Journal of Material Cycles and Waste Management (2023) 25:2925–2936
https://doi.org/10.1007/s10163-023-01726-4

ORIGINAL ARTICLE

Development of plant-friendly vermicompost using novel biotechnological methods

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Received: 14 September 2022 / Accepted: 8 June 2023 / Published online: 16 June 2023

Abstract

For the first time in the world, raw tea waste from tea plants was mineralized by rapid biotechnological methods using beneficial worms, enzymes (protease, lipase, dehydrogenase, hydrolase, urease, nitrogenase, cellulase) and microorganisms (*Aspergillus flavus, Bifidobacterium* spp. *Bacillus subtilis, Rhodotorula* spp., *Lactobacillus, Rhodopseudomas* spp.). Thus, biocompost technology was developed to create an exclusive organic tea fertilizer. The biocompost product was developed in a plant-friendly format for sustainable use of local resources. It was rich in organic material and free from diseases and pathogens, according to organic farming methods. It was also reliable in terms of heavy metal content. In the biocompost study, the application of five different compost mixtures and three different application methods were investigated. Worm + Plant Growth-Promoting Rhizobacteria + enzymes accelerated the mineralization of the compost among the studies, and the lowest C/N and OM % ratio was obtained. The other physical and chemical parameters were also in accordance with the ideal values determined in the literature. Also, the increase of nitrogen without nitrogen loss during the composting process shows that mineralization was successfully completed. In concluded, it can be suggested that the developed organic worm compost fertilizer can be used on agricultural land and in regenerative agriculture.

Keywords Vermicompost · Mineralization · Waste · Tea · Plant Growth-Promoting Rhizobacteria

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Abbreviations

(F1)	Factor 1
(F2)	Factor 2
(F3)	Factor 3
(FD)	Fertilizer Diversification
(OM)	Organic matter
(IC)	Initial composting
(OC)	Organic Carbon
(TP)	Total Phosphorus

Introduction

Long-term and intensive use of chemical fertilizers and agents change the resistance of the soil. This weakens and degrades the buffer system of the soil on agricultural land. Also, the biological layer, which is the immune system and forms the dynamic part of the living, is destroyed with time. Many cycles such as water, air circulation, nutrient cycling, automatic disease and pathogen control, and ion exchange that occur in nature and operate in a dynamic equilibrium are disrupted. As a result, soils that have lost their biodiversity lose their capacity and thus their productivity and efficiency. For this reason, applications that support biodiversity should be implemented quickly to strengthen the dynamic structure of the soil, increase its resilience and increase the buffer system [1–3]. It is therefore necessary to develop both production and consumption concepts to ensure global food security, reduce the use of external inputs, and reduce environmental damage. For this reason, production and consumption concepts are needed together and must be in balance with their ecological environment [4]. One approach that has recently received attention in the literature as a solution for a sustainable food system is regenerative agriculture (RA) [5, 6]. It was summarized from studies in 28 different articles by Schreefel [7]. For example, these conclusions were reached by dynamically developed [8], modified technology using organic farming methods [9], soil improvement [10], reducing greenhouse gas emissions and improving soil aeration [11], mitigating climate change [12], enhancing soil biodiversity by promoting soil biology [5] or "enhancing biodiversity" [13], such as enhancing soil carbon, expressed as soil organic matter buildup [9, 14–16]. Generally, the definition of RA is expressed as promoting and improving soil health, improving soil carbon, improving soil physical quality, and improving (soil) biodiversity. Sustainable agriculture increases crop production while maintaining soil quality, increases soil OM quality, and protects the environment, ensuring food availability without chemicals that are harmless to the public. Soil depletion disrupts ecosystem services and reduces ecosystem flexibility. Therefore, we need economically sustainable organic agriculture and a sustainable agricultural production method that minimizes environmental impact. Vermicompost contains many functional microbial groups such as cellulose and lignin decomposers, phosphate solubilizers, nitrogen fixers, and antibiotic producers [17].

Soil enzymes regulate ecosystem functioning and play a key role, especially in nutrient cycling [18]. Mineralization and transformation of nutrients are provided by enzymes secreted from the cell or by endogenous microbial cellular enzymes. Extracellular enzymes release organic and inorganic nutrients into the soil and either degrade or transform them [19]. These enzymes, known as soil enzymes, are critical for protecting soil fertility and health, and for protecting the environment by decomposing pollutant molecules [20, 21]. The composition, quantity, and microbial community of active soil enzymes determine the availability of nutrients and thus the health of the soil at a given time [22]. Microbial populations and processes affect soil fertility and structure in several ways, contributing to amendment, mineralization, and nutrient availability. Thus, it influences the effectiveness of nutrient cycling. They are responsible for the synthesis and decomposition of soil organic matter, which affects cation exchange capacity, N, S, and P reserves, soil acidity and toxicity, and soil water retention capacity. Each of these factors also has a positive effect on the main soil-related productivity constraints [23].

Currently, the government's attention is focused on the Circular Economy concept, a way to find methods and strategies to maximize resource recovery and minimize environmental impact [24]. Assessment of an appropriate integrated waste management plan targeting the Circular Economy based on the LCA method [24, 25]. In developing countries, large amounts of vegetable waste are often burned. This burning process pollutes the environment and contributes to climate change [26]. However, these burned crop residues contain high levels of nutrients, estimated to be 30–35% of N and P minerals and 70–80% of K added to the soil [27]. Vermicompost is therefore considered an environmentally friendly technology for the management of plant residues. Vermicomposting can be achieved by non-thermophilic decomposition of organic matter with the help of worms and microorganisms [28]. Vermicompost can add micronutrients to soil [29], improve soil physical properties [30], reduce soil salinity [31] and alkalinity [32]. In addition, vermicompost can increase the activity of soil enzymes such as acid phosphatase, acid invertase, and catalase, while affecting their activity [33].

