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The Integration of Phosphorus-Solubilizing Rhizobacteria, *Eisenia fetida* and Phosphorus Rock Improves the Availability of Assimilable Phosphorus in the Vermicompost

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Abstract: Due to increasing soil degradation caused by unsustainable agricultural practices and the continued demand for quality food for the human population, it is imperative to find sustainable strategies for high-quality food production. For this reason, the objective of the present study was to evaluate the interaction between the factors of consortium of phosphorus-solubilizing rhizobacteria, addition of phosphate rock and worm load in horse manure to produce an organic fertilizer fortified with phosphorus. For this, consortia of phosphate-solubilizing rhizobacteria of the genus *Bacillus* (*Bacillus aryabhattai*, *Bacillus subtilis* and *Bacillus cereus*) isolated from the rhizosphere of *Distichlis spicata* were inoculated. Igneous phosphate rock (0 and 2%) was added in the vermicomposting process (with 25 and 50 g of *E. fetida* worms per kg of horse manure). The results obtained show that there is a significant interaction between the factors of inoculation with bacterial consortia (1×10^8 CFU mL^{-1}), phosphate rock (2%) and earthworm biomass (50 g kg^{-1} of manure), and that this interaction promotes the production of assimilable forms of phosphorus for plants (such as monobasic phosphate ions $H_2PO_4{}^{-1}$ or dibasic phosphate ions $HPO_4{}^{-2}$) within the vermicomposting process, having as a product an organic substrate supplemented with the optimal nutritional requirements for the development and growth of crops. This work can serve as a basis to produce high-quality organic fertilizer. However, field studies are required in order to observe the impact of vermicompost on the yield and quality of the fruits, and it can be compared with other types of fertilizers and the relevance of their use in different types of climates.

Keywords: bacterial consortia; sustainable agriculture; phosphate; vermidegradation

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1. Introduction

The current food demand due to the accelerated growth of the human population is a worrying issue [1]. To satisfy this demand, excessive doses of agrochemicals are commonly applied to increase and accelerate crop yields, resulting in soil degradation [2,3]. Therefore, sustainable strategies are sought to replace these practices in order to obtain organic substrates rich in nutrients that satisfy the requirements of plants [4].

One of these strategies is the use of functional microorganisms for agriculture, such as plant-growth-promoting rhizobacteria (PGPR), which have demonstrated increases in crop yields [5]. The beneficial effect of these bacteria is that they are naturally part of the biological cycles of nutrients, facilitating their availability to plants in ionic forms. Among the promoter microorganisms are phosphate-solubilizing bacteria. These have an important role in the conversion to inorganic phosphorus from phosphate rock, thus increasing its bioavailability for the plant [6].

Phosphorus is considered an essential nutrient for the development of organisms (mainly in the form of phosphate). It is also part of the structure of nucleic acids, participates in different metabolic pathways and in energy storage, regulates enzymatic activity and signaling pathways through protein phosphorylation/dephosphorylation cycles and constitutes 0.2% of the dry weight of plants [7,8]. However, phosphates are considered one of the scarcest nutrients in soil intended for agricultural activities. To cover this deficit, one strategy is the addition of phosphate rock (PR) to manure-based organic substrates, allowing the microbiota to solubilize the phosphate and convert it into plant-available forms. This process can be carried out by different microorganisms, including bacteria belonging to the genera *Azospirillum*, *Acinetobacter*, *Alcaligenes*, *Arthrobacter*, *Bacillus*, *Pseudomonas*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Proteus*, *Rhizobium*, *Serratia*, and *Xanthomonas*, among others [9,10], which transform inorganic and insoluble organic phosphorus compounds into soluble forms [11,12].

Vermicomposting can convert polluting organic waste, such as raw manure, into value-added products [13]. These products can be used in different agricultural production systems (open field or under protected systems) as an option to produce higher-quality organic foods. From this perspective, the objective of the present study was to evaluate the interaction between the factors of consortium of phosphorus-solubilizing rhizobacteria, addition of phosphate rock and worm load in horse manure to produce an organic fertilizer fortified with phosphorus.

2. Materials and Methods

2.1. Experiment Preparation

We carried out the experiment in the greenhouses of the Facultad de Ciencias Biológicas of the Universidad Juárez del Estado de Durango (FCB-UJED) (25°35′14.08″ N 103°30′2.43″ W). Three factors were evaluated: phosphate rock (PR) (0 and 2% per kg of manure), consortium of phosphorus-solubilizing bacteria (PSB) (at a concentration of 0 and 1×10^8 CFU mL^{-1}) and earthworm biomass (*E. fetida*) (25 and 50 g of worms kg^{-1} of manure). We used plastic containers (0.55 × 0.40 × 0.20 m, length × width × height). The containers had plastic lids to protect the worms and maintain the moisture of the substrate, since conditions that are too humid can cause anaerobic environments that are harmful to earthworms [14] (Figure 1).

