







Article

Preparation, Characterization, and Testing of Compost Tea Derived from Seaweed and Fish Residues

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Abstract: Non-aerated compost tea (CT) was prepared from compost derived from rockweed (*Ascophyllum nodosum*) and fish (cod, common ling, haddock, saithe) residues that fermented in water. Electrical conductivity, pH, concentrations of dry matter, ash, C, macronutrients (N, P, K, Ca, and Mg), and micronutrients (Cu, Fe, Mn, Mo, and Zn) of CT prepared under different fermentation conditions were measured. The effects of process factors, i.e., water/compost mass ratio (4.2–9.8 g/g) and fermentation time (4.2–9.8 days = 100–236 h), on the physicochemical properties of CT were quantified using quadratic polynomial models. CT obtained at optimal levels of process factors (4.2 g/g and 5.6 days = 134 h) was tested for lettuce seed germination and seedling growth. Diluted CT (25% CT + 75% ultrapure water) improved seedling growth while achieving a high germination percentage (97%).

Keywords: circular economy; compost extract; fish residue; germination; lettuce; seaweed residue

1. Introduction

Compost tea (CT) is a fermented compost extract, rich in nutrients, organic molecules (humic acids, amino acids, phytohormones), and microorganisms (bacteria, fungi, protozoa), which can be beneficial to plants and used as a nutrient source and/or disease suppressor [1–4]. It is either aerated, prepared from a compost–water slurry that has been aerated during the fermentation, or non-aerated, derived from a slurry that has not been aerated or received minimal aeration [1,5–10].

The potential of CT to improve plant growth and health depends on various factors, e.g., compost feedstocks and age, fermentation conditions (aeration, water/compost ratio, duration, temperature, pH, nutrient additives), application technology (undiluted/diluted, to roots or/and leaves, equipment, timing, rate, adjuvants, specific microbial antagonists), and type of plant. Compost feedstocks used to prepare CT are typically agro-industrial,

municipal, and landscape residues [3,7,8,11,12]. The age of the compost is recommended to be between 2 and 12 months [3,8,9,13,14]. A mature compost typically releases higher amounts of soluble mineral nutrients and lower amounts of phytotoxic organic acids and heavy metals than an immature compost [3]. The levels of water (liquid)/compost (solid) ratio (R_{LS}) and fermentation time (t) are usually as follows: $R_{LS} = 3\text{--}10$ g/g for both aerated CT and non-aerated CT, $t = 1\text{--}7$ days for aerated CT, and $t = 3\text{--}21$ days for non-aerated CT [2,4,7,8,13,15,16]. The compost fermentation is commonly performed at $15\text{--}25$ °C [5,7–10,14]. Nutrient additives, e.g., molasses, glucose, sucrose, fish or kelp powder/slurry, yeast powder/extract, plant extracts, humic materials, and rock dust, can be added at the beginning or during fermentation process [1,6–8,16–19].

Numerous studies have reported positive effects of CTs on seed germination as well as on growth, development, and nutrient contents of different seedlings/plants, e.g., baby spinach [20], bean [2], chickpea [2], cowpea [21], cucumber [4,18], kohlrabi [11], lentil [17], lettuce [11,22,23], okra [24], pak choi [3,6], pea [2], pepper [15,25,26], potato [27,28], sweet corn [23], and tomato [12,18,22,29–31]. These beneficial effects of CTs are mainly due to their contents of mineral nutrients (especially N , P , K , Ca , Mg , Cu , Fe , Mn , Mo , and Zn), phytohormones (including auxins, cytokinins, gibberellins, abscisic acid and its metabolites), humic and fulvic acids, as well as to the presence of useful microorganisms [1,3,6,8,11,22,23,26,29].

There is debate about the necessity of aeration during CT preparation [5,7,8,10]. Preparation of aerated CT involves a shorter production time, whereas obtaining non-aerated CT is associated with lower energy consumption and cost [7,8,10]. Ingham (2005) [1] recommended aerated CT, but several other researchers concluded that, for a sufficiently long fermentation time (around 7 days), the effects of non-aerated CT on plant growth and health were similar or better than those of aerated CT [6,8,9].

Marin et al. [15] prepared aerated and non-aerated CTs from spent mushroom compost (SMC), grape marc compost (GMC), crop residue compost (CRC), and crop residue vermicompost (CRV). Fermentation tests were performed at 20 °C, $t = 5$ days, and $R_{LS} = 3\text{--}4$ g/g. The effects of aerated and non-aerated CTs diluted at $1/5$ in water on the growth of pepper (*Capsicum annuum* L.) seedlings were evaluated. Except for aerated CT from CRC, the mean values of the number of leaves (NL), dry masses of root (RDM), stem (SDM), leaf (LDM), and whole plant (PDM) were higher for treatments with aerated and non-aerated CTs than for the control treatment. Compared to the control treatment, the mean values of PDM were significantly higher for pepper plants treated with non-aerated CT from SMC, GMC, or CRV, and aerated CT from SMC. The mean values of relevant plant growth parameters, e.g., RDM , SDM , LDM , PDM , NL , leaf area, stem length, and stem base diameter, were generally higher for the treatment with non-aerated CT from SMC than for the other treatments.

Jarboui et al. [2] prepared non-aerated CT by fermenting compost from food waste (mainly fruits and vegetables) at 25 °C, $R_{LS} = 8$ g/g, and $t = 7$ days, using glucose as a nutrient additive. Diluted CT ($1/8$ in distilled water) applied to the roots improved the height, root diameter, and NL of bean (*Vicia faba* L.), chickpea (*Cicer arietinum* L.), and pea (*Pisum sativum* L.) seedlings.

Many studies have reported that non-aerated CT can prevent/control various diseases (including early/late/leaf blight, grey mold, apple scab, bacterial spot, damping-off, and powdery/downy mildew) of edible and ornamental crops, e.g., apple, cucumber, grape, lettuce, pepper, potato, strawberry, sugar beet, tomato, winter barley, geranium, and rose [5,7–10,14,32,33].

The marine sector produces significant amounts of materials that are still underutilized. Bone-rich residues from captured fish, which cannot be used for food or feed purposes, can be processed to obtain fertilizers, due to their high concentrations of macronutrients, especially N , P , and Ca [34–37]. Seaweed residues from extraction processes and seaweed deposited on beaches, which are rich in K , micronutrients, and growth activators, e.g., auxins, cytokines, are valuable sources of soil amendments, fertilizers, and biostimulants [35]. Well-balanced fertilizers for crop plants can be designed by composting seaweed and fish residual materials [35,36]. The application of marine waste-based compost and its

derived CT as soil amendments/fertilizers/biostimulants to horticultural crops deserves further attention.

In the present study, non-aerated CT was produced from composted industrial seaweed and fish residues. Relevant fermentation factors (i.e., water/compost mass ratio and fermentation time) were optimized, and CT obtained under optimal conditions was tested for germination of lettuce seeds and subsequent growth of lettuce seedlings. To our knowledge, there have been no published papers on the production and application of CT derived from marine residue-based compost. Therefore, the main novelty of this study is the utilization of seaweed and fish residues for the production of fertilizers/biostimulants for application in sustainable agriculture. These marine residues are currently underutilized and sometimes discarded, thus causing environmental concerns and loss of valuable nutrients and organic matter for soil/plants.

2. Materials and Methods

2.1. Marine Residues

In the composting step, seaweed and fish residual materials from the Norwegian industry were applied. Chemical extraction of dried and ground rockweed (*Ascophyllum nodosum*) to produce biostimulants resulted in a residual filter cake. This rockweed filter cake was a black paste with a content of dry matter (DM) of 25–30% [38,39]. The filter cake was mixed with residual material from the fish industry processing cod and similar fish species for the clipfish. Fish residues such as heads, skin, intestines, and backbones are commonly ground, acidified to pH < 4, and hydrolyzed in a tank. The upper layers of oil and soluble proteins were pumped out and applied as aquaculture feed. For the bone-rich sediment at the bottom of the hydrolysis tank (DM ≈ 50%), a sustainable application is still lacking. This acidified fish sediment was dried and then applied to produce compost. Fresh rockweed and fish residues are shown in Figure 1.



Figure 1. Fresh marine residues: (a) rockweed (*A. nodosum*) filter cake; (b) acidified fish sediment [38,39].

2.2. Compost Preparation

The compost was obtained by windrow composting of marine and non-marine residues according to a procedure described in previous reports [38,39]. The following volume percentages of residual materials were used: 40.8% rockweed filter cake, 9.2% acidified fish sediment, 39.5% woodchips, 6.6% cattle bedding, and 3.9% horse manure. A windrow consisting of marine residual materials, woodchips, cattle bedding, and horse manure is shown in Figure 2.



Figure 2. Windrow consisting of rockweed (*A. nodosum*) filter cake, acidified fish sediment, woodchips, cattle bedding material, and horse manure [38].

