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Foliar Supplementation of Recycled Phosphorus from Cattle Bone Meal Improves Soybean Growth Characteristics, Nutrient Content, and Chlorophyll Pigment Concentration

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Abstract: Plants can absorb only 30 to 40% of nutrients from the soil through the root system because the absorption process depends on soil properties, wheatear conditions, and the plant's species. Therefore, foliar fertilization using macro- and micronutrients has proven to be an excellent alternative. Herein, we evaluated the foliar application of a neutralized sulfuric bone meal hydrolysate (NSBMH) on soybean growth parameters, pod yield, nitrogen, phosphorus, and chlorophyll pigment concentrations under greenhouse conditions. A complete randomized block design was performed, and each block contained three treatments: 1% NSBMH, commercial fertilizer, and negative control. After 90 days of growth, soybean plants foliar-sprayed with 1% NSBMH improved significantly ($p < 0.05$) in terms of foliar area, plant fresh mass, plant dried mass, plant height, nitrogen, and chlorophyll $a + b$ concentrations, while trifoliar leaf number, pod number, and pod fresh and dried masses were higher but not significant, and phosphorus concentration maintained suitable levels when compared to the negative control treatment. Additionally, the 1% NSBMH group presented similar and higher values, but not significant ($p > 0.05$), on the evaluated traits versus the commercial fertilizer treatment. Consequently, cattle bone recycling for the obtainment of alternative phosphorus as neutralized sulfuric bone meal hydrolysate is an excellent choice because it encourages the reutilization of anthropogenic waste, such as cattle bone waste, which protects the environment and reduces the soil and foliar application of mineral phosphoric fertilizers and reduces dependency on the main unsustainable fertilizer suppliers.

Keywords: bone meal; foliar; fertilizer; hydrolysate; phosphorus; soybean

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1. Introduction

Plants generally uptake nutrients from soil and sediments through their root system. Unfortunately, just 30 to 40% of these nutrients are absorbed by plants because of the low efficiency usage of nutrients by a variety of plant species, the inefficient utilization of fertilizers in soils, different soil characteristics (e.g., pH, porosity, water content, and mineral content), and/or weather conditions [1,2]. Therefore, in order to overcome these drawbacks, the foliar application of macronutrients (N-P-K) and micronutrients (Zn, Mn, Fe, Cu, Bo) has proven to have positive effects on a variety of plants, for example: tomato, maize, wheat, soybean, among others [2–7]. In fact, maize plants can absorb up to 32% of total phosphorus when it is foliar-applied [8].

Brazil is the highest producer and exporter of soybeans; Brazil's 2022–2023 production and exportations of soybeans have been forecasted to be 139 MMT and 87 MMT (million metric tons), respectively [9]. Worldwide, soybean is an important oil seed crop because a variety of products can be obtained from it, such as oleochemicals, vegetable oil, and protein meal for human and animal feed [10–12]. Additionally, soybean by-products, such as hulls, okara, okara flour, and soymilk, can be used for the production of plant growth hormones, prebiotics, and bioethanol in biorefineries [10,13]. Therefore, in order to maintain the actual production levels of soybean, Brazil is importing around 85% of its total fertilizers and uses 40% of it exclusively for soybean production [9]. However, Brazil's soybean production has been affected in recent years because of the rises in fertilizer prices caused by the COVID-19 pandemic and the Russian invasion of Ukraine in February of 2022. This last factor is the primary concern, because Russia is the leading global supplier of fertilizers, and Brazil imports around one-quarter of its fertilizers from Russia [9].

Additionally, industrialized crops, such as soybean, require high amounts of nutrients, such as phosphorus, in order to improve crop yield per unit of area with the aim of achieving food security [14].

