lack of toxicity (Heavy metals—Residual Fraction; positive values on PC3), while DIG resulted associated to the bioavailable fractions of heavy metals (Heavy metals—Fraction 2; negative values on PC3). All the mixtures were located in the center of the plot, enlightening their intermediate level of biological stability (intermediate values of OD_{20}, BMP_{28} and Butyrate esterase; positive values on PC2); however, the different values on PC3 revealed a lower level of toxicity of MIX3 to MIX1 and MIX2, reflecting the germination test and the heavy metal fractionation results.
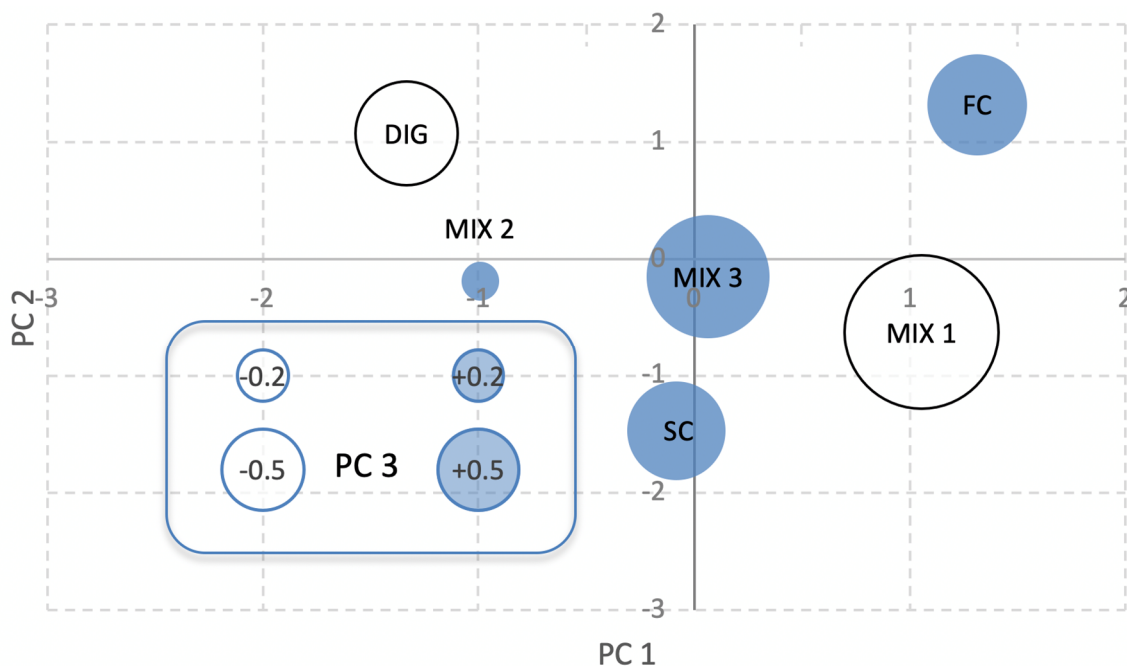
Figure 5. Principal component analysis. Bubble-plot of scores: PC 1 vs PC 2 and, PC 3 as area of each bubble.

Table 4. Principal component analysis. Principal component loadings.

	Factor 1	**Factor 2**	**Factor 3**
OD_{20}	−0.117	0.865 *	0.052
BMP_{28}	0.179	0.792 *	0.360
Butyrate esterase	0.287	0.901 *	−0.163
β-glucosidase	0.986 *	0.011	−0.026
Phosphatase	−0.915 *	−0.258	0.234
Arylsulphatase	−0.947 *	0.195	−0.150
Germination index	0.464	−0.539	0.576 *
Heavy metals – Fraction 1	−0.543	0.498	−0.515
Heavy metals – Fraction 2	0.117	0.077	−0.608 *
Heavy metals – Fraction 3	0.346	−0.642 *	0.122
Heavy metals – Residual Fraction	0.031	0.329	0.788 *
Explained variance	3.479	3.357	1.781
Total proportionality	0.316	0.305	0.162

* parameters used for PCA interpretation.

3.4. Digestate-Based Mixtures Quality Evaluation for Land Application

Notwithstanding the different origin of the starting materials, all the three mixtures presented excellent physical properties, with values comparable to an ideal growing media [63,64] (Table 5). Bulk Density (BD) and Total Pore Space (TPS) were comparable to values found in digestates as a constituent for growing media by Nesse et al. (2018) [65]. Moreover, the results about Air Capacity (AC), Water Content (WC) and, Easily Available Water (EAW) were in line with values proposed for growing media for container-grown tomato [66], thus confirming the suitability of the digestate-based mixtures for land application from a physical point of view.

The high nutrient concentration, in line with values reported by Montejo and collaborators (2015) [67] for municipal sludge compost, also confirmed the fertilizing potential of digestate-based

mixture for the agricultural sector (Table 5). This result was expected, given that the anaerobic digestion technology is considered one of the most effective technologies to recover nutrients from the input material, namely sewage sludges, that can be utilized as fertilizer [68].

