Influence of Microbial Inoculation of Igneous Rock Phosphate-Amended Cow and Pig Manures on Vermidegradation and Nutrient Release

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Abstract: Vermicomposting using Eisenia fetida has been shown to improve phosphorus mineralization from rock phosphate (RP). There is, however, a lack of information on the potential of integrating microbial inoculants like phosphate-solubilizing bacteria (PSB) during vermicomposting as a way of improving vermidegradation, and the release of nutrients from igneous RP-amended composts. This study evaluated the potential of using Eisenia fetida and Pseudomonas fluorescence in enhancing the vermidegradation, and nutrient release in igneous RP-amended cow and pig manure-based vermicomposts at a C/N ratio of 30. Compost maturity, nutrient and phytotoxicity parameters were measured to determine vermicompost quality. Final vermicompost results showed that the pig manure treatments achieved greater maturity as indicated by lower C/N of 10.3, high humification index (HI) of 7.6%, and humification ratio (HR) of 12.89% in the treatment with E. fetida and P. fluorescence. The inclusion of E. fetida alone in cow manure treatment resulted in the highest Olsen P of 2600 mg/kg, followed by the pig manure treatment with E. fetida only (2246.15 mg/kg). In conclusion, both E. fetida and P. fluorescence are necessary in the vermicomposting of RP-enriched pig and cow manure for efficient vermi-degradation and nutrient release.

Keywords: rock phosphate; earthworms; vermidegradation; phosphorus release; microbial inoculation

1. Introduction

The major challenge associated with world population growth has been the generation of huge quantities of solid waste. Currently, the global production of solid waste is estimated to be 1.3 billion tons per year and expected to double by 2025, with the organic wastes fraction ranking the highest among the solid waste generated [1]. Furthermore, the intensification in animal production for meat, eggs and dairy products has also resulted in drastic increases in the amounts of animal wastes produced [2]. Recycling of nutrients from solid waste through composting and vermicomposting have been studied to reduce the inappropriate disposals of these organic wastes in agricultural lands [2,3]. Furthermore, there has been a growing realization of the importance of organic nutrient sources in improving overall soil quality. Vermicomposting is one technology that has gained momentum in recent years as having the potential for converting both organic and inorganic waste materials into nutrient-rich fertilizer sources [4,5].

During the vermicomposting process, microorganisms in the gut of earthworms get their nourishment from the ingested waste and earthworms to stimulate the activity of microorganisms in soils through their microorganism-enriched casts [4,6]. Both the earthworms and microorganisms play
A critical role during the vermicomposting process [7]. This link between earthworms and microorganisms has resulted in several researchers exploring the possibility of using microbial inoculants to expedite the vermicomposting process. For example, Lukashe, et al. [4] conducted an experiment where cow manure-waste paper fly ash mixture was inoculated with P. fluorescence, and concluded that the interaction between E. fetida and P. fluorescence could optimize the vermi-degradation of the mixture and increase the nutrient release and biological activity. Though organic nutrient sources like vermicomposts have been reported to be superior to inorganic nutrient sources in improving overall soil chemical, biological and physical properties, their adoption in conventional agriculture has been limited by their low macro-nutrient concentrations [2,8].

After nitrogen, phosphorus is among the key macro-elements whose concentration is very low in organic materials [9]. When present in the soil in adequate quantities, P enhances crop growth, increases root growth, greater stalk strength and results in earlier crop maturity [10]. The world’s most important source of P is mined rock phosphate, a non-renewable resource. Therefore, unless cost-effective techniques to recycle P or new RP ore deposits are discovered, the decreasing supply of RP will threaten global food security [11]. When applied directly into soils, the dissolution of the apatite minerals in RP is influenced by soil properties such as pH, P and Ca concentration, P sorption capacity and organic matter content [12]. Therefore, direct application of RP in soils is more feasible in acidic soils than in alkaline soils, because of poor solubility of RP in alkaline soils. This low P dissolution is more pronounced in igneous RP, which is predominant in Southern Africa as opposed to the sedimentary RP [2].

Against this background, several researchers have investigated the potential of using RP as a P source in vermicomposts, while exploiting the higher nutrient dissolution within these composts [5,13]. Yan, et al. [14] reported that by applying 2% P as RP into rice straw vermicompost, the available P increased by 2.3-fold, and total P and humic substances also increased. In another study, Mupondi, et al. [5] observed that inclusion of RP at 7% resulted in the highest vermicompost quality. Moreover, Adhami, et al. [15] mixed sheep dung and leaf compost with E. fetida and RP powder at three levels, which included 0%, 6% raw RP and 2% modified RP, and observed the transformation of P from RP powder into various inorganic and organic P forms during vermicomposting. Generally, the studies reviewed here showed that the release of P during vermicomposting depended on the type of animal manure in the amendment used addition to the level of RP added.

The release of P during RP thermophilic composting can also be enhanced by inoculation with phosphate solubilizing bacteria (PSB). For example, Busato, et al. [16], evaluated the effect of PSB inoculation during RP composting on P solubilisation and bacterial community, and found that the inoculation of PSB during RP composting enhanced the availability of P. The enhanced P availability after the application of PSB, was attributed to the release of organic acids like oxaloacetic, fumaric, tartaric, glycolic, succinic, malic and citric acid by PSB [17]. It is thus clear that inoculation with PSB can enhance the release of P from RP during thermophilic composting. There is, however, lack of adequate information on how microbial inoculants such as the PSB combined with earthworms can be used to enhance vermi-degradation and inorganic P release in RP-enriched waste mixtures. The objectives of this study were thus to assess the effects of E. fetida and P. fluorescence on vermi-degradation of cow and pig manure RP-enriched vermicompost and inorganic P release.

2. Materials and Methods

2.1. Source of Materials

The study was performed at the University of Fort Hare research farm located in Alice Campus, Eastern Cape Province, South Africa. The white wastepaper used which was printed with black ink and not bleached, was collected from the University of Fort Hare, Alice campus and was shredded using a paper shredder. The cow manure used was collected from local kraals around the University of Fort Hare from cattle feeding exclusively on natural pasture. The cow manure was crushed to remove...
large lumps, air-dried, and stored under shade in a dry area. The pig manure was collected from a local piggery in Alice, where the pigs were fed exclusively on commercial feed. The pig manure collected had been mechanically squeezed to separate the solids from liquids, and these solids were then air-dried and stored under shade. Ground igneous RP was sourced from Phalaborwa located in the Limpopo province of South Africa. The igneous RP was supplied in a powder form which could pass through a 0.25 mm sieve, and its chemical properties were as follows: P\textsubscript{2}O\textsubscript{5}—40.3%, CaO—54.6%, MgO—0.26%, chromium—18.05 mg kg\(^{-1}\), cadmium—1.2 mg kg\(^{-1}\), copper—5.85 mg kg\(^{-1}\), and zinc—13.22 mg kg\(^{-1}\) and lead—6.05 mg kg\(^{-1}\). These chemical properties were provided through accredited methods and were availed to us by Forskor, Phalaborwa, South Africa. Selected chemical properties of the cow manure, pig manure and wastepaper used in this study are shown in Table 1.

| Chemical Property                  | Raw Material         |
|------------------------------------|----------------------|
|                                    | Cow Manure | Pig Manure | Wastepaper |
| pH (H\textsubscript{2}O)           | 8.53 ± 0.02*    | 6.52 ± 0.04 | 8.10 ± 0.10 |
| EC (\(\mu\)S/cm)                  | 4.00 ± 0.05      | 1.89 ± 2.83 | 1.80 ± 1.00 |
| Total C %                          | 22.84 ± 0.63     | 39.67 ± 1.37 | 37.96 ± 1.01 |
| Total N %                          | 2.05 ± 0.03      | 1.92 ± 0.04 | 0.04 ± 0.01 |
| C:N ratio                          | 11.14 ± 0.22     | 20.70 ± 0.94 | 998.94 ± 17.44 |
| Extractable NH\textsubscript{4} (mg/kg) | 31.410 ± 0.90 | 341.07 ± 26.5 | nd 1 |
| Extractable NO\textsubscript{3}/NO\textsubscript{2} (mg/kg) | 382.05 ± 63.71 | 97.03 ± 5.90 | nd |
| Olsen extractable P (mg/kg)        | 1479.19 ± 162.52 | 2135.17 ± 59. | nd |

* Values are mean ± standard deviation (n = 3); 1 nd = not detected.