Phosphorus is a vital nutrient for plant growth and development because it participates in cellular metabolism, such as through cell division, photosynthesis, and nutrient transport, among other processes; and in important cellular molecules, such as nucleic acids, adenosine triphosphate (ATP), and phospholipids [15–17]. Currently, most of the phosphorus used for soybean production comes from solid granulated phosphate-based products, such as mono-ammonium phosphate (MAP), di-ammonium phosphate (DAP), and triple superphosphate (TSP) [18–28], which are added periodically in order to maintain soluble phosphorus on soils; however, their extensive use can cause erosion and eutrophication [1]. Moreover, foliar application of mineral phosphoric fertilizers (because of their high concentration and high rate of utilization) can produce negative effects on plants, such as burns and/or necrotic spots on leaves. Consequently, foliar area, photosynthesis, dry weight, plant height, pod yield, and nutrient concentration, among other parameters, can be diminished [29–34].

According to Jayathilakan et al. [35], cattle bones represent 10% to 15% of the animal mass, which, after the slaughtering process, can be further processed for the obtainment of cattle bone meal [36]. Bone meals contain high phosphorus content (2.21–9.62% d.w.) [36] and have been used as an alternative phosphorus source in soils for the production of barley, oat, maize, and wheat [37–40]. However, the correct use of bone meals depends on soil pH, weather conditions, plant species, and method of application [39]. Thus, in order to overcome the limitations presented by bone meals and liberate the phosphorus present on them, chemical hydrolysis using a strong acid can be applied to release the fixed phosphorus on the bone meal into the liquid (hydrolysate), resulting in a renewable phosphorus fertilizer [41,42].

Nieto Monteros [43] developed a process for the recycling of phosphorus from cattle bone meal and proved its benefits as a liquid soil fertilizer for an alternative phosphorus source on soybean plants, but it can have some disadvantages as described above. Thus, here we evaluated the foliar supplementation of recycled phosphorus from cattle bone meal on soybean growth parameters, pod yield, nitrogen, phosphorus, and chlorophyll pigment concentrations under greenhouse conditions.

2. Materials and Methods

2.1. Fertilization Trail under Greenhouse Conditions

2.1.1. Greenhouse

The greenhouse is located at the Polytechnic Center of Federal University of Paraná (UFPR). The greenhouse is made of white plastic and its dimensions are 3.0 m wide, 6.0 m long, and 3.0 m height. Additionally, it has an automated temperature and humidity control and a fan.

2.1.2. Fertilizers

Table 1 contains the nitrogen and phosphorus composition of the fertilizers used in this study. The 1% NSMBH was obtained by mixing 10 milliliters of it with 20 mL of glycerin in deionized water; then, the solution was brought up to 1000 mL with deionized water. The commercial organo-mineral fertilizer (positive control) was prepared by mixing 10 mL of it in deionized water and brought up to 1000 mL (according to manufacture).

Table 1. Nitrogen and phosphorus composition of the fertilizers.

	Neutralized Sulfuric Bone Meal Hydrolysate (NSBMH) [43]	Commercial Organo-Mineral Fertilizer (Positive Control)
* Nitrogen (%)	3.28	2.38
** Phosphorus (%, as P_2O_5)	2.2	4.7

* Nitrogen was determined by Kjeldahl method. ** Phosphorus was determined by spectrophotometric molybdovanadate method.

2.1.3. Soybean Plants

In early September of 2021 (spring season) and under the greenhouse conditions of temperature and humidity of 30.5 °C and 51.6%, respectively, soybean seeds were sown in one-liter plastic cups which contained wet vermiculite (substrate). Then, one plantlet per cup was maintained for fertilization test.

2.1.4. Experiment

The experiment was performed during the spring season, September to November of 2021, in which the mean weather conditions of temperature, radiation, and precipitation were 17.81 °C, 1053.02 Kj m^{-2}, and 0.14 mm, respectively [44]. Greenhouse conditions of mean temperature and humidity were 28.6 °C and 61.9%, respectively.

A complete randomized block design experiment was developed in order to test each fertilizer. Three blocks were set; each block contained three treatments (1% NSBMH, commercial fertilizer, and negative control), and each treatment had five plants. Then, fertilization was performed on the 30th and 60th DAS (days after sowing) by spraying each fertilizer three times in the leaves, while nothing was sprayed in the negative control treatment. Watering was performed with running water when needed.

