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The Evaluation of Compost Maturity and Ammonium Toxicity Using Different Plant Species in a Germination Test

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Abstract: The determination of the maturity of compost and other organic fertilizers is very important because of possible phytotoxic or phytostimulating effects. The maturity of compost can be assessed on the basis of chemical analyses, and a germination test with different test plants is most often used to determine phytotoxicity. In this research, the maturity of compost produced from the plant residues subsequent to the maintenance of green public areas was assessed using the results of chemical analyses. Simultaneously, a germination test was carried out with the four test plant species (cucumber, garden cress, triticale, and barley) to determine the phytotoxicity of compost extract in a ratio of 1:2.5 v/v (1:3.3 w/v) and 1:10 v/v (1:13.3 w/v) and the three ammonium N solutions (in the concentrations of 200, 400, and 600 mg/L NH_4-N). According to the chemical properties of compost (primarily the C/N, NH_4-N/NO_3-N ratios, and the NH_4-N concentration) and the germination test with cucumber and garden cress, we may conclude that the tested compost was mature and that we did not expect a phytotoxic effect. The choice of a plant is very significant because the germination test with a compost extract demonstrated an undoubted phytostimulating effect on the garden cress and cucumber, with a more pronounced phytostimulating effect of the 1:10 than that of the 1:2.5 v/v compost extract. No such effect was detected on the monocotyledonous test plants triticale and barley since the 1:10 v/v extract had no significant effect, and the 1:2.5 v/v extract had a phytotoxic effect, moderate on the triticale and high on barley. The conclusion is that garden cress and cucumber are suitable test plants for the determination of compost's phytostimulative effect, but they are not suitable for the determination of phytotoxicity for monocotyledonous plants, especially if the cause of phytotoxicity is a non-ammonium component. Barley is the most suitable species for the determination of compost's non-ammonium phytotoxicity and nitrogen's ammonium-form phytostimulative or phytotoxic effect. It would be very useful to conduct a comparative germination test with the compost extracts in the ratios 1:2.5 and 1:10, whereby the 1:2.5 extract would be used as a test of phytotoxicity, and the 1:10 extract for the test of a phytostimulating effect.

Keywords: germination index; compost; ammonium nitrogen; garden cress; cucumber; barley; triticale

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1. Introduction

Various anthropogenic activities, escalating urbanization, industrialization, and economic growth lead to the production of huge quantities of solid waste around the globe. The management of this solid waste has now become an environmental and technical problem for all [1]. Compost is an organic fertilizer that can be safely applied in agriculture subsequent to the assessment of its stability and maturity [2]. Stability is usually defined in terms of the bioavailability of organic matter and exclusively refers to the resistance of compost organic matter to further degradation [3]. Thus, stability refers to a particular phase or

decomposition or the state of organic composting substances, which is related to the type of organic compounds present and the resulting biological activity in the materials [4,5]. Maturity is a term used to determine the level of phytotoxic substances in compost samples and the suitability of compost for plant growth. Also, maturity can be easily defined as a measure of composting completion [1,6]. Likewise, maturity is defined as the suitability of the material for plant growth and is often associated with a degree of compost humification [7]. Maturity is not described by a single method that is universally applied to all types of compost due to a variation in feedstock composition and composting procedures, so maturity is best assessed by measuring two or more tests, such as the physical, chemical, plant, and microbiological ones [8,9].

The total amount of soluble nitrogen in the organic mass decreases during composting and represents mineralization [10]. During maturation, the ammonium nitrogen levels decrease while the nitrate levels rise. The increased $N\text{-}NO_3^-/N\text{-}NH_4^+$ ratio is an indicator of compost maturity [11]. When an immature compost is incorporated into the soil, rapid decomposition of immature compost can cause a decrease in oxygen concentration in the root system and inhibit plant growth by producing phytotoxic substances based on organic acids, ethylene oxide, and ammonia [12,13].

Several plants were usually used in experiments to compare phytotoxicity. Garglio et al. (2002) used garden cress as an indicator, while Fauci et al. (2002) used pinto beans and tomatoes in a biological study of plant growth [14,15]. Smith and Hughes (2001) compared the germination of garden cress and cellulolytic activity [16]. Degradation of cellulose using filter paper as a substrate had a negative correlation with the fresh mass of cress roots. Although garden cress is very often used as an indicator plant worldwide, there are no universal plant species or a universal germination test procedure. Also, there are few data on which plant species are more sensitive to the toxic substances in compost than the garden cress. Warman (1999) compared the germination of garden cress, radish, and cabbage in the soil, compost–soil mixtures, and compost extracts and concluded that such tests were not sensitive enough to determine the differences between mature and immature composts [17].

The germination index (GI) is the best way to test the phytotoxicity of compost for plant growth because the results are quite simple and reliable [18]. A biological germination test is widely used to examine salinity, soil pathogens, toxic substances, and some other physical and chemical compost properties [19,20], which could be the main potential causes of phytotoxicity. Several researchers state that phytotoxic compounds are gradually removed during the composting process, which could explain the increase in GI with the composting time. The GI, which combines the measures of relative seed germination (G%) and relative root elongation (L%), was used to assess the compost toxicity [21–23]. It has been observed that a GI value of 80% indicated a disappearance of phytotoxins in composts [24]. Tiquia et al. (1996) used that value not only as an indicator of phytotoxicity disappearance but also as an indicator of compost maturity [22]. The GI is a maturity test based on the seed germination and initial plant growth using a liquid compost extract [24]. Compost is considered mature when the GI is higher than 60% compared to the control procedure with distilled water [25]. The GI is the most sensitive parameter used to assess the toxicity of compost to seedlings and to test whether the compost is mature [23,26,27]. Tang et al. (2006) state that the extract ratio is a very important factor influencing GI [28]. They demonstrated that an extract ratio of 10:1 was suitable for the estimation of GI changes during compost maturation. They also stated that different extraction ratios have produced different forms of GI change during the maturation process. The most popular germination test administered by researchers is the garden cress experiment [26,29]. According to them, compost is nontoxic when the germination is higher than 85% or when the weight of the plant seedlings is higher than 90%. In addition, the authors found that the GI at each time of composting did not manifest significant changes either when the extract was slightly diluted or when it was diluted by the distilled water to 75%. An increased GI indicates reduced phytotoxicity and, thus, a more mature product [22,23,30,31].

Phytotoxicity is one of the most important criteria for the assessment of maturity and suitability of compost for use in agriculture to avoid technological and environmental risks while incorporating the compost into the soil [3,7,22]. An immature compost also contains phytotoxic compounds such as heavy metals [21], phenolic compounds [32], ethylene and ammonia [21], increased salt accumulation [31], and organic acids [33] that could slow down the seed germination and plant growth. Phytotoxicity is best assessed by germination or growth testing [3,14], but the plant indicators must be carefully selected [34].

This study aimed to compare the maturity estimates of compost based on the results of the germination test conducted with the seeds of different species, with an assessment of maturity based on the chemical methods of substrate analysis. Also, this study aimed to compare the suitability of monocotyledonous and dicotyledonous plant species for the conduct of a germination test.

2. Materials and Methods

2.1. Compost Sampling

An initial raw material for the production of compost was a waste of green and woody plant parts collected during the maintenance of public green areas. The compost was prepared on a concrete dry surface, and then the plant waste was placed in a windrow with a width of 3 m, a height of 2 m, and a length of 20 m. The compost was sampled [35] after three months of windrow composting, with an occasional turning of the compost mass.

2.2. An Analysis of Compost's Physical Properties

As a part of research on the physical compost properties, compact density, water percentage, dry-matter percentage, ash content, and organic matter content were analyzed.

The compost's compact density was determined by laboratory measurements following EN 13040 and using cylinders of a known volume and mass. The compact density values are expressed in g/L or g/dm^3 [36].

The proportion of water and dry matter in the compost samples was determined by drying 100 g of fresh compost matter at 103 ± 2 °C to a constant weight according to EN 13040 [36]. The proportion of water and dry matter in compost is expressed as a percentage.

A laboratory procedure for the determination of the content of organic matter and ash in the compost samples is prescribed by the standard EN 13039 and is carried out by drying the compost samples for at least four hours at 103 ± 2 °C by successive annealing for six hours at 450 ± 10 °C in the annealing furnace and by weighing each additional annealing hour to a constant mass [37]. The organic matter and ash content in composts is expressed as a percentage (i.e., as a percentage of compost dry matter).

2.3. An Analysis of Chemical Compost Properties

The conducted research on the compost's chemical properties involved the following properties: pH value; electrical conductivity (EC); content of total carbon and nitrogen; C/N ratio; ratio of ammonium and nitrate form of nitrogen (NH_4-N/NO_3-N ratio); and total P and K content.