Mature *E. fetida* earthworms that had been feeding on a mixture of cow and wastepaper were obtained from the University of Fort Hare local wormery. Phosphate solubilizing bacteria were sourced from a local South African supplier Microbial Biological Fertilizer International (MBFi) (http://www.mbfi.co.za/biological-inoculants-2/). The vermireactors used were plastic truncated cone containers with a volume of 0.015 m\(^3\) having perforations underneath and on the lids.

### 2.2. Treatments, Experimental Design, and Experimental Establishment

The experiment was factorial with four factors, i.e., manure type (cow and pig manure); earthworm presence (with or without); PSB presence (with or without) and time (0, 2, 4, 6 and 10 weeks). This factorial design gave eight treatments as listed below, which were laid in a completely randomised design with three replications.

1. Cow manure (CM) with no *E. fetida* or *P. fluorescence* (Control)
2. Pig manure (PG) with no *E. fetida* or *P. fluorescence* (Control)
3. CM with *E. fetida* alone
4. PG with *E. fetida* alone
5. CM with *P. fluorescence* alone
6. PG with *P. fluorescence* alone
7. CM with *E. fetida* plus *P. fluorescence*
8. PG with *E. fetida* plus *P. fluorescence*

The cow and pig manure used in this study, were mixed with the shredded paper to adjust the C/N ratio to 30:1, as recommended by Mupondi, [18] whilst the RP was then added at 2% (w/w basis) to the organic waste materials as recommended by Unuofin and Mnkeni, [13]. The total weight of the mixture that was used was maintained at 3 kg (dry weight), with no extra feed being added during the experiment.

Before adding the earthworms and the *P. fluorescence*, the mixtures from all the treatments were allowed to pre-compost for 1 week to moisten the mixture and to eliminate volatile toxic gases [19].
After the pre-composting, the earthworms and the *P. fluorescence* were introduced in the respective treatments, and the substrate was adjusted to a moisture content of 60–70% as recommended by Mupondi, et al. [5]. Mature clitellate earthworms were introduced at 22.5 g-worm/kg dry substrate as recommended by Unuofin and Mnkeni, [13] while the *P. fluorescence* was inoculated at 50 mL/kg substrate having $1 \times 10^9$ CFU/mL as described by Das, et al. [20] and Busato, et al. [16]. Samples were collected at 0, 2, 4, 6 and 10 weeks, and oven dried before being ground using a mechanical grinder with a 1 mm sieve. The ground vermicompost samples were then analysed for several chemical and biological parameters as described below.

2.3. Chemical Properties

2.3.1. Total C and N Determination

The total C and nitrogen were determined on dry, ground samples using a LECO Truspec CN auto analyser [21].

2.3.2. Humic and Fulvic Acids

The humic and fulvic acid fractions in the composts were extracted using a method described by Sánchez-Monedero, et al. [22]. A 0.1 M NaOH solution was used as an extraction ratio of 1:20 (w/v), with the extracts being centrifuged at 8000 rpm equivalent to $8.2 \times 10^3$ g (relative centrifugal force). The supernatant was then divided into portions with one half being analyzed for total extractable carbon fraction ($C_{tEX}$). The other half was acidified using $\text{H}_2\text{SO}_4$ to a pH of 2 and then centrifuged at 8000 rpm after coagulation. After centrifuge, the supernatant was used for the analysis of fulvic acid ($C_{FA}$). The C concentrations in the supernatants were determined using the dichromate oxidation method, with the concentration of the humic acid ($C_{HA}$) fraction being calculated as the difference between ($C_{tEX}$) and ($C_{FA}$). Humification ratio (HR; Equation (1)) and Humification index (HI; Equation (2)) which are indices used for the evaluation of humification level in the compost were calculated as presented in the equations below; where C is total carbon in the vermicompost samples determined by dry combustion [21]:

$$\text{HR} \, (\%) = \frac{C_{tEX}}{C} \times 100 \quad (1)$$

$$\text{HI} \, (\%) = \frac{C_{HA}}{C} \times 100 \quad (2)$$

2.3.3. Inorganic Extractable Nitrogen (Ammonium, Nitrate, and Nitrite)

The mineral nitrogen was extracted using 0.5 M potassium sulphate (1:10 w/v) [23]. 50 mL of the 0.5 M potassium sulphate was added to 5 g sample, shaken for one hour in a reciprocal mechanical shaker at 180 rpm and then filtered using Whatman No. 2 filter paper for colorimetric analysis. The ammonium and nitrate concentration were determined using a UV/Vis spectrophotometer (Helios Gamma, Thermo Spectronic, Cambridge, United Kingdom), after the development of a blue color using salicylate-nitroprusside colorimetric method for ammonium-N, and a yellow color using 5% salicylic acid in concentrates sulphuric acid for nitrate and nitrite-N [24].

2.3.4. Olsen Extractable P

A solution of 0.5 M sodium hydrogen carbonate that had been adjusted to a pH of 8.5 using 1 M sodium hydroxide was used for extraction of Olsen P [25]. From the compost sample, 2.5 g was weighed and then extracted with 50 mL of 0.5 M NaHCO$_3$ solution and was shaken on a horizontal shaker for 30 min at 180 rpm. After shaking at this time, the sample extracts were filtered through Whatman$^{\text{TM}}$ No.42 filter paper, and the filtrates were adjusted to a pH of 5 with 2.5 mL sulphuric acid before the analysis. The P in the solution was analysed using the colourimetric ascorbic acid method. The ascorbic acid was dissolved in an ammonium molybdate solution that was prepared by mixing
2.5 M sulphuric acid with ammonium molybdate and potassium antimony tartrate. For the analysis of P, 10 mL aliquot was mixed with 8 mL of the ascorbic reagent in a 50 mL volumetric flask and filled to the mark with deionized water. The absorbance was measured at 880 nm after 10 min of standing, using UV spectrophotometer (Helios Gamma, Thermo Spectronic, Cambridge, United Kingdom), that had been calibrated using standards with the concentration range of 0–1.2 mgP/L treated in the same manner as the samples for the colourimetric ascorbic acid method [26].