2.2. Leaf Measurements

2.2.1. Foliar Area and Leaf Number

A week before each fertilization and a week before ending the experiment, the perimeter of the last fully formed trifoliate leaf was drawn on paper and then scanned, and the foliar area (cm^2) was obtained with the online version of Image J [45]. Then, after ninety days of growth, leaf number was counted for all the treatments.

2.2.2. Chlorophyll Pigment Concentration

A week before each fertilization and a week before harvesting, three circular discs with 23 mm diameter were cut from the middle of each leaf of the last fully expanded trifoliate. Pigments were extracted from these leaf discs with 8 mL of cold methanol in a mortar and pestle, and then the liquid was centrifuged at 2500 rpm for 10 min. The absorbance of the supernatant was recorded in a range of 750 to 600 nm in a Shimadzu UV-1601PC spectrophotometer, while methanol was used as blank [46]. Finally, chlorophyll pigments *a*, *b*, and *a* + *b* by area (cm^2) were calculated with the equations proposed by Porra; Thompson; Kriedemann [46]; and Fan et al. [47].

2.2.3. Nitrogen and Phosphorus Quantification

Sample Processing

After harvesting, plants from each treatment were put into a paper bag and air-force-dried at 65 °C for 72 h [24]. Then, the dried leaves from each treatment were hand-milled in a mortar and pestle and then passed through a TYLER/MESH 45 sieve. The passing material was used for nitrogen and phosphorus quantification.

Nitrogen

Nitrogen concentration and protein content were quantified by the automated Kjeldahl method (method 920.87) for soya beans and lupins [48,49]. The nitrogen-to-protein conversion factor used was 4.43 [50].

Phosphorus

Phosphorus as P_2O_5 (*w/w*) was quantified by the spectrophotometric molybdovanadate method [49].

2.3. Soybean Growth Measurements

2.3.1. Plant Height

After 90 days, with the help of a meter, the total plant height, as well as the shoot height and the root length, were measured.

2.3.2. Fresh and Dried Masses

Fresh and dried masses of soybean plants were obtained after 90 days. First, the total mass of the fresh plant was measured, then the separate shoot and root mass using an electric scale. After that, shoot and root were put into a paper bag and air-force-dried at 65 °C for 72 h [24], and then the dried mass was obtained.

2.3.3. Soybean Pod Yield

The number of pods from each treatment was counted on the day of the harvesting. After that, pod fresh mass was assessed using an analytical scale. Then, the pods were air force dried at 65 °C for 72 h, and later the dried mass was obtained.

2.4. Statistical Analysis

From each treatment, three samples were obtained for foliar area, trifoliar leaf number, pod number, and pod fresh and dried masses. Additionally, two samples from each treatment were used for chlorophyll pigment concentration. Moreover, one sample from each fertilizer treatment was used for nitrogen and phosphorus quantification in leaves, while for the negative control, a composite sample for each treatment was made from three samples. Additionally, total plant fresh and dried masses and plant height were obtained from all the treatments. Then, the collected data were processed in Microsoft Office Excel 2019 and Tukey's test (95% confidence) was performed for all the evaluated traits in order to establish significant differences between the means of the treatments using Minitab 19 Statistical Software.

Analysis of variance tables for the evaluated traits can be found in the Supplementary Materials.

3. Results

3.1. Foliar Area and Leave Number

Table 2 shows the mean values and improvement obtained for foliar area for each treatment after 30, 60, and 90 DAS under greenhouse conditions.

The treatment fertilized with 1% NSMBH registered a significant improvement ($p < 0.05$) in foliar area versus the negative control treatment for the 60th day (30 days after the first fertilization) and 90th day (30 days after the second fertilization) (Table 2), whereas when compared to the positive control, 1% NSMBH treatment registered a higher foliar area for the 60th day and a similar value for this trait at the 90th day, but were not significantly different ($p > 0.05$) (Table 2). In addition, 1% NSBMH treatment registered the higher foliar area at the 60th day (30 days after the first fertilization) which was significantly different ($p < 0.05$) from the 30th and 90th day within this treatment, while no significant difference ($p > 0.05$) was registered for this characteristic within the negative control and positive control treatments (Table 2).