A compost reaction—that is, the pH value—was determined in a suspension of fresh compost in the deionized water in a volume ratio of 1:5 (i.e., 60 mL of fresh sample and 300 mL of deionized water) after shaking it for 60 min on a shaker. The compost pH value was measured electrometrically (i.e., by the pH meters that measure a difference in electrical potential) according to the European standard EN 13037: 2011 [38]. Electrical conductivity was also measured (by a conductometer) in a suspension of fresh compost in the deionized water in a volume ratio of 1:5 (i.e., 60 mL of fresh sample and 300 mL of the deionized water) after shaking it for 60 min on a shaker, according to the European standard EN 13038: 2011 [39].

Organic carbon content was determined by wet composting [40]: 50 mg of a dry sample was weighed in the destruction cuvettes, filled with 5 mL of 0.27 M $K_2Cr_2O_7$ and

7.5 mL of concentrated H_2SO_4, destroyed for 30 min on a destruction block at 135 °C, quantitatively transferred to the volumetric flasks, and diluted with deionized water to a total volume of 100 mL. After 10 min of centrifugation at 2000 rpm, the organic carbon concentration was measured indirectly by spectrophotometry (i.e., by a spectrophotometric absorption measurement at 585 nm, involving a calibration with the standard glucose solutions). For the sake of laboratory determination of the total nitrogen concentration, the Kjeldahl digestion method was applied following the heating-provoked destruction of the sample with a mixture of acids. The total nitrogen content in the compost is expressed as a percentage. The C/N ratio was calculated using the data on total organic carbon and total nitrogen content.

The concentrations of two mineral forms of nitrogen (i.e., the NH_4-N and NO_3-N, respectively) were determined in the case of compost according to the standard EN 13652: 2001 [41]. In this analysis, 10 g of a fresh sample was used to determine the mineral forms of nitrogen. The results are expressed as g/kg NH_4-N and g/kg NO_3-N in dry matter or as mg/L NH_4-N and NO_3-N in fresh matter.

A total phosphorus concentration was determined by the phosphorus–molybdenum method in a sample compost solution prepared while destroying a dry sample by digestion with nitric and hydrochloric acid. The total potassium concentrations were measured in a stock solution subsequent to digestion with the nitric and hydrochloric acid using the inductively coupled plasma's optical emission spectrometry (ICP-OES). The measured concentrations of P and K are expressed in g/kg of compost dry matter.

2.4. An Analysis of Compost's Biological Properties

With regard to the biological properties of compost, a laboratory-based measurement of compost respiration intensity was performed, and a germination test was administered.

The respiration intensity of the compost was measured as a rate of CO_2 release from a fresh sample weighing 50 g after two days of incubation at room temperature. The emitted CO_2 was determined based on the neutralization of a part of the template with NaOH according to the TMECC 05.08-B method [42]. The results are presented in mg CO_2/g DM/day.

2.5. Germination Test

A modified germination test method developed by Zucconi and colleagues (1981), which uses compost extracts, was applied. This method combines the measurements of a shoot length and root growth [26]. The testing was performed on the four plant species. The germination test determined the influence of different solutions (i.e., the compost extracts and the ammonium carbonate solution) on the germination, shoot growth, and elongation of roots of the four plant species: garden cress (*Lepidium sativum* L.); cucumber (*Cucumis sativus* L.); barley (*Hordeum vulgare* L.); and triticale (*Triticosecale* Wittmack).

The following solutions were used in the germination test—namely, in the compost extracts: deionized water (control treatment); compost extract 1:2.5 (1:2.5 compost: deionized water *v*/*v*)—mark $CE_{2.5}$; compost extract 1:10 (1:10 compost: deionized water *v*/*v*)—mark CE_{10}; ammonium carbonate solution 200 mg/L (200 mg/L NH_4-N)—mark SOL-1; ammonium carbonate solution 400 mg/L (400 mg/L NH_4-N)—mark SOL-2; and ammonium carbonate solution 600 mg/L (600 mg/L NH_4-N)—mark SOL-3.

The compost extracts were prepared by weighing the required mass of compost to prepare an extract in a ratio of 1:10 *v*/*v* or 1:13.3 *w*/*v* (15.06 g in 200 mL) and an extract in a ratio of 1:2.5 *v*/*v* or 1:3.3 *w*/*v* (60.24 g in 200 mL). The weighed masses depended on a specific density of the compost (0.753 g/cm^3), and the planned ratios of 1:2.5 and 1:10 represented the ratios of compost volume and water. Two hundred mL of deionized water was added to the weighed mass of compost; the resulting suspension was stirred, and the compost extract was separated after 30 min.

The ammonium carbonate solutions were prepared by weighing a certain mass of ammonium carbonate and dissolving it in deionized water to a volume of 1000 mL. The solutions of 200 mg/L NH_4-N, 400 mg/L NH_4-N, and 600 mg/L NH_4-N were prepared.

The pH values of 7.81, 7.90, and 7.91 were measured in the prepared solutions, as were the conductivities amounting to 1.438 µS/cm, 2.83 µS/cm, and 4.15 µS/cm.

For the germination test, the seeds of four plant species were prepared and selected for the test (cucumber, garden cress, barley, and triticale). The seeds were washed according to the prescribed procedure, and 200 seeds of each plant species were prepared.

The Petri dishes were prepared, and filter paper was placed at the bottom of each of them. Five mL of a solution (water, two compost extracts, or three ammonium–carbonate solutions) were pipetted onto the filter paper, and ten seeds of a certain plant species were placed thereupon. A total of twenty-four Petri dishes (i.e., 6 different solutions × 4 replicates) were placed for each species, meaning that each treatment was set in four replicates.

The mass of each Petri dish was weighed and recorded, whereafter, the Petri dishes were transferred to the controlled conditions at 25 °C. After the expiration of the time allotted for germination (3 days, or 72 h), the number of germinated seeds in each Petri dish was recorded, and the root and shoot length of each plant were measured.

Based on the above data, the following parameters were statistically processed: germinated rate (GR) as a percentage of germinated seeds, root length per plant (RLP), root length index (RI), germination index (GI), shoot rate (SR) as a percentage of visible shoots, shoot length per plant (SLP), shoot length index (SI), and a vitality shoot index (MLSV).

The germinated rate (GR) was determined by counting all seeds on which the germination —that is, the initiation of a radicle development—was visible (signifying a percentage of germinated seeds out of a total of 10).

The root length per plant (RLP) involves measuring the length of each root in a single treatment and replication—that is, a Petri dish. It is expressed as a Root Length per Plant (RLP) = ∑RL/NGS (NGS = number of germinated seeds) in cm.

The root length index (RI) is a percentage difference between the root length of germinated seeds on the material under investigation and the root length of the control. The RI is expressed as a percentage: RI (%) = [(RLs1/RLc + RLs2/RLc + RLs3/RLc + RLs4/RLc)/4] × 100.

The GI was calculated as a product of two ratios: the ratio of the number of germinated seeds of each treatment and the control and the ratio of root length per plant of each treatment and the control: GI = (Germinated seeds/Germinated seeds in control) × (Root Length per Plant/Root Length per Plant in control) [43].

The shoot rate (SR) was determined by counting the seeds on which the initial development of coleoptile —that is, the shoot (expressed as a percentage of visible shoots out of a total of 10)—was visible.

The shoot length per plant (SLP) includes measuring the length of each shoot in a particular treatment and replication—namely, the Petri dish. It is expressed as a Shoot Length per Plant (SLP) = ∑SL/NS (NS = number of shoots visible) in cm.

The shoot length index (SI) is a percentual difference between the shoot length of visible shoots on a material under investigation and the shoot length of the control. The SI is expressed as a percentage: SI (%) = [SLs1/SLc + SLs2/SLc + SLs3/SLc + SLs4/SLc)/4] × 100.

The vitality shoot index, or Munoo–Liisa vitality index (MLSV), is an index calculated from the shoot rate and the shoot length (a modified formula according to EN 16086-2 [44]. The MLSV is expressed as a percentage: MLSV (%) = [[(SRs1 × SLs1) + (SRs2 × SLs2) + (SRs3 × SLs3) + (SRs4 × SLs4)]/[4 × (SRc × SLc)]] × 100.

2.6. Statistical Data Analysis

Statistical analysis was conducted using Microsoft Excel and Python scikit-learn library (version 3.11.9). Initially, each plant species was tested for data normality across the following variables: a germination rate (GR); root length per plant (RLP); root length index (RI); germination index (GI); shoot rate (SR); shoot length per plant (SLP); shoot length index (SI); and vitality shoot index (MLSV). Normality was tested within each species by administering the Shapiro–Wilk test, with a significance threshold of 0.05. If normality was confirmed, the ANOVA, followed by Tukey's HSD post hoc analysis, was performed to

assess the group differences across treatments. For the nonnormal data distributions, we have applied the nonparametric Kruskal–Wallis's test, supplemented by Dunn's post hoc test with Holm's adjustment for multiple comparisons. The average indicator values for each variable and treatment were calculated and stored, with the significant differences marked by the group letters.