2.4. Crop Phytotoxicity

Phytotoxicity was determined using a seed germination test on the samples collected at the end of this study. Liquid extracts were prepared from the two vermicompost mixtures with distilled water (1:10 w/v) as described by Ravindran, et al. [27]. Three test crops, i.e., tomato (Solanum lycopersicum), spinach (Spinacia oleracea L.), and radish (Raphanus raphanistrum subsp. sativus) were used for seed germination bioassay, and were sourced from freshly packed Starke Ayres seeds products. Two layers of filter papers were placed in a sterilized plastic petri dish, as 10mL of the liquid was added. In addition to different treatments, deionized water was used as a control. On top of the filter paper, ten seeds of each crop species were placed, and the Petri dishes used were then incubated at 25 degrees Celsius. After 5 days, the relative seed germination (RSG; Equation (3)) and relative root elongation (RRE; Equation (4)), germination index (GI; Equation (5)) were then calculated as follows:

\[
RSG (\%) = \frac{\text{Number of seeds germinated in the sample extract}}{\text{Number of seeds germinated in the control}} \times 100
\]

\[
RRE (\%) = \frac{\text{Mean root elongation in the sample extract}}{\text{Mean root elongation in the control}} \times 100
\]

\[
GI (\%) = \frac{(\% \text{ Seed germination}) \times (\% \text{ Root elongation})}{100}
\]

2.5. Earthworm Biomass

During vermicomposting, the E. fetida biomass was measured at 0, 2, 4, 6 and 10 weeks. At each sampling date, the composting mixture was emptied on to a plastic sheet, and 10 earthworms per pot were randomly removed manually and weighed. The weighted values of earthworms were determined as live weight after they were picked using the hand-sorting method and removal of any adhering material. After that, the earthworms were returned together with the composting mixture into their respective containers.

2.6. pH and Electrical Conductivity (EC)

The pH and the EC were determined in the water at the ratio of 1:10 (w/v) using a pH meter equipped with a glass electrode (Crison Instruments, Barcelona, Spain) [13].

2.7. Data Analysis

The data analysis was carried out with repeated measures analysis of variance (ANOVAR) and where sphericity assumption could not be met, then the Greenhouse—Geisser [28] was used as correction of the p-value. All data were analyzed using JMP version 14.0.1 statistical software (SAS Institute, Inc., Cary, NC, USA). Microsoft Excel (Microsoft, Washington, USA) was used for descriptive analysis and plotting of graphs.
3. Results

3.1. Effects of *E. fetida* and *P. fluorescence* Presence on Vermidegradation Efficiency

There was a significant interaction (*p* < 0.0001; Table 2) between time, earthworm presence, PSB inoculation and manure type, indicating that the effects of these factors were not consistent on changes in C/N ratio when averaged across the vermicomposting period. After 10 weeks of vermicomposting, the pig manure treatment with both *E. fetida* and PSB showed the lowest C/N ratio of 10.3, followed by the cow manure treatment with both *E. fetida* and PSB with the C/N ratio of 12.1 (Figure 1). By contrast, the cow and pig manure controls treatments only reached C/N ratios of 15.2 and 14.5, respectively.

Table 2. Repeated measure analysis (ANOVAR) for changes in biochemical properties of rock phosphate enriched cow manure and pig manure waste paper mixtures inoculated with *E. fetida* and *P. fluorescence* after 10 weeks of vermicomposting.

| Source of Variation | C/N Ratio | pH   | EC (mS/cm) | HR (%) | HI (%) | NH₄ (mg/kg) | NO₃ (mg/kg) | Olsen P (mg/kg) |
|---------------------|-----------|------|------------|--------|--------|-------------|-------------|---------------- |
| **Between subjects**|           |      |            |        |        |             |             |                |
| Earthworm presence (E) | 0.0020   | ns   | ns         | 0.0003 | 0.0324 | <0.0001     | 0.0008      | <0.0001       |
| PSB presence (P)     | Ns        | ns   | 0.0151     | <0.0001| <0.0001| 0.0131      | 0.0021      | 0.0202        |
| Manure type (M)      | <0.0001   | <0.0001 | <0.0001    | <0.0001| <0.0001| <0.0001     | <0.0001     | <0.0001       |
| **Within-subjects**  |           |      |            |        |        |             |             |                |
| Time (T)             | <0.0001   | <0.0001 | <0.0001    | <0.0001| <0.0001| <0.0001     | <0.0001     | 0.0002        |
| T × E                | <0.0001   | ns   | 0.0483     | ns     | <0.0001| <0.0001     | <0.0001     | 0.0001        |
| T × P                | 0.0241    | ns   | ns         | 0.0002 | <0.0001| <0.0001     | <0.0001     | 0.0011        |
| T × M                | Ns        | ns   | 0.0064     | 0.0092 | ns      | <0.0001     | <0.0001     | 0.0024        |
| T × E × P            | <0.0001   | ns   | 0.0004     | 0.0190 | <0.0001| 0.0228      | 0.0002      | 0.0009        |
| T × E × M            | 0.0105    | ns   | 0.0176     | ns     | <0.0001| 0.0007      | <0.0001     | ns            |
| T × P × M            | 0.0051    | ns   | <0.0001    | 0.0003 | <0.0001| <0.0001     | 0.0004      | <0.0001       |
| T × E × P × M        | 0.0072    | ns   | 0.0387     | ns     | 0.0038 | 0.0084      | <0.0001     | ns            |

ns = not significantly different at *p* > 0.05, HR = Humification ratio, HI = Humification Index.

![Figure 1](image_url) Changes in C/N ratio during vermicomposting of cow and pig manure amended with rock phosphate and *P. fluorescence*. Error bars indicate standard deviations.

The inconsistent changes in humification index (HI) and humification ratio (HR) observed in this study are explained by the significant interactions between the various factors as indicated in Table 2 above. For HI, the significant interaction (*p* < 0.0001; Table 2) between time, earthworm presence, PSB and manure type was observed. For cow manure-waste paper RP-enriched treatments, the highest HI at week 10 was...
observed in the control treatment, followed by the treatment with *P. fluorescence* only, with HI values of 4.3 and 3.7, respectively (Figure 2). For the pig manure-waste paper RP mixtures, the treatment with both *E. fetida* and *P. fluorescence* showed the highest percentage increase of 78.82%, with the control treatment showing the lowest percentage increase of 0.5%.

**Figure 2.** Effect of *E. fetida* and *P. fluorescence* on the humification index during the vermicomposting of RP-enriched cow and pig manure waste paper mixtures. Error bars indicate standard deviations.

Unlike the HI, changes in HR were significantly (*p* < 0.01) influenced by manure type, PSB presence and earthworm presence, with the significant interaction between all three factors indicating the inconsistent influence of these factors on changes in HR (Table 2). It was interesting to observe that after 10 weeks of vermicomposting there were no differences on HR between all cow manure-based treatments with the highest HR being the treatment with both *E. fetida* and PSB. However, for the pig manure-based treatments, there were significant differences between treatments, with the *E. fetida* plus *P. fluorescence* treatment showing the highest final HR of 12.89, followed by treatment with PSB only (9.60) and the treatment with *E. fetida* only (7.97) while the control had an HR of 7.75 (Figure 3).

**Figure 3.** Effect of *E. fetida* and *P. fluorescence* on the humification ratio during the vermicomposting of RP-enriched cow and pig manure waste paper mixtures. Error bars indicate standard deviations.

### 3.2. Effects of *E. fetida* and *P. fluorescence* Presence on Inorganic Nitrogen Content

There was a significant interaction (*p* < 0.001; Table 2) between time, PSB, and manure type, indicating the effects of these factors were not consistent on changes in ammonium concentration when averaged across the vermicomposting period. The ammonium content was higher on the pig manure treatments than cow manure treatments, and the highest ammonium content in the pig manure was observed in the treatment with PSB only (333.54 mg/kg), followed by the treatment with both
E. fetida plus PSB (253.85 mg/kg). Unlike the cow manure treatments were the ammonium content at the end of vermicomposting showed the same order of magnitude, the ammonium content in the pig manure treatment at the end of vermicomposting varied remarkably as it ranged from 187.85 mg/kg to 333.54 mg/kg. Among the cow manure treatments, the control showed the highest increase of 37.47%, while the ammonium content in the treatment with both E. fetida and PSB decreased from 41.31 mg/kg to 36.00 mg/kg (Figure 4). Across all the treatment combinations, the highest increase in ammonium concentration of 40.34% was observed in the pig manure treatment with PSB only.

