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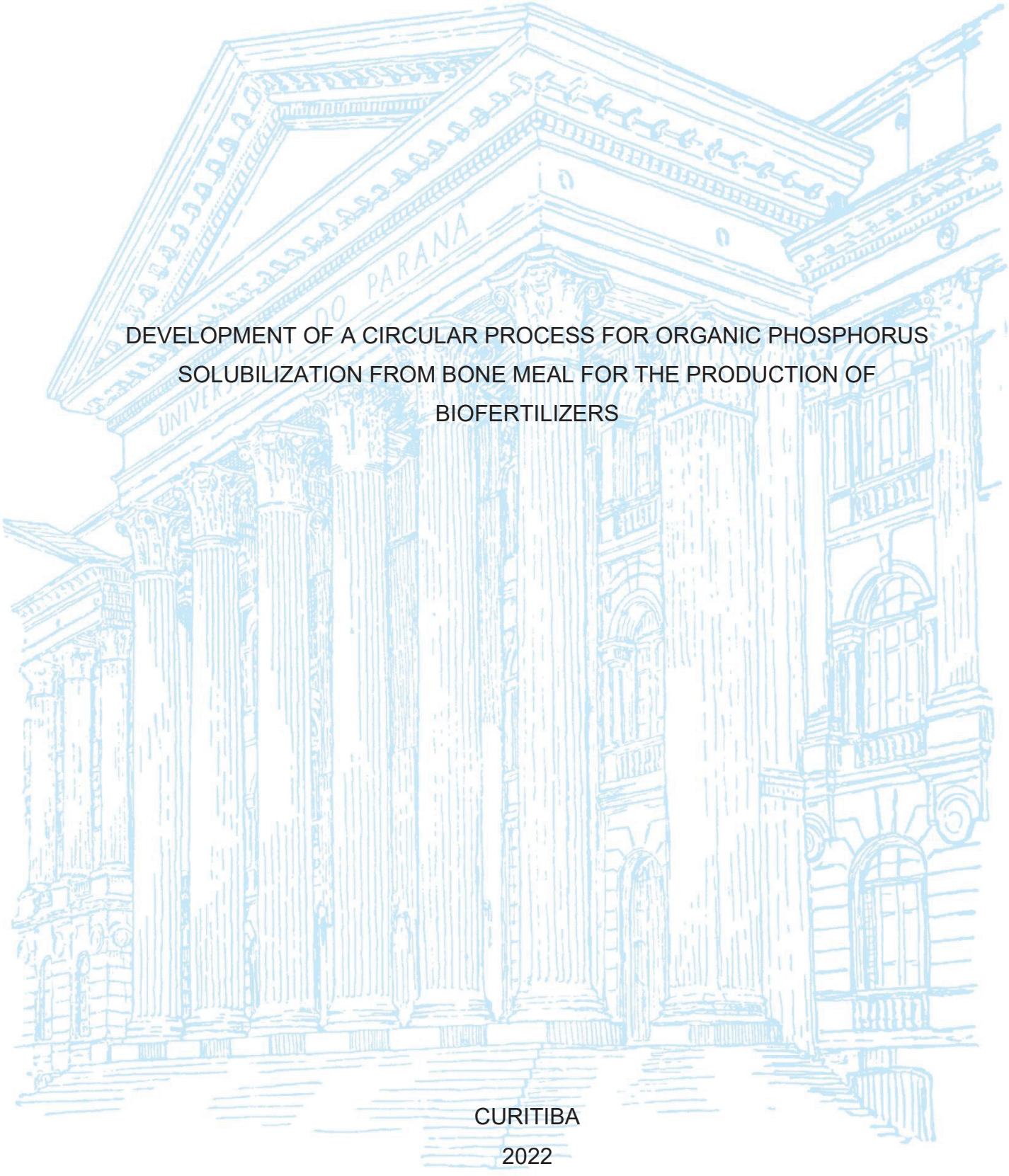

DEVELOPMENT OF A CIRCULAR PROCESS FOR ORGANIC PHOSPHORUS SOLUBILIZATION FROM BONE MEAL FOR THE PRODUCTION OF BIOFERTILIZERS

CURITIBA

2022

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DEVELOPMENT OF A CIRCULAR PROCESS FOR ORGANIC PHOSPHORUS SOLUBILIZATION FROM BONE MEAL FOR THE PRODUCTION OF BIOFERTILIZERS

Tese apresentada ao curso de Pós-Graduação em Engenharia de Bioprocessos e Biotecnologia, Setor de Tecnologia, Universidade Federal do Paraná, como requisito parcial à obtenção do grau de Doutor em Engenharia de Bioprocessos e Biotecnologia.

Orientador: Prof. Dr. Carlos Ricardo Soccol

Coorientadora: Dr.ª Rafaela de Oliveira Penha

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TERMO DE APROVAÇÃO

Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação ENGENHARIA DE BIOPROCESSOS E BIOTECNOLOGIA da Universidade Federal do Paraná foram convocados para realizar a arguição da tese de Doutorado de **DIEGO ALEJANDRO NIETO MONTEROS** intitulada: **DEVELOPMENT OF A CIRCULAR PROCESS FOR ORGANIC PHOSPHORUS SOLUBILIZATION FROM BONE MEAL FOR THE PRODUCTION OF BIOFERTILIZERS**, sob orientação do Prof. Dr. CARLOS RICARDO SOCCOL, que após terem inquirido o aluno e realizada a avaliação do trabalho, são de parecer pela sua APROVAÇÃO no rito de defesa.

A outorga do título de doutor está sujeita à homologação pelo colegiado, ao atendimento de todas as indicações e correções solicitadas pela banca e ao pleno atendimento das demandas regimentais do Programa de Pós-Graduação.

