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Microbiological Effectivity Evaluation of New Poultry Farming Organic Waste Recycling

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Abstract: Due to the intensification of the poultry sector, poultry manure is being produced in increasing quantities, and its on-site management is becoming a critical problem. Animal health problems can be solved by stricter the veterinary and environmental standards. The off-site coupled industrial chicken manure recycling technology (Hosoya compost tea) fundamentally affects the agricultural value of new organic-based products. Due to the limited information available on manure recycling technology-related microbiological changes, this was examined in this study. A pot experiment with a pepper test plant was set up, using two different soils (Arenosol, slightly humous Arenosol) and two different doses (irrigation once a week with 40 mL of compost tea: dose 1, D1; irrigation twice a week with 40 mL of compost tea: dose 2, D2) of compost tea. Compost tea raw materials, compost tea, and compost tea treated soils were tested. The products (granulated manure, compost tea) and their effects were characterized by the following parameters: aerobic bacterial count (log CFU/g), fluorescein diacetate activity (3',6'-diacetylfluorescein, FDA, µg FI/g soil), glucosidase enzyme activity (GIA; PNP/µmol/g), and identification of microorganisms in compost tea with matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS). Furthermore, we aimed to investigate how the microbiological indicators tested, and the effect of compost tea on the tested plant, could be interpreted. Based on our results, the microbiological characteristics of the treated soils showed an increase in enzyme activity, in the case of FDA an increase +0.26 µg FI/g soil at D1, while the GIA increased +1.28 PNP/µmol/g with slightly humous Arenosol soil and increased +2.44 PNP/µmol/g at D1; and the aerobic bacterial count increased +0.15 log CFU/g at D2, +0.35 log CFU/g with slightly humous Arenosol and +0.85 log CFU/g at W8. MALDI-TOF MS results showed that the dominant bacterial genera analyzed were *Bacillus* sp., *Lysinibacillus* sp., and *Pseudomonas* sp. Overall, the microbial inducers we investigated could be a good alternative for evaluating the effects of compost solutions in soil–plant systems. In both soil types, the total chlorophyll content of compost tea-treated pepper (*Capsicum annuum* L.) had increased as a result of compost tea. D1 is recommended for Arenosol and, D2 for slightly humous Arenosol soil.

Keywords: microbiology; poultry farming waste; organic waste

1. Introduction

The quick spread of intensive agricultural systems, the use of fertilizers, and rapid human population growth have had negative effects on soil fertility, mainly decreasing the soil organic carbon and the total soil nitrogen, and changing the composition of carbon and

nitrogen, owing to the loss of soil organic matter through erosion and leaching [1,2], and thus resulting in unsustainable soil degradation [3,4]. Soil is a finite natural resource that is under pressure of increasing consumption, rapid population growth, and agricultural intensification [5]. Increasing consumption and population growth are driving up yields per unit area in crop production, while poultry production is on the rise to provide an intensive source of protein [6]. Over the last few decades there have been rapid changes in livestock production, with 61% of pork, 81% of poultry, and 86% of eggs now produced on intensive, industrial farms [7]. Generally, there has been a rapid change in how animal products are produced, processed, consumed, and marketed. Growth in livestock production, in both developed and developing countries, has been led by poultry [8]; while eggs and poultry meat have become the main source of animal protein [9]. Recently, African swine fever led to an increase in demand for poultry meat. This market is further complicated by the increase in EU internal production, the UK's exit from the EU, and the increased quota for Ukrainian poultry meat. Broiler production is the dominant sector in poultry meat production, being the second most produced and consumed meat in the EU after pork [10]. In Europe, broiler farms with more than 100,000 places are very common. In 2013, 891.4 million broiler chickens were bred on more than two million farms in the EU. Farms with more than 100,000 birds account for 38% of the total poultry population. It is estimated that 90% of broilers in the EU are reared in intensive indoor systems [11,12]. These closed, intensive farming systems, with high animal density, indoor housing, and the use of fast-growing breeds obtained by genetic selection can facilitate the spread of epidemics and certain zoonotic diseases (H1N1, H5N1 influenza, brucellosis, salmonellosis, leptospirosis, avian influenza, etc.) [13]. Animal health problems can cause serious social, economic, and environmental damage, and in some cases can also pose a threat to human health [14]. The epidemics did not end with African swine fever, with the emergence of H5N1 avian influenza in early February 2020. The outbreak of avian influenza in Central Europe in December 2019 limited the production in 2020 in terms of volume and has posed a major challenge for the animal health system and the poultry sector. In the current situation (due to ever stricter animal health regulations) manure is an "environmental pollutant" that livestock farmers are trying to dispose of. At the same time, the concept of the circular economy highlights the inescapable role of animal manure in soil management; there has always been a fundamental link between livestock and crop production in agriculture. It is necessary to establish a system of biodegradable organic matter management that focuses on the cycling of organic matter to maintain soil fertility in the long term and that creates a shared interest between crop and livestock farmers in the use of animal manure [15].

There are various different types of poultry manure, such as deep litter manure, broiler manure, and hen manure. The ratio of litter to manure and the moisture content causes variation among manures from different poultry houses. The quality of manure can vary depending upon many factors, including the age and diet of the flock, the moisture content, and the age of the manure [16]. Secondary pollution is making landfills less and less suitable for organic waste [17], and tightening environmental regulations mean that the landfilling of organic waste and by-products is not an option [18]. The use of energy (biogas production) or material (composting) recovery methods to manage organic wastes and by-products is widespread around the world [19,20]. Composting is considered a favorable option in many developing countries due to the lower investment and operating costs, need for scientific expertise, and technical complexity [21,22]. One way to treat litter manure is composting, which increases the quality of raw manure and reduces the environmental risks [23,24]. During the composting process, the volume and weight of manure are reduced, pathogens and weed seeds are destroyed [25], unpleasant odors are reduced [26], and nutrients and organic matter are stabilized [27].

The use of compost in agriculture is very important because it contributes to the increase of soil fertility [28,29] and can also be crucial in the treatment of plant diseases [30]. There is a growing demand for compost to be further utilized as a raw material to produce compost tea. Compost tea is a concentrated microbial solution, which is made by extracting

nutrients and microorganisms from the compost using an extractant. Compost tea is made by mixing the compost with a distilled water (as a weak agent) and by incubating for a specified period of time, with or without active aeration (aerated compost tea, (ACT), or non-aerated compost tea, (NCT)), and produced with or without additives [31]. Compost teas made from matured compost contain high levels of bacteria (10^8 – 10^9 /mL). Aerated compost teas contain even higher amounts of bacteria (up to 10^{10} – 10^{11} /mL). However, if oxygen-deficient conditions exist during the production of a compost tea, the aerobic bacteria are no longer able to multiply and grow and die or enter to a dormant state [32]. The use of compost tea is widespread worldwide due to its wide applicability as a bio-stimulator [33,34]. The advantage of using compost tea instead of compost is that compost cannot be applied to the foliage by spraying or irrigation, and the availability of nutrients is better [35]. Several researchers have pointed to the effectiveness of organic manure, compost, and composting in horticultural technologies, and the positive effects of compost teas have been demonstrated on cumin [36], fennel [37], basil [38], pepper [34], lettuce [39], and tomatoes [40] with test plants. Compost tea reduces plant diseases [34,35], protects plant roots, provides nutrients to the plants, and improves plant health [41]; thus compost tea is a possible alternative to synthetic agents [38,39]. Following the application of these organic-based substances, the improvement in yield and quality can be attributed to the enhancement of the beneficial microbial communities in the soil, the improvement of plant mineral absorption, and the stimulation of phytohormones [42]. Various liquid fertilizers or their extracts are known to serve primarily as a source of soluble plant nutrients, growth promoters, and disease suppressants [43,44].