2.3. Compost Tea Preparation

Non-aerated CT was prepared by fermenting the compost derived from marine residues (10-month age) in demineralized water under different operation conditions. Liquid/solid mass ratio ($R_{LS} = 4.2\text{--}9.8$ g/g) and fermentation time ($t = 4.2\text{--}9.8$ days) were selected as process independent variables (factors). Based on a Central Composite Design (CCD) with 2 factors and 4 center point runs, 12 experimental runs were conducted at 5 levels of process factors [40]. The levels of dimensional and dimensionless factors for each experimental run are specified in Table 1, where the dimensionless factors (X_1 and X_2) were calculated using Equations (1) and (2) [40]. The mixture of compost and water was stirred once at the beginning of the fermentation process and then left in the dark at 25 °C. At the end of each experimental run, the slurry was filtered through a 0.020 mm sieve and the filtrate (CT) was analyzed. Images of the compost derived from marine residues and corresponding non-aerated CT are shown in Figure 3.

$$X_1 = \frac{R_{LS} - 7}{2} \quad (1)$$

$$X_2 = \frac{t - 7}{2} \quad (2)$$

Table 1. Levels of dimensional and dimensionless fermentation factors.

Run	R_{LS} (g/g)	t (Days)	X_1	X_2
1	5	5	−1	−1
2	5	9	−1	1
3	9	5	1	−1
4	9	9	1	1
5	7	7	0	0
6	7	7	0	0
7	4.2	7	−1.414	0
8	9.8	7	1.414	0
9	7	4.2	0	−1.414
10	7	9.8	0	1.414
11	7	7	0	0
12	7	7	0	0

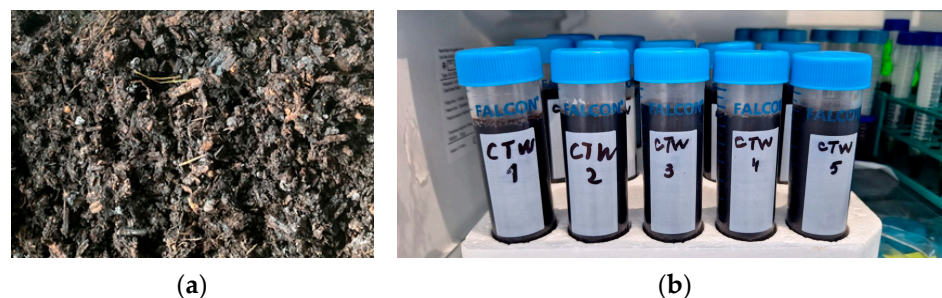


Figure 3. Compost obtained by composting rockweed (*A. nodosum*) filter cake, acidified fish sediment, woodchips, cattle bedding material, and horse manure (a) and derived non-aerated compost tea (CT) (b).

2.4. Compost Tea Analysis

Non-aerated CT obtained in each experimental run (Table 1) was analyzed in terms of electrical conductivity (*EC*), *pH*, concentrations of dry matter (*DM*), ash (*Ash*), carbon (*C*), macronutrients, and micronutrients. Analysis methods were detailed in previous papers [37,41–43]. *EC* and *pH* were measured using a Mettler Toledo SevenExcellence *pH/Conductivity Meter S470* (Mettler Toledo, Columbus, OH, USA). *DM* and *Ash* were determined using a Memmert UN110 oven (Mettmert GmbH, Schwabach, Germany) and a Nabertherm B150 oven (Nabertherm GmbH, Lilienthal, Germany), respectively. The percentages of carbon (*C*) and nitrogen (*N*) were measured using an EA3100 Elemental Analyzer (Eurovector SRL, Pavia, PV, Italy). Concentrations of phosphorus, potassium, calcium, magnesium, copper, iron, manganese, molybdenum, and zinc (*P*, *K*, *Ca*, *Mg*, *Cu*, *Fe*, *Mn*, *Mo*, and *Zn*) were determined after digestion with nitric acid and hydrogen peroxide (4/1 L/L), using an Agilent 7700 Series ICP-MS (Agilent Technologies, Santa Clara, CA, USA). All measurements were performed in triplicate.

2.5. Compost Tea Testing

Non-aerated CT was tested for lettuce seed germination and seedling growth. Before the tests, lettuce (*Lactuca sativa* L. var. *crispa* cv. ‘Lollo Rosso’) seeds were subjected to a thorough disinfection process using a 1% NaClO solution for 20 min, followed by rinsing with ultrapure water. Four treatments were applied: (T0) 100% ultrapure water (control); (T1) 25% CT + 75% ultrapure water; (T2) 50% CT + 50% ultrapure water; (T3) 100% CT. Each treatment was replicated 4 times (A, B, C, and D replicates), using 50 seeds per replicate (200 seeds per treatment). Two Petri dishes (19 cm diameter, 5 cm height) were used for each treatment, with two replicates per dish (A and B in a dish, C and D in the other dish), as shown in Figure 4. In Petri dishes, the seeds were placed evenly on moistened filter paper, 100 seeds in each dish. The same volume of diluted or non-diluted CT was used to wet the filter papers in each dish. The progress of seed germination and seedling growth was observed for 10 days in a controlled climate chamber maintained at 20 °C, with alternating light cycles mimicking natural conditions. The seeds were considered germinated when the radicle emerged from the seed coat and reached a length of 2 mm [44].

The following parameters were used to characterize the germination process and seedling growth (10 days after sowing): germination percentage (*GP*), mean germination time (*MGT*), germination speed (*GS*), seedling length (*SL*), seedling vigor index (*SVI*), seedling mass (*SM*), root length (*RL*), and total leaf surface area (*LA*). Characteristic parameters of lettuce seed germination (*GP*, *MGT*, and *GS*) were determined using Equations (3)–(5), where N_T represents the total number of tested seeds, t_i the time since sowing (1, 2, ..., 10 days, $i = 1, 2, \dots, 10$), $N_{g,i}$ the number of germinated seeds at t_i , and $N_{g,T} = N_{g,10}$ the total number of germinated seeds [45–47]. *SL*, *SM*, *RL*, and *LA* at $t_{10} = 10$ days were measured for the seedlings resulting from 50% of tested seeds, i.e., those from the upper half of each Petri dish (highlighted in red in Figure 4). *SVI* was calculated

depending on GP and SL using Equation (6) [17,47]. Images of seedling samples at $t_{10} = 10$ days are shown in Figure 5.

$$GP = 100 \frac{N_{g,T}}{N_T} \quad (3)$$

$$MGT = \frac{\sum_{i=1}^{10} N_{g,i} t_i}{N_{g,T}} \quad (4)$$

$$GS = \sum_{i=1}^{10} \frac{N_{g,i}}{t_i} \quad (5)$$

$$SVI = SL \frac{GP}{100} \quad (6)$$

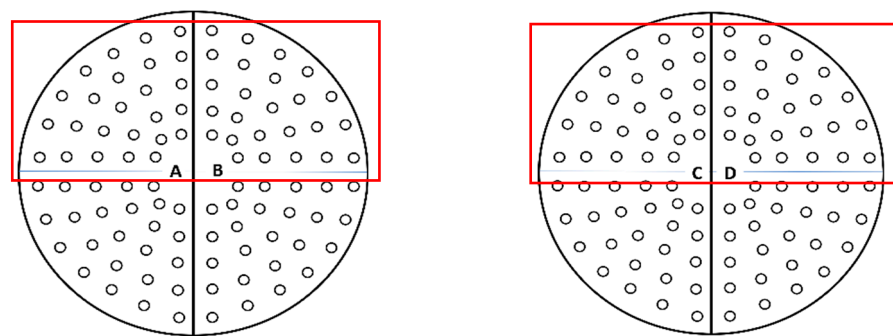


Figure 4. Schematic representation of Petri dishes for a treatment, using 4 replicates per treatment (A and B in a dish, C and D in the other dish) and 50 seeds per replicate.

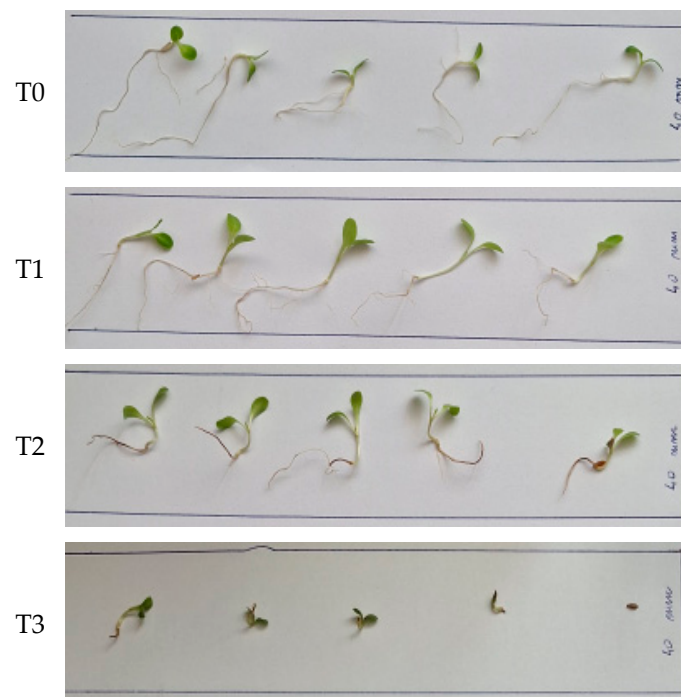


Figure 5. Samples of lettuce seedlings (10 days after sowing) for different treatments: (T0) 100% ultrapure water (control); (T1) 25% CT + 75% ultrapure water; (T2) 50% CT + 50% ultrapure water; (T3) 100% CT.