Table 2. Comparison of soybean foliar area (cm^2) between the treatments.

	Treatment					
Time	**Control (−)**		**Control (+)**		**1% NSBMH**	
Day	*** Mean ± S.D.**	**** Improvement (%)**	**Mean ± S.D.**	**Improvement (%)**	**Mean ± S.D.**	**S.D. (±)**
30	11.05 ± 1.88 Aa	-	9.78 ± 3.26 Aa	-	9.17 ± 0.85 Ba	2.22
60	11.92 ± 3.06 Ab	68.37	14.52 ± 3.24 Aab	38.22	20.07 ± 1.80 Aa	2.77
90	7.29 ± 0.34 Ab	52.53	11.38 ± 0.62 Aa	-	11.12 ± 0.60 Ba	0.53
S.D. (±)	2.08		2.67		1.19	

* Means that do not share a letter are significantly different according to Tukey's test ($p < 0.05$); capital letters are within the same treatment and lower-case letters among treatments. Mean = 3 ± S.D. ** Percentage of improvement of the NSBMH versus the negative control and positive control, respectively.

After ninety days of growth, no significant difference ($p > 0.05$) was registered between the treatments for the trifoliar leaf number. The 1% NSBMH treatment presented the higher trifoliar leaf number, followed by the positive control treatment and the negative control treatment as follows: 5.67 ± 0.88, 5.33 ± 1.20, and 4.11 ± 1.02. These represent a 6.38% and 37.96% improvement of the 1% NSBMH treatment over the positive and negative control treatments, respectively.

3.2. Chlorophyll Pigments Concentration

Table 3 shows the concentrations of chlorophyll pigments a, b, and a + b obtained after 30, 60, and 90 days of growth for the treatments that were foliar-fertilized with 1% NSBMH and the commercial fertilizer (positive control), and the negative control treatment under greenhouse conditions.

Table 3. Comparison of chlorophyll pigment concentration *a*, *b*, and *a* + *b* (ug cm^{-2}) between the treatments.

	Chl *a*			
Time	**Control (−)**	**Control (+)**	**1% NSBMH**	
Days	*** Mean ± S.D.**	**Mean ± S.D.**	**Mean ± S.D.**	**S.D. (±)**
30	34.597 ± 1.32 Aa	35.077 ± 2.59 Aa	36.287 ± 3.54 Aa	2.64
60	27.866 ± 3.31 Ba	27.445 ± 2.21 Ba	29.714 ± 2.12 Aa	2.60
90	26.405 ± 1.30 Bb	33.802 ± 3.23 ABa	33.494 ± 2.90 Aa	2.61
S.D. (±)	2.19	2.71	2.91	
	Chl *b*			
30	8.526 ± 0.48 Aa	8.512 ± 0.22 ABa	8.894 ± 0.92 Aa	0.61
60	7.295 ± 0.92 Aa	6.916 ± 0.39 Ba	7.778 ± 0.71 Aa	0.71
90	7.054 ± 0.56 Aa	9.176 ± 1.15 Aa	9.308 ± 1.06 Aa	0.96
S.D. (±)	0.68	0.71	0.91	
	Chl *a* + *b*			
30	43.123 ± 1.77 Aa	43.589 ± 2.77 Aa	45.181 ± 4.27 Aa	3.11
60	35.160 ± 4.15 Ba	34.361 ± 2.59 Ba	37.492 ± 2.83 Aa	3.26
90	33.458 ± 1.80 Bb	42.978 ± 4.37 Aa	42.802 ± 3.96 Aa	3.56
S.D. (±)	2.80	3.34	3.74	

* Means that do not share a letter are significantly different according to Tukey's test ($p < 0.05$); capital letters are within the same treatment and lower-case letters are among treatments. Mean = 3 ± S.D.

The concentration of chlorophyll pigments a and a + b increased significantly ($p < 0.05$) on 1% NSBMH treatment when compared to the negative control treatment just for the 90th day (30 days after the second fertilization), whereas versus the positive control treatment it presented similar values on chlorophyll pigment concentrations a, b, and a + b during the time of growth (Table 3).