![Figure 4](image-url)

**Figure 4.** Changes in ammonium during vermicomposting of cow and pig manure amended with rock phosphate and *P. fluorescence*. Error bars indicate standard deviations.

Regarding the nitrate concentration, the changes during vermicomposting were observed because of significant interaction (*p* < 0.001; Table 2) between time, earthworm presence and manure type. Opposite to the ammonium content, the cow manure relative treatments, showed the highest nitrate content compared to the pig manure treatments. However, the nitrate content increased for all the treatments except for the control treatment that showed a decrease from 275.92 mg/kg to 103.67 mg/kg (Figure 5). Among the treatments, the highest increase was observed in the treatment with both E. fetida and PSB with the percentage increase of 72.85% followed by the treatment with E. fetida only with the percentage increase of 63.22%. In all the pig manure relative treatments, the nitrate content decreased including the controlled treatment with no E. fetida and PSB.

![Figure 5](image-url)

**Figure 5.** Changes nitrate during vermicomposting of cow and pig manure amended with rock phosphate and *P. fluorescence*. Error bars indicate standard deviations.
3.3. Effects of *E. fetida* and *P. fluorescence* Presence on Olsen Extractable P

A significant interaction ($p < 0.001$; Table 2), between time, earthworm presence, PSB and manure type was observed, indicating the effects of these factors were not consistent on changes in Olsen P when averaged across the vermicomposting period. At week 6, the pig manure-waste paper RP-enriched treatment with *E. fetida* only had the highest P level of 3837.21 mg/kg amongst all the other treatments, followed by the pig manure and waste-paper RP-enriched treatment with both *E. fetida* and PSB (3051.2 mg/kg; Figure 6). At week 10, the Olsen extractable P for cow manure and waste-paper RP-enriched treatments ranged between 1150 and 2600 mg/kg and between 558.97 and 2246.154 mg/kg for pig manure and wastepaper RP-enriched treatments. When these are compared to the control treatments of both cow and pig manure-waste paper RP-enriched mixture, the cow and pig manure-waste paper RP-enriched mixture with *E. fetida* only showed a percentage difference of 54.44% and 75.1%, respectively. The cow manure RP-enriched treatments with *P. fluorescence* only, and both *E. fetida* and *P. fluorescence* peaked at week 6 (2933.33 mg/kg and 3342.636 mg/kg), while the treatment with *E. fetida* only reached its highest peak at week 10 (2600 mg/kg).

![Figure 6. Effect of *E. fetida* and *P. fluorescence* on Olsen extractable P during vermicomposting of cow and pig manure amended with rock phosphate and *P. fluorescence*. Error bars indicate standard deviations.](image)

3.4. Phytotoxicity Test

Each test crop evaluated showed a significant ($p < 0.05$) response to the eight treatments on relative seed germination (RSG), relative root elongation (RRE) and germination index (GI) percentage (Table 3). The RSG ranged between 43.3% and 100% in spinach; 96% to 112% in radish and between 48% to 88% in tomato (Table 3). Among the different treatment combinations and crops, the RSG with 100% and above were observed in the cow manure-waste paper RP-enriched control treatment for spinach with the RSG of 100% compared to the pig manure-waste paper RP-enriched control treatment with RSG of 43.3%. In radish the highest RSG was observed in the cow manure-waste paper RP-enriched control treatment with RSG of 112% compared to pig manure-waste paper RP-enriched control treatment with the RSG of 96.3%. The RRE for all the treatments ranged between 78.2% and 129%, 44% and 108%, and 71.4 % and 143.1% for spinach, radish, and tomato. RRE of 100% and above were recorded in three treatments in spinach, two in radish and six in tomato. In spinach, the highest RRE was observed in the cow manure-waste paper RP-enriched treatment with *E. fetida* only, with the RRE of 129% compared to both RP control treatments with RRE of 78.2% and 89%.
Table 3. The effects of *E. fetida* and *P. fluorescence* on the phytotoxicity of rock phosphate enriched cow manure (CM) and pig (PG) manure- waste paper mixtures.

| Treatments                          | Spinach | Radish | Tomato |
|-------------------------------------|---------|--------|--------|
|                                     | Percentage | RSG  | RRE  | GI  | Percentage | RSG  | RRE  | GI  | Percentage | RSG  | RRE  | GI  |
| CM: no worms, no PSB (Control)      | 100 a    | 78.2 c | 78.2 abc | 112 a | 48.7 c  | 55.2 b | 52.4 bc | 129 a | 67.4 a    |
| PG: no worms, no PSB (Control)      | 43.3 d   | 89 bc  | 38.5 f  | 96.3 a | 67.1 bc | 64.7 bc | 73.2 ab | 103 b | 75.9 a    |
| CM: *E. fetida* only                | 58.3 c   | 129 a  | 75 bc   | 100.4 a| 82.3 ab | 83.1 ab | 49 c   | 131.3 a| 64 a      |
| PG: *E. fetida* only                | 58.3     | 83.4 bc| 48.3 ef | 105 a  | 54 c    | 56.4 bc | 57 bc  | 103.3 b| 59 a      |
| CM: PSB only                        | 86.7 b   | 110 ab | 94.5 a  | 104 a  | 103 a   | 106.5 a | 48 c   | 105.2 b| 50.2 a    |
| PG: PSB only                        | 65 c     | 90 bc  | 58.3 de | 100.5 a| 108 a   | 112 a   | 61.3 bc| 143.1 a| 77 a      |
| CM: *E. fetida* plus PSB            | 65 c     | 127 a  | 83 ab   | 96 a   | 54 c    | 53.3 bc | 65.5 bc| 97.3 b | 64 a      |
| PG: *E. fetida* plus PSB            | 65 c     | 89.2 bc| 60 ede | 108.3 a| 44 c    | 49 c    | 88 a   | 143.1 a| 77 a      |

*Numbers that are followed by different letters for the same parameter are significantly different, according to LSD (p ≤ 0.05). RSG = relative seed germination, RRE = relative root elongation, GI = Germination Index.*

In radish, the highest RRE of 108% was observed in the pig manure-waste paper RP-enriched treatment with *P. fluorescence* only, compared to both RP-enriched control treatments with the RRE of 48.7% and 67.1%. However, in tomato the highest RRE of 143.1% was observed in the pig manure-waste paper RP-enriched treatment with *P. fluorescence* only compared to the cow manure, and pig manure-waste paper RP-enriched control treatments with RRE of 129% and 103%. The GI across all the treatment combinations ranged from 38.5% to 94.5% for spinach, 49% to 112% for radish and 50.2% to 77% for tomato. The GI of 80% and above was recorded in two treatments for spinach with the highest GI in the cow manure-waste paper RP-enriched treatment with *P. fluorescence* only with the GI of 94.5% compared to the cow and pig manure-waste paper RP-enriched mixture with GI of 78.2% and 38.5%. In radish, three treatments showed a GI of 80% and above, with the highest GI observed in the pig manure treatment-waste paper RP-enriched treatment with *P. fluorescence* only compared to the control treatments of cow and pig manure with GI of 55.2% and 64.7%.

### 3.5. The Effects of Manure Type and *P. fluorescence* Presence on *E. fetida* Biomass

There was a significant effect on the two-way interaction between time and manure type implying that the effect of manure type was not consistent when averaged across the vermicomposting time (Table 2). There were significant differences (*p < 0.05*) on *E. fetida* biomass between cow and pig manure (Table S1; Figure S1). On average, pig manure-waste paper RP-enriched mixture resulted in a maximum of 7.45 g of *E. fetida* biomass compared to 4.2g in cow manure-waste paper mixture, representing a 44% difference between the two manures. The *E. fetida* biomass peaked at week 6 for all the treatments, and thereafter either decreased or remained constant. Generally, *E. fetida* biomass was higher in the treatment where no *P. fluorescence* was added compared to where *P. fluorescence* was added, but this effect was not statistically significant.