The assumptions of data normality were tested by means of the Shapiro–Wilk test of normality, and most of the samples manifested a lack of normality (Table 1) and were, thus, analyzed by means of the nonparametric tests.

Table 1. The results of normality. "False" represents rejection of null hypothesis, that is, a violation of normality assumption.

	Garden Cress	Cucumber	Barley	Triticale
Germination rate (GR)	False	False	True	False
Root length per plant (RLP)	False	True	True	False
Root length index (RI)	False	True	True	False
Germination index (GI)	False	True	False	False
Shoot rate (SR)	False	False	False	False
Shoot length per plant (SLP)	False	False	False	True
Shoot length index (SI)	False	False	False	True
Vitality shoot index (MLSV)	True	False	False	False

For each species, the PCA was conducted to visualize the treatment differences across the plant-response indicators, using colors and shapes to distinguish the plant and treatment combinations. The eigenvectors were added to the PCA plot to depict each variable's contribution. A visualization of results was provided in a PCA biplot to highlight the treatment combinations and their effects on the variables, facilitating the interpretation of plant and treatment-specific effects across the compost and ammonium treatments.

3. Results

3.1. The Compost's Physical, Chemical, and Biological Properties

The physical, chemical, and biological properties of compost are listed and described by the average analytical values (Table 2). The average compost density was 0.753 g/cm^3; the average pH was 8.66, and the EC (electrical conductivity) was 2.37 mS/cm. The average moisture content in compost was 25.55%, signifying that the average dry matter was 72.45%. The average amount of ash was 74.03%, with an organic matter content of 25.97% and a C/N ratio of 9.93. The intensity of respiration as a biological property was 0.267 mg CO_2/g DM/day.

Table 2. The results of the analyses of physical, chemical, and biological compost properties.

Compost Properties	Value
Compact density (g/cm^3)	0.753
pH (1:5 *v*/*v*)	8.66
EC mS/cm	2.37
Moisture (%)	25.55
Dry matter (%)	72.45
Ashes (%)	74.03
Organic matter (%)	25.97
C/N ratio	9.93
Respiration intensity (mg CO_2/g DM/day)	0.267

The nitrogen content in the fresh compost was 97.94 mg/L (Table 3). The average concentration of the ammonium form of nitrogen was 4.04 mg/L NH_4^+-N, and the nitrate form was 93.90 mg/L NO_3-N within the average NH_4-N/NO_3-N ratio of 0.044.

Table 3. The content of total N, nitrate, and ammonium N (in mg/L and g/kg DM), and the total C, N, K, and P in compost (in g/kg DM).

NH_4^+ and NO_3-N Content	Value		Nutrient Content (g/kg DM)	Value
	mg/L FM	mg/kg DM		
NH_4^+-N + NO_3-N	97.94	179.5	C	117.00
NH_4^+-N	4.04	7.4	N	11.77
NO_3-N	93.90	172.1	K	18.51
NH_4-N/NO_3-N	0.044		P	4.42

The organic matter can also be represented as a proportion of organic carbon in the compost dry matter (Table 3), and the average carbon content in compost was 117.00 g/kg DM; nitrogen content amounted to 11.77 g/kg DM, potassium content to 18.51 g/kg DM, and the phosphorus content to 4.42 g/kg DM.

3.2. The Results of the Germination Tests

3.2.1. The Results of the Germination Test Using Cucumber

The compost extracts 1:2.5 ($CE_{2.5}$) and 1:10 (CE_{10}) significantly increased the cucumber GI (Table 4, Figure 1). At the same time, all ammonium–carbonate solutions significantly reduced the cucumber GI (Table 4) compared with compost extracts, but only the solution SOL-3 was significantly reduced compared with the control treatment. The highest cucumber GI was detected in the 1:10 (CE_{10}) compost extract, whereas the significantly lowest one was found in the SOL-3. In the SOL-2 and SOL-1, the GI was between the control and the SOL-3 but significantly higher than in the SOL-3.

Table 4. The results of cucumber germination test.

	Control	$CE_{2.5}$	CE_{10}	SOL-1	SOL-2	SOL-3
Germination rate (GR)	87.5 ^{A}	92.5 ^{A}	95.0 ^{A}	70.0 ^{A}	40.0 ^{A}	32.5 ^{B}
Root length per plant (RLP)	1.69 ^{A}	1.82 ^{A}	2.76 ^{A}	1.65 ^{A}	1.39 ^{A}	0.35 ^{B}
Root length index (RI)	102.25 ^{A}	109.84 ^{A}	167.01 ^{A}	99.96 ^{A}	83.87 ^{A}	21.08 ^{B}
Germination index (GI)	1.00 ^{B}	1.21 ^{A}	1.80 ^{A}	0.80 ^{B}	0.31 ^{B}	0.09 ^{C}
Shoot rate (SR)	60.0 ^{A}	60.0 ^{A}	87.5 ^{A}	47.5 ^{A}	0.0 ^{B}	0.0 ^{B}
Shoot length per plant (SLP)	0.47 ^{A}	1.12 ^{A}	1.29 ^{A}	0.31 ^{A}	0.0 ^{B}	0.0 ^{B}
Shoot length index (SI)	91.80 ^{B}	217.04 ^{A}	250.25 ^{A}	60.60 ^{B}	0.0 ^{B}	0.0 ^{B}
Vitality shoot index (MLSV)	100.00 ^{A}	268.55 ^{A}	364.52 ^{A}	51.61 ^{A}	0.0 ^{B}	0.0 ^{B}

The different letters denote significant differences at $\alpha = 0.05$ according to Dunn's test or Tukey's Honest significant difference test, following the tests of normality in Table 3. $CE_{2.5}$ = compost extract 1:2.5; CE_{10} = compost extract 1:10; SOL-1 = ammonium carbonate solution 200 mg/L; SOL-2 = amm. carbonate solution 400 mg/L; SOL-3 = amm. carbonate solution 600 mg/L.

The influence on root length per plant (RLP), root length index (RI), and germination rate (GR) were similar, with the highest values in the CE_{10} extract and the lowest ones in the SOL-3 solution, but only the SOL-3 solution had a statistically significant effect on the reduction in RI and RLP compared with all other experimental treatments.

A shoot length per plant (SLP), shoot length index (SI), and a vitality shoot index (MLSV), as the shoot indicators, resulted in higher values with regard to both compost extracts than with regard to the control, but only the SI was statistically significantly higher. There were no significant differences between the compost extracts (Table 5), although all values were higher for the 1:10 extract. No differences between the control and compost extracts were detected for shoot rate (SR).

On the contrary, the values of all these indicators (SR, SLP, SI, and MLSV) were lower for the ammonia solutions, but a statistically significant difference was established only concerning the SR for the SOL-2 and SOL-3 solutions, where there was no shoot emergence. At the same time, all shoot values were statistically significantly lower for the ammonia solutions SOL-2 and SOL-3 than the compost extracts, and only the SOL-1 solution resulted in an SI value significantly lower than the compost extracts.

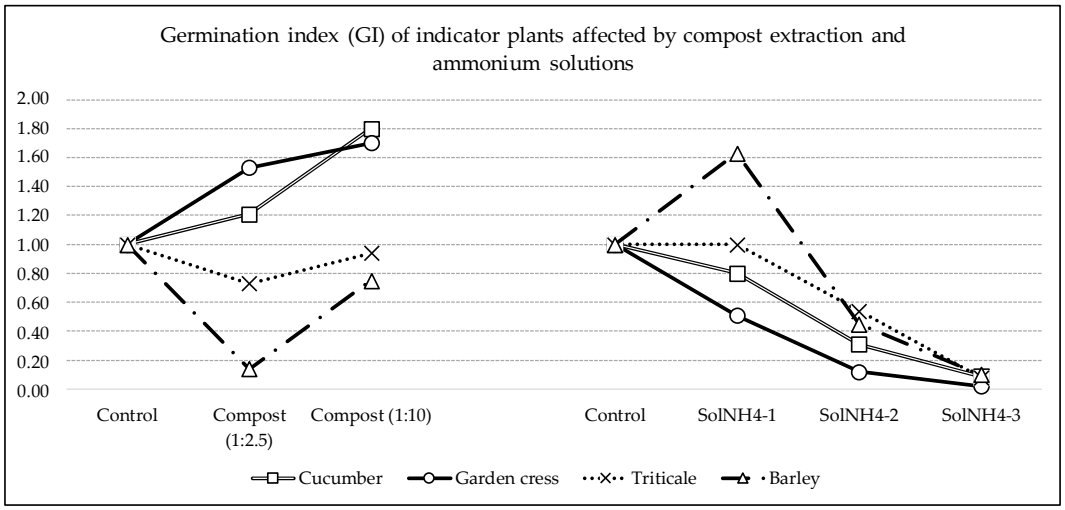

Figure 1. A comparison of the GI using the compost extracts or ammonium solutions.

Table 5. The results of the garden cress germination test.