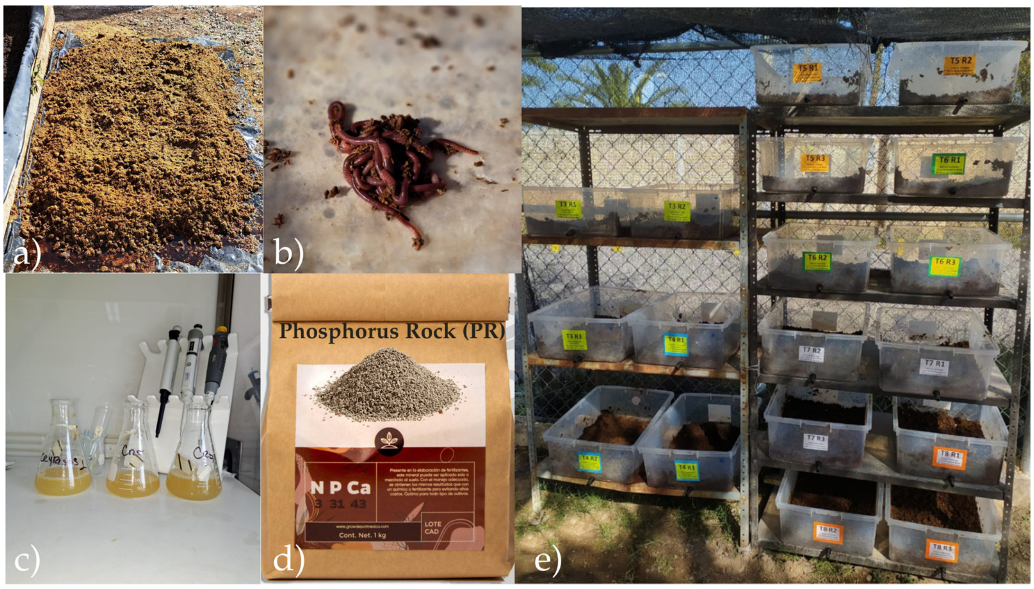

Figure 1. Establishment of the experiment and raw material used. (**a**) Horse manure, (**b**) earthworm (*E. fetida*), (**c**) phosphorus-solubilizing bacteria grown in LB nutrient broth. (**d**) Phosphorus rock and (**e**) experimental units (plastic containers).

In each plastic container, 3 kg of solarized top-quality alfalfa fodder-based horse manure (dry weight) obtained from a stable in the region of Coahuila was mixed (Figure 1a). Depending on the treatment, some containers were mixed with 2% PR (Brow Depot®) per kg of manure [15], commercial product obtained from the La Negra mine of Fosforita de México, S.A. de C.V. located in Pachuca, Hidalgo, at 20°57′55″ N 99°20′45″ W 1820 m. PR is characterized as phosphorite with high amounts of phosphate minerals of dark brown to ochre color, a density of 3.12 gr cm^{-3} and an insoluble solubility of (>0.01%). It also has a melting point of 1400 °C, a hardness of 5 Mohs, a pH of 7.25 and a phosphorus (P_2O_5) content of 29.07% [16].

The plastic containers were placed onto well-ventilated metal shelves. Also, the containers were inclined to leach the substrate solution and maintain the required moisture, which was monitored with a hygrometer. We placed juvenile *E. fetida* worms in plastic containers filled with horse manure with population densities of 25 and 50 g of worms per kg^{-1} of manure [17]. Moisture was maintained at 70% [18] during the vermicomposting period (60 days).

The bacteria used in this project were supplied by the Microbial Ecology Laboratory of the Faculty of Biological Sciences of the Universidad Juárez del Estado de Durango. As a bacterial consortium, we used strains of *Bacillus aryabhattai* (Cryrizos1), *Bacillus subtilis* (CR7) and *Bacillus cereus* (CR5). The activation of bacteria was carried out in liquid Luria Bertani (LB) culture medium; they were grown in a shaking incubator at 37 °C and 120 rpm for a period of 24 h. Once grown, we used a cell density of 1×10^8 CFU mL^{-1}, with a Neubauer chamber for counting.

2.2. Experimental Design

We used a factorial design where the biomasses of *E. fetida* (25 and 50 g kg^{-1} of manure), (Figure 1b) bacterial consortia (0 and 1×10^8 CFU mL^{-1}) (Figure 1c) and phosphate rock (0 and 2%) (Figure 1d) were considered as factors. As a result of the combination of these factors, a total of 8 treatments were obtained (Table 1) with three repetitions, for a total of 24 experimental units (Figure 1e).

Table 1. Treatments established in solarized horse manure.

Treatments	FACTOR 1. Phosphorus Rock (PR)	FACTOR 2. Biomass *E. fetida*	FACTOR 3. Cell Density of Bacterial Consortia
	(%)	g Worms per kg^{-1} Manure	CFU mL^{-1}
T1	2	25	1×10^8
T2	2	25	0
T3	0	25	1×10^8
T4	0	25	0
T5	2	50	1×10^8
T6	2	50	0
T7	0	50	1×10^8
T8	0	50	0

2.3. Sampling

In each experimental unit, sampling was carried out at three depth levels (approx. 6, 12 and 18 cm). All samples were mixed to obtain composite samples of 150 g of vermicompost per treatment. This was carried out at 30 and 60 days. The composite samples were analyzed at the Laboratorio Nacional de Servicios de Análisis de Aguas, Suelos, Plantas y Medio Ambiente del Centro Nacional de Investigación Disciplinaria en Relación Agua, Suelo, Planta, Atmósfera (CENID-RASPA) in Gómez Palacio, Durango, México.

2.4. Physicochemical Analysis

The samples were dried in an oven at a temperature of 45 °C until they reached a constant weight. They were subsequently ground (<2 mm) to obtain a homogeneous sample for the analysis of pH, electrical conductivity (EC), total nitrogen (TN), total phosphorus (TP), soluble phosphorus (SP), nitrate (NO_3^-) and ammonium (NH_4^+).

Electrical conductivity and pH were determined from a vermicompost–water suspension (1:10). This was shaken at 230 rpm for 30 min and allowed to settle for one hour before measuring pH and EC. A pocket pH/conductivity meter, model HI98129 (HANNA Instruments®), was used as described in [19]. Total nitrogen (TN) was measured by the standard digestion and distillation method with Kjeldahl equipment (Kelplus) using reagents (magnesium oxide for the determination of NH_4^+ and Devarda alloy for NO_3^- [20] to subsequently titrate with HCL (0.005 N) according to the NOM-021-RECNAT-2000 standard.