2.6. Data Processing

The values of CT properties obtained at different levels of fermentation factors were processed using Principal Component Analysis (PCA) [37,42,43,48]. The effects of fermentation process factors on physicochemical properties of CT were quantified using quadratic polynomial equations. The desirability function approach was used to optimize the process factors [40,49]. One-way ANOVA was applied to evaluate whether the treatments with diluted and undiluted CT had significant effects ($p < 0.05$) on the relevant characteristics of lettuce seed germination and seedling growth. Statistical analysis, modelling, and process factor optimization were performed using XLSTAT Version 2019.1 (Addinsoft, New York, NY, USA).

3. Results and Discussion

3.1. Compost Tea Characterization

Indicators of position (minimum, maximum, and mean values) and variability (standard deviation) of CT properties determined in triplicate in all experimental runs are summarized in Table 2. Tabulated data indicate lower variability of pH and C/N (coefficients of variation less than 4%) as well as higher variability of P , Ca , Mg , Cu , Mn , and Zn (coefficients of variation higher than 40%).

Table 2. Indicators of position and variability of CT properties measured in triplicate.

Symbol	Variable		Indicator		
	Units	Minimum Value (MIN)	Maximum Value (MAX)	Mean Value (m)	Standard Deviation (SD)
<i>EC</i>	dS/m	7.386	18.08	11.74	2.781
<i>pH</i>	-	7.777	8.295	7.996	0.163
<i>DM</i>	%	0.721	2.686	1.514	0.517
<i>Ash</i>	%	0.547	1.751	0.973	0.314
<i>C</i>	%	0.090	0.594	0.301	0.138
<i>N</i>	%	0.007	0.041	0.021	0.009
<i>C/N</i>	kg/kg	13.58	16.94	14.57	0.552
<i>P</i>	%	0.016	0.062	0.030	0.013
<i>K</i>	%	0.226	0.700	0.406	0.113
<i>Ca</i>	mg/kg	70.53	805.1	264.1	237.9
<i>Mg</i>	mg/kg	37.41	258.6	110.8	56.05
<i>Cu</i>	mg/kg	0.037	0.303	0.133	0.063
<i>Fe</i>	mg/kg	9.833	25.33	16.21	4.523
<i>Mn</i>	mg/kg	0.098	0.696	0.280	0.199
<i>Mo</i>	mg/kg	0.007	0.056	0.036	0.013
<i>Zn</i>	mg/kg	0.335	2.895	1.117	0.640

EC: electrical conductivity; *DM*: dry matter concentration; *Ash*: ash concentration; *C*: carbon concentration; *N*: nitrogen concentration; *P*: phosphorus concentration; *K*: potassium concentration; *Ca*: calcium concentration; *Mg*: magnesium concentration; *Cu*: copper concentration; *Fe*: iron concentration; *Mn*: manganese concentration; *Mo*: molybdenum concentration; *Zn*: zinc concentration.

Table 3 contains information reported by several authors regarding the physicochemical properties of CT, type of compost feedstock, and fermentation conditions. Tabulated data highlight the following:

- the mean values of *EC*, *K*, *Ca*, *Mg*, and *Zn* obtained in this study were significantly higher than those reported by Zaccardelli et al. [26] for aerated CT prepared from two types of compost derived from agro-industrial residual materials (wood, artichoke, fennel, and escarole residues), whereas the values of *pH*, *Cu*, and *Mn* were similar;
- the mean values of *EC*, *N*, *P*, *K*, *Ca*, *Mg*, and *Fe* were significantly higher than those found by Samet et al. [13] for aerated CT obtained from compost produced from olive mill wastewater, olive pomace, and coffee grounds, whereas the values of *pH* were similar;

- compared to aerated CT prepared by Morales-Corts et al. [31] from garden waste-based compost, the mean values of EC, Ca, Mg, Fe, Mn, and Zn obtained in this study were significantly higher, that of Cu was significantly lower, whereas the values of pH were similar;
- the mean values of EC, pH, C/N, and Mg were significantly higher than those reported by González-Hernández et al. [25,27,30] for aerated CT derived from garden waste-based compost, whereas those of Ca were similar;
- the mean values of EC, pH, and N were significantly higher than those found by Jarboui et al. [2] for non-aerated CT prepared from food waste-derived compost;
- the mean values of P and K were significantly higher than those obtained by Xu et al. [4] for non-aerated CTs derived from compost based on pig manure and rice straw, whereas those of N were similar.

Table 3. Comparison with the data reported in the related literature.

Variable	Zaccardelli et al. [26]	Samet et al. [13]	Morales-Corts et al. [31]	González-Hernández et al. [25,27,30]	Jarboui et al. [2]	Xu et al. [4]	This Study
EC (dS/m)	4.778 ± 0.500	7.61	2.6 ± 0.1	1.2 ± 0.1	3.12 ± 0.01	-	11.74 ± 2.78
pH	7.60 ± 0.16	8.00	7.81 ± 0.15	7.16 ± 0.15	6.93 ± 0.12	-	7.80 ± 0.16
N (%)	-	0.002 ± 0.000	-	-	0.006 ± 0.000	0.032	0.021 ± 0.009
C/N	-	-	-	7.1 ± 0.2	-	-	14.57 ± 0.55
P (mg/kg)	-	2.170 ± 0.254	-	-	-	239	301.5 ± 125.2
K (%)	0.142 ± 0.023	0.188 ± 0.018	-	-	-	0.108	0.406 ± 0.113
Ca (mg/kg)	21.8 ± 1.9	164.5 ± 34.3	50 ± 23	280 ± 17	-	-	264.1 ± 237.9
Mg (mg/kg)	37.8 ± 3.1	87.35 ± 15.59	27.5 ± 16.0	20 ± 14	-	-	110.8 ± 56.1
Cu (mg/kg)	0.16 ± 0.02	-	0.308 ± 0.047	-	-	-	0.133 ± 0.063
Fe (mg/kg)	-	3.746 ± 0.385	9.8 ± 2.1	-	-	-	16.21 ± 4.52
Mn (mg/kg)	0.45 ± 0.01	-	0.059 ± 0.019	-	-	-	0.280 ± 0.199
Zn (mg/kg)	0.15 ± 0.01	-	0.266 ± 0.025	-	-	-	1.117 ± 0.640
Compost feedstock	Agro-industrial residues	Olive residues and coffee grounds	Garden waste	Garden waste	Fruit and vegetable waste	Pig manure and rice straw	Rockweed and fish residues
Fermentation conditions	t = 7 days with aeration	R _{LS} = 5 g/g t = 7 days with aeration	20 °C R _{LS} = 5 L/L t = 14 days with aeration	20 °C R _{LS} = 5 L/L t = 5 days with aeration	25 °C R _{LS} = 8 g/g t = 7 days without aeration	20–25 °C R _{LS} = 8 g/g t = 7 days without aeration	20 °C R _{LS} = 4.2–9.8 g/g t = 4.2–9.8 days without aeration

EC: electrical conductivity; C: carbon concentration; N: nitrogen concentration; P: phosphorus concentration; K: potassium concentration; Ca: calcium concentration; Mg: magnesium concentration; Cu: copper concentration; Fe: iron concentration; Mn: manganese concentration; Zn: zinc concentration; variables are expressed as mean values ± SD and/or ranges of values (MIN–MAX); R_{LS}: liquid/solid ratio; t: fermentation temperature.

Accordingly, the mean values of EC (11.74 dS/m), N (0.021%), P (301.5 mg/kg), K (0.406%), Ca (264.1 mg/kg), Mg (110.8 mg/kg), Fe (16.21 mg/kg), and Zn (1.117 mg/kg) for non-aerated CT obtained in this study from marine residue-derived compost were generally significantly higher than those reported in the related literature for CT prepared from terrestrial residue-derived compost (Table 3). This is probably due to the high concentrations of macronutrients (N, P, K, Ca, and Mg) and micronutrients in seaweed and fish residues and their compost [34–37].

3.2. Results of PCA

A data matrix with 36 rows [number of triplicate samples corresponding to experimental runs 1, 2, . . . , 12 (CT1, CT2, . . . , CT12) in Table 1] and 15 columns (number of variables, including EC, pH, DM, Ash, C, N, P, K, Ca, Mg, Cu, Fe, Mn, Mo, and Zn) was used in PCA. The eigenvalues corresponding to the first two principal components (PCs), i.e., 11.63 for PC1 and 1.48 for PC2, were > 1 and these two PCs explained 87.4% (77.5% + 9.9%) of the total variance.