	Control	$CE_{2.5}$	CE_{10}	SOL-1	SOL-2	SOL-3
Germination rate (GR)	95.0 ^{A}	90.0 ^{A}	92.5 ^{A}	70.0 ^{A}	20.0 ^{A}	2.5 ^{B}
Root length per plant (RLP)	0.66 ^{A}	1.00 ^{A}	1.15 ^{A}	0.46 ^{A}	0.29 ^{A}	0.10 ^{B}
Root length index (RI)	99.98 ^{A}	152.66 ^{A}	174.27 ^{A}	69.90 ^{A}	44.02 ^{A}	15.20 ^{B}
Germination index (GI)	1.00 ^{A}	1.53 ^{A}	1.70 ^{A}	0.51 ^{A}	0.12 ^{B}	0.02 ^{B}
Shoot rate (SR)	85.0 ^{A}	90.0 ^{A}	92.5 ^{A}	70.0 ^{A}	20.0 ^{A}	2.5 ^{B}
Shoot length per plant (SLP)	0.50 ^{A}	0.56 ^{A}	0.61 ^{A}	0.70 ^{A}	0.39 ^{A}	0.08 ^{B}
Shoot length index (SI)	99.49 ^{A}	111.32 ^{A}	120.33 ^{A}	138.74 ^{A}	77.06 ^{A}	14.84 ^{B}
Vitality shoot index (MLSV)	100.0 ^{A}	113.19 ^{A}	124.73 ^{A}	106.0 ^{A}	20.88 ^{B}	1.65 ^{B}

The different letters denote significant differences at $\alpha = 0.05$ according to Dunn's test or Tukey's Honest significant difference test, following the tests of normality in Table 3. $CE_{2.5}$ = compost extract 1:2.5; CE_{10} = compost extract 1:10; SOL-1 = ammonium carbonate solution 200 mg/L; SOL-2 = amm. carbonate solution 400 mg/L; SOL-3 = amm. carbonate solution 600 mg/L.

3.2.2. The Results of the Germination Test Using Garden Cress

The germination tests with the garden cress obtained results similar to the test with cucumber because the indicators were generally higher for the compost extracts and lower for all ammonia solutions, with the exception of the effect of the SOL-1 on the SLP and SI (which was higher than in the control and compost extracts) and the MLSV (slightly higher than the control). However, there was not a single statistically significant difference between the control treatment, compost extract, and the SOL-1 treatment (Table 5), although the GI values were higher for the $CE_{2.5}$ and the highest for the CE_{10}. Also, ammonium carbonate solutions reduced the garden cress GI (Table 5) to 0.51 (SOL-1) and a significantly lower value of 0.12 (SOL-2), the lowest value being that of 0.02 (SOL-3).

The influence on the garden-cress root indicators GR, RLP, and RI was the same as the one with cucumber; all the lowest values were in the SOL-3 solution, and there were no significant differences between the $CE_{2.5}$ and the CE_{10} extracts, SOL-1, and SOL-2, although the compost extracts increased, and the ammonia solutions decreased the root indicator values compared to the control.

The SR, SLP, SI, and MLSV were higher in the 1:10 extract, slightly lower in the 1:2.5 extract, and even lower in the control, without significant differences between them. The indicators of the lowest ammonia solution (SOL-1) concentration were also in the same statistical range, in which the SR was lower, but the SLP and the SI were higher than in the control and compost extracts. However, the lowest and statistically significantly smaller values of the SR, SLP, SI, and MLSV were determined for the ammonia solution with the highest concentration (SOL-3).

3.2.3. The Results of the Germination Test Using Triticale

A pattern of the triticale germination test results was significantly different concerning the compost extracts than concerning cucumber and garden-cress results (Figure 1). The GR, RLP, RI, and GI had the highest values for the control (Table 6), slightly lower for the CE_{10}, and the lowest for the $CE_{2.5}$, but there were no statistically significant differences. All the root indicators and the GI for the ammonia solution of the lowest concentration (SOL-1) were in the same range as the control, lower for the SOL-2 (without significant differences) but the lowest for the SOL-3 ammonia solution.

Table 6. The results of the triticale germination test.

	Control	$CE_{2.5}$	CE_{10}	SOL-1	SOL-2	SOL-3
Germination rate (GR)	90.0 ^{A}	77.5 ^{A}	90.0 ^{A}	87.5 ^{A}	72.5 ^{A}	27.5 ^{B}
Root length per plant (RLP)	3.12 ^{A}	2.65 ^{A}	2.95 ^{A}	3.20 ^{A}	2.14 ^{A}	0.87 ^{B}
Root length index (RI)	100.00 ^{A}	85.00 ^{A}	94.69 ^{A}	102.73 ^{A}	68.58 ^{A}	27.81 ^{B}
Germination index (GI)	1.00 ^{A}	0.73 ^{A}	0.94 ^{A}	1.00 ^{A}	0.54 ^{A}	0.08 ^{B}
Shoot rate (SR)	82.5 ^{A}	75.0 ^{A}	90.0 ^{A}	87.5 ^{A}	70.0 ^{A}	27.5 ^{B}
Shoot length per plant (SLP)	1.82 ^{A}	2.21 ^{A}	1.87 ^{A}	2.12 ^{A}	1.58 ^{B}	0.70 ^{C}
Shoot length index (SI)	99.6 ^{A}	121.0 ^{A}	102.6 ^{A}	116.1 ^{A}	86.20 ^{B}	38.4 ^{C}
Vitality shoot index (MLSV)	100.0 ^{A}	110.8 ^{A}	111.0 ^{A}	123.4 ^{A}	71.6 ^{A}	12.4 ^{B}

The different letters denote significant differences at $\alpha = 0.05$ according to Dunn's test or Tukey's Honest significant difference test, following the tests of normality in Table 3. $CE_{2.5}$ = compost extract 1:2.5; CE_{10} = compost extract 1:10; SOL-1 = ammonium carbonate solution 200 mg/L; SOL-2 = amm. carbonate solution 400 mg/L; SOL-3 = amm. carbonate solution 600 mg/L.

The shoot-growth indicators SLP, SI, and MLSV had lower values for the control than for the compost extracts (except for the SR with regard to the $CE_{2.5}$) and the SOL-1 but without statistically significant differences. All shoot indicator values were lower in the SOL-2 treatment (with significantly lower SLP and SI values) compared to the control, compost extracts, and SOL-1, and all shoot indicators were statistically significantly at their lowest levels for the SOL-3 treatment.

3.2.4. The Results of the Germination Test Using Barley

The results of the barley germination test significantly differ from all other plant species tested (Figure 1). The highest GI was determined for the ammonia solution SOL-1, and all other solutions, the GI was lower than the control (Table 7). The GI for both compost extracts was lower than the control, and the GI was significantly lower than the control for the $EC_{2.5}$, in the range of the ammonia solution SOL-3. The GR was the highest for the control, in the same range as for the CE_{10} and the SOL-1, and significantly lower for the SOL-2, $CE_{2.5,}$ and the SOL-3.

Table 7. The results of the barley germination test.

	Control	$CE_{2.5}$	CE_{10}	SOL-1	SOL-2	SOL-3
Germination rate (GR)	87.5 ^{A}	27.5 ^{B}	67.5 ^{A}	77.5 ^{A}	32.5 ^{B}	10.0 ^{B}
Root length per plant (RLP)	1.40 ^{A}	0.59 ^{B}	1.28 ^{A}	2.53 ^{A}	1.92 ^{A}	0.63 ^{B}
Root length index (RI)	102.44 ^{A}	43.48 ^{B}	94.03 ^{A}	185.25 ^{A}	140.77 ^{A}	45.76 ^{B}
Germination index (GI)	1.00 ^{A}	0.14 ^{B}	0.75 ^{A}	1.63 ^{A}	0.45 ^{A}	0.10 ^{B}
Shoot rate (SR)	10.0 ^{A}	10.0 ^{A}	12.5 ^{A}	65.0 ^{A}	17.5 ^{A}	5.0 ^{B}
Shoot length per plant (SLP)	0.33 ^{A}	0.16 ^{A}	0.53 ^{A}	1.67 ^{A}	1.51 ^{A}	0.34 ^{A}
Shoot length index (SI)	50.0 ^{A}	25.0 ^{A}	80.77 ^{A}	257.5 ^{A}	232.7 ^{A}	51.9 ^{A}
Vitality shoot index (MLSV)	100.0 ^{B}	38.46 ^{B}	146.2 ^{B}	1646.2 ^{A}	384.6 ^{B}	103.9 ^{B}

The different letters denote significant differences at $\alpha = 0.05$ according to Dunn's test or Tukey's Honest significant difference test, following the tests of normality in Table 3. $CE_{2.5}$ = compost extract 1:2.5; CE_{10} = compost extract 1:10; SOL-1 = ammonium carbonate solution 200 mg/L; SOL-2 = amm. carbonate solution 400 mg/L; SOL-3 = amm. carbonate solution 600 mg/L.