Total phosphorus (TP) was determined by digesting 0.5 g of vermicompost sample using hot-plate crude-fiber digestion equipment according to NOM-F-90-S-1978 and the Hedley 1982 method modified by Tiessen in 1993 [21,22].

2.5. E. fetida Egg and Worm Count

Once the experiment was finished (60 days), the total biomass of the worms was weighed using a digital scale (Santul 5927) and the average weight of a single individual was obtained. The eggs were counted manually with a 20 cm straight stainless steel spatula.

2.6. Statistical Analysis

The data obtained were analyzed with the Anderson Darling normality test. For data corresponding to a normal distribution, we performed analysis of variance using the Statistical Analysis System software, version 9.4 (SAS. 2016). Tukey was used as a post hoc test ($p \leq 0.05$). For data that did not have a normal distribution, we performed Kruskal–Wallis tests.

3. Results and Discussion

3.1. Vermicompost Physicochemical Characteristics

The results obtained show that there is a significant interaction between the factors of inoculation with bacterial consortia (1×10^8 CFU mL^{-1}), phosphate rock (2%) and earthworm biomass (50 g kg^{-1} of manure), and that this interaction promotes the production of assimilable forms of phosphorus for plants (such as monobasic phosphate ions $(H_2PO_4)^{-1}$ or dibasic phosphate ions $(HPO_4)^{-2}$) within the vermicomposting process, having as a product an organic substrate supplemented with the optimal nutritional requirements for the development and growth of crops. The physicochemical characteristics of the vermicompost treatments at 60 days can be seen in Table 2. During this process, the moisture in the vermireactors was between 70 and 80%, providing a favorable environment for the development and multiplication of the *E. fetida* earthworms. Some authors indicate that the best conditions for the survival of *Eisenia fetida* range between 50 and 90% moisture in the substrate [23]. This is important because it is a crucial factor that would put the vermidegradation of the organic fertilizer at risk and alter the physical and chemical characteristics throughout the process [24].

Table 2. Physicochemical parameters analyzed based on the interaction of phosphorus rock (PR), consortium of phosphorus-solubilizing bacteria (PSB) and quantity of earthworms (*E. fetida*) with the vermidegradation of nutrients in the substrate at 60 days.

	pH	EC	TN	NO_3^-	NH_4^+	TP	SP
		(dS m^{-1})	%	ppm	ppm	%	ppm
Initial Manure Values	10	2.8	0.65	Nd	Nd	0.31	Nd
T1 (2% + 25 g + 1 × 10^8)	9.10 ± 0.04 a	4.2 ± 0.06 c	1.22 ± 0.08 a	481.66 ± 53.30 a	112.12 ± 25.41 ba	1.59 ± 0.08 a	350.26 ± 28.37 b
T2 (2% + 25 g)	8.83 ± 0.04 a	4.8 ± 0.69 b	1.43 ± 0.08 a	427.33 ± 48.78 a	148.61 ± 47.76 ba	1.95 ± 0.08 a	329.99 ± 35.34 cb
T3 (25 g + 1 × 10^8)	9.00 ± 0.19 a	8.3 ± 0.80 a	1.34 ± 0.05 a	390.33 ± 38.37 a	82.39 ± 15.85 b	0.51 ± 0.01 b	252.31 ± 11.66 d
T4 (25 g)	9.03 ± 0.22 a	8.0 ± 0.60 a	1.32 ± 0.14 a	397.00 ± 110.26 a	108.28 ± 15.01 ba	0.53 ± 0.04 b	269.12 ± 28.37 cd
T5 (2% + 50 g + 1 × 10^8)	9.14 ± 0.04 a	6.6 ± 1.02 bc	1.21 ± 0.07 a	472.66 ± 75.19 a	160.27 ± 30.29 a	1.62 ± 0.09 a	442.44 ± 17.29 a
T6 (2% + 50 g)	9.15 ± 0.03 a	4.1 ± 0.76 c	1.22 ± 0.07 a	481.00 ± 79.68 a	108.87 ± 31.73 ba	1.47 ± 0.06 a	325.70 ± 20.27 cb
T7 (50 g + 1 × 10^8)	9.00 ± 0.21 a	7.1 ± 0.65 a	1.19 ± 0.18 a	435.33 ± 115.31 a	101.51 ± 16.35 ba	0.55 ± 0.07 b	346.21 ± 24.32 b
T8 (50 g)	8.87 ± 0.04 a	7.5 ± 0.00 a	1.21 ± 0.13 a	368.33 ± 112.26 a	112.69 ± 13.96 ba	0.56 ± 0.04 b	236.73 ± 28.38 d

Means and standard deviations. Same letters in the column for each factor represent non-significant differences (Tukey, $p < 0.05$). Nd = not determined.