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RESUMO

O crescimento da população resulta em uma maior demanda de alimentos anualmente. Em consequência, a produção agrícola também demanda mais nutrientes como o fósforo, o mesmo que é um nutriente vital para o crescimento e desenvolvimento das plantas. Geralmente, o fósforo utilizado é obtido de minas de rocha fosfórica as mesmas que são altamente poluentes, por isso fontes alternativas de fósforo como a farinha de osso bovino precisam-se avaliar. Além disso, para o ano 2030 o Brasil vai-se tornar no maior produtor de carne bovina e soja. Então este trabalho tive como objetivo obter fósforo de farinha de osso bovino e avaliar o mesmo como uma fonte alternativa de fósforo em plantas de soja em condições de estufa. Como resultado, um hidrolisado sulfúrico neutralizado de farinha de osso bovino (NSBMH) foi obtido com teores de 3.40%N e 2.3%P_2O_5. Quando o hidrolisado foi utilizado em maior concentração (1/10) no solo, melhorou significativamente a área foliar, o número de folhas, a massa fresca da planta, a massa seca da planta, o comprimento da planta, o número de vagens, a massa fresca das vagens, e a massa seca das vagens em 271.02%, 248.58%, 420.60%, 646.21%, 68.69%, 664.43%, 937.78%, e 1,021.75%, respetivamente. Também, o teor de nitrogênio (0.0405±0.003gN g^{-1}) e a percentagem de proteína (17.97%) forem maiores em o grupo fertilizado com o NSBMH, enquanto os teores de fósforo (0.00212±0.0003gP_2O_5 g^{-1}) e clorofila (Chl *a*+*b* = 41.037±2.55ug cm^{-2}) tiverem níveis aceitáveis versus o controle negativo. Além disso, quando as plantas de soja forem fertilizadas por spray com 1% de NSMBH melhorou significativamente a área foliar (52.53%), a massa fresca da planta (103.88%), a massa seca da planta (182.96%), o comprimento da planta (36.83%), e o teor de clorofila (27.93%) quando comparado ao controle negativo. Em conclusão, o hidrolisado sulfúrico neutralizado de farinha de osso bovino desenvolvido neste trabalho é uma excelente fonte alternativa de fósforo o qual ajudará ao Brasil a reduzir sua dependência em fontes estrangeiras de fósforo mineral, tornando-se no primeiro país agro sustentável do mundo.

Palavras chave: Área foliar. Clorofila. Farinha de osso. Hidrolisado. Fósforo. Soja.

ABSTRACT

The growing population demands more food annually. Consequently, crop production needs more nutrient sources such as phosphorus. Phosphorus is an essential nutrient for plant growth and development and usually comes from mining industries which are highly contaminant and to reduce its consumption alternative phosphorus sources like cattle bone meal are being used. Additionally, Brazil will become the major producer of cattle meat and soybean by 2030. So, this work aimed to obtain phosphorus from cattle bone meal and evaluate it as an alternative phosphorus source for soybean plants under greenhouse conditions. As a result, a neutralized sulfuric bone meal hydrolysate (NSBMH) was developed containing 3.40%N and 2.3%P_2O_5. When the NSBMH was used in its high concentration (1/10) as soil fertilizer, it improved significantly foliar area, trifoliar leaves number, plant fresh mass, plant dried mass, plant height, pod number, pod fresh mass, and pod dried mass by 271.02%, 248.58%, 420.60%, 646.21%, 68.69%, 664.43%, 937.78%, and 1,021.75%, respectively, against the negative control. Also, nitrogen concentration (0.0405±0.003gN g^{-1}) and protein content (17.97%) in plants fertilized with NSBMH were greater when compared to the negative control, while phosphorus concentration (0.00212±0.0003gP_2O_5 g^{-1}) and chlorophyll pigment concentration (Chl *a*+*b* = 41.037±2.55ug cm^{-2}) maintained suitable levels. Additionally, foliar fertilization of soybean plants with 1% NSBMH improved significantly foliar area (52.53%), plant fresh mass (103.88%), plant dried mass (182.96%), plant height (36.83%), and chlorophyll pigment concentration *a*+*b* (27.93%) when compared to the negative control group. In conclusion, the neutralized sulfuric bone meal hydrolysate developed in this study is an excellent alternative as phosphorus nutrient source which will allow Brazil to reduce its dependency on foreign phosphoric fertilizer and become the first sustainable agroindustry.

Key-words: Bone meal. Chlorophyll. Foliar area. Hydrolysate. Phosphorus. Soybean.

SUMMARY

1 INTRODUCTION

Food demand is directly linked to population growth and by 2030 world population will be 8.5 billion people; consequently, crop and livestock production will increase too. Additionally, crop industry will demand fertilizers (N-P-K) in order to meet food supply and livestock industry will generate high amounts of residues (e. g. cattle bones) that must be handle correctly in order to prevent environmental pollution. As an example, Brazil is the second world cattle meat producer and by 2030 will become the world first soybean producer (OECD-FAO Agricultural Outlook 2021-2030, 2021; World Food and Agriculture - Statistical Yearbook 2020, 2020).

Phosphorus is an essential nutrient source for plant growth and development, and it usually comes from mine ores (as mineral phosphorus) which are highly contaminant (EUROPEAN PHOSPHATE FERTILIZER ALLIANCE, 2019; FLORIDA INDUSTRIAL AND PHOSPHATE RESEARCH INSTITUTE, 2019; RETA et al., 2018). Also, its incorrect use in crop lands are causing environmental problems such as erosion and eutrophication (HELYAR, 1998). In order to reduce the utilization of mineral phosphoric fertilizers, alternative phosphorus sources such cattle meat bone meal (MBM) can be used because its high phosphorus content (MÖLLER, 2015). Thus, in order to reduce the dependency on mineral phosphoric fertilizers and reduce the environmental impact generated by the by-products from the livestock industry, alternative phosphorus fertilizers can be developed and used on industrialized crops such as soybean.

This thesis is divided in three chapters. The first chapter evaluated different concentrations of bone meal hydrolysate on soybean growth and chlorophyll pigment content, in the second chapter the bone meal hydrolysate was compared versus a commercial fertilizer on soybean growth, productivity, and chlorophyll pigment content, and in the third chapter the potentiality of bone meal hydrolysate as foliar fertilizer on soybean growth and chlorophyll pigment content was evaluated, all of them under greenhouse conditions.

2 OBJECTIVES

2.1 MAIN OBJECTIVE

To develop a process for the obtainment of phosphorus from cattle bone meal and evaluate its product as an alternative phosphorus source for a sustainable agriculture.