The results presented in Figure 6 (PCA bi-plot), Table 4 (factor loadings), and Table 5 (correlation matrix) highlight the following:

- depending on significant levels of factor loadings (highlighted in bold in Table 4), the most important variables are *EC*, *DM*, *Ash*, *C*, *N*, *P*, *K*, *Ca*, *Mg*, *Cu*, *Fe*, *Mn*, *Mo*, and *Zn* for PC1 as well as *pH* for PC2;
- CT1 and CT7 samples obtained in the experimental runs 1 ($X_1 = -1$ and $X_2 = -1$) and 7 ($X_1 = -1.414$ and $X_2 = 0$) have higher values of *EC*, *DM*, *Ash*, *C*, *N*, *P*, *K*, *Ca*, *Mg*, *Cu*, *Fe*, *Mn*, *Mo*, and *Zn* than the other samples [discrimination on PC1 between CT1 and CT7 (inside the blue ellipse in Figure 6) and the other samples (inside the green ellipse), especially CT2 ($X_1 = -1$ and $X_2 = 1$), CT4 ($X_1 = 1$ and $X_2 = 1$), CT8 ($X_1 = 1.414$ and $X_2 = 0$), and CT10 ($X_1 = 0$ and $X_2 = 1.414$)];
- CT4 samples obtained in the experimental run 4 ($X_1 = 1$ and $X_2 = 1$) have higher values of *pH* than CT2, CT3, CT5, CT6, CT8–CT12 samples (discrimination on PC2 between CT4 and the other samples inside the green ellipse in Figure 6);
- *EC*, *DM*, *Ash*, *C*, *N*, *P*, *K*, *Ca*, *Mg*, *Cu*, *Fe*, *Mn*, *Mo*, and *Zn* are directly correlated and the corresponding correlation coefficients ($0.394 \leq r \leq 0.999$) are significant at $\alpha = 0.05$ (Table 5); *pH* is directly correlated with *Ash*, *P*, *Ca*, *Cu*, *Fe*, *Mn*, and *Zn*, with the corresponding correlation coefficients ($0.358 \leq r \leq 0.667$) being significant at $\alpha = 0.05$.

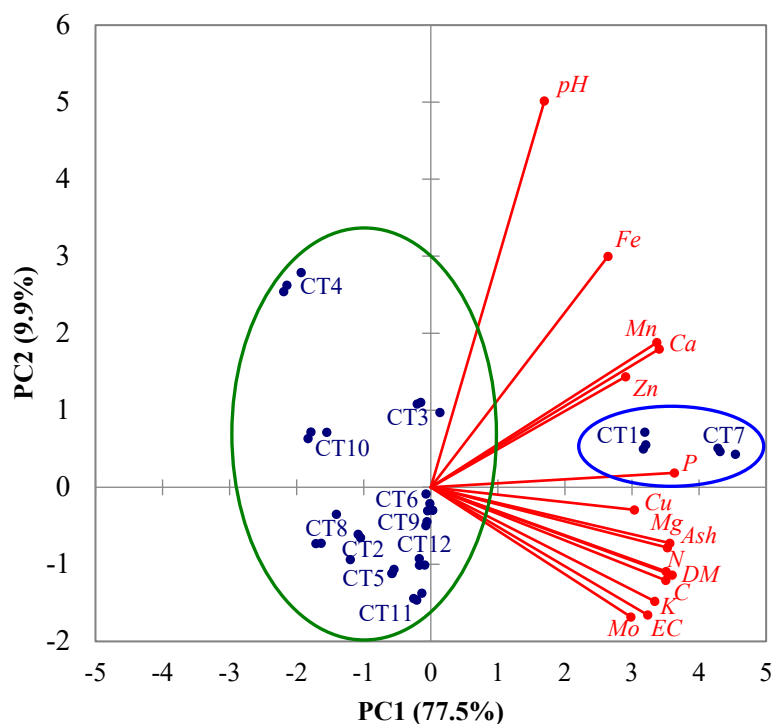


Figure 6. Projections of variables (*EC*, *pH*, *DM*, *Ash*, *C*, *N*, *P*, *K*, *Ca*, *Mg*, *Cu*, *Fe*, *Mn*, *Mo*, and *Zn*) and samples (CT1, CT2, . . . , CT12) on the factor-plane PC1–PC2. *EC*: electrical conductivity; *DM*: dry matter concentration; *Ash*: ash concentration; *C*: carbon concentration; *N*: nitrogen concentration; *P*: phosphorus concentration; *K*: potassium concentration; *Ca*: calcium concentration; *Mg*: magnesium concentration; *Cu*: copper concentration; *Fe*: iron concentration; *Mn*: manganese concentration; *Mo*: molybdenum concentration; *Zn*: zinc concentration; CT: compost tea.

Table 4. Factor loadings.

No.	Variable		Principal Component	
	Name	Symbol	PC1	PC2
1	Electrical conductivity	<i>EC</i>	0.88	−0.27
2	pH	<i>pH</i>	0.46	0.82
3	Dry matter concentration	<i>DM</i>	0.98	−0.19
4	Ash concentration	<i>Ash</i>	0.96	−0.13
5	Carbon concentration	<i>C</i>	0.95	−0.20
6	Nitrogen concentration	<i>N</i>	0.96	−0.18
7	Phosphorus concentration	<i>P</i>	0.99	0.03
8	Potassium concentration	<i>K</i>	0.91	−0.24
9	Calcium concentration	<i>Ca</i>	0.93	0.29
10	Magnesium concentration	<i>Mg</i>	0.97	−0.12
11	Copper concentration	<i>Cu</i>	0.83	−0.05
12	Iron concentration	<i>Fe</i>	0.72	0.49
13	Manganese concentration	<i>Mn</i>	0.92	0.31
14	Molybdenum concentration	<i>Mo</i>	0.81	−0.27
15	Zinc concentration	<i>Zn</i>	0.79	0.23

Significant values of factor loadings are highlighted in bold.

Table 5. Correlation matrix.

Variable	<i>EC</i>	<i>pH</i>	<i>DM</i>	<i>Ash</i>	<i>C</i>	<i>N</i>	<i>P</i>	<i>K</i>	<i>Ca</i>	<i>Mg</i>	<i>Cu</i>	<i>Fe</i>	<i>Mn</i>	<i>Mo</i>	<i>Zn</i>
<i>EC</i>	1.000	0.230	0.930	0.970	0.821	0.812	0.895	0.989	0.749	0.862	0.626	0.504	0.682	0.680	0.598
<i>pH</i>	0.230	1.000	0.306	0.358	0.271	0.278	0.478	0.269	0.618	0.324	0.394	0.667	0.651	0.182	0.469
<i>DM</i>	0.930	0.306	1.000	0.975	0.970	0.967	0.970	0.949	0.853	0.977	0.797	0.626	0.835	0.822	0.708
<i>Ash</i>	0.970	0.358	0.975	1.000	0.898	0.893	0.960	0.972	0.866	0.931	0.718	0.630	0.820	0.754	0.719
<i>C</i>	0.821	0.271	0.970	0.898	1.000	0.999	0.925	0.857	0.807	0.975	0.846	0.619	0.825	0.864	0.673
<i>N</i>	0.812	0.278	0.967	0.893	0.999	1.000	0.928	0.849	0.816	0.975	0.844	0.634	0.832	0.859	0.684
<i>P</i>	0.895	0.478	0.970	0.960	0.925	0.928	1.000	0.917	0.937	0.960	0.783	0.731	0.914	0.745	0.769
<i>K</i>	0.989	0.269	0.949	0.972	0.857	0.849	0.917	1.000	0.771	0.898	0.689	0.542	0.718	0.705	0.621
<i>Ca</i>	0.749	0.618	0.853	0.866	0.807	0.816	0.937	0.771	1.000	0.860	0.700	0.775	0.971	0.640	0.860
<i>Mg</i>	0.862	0.324	0.977	0.931	0.975	0.975	0.960	0.898	0.860	1.000	0.808	0.704	0.841	0.777	0.696
<i>Cu</i>	0.626	0.394	0.797	0.718	0.846	0.844	0.783	0.689	0.700	0.808	1.000	0.495	0.792	0.740	0.562
<i>Fe</i>	0.504	0.667	0.626	0.630	0.619	0.634	0.731	0.542	0.775	0.704	0.495	1.000	0.729	0.408	0.568
<i>Mn</i>	0.682	0.651	0.835	0.820	0.825	0.832	0.914	0.718	0.971	0.841	0.792	0.729	1.000	0.675	0.836
<i>Mo</i>	0.680	0.182	0.822	0.754	0.864	0.859	0.745	0.705	0.640	0.777	0.740	0.408	0.675	1.000	0.665
<i>Zn</i>	0.598	0.469	0.708	0.719	0.673	0.684	0.769	0.621	0.860	0.696	0.562	0.568	0.836	0.665	1.000

EC: electrical conductivity; *DM*: dry matter concentration; *Ash*: ash concentration; *C*: carbon concentration; *N*: nitrogen concentration; *P*: phosphorus concentration; *K*: potassium concentration; *Ca*: calcium concentration; *Mg*: magnesium concentration; *Cu*: copper concentration; *Fe*: iron concentration; *Mn*: manganese concentration; *Mo*: molybdenum concentration; *Zn*: zinc concentration. Values in bold of correlation coefficient (*r*) are different from 0 with a significance level $\alpha = 0.05$.