Based on the above, in this study we investigated the specific products produced by a novel approach to organic waste utilization technology. A pot experiment with a pepper test plant was set up, using two different soils (Arenosol, slightly humous Arenosol) and two different doses (irrigation once a week with 40 mL of compost tea: dose 1, D1; irrigation twice a week with 40 mL of compost tea: dose 2, D2) of compost tea. Compost tea raw materials, compost tea, and compost tea treated soils were tested. The products (granulated manure, compost tea) and their effects were characterized by the following parameters: aerobic bacterial count (log CFU/g), fluorescein diacetate activity (FDA, $\mu\text{g FI/g soil}$), glucosidase enzyme activity (GIA; PNP/ $\mu\text{mol/g}$), and the identification of microorganisms in compost tea with MALDI-TOF MS. Furthermore, we aimed to investigate how the microbiological indicators tested and the effects of compost tea on the tested plants could be interpreted.

2. Materials and Methods

The examined product from which compost tea was produced, consisted of a mixture of broiler manure, hen manure, and straw pellets, which were treated by intensive composting with meat meal as an additive. The process was carried out under standard, controlled conditions from hatching, through feed production and mixing, to manure processing. A schematic of the process is shown in Figure 1.

The composting plant uses deep litter broiler manure and hen manure from a downstream hatchery as feedstock, and 60,000 tons of manure are produced annually at the company's various sites. The parent breeding is carried out with colored breeding pairs and the broiler is kept with a white meat hybrid Ross 308, and straw bedding is used for both breeds. The breeding is carried out with colored breeding pairs and the broiler is kept with a white meat Ross 308 hybrid. Both on the breeding farms and on the broiler farms, litter is produced with heat-treated straw pellets, whose high absorption capacity results not only in excellent litter quality but also in a low moisture, dry, and deep manure. In the manure processing plant, where the product under study originated, three continuous mode manure fermenters of the HOSOYA [45] (Hosoya Ltd., Kanagawa, Japan) type are operated, with controlled and regulated fermentation [46,47]. The fermenters with a stirring machine are used to store 30 tons of raw material every day of the year (1.5 tons of raw material (a mixture of broiler, hen manure, and straw pellets) is stored,

which represents 953.84 kg of broiler manure, 476.93 kg of hen manure, and 69.23 kg of straw pellets (Table S1). The 2/3:1/3 mixing ratio of the raw materials (deep layer broiler manure and hen manure) is necessary because our preliminary studies showed that (at least) 1/3 of chicken manure is needed for composting of broiler manure, whose microbial composition helps to start the fermentation process.

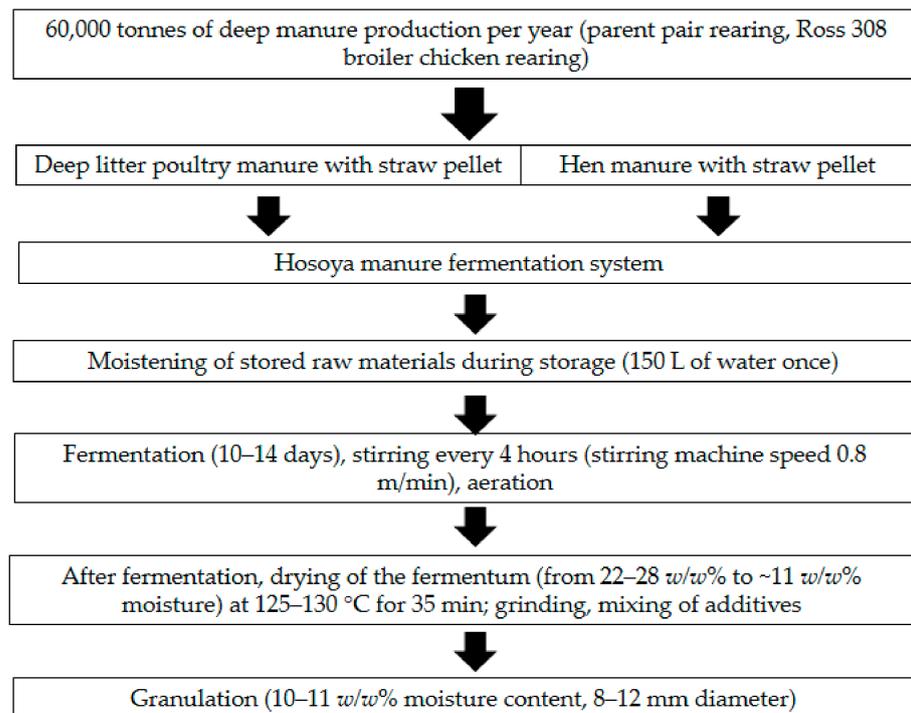


Figure 1. The schematic of the poultry manure utilization process.

Fermentation is carried out by air injection, mixing the raw material, and turning it over every 4 h, for 10 to 14 days. The incoming raw material has a moisture content of 20–40 *w/w%*, depending on the type of raw material. In order for fermentation to start and be intensive, the raw material must be adjusted to a moisture content of 40–45 *w/w%* by adding water. The raw material is moistened once with 150 L of water when it is fed into the fermenters. At the end of fermentation (after 10 to 14 days) the moisture content of the fermented fermentate is reduced to 22–28 *w/w%*. The next technological process is the intensive fluid bed drying (Tema Process, Wapenveld, The Netherlands) (125–130 °C, 25–30 min) of the fermentum from 22–28 *w/w%* to 10–11 *w/w%* moisture. The prolonged exposure to heat above 125 °C kills all pathogenic bacteria. The dried fermentum is ground into a powder fraction and becomes the raw material for further finished products. The final product is packaged in granulated form, but before the granulation process is carried out, the natural fermentum is supplemented with complex organic nutrients as a raw material.

2.1. Compost Tea Preparation

The characteristics of the product used to prepare compost tea are detailed in Table 1.

The equipment used in the production of compost tea was sterilized in an AE-75 DRY autoclave (Raypa Ltd., Barcelona, Spain) with wet steam sterilization. After sterilization, the composted granulated manure mixture was weighed in a 0.7 l volume glass container. For the preparation of compost tea, distilled water was used as a weak agent. Compost tea was prepared in a 1/10 (*w/v*) compost to water ratio (CWR) based on Islam et al. [48] and Zhang et al. [49], and using an Unimax 1010 shaker (Heidolph Instruments GmbH & Co, Schwabach, Germany) at 130 rpm for 48 h; the compost tea temperature was set at 35 °C (Table S2), after the extraction time the compost tea was filtered through a filter

paper (12–15 μm , VWR International, Debrecen, Hungary), and the filtered tea was used for further processing.

Due to the concentration of the compost tea and the nutrient requirements of the test plant, the suspension was applied to the peppers at a five-fold dilution.

Table 1. Characteristics of evaluated product.

Parameters	Value
Dry matter content ($w/w\%$)	87.60
Moisture content ($w/w\%$)	12.40
pH	6.99
Organic matter content ($w/w\%$)	73.03
Nitrogen-content ($w/w\%$)	4.86
Phosphorus pentoxide content ($w/w\%$)	6.88
Potassium oxide content ($w/w\%$)	4.04

2.2. Compost Tea Treatments and Design of Pot Experiments

The selected test plant for testing the effect of compost tea was white sweet pepper (*Capsicum annuum* L.). During the experiments, 1 kg of slightly humous Arenosol (SHA) and Arenosol (A) soil was weighed into pots and the pepper seedlings were planted.

The selected soils are classified as “Arenosols” according to the World Base Reference of Soil Resources (WRB). Soils in this category are characterized by low water holding capacity, high water permeability, and low nutrient content, all of which lead to rapid water stress [50]. Soils that are poorly vegetated and susceptible to wind erosion belong to this major group [51]. The main characteristics of the sub-categorized soils are presented in Table 2.

Table 2. Characteristics of the soils used for the experiments.