2.2 SECONDARY OBJECTIVES

- To develop a process for the solubilization of phosphorus from cattle bone meal;
- To test different concentrations of bone meal hydrolysate on soybean morphological parameters under greenhouse conditions;
- To test different concentrations of bone meal hydrolysate on chlorophyll pigments *a*, *b*, and *a+b* content of soybean plants under greenhouse conditions;
- To evaluate the best concentration of bone meal hydrolysate versus a commercial fertilizer on soybean morphological parameters and chlorophyll pigment content under greenhouse conditions;
- To develop a foliar fertilizer from bone meal hydrolysate and asses it on soybean morphological parameters and chlorophyll pigment content under greenhouse conditions.

3 CHAPTER 1 – EVALUATION OF CONSECUTIVE FERTILIZATIONS WITH NEUTRALIZED SULFURIC BONE MEAL HYDROLYSATE ON SOYBEAN (*GLYCINE MAX*) GROWTH AND CHLOROPHYLL PYGMENT CONTENT

ABSTRACT

Food demand will increase due to population growth and, consequently, crop production will require different sources of essential nutrients such as phosphorus. Phosphorus usually comes from the mining industries which are highly contaminant and to reduce its consumption alternative phosphorus sources like cattle bone meal are being used. However, the correct use of bone meals depends on its physical and chemical characteristics, soil properties, plant species, and environmental factors. Thus, here we evaluated the effect of consecutive fertilizations with neutralized sulfuric bone meal hydrolysate (NSBMH) on soybean (*Glycine max*) growth and chlorophyll pigment content under greenhouse conditions. From July to November 2020 soybean plants were grown and fertilized with NSBMH (3.40%N and 2.3%P_2O_5) at three levels: diluted 10 times (high), 50 times (middle), and 100 times (low), plus a control (unfertilized). A hundred and twenty days after sowing, soybean growth under the high concentration of NSBMH showed significant ($p<0.05$) improvement for trifoliar leaves number, foliar area, plant fresh and dried masses, plant height, and chlorophyll pigment content *a*+*b* by 625%, 314%, 498%, 1110%, 42%, and 229%, respectively. Therefore, the use of NSBMH as an alternative phosphorus nutrient source will improve soybean agronomical characteristics and reduce phosphorus dependency from mineral sources.

Key-words: Bone meal. Chlorophyll. Foliar area. Hydrolysate. Phosphorus.

3.1 INTRODUCTION

Phosphorus is a finite source which is mainly present as phosphate rock around the world.

During phosphate rock mining and phosphoric fertilizer manufacture soil and water can be polluted by the presence of hazardous materials (e. g. arsenic, chromium, lead, mercury, cadmium, uranium, and thorium) and by-products such phosphogypsum (containing radium) which is generated from the reaction of sulfuric acid with phosphate rock for the obtainment of phosphoric acid (EUROPEAN PHOSPHATE FERTILIZER ALLIANCE, 2019; FLORIDA INDUSTRIAL AND PHOSPHATE RESEARCH INSTITUTE, 2019; RETA et al., 2018).

According to the United Nations, the world population is projected to increase to 9.7 billion people by 2050 (UNITED NATIONS, 2019). As consequence, food demand, crop production, and fertilizers nutrient demand such as nitrogen, phosphorus, and potassium will increase as well.

Phosphorus plays an important role in cellular metabolism and structure of living organisms. For example, in plants phosphorus participates in cell division, regulation of metabolic pathways, sugars breakdown, photosynthesis, and nutrient transport. Also, phosphorus is a vital element for the biosynthesis of macromolecules such as: nucleic acids (e. g. DNA and RNA), adenosine triphosphate (ATP), and phospholipids (BEHERA et al., 2014; KARUNANITHI et al., 2015; SHARMA et al., 2013).

Nowadays, most of the phosphorus needed for crop production come from solid granulated phosphate-based products such as mono-ammonium phosphate (MAP), di-ammonium phosphate (DAP), and triple superphosphate (TSP) (REETZ; IMPR. POINT 44), 2016); however, they must be added periodically in order to maintain soluble phosphorus on soils because phosphorus mobility can be reduced or increased under extreme weather conditions, such as long dry or wet seasons, consequently affecting soil (e. g. erosion) and water bodies (e. g. eutrophication), respectively (HELYAR, 1998).

Besides crop production, high quality and quantity of phosphorus enters the environment through other anthropogenic processes like sewerage systems, paper mills, industrial inorganic chemicals, pulp mills, organic fibers synthesis, meat production and packaging (e. g. cattle, pig, and poultry), among others (ENVIRONMENTAL PROTECTION AGENCY, 2021; LEINWEBER et al., 2018). Thus,

in order to minimize soil and water pollution caused by anthropogenic phosphorus novel thermal, physical, chemical and/or biological processes are being applied to reduce its environmental impact and as consequence generating a circular economy (KARUNANITHI et al., 2015; SUN et al., 2018; TARAYRE et al., 2016).

During cattle meat production the main by-products are muscle (63-75%), bone (13-18%), and fatty tissue (12-19%) (FAO, 1996; JAYATHILAKAN et al., 2012). As a matter of fact, 67.35 million tons of cattle meat were produced worldwide until 2018 and Brazil was the second largest producer with 9.90 million tons (FAO, 2020). Around 130 billion kg of animal bone waste are generated each year by the slaughter industry and it is considered an alarming environmental problem, especially with the increasing meat consumption (HUSSAIN et al., 2021). Furthermore, after cattle slaughtering bones can be used in a variety of ways and one of them is for the obtainment of bone meal (BM, composed only on bone), meat bone meal (MBM, composed of bone and meat residues attached to it), and/or MBM ashes, each one containing high amounts of phosphorus 5.24-16.5%d.w., 2.21-9.62%d.w., and 6.07-18.9%d.w., respectively (MOLLER, 2015; SAEID et al., 2014). Because of that bone meals have been applied as an alternative phosphorus nutrient source on a variety of crops like barley, oat, maize, and wheat (CHEN et al., 2011; NOGALSKA; ZALEWSKA, 2013); however, no investigations have demonstrated its effect on soybean (*G. max*) plants, which is Brazil's major crop. According to FAO, the country produced 117.89 million tons of soybean on 2018, being the second largest producer around the world (FAO, 2020). Nonetheless, the correct use of animal meals as fertilizers depends on its nutrient content (N-P-K), its particle size, the quantity used and application method, soil pH, plant species, and weather conditions (NOGALSKA; ZALEWSKA, 2013).