3.3. Prediction of Process Responses

Statistical models described by Equation (7) link the predicted process responses ($Y_{j,pr}$, $j = 1, \dots, 15$), i.e., EC_{pr} , pH_{pr} , DM_{pr} , Ash_{pr} , C_{pr} , N_{pr} , P_{pr} , K_{pr} , Ca_{pr} , Mg_{pr} , Cu_{pr} , Fe_{pr} , Mn_{pr} , Mo_{pr} , and Zn_{pr} , to X_1 , X_1^2 , X_2 , X_2^2 , and X_1X_2 .

$$Y_{j,pr} = \alpha_{0j} + \alpha_{1j}X_1 + \alpha_{11j}X_1^2 + \alpha_{2j}X_2 + \alpha_{22j}X_2^2 + \alpha_{12j}X_1X_2, \quad j = 1 \dots 15 \quad (7)$$

Regression coefficients in Equation (7), i.e., α_{0j} , α_{1j} , α_{11j} , α_{2j} , α_{22j} , and α_{12j} , were determined from mean experimental values (corresponding to triplicate measurements) of fermentation process responses ($Y_{j,m}$, $j = 1, \dots, 15$), which are summarized in Tables 6 and 7. The values of regression coefficients, determination coefficient (R_j^2), *F* statistic (F_j), and p_j -value for F_j , which are specified in Tables 6 and 7, highlight the following relevant aspects:

- pH_{pr} and Mn_{pr} do not vary significantly with X_1 , X_1^2 , X_2 , X_2^2 , or X_1X_2 ($0.360 \leq R_j^2 \leq 0.693$, $0.675 \leq F_j \leq 2.708$, and $0.129 \leq p_j \leq 0.658$ for $j = 2, 14$);
- EC_{pr} , DM_{pr} , Ash_{pr} , C_{pr} , N_{pr} , P_{pr} , K_{pr} , Ca_{pr} , Mg_{pr} , Fe_{pr} , Mo_{pr} , and Zn_{pr} vary significantly with at least one of X_1 , X_1^2 , X_2 , X_2^2 , and X_1X_2 , and there is a good agreement

between experimental and predicted values of process responses ($0.809 \leq R_j^2 \leq 0.979$, $5.080 \leq F_j \leq 54.87$, and $0.0001 \leq p_j \leq 0.036$ for $j = 1, 3, \dots, 10, 12, 13, 15$); EC_{pr} increases significantly with an increase in X_1^2 and a decrease in X_1 , X_2 , and X_2^2 ; DM_{pr} , C_{pr} , N_{pr} , P_{pr} , K_{pr} , and Mg_{pr} increase significantly with a decrease in X_1 and X_2 ; Ash_{pr} and Zn_{pr} increase significantly with an increase in X_1^2 and a decrease in X_1 and X_2 ; Ca_{pr} increases significantly with an increase in X_1^2 and a decrease in X_1 ; Fe_{pr} increases significantly with an increase in X_1X_2 ; Mo_{pr} increases significantly with a decrease in X_1 , X_2 , and X_2^2 ;

- Cu_{pr} increases significantly with a decrease in X_2 , but the statistical model defined by Equation (7) for $j = 11$ is statistically non-significant ($F = 2.326$ and $p = 0.167$).

Table 6. Mean experimental values (corresponding to triplicate measurements) of fermentation process responses ($Y_{j,m}$, $j = 1, \dots, 8$) and related values of regression coefficients, determination coefficient (R_j^2), F statistic (F_j), and p_j -value for F_j at different levels of dimensionless process factors.

Run	X_1	X_2	$Y_{j,m}$							
			$j = 1$	$j = 2$	$j = 3$	$j = 4$	$j = 5$	$j = 6$	$j = 7$	$j = 8$
			EC_m (dS/m)	pH_m	DM_m (%)	Ash_m (%)	C_m (%)	N_m (%)	P_m (%)	K_m (%)
1	-1	-1	15.16	8.078	2.312	1.460	0.502	0.035	0.052	0.532
2	-1	1	13.32	7.920	1.281	1.001	0.153	0.010	0.024	0.443
3	1	-1	9.380	8.164	1.340	0.812	0.289	0.020	0.028	0.323
4	1	1	7.412	8.225	0.725	0.553	0.093	0.007	0.017	0.233
5	0	0	11.75	7.815	1.471	0.932	0.296	0.020	0.027	0.388
6	0	0	11.65	8.017	1.564	0.976	0.328	0.022	0.030	0.393
7	-1.414	0	18.01	8.285	2.680	1.715	0.589	0.040	0.059	0.684
8	1.414	0	8.615	7.785	1.085	0.675	0.229	0.016	0.016	0.296
9	0	-1.414	11.65	7.998	1.540	0.921	0.330	0.023	0.030	0.413
10	0	1.414	10.12	7.980	1.047	0.729	0.147	0.010	0.023	0.338
11	0	0	11.90	7.783	1.570	0.925	0.336	0.023	0.029	0.421
12	0	0	11.93	7.897	1.558	0.972	0.325	0.022	0.028	0.412
	a_{0j}		11.81	7.878	1.541	0.951	0.321	0.022	0.028	0.403
	a_{1j}		-3.121	-0.040	-0.473	-0.321	-0.100	-0.007	-0.011	-0.121
	a_{11j}		0.556	0.100	0.128	0.108	0.028	0.002	0.004	0.031
	a_{2j}		-0.746	-0.015	-0.293	-0.124	-0.100	-0.007	-0.006	-0.036
	a_{22j}		-0.655	0.077	-0.167	-0.076	-0.057	-0.004	-0.001	-0.026
	a_{12j}		-0.032	0.055	0.104	0.050	0.038	0.003	0.004	-0.000
	R_j^2		0.979	0.360	0.921	0.956	0.880	0.877	0.877	0.942
	F_j		54.87	0.675	14.03	26.12	8.811	8.588	8.564	19.65
	p_j		0.000	0.658	0.003	0.001	0.010	0.010	0.011	0.001

EC: electrical conductivity; *DM*: dry matter concentration; *Ash*: ash concentration; *C*: carbon concentration; *N*: nitrogen concentration; *P*: phosphorus concentration; *K*: potassium concentration. Statistically significant regression coefficients are highlighted in bold.

Table 7. Mean experimental values (corresponding to triplicate measurements) of fermentation process responses ($Y_{j,m}$, $j = 9, \dots, 15$) and related values of regression coefficients, determination coefficient (R_j^2), F statistic (F_j), and p_j -value for F_j at different levels of dimensionless process factors.

Run	X_1	X_2	$Y_{j,m}$						
			$j = 9$	$j = 10$	$j = 11$	$j = 12$	$j = 13$	$j = 14$	$j = 15$
			Ca_m (mg/kg)	Mg_m (mg/kg)	Cu_m (mg/kg)	Fe_m (mg/kg)	Mn_m (mg/kg)	Mo_m (mg/kg)	Zn_m (mg/kg)
1	-1	-1	779.7	198.1	0.179	23.71	0.658	0.053	2.509
2	-1	1	141.7	56.72	0.056	10.06	0.106	0.032	1.092
3	1	-1	326.9	83.52	0.188	12.59	0.448	0.042	1.564

Table 7. Cont.

Run	X_1	X_2	$Y_{j,m}$						
			$j = 9$	$j = 10$	$j = 11$	$j = 12$	$j = 13$	$j = 14$	$j = 15$
			Ca_m (mg/kg)	Mg_m (mg/kg)	Cu_m (mg/kg)	Fe_m (mg/kg)	Mn_m (mg/kg)	Mo_m (mg/kg)	Zn_m (mg/kg)
4	1	1	134.3	38.63	0.050	21.00	0.149	0.020	0.770
5	0	0	150.6	108.6	0.118	13.48	0.182	0.032	0.726
6	0	0	231.8	120.5	0.131	15.49	0.255	0.037	0.820
7	-1.414	0	759.7	240.1	0.265	25.16	0.692	0.053	2.029
8	1.414	0	85.64	80.34	0.096	12.57	0.116	0.031	0.967
9	0	-1.414	173.7	114.8	0.141	15.32	0.217	0.039	0.861
10	0	1.414	154.4	64.73	0.084	14.43	0.187	0.008	0.430
11	0	0	122.0	115.3	0.133	14.92	0.176	0.041	0.870
12	0	0	108.2	107.9	0.153	15.77	0.178	0.043	0.761
	a_{0j}		153.2	113.1	0.134	14.91	0.198	0.038	0.794
	a_{1j}		-176.7	-44.83	-0.029	-2.250	-0.123	-0.007	-0.346
	a_{11j}		147.8	15.89	0.016	1.967	0.113	0.003	0.455
	a_{2j}		-107.3	-32.14	-0.043	-0.812	-0.112	-0.011	-0.353
	a_{22j}		18.51	-19.34	-0.018	-0.027	0.011	-0.006	0.028
	a_{12j}		111.4	24.12	-0.003	5.517	0.063	0.000	0.156
	R_j^2		0.809	0.869	0.660	0.815	0.693	0.943	0.833
	F_j		5.080	7.993	2.326	5.282	2.708	20.01	6.005
	p_j		0.036	0.013	0.167	0.033	0.129	0.001	0.025

Ca: calcium concentration; Mg: magnesium concentration; Cu: copper concentration; Fe: iron concentration; Mn: manganese concentration; Mo: molybdenum concentration; Zn: zinc concentration. Statistically significant regression coefficients are highlighted in bold.