In 2018, according to the FAO report, Global consumption and production of tea is expected to continue to increase over the next decade due to high demand in developing and emerging economies. According to the properties of tea has increased awareness of the effect of anti-inflammatory agents, antioxidants, and weight loss. In parallel with these production increases, the processing of fresh tea leaves into black tea in tea factories generates solid waste, which consists of trash, fibers and dust. For every 100 kg of green shoots harvested after processing at the factory, about 22 kg of black tea is produced. However, only about 82% of this tea can be marketed, and the rest is thrown away. Waste from the tea factory accumulates in large quantities and weighs on average about 4% of the green shoots [34]. This waste from tea factories is increasing day by day. However, the areas where these wastes are used are practically insufficient for the use of wastes in sustainable agriculture. The difference in biocompost technology and our study is to promote mineralization process, using enzymes, microorganisms, and worms together in tea vermicomposting experimentation. In such a way, organic tea vermicompost was developed with enzymes for the first time.In this decomposition technology, mineralization was carried out with the help of beneficial worms, enzymes and microorganisms using rapid and biotechnological methods.

Material and method

In this research, 60 tons of raw tea waste transported from tea factories in Turkey, were conducted in composting facilities in the Kandıra district of the Kocaeli province in 2019–2020. It was planned in accordance with the composting procedure in the transfer pile. Long stakes were formed from compost prepared using this method. These piles were mixed regularly during composting to determine water content, organic matter, pH, porosity, micro-organism, C/N ratio, etc. homogeneous in terms of parameters. The composting piles were aerated and mixed on days 3, 14, 27, 39, 54, 69, and 76. A total of seven measures (mixing processes) were performed. The compost was mineralized in two stages. In the first stage, the mixture (raw material and waste from mushroom cultivation) was decomposed by bacterial activity, and no worms were added at the beginning of the composting process. Then, the mixture was brought to high temperature once under temperature control by the automation system so that the compost temperature did not exceed 60 °C. It took 1 day for the temperatures to reach the 60 °C control points. As for compost hygiene, all mixtures remained at 55 °C for more than 4 days, after which the temperature started to decrease. In the second phase, worms were added when the temperature of the mixture was between 40 and 45 °C, and all piles were maintained at 40 to 45 °C throughout. Each compost pile contained a total of 2700 ± 150 kg of the mixture.

The solid compost fertilizer was developed as a standard solid organic fertilizer in accordance with the Regulation on the Principles and Implementation of Organic Agriculture in Turkey [35]. These regulations cover the production, import and marketing of organic, organic and soil improvement fertilizers, as well as microbial, enzymatic and other agricultural products.

Fertilizer production process

Generation of isolated bacteria

Bacterial isolates (*Aspergillus Flavus, Bifidobacterium* spp. *Bacillus subtilis, Rhodotorula* spp.,*Lactobacillus,Rhodopseudomas* spp.) used in the study were obtained from glycerol stock cultures stored at −80 °C. The culture medium required for bacterial growth was prepared. A two-hour incubation period at 30 ºC was provided. Depending on the operating conditions, 10^8 cfu/ml were fixed in the soil. Initial and final product analyses of tea compost samples were performed, and stabilization studies were continued based on the results.

Fertilizer diversification

The raw tea waste from the tea factory was sieved through a 4 mm sieve. Stacks of 45 beds were then performed with 5 different tea waste ratios, 3 replicates, and 3 different factors. After three months of incubation with all composting factors, the changes in the initial tea waste content over time were analyzed for the final compost.

The study with 3 factors was conducted as follows;

Factor 1 (F1D) raw tea waste + Mushroom cultivation waste + worm (50,000 pieces ton^{-1}).

(F1D 1): 70% raw tea waste + 30% Mushroom cultivation waste + worm (50,000 pieces ton^{-1}).

(F1D 2): 60% raw tea waste + 40%Mushroom cultivation waste + worm (50,000 pieces ton^{-1}).

(F1D 3): 50% raw tea waste + 50% Mushroom cultivation waste + worm (50,000 pieces ton^{-1}).

(F1D 4): 40% raw tea waste + 60% Mushroom cultivation waste + worm (50,000 pieces ton^{-1}).

(F1D 5): 30% raw tea waste + 70% Mushroom cultivation waste + worm (50,000 pieces ton^{-1}).

Stacks of F1D is shown in Fig. 1

Factor 2 (F2D): Raw tea waste + Mushroom cultivation waste + worm + PGPR (*Aspergillus flavus, Bifidobacterium* spp. *Bacillus subtilis, Rhodotorula* spp., *Lactobacillus, Rhodopseudomas* spp.,). PGPR prepared to have a microbial load of 1×10^9 cfu ml^{-1}, were inoculated by spraying into stacks of raw tea waste + Mushroom + worm with 150 L of water. Stacks of F2D was shown in Fig. 2

(F2D1): 70% raw tea waste + 30% Mushroom cultivation waste + worm (50,000 pieces ton^{-1}) + PGPR.

(F2D2): 60% raw tea waste + 40% Mushroom cultivation waste + worm (50,000 pieces ton^{-1}) + PGPR.

Fig. 1 Image of stacks of F1D beds

Fig. 2 Image of stacks of F2D beds

(F2D3): 50% raw tea waste + 50% Mushroom cultivation waste + worm (50,000 pieces ton^{-1}) + PGPR.

(F2D4): 40% raw tea waste + 60% Mushroom cultivation waste + worm (50,000 pieces ton^{-1}) + PGPR.

(F2D5): 30% raw tea waste + 70% Mushroom cultivation waste + worm (50,000 pieces ton^{-1}) + PGPR.

Factor 3 (F3D): Raw tea waste + Mushroom cultivation waste + worm + PGPR + enzymes with product number (protease (ATCC 1621), lipase (ATCC 87190), dehydrogenase (ATCC 8585), hydrolase(ATCC 24725), urease (ATCC 9029), nitrogenase (ATCC29413), cellulase (ATCC 1513)). The enzymes in 150 L water, adjusted to an enzyme concentration of 80 EU ml^{-1}, were mixed by spraying into stacks of raw tea waste + Mushroom + worm + PGPR (microbial load of 1×10^9 cfu ml^{-1} with 150 L). Stacks of F3D was shown in Fig. 3

(F3D1): 70% raw tea waste + 30% Mushroom cultivation waste + worm (50,000 pieces ton^{-1}) + PGPR + Enzymes.

(F3D2): 60% raw tea waste + 40% Mushroom cultivation waste + worm (50,000 pieces ton^{-1}) + PGPR + Enzymes.