The RLP and the RI were the highest for the ammonia solution SOL-1 (Table 7), in the same range as for solution SOL-2, whereas it was significantly lower for the SOL-3 and the lowest for the $CE_{2.5}$.

The highest SR, SLP, SI, and MLSV were determined for the SOL-1 solution, followed by the SOL-2 and CE_{10}, while the significant differences were only established in the SR value, which was significantly lower for SOL-3 treatment, and the MLSV, which, on the other hand, was significantly higher for the SOL-1.

3.3. A Comparison of Indicator Plants

According to the results of the germination test, the compost extract in a ratio of 1:2.5 ($CE_{2.5}$) had a significantly different effect on the four tested species, with the most positive effect on the GI and the RI of the garden cress (Table 5), the SI and MLSV shoot indicators of cucumber (Table 4), and the SLP of triticale (Table 6), but the most inhibitory effect was recorded on the barley root and shoot (Table 7).

The highest sensitivity to the CE2.5 extract was detected for barley, with the lowest values of all indicators, significantly lower than that of all the indicators for triticale (except the SI and the MLSV), but four of the indicator values (the GR, RI, GI, and the SR, respectively) were also significantly lower than those for cucumber and the garden cress. On average, the compost extract CE_{10} resulted in the highest indicator values for cucumber (Table 4) and the lowest indicator values for barley (Table 7), like those of the $CE_{2.5}$.

For the compost extract 1:10, the statistically significantly lowest values of GI, GR, and SR were determined for barley, while there were no differences between the GI, GR, and SR values among the other indicator plants. However, the RLP and the SLP for the garden cress were slightly higher (without significant differences) than for barley but significantly lower than for cucumber and triticale.

The germination test with the SOL-1 resulted in significant differences between the tested plant species (Tables 4–7). The highest GI was determined for barley (Table 7), being significantly lower for triticale (Table 6), cucumber (Table 4), and garden cress (Table 5). The RI was significantly at its lowest value for the garden cress, while there were no significant differences in the GR of the tested plants. However, the RLP values differed significantly, as the highest value was determined for triticale, significantly lower for barley, repeatedly significantly lower for cucumber, and statistically significantly at its lowest level for the garden cress.

All shoot indicators were at their lowest for cucumber and at their highest for triticale (the SR and the SLP) or barley (the SI and the MLSV).

The ammonium solution 2 (SOL-2) also resulted in significant differences between the tested plant species (Tables 4–7). The highest GI was determined for triticale and barley, slightly lower for cucumber, and significantly at its lowest for the garden cress. There was a similar relationship established concerning the RLP values, while the GR was also at its highest value for triticale and at a statistically significantly lower value for barley and garden cress.

The pattern of RI values was significantly different: it was the highest for barley, followed by cucumber and triticale, and significantly lower only for garden cress.

The shoot indicators were at their highest values for barley (the SI and the MLSV) or triticale (the SR and the SLP) and at a lower (but not significantly decreased) value for garden cress. The most pronounced inhibitory effect of the SOL-2 was exerted on the cucumber shoot because there were no shoots, and all indicator values were at their lowest.

There were no significant differences in the values of the GI, RI, and SI indicators in the treatment with the SOL-3 solution (Tables 3–6). The highest GR was determined for cucumber and triticale, whereas the significantly lowest one was recorded for the garden cress.

Also, some differences in shoot indicators were detected because the statistically significantly lowest values of the SR, SLP, and MLSV were determined for cucumber, whereas there were no significant differences between the other plants.

Principal Component Analysis (PCA)

The first two principal components (PCs) explained 78.04% of the total variance (Figure 2). The loadings of the PCs showed the highest influence of GI (0.39) and RI (0.38) on grouping on the PC1 axis and the highest influence of SR (−0.38) and SI (0.36) on grouping along PC2.

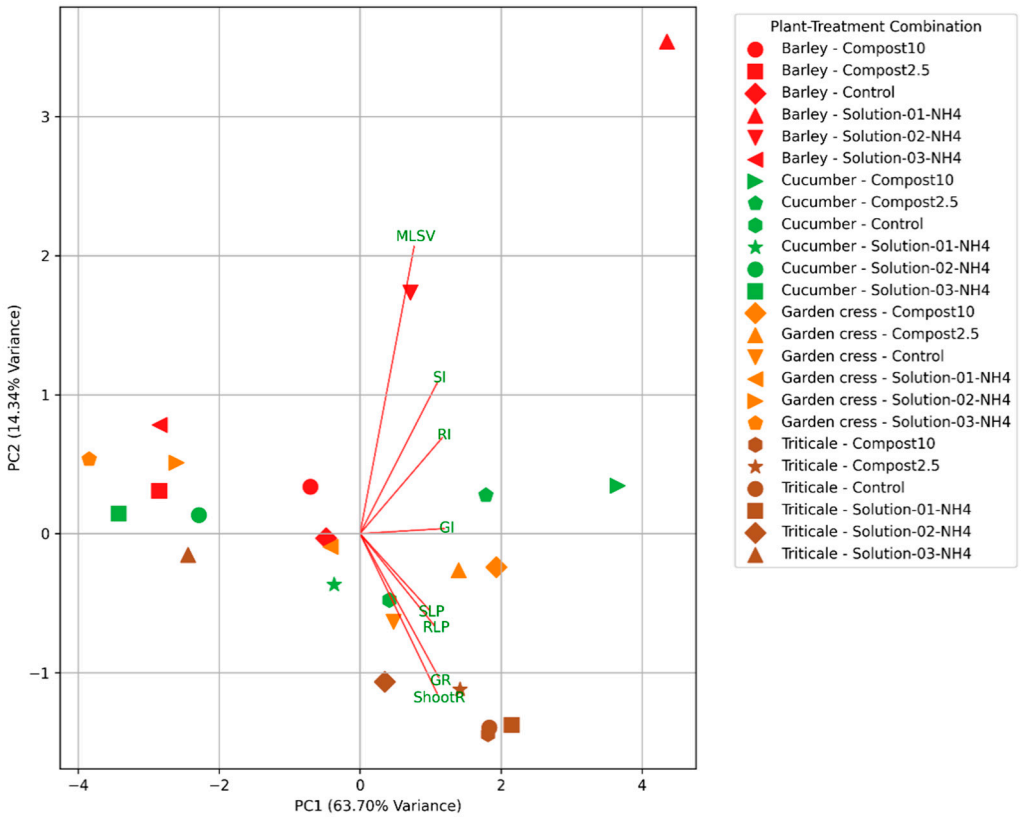

Figure 2. The results of principal component analysis (PCA).

The most distinct grouping was observed in all the triticale samples at the lower side of the PC2, while other plant species were scattered with treatment variations.

4. Discussion

In this research, the results of the laboratory analysis of compost and germination test were combined and compared as the indicators of compost maturity and potential phytotoxicity on the four tested plant species (cucumber, garden cress, triticale, and barley). Also, with the aim of measuring the phytotoxic or phytostimulating effect, the solutions of different concentrations of ammonium nitrogen were used.

As a very important factor [45,46] at the end of composting, the pH value should be in an acceptable range for the plants. In this study, an average pH value of 8.66 was measured, which means that the pH was in an acceptable range, although it was very close to the upper limit values. A stable and mature compost should have a pH value within the acceptable range of 5.5–9.0 [47].

Electrical conductivity (EC) can serve as a measure of soluble nutrients, cations, and anions, and a lower EC can be a result of a lower content of cations in the soil [1]. Nonetheless, when the salinity of the soil (EC) is ≥4 mS/cm, the soil is considered saline, with potential salt stress, especially for the glycophytes [48]. The composts obtained from municipal waste have high salt concentrations, which, in addition to the inhibition of plant growth, negatively affects the soil structure [49], but the EC of 5 mS/cm is the upper limit for a substrate in container production [50]. In this study, a conductivity of 2.37 mS/cm was determined, which, according to the other results [50,51], should not cause phytotoxicity.

As most of the nitrogen is found in organic form, bound in the structure of proteins and simple peptides, an intensive decomposition of organic matter in the first weeks of composting leads to ammonification and later to nitrification, but in the conditions of good aeration and subsequent to a decrease the temperature of the compost pile was lowered below 40 °C [52]. An optimal C/N ratio in the compost is considered to be 25/1 to 35/1 [2,53]. However, there is a possibility of the production of high-quality compost at the lower C/N ratios, and good examples are pig manure with wood sawdust, with the C/N amounting to 15 [54], greenbelt waste and food-industry waste, with the C/N amounting to 19 [55], pig manure with the rice straw [56], and chicken manure with the wood sawdust [57], with a C/N ratio amounting to 20. Since the average C/N ratio for the analyzed compost was 9.93, the compost could be evaluated as a mature organic fertilizer.