### 3.6. The Effect of *E. fetida* and *P. fluorescence* Inoculation on Changes in pH and EC

There was a significant interaction (*p < 0.038; Table 2*) between time, earthworm presence, PSB inoculation and manure type, indicating that the effects of these factors were not consistent on changes in EC when averaged across the vermicomposting period. There was a significant difference (*p < 0.05*) in pH between the two manure types (Table 2). However, neither earthworm presence nor PSB inoculation significantly influenced changes in pH in this study. Generally, there was minimal variation in the pH of the two manure types throughout the vermicomposting period (Figure S2). For the cow manure treatments, pH ranged from 8.52 to 8.99 while the pH for the pig manure treatments ranged from 7.21 to 7.73. There were significant differences (*p < 0.01; Table 2*) on the average EC between the cow and pig manure mixtures (Figure S3). The highest increase from week 0 to week 10 was observed in the cow manure control treatment (1.28), followed by the pig manure treatment with an EC value of 1.09. Also, the presence of PSB significantly (*p < 0.01*) influenced the changes in EC, with the average EC in cow manure with PSB being 1.92, and that for pig manure being 1.52. At week 10, in all the
treatment combinations in both cow and pig manure, the EC was significantly higher compared to values at week 0.

4. Discussion

4.1. The Effect of P. fluorescence Inoculation on the E. fetida Biomass

Earthworm growth is a biological indicator that can be used to monitor the vermicomposting process [29]. The higher earthworm biomass observed in pig manure compared to cow manure-waste paper RP-enriched vermicompost could be due to differences in the availability of nutrients in the mixture [30]. Generally, pig manure treatments maintained higher levels of NH$_4$-N and Olsen extractable P than cow manure-based treatments (Table 1) and possibly other nutrients that were not analysed. This implies that higher nutrients concentrations in pig manure could have explained at least partially the much higher earthworm biomass treatments observed in pig manure treatments than in cow manure-based treatments, as the pH, EC, nitrates and ammonium in the pig manure showed favourable conditions for the growth of earthworms (Table 1). The lack of significant differences after the inoculation with P. fluorescence in both cow and pig manure-based RP vermicompost shows that the cow and pig manure enriched RP is enough for the growth of E. fetida during vermicomposting.

The weight loss of earthworms in both cow and pig manure vermicompost mixture recorded between week 6 and week 10 (Figure S1) could be attributed to a possible decline in water-soluble carbon in the vermicompost mixtures with time [18]. The rapid degradation of organic waste during vermicomposting could also result in the loss of N due to leaching, among other things, which in turn could according to Bernal, et al. [31] affect the E. fetida growth. In a related study, de Lima Rodrigues, et al. [32], observed a decrease in earthworm density at the end of vermicomposting using cattle manure mixed with tannery sludge that had been amended with rock powder. In their study, the decline of earthworm was attributed to the decrease in the availability of the substrate in all the treatments at the end of the vermicomposting.

4.2. Effects of E. fetida and P. fluorescence on Changes in pH and EC During RP-enriched Vermicomposting

No clear treatment effect on differences in pH were observed in either cow manure or pig manure treatments. These findings corroborated previous findings by Yan, et al. [14] and Unuofin, et al. [33], who also reported that changes in pH during RP-enriched manure-waste paper mixtures vermicomposting using E. fetida to be minimal and with no consistency. The slight variations in pH could be due to the application of RP and P. fluorescence, which is likely due to the compost buffering system, which resulted in slight changes in pH [33,34].

Electrical conductivity (EC) is regarded as a good indicator of the safety and agricultural suitability of vermicompost mixtures [35]. However, the magnitude of EC in all the treatment combinations was less than 4 mS/cm, and thus, its use is unlikely to result in salinity hazards [36]. The observed increase in EC at the end of vermicomposting for each manure type was most probably due to the release of phosphate and soluble salts like ammonium, caused by the decomposition of the readily biodegradable organic mixture by earthworms and microbes [37]. The results of EC agree with the findings of other researchers who also observed increased EC levels at the end of vermicomposting [38–41]. The increased EC in the present study can be explained by the degradation of organic matter [38], and the release of soluble and inorganic salts like ammonium salts, phosphates and nitrates [42]. Therefore, the increased EC is an indication that the vermicomposting process has accelerated the mineralization of organic matter and have transformed insoluble particles into soluble forms [43].

4.3. Effects of E. fetida and P. fluorescence on Humification Parameters During RP-enriched Vermicomposting

The C/N ratio reflects the decomposition of the waste material and is a widely used maturity indicator for monitoring vermicomposting [2]. The loss of organic carbon in the final RP vermicompost mixture is because of high CO$_2$ emission due to mineralization of carbon from the substrate through
respiration by both earthworms and microbes. Also, an increase in the production of nitrogenuous excreta can increase N content resulting in the decreased C/N ratio [44,45]. The findings of C/N in the present study confirms the findings of Unuofin and Mnkeni, [13] and Malafaia, et al. [46] which observed decreased C/N ration during cow manure RP-enriched vermicomposting and during vermicomposting of different tanning sludge mixed with cattle dung. The results for C/N ratio (Figure 1) proved that the incorporation of *E. fetida* only, *P. fluorescence* alone and in combination, accelerated the vermi-degradation of RP-enriched cow and pig manure-waste paper mixtures. This shows that the degradation was faster when both *E. fetida* and *P. fluorescence* were added and that the mixtures can be used to enhance the rate of biodegradation of cow and pig manure-waste paper RP mixtures using *E. fetida*. A C/N ratio of less than 20 is believed to indicate a progressive degree of the solubilization of organic matter and satisfactory degree of compost maturity [47,48]. In all the treatment combinations at the end of vermicomposting, the C/N ratio in the present study ranged from 10.3 to 15.8 which is less than 20, suggesting that compost maturity was achieved in all treatment combinations.

**HI and HR**

Both the HI and HR increased during vermicomposting, which is in agreement with the findings of Mupondi, et al. [5] who vermicomposted cow manure-waste paper mixture at different RP rates using *E. fetida*. In all the cow and pig manure-waste paper RP-enriched treatments, except for the pig manure control treatment, the humification Index (HI) increased with time consistent with observations in other studies where the cow manure-waste paper RP-enriched vermicompost was tested using *E. fetida* [13]. In their study, the found an increased HI during cow manure-waste paper RP-enriched vermicompost, which indicated that all treatment combinations resulted in a high degree of humification as also indicated by the C/N values. These HI findings further showed that the presence of *E. fetida* and *P. fluorescence* enhanced the maturation of the vermicomposts. This is likely due to the increased rate of decomposition of the organic substrate mixture by the *E. fetida* which produce numerous bioactive humic substances, and the increased humification level in organic matter is mostly associated with the higher agricultural value of the final vermicompost. In the present study, all the treatments at week 10 had HI values greater than 3.5% with the highest value observed in the treatment with both *E. fetida* and *P. fluorescence* in the pig manure-waste paper RP-enriched mixture. According to Bernal, et al. [31] a mature compost is expected to have a HI of greater than 3.5%. Therefore, the results of the present study shows that the earthworms, microorganisms and the composition of humus are closely related to soil fertility [49], and in this case the mixture can be used as supplement for nutrient deficient soils.