(F3D3): 50% raw tea waste + 50% Mushroom cultivation waste + worm (50,000 pieces ton^{-1}) + PGPR + Enzymes.

(F3D4): 40% raw tea waste + 60% Mushroom cultivation waste + worm (50,000 pieces ton^{-1}) + PGPR + Enzymes.

Fig. 3 Image of stacks of F3D beds

(F3D5): 30% raw tea waste + 70% Mushroom cultivation waste + worm (50,000 pieces ton^{-1}) + PGPR + Enzymes.

Physical and chemical analyses of the obtained compost manure

Vermicompost samples were passed through a 2 mm sieve before physical and chemical analysis before the analyzes. Total carbon, OC, and OM was determined using the Smith-Weldon method as described by Nelson and Sommers [36]. pH, EC and moisture content were directly determined using vermicompost samples. EC was measured in saturation extracts according to Rhoades [37]. The undried compost sample was taken in the hand and then pressure was applied by closing the fingers into fists. There was not any leaching of water between the fingers during the process. However, when the sample compressed under light pressure to vermicompost was crumbled. but, it was not deformed [38]. Vermicompost pH determinations were done in 1:2 extracts according to McLean [39] The moisture content of the samples was determined by oven drying at 105 °C (Nüve-FN400). The Kjeldahl method [40] and a Vapodest 10 Rapid Kjeldahl Distillation Unit (Gerhardt, Konigswinter, Germany) were used to determine total N while plant-available P was determined using the sodium bicarbonate method of Olsen [41], and other macro and micro nutrients were determined after wet digestion of dried and ground subsamples using a HNO_3-H_2O_2 acid mixture (2:3 v/v) with three steps (first step; 145 °C, 75% radio-frequecy power (RF), 5 min; second step; 180 °C, 90% RF, 10 min; and third step; 100 °C, 40% RF, 10 min) in microwave digestion (Bergof Speedwave Microwave Digestion Equipment MWS-2; Berghof Products and Instruments, Eningen, Germany) and readings were performed in an ICP OES (Inductively Couple Plasma spectrophotometer) (Perkin-Elmer, Optima 4300 DV, ICP/OES, Shelton, CT 06484–4794, USA) for mineral contents [42]. Cation exchange capacity was determined by ammonium acetate saturation method [43]. One gram of oven dried compost was saturated with 10 mL of Neutral

Normal Ammonium acetate (NH_4OAc) and was shaken for 1 h. The sample was filtered through Whatman no. 1 filter paper and washed with three to four ethanol washing. The sample was distilled with Kel-plus auto-analyser after ethanol washing. The exchangeable ammonium was titrated against standard 0.02N H_2SO_4 and CEC was calculated.

Statistical analyzes

The data obtained were analyzed through the SPSS 22 program. The data were subjected to an analysis of variance (ANOVA) and the differences in averages were determined by the Duncan multiple range test ($p < 0.05$) (DMRT).

Results

The changes that occurred after the three-month mineralization of waste from composting methods F1D, F2D and F3D with different ratios were analyzed. In addition, the changes in the initial content of the raw tea waste over time for the final compost were also analyzed (Tables 1, 2, and 3).

After three months of F1D mineralization, the changes in the final compost were analyzed. In addition, the original pre-composting raw tea waste values (IC) is presented in Table 1.

After three months of mineralization, organic matter content (% OM) according to IC showed decreases in the rate of F1D2 (1.14%), F1D3 (2.52%), F1D4 (3.80%), and F1D5 (5.35%). However, after mineralization of F1D1, there was no significant change in OM %. As a result of mineralization, total N concentration increased in F1D1 (0.41%), F1D2 (0.41%), F1D3 (2.04%), F1D4 (6.49%), and F1D5 (10.20%) according to IC. Total P concentration increased in F1D1 (14.29%), F1D2 (97.14%), F1D3 (134.29%), F1D4 (67.29%), and F1D5 (268.57%). Total %K increased in F1D1 (0.00%), F1D2 (3.37%), F1D3 (6.18%), F1D4 (15.24%), and F1D5 (21.91%) compared to IC.

Water soluble concentration of NH_4^+, NO_3^-, P, and K increased in F1D1 (11.38- 3.52- 2.96- 3.94%), F1D2 (34.21-28.20–19.30- 19.28%), F1D3 (81.74–49.19–62.26- 43.54%), F1D4 (160.56- 38.67- 46.06- 33.76%), and F1D5 (279.69-108.52- 105.93- 123.44%) according to IC.

The pH was (6.45 ± 0.44) for IC, while F1D1 (6.57 ± 0.4), F1D2 (6.68 ± 0.35), F1D3 (6.79 ± 0.3), F1D4 (7.3 ± 0.16), F1D5 (7.57 ± 0.45). Depending on the mixing ratio decreased changes in specific gravity, FD1 (4.23%), FD2

Table 1 Changes in fertilizer parameters (F1D) after three months of mineralization of raw tea waste at different rates (n = 5)