3.1.1. pH

The pH values did not show significant differences between treatments ($p \leq 0.05$) at 60 days (Table 2). However, the highest values were the treatments containing PR, T6 (2% + 50 g, 0 CFU), T5 (2% + 50g + 1 $\times$ 10^8 CFU) and T1 (2% + 25 g + 1 $\times$ 10^8 CFU), with 9.15 $\pm$ 0.03, 9.14 $\pm$ 0.04 and 9.10 $\pm$ 0.04 respectively. These values were high with respect to the NMX-FF-109-SCFI-2007 standard; however, this work was carried out under the standards and parameters established by the USDA [25]. This specifies the parameters of a quality vermicompost, and the pH values should vary from 5.5 to 8.5. The probable cause of high pH values may be due to higher pH values of the pre-composted horse manure, which registered an initial alkaline value of 10. Different authors have mentioned that the pH of horse manure can vary depending on the type of feed, having a more acidic pH (5.7~) when the feed is enriched with grains and a more neutral pH (6.7~) when the horses are fed with grass and forage [26]. In addition, no studies have been reported with pH values higher than 8, so the high pH values in all treatments may be due to the initial pH of the horse manure, which was 10. However, it should be noted that the pH variable decreased from the beginning of the experiment. After 30 days of sampling, it decreased to 9.8, and at 60 days, the pH value was 8.83, slightly alkaline. This may be because the bacteria and number of worms contributed to the decrease in the pH of the medium towards either neutrality or acidity throughout the process [27,28]. Some authors [19,29] have mentioned that the metabolism of earthworms decreases the pH in the surface layer of the soil (Horizon A) or in the vermicompost due to the processes of bioturbation and/or vermidegradation (mineralization of nitrogen into nitrates and nitrites and phosphorus into orthophosphates) and the bioconversion of organic matter into other organic acids. In addition, the metabolism of phosphorus-solubilizing bacteria released organic acids, causing decreases in the pH of the substrate and greater permeability [30,31]. Also, it was demonstrated in [32] that microbes become additives for the reduction in solid waste in the composting process. This confirms that the vermidegradation process was successful, significantly reducing the pH noted in Figure 2 and supporting the Table 3 data that show the interactions among pH and the factors. There were significant differences according to the factors and their interactions. Regarding the separate analyses of the factors, for the worm gram factor (25 and 50 g), no significant differences were found ($p > 0.05$) in the two periods evaluated (30 and 60 days). For the phosphorus rock factor (0 and 2%), significant differences were recorded ($p \leq 0.05$) at 30 days, with a higher pH value for 2% (9.79), but not at 60 days. For the bacterial consortia factor (0 and 1 $\times$ 10^8 CFU), significant differences ($p \leq 0.05$) were recorded at 30 days, registering values of 9.62 and 9.70, respectively; on the contrary, no significant differences were recorded for this factor at 60 days. Regarding the interaction between the factors, an interaction between factors was recorded 60 days after the start of the experiment (Table 3, Figure 3a). On the contrary, there was no interaction between factors at the first sampling time (30 days). The values closest to neutral resulted from treatments T2 (2% + 25 g + 0 CFU) and T8 (0% + 50 g + 0 CFU), with 8.83 $\pm$ 0.04 and 8.87 $\pm$ 0.04. However, if the process of vermidegradation were carried out for 90 days, we could obtain more desirable pH values between 7.8 and 8.5, which are reported to be optimal for worm growth and microbial activity [33].

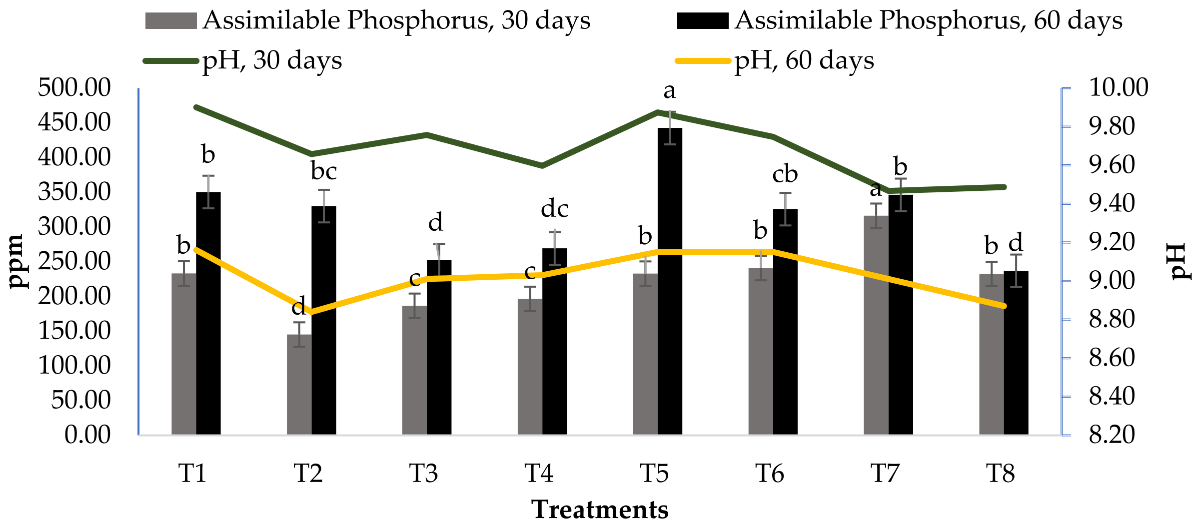

Figure 2. Statistical analysis of the behavior of the assimilable phosphorus and pH in the treatments. Bars with different letters are statistically different according to Tukey's test ($p \leq 0.05$).