So, in this study we evaluated the effect of consecutive fertilizations with neutralized sulfuric bone meal hydrolysate on soybean (*G. max*) growth and chlorophyll pigment content under greenhouse conditions with the aim of reducing mineral phosphate-based fertilizers dependency and enhance the availability of phosphorus and other nutrients present on cattle bone meal.

3.2 MATERIALS AND METHODS

3.2.1 Sulfuric bone meal hydrolysate

In a 250mL Erlenmeyer flask, 40g of commercial cattle bone meal (<425mm particle size) was mixed with 200mL of 2.5M H_2SO_4. Then the hydrolysis took place in an orbital shaker at 25°C and 120rpm, during 60min (modified from Xia; Ren; Gao (2011)). After that, the liquid fraction was recovered and neutralized to pH=6.5 with NH_4OH. Finally, the neutralized sulfuric bone meal hydrolysate (NSBMH) containing 3.40%N (w/w) (Kjeldhal method) and 2.3%P_2O_5 (w/w) (spectrophotometric molybdovanadate method) was diluted 10 (high), 50 (middle), and 100 (low) times with deionized water.

3.2.2 Greenhouse test and fertilization

Five hundred milliliters plastic cups were filled with 40g of dried vermiculite (substrate) which then was soaked with deionized water prior to sowing. Twenty-four hours later, soybean seeds (*G. max*) were sowed (1 seed/cup). Each dilution and the control had five replicates with five cups each. Then, 28 days after sowing (DAS) the first fertilization was done by adding 10mL of each dilution to the vermiculite with a micropipette and deionized water was used for control (not fertilized). After each 21 days fertilization was performed (total of 5 fertilizations). All replicates were watered when needed with deionized water. This experiment was performed under greenhouse conditions from July to November of 2020 (120 days) (FIGURE 1).

3.2.3 Growth measurements

3.2.3.1 Leaf number and foliar area

A week before ending the experiment leaf number and foliar area were assessed. Fully formed trifoliar leaves number was counted for all the replicates of each dilution and the control.

Then, the perimeter of the central leaf of the 3th fully expanded trifoliate leaf (top to bottom) was drawn on a paper (three randomly selected samples from each

replicate of each dilution and the control were taken), then scanned and foliar area (cm^2) was obtained with the online version of ImageJ software (RASBAND, 2012) (FIGURE 2).

3.2.3.2 Fresh and dried masses

Fresh and dried masses of soybean plants were obtained after 120 days for all the replicates of each dilution and the control. 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 putted in a paper bag and air force dried at 65°C during 72h (SOARES et al., 2016), then the dried mass was obtained.

3.2.3.3 Plant height

After 120 days with the help of a meter the total plant height, as well as the shoot height and the root length were measured for all the replicates of each dilution and the control.

3.2.4 Chlorophyll pigments

A week before experiment ended, circular discs with 23mm diameter were cut from the central leaf of the 3th fully expanded trifoliate leaf (top to bottom) (one randomly selected sample from three replicates of each dilution and the control were used). Pigments were extracted from these leaf discs with 10mL of cold methanol in a mortar and pestle, then the liquid was centrifuged at 2500rpm during 10min, and the supernatant was used to record the absorbance in the range of 750 to 600nm, methanol was used as blank (PORRA; THOMPSON; KRIEDEMANN, 1989) (FIGURE 2). Finally, chlorophyll pigments *a*, *b*, and *a+b* by area (cm^2) were calculated with the equations proposed by Porra; Thompson; Kriedemann (1989) and Fan et al., (2018).

3.2.5 Statistical analysis

Data processing was developed on Microsoft Office Excel 2019 and Tukey's test (95% confidence) was performed for trifoliar leaves number, foliar area, fresh and

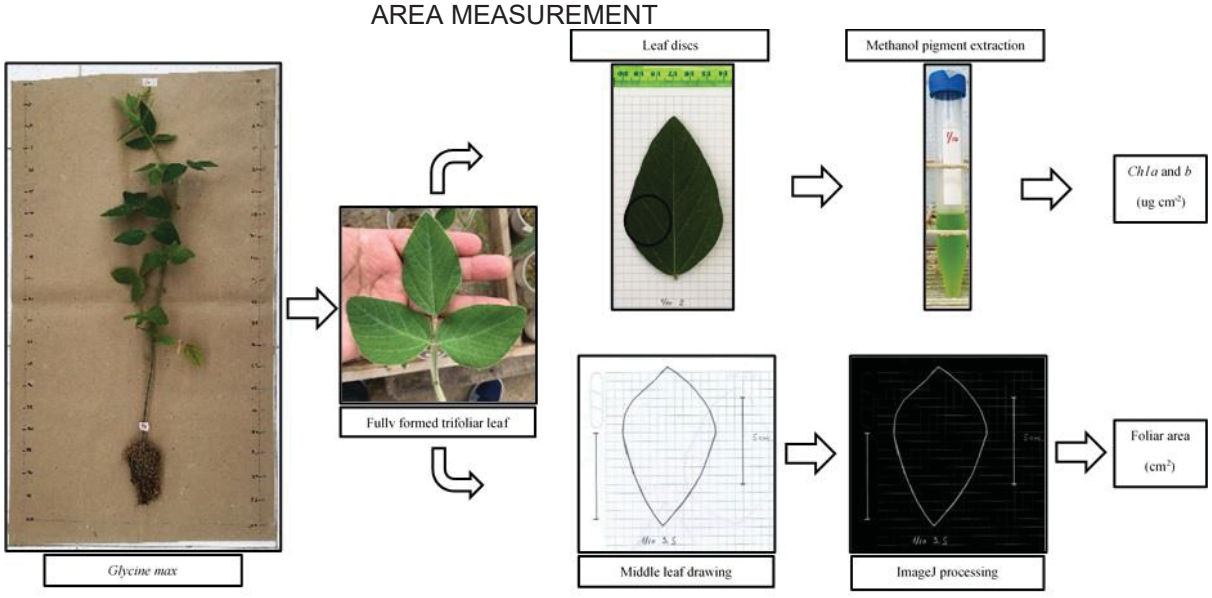
dried masses, plant height, and chlorophyll pigments by area (*a*, *b*, *a*+*b* cm^{-2}) in order to establish significant differences between experiments means using Minitab 19 Statistical Software.