In addition, chlorophyll pigment concentrations a, b, and a + b changed over the time of growth within each treatment (Table 3). The negative control treatment presented a significant decrease ($p < 0.05$) on chlorophyll pigment concentrations a and a + b for the 60th and 90th day when compared to the 30th day. Additionally, a similar trend was registered for the positive control treatment where a significant decrease ($p < 0.05$) on the concentration of chlorophyll pigments a and a + b was noticed just for the 60th day (30 days after the first fertilization) when compared to the 30th and 90th day. Additionally, within the positive control treatment, chlorophyll pigment concentration b increased significantly ($p < 0.05$) for the 90th day (30 days after the second fertilization) when compared to the 60th day; however, this result was not significantly different when compared to the concentration obtained at 30 DAS. On the contrary, 1% NSBMH treatment did not present significant variations for chlorophyll pigment concentration during the time of growth.

3.3. Nitrogen and Phosphorus Concentration

Nitrogen concentration was significantly different ($p < 0.05$) between the 1% NSBMH and the commercial fertilizer treatments when compared to the negative control treatment, whereas the 1% NSBMH treatment reached similar values than the commercial fertilizer treatment after 90 days of growth (Table 4). Consequently, nitrogen and protein percentage were higher on the 1% NSBMH treatment, followed by the commercial fertilizer treatment and the negative control treatment (Table 4).

Table 4. Comparison of nitrogen concentration, % N, % protein, and phosphorus concentration (as P_2O_5) between treatments.

Treatment	* Mean ± S.D. ($gN\ g^{-1}$)	%N	% Protein (%N × 4.43)	Mean ± S.D. ($gP_2O_5\ g^{-1}$)	$\%P_2O_5$
** Control (−)	0.0270 ± 0.001 b	2.70	11.98	0.00145 ± 0.0002 a	0.15
*** Control (+)	0.0303 ± 0.001 a	3.03	13.41	0.00140 ± 0.0001 a	0.14
1% NSBMH	0.0304 ± 0.001 a	3.04	13.48	0.00136 ± 0.0001 a	0.14

* Means that do not share a letter in the same column are significantly different according to Tukey's test ($p < 0.05$). Mean = 3 ± S.D. ** Control (−): compose sample of three randomly selected samples from each block. *** Control (+): commercial fertilizer.

Phosphorus concentration (as P_2O_5) did not present a significant difference ($p > 0.05$) between the treatments after 90 days of growth (Table 3).

3.4. Soybean Growth Characteristics

3.4.1. Plant Height

Ninety days after growth, the 1% NSBMH treatment significantly improved ($p < 0.05$) shoot and plant height versus the negative control treatment, and attained higher values for shoot and plant height when compared to the positive control treatment, but were not significantly different (Table 5).

3.4.2. Plant Fresh and Dried Masses

After 90 days of growth, the 1% NSBMH treatment significantly improved ($p < 0.05$) plant fresh and dried masses (except for root fresh mass) versus the negative control treatment (Table 5). Additionally, 1% NSBMH treatment reached higher values on plant fresh and dried masses versus the positive control treatment, and presented a significant difference ($p < 0.05$) for shoot dried mass (Table 5).

3.4.3. Soybean Pod Yield

After 90 days of growth, the 1% NSBMH treatment showed higher values for pod number and pod fresh and dried masses versus the negative control treatment, and reached similar numbers for these parameters when compared to the commercial fertilizer treatment (Table 5). However, no significant difference ($p > 0.05$) was noticed for these parameters between the treatments (Table 5).

Table 5. Comparison of agronomical traits between the treatments.