3.4. Optimization of Fermentation Process Conditions

Optimization of fermentation process factors, aiming at maximizing the process responses in terms of EC_{pr} , DM_{pr} , Ash_{pr} , C_{pr} , N_{pr} , P_{pr} , K_{pr} , Ca_{pr} , Mg_{pr} , Fe_{pr} , Mo_{pr} , and Zn_{pr} was performed based on the desirability function approach. The optimal levels of dimensionless factors were $X_{1,opt} = -1.414$ and $X_{2,opt} = -0.707$, corresponding to $R_{LS,opt} = 4.2$ g/g and $t_{opt} = 5.6$ days = 134 h, and the value of desirability function at $X_{1,opt}$ and $X_{2,opt}$ was 0.988. The values of the process responses predicted by Equation (7) at $X_{1,opt}$ and $X_{2,opt}$, i.e., $Y_{j,pr,opt}$ ($j = 1, \dots, 15$), are summarized in Table 8.

Table 8. Predicted and experimental values of fermentation responses under optimal process conditions.

j	Variable		Optimal Value		Percentage Prediction Error ϵ_j (%)
	Symbol	Units	Predicted	Experimental	
			$Y_{j,pr,opt}$	$Y_{j,m,opt} \pm SD_j$	
1	EC	dS/m	17.50	16.92 ± 1.51	-3.4
2	pH	-	8.239	8.463 ± 0.359	2.7
3	DM	%	2.694	2.658 ± 0.227	-1.3
4	Ash	%	1.721	1.755 ± 0.100	2.0
5	C	%	0.599	0.606 ± 0.032	1.2
6	N	%	0.042	0.041 ± 0.002	-1.9
7	P	%	0.059	0.060 ± 0.001	1.8
8	K	%	0.649	0.628 ± 0.038	-3.3
9	Ca	mg/kg	895.2	887.8 ± 14.3	-0.8
10	Mg	mg/kg	245.5	249.0 ± 7.6	1.4
11	Cu	mg/kg	0.225	0.232 ± 0.042	3.0
12	Fe	mg/kg	28.10	29.34 ± 3.18	4.2
13	Mn	mg/kg	0.746	0.720 ± 0.053	-3.5
14	Mo	mg/kg	0.059	0.058 ± 0.002	-1.8
15	Zn	mg/kg	2.613	2.516 ± 0.116	-3.8

EC: electrical conductivity; DM: dry matter concentration; Ash: ash concentration; C: carbon concentration; N: nitrogen concentration; P: phosphorus concentration; K: potassium concentration; Ca: calcium concentration; Mg: magnesium concentration; Cu: copper concentration; Fe: iron concentration; Mn: manganese concentration; Mo: molybdenum concentration; Zn: zinc concentration.

3.5. Validation of Statistical Models

To validate the statistical models defined by Equation (7), three fermentation experiments were performed at optimal levels of process factors ($R_{LS,opt} = 4.2$ g/g and $t_{opt} = 5.6$ days = 134 h). The mean values of experimental responses at $R_{LS,opt}$ and t_{opt} , i.e., $Y_{j,m,opt}$ ($j = 1, \dots, 15$), related standard deviations (SD_j), and values of percentage prediction error (ε_j) defined by Equation (8) are presented in Table 8. The values of percentage prediction error ($-3.8\% \leq \varepsilon_j \leq 4.2\%$) and results of equal and unequal variance t -test ($p_j \geq 0.07$) indicate that $Y_{j,m,opt}$ and $Y_{j,pr,opt}$ were not significantly different, which proves the validity of statistical models described by Equation (7).

$$\varepsilon_j = 100 \frac{Y_{j,m,opt} - Y_{j,pr,opt}}{Y_{j,m,opt}}, \quad j = 1 \dots 15 \quad (8)$$

3.6. Testing Compost Tea for Lettuce Seed Germination and Seedling Growth

Non-aerated CT obtained at optimal levels of process factors ($R_{LS,opt} = 4.2$ g/g and $t_{opt} = 5.6$ days = 134 h) was tested for lettuce seed germination and seedling growth. Images of lettuce seedling, 10 days after sowing, are shown in Figure 7. The mean values (for four replicates) of relevant characteristics of germination and seedling growth for treatments T0 (100% ultrapure water) (control), T1 (25% CT + 75% ultrapure water), T2 (50% CT + 50% ultrapure water), and T3 (100% CT) are summarized in Table 9.

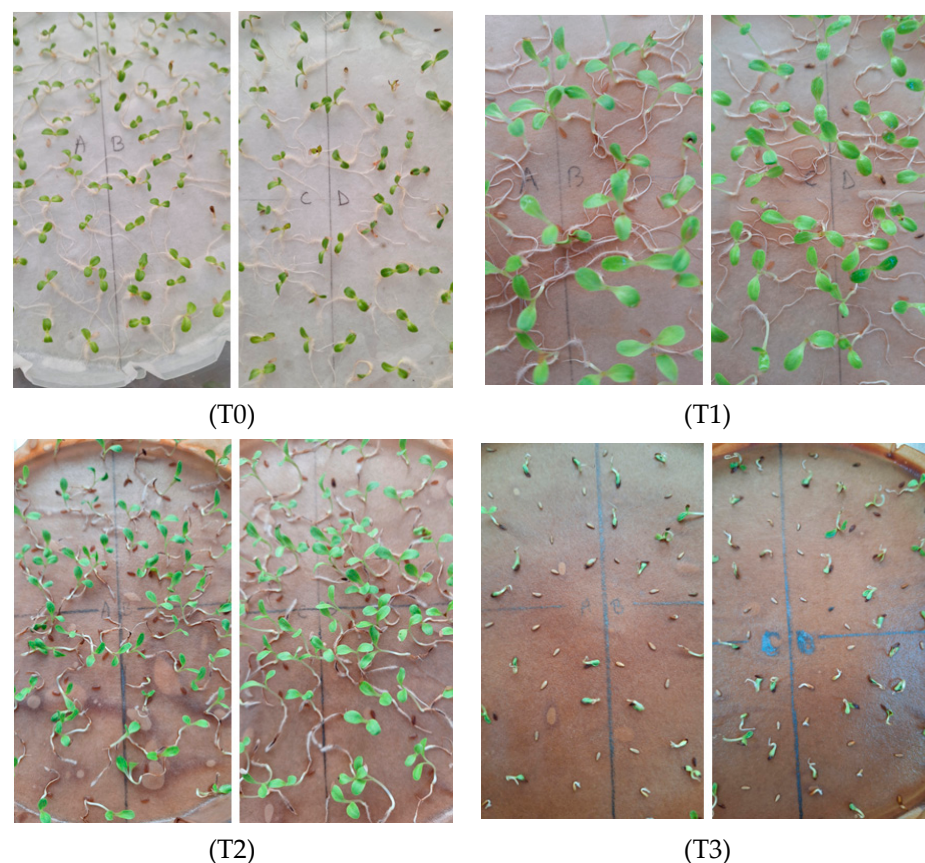


Figure 7. Lettuce seedlings (10 days after sowing) for different treatments: (T0) 100% ultrapure water (control); (T1) 25% CT + 75% ultrapure water; (T2) 50% CT + 50% ultrapure water; (T3) 100% CT. Two Petri dishes were used for each treatment with two replicates (50 seeds per replicate) per dish (A and B in a dish, C and D in the other dish).

Table 9. Mean values of relevant variables of lettuce seed germination and seedling growth.

Variable	Treatment			
	T0	T1	T2	T3
Germination percentage, <i>GP</i> (%)	98 a	97 a	97 a	73 b
Mean germination time, <i>MGT</i> (day)	1.2 d	1.6 c	2.2 b	5.2 a
Germination speed, <i>GS</i> (day ⁻¹)	44.5 a	34.7 b	25.5 c	7.9 d
Seedling length, <i>SL</i> (cm)	3.92 b	4.95 a	3.75 b	1.98 c
Seedling vigor index (<i>SVI</i>) (cm)	3.84 b	4.79 a	3.63 b	1.47 c
Seedling mass, <i>SM</i> (g)	0.30 b	0.37 a	0.33 b	0.11 c
Root length, <i>RL</i> (cm)	3.18 a	3.80 a	2.21 b	0.75 c
Total leaf surface area, <i>LA</i> (cm ²)	0.29 c	0.48 a	0.35 b	0.10 d

(T0) 100% ultrapure water (control); (T1) 25% CT + 75% ultrapure water; (T2) 50% CT + 50% ultrapure water; (T3) 100% CT. Different letters indicate a significant difference between treatments.