F1D	IC ± SD	F1D1 ± SD	F1D2 ± SD	F1D3 ± SD	F1D4 ± SD	F1D5 ± SD
OM, %	79.51 ± 0.31	79.8 ± 0.35	78.6 ± 0.3	77.51 ± 0.23	76.6 ± 0.25	75.26 ± 0.27
Total (N), %	2.45 ± 0.13	2.46 ± 0.25	2.46 ± 0.25	2.50 ± 0.28	2.62 ± 0.27	2.70 ± 0.25
Total P (P_2O_5), %	0.35 ± 0.15	0.40 ± 0.02	0.69 ± 0.18	0.82 ± 0.17	1.07 ± 0.07	1.29 ± 0.05
Total K (K_2O)	1.78 ± 0.19	1.78 ± 0.22	1.84 ± 0.13	1.89 ± 0.3	2.10 ± 0.22	2.17 ± 0.17
Water soluble NH_4^+ (mg kg^{-1})	55.43 ± 0.31	61.74 ± 0.25	74.39 ± 0.19	100.74 ± 0.91	140.55 ± 2.36	210.46 ± 1.57
Water soluble NO_3^- (mg kg^{-1})	68.76 ± 2.23	71.18 ± 2.02	88.15 ± 2.95	102.58 ± 1.93	112.11 ± 3.39	143.38 ± 5.48
Water soluble P (mg kg^{-1})	18.55 ± 1.46	19.10 ± 1.00	22.13 ± 0.92	30.10 ± 3.10	34.39 ± 2.40	38.20 ± 1.41
Water soluble K (mg kg^{-1})	1980 ± 53	2057.99 ± 15.99	2361.80 ± 34.2	2842.00 ± 29.00	2989.00 ± 11.00	4424.11 ± 22.54
Specific gravity (2 mm size) g/cm^3	1.42 ± 0.04	1.36 ± 0.07	1.26 ± 0.14	1.17 ± 0.20	1.08 ± 0.24	0.93 ± 0.16
Volume weight (2 mm size) g/cm^3	0.12 ± 0.04	0.11 ± 0.04	0.09 ± 0.01	0.08 ± 0.01	0.07 ± 0.01	0.08 ± 0.01
Water buffer capacity, %	2.88 ± 0.16	2.83 ± 0.17	3.11 ± 0.15	3.20 ± 0.15	3.31 ± 0.11	3.39 ± 0.30
readily available water;%	25.11 ± 4.41	26.03 ± 4.72	27.24 ± 2.57	33.02 ± 0.49	35.49 ± 1.54	38.08 ± 4.16
Volumetric water content, % pF = 0	62.33 ± 3.51	63.21 ± 3.21	65.14 ± 1.82	66.30 ± 0.96	67.55 ± 0.13	70.5 ± 3.10
Volumetric water content, % pF = 1,0	30.55 ± 3.85	32.47 ± 2.57	33.20 ± 2.27	35.40 ± 0.45	36.45 ± 6.65	38.32 ± 3.51
volumetric water content, % pF = 1,7	25.88 ± 1.61	25.90 ± 1.32	25.99 ± 1.99	27.59 ± 0.72	29.29 ± 0.86	30.29 ± 2.00
Volumetric water content, % pF = 2,0	23.42 ± 1.76	23.41 ± 2.06	24.48 ± 1.33	25.84 ± 0.19	26.44 ± 0.38	27.46 ± 1.46
Cation exchange capacity (me/100 g)	165.50 ± 0.72	167.16 ± 0.82	175.77 ± 9.57	170.96 ± 6.35	145.43 ± 18.12	172.49 ± 5.92
pH (1:10)	6.45 ± 0.44	6.57 ± 0.40	6.68 ± 0.35	6.79 ± 0.30	7.30 ± 0.16	7.57 ± 0.45
Organic C, %	40.87 ± 2.25	44.83 ± 1.26	44.16 ± 0.91	43.54 ± 0.59	43.03 ± 0.38	42.28 ± 0.69
C/N, %	16.68 ± 0.39	18.22 ± 1.09	17.95 ± 1.09	17.42 ± 0.93	16.40 ± 0.37	15.66 ± 0.88
EC (mS/cm)	1.55 ± 0.01	1.52 ± 0.28	1.54 ± 0.03	1.57 ± 0.01	1.58 ± 0.06	1.58 ± 0.07
Total (Fe), (mg kg^{-1})	7.79 ± 0.93	7.76 ± 0.34	8.66 ± 0.25	8.82 ± 0.54	9.36 ± 1.32	9.90 ± 1.06
Total (Zn), (mg kg^{-1})	2.33 ± 0.19	2.05 ± 0.08	2.30 ± 0.22	2.65 ± 0.14	2.75 ± 0.24	3.01 ± 0.50
Total (Cu), (mg kg^{-1})	49.77 ± 8.26	54.00 ± 5.68	55.01 ± 6.10	58.61 ± 4.53	64.54 ± 1.40	66.26 ± 4.32

Table 2 Changes in fertilizer parameters (F2D) after three months of mineralization of raw tea waste at different rates (n = 5)