		Treatment				
		Control (−)		Control (+)		1% NSBMH
Parameter		*** Mean ± S.D.**	**** Improvement (%)**	**Mean ± S.D.**	**Improvement (%)**	**Mean ± S.D.**
Plant height (cm)	Shoot	49.10 ± 1.05 b	45.42	65.50 ± 5.05 a	9.01	71.40 ± 1.65 a
	Root	34.80 ± 1.71 b	24.71	45.30 ± 6.60 a	-	43.40 ± 2.35 ab
	Plant	83.90 ± 2.29 b	36.83	110.80 ± 9.01 a	3.61	114.80 ± 1.87 a
Fresh mass (g)	Shoot	5.99 ± 0.79 b	157.76	12.57 ± 2.33 a	22.83	15.44 ± 0.32 a
	Root	10.49 ± 0.59 a	73.21	16.27 ± 4.31 a	11.68	18.17 ± 5.65 a
	Plant	16.48 ± 1.06 b	103.88	28.84 ± 6.18 ab	16.50	33.60 ± 5.96 a
Dry mass (g)	Shoot	1.36 ± 0.19 c	208.09	3.25 ± 0.52 b	28.92	4.19 ± 0.23 a
	Root	1.34 ± 0.09 b	157.46	2.76 ± 0.71 ab	25.00	3.45 ± 1.07 a
	Plant	2.70 ± 0.24 b	182.96	6.01 ± 1.18 a	27.12	7.64 ± 1.29 a
Pod number (plant^{-1})		1.89 ± 0.51 a	70.37	3.00 ± 2.60 a	7.33	3.22 ± 2.80 a
Pod fresh mass (plant^{-1})		0.86 ± 0.41 a	68.60	1.57 ± 1.61 a	-	1.45 ± 1.26 a
Pod dried mass (plant^{-1})		0.16 ± 0.08 a	50.00	0.28 ± 0.30 a	-	0.24 ± 0.21 a

* Means that do not share a letter in the same line are significantly different according to Tukey's test ($p < 0.05$). Mean = 3 ± S.D. ** Percentage of improvement of the NSBMH treatment versus the negative control and positive control treatments, respectively. "-": no improvement.

4. Discussion

After 90 days, soybean plants grown under greenhouse conditions and foliar-sprayed with 1% NSBMH significantly improved in terms of foliar area, plant height, plant fresh mass, plant dried mass, nitrogen, and chlorophyll $a + b$ concentrations, while trifoliar leaf number, pod number, and pod fresh and dried masses increased but not significantly, and phosphorus concentration presented a slight decrease when compared to the negative control treatment, whereas versus the commercial fertilizer treatment it attained similar or higher improvement on the evaluated traits, and on the chemical and biochemical parameters. These results have potential and we recommend further trials in the field.

Leaf number increase was favored by the foliar supplementation of nitrogen and phosphorus, which were immediately used by the plants for growth and development. Our results are in accordance with those obtained by Sharifi et al. [33], who reported a 1 to 5% improvement on leaf number when compared to the positive control group. However, Sharifi et al. [33] used a water-soluble fertilizer composed of urea, single-super phosphate, and muriate of potash as an N-P-K nutrient source and utilized it as a basal fertilizer (before sowing) and as a foliar fertilizer during different growth stages of soybean plants.

Foliar area enlargement and maintenance were improved by the foliar supply of N and P as well. These nutrients were quickly translocated within the leaves and utilized for growth and development. These results are in agreement with Boote et al. [30], who observed a leaf area extension after foliar spraying of potassium polyphosphate during the seed-filling period of soybean when compared to the non-foliar-sprayed group. However, the leaf area extension registered by Boote et al. [30] lasted only one day, while ours lasted sixty days. On the contrary, Haq [29] registered a decrease between 2.21 to 3.79% on the soybean foliar area of the group foliar-sprayed with a commercial fertilizer 3-8-15 (N-P-K) composed of H_3PO_4, liquid ammonia, and KOH. The short period extension on leaf area observed by Boote et al. [30] and the decrease on foliar area registered by Haq [29] can be explained by burning and necrotic spots caused by the fertilizer salts and the application rate used by these authors, as suggested by Boote et al. [30]. These negative effects were not present in our treatment that was foliar-sprayed with 1% NSBMH because the 1% NSBMH foliar fertilizer was applied twice with a 30-day gap between each application and contained a well-balanced amount of nutrients (N-P), an optimum pH, and glycerin. This last factor, according to Barèl [51], when present in foliar fertilizers, prevents sprays from drying completely from the leaf surface, which at the same time diminishes the appearance of burning and necrotic spots on leaves caused by foliar fertilization. Additionally, when glycerin is part of foliar fertilizers containing N-P-K nutrients, it can increase by seven-fold the absorption of phosphorus by leaves, which allows them to utilize this vital nutrient immediately for growth and development [51].