The results of the Seedling Growth Index (SGI), a test to detect the phytotoxicity and the biomass growth on selected plant species (*Lepidium sativum* L., *Cucumis sativus* L. and *Lolium perenne* L.), confirmed the fertilizer potential of the digestate-based mixtures (Table 5). This fact was particularly relevant for MIX3, which presented not only values that indicate the lack of toxicity (SGI > 60%), but values which denote a stimulant effect on vegetation (SGI > 90%) [30,69].

Table 5. Physical, nutritional and toxicological properties. Dry Bulk Density (BD, g/cm^3); Particle Density (PD, g/cm^3); Total Pore Space (TPS, % v/v); Air Capacity (AC, % v/v); Water Content (WC, % v/v); Easily Available Water (EAW, % v/v); Water Buffer Capacity (WBC, % v/v); Total Nitrogen (N, % N/ds); Total Phosphorus (P, %P/ds); Total Potassium (K, %K/ds); Total Calcium (CaO, g CaO/kg ds); Total Magnesium (MgO, g MgO/kg ds); Seedling growth indices with *Lepidium sativum* (SGI $_{Lepidium\ sativum}$, %), *Cucumis sativus* (SGI $_{Cucumis\ sativus}$, %) and *Lolium perenne* (SGI $_{Lolium\ perenne}$, %).

	Mix 1	Mix 2	Mix 3
BD	0.24 ± 0.01 a	0.33 ± 0.01 c	0.29 ± 0.01 b
PD	1.81 ± 0.01 a	1.82± 0.0 a	1.87 ± 0.01 b
TPS	86.7 ± 0.03 b	81.7 ± 0.04 a	84.8 ± 0.03 ab
AC	41.0 ± 2.83 b	44.1 ± 2.23 b	34.6 ± 2.21 a
WC	26.3 ± 2.83 b	20.3 ±2.83 a	26.4 ± 2.21 b
EAW	19.6 ± 0.47 ab	17.3 ± 1.66 a	23.8 ± 0.50 b
WBC	1.06 ± 0.24 a	3.00 ± 0.33 b	0.57 ± 0.40 a
TN	4.27 ± 0.43 b	3.19 ± 0.32 a	3.14 ± 0.31 a
P	1.09 ± 0.16 c	0.782 ± 0.12 b	0.536 ± 0.08 a
K	0.740 ± 0.11 a	0.990 ± 0.15 b	0.980 ± 0.15 b
MgO	6.27 ± 0.94 a	6.38 ± 0.96 a	10.9 ± 1.60 b
CaO	11.9 ± 1.8 a	18.2 ± 2.7 b	29.6 ± 4.4 c
SGI $_{Lepidium\ sativum}$	65.8 ± 7.9 a	68.8 ± 8.2 a	113 ± 13.6 b
SGI $_{Cucumis\ sativus}$	82.9 ± 8.9 a	102 ± 12.3 ab	104 ± 12.5 b
SGI $_{Lolium\ perenne}$	66.3 ± 7.9 a	92.8 ± 11.1 b	93.3 ± 11.2 b

Note: Different letters mean values significantly different ($p < 0.05$) between samples (N = 3).

3.5. Practical Implications of This Study

Besides providing the 11 different component material categories (CMC) and their specific requirements for the production of EU fertilizer products, the EU Regulation on fertilizers (Fertilizer Regulation, 1009/2019), also include acceptable production as well as mandatory process parameters. CMC 5 (other digestate than fresh crop digestate) includes biowaste resulting from separate biowaste collection at source, certain animal by-products of categories 2 and 3 according to Regulation (EC) No 1069/2009 and living or dead organisms or parts; however, the use of the organic fraction of mixed municipal household waste as well as the use of sewage sludge, industrial sludge and dredging sludge is not permitted. Moreover, the Regulation establishes threshold values for several organic and inorganic contaminants in the fertilizing products, in order to minimize the risk to human, animal or plant health, to safety or to the environment, given that the contaminants can enter in the food chain as well as accumulate into the environment. However, the threshold level for heavy metals and organic contaminants, such as PAH, are established on the basis of total content of the materials, and not on the bioavailable fractions.

For what concerns the practical application of the findings of this study, it was evident that the quality of the digestate-based mixtures responded to the threshold values proposed in the national and international standard (Table 6).

Table 6. Different legislations on compost quality and comparison with the obtained values for the starting materials and digestate-based mixtures.