FIGURE 1 - GREENHOUSE GROWTH OF SOYBEAN (*Glycine max*) PLANTS UNDER CONSECUTIVE FERTILIZATIONS WITH NSBMH

	Sowing						End
DAS	0	28	49	70	91	112	120
Fertilization	-	1st	2nd	3rd	4th	5th	-
Volume (mL)	-	10	10	10	10	10	-
°C/%H	26.3/56.9	36.5/30.6	35.3/34.5	20.4/91.9	18.0/88.7	29.7/52.7	36.6/55.8
Month/Year	Jul/2020	Aug/2020	Sep/2020	Oct/2020	Oct/2020	Nov/2020	Nov/2020

SOURCE: The author (2022).

FIGURE 2 - FLOW CHART FOR CHLOROPHYLL PIGMENT EXTRACTION AND FOLIAR AREA MEASUREMENT



SOURCE: The author (2022).

3.3 RESULTS

3.3.1 Phosphorus recovery from bone meal

TABLE 1 summarizes the quantity of solubilized phosphorus (as g P_2O_5) from bone meal.

TABLE 1 – SOLUBILIZED PHOSPHORUS (AS g P_2O_5) AFTER SULFURIC HYDROLYSIS

	Hydrolysis		
		Mass (g)	g P_2O_5	
	Bone meal	40	7.73	
	2.5M H2SO4	226.82	-	
	Total (Hydrolysate)	266.82	-	
	Separation			
		Mass (g)	g P_2O_5	% Recovery
	Hydrolysate	265.37	-	
Filtration	Bone meal after hydrolysis	30.4	1.42	
Decantation	Bone meal hydrolysate	97.73	4.86	62,87
	Precipitate	51.44	1.73	
	Losses	85.8	-	
	Total	265.37	-	

SOURCE: The author (2022).

3.3.2 Soybean growth

3.3.2.1 Leaf number and foliar area

The number of trifoliar leaves was significantly different ($p<0.05$) among the experiments and the control (except between middle and low concentrations) (TABLE 2). The higher number of trifoliar leaves was obtained with the high (8.12±1.34) concentration of NSBMH followed by the middle (3.6±0.38), low (2.48±0.23), and control (1.12±0.23). This means an improvement of 625%, 221.43%, and 121.43% after using high, middle, and low concentrations of NSBMH, respectively.

As well, foliar area was significantly different ($p<0.05$) among the experiments and control (except between middle and low concentrations) (TABLE 2, FIGURE 3b). The smaller foliar area was obtained in the control (6.46±0.81cm^2) followed by the low (13.45±0.65cm^2), middle (14.81±1.08cm^2), and high (26.73±2.19cm^2) concentrations of NSBMH, meaning an improvement of 108.20%, 129.26%, and 313.78%, respectively.

3.3.2.2 Fresh mass

Shoot fresh mass was significantly different ($p<0.05$) among the experiments and the control (TABLE 2). The control (1.78±0.15 g plant^{-1}) had the lowest shoot fresh mass followed by the low (4.45±0.17g plant^{-1}), middle (6.16±0.35g plant^{-1}), and high (18.30 ± 1.01g plant^{-1}) concentrations, improving this feature in 150%, 246.07%, and 928.09%, respectively.

Root fresh mass was significantly different ($p<0.05$) among the experiments and the control (except between middle and low concentrations, and low concentration and the control) (TABLE 2). Root fresh mass increased from the control (5.22±10.33g plant^{-1}) followed by the low (9.93±1.26g plant^{-1}), middle (12.29±1.71g plant^{-1}), and high (23.60±5.31g plant^{-1}) concentrations which means a 90.23%, 135.44%, and 352.11% improvement of this characteristic, respectively.

The total plant fresh mass was significantly ($p<0.05$) different among the experiments and the control (except between middle and low concentrations) (TABLE 2). The highest total plant fresh mass was obtained with the high (41.90±5.87g plant^{-1}) concentration and decreased with the middle (18.45±1.70g plant^{-1}) and low (14.38±1.21g plant^{-1}) concentrations, being the control (7.01±0.42g plant^{-1}) the smallest mass obtained. Total plant fresh mass was improved by 497.72%, 163.20%, and 105.14% for the high, middle, and low concentrations of NSBMH, respectively.

3.3.2.3 Dried mass

Shoot dried mass was significantly different ($p<0.05$) among the experiments and the control (TABLE 2). Shoot dried mass increased from the control (0.57±0.05g plant^{-1}) followed by the low (1.41±0.07g plant^{-1}), middle (1.92±0.13g plant^{-1}), and high (5.49±0.32g plant^{-1}) concentrations which means an improvement of this feature in 147.37%, 236.84%, and 863.16%, respectively.

Root dried mass was significantly different ($p<0.05$) among the experiments and the control (except between middle and low concentrations, and low concentration and the control) (TABLE 2). Root dried mass increased from the control (0.46±0.06g plant^{-1}) followed by the low (1.47±0.22g plant^{-1}), middle (2.53±0.65g plant^{-1}), and high (6.85±2.06g plant^{-1}) concentrations which means a 219.57%, 450%, and 1389.13% improvement, respectively.

The total plant dried mass was significantly different ($p<0.05$) among the experiments and the control (except between middle and low concentrations, and low concentration and the control) (TABLE 2). The highest total plant dried mass was obtained with the high (12.35±2.14g plant^{-1}) concentration and decreased with the middle (4.44±0.61g plant^{-1}), and low (2.89±0.17g plant^{-1}) concentrations, being the control (1.02±0.10g plant^{-1}) the smallest dried mass obtained. Total plant dried mass was improved by 1110.78%, 335.29%, and 183.33% for the high, middle, and low concentrations of NSBMH, respectively.