Soybean leaves presented a high nitrogen concentration, which was a result of the foliar application of 1% NSBMH that contained soluble nitrogen. Consequently, nitrogen percentage and protein content were higher too. These results are in agreement with Haq and Mallarino [52], who registered a higher nitrogen concentration than ours after the foliar application of a commercial fertilizer 3-8-15 (N-P-K) during the vegetative stage of soybean plants on field trials. On the contrary, Parker and Boswell [34] and Milanez De Rezende et al. [31] registered a decline in nitrogen concentration in leaves after the foliar application of urea K-polyphosphate mix and NH_4-polyphosphate mix, and Quimifol P30, respectively, during the reproductive stage of soybean plants. The decrease in nitrogen concentration could be an effect of its translocation from leaves to pods and seeds [34].

Phosphorus concentration in soybean leaves did not present significant variation, even after the foliar application of 1% NSBMH, which contained soluble phosphorus. Our findings are in accordance with those of Haq and Mallarino [52] and Milanez De Rezende et al. [31], who did not find significant variation in phosphorus concentration in leaves after the foliar application of the commercial fertilizers 3-8-15 (N-P-K) and Quimifol P30, respectively, during the vegetative stage of soybean plants on field trials. The lack of difference on phosphorus concentration registered in our study and others could be an effect of plant age and nutrient (N and P) movement, where nutrients move from leaves to pods and seeds [11,53]. Additionally, under phosphorus scarcity (in our case, the unfertilized group), legumes such as soybean activate their regulatory systems and adaptability characteristics, such as phosphorus accumulation in nodules and/or phosphorus recycling from organic phosphorus (present in different plant organs such as leaves), in order to maintain phosphorus homeostasis within the plant [20,54]. Additionally, Görlach et al. [5] demonstrated that phosphorus is rapidly translocated within the leaves and then transported to the demanding sites inside the plant for its usage in different cell processes, such as energy production, cell division, and lipid and protein biosynthesis. This, according to Görlach et al. [5], occurred within the first 6h after the foliar supply of phosphorus to maize plants grown under phosphorus deficiency in hydroponic conditions. Therefore, we hypothesized that the phosphorus supplied through the foliar fertilizations with 1% NSBMH on days 30 and 60 was easily available, which was used immediately by soybean plants for trifoliar leaf increment, enlargement, and maintenance (as consequence foliar area too), for the maintenance and enhancement of chlorophyll pigment concentration, for the augmentation of fresh and dried masses, for the increase of plant height, and for the formation and weight increase of pods.

Chlorophyll pigment concentrations *a* and *a* + *b* were improved by 26.85% and 27.93%, respectively, when comparing the 1% NSBMH treatment versus the negative control treatment. This was the result of the foliar supplementation of easily assimilable nitrogen and phosphorus, which enhanced foliar area enlargement, and the incrementation and maintenance of suitable amounts of nitrogen and phosphorus in leaves. Our results are in agreement with Sharifi et al. [33], who registered an improvement between 14.14%

to 47.42% on chlorophyll pigment content after the foliar application of a water-soluble fertilizer composed of urea, single-super phosphate, and muriate of potash as an N-P-K nutrient source during different stages of soybean growth.