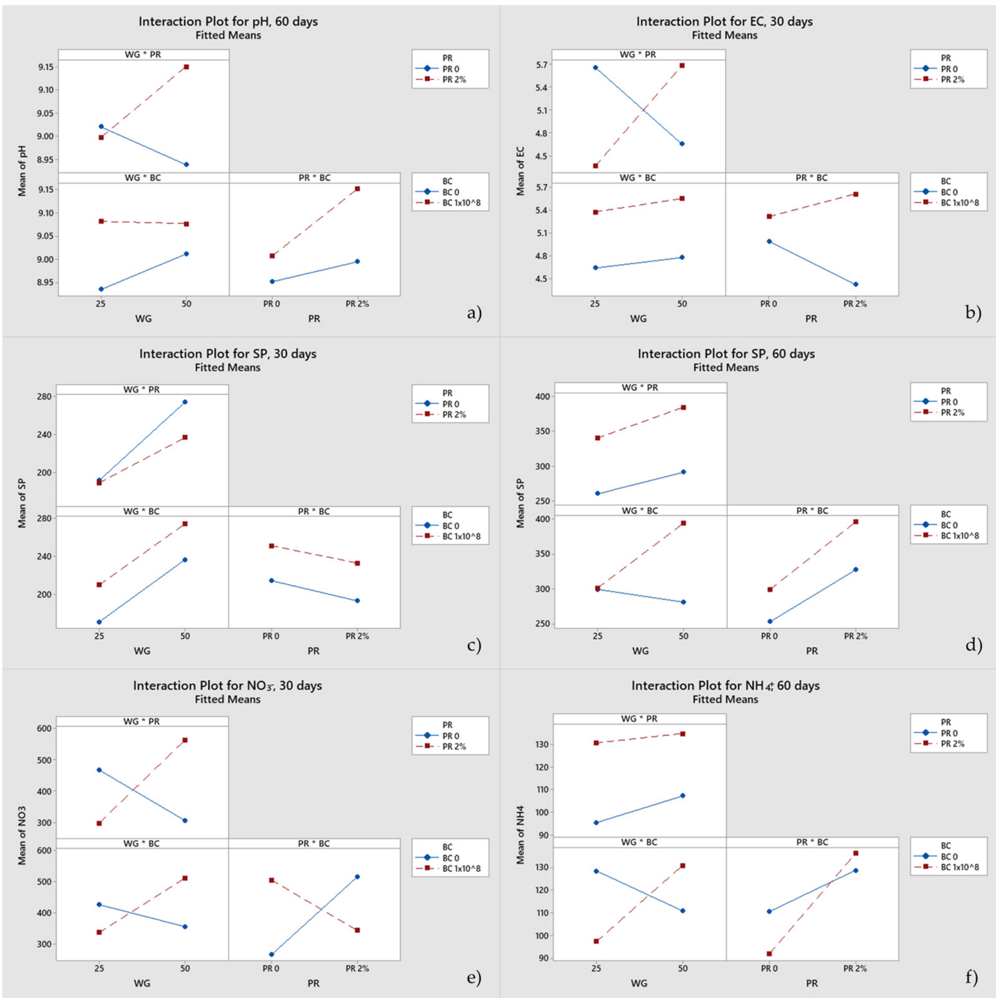

Figure 3. Graphs representing the interaction between the factors of worm grams (WG), phosphoric rock (PR) and bacterial consortia (BC) and which were significant ($p \leq 0.05$) in the factor analysis of biodegradation and accumulation of nutrients in the vermicomposting process. (**a**) pH at 60 days, (**b**) electrical conductivity (EC) at 30 days, (**c**) soluble phosphorus (SP) at 30 days, (**d**) Soluble phosphorus (SP) at 60 days, (**e**) Nitrates (NO_3^-) at 30 days and (**f**) Ammonium (NH_4^+) at 60 days.

Table 3. Factor analysis of the effects of phosphorus rock (PR), consortium of phosphorus-solubilizing bacteria (PSB) and amount of earthworms (*E. fetida*) and their interaction (WG × PR and BC) on the vermidegradation of nutrients in the substrate.

Factor	**pH**		**EC**		**TN**		**NO_3^-**		**NH_4^+**		**TP**		**SP**	
			(dS m^{-1})		**%**		**ppm**		**ppm**		**%**		**ppm**	
Worm grams (WG)														
	30 days	60 days	30 days	60 days	30 days	60 days	30 days	60 days	30 days	60 days	30 days	60 days	30 days	60 days
25 g	9.72 ± 0.04 a	9.0 ± 0.0 a	5.09 ± 0.04 b	6.3 ± 0.0 a	1.2 ± 0.05 a	1.3 ± 0.05 a	381 ± 26 b	439 ± 7.5 a	77 ± 11 b	121 ± 4.0 a	0.84 ± 0.0 a	1.2 ± 0.1 a	190 ± 33 b	300 ± 15 b
50 g	9.64 ± 2.96 a	9.0 ± 0.0 a	5.16 ± 0.09 a	6.3 ± 0.0 a	1.1 ± 0.05 a	1.2 ± 0.00 b	434 ± 27 a	424 ± 7.5 a	99 ± 11 a	113 ± 4.0 a	0.81 ± 0.0 a	1.0 ± 0.1 a	255 ± 33 a	308 ± 16 a
Phosphorus rock (PR)														
	30 days	60 days	30 days	60 days	30 days	60 days	30 days	60 days	30 days	60 days	30 days	60 days	30 days	60 days
0%	9.58 ± 0.0 b	8.9 ± 0.0 a	5.15 ± 0.0 a	5.9 ± 0.4 a	1.1 ± 0.0 a	1.3 ± 0.0 a	386 ± 22 b	397 ± 34 a	106 ± 18 a	101 ± 15 b	0.46 ± 0.4 b	0.5 ± 0.6 b	232 ± 9.5 a	276 ± 43 b
2%	9.79 ± 0.0 a	9.0 ± 0.0 a	5.02 ± 0.0 a	6.7 ± 0.4 a	1.2 ± 0.0 a	1.3 ± 0.0 a	430 ± 22 a	465 ± 34 a	70 ± 18 b	132 ± 15 a	1.19 ± 0.4 a	1.7 ± 0.6 a	213 ± 9.5 a	362 ± 43 a
Bacterial consortia (BC)														
	30 days	60 days	30 days	60 days	30 days	60 days	30 days	60 days	30 days	60 days	30 days	60 days	30 days	60 days
0 CFU	9.62 ± 0.0 b	8.9 ± 0.0 a	4.71 ± 0.3 b	6.1 ± 0.2 a	1.2 ±0.0 a	1.2 ± 0.0 a	391 ± 17 b	418 ± 13 a	74 ± 14 b	114 ± 2.5 a	0.83 ± 0.0 a	1.0 ± 0.1 a	203 ± 19 b	290 ± 28 b
1 × 10^8 CFU	9.70 ± 0.0 a	9.0 ± 0.0 a	5.46 ± 0.3 a	6.5 ± 0.2 a	1.1 ± 0.0 a	1.3 ± 0.0 a	425 ± 17 a	445 ± 13 a	102 ±14 a	119 ± 2.5 a	0.81 ± 0.0 a	1.1 ± 0.1 a	242 ± 19 a	348 ± 29 a
WG × PR × BC														
	ns	*	*	ns	*	ns	*	ns	ns	*	*	*	*	ns

Means and standard deviation. Equal letters for each factor represent non-significant differences. * = significant difference; ns = non-significant difference according to Tukey test ($p \leq 0.05$).