Measured Parameters	Slightly Humous Arenosol	Arenosol
pH (KCl-extract)	5.76	6.13
Total water soluble salts ($w/w\%$)	0.02	0.05
Carbonate content ($w/w\%$)	<0.100	<0.100
Organic carbon content (humus content) ($w/w\%$)	1.57	0.67
Phosphorus-pentoxide (mg/kg) (AL-extract)	176	131.2
Potassium-oxide (mg/kg) (AL-extract)	351	177.96
Nitrate (mg/kg) (KCl-extract)	12.3	7.42

The Arenosol soils are characterized by a partially developed topsoil layer with low humus content and no subsurface clay accumulation. In addition, the aggregate thickness of the finer textured layers is less than 15 cm, “the proportion of coarse debris within ≤ 100 cm of the mineral soil surface is $<40 v/v\%$ in all layers” [50]. Arenosol and slightly humous Arenosol soils are also characterized by rapid mineralization of organic matter, lack of organic colloid, poor water management, poor nutrient supply, and drought sensitivity. The conditions necessary for biological soil formation processes in the formation of these main soil types are only present for a short period of time and their impact is therefore limited. Of the soils selected, Arenosol soil had inferior properties compared to the humic sandy soil, as reflected in the parameters tested. There was a minimal difference in pH, as well as a higher total water-soluble salinity in the Arenosol soil.

Each treatment was set up in three replicates. In the pot experiments, 40–40 mL of a five-fold diluted compost tea was applied by seeding once (Dose 1, D1) and twice (Dose

2, D2) a week. On the other days of the week, the seedlings were irrigated with distilled water to a level of 70 *w/w*% of field water capacity. The experiment was terminated at the fourth (Week 4, W4) and eighth weeks (Week 8, W8).

2.3. Microbiological Analysis

The following microbiological parameters were used to examine the granulated product, compost tea produced, and compost tea treated soils (Table 3).

Table 3. Evaluated microbiological indicators.

Measured Microbiological Indicators	Tested Samples	References
Fluorescein diacetate hydrolysis activity (FDA)	Granulated product	[52,53]
	Compost tea	
	Soils treated with compost tea	
Most probable number of microorganisms (aerobic bacteria: nutrient medium)	Granulated product	[54]
	Compost tea	
	Soils treated with compost tea	
β -Glucosidase enzyme activity (GIA)	Granulated product	[55]
	Compost tea	
	Soils treated with compost tea	
MALDI-TOF MS	Compost tea	[56]

Monitoring of the total microbial activity is a suitable method for measuring organic matter cycling, as more than 90% of the energy passes through microbial degraders. The FDA enzyme assay is one that shows how many microorganisms in the soil are engaged in life activity, i.e., degradative (catabolic) activity. The FDA test can be used to show the livingness of the soil and its actual functionality [51]. The β -glucosidase enzyme activity assay was chosen because it is generally positively correlated with soil organic matter content.

2.4. Identification of Microorganisms in Compost Tea

To identify the microorganisms in compost tea, a sterilized pelleted sample of a mixture of deep litter broiler manure, deep litter hen manure, and straw pellets (953.84 kg broiler manure, 476.92 kg chicken manure and 69.23 kg straw pellets) was used, which had been subjected to a 14-day composting process; 1:10 CWR, 48 h extraction time, 35 °C extraction temperature; the compost tea was prepared and filtered on filter paper (12–15 μ m, VWR International, Debrecen, Hungary) prior to the tests and refrigerated at +4 °C until the tests. The identification procedure was performed once.

Single colonies from freshly grown isolates from given solid medium were picked in duplicate on a ground-steel target plate. Afterwards, 1 μ L formic acid solution was added to each spot, then 1 μ L of matrix solution was pipetted onto each spot, and the plate was air dried at room temperature. Mass spectra were generated with a Microflex Biotyper (Bruker Daltonics, Billerica, MA, USA) using the standard settings. In case of each sample, mass fingerprints were acquired using flexControl version 3.0 software (Bruker Daltonics, Billerica, MA, USA), analyzed over a mass range from 2000 to 20,000 Dalton, and compared with the Bruker Daltonics database. This software generates a result list with score values suggesting the reliability of identification. The received score values are interpreted as unreliable identification when a score is lower than 1.7, as a probable genus identification when a score is between 1.7 and 1.99, and as a secure genus identification when a score is >2.0. The results were probable and highly probable species identification for 2.0–2.29 and \geq 2.3, respectively [56].

2.5. Examination on the Pepper Test Plant

The pot experiments were terminated after 4 and 8 weeks, and the height of the plants (cm) was measured according to Slezák [57], while the total chlorophyll content ($\mu\text{g/g}$) was determined and calculated according to Szabó et al. [58].

2.6. Statistical Analysis

Statistical analyzes were performed using R software in an R Studio user environment (version 3.6.2.) [59]. The Shapiro–Wilk normality test was used to examine the distribution of the data, and then the type of test to be used for further analyzes was selected as a function of the distribution. To verify statistical differences between the different treatments, one-way analysis (Duncan-test) of variance was used at a $p < 0.05$ level of significance.

3. Results

3.1. Microbiological and Chemical Characteristics of Broiler and Hen Manure

The dry matter content ($w/w\%$) of broiler manure (65–70 $w/w\%$) and hen manure (63–67 $w/w\%$) was high due to the breeding technology, as the dry matter content of the manure decreases at the end of the 6-week rotation for broiler manure. The pH of the manures was slightly alkaline to neutral (in the case of broiler manure the pH was 6.91–7.40, and in the case of hen manure the pH was 6.59–6.82), while the specific conductivity (11.10–12.78 mS/cm) and total nitrogen content (2.14–2.75 $w/w\%$) were almost the same. The organic matter content, was higher in hen manure (66.18 $w/w\%$) compared to broiler manure (58.81 $w/w\%$). The difference in breeding technology also showed a higher biological activity for chicken manure.

In addition to the physical and chemical characteristics of the broiler and chicken manure, the microbiological characteristics were also investigated. The number of microorganisms in poultry manure is very high, up to 10^{10} CFU/g (Colony Forming Unit/g), and Gram-positive bacteria (*Actinomycetes* sp., *Bacillus* sp.) account for 90%. The presence of *Actinomycetes* sp. and *Lactobacillus* sp. is beneficial because they prevent the development of pathogens in the manure [60]. The aerobic bacteria in poultry manure were of the order of 10^9 CFU/g [61]. In the poultry manure, aerobic bacteria of the genus *Enterococcus* were predominant. The bacterial plate count of digestible aerobic bacteria in chicken manure was of the order of 10^8 CFU/g, while in broiler manure the aerobic bacteria count was three orders of magnitude, or a thousand times, less (10^5 CFU/g). In contrast, the most probable numbers of microorganisms in both the broiler manure and hen manure were of the same order of magnitude (10^3 CFU/g).

3.2. Microbiological Characteristics of the Evaluated Product and Compost Tea

Compost tea produced from granulated manure had a slightly acidic-neutral pH (pH 6.59 ± 0.06), which was similar to that of a mixture of broiler manure and hen manure. The electrical conductivity of the compost tea was high (14.82 ± 0.05 mS/cm), which can be explained by the high electrical conductivity of the two types of manure and the high content of water soluble salt in the manure. The nitrate concentration (1002.22 ± 40.55 mg/L) and the ammonium concentration (1426.67 ± 46.90 mg/L) in compost tea were high, indicating that the non-aerated system was not anaerobic, but oxidative. The high potassium concentration (1000.00 ± 223.61 mg/L) can be explained by the fact that potassium is highly soluble in aqueous media, yet the phosphate concentration is the lowest of the ions tested, which can be explained by the low phosphorus concentration in the initial compost and the low water-soluble phosphorus concentration (Table S1).

In general, the biological activity of the starting products in a solid form was higher than that of the extracted compost tea made from them (Table 4).

Table 4. Microbiological characteristics of evaluated product and compost tea.

Indicators	Evaluated Product	Compost Tea
FDA ($\mu\text{g Fl/g soil}$) *	16.74	12.41
GIA (PNP/ $\mu\text{mol/g}$)	174.98	6.67
Culturable aerobic bacteria ($\log_{10}\text{CFU/g}$)	5.97	6.36

* $\mu\text{g Fl/g soil}$: $\mu\text{g Fluorescein/g soil}$.