In all the treatment combinations, the HR was greater than 7, which, according to Bernal, et al. [31] signifies a matured compost mixture. The presence of *E. fetida* and *P. fluorescence* showed greater HR, followed by cow manure-waste paper RP-enriched treatment with both *E. fetida* and *P. fluorescence*. However in most studies, the HI and HR at the beginning of vermicomposting is usually the same in all the treatments, and the initial difference in this study among the treatment is likely due to differences in chemical composition of vermicompost mixtures.

**4.4. Effects of *E. fetida* and *P. fluorescence* on the Ammonium and Nitrates During RP-Enriched Vermicomposting**

During vermicomposting, the nitrogen balance is usually regulated by the nitrification process which enhances the quality of vermicompost. The high nitrate content of cow manure RP-enriched vermicompost compared to low ammonium content indicated that the process of ammonification and nitrification was not hindered. Similar observations were reported by Mupondi, et al. [5] who reported decreased ammonium, with a corresponding increase in nitrate content during RP-enriched cow manure vermicomposting. However, they associated the increased nitrogen mineralization with increased biodegradation and P availability from the added RP because of higher biological sorption. Based on Nigussie, et al. [50], the high nitrate content implies low nitrate loss in the vermicompost mixture. Additionally, the use of *E. fetida* alone which showed high nitrate content compared to other
treatments in cow manure RP vermicompost can be explained by the presence of earthworm, which is well known for its impact of increasing nitrate content [51]. The different trends in both ammonium and nitrate of the cow manure and pig manure can be explained by their initial concentration of ammonium and nitrate (Table 1). For instance, it was noted by De Guardia, et al. [52] that, the nitrification ability is an intrinsic property of the waste since the rate of nitrification is dependent on the waste type.

4.5. Effects of E. fetida and P. fluorescence on the Olsen Extractable P During RP-enriched Vermicomposting

Olsen P is a very important chemical property as it represents the mineralized portion of organic P in the vermicompost mixture. In the vermicompost experiment conducted by Getachew, et al. [53] to investigate the potential of E. fetida and Eudrilus eugeniae (Debrezeit and Keshmando) on the decomposition of various organic waste through the production of nutrient-rich vermicompost and worm biomass, the Olsen P content in the treatment with E. fetida was high compared to Olsen P produced by the two local earthworms. The release of P in these treatments can be explained by the earthworm gut phosphatase and P solubilizing microorganisms in the worm cast [54]. Their results agree with the findings of the present study, were high Olsen P was observed in the cow and pig manure RP-enriched treatment with E. fetida only compared to control treatment. In another study, Schaik, et al. [55] conducted an experiment, where they investigated the practicality of vermicomposting septic tank waste using E. fetida. In their study, they noticed an increase of Olsen P in all the treatments during vermicomposting, until day 89 after which a steady state was reached. The observed increase in P in the vermicomposting mixtures due to the enhanced dissolution and the mineralization of P from RP could have been facilitated by organic acids released during organic waste decomposition [5,14,33]. The release of the organic acids during organic waste mixture decomposition is facilitated by E. fetida, and the presence of P. fluorescence. The organic acids have carboxylic acid and the phenolic groups that can chelate with Ca 2+ ions from the apatite minerals in RP thereby enhancing the dissolution and the mineralization of P from RP [34] (Ca_{10}(PO_4)_{6}F_2 + OH^- + H^+ → 10 Ca^{2+} + 6PO_4^{3-} + 2F^- + H_2O). The observed increase in humic acid reflected by the high HI in week 10 (Figure 3) could also have contributed to the improved mineralization and dissolution of P. Furthermore, the increased Olsen P in cow and pig manure RP-enriched mixture with E. fetida only like decreased C/N ratio, might be due to the accelerated humification that is promoted by earthworms during vermicompost [44,56]. This is because these humic acids also have carboxylic acid and the phenolic groups like those found in simple organic acids, which have the ability of chelating Ca 2+ ions from the apatite minerals in RP and thus enhance the dissolution and the mineralization of P from RP [34].

4.6. Effects of E. fetida and P. fluorescence on the Phytotoxicity of Vermicomposting Cow and Pig Manures Mixtures Enriched with RP

It is generally accepted that for reliable and complete characterization of compost quality for safe use in agriculture, it is crucial to also evaluate compost stability [57]. Incomplete composting is known for its effect of limiting the degradation of phytotoxic synthetic compounds and intermediates [58]. It is, therefore, essential for vermicomposts to be evaluated for their phytotoxic properties. The GI index of more than 50% was observed in most treatments, and according to Jodice, [59], this indicates matured compost with low levels of phytotoxicity. The GI of less than 50% in radish plant, that was planted using pig manure RP vermicompost with both E. fetida and P. fluorescence, indicates higher phytotoxicity [60]. The high phytotoxicity observed, could be because of high ammonium content observed in the treatment (Figure 4), since radish is considered sensitive to high ammonium content. For instance, in the study conducted by Olivera Viciedo, et al. [61] on the response of radish to difference ammonical nitrogen concentrations in absence and presence of silicon, observed highest sensitivity in treatments without silicon. In the cow manure treatment with E. fetida only, the highest germination observed agree with findings of Bernal, et al. [62] who found increased GI value of radish from between 24 to 120 h of germination using cow manure vermicompost using E. fetida. Therefore, the results showed low levels of phytotoxic substances.
5. Conclusions

The addition of both *E. fetida* and *P. fluorescens* during cow and pig manure-waste paper RP-enriched vermicomposting, significantly influenced biodegradation, and nutrient release, though the effects of these factors were not consistent between the two manures evaluated in this study. However, there was greater humification and P release from the RP-enriched pig manure than from the cow manure. This was reflected in greater reduction of the C/N ratio, higher humification parameters (HI and HR), and higher levels of Olsen extractable P. Inoculation with *P. fluorescens* further enhanced the maturation of the vermicomposting mixtures as well as the release of P from these mixtures. This indicated that inoculation with *P. fluorescens* could be used to optimize the vermicomposting of RP-enriched animal manure-waste paper mixtures and possibly other waste mixtures as well.

**Supplementary Materials:** The following are available online at http://www.mdpi.com/2073-4395/10/10/1587, Figures S1–S3: Changes in earthworm biomass (*E. fetida*), pH and EC during vermicomposting of cow and pig manure amended with rock phosphate and inoculated with *P. fluorescens*, Table S1: Repeated measure analysis (ANOVAR) for changes in earthworm biomass in rock phosphate enriched cow manure and pig manure waste paper mixtures inoculated with *P. fluorescens* after 10 weeks of vermicomposting.

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**References**

1. Hoornweg, D.; Bhada-Tata, P. *What a Waste: A Global Review of Solid Waste Management*; World Bank: Washington, DC, USA, 2012; Volume 15, p. 116.

2. Mupambwa, H.A.; Mnkeni, P.N.S. Optimizing the vermicomposting of organic wastes amended with inorganic materials for production of nutrient-rich organic fertilizers: A review. *Environ. Sci. Pollut. Res.* 2018, 25, 10577–10595. [CrossRef] [PubMed]

3. Gómez-Brandón, M.; Juárez, M.F.D.; Domínguez, J.; Insam, H. Animal manures: Recycling and management technologies. In *Biomass Now-Cultivation and Utilization*; InTech: Rijeka, Croatia, 2013; pp. 237–272.