F2D	IC ± SD	F2D1 ± SD	F2D2 ± SD	F2D3 ± SD	F2D4 ± SD	F2D5 ± SD
OM, %	79.51 ± 0.31	77.65 ± 8.42	73.82 ± 5.05	64.26 ± 10.53	62.25 ± 1.27	57.89 ± 5.80
Total (N), %	2.45 ± 0.13	2.47 ± 0.15	2.47 ± 0.18	2.59 ± 0.09	2.78 ± 0.09	2.82 ± 0.15
Total P (P_2O_5), %	0.35 ± 0.15	0.51 ± 0.18	0.71 ± 0.10	0.90 ± 0.10	1.10 ± 0.26	1.34 ± 0.50
Total K (K_2O)	1.78 ± 0.19	1.80 ± 0.21	1.86 ± 0.19	2.00 ± 0.08	2.1 ± 0.01	2.33 ± 0.24
Water soluble NH_4^+ (mg kg^{-1})	55.43 ± 0.31	73.25 ± 6.44	86.12 ± 10.89	107.90 ± 19.80	147.50 ± 9.90	316.80 ± 8.40
Water soluble NO_3^- (mg kg^{-1})	68.76 ± 2.23	93.04 ± 9.02	100.15 ± 12.58	110.80 ± 17.90	124.60 ± 24.80	153.45 ± 39.23
Water soluble P (mg kg^{-1})	18.55 ± 1.46	20.11 ± 4.91	23.35 ± 0.83	31.60 ± 4.172	36.12 ± 4.70	39.60 ± 2.7
Water soluble K (mg kg^{-1})	1980.00 ± 53.00	2100.00 ± 50.00	2410.00 ± 45.00	2900.00 ± 50.00	3050.00 ± 75.00	4514.4 ± 92.8
Specific gravity (2 mm size) g/cm^3	1.42 ± 0.04	1.39 ± 0.06	1.29 ± 0.11	1.19 ± 0.16	1.10 ± 0.20	0.95 ± 0.28
Volume weight (2 mm size) g/cm^3	0.12 ± 0.04	0.11 ± 0.05	0.09 ± 0.06	0.08 ± 0.01	0.07 ± 0.01	0.08 ± 0.02
Water buffer capacity, %	2.88 ± 0.16	2.89 ± 0.42	3.10 ± 0.20	3.31 ± 0.05	3.41 ± 0.19	2.33 ± 0.43
Readily available water;%	25.11 ± 4.41	23.89 ± 4.65	27.31 ± 5.98	34.43 ± 3.87	38.14 ± 3.80	42.32 ± 4.02
Volumetric water content, % pF = 0	62.33 ± 3.51	64.50 ± 2.72	67.13 ± 2.20	67.49 ± 0.64	69.75 ± 2.37	72.96 ± 6.23
Volumetric water content, % pF = 1,0	30.55 ± 3.85	33.13 ± 1.95	33.88 ± 2.70	36.12 ± 1.69	37.19 ± 2.20	39.11 ± 4.94
Volumetric water content, % pF = 1,7	25.88 ± 1.61	26.43 ± 1.70	26.52 ± 1.61	29.17 ± 1.04	29.89 ± 1.76	30.91 ± 2.78
Volumetric water content, % pF = 2,0	23.42 ± 1.76	23.89 ± 1.72	24.98 ± 0.63	26.37 ± 0.76	26.98 ± 1.37	28.02 ± 2.41
Cation exchange capacity (me/100 g)	165.50 ± 0.72	170.57 ± 1.20	180.38 ± 8.61	177.51 ± 5.74	150.44 ± 21.33	186.22 ± 14.45
pH (1:10)	6.45 ± 0.44	6.70 ± 0.31	6.82 ± 0.19	6.93 ± 0.08	7.45 ± 0.44	7.72 ± 0.71
Organic C, %	40.87 ± 2.25	41.62 ± 6.04	41.47 ± 6.89	35.10 ± 1.67	34.71 ± 1.56	24.26 ± 6.02
C/N, %	16.68 ± 0.39	16.85 ± 3.18	16.79 ± 3.66	13.55 ± 1.46	12.49 ± 0.21	8.22 ± 0.39
EC (mS/cm)	1.55 ± 0.01	1.55 ± 0.03	1.57 ± 0.01	1.60 ± 0.02	1.61 ± 0.03	1.61 ± 0.03
Total (Fe), (mg kg^{-1})	7.79 ± 0.93	7.92 ± 0.95	8.84 ± 0.03	9.00 ± 0.13	9.55 ± 0.68	10.10 ± 1.23
Total (Zn), (mg kg^{-1})	2.33 ± 0.19	2.09 ± 0.47	2.35 ± 0.21	2.70 ± 0.14	2.81 ± 0.25	3.07 ± 0.51
Total (Cu), (mg kg^{-1})	49.77 ± 8.26	55.10 ± 5.80	56.13 ± 6.23	59.81 ± 4.62	65.86 ± 1.43	67.62 ± 4.42

(11.27%), F1D3 (17.61%), F1D4 (31.48%), and F1D5 (34.51%) according to IC.

A decrease in volume weight was observed with F1D1 (8.33%), F1D2 (25.00%), F1D3 (33.33%), F1D4 (71.43%) and F1D5 (33.33%). Water buffering capacity increased with F1D2 (7.99%), F1D3 (11.11%), F1D4 (12.99%), and F1D5 (17.71%). However, F1D1 decreased by 1.74% according to IC.

The percentage of readily available water increased in F1D1 (3.66%), F1D2 (8.48%), F1D3 (31.50%), F1D4 (29.25%), F1D5 (51.65%) compared to IC. The cation exchange capacity was increased by F1D1 (1%), F1D2 (6.21%), F1D3 (3.30%), F1D5 (4.22%) and decreased by F1D4 (13.80%). Organic C concentration was increased by F1D1 (9.69%), F1D2 (8.05%), F1D3 (6.53%), F1D4 (5.02%) and F1D5 (3.45%). The C/N content (%) of F1D1 (9.23%), F1D2 (7.61%), and F1D3 (4.44%) elevated compared to IC. However, F1D4 (1.71%) and F1D5 (6.12%) reduced. EC values (mS/cm) were measured as 1.52 ± 0.28 in F1D1, 1.54 ± 0.03 in F1D2, 1.57 ± 0.01 in F1D3, 1.58 ± 0.06 in F1D4, and 1.58 ± 0.07 in F1D5. Total Fe concentration (mg kg^{-1}) increased in F1D2 (1.17%), F1D3 (13.22%), F1D4 (16.77%), F1D5 (27.09%) and decreased in F1D1 (0.39%) compared to IC. While total Zn concentration (mg kg^{-1}) increased in F1D3 (13.73%), F1D4 (15.27%), F1D5 (29.18%), a decrease was observed in F1D1 (12.02%), F1D2 (1.29%). Increases in total Cu concentration (mg kg^{-1}) were observed in FD1 (8.50%), FD2 (10.53%), FD3 (17.76%), F1D4 (22.89%) and F1D5 (33.13%) compared to IC.

After three months of F2D mineralization, the changes in the final compost were analyzed. The changes at the end of the mineralization period with the F2D application were evaluated according to IC are shown in the Table 2.

There was a decrease in % OM, F2D1 (2.34%), F2D2 (7.16%), F2D3 (19.18%), F2D4 (27.73%), and F2D5 (27.19%).

Increases in total % N, % P and % K concentrations occurred in F2D1 (0.82- 45.71- 1.12%), F2D2 (0.82- 102.86- 4.49%), F2D3 (5.71–157.14–12.36%), F2D4 (11.87–68.18–15.24%), F2D5 (15.10- 282.86- 30.90%). Water soluble NH_4^+, NO_3^-, P, and K content raised in F2D1 (32.15- 35.31–8.41- 6.06%), F2D2(55.37- 45.65- 25.88–21.72%), F2D3 (94.66- 61.14- 70.35- 46.46%), F2D4 (62.42- 44.82- 48.64- 35.08%) in F2D5 (471.53- 123.17- 113.48- 128.00%) compared to IC.