Soybean growth characteristics, such as plant fresh and dried mass and plant height, were positively affected by the foliar supplementation of soluble nitrogen and phosphorus, which were quickly translocated inside the leaves and used for its multiplication, enlargement, and maintenance, resulting in a foliar area increment as well. This allowed plants to receive more nutrients for storage and for its immediate usage for chlorophyll pigment production and increase, which are essential for the photosynthesis process. Therefore, the synergistic effect caused by a larger foliar area, suitable N and P concentrations in leaves, and a higher chlorophyll pigment concentration allowed plants to intercept and transform sunlight into chemical energy, which was then used for cell division and for the production of biomolecules (e.g., protein content) through CO_2 fixation, during the photosynthesis process, resulting in a total plant height increase, and a total plant fresh and dried mass augmentation. Our results for dried mass are in agreement with Mannan [32], who reported, after >120 days, a total dry weight improvement in soybean plants of 32.64% and 19.93% versus negative and positive control groups, respectively, when using a N-P-K-Mg mineral foliar fertilizer composed of sodium dihydrogen orthophosphate as the phosphorus source. Additionally, our findings regarding plant height are in accordance with Mandic [55], who found that the foliar fertilization during the reproductive stage of soybean with two different commercial fertilizers, Wuxal and Ferticare I, improved total plant height by 15.68% and 17.61%, respectively, when compared to their negative control group. Additionally, Mannan [32] registered a total plant height improvement of 7.16% when compared to their negative control group.

Soybean yield, reported here as pod number and pod fresh and dried mass, was improved by the synergistic effect caused by the foliar supplementation of easily available nitrogen and phosphorus, by the reserves of nitrogen (e.g., protein content) and phosphorus present in leaves, and by the biomass (e.g., dried mass) generated during soybean growth through the photosynthesis process. The nutrients (e.g., N, P, protein, carbohydrates) present in soybean plants, especially in leaves, were used first for the generation of pods, which then increased its weight by the formation and filling with seeds. Our findings, regarding to pod number, are in agreement with other authors that reported improvement on this trait after >110 days, in field trials, and after the evaluation of a diverse mineral foliar fertilizers (containing phosphorus) during the flowering and/or pod filling stages of soybean. For example, Vinoth Kumar et al. [56] registered a 30.70% and 27.16% pod number improvement over their negative control group after foliar spraying with a 1% urea phosphate and 1% mono-ammonium phosphate, respectively. Additionally, Mannan [32] obtained an 8.90% pod improvement versus their negative control group after the foliar application of a N-P-K-Mg fertilizer composed on urea, sodium dihydrogen phosphate, potassium chloride, and hydrated magnesium chloride. Moreover, Mandic et al. [55] recorded a 23.62% and 28.26% pod improvement after foliar spraying with the commercial fertilizers Wuxal and Ferticare I, respectively, when compared to their negative control group. Furthermore, Sharifi et al. [33] noticed a pod improvement from 1.16% to 34.80% versus their control group after foliar spraying with a water-soluble fertilizer composed of urea, single-super phosphate, and muriate of potash as the N-P-K nutrient source. In addition, da Silva Domingos [57] reported a 33.33% pod improvement versus their negative control group after the foliar application of a P-Ca-K-B fertilizer.

In summary, soybean growth characteristics, pod yield, nutrient content, and chlorophyll pigment concentration were improved by the foliar supplementation with 1% NSBMH. Therefore, the 1% NSBMH foliar fertilizer is an excellent alternative when compared to the actual commercialized foliar fertilizers because (1) it has a well-balanced nitrogen and phosphorus composition (1% from the original neutralized sulfuric bone meal hydrolysate), (2) its pH is suitable (pH = 6.5), (3) it has a humectant agent (2% glycerin), and (4) it is a source of easily assimilable nitrogen and phosphorus (cattle bone meal as the only phosphorus source). Additionally, the 1% NSBMH foliar fertilizer enhances its performance when it is used twice: first application at 30 DAS and second application at 60 DAS, with a 30-day span between each application.

5. Conclusions

Foliar fertilization with recycled phosphorus from cattle bone meal improved significantly when compared to the negative control treatment, soybean foliar area, plant fresh mass, plant dried mass, plant height, nitrogen concentration and protein content on leaves, and chlorophyll pigment concentration $a + b$, while it increased trifoliar leaf number, pod number, and pod fresh and dried masses, and maintained a suitable phosphorus concentration on leaves. Consequently, cattle bone recycling is an excellent choice for phosphorus obtainment, which at the same time reduces the environmental pollution generated from cattle bone waste and mineral phosphoric fertilizers.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/su15086582/s1. Analysis of variance tables for the evaluated traits.

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