3.3.2.4 Plant height

Shoot height was significantly different ($p<0.05$) between all the experiments vs the control; however, no significant difference was observed between the results of the different concentrations (TABLE 2, FIGURE 3a). The control showed the smallest shoot height (40.94±3.07cm plant^{-1}) followed by the low (62.12±3.13cm plant^{-1}), middle (62.08±3.84cm plant^{-1}), and high (66.40±5.40cm plant^{-1}) concentrations meaning an improvement of 51.73%, 51.64%, and 62.19%, respectively. Moreover, there was not significantly difference ($p>0.05$) for root length between experiments vs the control (TABLE 2). Finally, no significant difference was found in total plant fresh height between the different concentrations (low, middle, and high); however, all of them presented significant better results when compared to the control (TABLE 2). The highest plant height was achieved with the high (87.32±6.31 cm plant^{-1}) concentration followed by the low (83.44±3.42cm plant^{-1}) and middle (82.65±3.58cm plant^{-1}) concentrations, being the smallest the control (61.60±3.30cm plant^{-1}). Total plant fresh height was improved by 41.75%, 34.17%, and 35.45% for the high, middle, and low concentrations of NSBMH, respectively.

3.3.3 Chlorophyll pigments concentration

Chlorophyll *a* concentration was significantly different ($p<0.05$) between the high concentration of NSBMH and the rest of the experiments and the control, as well (TABLE 2, FIGURE 3c). The highest chlorophyll *a* concentration was achieved with the high (35.20±6.03ug cm^{-2}) concentration of NSBMH followed by the middle (18.14±2.16ug cm^{-2}), low (10.16±0.31ug cm^{-2}), and the control (10.71±1.98ug cm^{-2}).

Chlorophyll *b* concentration was significantly different ($p<0.05$) between the high and the low concentration of NSBMH and the control, too (TABLE 2, FIGURE 3c). The lowest chlorophyll *b* concentration was observed using the low NSBMH concentration (3.06±0.21ug cm^{-2}), followed by the middle (6.69±2.44ug cm^{-2}), and high (10.30±1.34ug cm^{-2}) concentration. The control presented a concentration of chlorophyll *b* of 3.11±0.40ug cm^{-2}.

Chlorophyll *a+b* concentration was significantly different ($p<0.05$) between the experiments and the control (except among the low concentration and the control) (TABLE 2, FIGURE 3c). The highest chlorophyll *a+b* concentration was achieved with the high (45.50±7.36ug cm^{-2}) concentration of NSBMH followed by the middle concentration (24.82±2.20ug cm^{-2}), the control (13.82±2.38ug cm^{-2}), and the low concentration (13.22±0.51ug cm^{-2}).

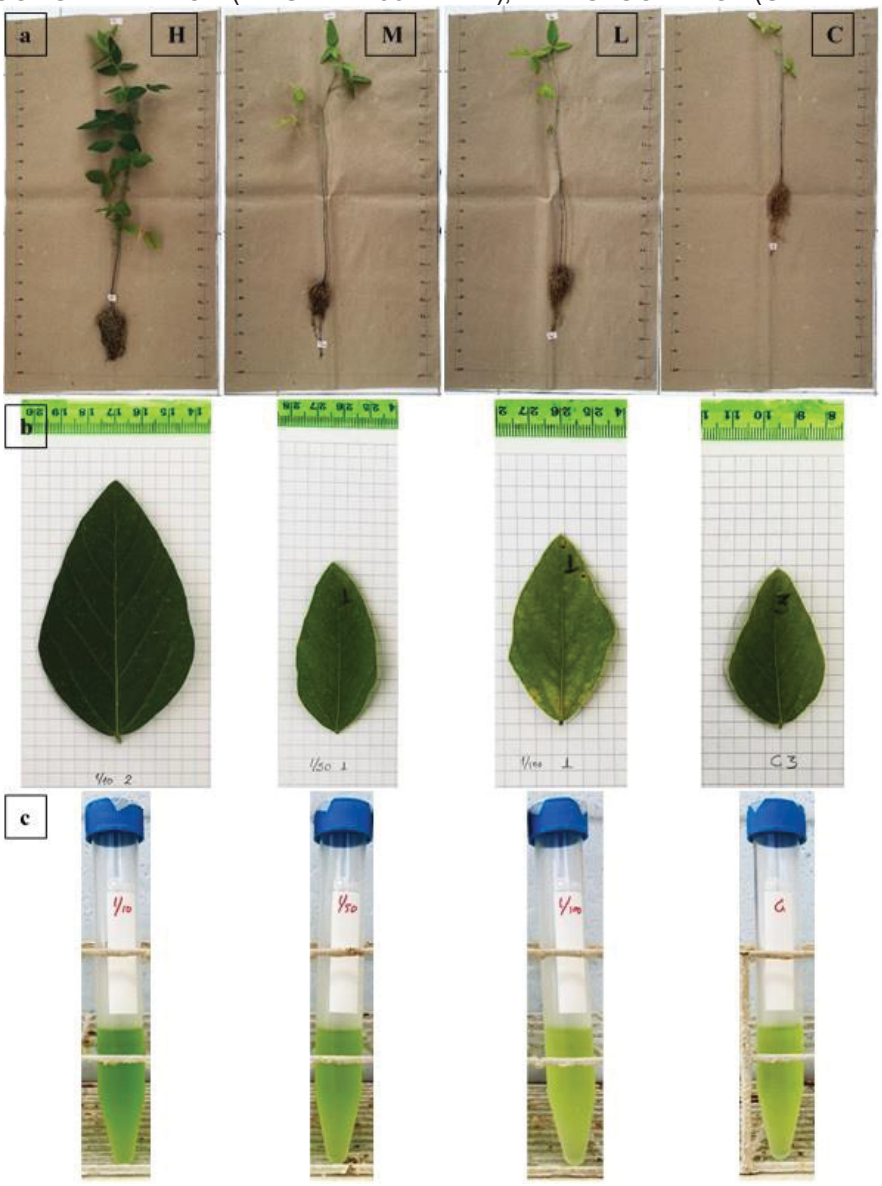
The high and middle concentrations of neutralized sulfuric bone meal hydrolysate improved chl *a* concentration by 228.66% and 69.37%, chl *b* concentration by 231.19% and 115.11%, and chl *a+b* concentration by 229.23% and 79.59%, respectively.