The pH of F2D1, F2D2, F2D3, F2D4 and F2D5 were 6.7 ± 0.31, 6.82 ± 0.19, 6.93 ± 0.08, (7.45 ± 0.44 and 7.72 ± 0.71, respectively. Increases in EC values (mS/

Table 3 Changes in fertilizer parameters (F3D) after three months of mineralization of raw tea waste at different rates (n = 5)

F3D	IC ± SD	F3D1 ± SD	F3D2 ± SD	F3D3 ± SD	F3D4 ± SD	F3D5 ± SD
OM, %	79.51 ± 0.31	60.50 ± 3.90	56.40 ± 3.40	50.20 ± 6.93	47.70 ± 3.85	45.30 ± 6.89
Total (N), %	2.45 ± 0.13	2.49 ± 0.17	2.50 ± 0.19	2.62 ± 0.10	2.78 ± 0.04	2.95 ± 0.22
Total P (P_2O_5), %	0.35 ± 0.15	0.52 ± 0.17	0.78 ± 0.17	0.92 ± 0.10	1.20 ± 0.34	1.35 ± 0.45
Total K (K_2O)	1.78 ± 0.19	1.88 ± 0.21	1.95 ± 0.18	2.10 ± 0.06	2.22 ± 0.06	2.35 ± 0.19
Water soluble NH_4^+ (mg kg^{-1})	55.43 ± 0.31	175.00 ± 6.50	188.00 ± 11.00	210.00 ± 20.00	250.00 ± 10.00	320.00 ± 10.00
Water soluble NO_3 (mg kg^{-1})	68.76 ± 2.23	96 ± 10.50	105.00 ± 15.00	117.00 ± 21.00	128.00 ± 26.50	155.00 ± 40.00
Water soluble P (mg kg^{-1})	18.55 ± 1.46	21.00 ± 5.33	25.00 ± 0.00	33.00 ± 4.00	38.00 ± 5.82	40.00 ± 2.50
Water soluble K (mg kg^{-1})	1980.00 ± 53.00	2210.00 ± 5.00	2450.00 ± 25.00	2980.00 ± 10.00	3100.00 ± 50.00	4560.00 ± 70.00
Specific gravity (2 mm size) g/cm^3	1.42 ± 0.04	1.39 ± 0.06	1.27 ± 0.12	1.15 ± 0.18	1.02 ± 0.24	0.96 ± 0.27
Volume weight (2 mm size) g/cm^3	0.12 ± 0.04	0.12 ± 0.04	0.1 ± 0.05	0.09 ± 0.01	0.08 ± 0.00	0.08 ± 0.02
Water buffer capacity, %	2.88 ± 0.16	2.92 ± 0.51	3.10 ± 0.33	3.55 ± 0.16	3.75 ± 0.45	2.37 ± 0.42
Readily available water;%	25.11 ± 4.41	24.1 ± 5.37	28.60 ± 6.35	35.70 ± 3.97	40.55 ± 5.26	42.75 ± 3.08
Volumetric water content, % pF = 0	62.33 ± 3.51	65.20 ± 3.95	68.70 ± 2.575	70.20 ± 0.52	72.50 ± 4.07	73.70 ± 6.22
Volumetric water content, % pF = 1,0	30.55 ± 3.85	33.70 ± 2.4	35.20 ± 2.35	37.40 ± 2.09	38.10 ± 2.64	39.50 ± 4.76
Volumetric water content, % pF = 1,7	25.88 ± 1.61	26.70 ± 1.80	27.80 ± 0.70	29.30 ± 0.80	30.11 ± 1.61	31.22 ± 2.72
Volumetric water content, % pF = 2,0	23.42 ± 1.76	24.10 ± 1.65	25.20 ± 0.55	26.30 ± 0.55	27.20 ± 1.45	28.30 ± 2.55
Cation exchange capacity (me/100 g)	165.50 ± 0.72	174.30 ± 0.87	188.20 ± 13.03	179.30 ± 4.13	155.60 ± 19.57	188.10 ± 12.93
pH (1:10)	6.45 ± 0.44	6.77 ± 0.3	6.88 ± 0.19	7.00 ± 0.07	7.50 ± 0.43	7.80 ± 0.73
Organic C, %	40.87 ± 2.25	35.00 ± 5.86	31.50 ± 3.34	27.60 ± 0.00	25.40 ± 2.20	24.50 ± 2.07
C/N, %	16.68 ± 0.39	14.06 ± 2.97	12.60 ± 2.01	10.53 ± 0.56	9.14 ± 1.02	8.31 ± 0.35
EC (mS/cm)	1.55 ± 0.01	1.58 ± 0.02	1.59 ± 0.01	1.62 ± 0.02	1.63 ± 0.03	1.63 ± 0.03
Total (Fe), (mg kg^{-1})	7.79 ± 0.93	7.90 ± 0.9	8.16 ± 0.65	9.20 ± 0.4	9.55 ± 0.752	10.20 ± 1.40
Total (Zn), (mg kg^{-1})	2.33 ± 0.19	2.42 ± 0.27	2.58 ± 0.11	2.79 ± 0.10	2.90 ± 0.21	3.10 ± 0.41
Total (Cu), (mg kg^{-1})	49.77 ± 8.26	55.66 ± 6.10	58.70 ± 4.59	60.42 ± 4.4	65.74 ± 0.92	68.30 ± 4.05

cm) were observed in F2D1 (- 0%), F2D2 (1.29%), F2D3 (3.23%), F2D4 (3.73%), F2D5 (3.87%) compared to IC. While an increase was observed in cationic exchange capacity of F2D1 (3.06%), F2D2 (8.99%), F2D3 (7.26%), F2D5 (12.52%), a decrease was observed in F2D4 (10.01%). Decreases in specific gravity and volume weights were 2.11–8.33% in F2D1, 9.15–25.00% in F2D2, 16.20- 33.33% in F2D3, 29.09- 71.43% in F2D4 and 33.10–33.33% in F2D5, respectively compared to IC. F2D1, F2D2, F2D3, F2D4, and F2D5 treatments raised water buffer capacity by 0.35%, 7.64%, 14.93%, 15.54% and 18.89% compared to IC. While there was an increase in readily available water (%) for F2D2 (8.76%), F2D3 (37.12%), F2D4 (34.16%), F2D5 (68.54%), a decrease was observed for F2D1 (4.86%) compared to IC.

Organic % C concentrations increased by 1.84% in F2D1 and 1.47% in F2D2. However, decreases were observed in F2D3 (14.12%), F2D4 (17.75%), and F2D5 (40.64%). C/N (%) for F2D1 (1.02%) and F2D2 (0.66%) increased, while F2D3 (18.76%), F2D4 (33.55%), and F2D5 (50.72%) decreased compared to IC.