CT had a negative effect on the germination of lettuce seeds. Even though there were no statistically significant differences ($p > 0.05$) between the values of germination percentage (*GP*) for treatments T0 (98%), T1 (97%), and T2 (97%), CT application delayed the germination, i.e., mean germination time (*MGT*) increased and germination speed (*GS*) decreased with an increase in CT concentration from 25% to 100%.

However, the treatment T1 (25% CT) had significant ($p < 0.05$) positive effects on seedling length (*SL*), seedling vigor index (*SVI*), seedling mass (*SM*), and total leaf surface area (*LA*) compared with the other treatments. Moreover, the treatment T3 (100% CT) had significant adverse effects on seed germination and seedling growth characteristics.

Consequently, it is possible to add diluted CT (25% CT + 75% ultrapure water) to lettuce growth medium to improve seedling growth while obtaining a high level of *GP* as well as reasonable values of *MGT* and *GS*.

Seed germination is a critical process in the growth cycle of a plant, as it can significantly affect seedling establishment and plant production [50]. This process begins with imbibition, i.e., the water uptake by the seed, and ends with radicle protrusion [51]. Germination is regulated by internal factors, e.g., hormones (gibberellins, abscisic acid, auxins, cytokinins), proteins, seed age, size, and structural components, and external factors, including salinity, temperature, acidity, light, and nutrient and moisture concentration [51]. Salinity is a major stress that may negatively affect the germination process by decreasing the amount of gibberellins (that stimulate germination), increasing the amount of abscisic acid (that promotes seed dormancy and inhibits germination), and altering membrane permeability and water uptake [51]. Depending on the salt tolerance of the plant, salinity can cause the inhibition of seed germination or a decrease in *GP* and an increase in *MGT* [50–52]. Lettuce is a salinity sensitive plant (glycophyte) and its seed germination can be delayed or inhibited, even under conditions of moderate salinity ($EC = 4–8$ dS/m), by both osmotic stress and ionic toxicity stress (caused by excess Na^+ and Cl^-) [50–52].

Nasri et al. [50] studied the effects of NaCl concentration (0–150 mM, corresponding to $EC = 0–16$ dS/m) on *GP* for four lettuce varieties (Romaine, Augusta, Vista, and Verte). For the Romaine variety, the values of *GP* were similar (92–93%) for $EC = 0–10.6$ dS/m and about 45% higher than the value obtained for $EC = 16$ dS/m, whereas for the other varieties *GP* decreased with an increase in *EC*. Moreover, for the Vista and Verte varieties, germination was inhibited at higher levels of *EC* (16 dS/m for Vista, 10.6 dS/m and 16 dS/m for Verte). Inhibition of seed germination could be an effect of altered activity of hydrolytic enzymes, e.g., phytase [50]. Rosas et al. [52] evaluated the influence of NaCl concentration (0–100 mM, corresponding to $EC = 0–10.6$ dS/m) on lettuce seed germination. For *EC* levels higher than 2.8 dS/m, they found a decrease in *GP* and *GS* with an increase in *EC*. Germination can be delayed by a forced dormancy, caused by a decrease in water uptake by the seeds, which has negative effects on cell elongation and division [52]. These findings are consistent with those obtained in our study. The mean value of *EC* for undiluted CT (treatment T3) was 16.9 dS/m (Table 8). Assuming $EC = 0$ dS/m in ultrapure water, this

implies *EC* values of 4.23 dS/m and 8.46 dS/m for diluted CT in the treatments T1 and T2. The values of *GP* were similar (97–98%) for *EC* = 0–8.46 dS/m (treatments T0–T2) and about 30% higher than the value obtained for *EC* = 16.9 dS/m (treatment T3). For the treatments T1–T3, *MGT* increased and *GS* decreased with an increase in *EC* (4.23–16.9 dS/m).

Salinity can have significant negative effects on seedling/plant growth and development. Nasri et al. [50] evaluated the influence of salinity on the lettuce seedling growth, 4 days after sowing. In the presence of 100 mM NaCl (*EC* = 10.6 dS/m), masses and lengths of radicles and shoots were diminished, the decrease being more pronounced in the Vista variety (more salt sensitive) than in the Romaine variety (more salt tolerant). Rosas et al. [52] reported a decrease in *SL* and *RL* of lettuce seedlings (7 days after sowing) with an increase in *EC* (2.8–10.6 dS/m). In our study, the treatment T1 (*EC* = 4.23 dS/m) had a significant beneficial effect on *SL*, *SVI*, *SM*, and *LA* compared to the other treatments, whereas the treatments T0 (*EC* = 0 dS/m) and T1 had similar positive effects on *RL*. Moreover, all seedling growth characteristics (*SL*, *SVI*, *SM*, *RL*, and *LA*) decreased significantly with an increase in *EC* (4.23–16.9 dS/m). Ünlükara et al. [53] studied the influence of irrigation water salinity on the growth and yield of lettuce (*Lactuca sativa* L.). *EC* in the growing medium (soil) increased from 1.3 to 11.8 dS/m and the yield decreased from 144.8 to 30.6 g per lettuce with an increase in water salinity from 0.75 to 7.0 dS/m. A similar trend was observed for okra (*Abelmoschus esculentus* L.) [54].

In a review on salt stress in crop plants, stress was assessed using sodium chloride (NaCl) solutions of 80–150 mM, corresponding to about 9–16 dS/m [55]. At such concentration levels, leaf and root elongation decreased. Plants seem to cope with high concentrations up to a certain level, being able to maintain much lower concentrations of toxic ions inside the plant cells than in the saline root environment. However, when the mechanisms to avoid toxic levels are exceeded, the plant dies by dehydration (when salt accumulates in cell walls, causing cell shrinkage) or poisoning (when cell cytoplasm concentrations become too high for enzyme activity) [55]. The salt concentrations in the treatments T2 and T3 applied in our study, corresponding to about 8.5 and 17 dS/m, clearly had negative effects, highlighting the risk of applying too strong fertilizer solutions to sensitive crops.

Macronutrients, micronutrients, humic and fulvic acids, phytohormones, or other microbial metabolites present in CT are responsible for higher levels of *GP* and enhanced seedling growth [22,29,56–58]. The availability and balance of essential mineral nutrients in the growing substrate play a crucial role in the germination process and subsequent seedling/plant growth and development [57,59]. All essential nutrients are equally important to plants and an imbalance or an excess of nutrients in the substrate solution can significantly affect germination and growth stages [57,60,61].

Determining optimal levels of *N*, *P*, and *K* in the growing substrate is essential for proper seed/seedling/plant growth and development. For lettuce grown in hydroponics, the *N* supply should be 100–150 mg/L [62]. In our study, the mean value of *N* for undiluted CT (treatment T3) was 0.041% \approx 410 mg/L (Table 8) and those for diluted CT were about 102.5 and 205 mg/L in the treatments T1 and T2. Accordingly, the level of *N* for the beneficial treatment T1 is within the recommended value range. The mean values of *P* and *K* were about 600 and 6280 mg/L in treatment T3, 150 and 1570 mg/L in treatment T1, and 300 and 3140 mg/L in treatment T2. Xu et al. [61] studied the effect of five levels of *K* (0, 3, 6, 9, and 12 mM, corresponding to 0–468 mg/L) on apple dwarf rootstock seedling (M9T337) growth. Root and shoot dry masses were significantly higher for a *K* supply of 6 mM (234 mg/L) than for the other treatments. Moreover, this optimal level of *K* led to an increase in *N* use efficiency (NUE). Karimjojeni et al. [56] evaluated the effect of five levels of KNO_3 concentration (0, 0.2, 2, 20, and 200 mM, corresponding to *K* = 0–7800 mg/L) on *GP* of perennial pepperweed (*Lepidium latifolium*) seeds and found an optimal level of 20 mM (*K* = 780 mg/L). Niu et al. [63] hydroponically grew two eucalyptus species (*Eucalyptus dunnii* and *Corymbia citriodora*) at six levels of *P* (0, 0.01, 0.1, 0.5, 1, and 2 mM, corresponding to 0–62 mg/L). Eucalyptus seedlings had optimal growth for *P* levels of 0.1–1 mM (3.1–31 mg/L).

Yap et al. [64] studied the influence of *Ca* in nutrient solution (150, 250, and 350 mg/L) on hydroponic lettuce (*Lactuca sativa* L.) growth. They reported that a *Ca* level of 150 mg/L improved lettuce growth and reduced tip burn compared to the other treatments. This optimal level is quite close to the mean level of *Ca* corresponding to treatment T1 in this study, i.e., $Ca_m = 222$ mg/kg, for which the highest values of growth characteristics were obtained. Higher levels of *Ca* result in reduced *Mg* uptake, which is one of the causes of slower plant growth.