The detectable values of the aerobic bacteria count were very high in the evaluated product, within which only one order of magnitude difference could be detected. Due to the magnitude of the variance, an order of magnitude (10-fold) difference also occurred within the treatment. However, the germ count values of aerobic bacteria were not proportional to the activities of any of the enzymes tested. The added bacteria did not correlate with β -glucosidase activity (G1A) or FDA, which indicates sugar metabolism, so they did not indicate active metabolism. However, their presence was confirmed by this method, which may suggest that they may be activated during subsequent use.

The biological activity of the starting products in solid form was higher than that of the extracted compost tea made from this. Non-aerated compost teas usually have no added nutrients other than the starting material. In these compost teas, there is a high probability that the solution will be low in oxygen or anoxic, anaerobic.

However, it was observed, that after 10-fold dilution, the difference in the FDA measurement did not become ten times smaller, as would be expected from the degree of dilution, but much higher values were obtained. FDA activity, which indicates degradable metabolism, is a good indication of the role of microbes in the breakdown of organic matter. However, the low values of GIA indicated that the sugar utilization capacity was not high or may have already been incorporated into the body weight of microorganisms engaged in catabolic activity. The numbers are expressed in logarithms based on 10, so the differences between the individual numbers in the germ count show 10–100 \times differences between the samples according to the logarithm.

The preparation of compost tea was thus primarily conducive to the growth of bacteria, although the growth did not reach an order of magnitude of 0.5 compared to the original starting products. The aerobic bacterial count of the products and the solutions did not follow the previous trends, as in the present case compost tea soluble and relatively easy-to-absorb nutrients were revealed and ideal conditions created primarily for bacterial growth. However, high aerobic bacterial germ counts do not correlate with enzyme activities.

On the other hand, the enzymes are specific and selectively show the activity of the participating microbes, so only a certain part of the microorganisms can be functional, depending on the enzyme measured. In addition, soils and compost are not only inhabited by bacteria, but also by so-called whole soil food web organisms. The soil–food web includes not only bacteria and fungi, but also nematodes, plant roots, algae, and some macroscopic animals. Microorganisms in the compost tea were identified using MALDI-TOF MS. This method provides efficient, rapid, and accurate results for the determination of protein mass spectra, and is thus useful for the identification of microorganism strains and the evaluation of their relatedness [56]. Nevertheless, this technology has not yet been used for the identification of microorganisms in compost tea. Based on this, our aim was to investigate how the agglomeration process affects the microbial composition and thus the microbiological properties of compost tea. The microbial communities of the compost tea were predominantly bacteria. The dominant bacterial genera were analyzed as *Bacillus* sp., *Lysinibacillus* sp., and *Pseudomonas* sp. These identified bacterial genera were found in the range of 10^3 – 10^4 CFU/mL in the compost solution. The results show that the heat treatment in the production process significantly reduced the number of bacteria, which in result reduced the environmental risk of the manure.

3.3. Microbiological Characteristics of Soils Treated with Compost Tea

Among the enzymes, the activity of the fluorescein diacetate (FDA) enzyme was examined. In general, the slightly humous Arenosol showed (Figure 2.) higher values, as did the compost tea with which the treatments were performed. Outliers may also have been influenced by the water content of the soil. This is because the values of FDA activity can also be strongly influenced by abiotic environmental factors, such as the water and organic matter content of a given soil and other soil physical properties. Higher values were observed in the 8-week-old, older samples, indicating an increase in FDA enzyme activity with the age of the plants; in accordance with previous experience and literature data [59].

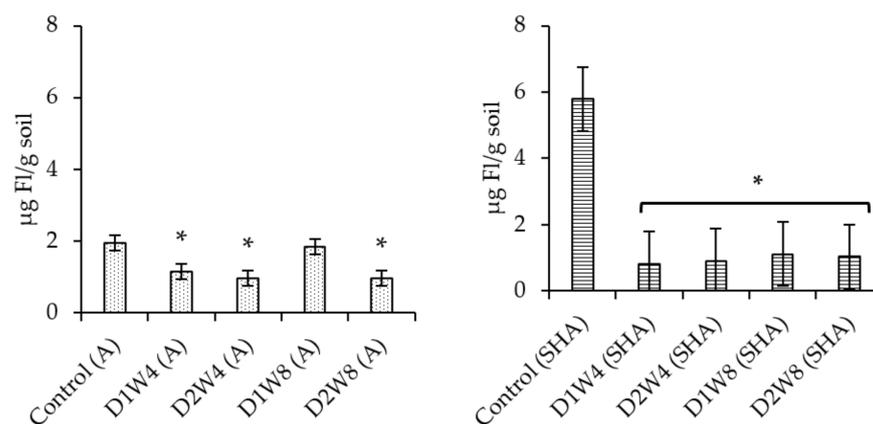


Figure 2. FDA enzyme activity of Arenosol and slightly humous Arenosol. The codes mean the following treatments: Control (A): Control Arenosol. D1W4 (A): Dose 1 week 4 (Arenosol). D2W4 (A): Dose 2 week 4 (Arenosol). D1W8 (A): Dose 1 week 8 (Arenosol). D2W8 (A): Dose 2 week 8 (Arenosol). Control (SHA): Control slightly humous Arenosol. D1W4 (SHA): Dose 1 week 4 (slightly humous Arenosol). D2W4 (SHA): Dose 2 week 4 (slightly humous Arenosol). D1W8 (SHA): Dose 1 week 8 (slightly humous Arenosol). D2W8 (SHA): Dose 2 week 8 (slightly humous Arenosol). * Indicates significant difference at $p < 0.05$ (calculated by Duncan-test) between control and compost tea treated soils.

Lower enzyme activity values could be detected in the treated soil without plants, as plants increase enzyme activities. When mixing compost tea into the soil, it was also observed that dilution of compost products with high enzyme activity occurred. A significantly lower microbial activity could be detected in the Arenosol than in the slightly humous Arenosol soil. However, when the values of the two soils were averaged from a plant point of view, the peppers increased the FDA enzyme activity values. There was less activity in compost tea treated soils.

The level of FDA activity was found to be uniform in the soils at weeks 4 and 8 of the growing season, although after week 8 the activity tended to be lower. In the control, the value tended to be higher, but due to the large standard deviation, we could not detect an appreciable difference between the control and treated soils. However, the activity of the original granulated product was significantly higher than these.

It can be stated that the activity of the two soils was similar, although significantly larger standard deviations were obtained in the Arenosol compared to the slightly humous Arenosol soil (Figure 3). With the treatment of the product, it was necessary to increase the intake of organic matter in the Arenosol, and thus increase the activity. However, increasing the product dose did not result in a proportionally higher FDA activity. The values of the samples taken after 8 weeks tended to be higher, which is proportional to the age and growth of the plant, but this could also be detected only as a tendency, it did not prove to be significant, due to the large standard deviations. FDA activity is a good indicator of the microbiological and degradative activity of soils. Studies have demonstrated the high

activity value found in original compost. This became lower immediately after mixing into the soil, as a result of the obvious dilution. This was not significantly increased by doubling the dose. However, it can also be stated that the activity could be increased to sand-like values in Arenosol, which were significantly poorer in organic matter than before. The pepper test plants also increased the FDA values of the soils at the same level, which tended to improve with the physiological condition of the plants with age. A similar conclusion can be drawn for the enzyme glucosidase as for the examination of FDA enzyme activity.