Total Fe and Cu concentrations increased with F2D1 (1.67–10.71%), F2D2 (13.48–12.78%), F2D3 (15.53–20.17%), F2D4 (18.43–24.43%) and F2D5 (29.65–35.86%). Although the total Zn concentration increased in F2D2 (0.86%), F2D3 (15.88%), F2D4 (17.08%) and F2D5 (31.76%), a decrease was observed in F2D1 (10.30%) compared to IC.

After three months of mineralization, the changes in the final compost were analyzed according to the F3D. The changes at the end of the mineralization period with the F3D application were evaluated according to IC are shown in the Table 3.

In regard to OM content, decreases were observed in F3D1 (23.91%), F3D2 (29.07%), F3D3 (36.86%), F3D4 (66.69%), and F3D5 (43.03%) compared to IC. The total concentrations in % N, P, K, F3D1 (1.63–48.57–5.62%), F3D2 (2.04–122.86–9.55%), F3D3 (6.94–162.86–17.98%), F3D4 (11.87–70.83–19.82%), and F3D5 (20.41–285.71–32.02%) increased.

For water soluble NH_4^+, NO_3^-, P, K concentrations increased in F3D1 (215.71- 39.62–13.21–11.62%), F3D2 (239.17- 52.71–34.77–23.74%), F3D3 (278.86–70.16-77.90–50.51%), F3D4 (77.83- 46.28- 51.18- 36.13%) and F3D5 (477.30–125.42- 115.63- 130.30%). The pH values were determined as 6.77 ± 0.31 in F3D1, 6.88 ± 0.19 in F3D2, 7.00 ± 0.07 in F3D3, 7.50 ± 0.43 in F3D4 and 7.80 ± 0.73 in F3D5.

F3D1, F3D2, F3D3, F3D4 and F3D5 treatments elevated the EC values by 1.94%, 2.58%, 4.52%, 4.91% and 5.16%, respectively compared to IC. While cation exchange capacities increased in F3D1 (5.32%), F3D2 (13.72%), F3D3 (8.34%) and F3D5 (13.66%), a 6.36% decrease in mineralization was observed in F3D4. Decreases were observed in the specific gravity and volume weight changes of F3D1 (2.11–0.00%), F3D2 (10.56–16.67%), F3D3 (19.01–25.00%), F3D4 (39.22–50.00%), and F3D5 (32.39–33.33%) according to IC.

The water buffer capacities were increased according to IC: F3D1 (1.39%), F3D2 (7.64%), F3D3 (23.26%), and F3D4 (30.20%). However, mineralization of F3D5 resulted in a decrease of 17.71%. the percentage of easily accessible water (%) increased according to IC; F3D1 (0.35%), F3D2 (14.13%), F3D3 (42.17%), F3D4 (61.48%), and F3D5 (70.25%). However, F3D1 showed a decrease (4.02%).

Changes in % OC concentrations resulted in decreases in F3D1 (14.36%), F3D2 (22.93%), F3D3 (32.47%), F3D4 (60.91%), and F3D5 (40.05%). According to the mineralization results, decreases were obtained in the ratio C/N (%), F3D1 (15.71%), F3D2 (24.46%), F3D3 (36.87%), F3D4 (82.49%), and F3D5 (50.18%) compared to the IC. The changes in total concentrations of Fe, Zn and Cu are respectively 1.41–3.86–11.83% for F3D1, 4.75–10.73–17.94% for F3D2, for 18.10–19.74- 21.40%F3D3 and 18.43–19.66–24.29% for F3D4, while increases in the rate of 30.94- 33.05- 37.23% for F3D5 were obtained.

Discussion

During the mineralization process, the decrease in the concentrations of OM and organic carbon (OC) in the assessment of compost maturity indicates the degradation and effective decomposition of OM. After three months of mineralization of the raw tea waste, there were statistically significant differences between factors F1, F2, and F3, relative to the original value. There were also statistically significant differences between them depending on the mixing ratios ($p < 0.05$).

After three months of mineralization, the lowest values OM % and OC % were obtained by the F3D4 and F3D5. These decreases in OM % and OC % concentrations were F3D4 (40%, 38%) and F3D5 (43%, 40%) in comparison with the IC value. The reason for the decrease in the percentage of OM during composting or vermicomposting can be decomposition of organic matter. Another reason is that worms and microbes in the gut of earthworms can use OM as a source of energy to build their cell structures [44]. Therefore, the loss of OM during vermicomposting may be due to the synergistic effects of worms and microorganisms [45]. In addition, another study showed a decrease in OC after composting tea leaves with vermicompost [46]. It was also reported that there was a decrease in OC and OM after vermicomposting of different types of biodegradable wastes [47, 48]. The results of this study were consistent with previous studies.

As a result of mineralization, the highest values of total N concentration (%), water-soluble NH_4^+ and water-soluble NO_3^- concentration were obtained in F3D4 and F3D5 measured against the original waste values. While these increases occurred in F3D4 (14%, 355%, 88%) and F3D5 (20%, 482%, 128%), they achieved the lowest C/N ratios. These losses in C/N ratio were 46% and 50% lower than the baseline values, respectively. The reduction in C/N ratio may be due to the emission of carbon dioxide into the worm gut and the addition of nitrogenous substances during vermicompost production, as well as increased mineralization by microbial and enzymatic activities [49]. In addition, the decrease in C/N ratio is also indicative of the increased rate of humification of organic matter [50]. There are probably two reasons why the nitrogen content in worm composts is higher than in raw materials. These factors may be in the form of reduced organic carbon, carbon dioxide and mucus, nitrogen excreted substances, growth stimulating hormones and enzymes, and nitrogen additions from worms [51]. Another reason for this high nitrogen concentration may be earthworm activity and mineralization of organic matter. The high NH_4^+ content in the liquid separated from the decay product enhances N uptake by plants. Again, a higher total nitrogen content is associated with a lower OC concentration. As a result, lower nitrogen immobilization, faster soil infiltration, and higher short-term effect of nitrogen fertilization after field propagation lead to better control of applied nitrogen [52]. The C/N ratio is an important parameter for determining the maturity and stability of vermicompost. Decrease in C/N ratio in the finished vermicompost indicates rapid mineralization and decomposition of the original raw material and is one of the most commonly used parameters for stability and maturity of organic wastes [53–55]. Moreover, the C/N ratio < 12 is also preferred for agricultural purposes [56]. As a result of F3D4 and F3D5 mineralization in F3 factor, the C/N ratio was 9.14 and 8.31, respectively. These values indicate that F3D4 and F3D5 with F3 mineralization worm compost are readily available for agricultural use.