3.1.2. Electric Conductivity (EC)

For electrical conductivity, significant differences were recorded between treatments ($p \leq 0.05$) at 60 days, with treatments T3 (0% + 25 g + 1 $\times$ 10^8 CFU), T4 (0% + 25 g + 0 CFU), T8 (0% + 50 g + 0 CFU) and T7 (0% + 50 g + 1 $\times$ 10^8 CFU) showing the highest EC values (8.3, 8.0, 7.5 and 7.1 dS m^{-1}, respectively) (Table 2). Like the pH values, the EC values were high according to NMX-FF-109-SCFI-2007 (regarding the quality specifications of worm humus produced in Mexico), which establishes that the permissible EC value is less than 4 (dS m^{-1}), while for other countries, this value may be less than 8.2 (dS m^{-1}) [34,35]. Nevertheless, the initial electrical conductivity value was 2.8 dS m^{-1}, showing an increase in all treatments towards the end of the experiment, with treatment T3 (0% + 25 g + 1 $\times$ 10^8 CFU) being the one with the highest EC value. These variations could be attributed to the mineralization of organic matter produced by *E. fetida* organisms and inoculations with bacterial consortia [36]. In addition, some authors have mentioned that EC is undoubtedly the most impactful factor that favors the acceptability of the final product, considering that it is a good indicator of safety and suitability for agricultural purposes [37]. Regarding the separate analyses of factors, it was found that for the worm grams factor (25 and 50 g), significant differences were recorded ($p \leq 0.05$) at 30 days, with the highest value being 5.16 dS m^{-1}; however, no statistical differences were recorded at 60 days. For the phosphate rock factor (0 and 2%), no significant differences were recorded ($p > 0.05$) at either of the two sampling times. For the bacterial consortium factor (0 and 1 $\times$ 10^8 CFU), significant differences were recorded ($p \leq 0.05$) at 30 days, recording values of 4.71 and 5.46, respectively; on the contrary, no significant differences were recorded for this factor at 60 days. Regarding the interaction between factors, it was found that there was an interaction between factors at the first sampling time (30 days), but on the contrary, no interaction between factors was recorded 60 days after the experiment began (Table 3, Figure 3b).

3.1.3. Total Nitrogen, NO_3^- and NH_4^+

For the total nitrogen (TN), there were no significant differences between treatments ($p > 0.05$). However, it is important to highlight that the simple vermicomposting process increased the TN values towards the end of the experiment, since the initial TN value of the horse manure was 0.65% and at the end of the experiment, it ranged between 1.19 and 1.43% (Table 2). This could be because, as some researchers have stated, nitrogen concentrations at the end of vermicomposting are determined by the amount of nitrogen provided by the substrate material used [38,39]. Other factors that can contribute to the mineralization of the substrate are the feeding of microorganisms and worms, the release of mucus, other nitrogenous excretions and the death of worms [40]. Regarding the separate analyses of the factors, it was determined that, for the worm grams factor (25 and 50 g), no significant differences were recorded ($p > 0.05$) in the first period evaluated (30 days). On the contrary, significant differences ($p \leq 0.05$) were determined at the 60-day sampling, registering 1.3% for the 25 g. For the percentage of phosphate rock (0 and 2%) and bacterial consortium (0 and 1 $\times$ 10^8 CFU) factors, no significant differences were recorded ($p \leq 0.05$) in either of the periods evaluated (30 and 60 days) (Table 3). Specifically, for nitrate (NO_3^-) there were no significant differences between treatments ($p > 0.05$). The range of concentrations between treatments ranged from 368.3 to 481.6 ppm (Table 2). Regarding the separate analyses of the factors, it was determined that, for the worm grams factor (25 and 50 g), significant differences were recorded ($p \leq 0.05$) in the first period evaluated (30 days), with the 50 g load registering the highest concentration of NO_3^- (434 ppm). For the factors of percentage of phosphate rock (0 and 2%) and bacterial consortia (0 and 1 $\times$ 10^8 CFU), significant differences were recorded ($p \leq 0.05$) at 30 days. Regarding the interaction between factors, it was found that there was an interaction between factors at the first sampling time (30 days), but on the contrary, no interaction between factors was recorded 60 days after the experiment began (Table 3, Figure 3e). Regarding ammonium NH_4^+, according to the analysis of variance, there were significant differences between the treatments ($p \leq 0.05$), with treatment 5 being ($2\% + 50$ g $+ 1 \times 10^8$) the one that registered the highest concentration of ammonium (160 ppm), while treatment 3 (25 g $+ 1 \times 10^8$) was the one that registered the lowest concentration of this ion (82.39 ppm) (Table 2). This may be proof of the ability of these bacterial consortia to transform ammonia, a product of worms' metabolism, into ammonium. Regarding the separate analyses of the factors, it was determined that, for worm grams factor (25 and 50 g), there were significant differences ($p \leq 0.05$) in the first period evaluated (30 days), with the load of 50 g being the one that registered a higher concentration of NH_4^+ (99 ppm). At 60 days, there were no significant differences. For the percentage of phosphate rock (0 and 2%) and bacterial consortia (0 and 1×10^8 CFU) factors, significant differences ($p \leq 0.05$) were recorded in both periods evaluated. Regarding the interaction between factors, it was found that there was an interaction between factors at the second sampling time (60 days), but on the contrary, no interaction between the factors was recorded 30 days after the start of the experiment (Table 3, Figure 3f).