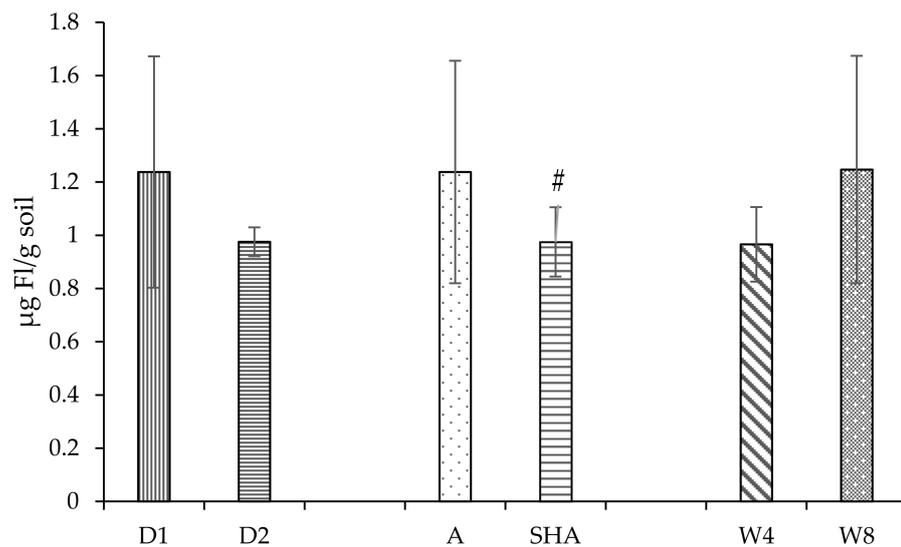


Figure 3. Summary of FDA enzyme activity results by treatments (doses), soil types, sampling weeks. The codes are as follows: D1: Dose 1. D2: Dose 2. A: Arenosol. SHA: slightly humous Arenosol. W4: Week 4. W8: Week 8. # indicates significant difference at $p < 0.05$ (calculated by Duncan-test) between Arenosol and slightly humous Arenosol soils.

In the study of glucosidase enzyme, the sand soil values improved the most between the two soils, as organic matter supplementation had a greater effect on increasing the initial activity in Arenosol (Figures 4 and 5).

The untreated activity was markedly increased by the pepper, but the measured enzyme activity was balanced. β -glucosidase activity values also increased with the age of the plants, this dose-effect of the product was only proven in D2.

The number of aerobic bacteria also supports the above mentioned observations (Figure 6). Even in the case of the control soil, it can be seen that the values of the Arenosol are 0.5–1 orders of magnitude lower compared to the slightly humous Arenosol, which is not surprising in the case of Arenosol. Here it is more noticeable than the number of aerobic bacteria increased after the application of compost tea, and this was also confirmed by previous enzyme studies.

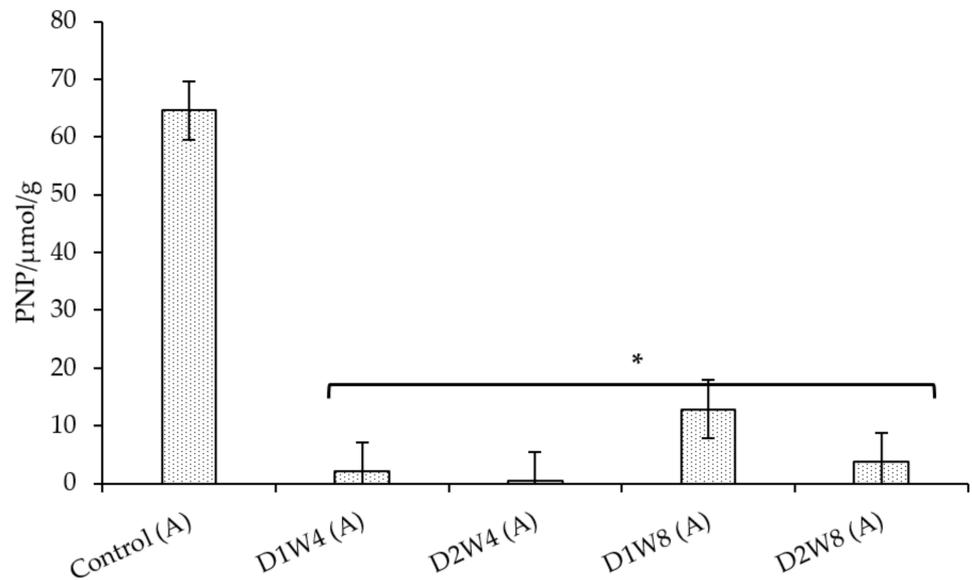


Figure 4. Glucosidase enzyme activity (GIA) of Arenosol. The codes mean the following treatments: Control (A): Control Arenosol. D1W4 (A): Dose 1 week 4 (Arenosol). D2W4 (A): Dose 2 week 4 (Arenosol). D1W8 (A): Dose 1 week 8 (Arenosol). D2W8 (A): Dose 2 week 8 (Arenosol). * indicates significant difference at $p < 0.05$ (calculated by Duncan-test) between control and compost tea treated soils.

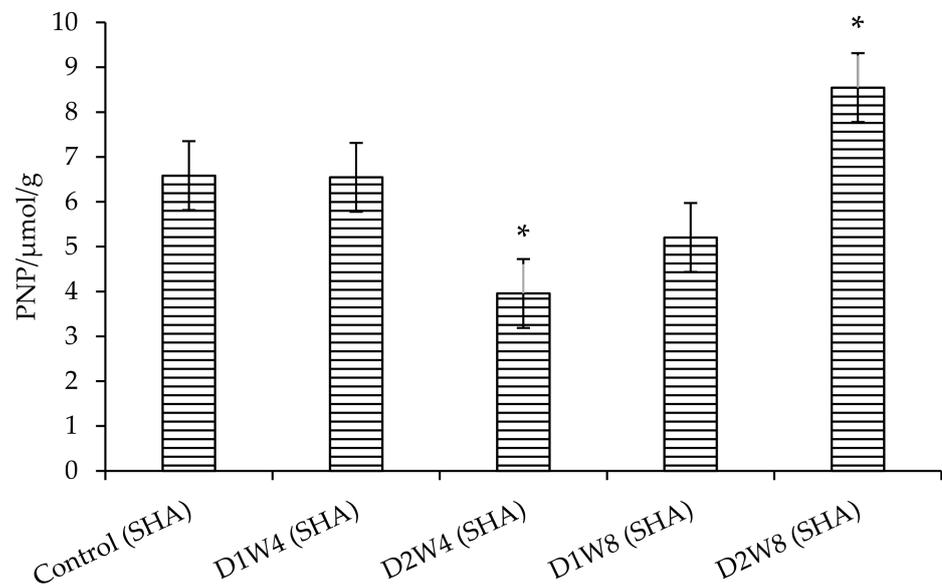


Figure 5. Glucosidase enzyme activity (GIA) of slightly humous Arenosol control (SHA): Control slightly humous Arenosol. D1W4 (SHA): Dose 1 week 4 (slightly humous Arenosol). D2W4 (SHA): Dose 2 week 4 (slightly humous Arenosol). D1W8 (SHA): Dose 1 week 8 (slightly humous Arenosol). D2W8 (SHA): Dose 2 week 8 (slightly humous Arenosol). * indicates significant difference at $p < 0.05$ (calculated by Duncan-test) between control and compost tea treated soils.