The pH and EC are also important in assessing the maturity and quality of vermicompost. During the vermicomposting process, pH often changes from alkaline to neutral [57]. As NH_4^+ concentration increases and pH increases, the C/N ratio decreases. In addition, the change in pH to acidic or neutral, the formation of organic acids leading to the production of ammonium ions and humic acids, may be a result of the mineralization of organic wastes [58] In addition, changes in the pH of the final vermicompost are due to the decomposition of organic wastes into organic acids

[59]. It has been reported that most plants, the pH range of vermicompost between 6 and 8.5 is the most appropriate to assure compatibility [60]. Following these mineralization processes, the pH of vermicompost was provided in the perfect ranges reported in the literature.

EC measures the salinity of organic matter and is a good indicator of the quality of vermicompost used in agriculture [61]. The decrease in EC during vermicomposting may be the result of precipitation or leaching of soluble salts and mineralization of organic acids [56]. It has also been observed that the maximum EC tolerance level of plants is 4.0 $mScm^{-1}$ and below this EC value fertilizer can be applied to the soil for plant growth and development [62]. Due to the mineralization process, soluble salts are produced during vermicomposting, which increases the EC value. As a result, the increase in EC values indicates that the soluble salts are within the range applicable to the soil without precipitation and leaching. In this study, the increased EC results within the limits indicated that good mineralization of raw tea waste was achieved by vermicomposting.

Worms are capable of converting insoluble P to soluble P during vermicomposting. In particular, the gut of worms contains P-soluble microorganisms that are often involved in the P-mineralization process [63]. Materials treated with worms have significant P concentration compared to the raw material [64]. The F3 factor on the F3D4 and F3D5 mixtures obtained the highest total P values after three months of mineralization (TP). Increases occurred at TP (243%, 286%). Increases in soluble P were also obtained (11%, 122%). Studies on vermicompost have indicated similar increases in TP [65]. In composting with worms and fly ash, the effect of the phosphate solubilizer *Pseudomonas fluorescens* in cattle manure was found to enhance the conversion of insoluble P compounds to releasable forms [66]. The impact of the pyroligneous acid-assisted biomass ash vermicompost on dry beans through climatic and agroecosystem changes indicated that the soil application of nutrient-rich biomass ash vermicompost not only has a beneficial effect on crop production, but also provides viable alternative recycling options to biomass ash in sustainable crop production systems [67].

Increased microbial communities in the worm gut during the vermicomposting process may enrich K concentrations [68]. The highest total K and soluble K values were obtained as a result of three months of mineralization of the F3 application for F3D4 and F3D5 mixtures. These increases were observed for total potassium (TK) (25%, 32%) and soluble K (57%, 130%). Various environmental researchers have reported that earthworms play an important role in increasing K while converting various bio-wastes with livestock manure into vermicompost [69, 70]. The result of this composting study was that the concentration of P and K in compost of F3D was positively correlated with mineralization of OM ($p < 0.001$). In addition, after three months of mineralization, volume, and mass reduction, according to IC, occurred at a rate of F3D4 (33–28.17%) and F3D5 (33–32.39%). Microbial activity, worms and enzymes may be responsible for this. Similar results indicated that the reduction in mass and volume during vermicompost production was due to microbial activity [71].

In this study, increased Fe, Zn, and Cu concentrations were found in F3D4 (23%, 25%, 33%) and F3D5 (31%, 33%, 39%) compared with IC. However, these increases were not at levels that would cause phytotoxicity and were within plant growth limits. It has been suggested that the increase in heavy metal content may result from a reduction in the weight and volume of substrates during organic matter degradation [72, 73]. Low concentrations of some heavy metals may be necessary for plant growth. However, higher concentrations of heavy metals are potentially harmful to plant growth [74]. In addition, the increase in Fe concentration can be considered positive as it is a metal ion essential for plant growth [75].

Conclusion

In this study on raw tea waste, the biomineralization process resulted in the production of high quality vermicompost with a high content of plant-available nutrients. After the application of microorganisms, enzymes, and worms to raw tea waste in different amounts, mineralization accelerated more than raw tea waste + mushroom cultivation waste + worm (F1D) applications. Concentrations of the major nutrients, nitrogen, phosphorus, and potassium differed significantly. In addition, significant quantitative improvements were observed for N and P. As a result, the tea was converted into a valuable source of organic food for crop production, along with microorganisms and enzymes added to vermicomposting. In agreement with the obtained results, the lowest C/N ratio and the lowest OM % content were obtained with the worming activities of the enzyme and microorganism applications in the F3D4 and F3D5 mixing ratios. The increase in the amount of N without nitrogen losses during the composting process shows that mineralization was successful. In this study, beneficial microorganisms and enzymes used in addition to worm activity turned tea waste into a high quality biofertilizer product. As a result of the increase in tea production due to the growing demand for tea, raw tea waste may become a common environmental problem. Therefore, it will be important to utilize these wastes in regenerative agriculture by turning them into biofertilizers in terms of waste management. The results of this study have shown that tea waste can be readily used in regenerative agriculture. As a result of the biomineralization process with earthworms, enzymes, and microorganisms, it is necessary to study the contribution of composts F3D4 and F3D5, which have a high biofertilizer value, to the products. In particular, the mixing ratio of F3D4 is recommended for better waste management.

Funding "This research was funded by General Directorate of Agricultural Research And Policies, Turkey, grant number" TAGEM/ 17 / AR-GE / 17.

Data availability Not applicable.

Declarations

Conflict of interest We know of no conflicts of interest associated with this publication, and there has been no significant financial support for this work that could have influenced its outcome. As Corresponding Author, I confirm that the manuscript has been read and approved for submission by all the named authors.

Ethical approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

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