4. Lukashe, N.S.; Mupambwa, H.A.; Green, E.; Mnkeni, P.N.S. Inoculation of fly ash amended vermicompost with phosphate solubilizing bacteria (*Pseudomonas fluorescens*) and its influence on vermi-degradation, nutrient release and biological activity. *Waste Manag.* 2019, 83, 14–22. [CrossRef]

5. Mupondi, L.T.; Mnkeni, P.N.S.; Muchaonyerwa, P.; Mupambwa, H.A. Vermicomposting manure-paper mixture with igneous rock phosphate enhances biodegradation, phosphorus bioavailability and reduces heavy metal concentrations. *Heliyon* 2018, 4, e00749. [CrossRef] [PubMed]

6. Ali, U.; Sajid, N.; Khalid, A.; Riaz, L.; Rabbani, M.M.; Syed, J.H.; Malik, R.N. a review on vermicomposting of organic wastes. *Environ. Prog. Sustain. Energy* 2015, 34, 1050–1062. [CrossRef]

7. Kumar, A.; Gupta, R.K.; Kumar, S.; Kumar, S. Nutrient variations in vermicompost prepared from different types of straw wastes. *Forage Res.* 2017, 42, 267–270.

8. Baversiha, N.; Parvanak, K.; Nasrabadi, M. Reduction of adverse environmental impacts caused by urban sewage: Application of green soil fertilizers. *Ukr. J. Ecol.* 2018, 8, 437–440. [CrossRef]

9. Novo, D.L.; Pereira, R.M.; Costa, V.C.; Hartwig, C.A.; Mesko, M.F. a novel and eco-friendly analytical method for phosphorus and sulfur determination in animal feed. *Food Chem.* 2018, 246, 422–427. [CrossRef] [PubMed]

10. Meyer, J.; Rein, P.; Turner, P.; Mathias, K.; McGregor, C. *Good Management Practices for the Cane Sugar Industry (Final)*; Produced for the International Finance Corporation (IFC): Johannesburg, South Africa, 2011; pp. 200–206.
11. Wendling, L.A.; Blomberg, P.; Sarlin, T.; Priha, O.; Arnold, M. Phosphorus sorption and recovery using mineral-based materials: Sorption mechanisms and potential phytoavailability. *Appl. Geochem.* **2013**, *37*, 157–169. [CrossRef]

12. Roy, T.; Biswas, D.R.; Datta, S.C.; Sarkar, A. Phosphorus release from rock phosphate as influenced by organic acid loaded nanoclay polymer composites in an alfsol. *Proc. Natl. Acad. Sci. India Sect. B Biol. Sci.* **2018**, *88*, 121–132. [CrossRef]

13. Unuofin, F.O.; Mnkeni, P.N.S. Optimization of Eisenia fetida stocking density for the bioconversion of rock phosphate enriched cow dung–waste paper mixtures. *Waste Manag.* **2014**, *34*, 2000–2006. [CrossRef]

14. Yan, Y.W.; Nor Azwady, A.A.; Zulkifli, H.S.; Muskhazli, M.; Suraini, A.A.; Teng, S.K. Enhancement of plant nutrient contents in rice straw vermicompost through the addition of rock phosphate. *Acta Biol. Malays.* **2012**, *1*, 41–45. [CrossRef]

15. Adhami, E.; Hosseini, S.; Owliaie, H. Forms of phosphorus of vermicompost produced from leaf compost and sheep dung enriched with rock phosphate. *Int. J. Recycl. Org.* **2014**, *3*, 5. [CrossRef]

16. Busato, J.G.; Lima, L.S.; Aguilar, N.O.; Canelas, L.P.; Olives, F.L. Changes in labile phosphorus forms during maturation of vermicompost enriched with phosphorus-solubilizing and diazotrophic bacteria. *Bioresour. Technol.* **2012**, *110*, 390–395. [CrossRef]

17. Krishnaraj, P.U.; Dahale, S. Mineral phosphate solubilization: Concepts and prospects in sustainable agriculture. *Proc. Indian Natl. Sci. Acad.* **2014**, *80*, 389–405. [CrossRef]

18. Mupondi, L.T. Improving Sanitization and Fertiliser Value of Dairy Manure and Waste Paper Mixtures Enriched with Rock Phosphate through Combined Thermophilic Composting and Vermicomposting. Ph.D. Thesis, University of Fort Hare, Alice, South Africa, 2010.

19. Mupambwa, H.A.; Mnkeni, P.N.S. Optimization of fly ash incorporation into cow dung–waste paper mixture for enhanced vermicomposting and nutrient release. *J. Environ. Qual.* **2015**, *44*, 972–981. [CrossRef]

20. Das, D.; Battacharya, P.; Gosh, B.C.; Banik, P. Bioconversion and biodynamics of Eisenia fetida in different organic wastes through microbially enriched vermicomposting technologies. *Ecol. Eng.* **2016**, *86*, 154–161. [CrossRef]

21. LECO. *Tuspec CN Carbon/Nitrogen Determinator Instructors Manual*; LECO Corporation: St Joseph, MI, USA, 2003.

22. Sánchez-Monedero, M.A.; Roig, A.; Martínez-Pardo, C.; Cegarra, J.; Paredes, C. A microanalysis method for determining total organic carbon in extracts of humic substances. Relationships between total organic carbon and oxidable carbon. *Bioresour. Technol.* **1996**, *57*, 291–295. [CrossRef]

23. Maynard, D.G.; Kalra, Y.P.; Crumbaugh, J.A. Nitrate and exchangeable ammonium nitrogen. In *Soil Sampling and Methods of Analysis*, 2nd ed.; Carter, M.R., Gregorich, E.G., Eds.; CRC Press: Boca Raton, FL, USA, 2006; pp. 71–80.

24. Okalebo, J.R.; Gathua, K.W.; Woomer, P.L. Laboratory Methods of Soil and Plant Analysis: a Working Manual; TSBF-KARI-UNESCO: Nairobi, Kenya, 2002.

25. Schoenau, J.J.; O’Halloran, I.P. Sodium bicarbonate-extractable phosphorus. In *Soil Sampling and Methods of Analysis*; Taylor Francis Group: Abingdon, UK, 2008; Volume 2.

26. Kuo, S. Methods of Soil Analysis. Part 3: Chemical methods; Soil Science Society of America: Madison, WI, USA, 1996.

27. Ravindran, B.; Contreras-Ramos, S.M.; Wong, J.W.C.; Selvam, A.; Sekaran, G. Nutrient and enzymatic changes of hydrolysed tannery solid waste treated with epigeic earthworm Eudrilus eugeniae and phytotoxicity assessment on selected commercial crops. *Environ. Sci. Pollut. Res.* **2014**, *21*, 641–651. [CrossRef]

28. Greenhouse, S.W.; Geisser, S. On methods in the analysis of profile data. *Psychometrika* **1959**, *24*, 95–112. [CrossRef] [PubMed]

29. Garg, V.K.; Suthar, S.; Yadav, A. Management offood industry waste employing vermicomposting technology. *Bioresour. Technol.* **2012**, *116*, 437–443. [CrossRef]

30. Suthar, S.; Mutiyar, P.K.; Singh, V. Vermicomposting of milk processing industry sludge spiked with plant wastes. *Bioresour. Technol.* **2012**, *116*, 214–219. [CrossRef] [PubMed]

31. 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] [PubMed]

32. De Lima Rodrigues, A.S.; Mesak, C.; Silva, M.L.G.; Silva, G.S.; Leandro, W.M.; Malafaia, G. Organic waste vermicomposting through the addition of rock dust inoculated with domestic sewage wastewater. *J. Environ. Manag.* **2017**, *196*, 651–658. [CrossRef] [PubMed]

33. Unuofin, F.O.; Siswana, M.; Cishe, E.N. Enhancing rock phosphate integration rate for fast bio-transformation of cow-dung waste-paper mixtures to organic fertilizer. *SpringerPlus* **2016**, *5*, 1986. [CrossRef] [PubMed]
34. Panhwar, Q.A.; Jusop, S.; Naher, U.A.; Othman, R.; Razi, M.I. Application of potential phosphate-solubilizing bacteria and organic acids on phosphate solubilization from phosphate rock in aerobic rice. *Sci. World J.* 2013, 2013, 272409. [CrossRef]