Nitrogen transformation is a rather complicated process that simultaneously depends on many aspects, such as the pH, temperature, C/N ratio, and starting materials [2,58]. According to the NH_4-N/NO_3-N ratio, the maturity threshold of an organic fertilizer is <0.16 [59], which means that there should be 6.25 times more nitrate than the ammonium form of nitrogen in a mature organic fertilizer. The average NH_4-N/NO_3-N ratio in the tested compost was 0.044, and twenty-three times more nitrate (93.9 mg/L) than the ammonia (4.04 mg/L) nitrogen was found. Thus, the evaluation of compost maturity according to the NH_4-N/NO_3-N ratio was that the compost was a mature organic fertilizer.

The stability of an organic fertilizer is measured by a respiration rate (i.e., by a CO_2 release in mg/g of fertilizer/day). The intensity of respiration is used to assess the stability of compost [60]. The respiration intensity of compost was determined to average 0.267 mg CO_2/g DM/day. Since the respiration intensity was <1, the assessment was that the compost was very stable—that is, it was a mature, finished compost without continuing decomposition, odor, or potential phytotoxicity.

A germination test and a germination index were used to investigate a possible phytotoxic effect of the compost as substrate [25,60]. An immature compost may contain phytotoxic components (toxic to plants) that inhibit seed germination, especially in highly sensitive seeds. The phytotoxicity of organic fertilizer, substrate, or other medium or solution is interpreted from being highly phytotoxic to producing a phytostimulant activity based on the following GI values [18,25,45,61]: a GI < 0.50 signifies high phytotoxicity; a GI of 0.50–0.80 signifies moderate phytotoxicity; a GI of 0.80–1.00 signifies no phytotoxicity; and a GI > 1.00 signifies a phytostimulative effect. However, the results obtained by the GI index research should be interpreted carefully because they are influenced by a seed type and compost source [20,62]. The application of immature compost causes negative effects on the seed germination, growth, and development of plants since an immature compost, among other effects, could cause a high microbiological activity that reduces the oxygen concentration in the soil and blocks (i.e., microbiologically fixes) the existing available nitrogen [30,34].

Among the four tested plant species as an average for all the tested treatments in the germination test experiment (i.e., the compost extracts and ammonium solutions), barley had the lowest GI of 0.62, which means that a moderate phytotoxicity to barley was present. Also, average moderate phytotoxicity was present in the triticale (GI = 0.72) and garden cress (GI = 0.78). The average GI for cucumber was 0.84, which meant that no phytotoxicity was determined. A final evaluation was that there was an average phytotoxic effect present in all the treated treatments—namely, in three out of the four tested plant species.

However, it is an average of all treatments that hides a significantly different effect of compost extract and ammonia solutions. Thus, the highest average GI for two different compost extracts was determined for the garden cress (1.62) and cucumber (1.51), which detected a phytostimulating effect. A highly phytotoxic effect was determined for barley (with a GI average of 0.45), and no phytotoxic effect was determined for the triticale (with an average GI amounting to 0.84).

On the other hand, the effect of the ammonia solutions was the opposite because, on average, moderate phytotoxicity was detected in all solutions for barley (0.73) and triticale

(0.54), whereas high phytotoxicity was detected for cucumber (0.40) and the garden cress (0.22), which means that we could have expected the differences in the effects of compost extracts and ammonia solutions on the tested plant species, as well as, perhaps, a different reaction of the tested plant species.

In cucumber, the compost extract in the ratio of 1:2.5 v/v (a less diluted compost) had a significant effect on the GI, acting as a phytostimulator (1.21), and the ammonium solution of the lowest concentration (200 mg/L NH_4-N) acted in a phytotoxic (0.80) direction. However, a compost extract in a ratio of 1:10 v/v (a more diluted compost) undoubtedly resulted in a strong phytostimulating effect (GI = 1.80) and a more concentrated ammonium–nitrogen solution with a high phytotoxic (GI = 0.31 and 0.09) effect. Hereby, we may conclude that the tested compost had a pronounced phytostimulative effect on cucumber and that the phytotoxicity of the ammonium solution for cucumber was significantly lower than 400 mg/L NH_4-N, probably much closer to 200 than to 400 mg/L.

We may derive a similar conclusion on the basis of a shoot-length index since a pronounced phytostimulative effect of both compost extracts on the SI was determined, while there were no shoots at all in the treatments with 400 and 600 mg/L NH4-N.

A very similar finding was also detected for the garden cress: both compost extracts had a phytostimulating effect because the GI was 1.53 and 1.70 (although a statistical significance was not proven due to variability), while the GI was already at the limit of high phytotoxicity in the treatment with the lowest concentration of ammonia solution (GI = 0.51).

In general, the stimulatory effect of compost extracts and an inhibitory effect of ammonia solutions on the GI of cucumber and the garden cress are clear, with a more pronounced effect of ammonia solutions on the garden cress.

The difference between the garden cress and cucumber is visible in a comparison of shoot-growth indicators. Namely, the compost extracts did not stimulate the growth of the garden cress as much as they did for cucumber. Although the SOL-2 and SOL-3 (the solutions with higher concentrations of ammonia) had an extremely phytotoxic effect on the garden-cress shoots; there were still the garden-cress shoots different from the cucumber shoots.

The reaction of triticale and barley in the germination test was significantly different from that of the garden cress and cucumber. First, the compost extract at a ratio of 1:2.5 was moderately phytotoxic (0.73) to triticale and highly phytotoxic (0.14) to barley, having reduced the GR, RLP, and RI, especially in barley. By diluting the compost extract (a ratio of 1:10), this phytotoxic effect completely disappeared in the triticale (GI = 0.94) and was significantly mitigated in barley (GI = 0.75), with a neutral to mild stimulatory effect on the triticale and barley shoots.

At the same time, the SOL-1 had no significant effect on the triticale but had a phytostimulating effect on the GI (1.63), length, index, and vitality of barley roots when compared to the compost extract at a ratio of 1:2.5 and an even more pronounced stimulating effect on the barley shoots' vitality. As expected, the SOL-2 and the SOL-3 solutions had an inhibitory effect on the root and shoot of the triticale, but a phytotoxic effect was much lower on barley than on the triticale.

A multiple-regression analysis proved that the NH_4^+-N content was an important factor influencing the seed germination and root growth of the selected plant species [63]. In a study by Cheung et al. (1989), Chinese cabbage was the most sensitive species to metal toxicity and was recommended as a test species to assess the toxicity of heavy metals [64]. We may conclude that the garden cress and cucumber were the most suitable and barley a less suitable species for the determination of phytotoxicity of the ammonium form of nitrogen. However, barley, as a test plant, was very suitable for the determination of some other phytotoxicity because it reacted very sensitively to the compost extract in the ratio of 1:2.5, which was almost absent in the ratio of 1:10. On the other hand, the garden crass and cucumber did not react so sensitively to the compost–extract ratio, but there were other compounds that have contributed to a phytotoxic effect, such as the ammonium form of nitrogen [65].

Regarding the influence of ammonium–carbonate solutions on the GI, we may conclude that we have obtained the expected results. Namely, the maturity threshold of organic fertilizer is considered to be <400 mg/kg NH_4-N [5], which is the concentration of NH_4-N in Solution 2. Consequently, Solution 2 had an averagely high phytotoxic effect (GI = 0.36), as well as Solution 3 (GI = 0.07). Similar to the compost extract, Solution 1 (with 200 mg/kg NH_4-N), on average, had no phytotoxic effect (GI = 0.99) with regard to all four tested species, which can also be explained by the possible different reactions of the analyzed plant species to the ammonium form of nitrogen as a phytotoxic component. Also, it is important to conclude that according to these results, a limit value of 400 mg/kg NH_4-N is not a good indicator of phytotoxicity for all species, as demonstrated by the example of the garden cress. This may be related to the research conducted by Cheung et al. (1989), who reported that the seeds of root crops, cereals, and legumes, which contained large amounts of food supplies, would be less sensitive to toxicity than the seeds of deciduous plants with a lesser food supply [64]. Chinese cabbage and the Chinese spinach seeds are the most sensitive species, probably because their seeds are very small.

5. Conclusions

The interpretation of maturity of the tested compost is in accordance with the results of the germination test with cucumber and the garden cress, but the compost can still be phytotoxic to other plant species, especially barley. By evaluating the chemical properties of the compost, we can conclude that the tested compost was mature, but the pH value above 8.5 and the established conductivity left the space for a possibility of a phytotoxic effect. A very low intensity of microbiological respiration indicated that the tested compost was stable.

A compost extract proved an undoubted phytostimulating effect on the garden cress and cucumber, with a more pronounced phytostimulating effect of the compost extract in the ratio of 1:10 than that of the ratio 1:2.5. However, no such effect was detected on the monocotyledonous test plants triticale and barley, the 1:10 extract had no significant effect, while the 1:2.5 extract had a phytotoxic effect on barley.