Some metals like *Zn* and *Fe* are essential for plants, but they can become toxic at high concentrations [57]. Their toxic effect is more significant in the growth stage than in the germination stage, because the absorption of minerals is much more accentuated with the appearance of the radicle [60]. Levels of *Zn* in the nutrient solution of 0.05–0.50 mg/L usually meet the needs of most crops [65,66]. Higher levels of *Zn* can produce physiological and biochemical dysfunctions that can lead to a slow plant growth by hindering uptake of water and essential nutrients (*N*, *P*, *K*, *Ca*, *Mg*, *Fe*), affecting carbohydrate metabolism, lowering the rate of photosynthesis, and causing oxidative damage to cell membranes [66,67]. In this study, treatment T1 with 25% CT ($Zn_m = 0.629$ mg/kg) led to significantly higher levels of seedling growth characteristics than treatments with 50% CT ($Zn_m = 1.258$ mg/kg) and undiluted CT ($Zn_m = 2.516$ mg/kg).

The mean values of macronutrient and micronutrient concentrations in diluted and undiluted CT used in treatments T1, T2, and T3 are summarized in Table 10. The results represented in Figure 8, i.e., characteristic variables of lettuce seed germination and seedling growth vs. *N*, indicate the following:

- the value of *GP* for $N = 410.0$ mg/kg (treatment T3 with undiluted CT), i.e., 73%, is significantly lower than the values obtained by applying the other treatments ($N = 0$ –205.0 mg/kg), which are almost equal ($GP = 97$ –98%);
- *MGT* increases linearly with *N* ($R^2 = 0.9223$) and *GS* decreases linearly with *N* ($R^2 = 0.9994$) for values of *N* ranging from 0 (treatment T0) to 410.0 mg/kg (treatment T3);
- *SL*, *SVI*, *SM*, *RL*, and *LA* decrease linearly with *N* ($R^2 = 0.9542$ – 0.9997) for values of *N* ranging from 102.5 (treatment T1) to 410.0 mg/kg (treatment T3).

Table 10. Mean values of nutrient concentrations in diluted and undiluted CT.

No.	Variable		Treatment		
	Symbol	Units	T1	T2	T3
1	<i>N</i>	mg/kg	102.5	205.0	410.0
2	<i>P</i>	mg/kg	150.0	300.0	600.0
3	<i>K</i>	mg/kg	1570	3140	6280
4	<i>Ca</i>	mg/kg	222.0	443.9	887.8
5	<i>Mg</i>	mg/kg	62.25	124.5	249.0
6	<i>Cu</i>	mg/kg	0.058	0.116	0.232
7	<i>Fe</i>	mg/kg	7.335	14.67	29.34
8	<i>Mn</i>	mg/kg	0.180	0.360	0.720
9	<i>Mo</i>	mg/kg	0.015	0.029	0.058
10	<i>Zn</i>	mg/kg	0.629	1.258	2.516

N: nitrogen concentration; *P*: phosphorus concentration; *K*: potassium concentration; *Ca*: calcium concentration; *Mg*: magnesium concentration; *Cu*: copper concentration; *Fe*: iron concentration; *Mn*: manganese concentration; *Mo*: molybdenum concentration; *Zn*: zinc concentration. (T1) 25% CT + 75% ultrapure water; (T2) 50% CT + 50% ultrapure water; (T3) 100% CT.

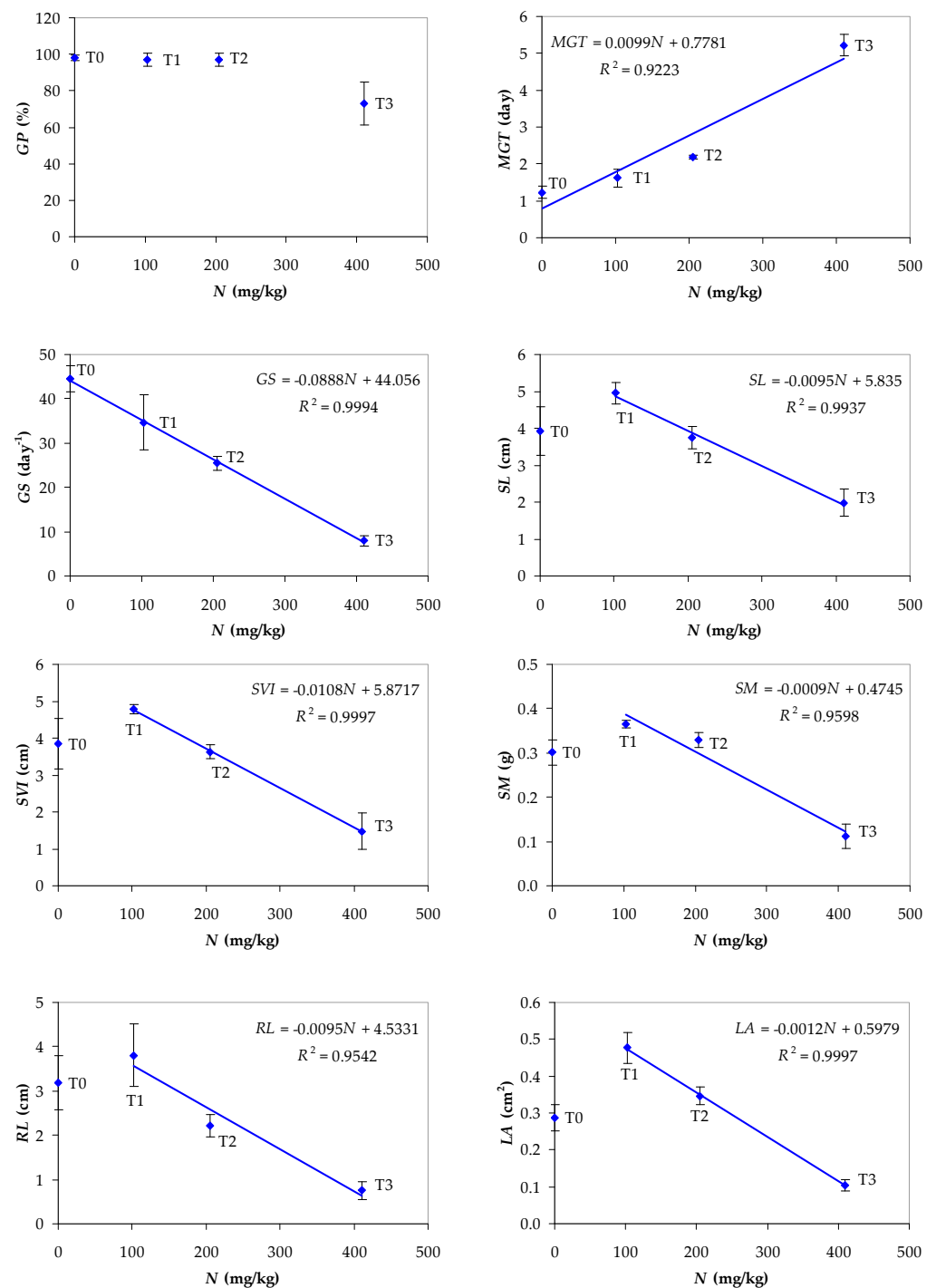


Figure 8. Characteristic variables (mean values \pm SD) of lettuce seed germination and seedling growth vs. nitrogen concentration (N) in different CT treatments: (T0) 100% ultrapure water (control); (T1) 25% CT + 75% ultrapure water; (T2) 50% CT + 50% ultrapure water; (T3) 100% CT. GP: germination percentage; MGT: mean germination time; GS: germination speed; SL: seedling length; SVI: seedling vigor index; SM: seedling mass; RL: root length; LA: total leaf surface area.

The data shown in Figure 8 suggest that a dilution higher than 75% water could have beneficial effects on the characteristics of lettuce seed germination and seedling growth. The effects of the concentrations of the other nutrients as well as of *EC*, *DM*, and *Ash* on germination and seedling growth processes are similar to those of *N*.

4. Conclusions

Preparation of non-aerated compost tea (CT) from compost derived from rockweed and fish residues, its characterization, and testing for lettuce germination and seedling growth were presented in this paper.

CT was prepared by fermenting compost derived from marine residues under different working conditions. Effects of fermentation process factors, i.e., water/compost mass ratio ($R_{LS} = 4.2\text{--}9.8$ g/g) and fermentation time ($t = 4.2\text{--}9.8$ days), on the physicochemical properties of CT were quantified using quadratic polynomial models. There was a good agreement between the experimental and predicted values of electrical conductivity, dry matter concentration, ash concentration, *C*, *N*, *P*, *K*, *Ca*, *Mg*, *Fe*, *Mo*, and *Zn* concentrations. Optimization of fermentation process factors, aiming at maximizing relevant process responses, was based on the desirability function approach.

CT obtained at optimal levels of process factors ($R_{LS,opt} = 4.2$ g/g and $t_{opt} = 5.6$ days = 134 h) was tested for lettuce seed germination and seedling growth. The results of tests performed for 10 days indicated that diluted CT (25% CT + 75% ultrapure water) can be added to lettuce growth medium to improve seedling growth while achieving a high germination percentage.

To avoid too high salinity, CT should be diluted with 75% water. This gives a solution with an acceptable concentration of N (102.5 mg/L) for growing lettuce and high concentrations of P (150 mg/L) and K (1570 mg/L). The pH was high (> 8), which would hamper application of CT as the only fertilizer applied over time, because the high pH would limit the uptake of micronutrients by plants.

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