35. Singh, S.; Singh, J.; Vig, A.P. Earthworm as ecological engineers to change the physico-chemical properties of soil: Soil vs. vermicast. *Ecol. Eng.* 2016, 90, 1–5. [CrossRef]

36. García-Gómez, A.; Bernal, M.P.; Roig, A. Organic matter fractions involved in degradation and humification processes during composting. *Compost Sci. Util.* 2005, 13, 127e135. [CrossRef]

37. He, X.; Zhang, Y.; Shen, M.; Zeng, G.; Zhou, M.; Li, M. Effect of vermicomposting on concentration and speciation of heavy metals in sewage sludge with additive materials. *Bioresour. Technol.* 2016, 218, 867–873. [CrossRef]

38. Sharma, K.; Garg, V.K. Comparative analysis of vermicompost quality produced from rice straw and paper waste employing earthworm *Eisenia fetida* (Sav.). *Bioresour. Technol.* 2018, 250, 708–715. [CrossRef]

39. Negi, R.; Suthar, S. Degradation of paper mill wastewater sludge and cow dung by brown-rot fungi *Oligoporus placenta* and earthworm (*Eisenia fetida*) during vermicomposting. *J. Clean. Prod.* 2018, 201, 842–852. [CrossRef]

40. Cai, L.; Gong, X.; Sun, X.; Li, S.; Yu, X. Comparison of chemical and microbiological changes during the aerobic composting and vermicomposting of green waste. *PloS ONE* 2018, 13, e0207494. [CrossRef] [PubMed]

41. Yadav, A.; Garg, V.K. Biotransformation of bakery industry sludge into valuable product using vermicomposting. *Bioresour. Technol.* 2019, 274, 512–517. [CrossRef] [PubMed]

42. Lazcano, C.; Gómez-Brandón, M.; Dominguez, J. Comparison of the effectiveness of composting and vermicomposting for the biological stabilization of cattle manure. *Chemosphere* 2008, 72, 1013–1019. [CrossRef] [PubMed]

43. Liu, J.; Lu, Z.; Yang, J.; Xing, M.; Yu, F.; Guo, M. Effect of earthworms on the performance and microbiological communities of excess sludge treatment process in vermicompost. *Bioresour. Technol.* 2012, 117, 214–221. [CrossRef]

44. Vig, A.P.; Singh, J.; Wani, S.H.; Dhaliwal, S.S. Vermicomposting of tannery sludge mixed with cattle dung into valuable manure using earthworm *Eisenia fetida* (Savigny). *Bioresour. Technol.* 2011, 102, 7941–7945. [CrossRef]

45. Hait, S.; Tare, V. Vermistabilization of primary sewage sludge. *Bioresour. Technol.* 2011, 102, 2812–2820. [CrossRef]

46. Malafia, G.; da Costa Estrela, D.; Guimarães, A.T.B.; de Araújo, F.G.; Leandro, W.M.; de Lima Rodrigues, A.S. Vermicomposting of different types of tanning sludge (liming and primary) mixed with cattle dung. *Ecol. Eng.* 2015, 85, 301–306. [CrossRef]

47. Soobhany, N.; Mohee, R.; Garg, V.K. Experimental process monitoring and potential of *Eudrilus eugeniae* in the vermicomposting of organic solid waste in Mauritius. *Ecol. Eng.* 2015, 84, 149–158. [CrossRef]

48. Ravindran, B.; Mupambwa, H.A.; Silwana, S.; Mnkeni, P.N. Assessment of nutrient quality, heavy metals and phytotoxic properties of chicken manure on selected commercial vegetable crops. *Heligen* 2017, 3, e00493. [CrossRef]

49. Helitha, A.M.; Manivannan, S. Enhancement of Humus Composition by Earthworms during Biotransformation of Coffee Pulp Amended with Sugar Industrial Wastes. *Int. J. Zool. Appl. Biosci.* 2018, 3, 82–88.

50. Nigussie, A.; Kuyper, T.W.; Bruun, S.; de Neergaard, A. Vermicomposting as a technology for reducing nitrogen losses and greenhouse gas emissions from small-scale composting. *J. Clean. Prod.* 2016, 139, 429–439. [CrossRef]

51. Huang, K.; Li, F.; Wei, Y.; Chen, X.; Fu, X. Changes of bacterial and fungal community compositions during vermicomposting of vegetable wastes by *Eisenia fetida*. *Bioresour. Technol.* 2013, 150, 235–241. [CrossRef]

52. De Guardia, A.; Mallard, P.; Teglia, C.; Marin, A.; Le Pape, C.; Benoist, J.C.; Petiot, C. Comparison of five organic wastes regarding their behaviour during composting: Part 2, nitrogen dynamic. *Waste Manag.* 2010, 30, 415–425. [CrossRef] [PubMed]

53. Getachew, Z.; Adisu, T.; Abele, L.; Anbessa, B. Vermicompost Potential of Common Earthworms (*Eudrilus eugeniae*) and Red Wiggler (*Eisenia fetida*) Worm on the Decomposition of Various Organic Wastes; Assosa, Ethiopia. *Int. J. Plant Soil Sci.* 2018, 24, 1–13. [CrossRef]

54. Aalok, A.; Tripathi, A.K. Composting-Vermicomposting of different types of leaves using earthworm species *Eisenia fetida*. *Dyn. Soil Dyn. Plant* 2010, 4, 139–144.

55. Schai, A.V.; Prosser, J.; Graham, D.; Xue, J.; Booth, L. The Suitability of Using Vermicomposting for the Stabilization of Septic Tank Waste. *J. Bioremediat. Biodegrad.* 2016, 7, 368. [CrossRef]

56. Dores-Silva, P.R.; Landgraf, M.D.; Rezende, M.O. Acompanhamento químico da vermicompostagem de lodo de esgoto doméstico. *Quimica Nova* 2011, 34, 956–961. [CrossRef]

57. Cesaro, A.; Belgiojorno, V.; Guida, M. Compost from organic solid waste: Quality assessment and European regulations for its sustainable use. *Resour. Conserv. Recycl.* 2015, 94, 72–79. [CrossRef]

58. Getahun, T.; Nigussie, A.; Entele, T.; Van Gerven, T.; Van der Bruggen, B. Effect of turning frequencies on composting biodegradable municipal solid waste quality. *Resour. Conserv. Recycl.* 2012, 65, 79–84. [CrossRef]
59. Jodice, R. Chemical and biological parameters for evaluating compost quality. In Proceedings of the International Symposium on Compost Production and Use, San Michele all’Adige, Italy, 20–23 June 1989; pp. 20–23.

60. Zucconi, F.; Monaco, A.; Forte, M.; De Bertoldi, M. Phytotoxins during the stabilization of organic matter. In Composting of Agricultural and Other Wastes; Gasser, J.K.R., Ed.; Elsevier: London, UK, 1985; pp. 73–85.

61. Olivera Viciedo, D.; de Mello Prado, R.; Lizzano Toledo, R.; Nascimento dos Santos, L.C.; Peña Calzada, K. Response of radish seedlings (Raphanus sativus L.) to different concentrations of ammoniacal nitrogen in absence and presence of silicon. Agronomía Colombiana 2017, 35, 198–204. [CrossRef]

62. Bernal, D.A.; Hernández, M.A.L.; Osben, H.R.B.; Ramos, S.M.C.; Mora, M. Vermicompost as an alternative of management for water hyacinth. Revista Internacional de Contaminación Ambiental 2016, 32, 425–433. [CrossRef]

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