The phytotoxicity of ammonium solution for cucumber and the garden cress was already at significantly lower concentrations than 400 mg/L NH_4-N, probably much closer to 200 mg/L, which is not toxic for triticale and is even stimulating for barley. The garden cress and cucumber were the suitable test plants for the determination of the phytostimulative effect of compost with the low concentrations of NH_4-N and NH_4-N/NO_3-N ratios, but they were not suitable for the determination of phytotoxicity for monocotyledonous plants, especially if the cause of phytotoxicity was a non-ammonium component. Barley was the most suitable species for the determination of the non-ammonium phytotoxic components in the compost and the phytostimulative or phytotoxic effect of the ammonium form of nitrogen.

A comparative germination test with the compost extracts in the ratio of 1:2.5 could be used as a test for phytotoxicity and the 1:10 extract for a phytostimulating effect.

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References

1. Galić, L.; Špoljarević, M.; Auriga, A.; Ravnjak, B.; Vinković, T.; Lončarić, Z. Combining Selenium Biofortification with Vermicompost Growing Media in Lamb's Lettuce (*Valerianella locusta* L. Laterr). *Agriculture* **2021**, *11*, 1072. [CrossRef]
2. Nemet, F.; Perić, K.; Lončarić, Z. Microbiological Activities in the Composting Process: A Review. *Columella J. Agric. Environ. Sci.* **2021**, *8*, 41–53. [CrossRef]
3. Brewer, L.J.; Sullivan, D.M. Maturity and Stability Evaluation of Composted Yard Trimmings. *Compos. Sci. Util.* **2003**, *11*, 96–112. [CrossRef]
4. Eiland, F.; Klamer, M.; Lind, A.M.; Leth, M.; Bååth, E. Influence of Initial C/N Ratio on Chemical and Microbial Composition during Long Term Composting of Straw. *Microb. Ecol.* **2001**, *41*, 272–280. [CrossRef] [PubMed]
5. Tiquia, S.M. Microbiological Parameters as Indicators of Compost Maturity. *J. Appl. Microbiol.* **2005**, *99*, 816–828. [CrossRef] [PubMed]
6. Aslam, D.N.; Horwath, W.; VanderGheynst, J.S. Comparison of Several Maturity Indicators for Estimating Phytotoxicity in Compost-Amended Soil. *Waste Manag.* **2008**, *28*, 2070–2076. [CrossRef]
7. Cooperband, L.R.; Stone, A.G.; Fryda, M.R.; Ravet, J.L. Relating Compost Measures of Stability and Maturity to Plant Growth. *Compos. Sci. Util.* **2003**, *11*, 113–124. [CrossRef]
8. He, X.-T.; Logan, T.J.; Traina, S.J. Physical and Chemical Characteristics of Selected U.S. Municipal Solid Waste Composts. *Am. Soc. Agron. Crop Sci. Soc. Am. Soil Sci. Soc. Am.* **1995**, *24*, 543–552. [CrossRef]
9. Itävaara, M.; Vikman, M.; Liisa, M.; Vuorinen, A. Maturity Tests for Composts—Verification of a Test Scheme for Assessing Maturity. *Compos. Sci. Util.* **2010**, *18*, 174–183. [CrossRef]
10. Chefetz, B.; Hadar, Y.; Chen, Y.; Chefetz, B.; Chen, Y.; Hadar, P. Dissolved Organic Carbon Fractions Formed during Composting of Municipal Solid Waste: Properties and Significance Gelöster Organisch Gebundener Kohlenstoff Bei Der Kompostierung von Hausmüll: Eigenschaften Und Anteil Einzelner Fraktionen. *Acta Hydrochim. Hydrobiol.* **1998**, *26*, 172–179. [CrossRef]
11. Riffaldi, R.; Levi-Minzi, R.; Pera, A.; de Bertoldi, M. Evalution of Compost Maturity by Means of Chemical and Microbial Analyses. *Waste Manag. Res.* **1986**, *4*, 387–396. [CrossRef]
12. Chikae, M.; Ikeda, R.; Kerman, K.; Morita, Y.; Tamiya, E. Estimation of Maturity of Compost from Food Wastes and Agro-Residues by Multiple Regression Analysis. *Bioresour. Technol.* **2006**, *97*, 1979–1985. [CrossRef] [PubMed]
13. Gómez-Brandón, M.; Lazcano, C.; Domínguez, J. The Evaluation of Stability and Maturity during the Composting of Cattle Manure. *Chemosphere* **2008**, *70*, 436–444. [CrossRef] [PubMed]
14. Gariglio, N.F.; Buyatti, M.A.; Pilatti, R.A.; Russia, D.E.G.; Acosta, M.R. Use of a Germination Bioassay to Test Compost Maturity of Willow (*Salix* sp.) Sawdust. *N. Z. J. Crop Hortic. Sci.* **2002**, *30*, 135–139. [CrossRef]
15. Fauci, M.; Bezdicek, D.F.; Caldwell, D.; Finch, R. Development of Plant Bioassay to Detect Herbicide Contamination of Compost at or below Practical Analytical Detection Limits. *Bull. Environ. Contam. Toxicol.* **2002**, *68*, 79–85. [CrossRef]
16. Smith, D.C.; Hughes, J.C. A Simple Test to Determine Cellulolytic Activity as Indicator of Compost Maturity. *Commun. Soil Sci. Plant Anal.* **2001**, *32*, 1735–1749. [CrossRef]
17. Warman, P.R. Evaluation of Seed Germination and Growth Tests for Assessing Compost Maturity. *Compos. Sci. Util.* **1999**, *7*, 33–37. [CrossRef]
18. Lončarić, Z.; Parađiković, N.; Popović, B.; Lončarić, R.; Kanisek, J. *Gnojidba Povrća, Organska Gnojiva i Kompostiranje*; Poljoprivredni fakultet Sveučilišta Josipa Jurja Strossmayera u Osijeku: Osijek, Hrvatska, 2015.
19. Zucconi, F.; Monaco, A.; Forte, M.; de Bertoldi, M. Phytotoxins during the Stabilization of Organic Matter. In *Composting of Agricultural and Other Wastes*; Elsevier Applied Science Publishers Ltd.: Barking, UK, 1985; pp. 73–85.
20. Gajdoš, R. Effects of Two Composts and Seven Commercial Cultivation Media on Germination and Yield. *Compos. Sci. Util.* **1997**, *5*, 16–37. [CrossRef]
21. Tam, N.F.Y.; Tiquia, S. Assessing Toxicity of Spent Pig Litter Using a Seed Germination Technique. *Resour. Conserv. Recycl.* **1994**, *11*, 261–274. [CrossRef]
22. Tiquia, S.M.; Tama, N.F.Y.; Hodgkiss, I.J. Effects of Composting on Phytotoxicity of Spent Pig-Manure Sawdust Litter. *Environ. Pollut.* **1996**, *93*, 249–256. [CrossRef]
23. Wong, J.W.C.; Mak, K.F.; Chan, N.W.; Lam, A.; Fang, M.; Zhou, L.X.; Wu, Q.T.; Liao, X.D. Co-Composting of Soybean Residues and Leaves in Hong Kong. *Bioresour. Technol.* **2001**, *76*, 99–106. [CrossRef] [PubMed]
24. Zucconi, F.; Pera, A.; Forte, M.; de Bertoldi, M. Evaluating Toxicity of Immature Compost. *BioCycle* **1981**, *22*, 54–57.
25. Soriano-Disla, J.M.; Gómez, I.; Navarro-Pedreño, J. Assessment of the Evolution and Efficiency of a Full-Scale Sewage Sludge Composting Process. *Compos. Sci. Util.* **2010**, *18*, 81–88. [CrossRef]
26. Zucconi, F.; Forte, M.; Monaco, A.; de Bertoldi, M. Biological Evaluation of Compost Maturity. *BioCycle* **1981**, *22*, 27–29.