For the total nitrogen (TN), there were no significant differences between treatments ($p > 0.05$). However, it is important to highlight that the simple vermicomposting process increased the TN values towards the end of the experiment, since the initial TN value of the horse manure was 0.65% and at the end of the experiment, it ranged between 1.19 and 1.43% (Table 2). This could be because, as some researchers have stated, nitrogen concentrations at the end of vermicomposting are determined by the amount of nitrogen provided by the substrate material used [38,39]. Other factors that can contribute to the mineralization of the substrate are the feeding of microorganisms and worms, the release of mucus, other nitrogenous excretions and the death of worms [40]. Regarding the separate analyses of the factors, it was determined that, for the worm grams factor (25 and 50 g), no significant differences were recorded ($p > 0.05$) in the first period evaluated (30 days). On the contrary, significant differences ($p \leq 0.05$) were determined at the 60-day sampling, registering 1.3% for the 25 g. For the percentage of phosphate rock (0 and 2%) and bacterial consortium (0 and 1×10^8 CFU) factors, no significant differences were recorded ($p \leq 0.05$) in either of the periods evaluated (30 and 60 days) (Table 3). Specifically, for nitrate (NO_3^-) there were no significant differences between treatments ($p > 0.05$). The range of concentrations between treatments ranged from 368.3 to 481.6 ppm (Table 2). Regarding the separate analyses of the factors, it was determined that, for the worm grams factor (25 and 50 g), significant differences were recorded ($p \leq 0.05$) in the first period evaluated (30 days), with the 50 g load registering the highest concentration of NO_3^- (434 ppm). For the factors of percentage of phosphate rock (0 and 2%) and bacterial consortia (0 and 1×10^8 CFU), significant differences were recorded ($p \leq 0.05$) at 30 days. Regarding the interaction between factors, it was found that there was an interaction between factors at the first sampling time (30 days), but on the contrary, no interaction between factors was recorded 60 days after the experiment began (Table 3, Figure 3e). Regarding ammonium NH_4^+, according to the analysis of variance, there were significant differences between the treatments ($p \leq 0.05$), with treatment 5 being ($2\% + 50$ g $+ 1 \times 10^8$) the one that registered the highest concentration of ammonium (160 ppm), while treatment 3 (25 g $+ 1 \times 10^8$) was the one that registered the lowest concentration of this ion (82.39 ppm) (Table 2). This may be proof of the ability of these bacterial consortia to transform ammonia, a product of worms' metabolism, into ammonium. Regarding the separate analyses of the factors, it was determined that, for worm grams factor (25 and 50 g), there were significant differences ($p \leq 0.05$) in the first period evaluated (30 days), with the load of 50 g being the one that registered a higher concentration of NH_4^+ (99 ppm). At 60 days, there were no significant differences. For the percentage of phosphate rock (0 and 2%) and bacterial consortia (0 and 1×10^8 CFU) factors, significant differences ($p \leq 0.05$) were recorded in both periods evaluated. Regarding the interaction between factors, it was found that there was an interaction between factors at the second sampling time (60 days), but on the contrary, no interaction between the factors was recorded 30 days after the start of the experiment (Table 3, Figure 3f).

3.1.4. Total Phosphorus and Assimilable Soluble Phosphorus

As time goes by, it becomes more important to integrate strategies to improve crop nutrition and yield in a more sustainable way. In this sense, phosphate rock (PR) has been of great importance, since it is among the few inorganic compost amendments that are allowed in organic agriculture [41]. Regarding the total phosphorus variable (TP), significant differences were obtained between treatments ($p \leq 0.05$), resulting in a higher percentage in those to which phosphate rock was added (T1 (2% + 25 g + 1 $\times$ 10^8 CFU), T2 (2% + 25 g + 0 CFU), T5 (2% + 50 g + 1 $\times$ 10^8 CFU) and T6 (2% + 50 g + 0 CFU)). It is important to mention that the simple addition of phosphate rock (2%) to horse manure drastically increased the content of this element. The initial TP value recorded in horse manure was 0.31%, and this increased to 1.91% for T2 (2% + 25 g + 0 CFU) (Table 2). This confirms that increases in total phosphorus in the vermicompost are directly related to the supply of phosphate rock in the treatments. According to the separate analyses of the factors, it was found that for the worm grams factor (25 and 50 g), there were no significant differences ($p > 0.05$) in any of the periods evaluated (30 and 60 days); however, it is important to mention that it increased, on average, from 0.82% to 1.1% in the TP content towards the end of the experiment. However, it is important to highlight that this phosphorus is not completely assimilated by crops. Phosphorus assimilated by plants occurs in the forms of dihydrogen phosphate ($H_2PO_4{}^{-1}$) and hydrogen phosphate ($HPO_4{}^{-2}$). For the percentage of phosphate rock factor (0 and 2%), obvious significant differences were recorded in both periods evaluated. For the treatments with 2% of phosphate rock, a value of 1.19 was reached at 30 days and a value of 1.7 was reached at 60 days. The bacterial consortia factor (0 and 1 $\times$ 10^8 CFU) behaved in a similar way to the grams of worm factor. Regarding the interaction between factors, it was found that there was an interaction between factors at both evaluation times (Table 3). One of the most important variables and objects of study in the present investigation was the presence of assimilable phosphorus in the designed vermicomposts. According to the ANOVA, significant differences ($p \leq 0.05$) were found between the treatments; the T5 treatment (2% + 50 g + 1 $\times$ 10^8) had the highest quantified concentration of soluble phosphorus with 442.44 ppm. This was mainly due to the biodegradation and solubilization processes carried out by the worms in interaction with the bacterial consortium in the substrate [42]. Regarding the separate analyses of the factors, it was determined that for the grams of earthworm factor (25 and 50 g), significant differences were recorded ($p \leq 0.05$) for both periods evaluated (30 and 60 days), highlighting that the highest values were quantified for the 50 g load. This is supported by studies that have shown that the joint action between bacteria and worms increases the solubilization of phosphorus due to the intestinal phosphatases of the worms [43]. This alkaline phosphatase (esterase group phosphatase) from worm feces is directly involved in the phosphorus cycle [44]. For the percentage of phosphate rock factor (0 and 2%), significant differences were recorded only for the 60-day period, reaching an average value of 362 ppm for the 2% load. For the bacterial consortia factor (0 and 1 $\times$ 10^8 CFU), significant differences were recorded in both periods, highlighting that for the 30-day period, the treatments with bacterial load reached 242 ppm, and for the 60-day period, a concentration of 348 ppm was reached. Also, phosphorus-solubilizing bacteria release organic acids that reduce pH and improve permeability, since these organic acids are byproducts of microbial fermentation produced by oxidative respiration, while glucose is used as a carbon source [30]. This is why a positive relationship was confirmed between the release of organic acids and the segregation of phosphatases, with an effect on the solubilization of phosphate rock. Regarding the interaction between factors, it was determined that if there was an interaction at 30 days, this was not the case for 60-day evaluation between factors evaluated at both times (Table 3, Figure 3c,d).