TABLE 2 - STATISTICAL DIFFERENCES AND IMPROVEMENT (%) OF TRIFOLIAR LEAVES NUMBER, FOLIAR AREA, FRESH AND DRIED MASSES, FRESH PLANT HEIGHT, AND CHLOROPHYLL PIGMENTS CONCENTRATION OF SOYBEAN (*G. max*) AFTER CONSECUTIVE FERTILIZATIONS WITH DILUTED NSBMH

			Experiment						
			[1/10]	***Imp*	[1/50]	*Imp*	[1/100]	*Imp*	Control
Parameter		**n**	**Mean ± S.D.**	*%*	**Mean ± S.D.**	*%*	**Mean ± S.D.**	*%*	**Mean ± S.D.**
Trifoliar leaves (N°)		5	8.12 ± 1.34***a***	*625.00*	3.6 ± 0.38***b***	*221.43*	2.48 ± 0.23***b***	*121.43*	1.12 ± 0.23***c***
Foliar area (cm^2)		5	26.73 ± 2.19***a***	*313.78*	14.81 ± 1.08***b***	*129.26*	13.45 ± 0.65***b***	*108.20*	6.46 ± 0.81***c***
Fresh mass (g)	Shoot	5	18.30 ± 1.01***a***	*928.09*	6.16 ± 0.35***b***	*246.07*	4.45 ± 0.17***c***	*150.00*	1.78 ± 0.15***d***
	Root		23.60 ± 5.31***a***	*352.11*	12.29 ± 1.71***b***	*135.44*	9.93 ± 1.26***bc***	*90.23*	5.22 ± 0.33***c***
	Plant		41.90 ± 5.87***a***	*497.72*	18.45 ± 1.70***b***	*163.20*	14.38 ± 1.21***b***	*105.14*	7.01 ± 0.42***c***
Dry mass (g)	Shoot	5	5.49 ± 0.32***a***	*863.16*	1.92 ± 0.13***b***	*236.84*	1.41 ± 0.07***c***	*147.37*	0.57 ± 0.05***d***
	Root		6.85 ± 2.06***a***	*1389.13*	2.53 ± 0.65***b***	*450.00*	1.47 ± 0.22***bc***	*219.57*	0.46 ± 0.06***c***
	Plant		12.35 ± 2.14***a***	*1110.78*	4.44 ± 0.61***b***	*335.29*	2.89 ± 0.17***bc***	*183.33*	1.02 ± 0.10***c***
Plant height (cm)	Shoot	5	66.40 ± 5.40***a***	*62.19*	62.08 ± 3.84***a***	*51.64*	62.12 ± 3.13***a***	*51.73*	40.94 ± 3.07***b***
	Root		20.92 ± 1.54***a***	*1.26*	20.57 ± 2.06***a***	-	21.32 ± 1.74***a***	*3.19*	20.66 ± 3.12***a***
	Plant		87.32 ± 6.31***a***	*41.75*	82.65 ± 3.58***a***	*34.17*	83.44 ± 3.42***a***	*35.45*	61.60 ± 3.30***b***
Chlorophyll pigments (ug/cm^2)	*a*	3	35.20 ± 6.03***a***	*228.66*	18.14 ± 2.16***b***	*69.37*	10.16 ± 0.31***b***	-	10.71 ± 1.98***b***
	b		10.30 ± 1.34***a***	*231.19*	6.69 ± 2.44***ab***	*115.11*	3.06 ± 0.21***b***	-	3.11 ± 0.40***b***
	a+b		45.50 ± 7.36***a***	*229.23*	24.82 ± 2.20***b***	*79.59*	13.22 ± 0.51***c***	-	13.82 ± 2.38***c***

**Means that do not share a letter in the same line are significantly different according to Tukey's test ($p<0.05$).*
***Percentage of improvement versus the control.*
SOURCE: The author (2022).

FIGURE 3 - DIFFERENCES ON MORPHOLOGICAL CHARACTERISTICS (A), LEAF AREA (B), AND CHLOROPHYLL PIGMENTS (C) BETWEEN SOYBEAN PLANTS FERTILIZED WITH DIFFERENT DILUTIONS OF NEUTRALIZED SULFURIC BONE MEAL HYDROLYSATE. H: HIGH CONCENTRATION (DILUTED 10 TIMES), M: MIDDLE CONCENTRATION (DILUTED 50 TIMES), L: LOW CONCENTRATION (DILUTED 100 TIMES), AND C: CONTROL (UNFERTILIZED)



SOURCE: The author (2022).

3.4 DISCUSSION

3.4.1 Phosphorus recovery from bone meal

Here we presented for the first time that phosphorus (as P_2O_5) can be solubilized from bone meal. The percentage of phosphorus recovery (solubilized phosphorus as P_2O_5) that we registered (62,87%) was lower when sulfuric acid was used for the solubilization of phosphorus from sewage sludge ash (66-99% of phosphorus recovery) and biochar (90% of phosphorus recovery) (KARUNANITHI et al., 2015). The difference observed are because ashes and biochar have been thermally pre-treated (incineration of organic matter) which allows to release more easily nutrients (e. g. phosphorus) when are attacked by an acid.

Recycling bone meal for the production of bone meal hydrolysate which contain soluble phosphorus can be economically an option. For example, 1.0 L of bone meal hydrolysate will require around 0.46 Kg of bone meal, and the actual cost of 1.0 Kg of bone meal is 1.55 US dollars (VERDE MANIA, 2022), meaning that 1.0 L of bone meal hydrolysate will cost nearly 0.71 US dollars whereas 1.0 L of phosphorus liquid fertilized can be found in the market by 28.0 US dollars (GREEN LEAF AQUARIUMS, 2022).