27. Meunchang, S.; Panichsakpatana, S.; Weaver, R.W. Co-Composting of Filter Cake and Bagasse; by-Products from a Sugar Mill. *Bioresour. Technol.* **2005**, *96*, 437–442. [CrossRef]
28. Tang, J.C.; Maie, N.; Tada, Y.; Katayama, A. Characterization of the Maturing Process of Cattle Manure Compost. *Process Biochem.* **2006**, *41*, 380–389. [CrossRef]
29. Erhart, E.; Burian, K. Evaluating Quality and Suppressiveness of Austrian Biowaste Composts. *Compos. Sci. Util.* **1997**, *5*, 15–24. [CrossRef]
30. Bernai, M.P.; Paredes, C.; Sánchez-Monedero, M.A.; Cegarra, J. Maturity and Stability Parameters of Composts Prepared with a Wide Range of Organic Wastes. *Bioresour. Technol.* **1998**, *63*, 91–99. [CrossRef]
31. Tiquia, S.M.; Tam, N.F.Y. Elimination of Phytotoxicity during Co-Composting of Spent Pig-Manure Sawdust Litter and Pig Sludge. *Bioresour. Technol.* **1998**, *65*, 43–49. [CrossRef]
32. Wong, M.H. Phytotoxicity of Refuse Compost During the Process of Maturation. *Environ. Pollut. (Ser. A)* **1985**, *37*, 159–174. [CrossRef]
33. Manios, V.I.; Tsikalas, P.E.; Siminis, H.I.; Verdonck, O. Phytotoxicity of Olive Tree Leaf Compost in Relation to the Organic Acid Concentration. *Biol. Wastes* **1989**, *27*, 307–317. [CrossRef]
34. Emino, E.R.; Warman, P.R. Biological Assay for Compost Quality. *Compos. Sci. Util.* **2004**, *12*, 342–348. [CrossRef]
35. *EN 12579:2013*; Soil Improvers and Growing Media—Sampling. European Standard: Pilsen, Czech Republic, 2013.
36. *EN 13040:2007*; Soil Improvers and Growing Media—Sample Preparation for Chemical and Physical Tests, Determination of Dry Matter Content, Moisture Content and Laboratory Compacted Bulk Density. European Standard: Pilsen, Czech Republic, 2007. Available online: https://standards.iteh.ai/catalog/standards/cen/f65ff417-8656-4f00-a840-a6ca1846fb6f/en-13040-2007 (accessed on 15 November 2021).
37. *EN 13039:2011*; Soil Improvers and Growing Media—Determination of Organic Matter Content and Ash. European Standard: Pilsen, Czech Republic, 2011. Available online: https://standards.iteh.ai/catalog/standards/cen/825ecadd-2c72-4dc9-a38c-1e4bdeac857f/en-13039-2011 (accessed on 15 November 2021).
38. *EN 13037:2011*; Soil Improvers and Growing Media—Determination of PH. European Standard: Pilsen, Czech Republic, 2011. Available online: https://standards.iteh.ai/catalog/standards/cen/a127e814-60b7-42b0-80c7-453c28968d64/en-13037-2011 (accessed on 15 November 2021).
39. *EN 13038:2011*; Soil Improvers and Growing Media—Determination of Electrical Conductivity. European Standard: Pilsen, Czech Republic, 2011. Available online: https://standards.iteh.ai/catalog/standards/cen/121ac421-7003-471a-b75d-73eb4b8d784d/en-13038-2011 (accessed on 15 November 2021).
40. *ISO 14235:1998*; Soil Quality—Determination of Organic Carbon by Sulfochromic Oxidation. International Organization for Standardization: Geneva, Switzerland, 1998. Available online: https://standards.iteh.ai/catalog/standards/iso/3c00de65-23aa-4314-bd1f-c2a24c070565/iso-14235-1998 (accessed on 15 November 2021).
41. *EN 13652:2001*; Soil Improvers and Growing Media—Extraction of Water Soluble Nutrients and Elements. European Standard: Pilsen, Czech Republic, 2001. Available online: https://standards.iteh.ai/catalog/standards/cen/a27867c1-a6a0-426e-b4cf-e6eaea48975d/en-13652-2001 (accessed on 15 November 2021).
42. Thompson, W.H.; Leege, P.; Millner, P.; Watson, M. *Test Methods for the Examination of Composting and Compost*; The United States Composting Council Research and Education Foundation: New York, NY, USA; The United States Department of Agriculture: New York, NY, USA, 2001.
43. Malakahmad, A.; Idrus, N.B.; Abualqumboz, M.S.; Yavari, S.; Kutty, S.R.M. In-Vessel Co-Composting of Yard Waste and Food Waste: An Approach for Sustainable Waste Management in Cameron Highlands, Malaysia. *Int. J. Recycl. Org. Waste Agric.* **2017**, *6*, 149–157. [CrossRef]
44. *EN 16086-2:2012*; Soil Improvers and Growing Media—Determination of Plant Response—Part 2: Petri Dish Test Using Cress. European Standard: Pilsen, Czech Republic, 2012.
45. Selim, S.M.; Zayed, M.S.; Atta, H.M. Evaluation of Phytotoxicity of Compost during Composting Process. *Nat. Sci.* **2011**, *10*, 69–77.
46. Buck, R.P.; Rondinini, S.; Covington, A.K.; Baucke, F.G.K.; Brett, C.M.; Camoes, M.F.; Wilson, G.S. Measurement of PH. Definition, Standards, and Procedures (IUPAC Recommendations 2002). *Pure Appl. Chem.* **2002**, *74*, 2169–2200. [CrossRef]
47. Monaghan, J.M.; Beacham, A.M. Salad Vegetable Crop. In *Encyclopedia of Applied Plant Sciences*, 2nd ed.; Academic Press: Cambridge, MA, USA, 2017; Volume 3.
48. Smith, J.L.; Doran, J.W. Measurement and Use of PH and Electrical Conductivity for Soil Quality Analysis. *Methods Assess. Soil Qual.* **1996**, *49*, 169–185. [CrossRef]
49. Miller, M. Fulfilling special needs of nurseries. *BioCycle* **2001**, *4*, 55–59.
50. Vukobratović, M.; Lončarić, Z.; Vukobratović, Ž.; Mužić, M. Use of Composted Manure as Substrate for Lettuce and Cucumber Seedlings. *Waste Biomass Valoriz.* **2018**, *9*, 25–31. [CrossRef]
51. Hargreaves, J.C.; Adl, M.S.; Warman, P.R. A Review of the Use of Composted Municipal Solid Waste in Agriculture. *Agric. Ecosyst. Environ.* **2008**, *123*, 1–14. [CrossRef]
52. Sánchez-Monedero, M.A.; Roig, A.; Cegarra, J.; Bernal, M.P.; Noguera, P.; Abad, M.; Anton, A. Compost as Media Constituents for Vegetable Transplant Production. *Compos. Sci. Util.* **2004**, *12*, 161–168. [CrossRef]

53. Guo, R.; Li, G.; Jiang, T.; Schuchardt, F.; Chen, T.; Zhao, Y.; Shen, Y. Effect of Aeration Rate, C/N Ratio and Moisture Content on the Stability and Maturity of Compost. *Bioresour. Technol.* **2012**, *112*, 171–178. [CrossRef] [PubMed]
54. Huang, G.F.; Wong, J.W.C.; Wu, Q.T.; Nagar, B.B. Effect of C/N on Composting of Pig Manure with Sawdust. *Waste Manag.* **2004**, *24*, 805–813. [CrossRef] [PubMed]
55. Kumar, M.; Ou, Y.L.; Lin, J.G. Co-Composting of Green Waste and Food Waste at Low C/N Ratio. *Waste Manag.* **2010**, *30*, 602–609. [CrossRef] [PubMed]
56. Zhu, N. Effect of Low Initial C/N Ratio on Aerobic Composting of Swine Manure with Rice Straw. *Bioresour. Technol.* **2007**, *98*, 9–13. [CrossRef] [PubMed]
57. Ogunwande, G.A.; Osunade, J.A.; Adekalu, K.O.; Ogunjimi, L.A.O. Nitrogen Loss in Chicken Litter Compost as Affected by Carbon to Nitrogen Ratio and Turning Frequency. *Bioresour. Technol.* **2008**, *99*, 7495–7503. [CrossRef]
58. Bernal, M.P.; Alburquerque, J.A.; Moral, R. Composting of Animal Manures and Chemical Criteria for Compost Maturity Assessment. *A Review. Bioresour. Technol.* **2009**, *100*, 5444–5453. [CrossRef]
59. William, F.B.; Manager, P. *Compost Quality Standards and Guidelines Final Report*; New York State Association of Recyclers: New York, NY, USA, 2000.
60. Chodak, M.; Borken, W.; Ludwig, B.; Beese, F. Effect of Temperature on the Mineralization of C and N of Fresh and Mature Compost in Sandy Material. *J. Plant Nutr. Soil Sci.* **2001**, *164*, 289–294. [CrossRef]
61. Alburquerque, J.A.; Gonzálvez, J.; García, D.; Cegarra, J. Measuring Detoxification and Maturity in Compost Made from "Alperujo", the Solid by-Product of Extracting Olive Oil by the Two-Phase Centrifugation System. *Chemosphere* **2006**, *64*, 470–477. [CrossRef]
62. Barral, M.T.; Paradelo, N. A Review on the Use of Phytotoxicity as a Compost Quality Indicator. *Dyn. Soil Dyn. Plant* **2011**, *5*, 36–44.
63. Chanyasak, V.; Hirai, M.; Kubota, H. Changes of Chemical Components and Nitrogen Transformation in Water Extracts during Composting of Garbage. *J. Ferment. Technol.* **1982**, *60*, 439–446.
64. Cheung, Y.H.; Wong, M.H.; Tam, N.F.Y. Root and Shoot Elongation as an Assessment of Heavy Metal Toxicity and 'Zn Equivalent Value' of Edible Crops. *Hydrobiologia* **1989**, *188*, 377–383. [CrossRef]
65. Ozores-Hampton, M. Compost as an Alternative Weed Control Method: Municipal Waste Compost Production and Utilization for Horticultural Crops. *HortScience* **1998**, *33*, 938–940. [CrossRef]