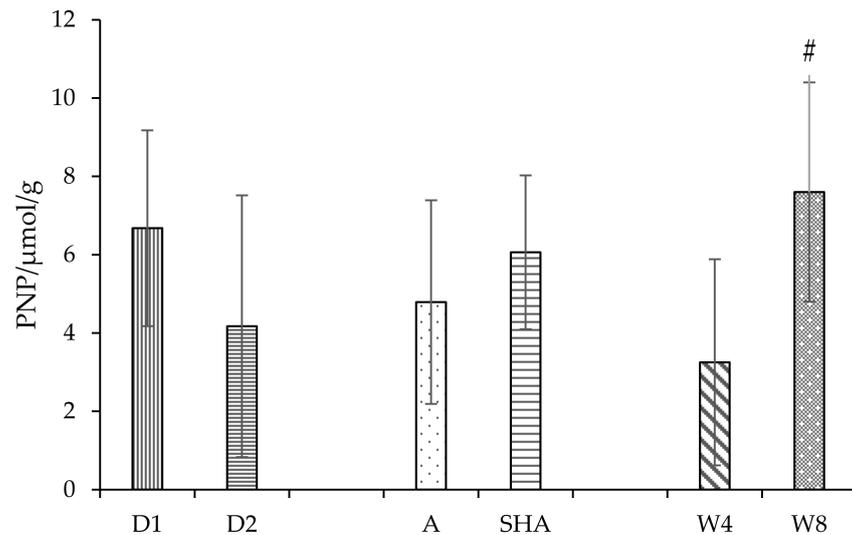


Figure 6. Summary of GLA enzyme activity results by treatments (doses), soil types, and sampling weeks. The codes are as follows: D1: Dose 1. D2: Dose 2. A: Arenosol. SHA: slightly humous Arenosol. W4: Week 4. W8: Week 8. # indicates significant difference at $p < 0.05$ (calculated by Duncan-test) between W4 and W8.

It can be stated that the values of the aerobic bacterial count became higher as a result of the treatment (Figure 7). In the treated soil, the effect of the treatment also showed improvements (Figure 8). However, peppers were also able to raise germ count values. Similarly, no single or double dose of compost tea caused an increase in germ count, although the detectable values could rise to the same level as the compost tea.

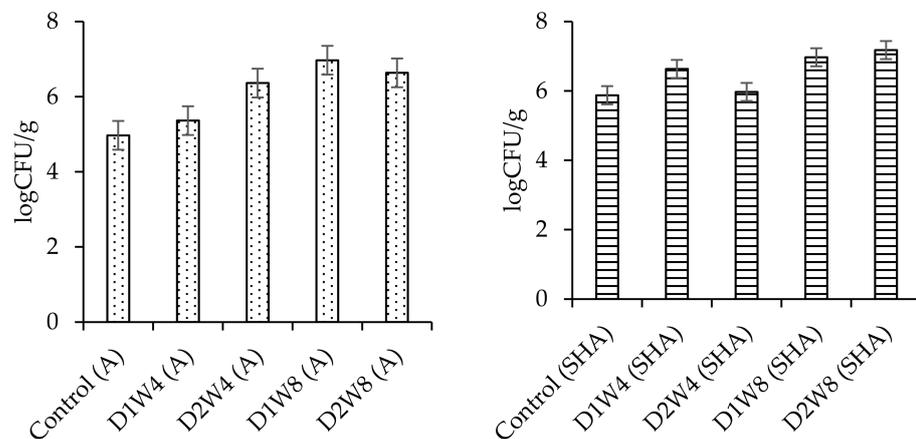


Figure 7. Number of aerobic bacteria, number (log CFU/g) of Arenosol and slightly humous Arenosol. The codes means the following treatments: Control (A): Control Arenosol. D1W4 (A): Dose 1 week 4 (Arenosol). D2W4 (A): Dose 2 week 4 (Arenosol). D1W8 (A): Dose 1 week 8 (Arenosol). D2W8 (A): Dose 2 week 8 (Arenosol). Control (SHA): Control slightly humous Arenosol. D1W4 (SHA): Dose 1 week 4 (slightly humous Arenosol). D2W4 (SHA): Dose 2 week 4 (slightly humous Arenosol). D1W8 (SHA): Dose 1 week 8 (slightly humous Arenosol). D2W8 (SHA): Dose 2 week 8 (slightly humous Arenosol).

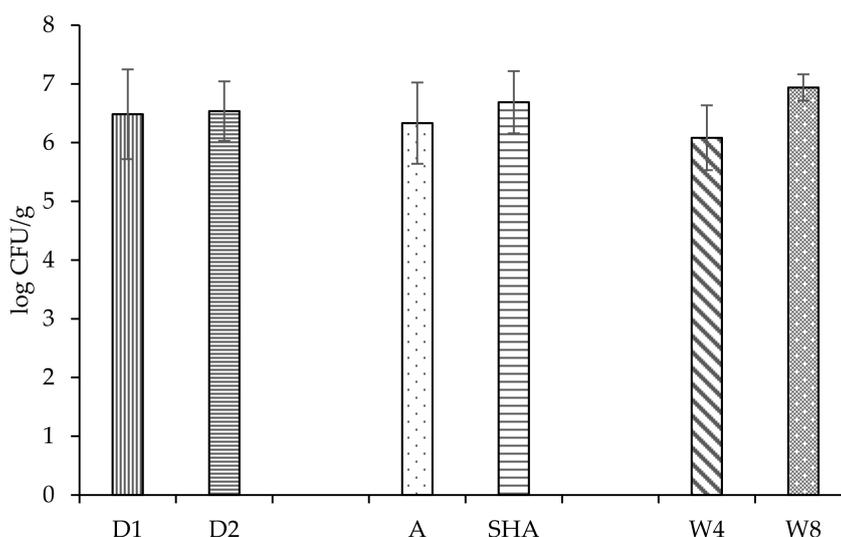


Figure 8. Summary of aerobic bacteria number (log CFU/g) results by treatments (Doses), soil types, and sampling weeks. The codes are as follows: D1: Dose 1. D2: Dose 2. A: Arenosol. SHA: slightly humous Arenosol. W4: Week 4. W8: Week 8.

The germ counts that could be cultured can be compared in a manner proportionate to the results of the enzyme assay, especially with FDA activity. Examination of germ counts showed that the number of aerobic microorganisms increased with compost tea treatment. The peppers were able to utilize the product and the values also improved with the age of the plant. At the application dose, even the first dose caused an increase, the double dose did not give a better result.

3.4. Effect of Compost Tea on the Pepper Test Plant

In addition to the microbiological measurements, the effect of compost tea on the pepper was determined by measuring plant shoot length (cm) and total chlorophyll content ($\mu\text{g/g}$).

At the fourth week for D2 of Arenosol soil, the longest average plant shoot length (41.00 ± 2.65 cm) was measured (Figure 9). This treatment was significantly different from the control when the fourth week of treatment is considered. At W8, the pepper shoot length varied between 37.67 ± 2.89 cm and 40.33 ± 2.08 cm. D2W4, D1W8, and D2W8 were statistically in the same group, indicating that the eighth week of treatment did not result in significantly longer shoot length. There was no significant difference in plant shoot length between treatments (Table S2).

In Arenosol soil (Figure 10), the total chlorophyll content of plants increased as a result of the treatments compared to the control.

For the fourth week of treatments, the D2W4 had the highest total chlorophyll content (4135.49 ± 344.17 $\mu\text{g/g}$). This was the only treatment that was significantly different from the control and the other treatments in both the fourth and eighth week. The lowest total chlorophyll content was measured for D1W8 (3028.96 ± 356.25 $\mu\text{g/g}$). All treatments except D2W4 were in the same statistic group as the control (Table S3). In W8 of measurements, the total chlorophyll content of the treated plants was lower than in the W4.

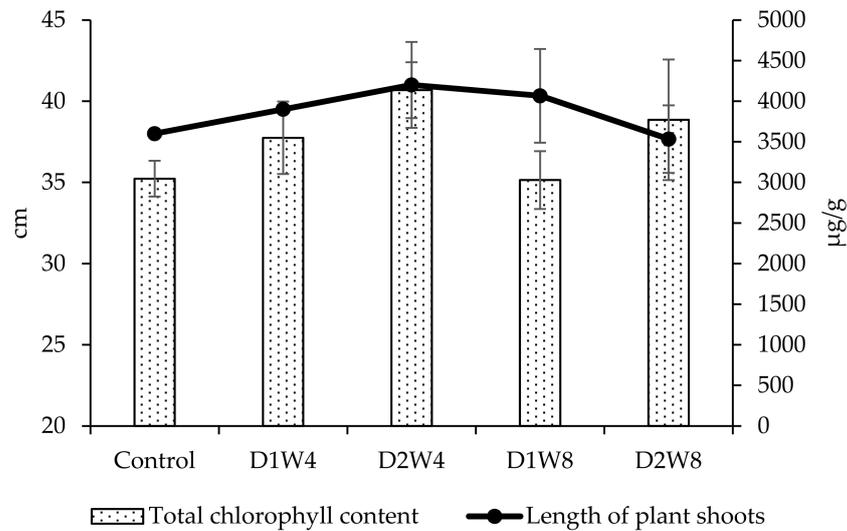


Figure 9. Changes in total chlorophyll content and plant shoot length of Arenosol soil. The codes are as follows: D1W4: Dose 1 Week 4. D2W4: Dose 2 Week 4. D1W8: Dose 1 Week 8. D2W8: Dose 2 Week 8.