Indicator	Unit Measure	End of Waste Criteria [1]	EU Ecolabel [2]	Italian Regulation 75/2010 [3]		EU Fertilizer Regulation [4]		DIG	FC	SC	MIX 1	MIX 2	MIX 3
			Soil Improver	Green-Based Compost	Mixed-Based Compost	Compost	Digestate						
Volatile solids	%	>15	>15 *					74.9	69.2	46.1	63.8	65.9	58.4
Respirometric index	mmol O_2/kg VS h	<25	<15			<25		18.0 +	24.0 +	3.0 +	11.0 +	17.3 +	10.0 +
Self-heating factor	Rottengrad	≥III					≥III	IV	I	IV	IV	I	I
Zn	mg/kg dw	<600	<300	<500		<800 ***		847	128	129	546	272	240
Cd	mg/kg dw	<1.5	<1	<1.5		<2.0 ***		1.40	0.298	0.380	0.940	0.471	0.454
Ni	mg/kg dw	<50	<50	<100		<50 ***		33.7	26.0	56.6	46.0	24.1	43.5
Pb	mg/kg dw	<120	<100	<140		<120 ***		61.5	23.4	25.7	43.6	31.1	34.6
Cr	mg/kg dw	<100	<100					65	85	146	106	88	123
Cr (VI)	mg/kg dw			<0.5		<2 ***		<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Cu	mg/kg dw	<200	<100	<230		<300 ***		433	90	100	274	154	131
Hg	mg/kg dw	<1	<1	<1.5		<1 ***		0.8	<0.5	<0.5	0.5	<0.5	<0.5
As	mg/kg dw							6.0	2.0	3.2	4.7	2.3	2.6
As (in)	mg/kg dw					<40 ***		nd	nd	nd	nd	nd	nd
PAH	mg/kg dw					<6	<6	<0.01	0.67	0.43	0.30	0.55	0.75
Salmonella	MPN/25 g	Absent	Absent	Absent		Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
E. coli	CFU/g	<10^3	<10^3	<10^3		<10^3	<10^3	<10	<10	<10^3	<10^3	<10^3	<10^3
Germination index (UNI)	%		**	>60				88	84	79	90	70	99
Moisture content	%		<75 *	<50				78.0	16.0	56.9	66.0	49.8	49.6
Total organic carbon	% dw			>20				41.2	38.9	27.3	36.7	41.4	34.5
Humic carbon	% dw			>2.5	>7			16.8	11.1	11.6	13.7	14.1	12.7
C/N ratio				<50	<25			7.0	13.0	12.0	9.0	13.0	11.0
Org N/TN ratio	%			>80	>80			73.2	94.6	99.4	81.2	86.8	94.5
pH			4–7	6–8.5	6–8.8			8.12	7.29	8.26	8.09	7.04	7.25

nd: not determined in this study; + The unit of measurement for this study is expressed in mgO_2/g VS*h i.e., different from EU Regulation 2019/1009; [1] Report EUR 26425 EN. 2014. Hans Saveyn and Peter Eder. End-of-waste criteria for biodegradable waste subjected to biological treatment (compost and digestate); [2] COMMISSION DECISION (EU) 2015/2099 of 18 November 2015 the ecological criteria for the award of the EU Ecolabel for growing media, soil improvers and mulch; [3] Reorganization and review of the fertilizer regulations (Riordino e revisione della disciplina in materia di fertilizzanti) Decreto legislativo 75/2010; [4] Fertilizer Regulation (1009/2019) of the European Parliament and of the Council laying down rules on the making available on the market of EU fertilizing products and amending Regulations (EC) No 1069/2009 and (EC) No 1107/2009 and repealing Regulation (EC) No 2003/2003; * for soil improver and mulches; ** no adverse effect on Chinese cabbage; *** organic soil improver.

The biological stability, the physical-chemical characteristics, and the pollutant content of MIX2 and MIX3 were consistent with the threshold values reported in EU fertilizer decree for CE marked fertilizers (Fertilizer Regulation, 1009/2019), even though the presence of digestate for organic improver was not allowed.

Moreover, the values established by the End of Waste criteria for biodegradable waste (compost) were attended; the digestate-based mixtures, in general, also responded to the Italian regulation (National legislation DL 75/2010) for compost preparation (green-based compost and mixed-based compost). Only MIX 1 did not respond to the threshold value for Cu (274 mg/kg dw, with threshold value of 230 mg/kg for compost preparation); however, it was clearly proven from the fractionation results that only 8.21% was in the most bioavailable fractions. Considering also properties established for the growing media sector, the values for the EU Ecolabel certification (for soil improver) and requirements as mixed growing media in Italian regulation (National legislation DL 75/2010) were respected.

The evidence found in this study highlighted that the strategy of mixing sludge digestates with composts allowed to mitigate the environmental risk posed by each starting material and to valorize their nutrient content.

4. Conclusions

The present work proposes an innovative wastewater digestate management system that foresees the mixing of sludge digestates with the compost produced by the organic fraction of municipal solid waste in order to valorize their nutrient content for land application.

The digestate-based mixtures responded to the main threshold values of biological stability, with the exception of self-heating factor, proposed in the EC fertilizer decree for CE marked fertilizers (Fertilizer Regulation 1009/2019). Results about heavy metal fractionation highlighted that the use of the digestate-based mixtures will not pose any environmental risk. The physical and nutritional properties of the digestate-based mixtures were comparable with values usually found for high quality composts.

The evidence found in this study highlighted that the strategy of mixing of sludge digestates with the composts allowed to mitigate the environmental risk posed by each starting material and to valorize their nutrient content.

For what concerns the practical application of the findings of this study, it was evident that the quality of the digestate-based mixtures mainly depends on the basis of the quality of the compost used for mixing. Future studies are needed in order to evaluate the effect of the application of digestate-based mixtures at field level.

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