3.1.5. *E. fetida* Earthworm Biomass and Number of Eggs

According to the Kruskal–Wallis test, significant differences were recorded in the two variables analyzed, i.e., earthworm biomass (H-Value = 18.05, $p = 0.012$) and number of egg capsules (H-Value = 21.26, p = 0.003). The treatment that led to a greater biomass of *E. fetida* was treatment T6 (2% + 50 g + 0 CFU), with an average weight per individual of 1.03 ± 0.3 g (Table 4), while treatment T1 (2% + 25 g + 1 × 10^8 CFU) was the one that presented the lowest biomass, with 0.23 ± 0.06 g. For the number of eggs variable, the treatment that had the greatest impact was treatment T8 (0% + 50 g + 0 CFU), with an average of 384 ± 65.29, while the treatment that recorded the lowest number of eggs was T5 (2% + 50 g + 1 × 10^8), with an average of 132. Curiously, the treatments to which bacterial consortia were not added were those that presented a higher biomass and number of eggs. This is because, as some authors [45,46] have mentioned, the metabolic activity of worms decreases with the presence of bacterial communities. However, the survival of the organisms is not affected. In this research, it is shown that the presence of the bacterial consortium, beyond having a limiting effect on the reproduction of the worms, participates efficiently in the process of nutrient bioavailability.

Table 4. Results of the KruskalWallis test determining the differences in biomass and *E. fetida* eggs between treatments.

	Worm Biomass H-Value = 18.05 p = 0.012				Egg Capsules H-Value = 21.26 p = 0.003			
Treatment	**Mean (g)**	**Median**	**Mean Rank**	**Z-Value**	**Mean**	**Median**	**Mean Rank**	**Z-Value**
T1 (2% + 25 g + 1 × 10^8)	0.23 ± 0.06	0.23529	2.0	−2.75	160 ± 37.70	156	14.5	−1.87
T2 (2% + 25 g)	0.83 ± 0.08	0.83333	20.3	2.05	216 ± 53.70	216	27.2	0.5
T3 (25 g + 1 × 10^8)	0.52 ± 0.11	0.58333	10.3	−0.57	168 ± 77.40	144	16.5	−1.5
T4 (25 g)	0.51 ± 0.12	0.43750	10.7	−0.48	221.33 ± 26.85	220	27.8	0.62
T5 (2% + 50 g + 1 × 10^8)	0.47 ± 0.13	0.38462	8.8	−0.96	180 ± 103.51	132	17.2	−1.37
T6 (2% + 50 g)	1.03 ± 0.3 *	1.00000	22.7 *	2.66	192 ± 42.93	192	20.8	−0.69
T7 (50 g + 1 × 10^8)	0.54 ± 0.13	0.54000	11.2	−0.35	214 ± 43.04	210	26.5	0.37
T8 (50 g)	0.62 ± 0.08	0.58974	14.0	0.39	384 ± 65.29	348	45.5 *	3.93
Overall			12.5				24.5	

* = significant difference.

4. Conclusions

This study evaluated the potential of phosphorus-solubilizing rhizobacteria to enhance the degradation and nutrient release from phosphate rocks in vermicompost based on horse manure, aiming to create an organic fertilizer enriched with soluble phosphorus. While total nitrogen and NO_3^- values did not show significant differences across treatments, NH_4^+ levels correlated with treatments containing higher worm populations (T5, T6, T7 and T8). Notably, the results underscored the importance and potential of phosphorus-solubilizing rhizobacteria and their interaction with worms in the promotion of soluble phosphorus availability in the substrate (vermicompost from horse manure). Treatments that included phosphate rock and bacterial consortia achieved high levels of $(H_2PO_4)^{-1}$. In addition to the above, the treatment with the highest worm load was the one that presented the highest amount of soluble phosphorus (T5). This positive interaction between worms and bacteria enhanced phosphorus solubilization, highlighting the potential to produce vermicompost suitable for phosphorus-deficient soils. Such vermicompost could significantly improve soil nutritional characteristics, thereby boosting crop yields. The findings of this research provide a foundation for producing high-quality organic fertilizer that contributes to environmental sustainability. Future research should address the optimal application rates of this product and involve field trials to compare yields with other fertilizers, assessing its relevance across various crops and climates.

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