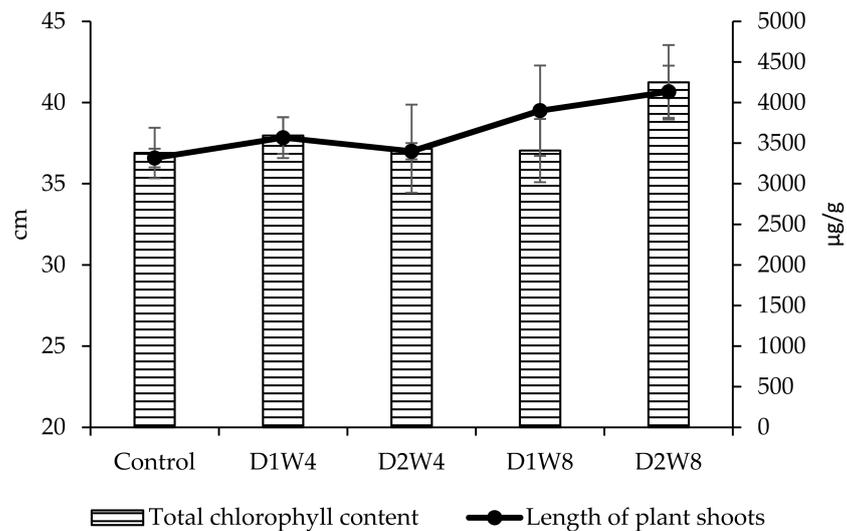


Figure 10. Changes in total chlorophyll content and plant shoot length of slightly humous Arenosol soil. The codes are as follows: D1W4: Dose 1 Week 4. D2W4: Dose 2 Week 4. D1W8: Dose 1 Week 8. D2W8: Dose 2 Week 8.

On in the slightly humous Arenosol did the length of the pepper shoots also increase with increasing dose in the fourth week (Figure 10). The fourth week treatments were significantly different from the control, but the treatments were statistically in the same group, with no significant difference between D1 and D2 (Table S3). Similar results were obtained for the eighth week treatment, as the treatments were statistically in the same group, but there was a significant difference between the fourth and eighth week treatments. The longest shoot length was measured for D2W8 (40.66 ± 1.61 cm), and the lowest shoot length was measured for the control (36.58 ± 0.58 cm). These results demonstrate the positive effect of compost tea on the pepper.

In the slightly humous Arenosol (Figure 10), there was no increase in total chlorophyll content compared to the control as a result of the treatments (Table S3). The fourth week

treatments formed a statistical group with the control, no significant increase in total chlorophyll content was detected between these treatments. The eighth week treatments were significantly different from each other and from the fourth week treatments. The highest total chlorophyll content was measured for D2W8 ($4249.62 \pm 458.76 \mu\text{g/g}$), and the lowest total chlorophyll content was measured for the control ($3379.17 \pm 310.64 \mu\text{g/g}$). These results demonstrate that the compost solution applied on slightly humous Arenosol soil had a positive effect on the total chlorophyll content of the pepper.

In the Arenosol soil, there was no significant difference compared to the control (Figure S1), but in the slightly humous Arenosol, the effect of the treatments was statistically proven by W8 (Figure S2).

4. Discussion

In the case of industrial organic matter management technologies, less attention is paid to microbiology, and the physical, chemical, and engineering approach predominates. However, when using organic materials and fertilizers, the whole process must be considered, so it is important to examine the microbiology of the raw materials and the products made from them. The starting point for our investigations was that compost tea made from materials from conventional technology had been investigated before [62–65], but compost tea made from industrial compost had previously not been prepared, investigated, and tested

The bacterial plate counts of cultivable aerobic bacteria in hen manure were of the order of 10^8 g, while in broiler manure the aerobic bacteria count was three orders of magnitude, or a thousand times, less (10^5 CFU/g). In contrast, the most probable number of microorganisms in both broiler manure and hen manure were of the same order of magnitude (10^3 CFU/g). Chen-Jiang [58], investigated the microbiological composition of organic nutrient supplements in poultry manure and poultry manure-based organic nutrient supplements. Their results showed that the concentration of microorganisms was 10^{10} CFU/g, and 90% of the microbial community was composed of Gram-positive bacteria, e.g., *Clostridia*, *Bacilli*, *Lactobacilli*.

Based on our results, the biological activity of the starting products was found to be higher (FDA: $+4.33 \mu\text{g/g}$, GIA: $+168.31 \text{ PNP}/\mu\text{mol/g}$) than that of the compost tea made from them. The aerobic bacterial counts of the products and compost tea did not follow these trends, since in this case the solution had a higher value compared to the product. The most probable reason for this is that with compost tea, relatively easily absorbed nutrients were revealed and ideal conditions were created for bacterial growth in particular. The low oxygen levels in non-aerated compost tea, which results in anaerobic conditions, can lead to aerobic microorganisms becoming inactive and anaerobic microbes multiplying. Short anaerobic periods can increase the diversity if aerobic organisms do not die or become inactive [36]. Long anaerobic conditions mean that many organisms become inactive or die, and nutrients are lost. However, microbial nutrients added to solutions should be used with caution, as studies [43] have shown that the addition of molasses or other simple sugars to compost tea can lead to the growth of *Escherichia coli*, *Salmonella*, and *Listeria*. Compost solutions are dominated by bacteria: aerated compost solutions are dominated by aerobic bacteria, while non-aerated compost tea are dominated by facultative anaerobic bacteria. The compost used should be stable and pathogen-free [31,32]. A previous study [66] showed that nitrogen-rich feedstocks, such as composts containing manure, result in compost tea with a higher bacterial content. Kim et al. [39] also reported that the microbial communities in different compost teas were predominantly bacteria [67]. Aerated compost tea was characterized by a predominance of aerobic bacteria, yet non-aerated compost teas were dominated by facultative anaerobic organisms [31]. The dominant bacterial phylums detected in the non-aerated compost tea were *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, *Proteobacteria*, *Verrucomicrobia*, *Chloroflexi*, *Planctomycetes*, and *Acidobacteria* [68]. In studies by González-Hernández et al. [68], the most abundant group of microorganisms

in compost tea was total aerobic bacteria (2.0×10^7 CFU/mL), followed by *N*-fixing bacteria (1.4×10^5 CFU/mL) and *Actinobacteria* (7.4×10^4 CFU/mL).

For products containing living microorganisms, it is important to preserve the survival of microbes for as long as possible, at least until the actual use. It is necessary to start from the highest possible cell counts in order to maintain an adequate and efficient cell count in the compost tea despite the destruction caused by environmental stress factors. The product under test was formulated in a dry state or with a very low water content, and therefore requires micro-organisms that can survive in a 'dormant', inactive state and that will revive after application when the environmental conditions (temperature, water content) are ideal for their functionality.

Several studies have been conducted on the microbial characterization of compost tea [48,69], using 16S rDNA sequencing [70], plate count, or MPN [71] methods to determine the density of cultivable bacteria found in compost tea. The MALDI-TOF method used to identify microorganisms in compost tea is considered a new technology. Based on our results, the MALDI-TOF method may be a good alternative for species-level identification of microorganisms in compost teas. The results obtained were in agreement with the literature, as previous studies showed that the following groups of microorganisms were found in compost tea: *Bacillus*, *Pseudomonas*, *Micrococcus*, *Staphylococcus*, *Clavibacter*, *Lactobacillus*, and other bacterial species [71], as well as *Actinomycetes*, yeasts, and *Trichoderma* sp., *Aspergillus* sp., *Penicillium* sp., and other filamentous fungi species [71]. With the MALDI-TOF MS method we can identify not only beneficial, but also pathogenic, microorganisms at the species level; but in our case this was not a problem, because the heat treatment (125–130 °C) applied during composting and granulation significantly reduced the potential risk.

FDA activity is a good indicator of the microbiological and degradative activity of soils. The FDA activity values can also be strongly influenced by abiotic environmental factors, such as the water and organic matter content of the soil and other soil physical properties. Measurements demonstrated a high activity value (16.74 µg/g) in the granulated product. This was lowered immediately after mixing into the soil as a result of apparent dilution. This value was not significantly increased by doubling the dose. Although it was possible to increase the activity in Arenosol, which was originally much poorer in organic matter, to values similar to those in slightly humous Arenosol. Furthermore, the pepper test plant increased the FDA values of the soils to the same level, which also tended to improve with the age of the plant physiologically. Similar results were obtained by Elbl et al. [72], who found that compost applied at higher doses had a positive effect on FDA growth (+95% increase compared to the control). Furthermore, according to Komilis et al. [73], the hydrolysis of FDA can be used as an indicator of microbial activity in relation to the state of the soil environment. Based on their results, they concluded that the application of organic fertilizers has positive effects on soil enzyme activity. Tian et al. [74] reported that FDA is widely accepted as an accurate and simple method for determining total soil microbial activity, and observed a direct effect of organic matter application in the form of compost on the increase of soil microbial activity.

The decreasing trend of the FDA is probably explained by the fact that only a single snapshot was available during the tests, and it is possible that the enzyme peak in the compost tea had already occurred long before and was already low at the time of measurement, while in the untreated soil it occurred later. This faster peak may have occurred because the compost tea has a rapid mineralization process and the microbes multiply rapidly and then undergo a rapid death; hence the low FDA enzyme activity at that time. Alidadi et al. [75] obtained similar results in their study of dehydrogenase enzyme activity, as the enzyme activity decreased from day 75 of composting (maturation phase). Lazcano et al. [76] found that a high activity level of microorganisms is due to the high amount of water-soluble carbon in their starting substrates. The stabilization of dehydrogenase activity is attributed to the complete degradation of available organic matter [77]. This point therefore represents the maturation time of the compost. Based on these results, in

our case, the readily available nutrients in the compost tea were taken up by the plant, leaving no available nutrients for the microorganisms in the soil.

For the β -glucosidase enzyme, sandy soil showed the greatest improvement of the two soil types, as organic matter supplementation had a greater effect on increasing the initial activity in sand. The glucosidase activity without treatment was greatly increased by pepper (+4.35 PNP/ $\mu\text{mol/g}$), but the enzyme activity was balanced by treatment. The glucosidase activity values also increased with plant age, but the dose effect remains unproven. Results from Vinhal-Freitas et al. [78] showed that the decrease in β -glucosidase enzyme activity was significantly influenced by mixing compost into the soil and that the enzyme activity was higher (+10–15 PNP/ $\mu\text{mol/g}$) with higher (20 g/kg) compost dosages. An increase in β -glucosidase activity after application of compost consisting of municipal solid residues was reported by several authors [79,80]. The detection of the enzyme β -glucosidase is related to the breakdown of cellulose synthesized by fungi, bacteria, and other soil organisms. Compost, however, is a stable organic waste (compared to, for example, uncomposted residues) that provides more resistant C compounds [81] and which is hydrolyzed more slowly by the enzymes.

Aerobic bacterial counts are comparable to enzyme assay results, especially FDA activity. Our results showed that the number of aerobic microorganisms increased with the effect of compost tea treatment. The peppers were able to utilize the applied compost tea in a sonic manner and the values improved with the age of the plant. At the application dose, the first dose already caused an increase (+0.15 log CFU/g), the double dose did not give better results, as was expected. It can be concluded that the activity of the two soils was similar, however, the results showed that it was the sandy soil that needed to be treated with the product to increase the organic matter input, and thus the activity. Bacteria such as *Enterobacteria* sp., *Nitrobacter* sp., *Pseudomonads* sp., *Bacillus* sp., *Staphylococcus* sp. and various *Actinomycetes* sp., as well as fungi such as *Trichoderma* sp. have been isolated from properly matured composts [82]. Subgroups of these species, known as “facultative anaerobes”, live in low oxygen environments but can also grow under aerobic conditions.

The results of Sifatullah et al. [83] showed that anaerobic tea had a higher bacterial count (4×10^{10} , 4.2×10^{10} , 4.3×10^{10} logCFU/g) than aerated compost tea. The observed high microbial counts were due to the closed container anaerobic compost tea, which may be useful for disease control.

Considering the fertilizing effect of compost tea, Wang et al. [84] reported there was no effect on the yield of zucchini using a chicken manure based vermicompost tea (1:10, w/v, continuously aerated for 12 h) as fertilizer. Moreover, Hewidy et al. [85] showed no effect on broccoli yield of a compost tea obtained from the organic fraction of municipal solid wastes and pruning residues (1:5, w/v, continuously aerated for 24 h).

The electrical conductivity of the compost tea applied to the peppers was high (14.82 mS/cm), which resulted in a concentrated soil solution, and which may also cause yellowing of leaf edges. This may be the reason for the increase in total chlorophyll content at the four-week eradication period, whereas the eight-week eradication period was characterized by a decrease in chlorophyll content. Nitrogen deficiencies may also have developed during treatments, which may have resulted in chlorotic yellowing [86]. In the Arenosol soil, there was no significant differences compared to the control, but in the slightly humous Arenosol, the effect of the treatments was statistically proved by W8. Similar results for total chlorophyll content were reported by Pérez-López et al. [87], who observed that the use of composted manure increased the total chlorophyll content of sweet peppers (*Capsicum annuum* L.).

In this case study we evaluated a new technological approach for poultry farming organic waste. We investigated the effect of a compost tea made from a mixture of poultry manure and hen manure on a test pepper (*Capsicum annuum* L.) plant grown in a pot experiment. We investigated the potential microbiological indicators of the new utilization process and how the microbiological indicators and the mechanism of action of compost tea can be interpreted in the case of the indicator plant.

Overall, compost solutions can be used to keep organic fertilizers in circulation, and thus products suitable for organic nutrient replenishment can be used instead of fertilizers. One of the main objectives of the European Union's Green Deal [88] is to promote the efficient use of resources through the transition to a clean, circular economy, and one of the ways of achieving this in agricultural practice is to develop products based on organic fertilizers.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/agriculture11070683/s1>, Table S1: Characteristics of evaluated product base materials (Broiler and hen manure), Table S2: Changes of plant shoot length (cm) and total chlorophyll content ($\mu\text{g/g}$) in Arenosol soil, week 4 and week 8 of the treatments ($p < 0.05$)*, Table S3: Changes of plant shoot length (cm) and total chlorophyll content ($\mu\text{g/g}$) in slightly humous Arenosol soil, week 4 and week 8 of the treatments ($p < 0.05$)*, Figure S1: Summary of total shoot length (cm) results by treatments (Doses), soil types, sampling weeks. The codes are as follows: D1: Dose 1. D2: Dose 2. A: Arenosol. SHA: slightly humous Arenosol. W4: Week 4. W8: Week 8, Figure S2. Summary of total chlorophyll content ($\mu\text{g/g}$) results by treatments (Doses), soil types, sampling weeks. The codes are as follows: D1: Dose 1. D2: Dose 2. A: Arenosol. SHA: Slightly humous Arenosol. W4: Week 4. W8: Week 8.

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