3.4.2 Soybean growth

3.4.2.1 Leaf number and foliar area

The utilization and rate of application of diluted NSBMH showed a positive effect on leaf number and foliar area, and its potential was better seen when it was used at its high concentration. This high concentration of NSBMH had a high well-balanced amount of phosphorus (from bone meal), nitrogen (from bone meal and NH_4OH), and sulphur (from H_2SO_4), which were absorbed by the root system of soybean plants and then easily translocated to the shoot where they were metabolized for the formation of new leaves and the maintenance of the older ones, also allowing a foliar area to increase by the proliferation of cells and consequently larger leaves.

Leaf number is a vital characteristic of plant growth and development. In this study we demonstrated that the utilization and rate of application of NSBMH enhanced

the number of healthy trifoliar leaves and reduced its abscission after 120 DAS, especially when NSBMH was used in its high concentration (high amount of phosphorus as well). In this case, healthier and long-lasting leaves were obtained whereas this positive effect was reduced when NSBMH concentration was middle and low (TABLE 2, FIGURE 3). This result is in accordance with Kamran et al. (2018) where soybean leaf number was improved by 51% and 19% after 42 days of growth when $(NH_4)_3PO_4$ (soluble) and $Ca(PO_4)_3(OH)$ (slowly soluble) were used as P source at their highest level. Furthermore, soybean leaf number increased when phosphorus concentration (as inorganic phosphate in Hoagland's nutrient solution) was augmented from low (10.1 leaves) to middle (26.9 leaves) to high (36.4 leaves) after 110 DAS (SINGH et al., 2014). Also, after 104 DAS leaf number of soybean plants increased by 10.82, 11.34, and 11.38 (control=10.30) after soil fertilization with 15Kg P ha^{-1} (low), 30Kg P ha^{-1} (middle), and 50Kg P ha^{-1} (high) doses of phosphorus, respectively (AKTER et al., 2013). Finally, the NSBMH diluted 10 times improved the leaf number by 625% versus the negative control whereas others investigations registered a leaf number improvement of 51% (KAMRAN et al., 2018) and 10,48% (AKTER et al., 2013) when mineral phosphorus was used versus the negative control.

Foliar area is an important morphological feature because allows carbon assimilation and light interception (HUSSAIN et al., 2019). In our investigation as the concentration of NSBMH increased the foliar area of soybean plants did it too, having the maximum foliar area with the high concentration of NSBMH (high concentration of phosphorus) (TABLE 2, FIGURE 3). Our results are in agreement with He et al. (2019), who obtained an increment in foliar area of soybean plants after 147 DAS when using middle (60mg P Kg^{-1}) and high (120mg P Kg^{-1}) concentrations of ammonium dihydrogen phosphate as P nutrient source. Moreover, foliar area of different soybean cultivars reached its maximum at pod-bearing stage when using a high concentration of phosphorus (450Kg ha^{-1} diammonium phosphate) (AO et al., 2013). Finally, when the NSBMH was used in its high concentration improved foliar area by 313,78 versus the negative control whereas He et al. (2019) registered a 50% improvement when using mineral phosphorus versus the unfertilized experiment.

Therefore, using a high concentration of NSBMH enhanced leaf number and foliar area in soybean plants as described above. So, coupling these two morphological traits will improve soybean plants growth because a higher leaf number plus larger

foliar area will enable to increase light absorption and CO_2 fixation, consequently increasing plant dry matter and productivity.

3.4.2.2 Fresh and dried masses

Fresh and dry masses are important morphological characteristics that reflects plant nutrition during a fertilizer evaluation and its utilization.

In this investigation we demonstrated that the increment from low to middle to high concentration of NSBMH (high amount of phosphorus as well) augmented the fresh and dried masses of shoot, root, and total plant after 120 DAS (TABLE 2). This course was also observed by Akter et al. (2013). The authors registered after 104 DAS an average of plant fresh weight of 88.62g, 90.73g, and 93.57g when using phosphorus concentrations of 0Kg P ha^{-1} (none), 15Kg P ha^{-1} (low), and 30Kg P ha^{-1} (middle), respectively, showing a correlation between increase of plant weight and phosphorus concentration. Finally, our plant fresh mass improved by 497,72% when using the NSBMH diluted ten times versus the negative control whereas Akter et al. (2013) registered a 4,62% improvement when using mineral phosphorus versus the negative control.

Dried mass increase was also observed when phosphorus concentration augmented. Milton et al. (1991) noted after 60 DAS that shoot dry matter of soybean plants in Hoagland's nutrient solution was 3.62±0.19g, 6.60±0.28g, 7.19±0.38g, using 0.1ppm P (low), 0.2ppm P (middle), and 0.3ppm P (high), respectively, and root dry mass was 0.86±0.04g, 1.70±0.08g, and 22.2±0.12g at the same P concentrations. Also, Pan et al. (2008) registered after 45 days of germination an increment on shoot (1.81±0.22g $plant^{-1}$) and root (0.63±0.06 g $plant^{-1}$) dried weights of 96 genotypes of soybean plants after using 340mg Kg^{-1} KH_2PO_4 (high) as phosphorus nutrient source. In addition, total plant dry matter of three different soybean cultivars were above 150g $plant^{-1}$ than controls after using a high dosage (450Kg ha^{-1}) of diammonium phosphate as fertilizer (AO et al., 2013). Moreover, dry matter augmented after 150 DAS on soybean plants with high and low P efficiencies by 18.8% and 24.5% at 82.5Kg P ha^{-1} (middle), and by 16.0% and 25.9% at 165Kg P ha^{-1}(high), respectively (AO et al., 2014). Furthermore, shoot and root dried weights of soybean plants increased after 42 days of growth from 0.3g pot^{-1} and 0.05g pot^{-1} at 100mg Kg^{-1} (low) by 0.4g pot^{-1} and 0.1g pot^{-1} at 200mg Kg^{-1} (high) when $(NH_4)_3PO_4$ (soluble) was utilized (KAMRAN et

al., 2018). Additionally, the maximum total dry weight of soybean plants after 118 DAS at 28°C was 180.1g plant^{-1} when sufficient triple superphosphate (0.50 mM) was supplied in a modified Hoagland's nutrient solution as fertigation from 4 to 6 times a day in excess (SINGH et al., 2018). Furthermore, after >120 DAS total soybean dry weight was 30g plant^{-1} when 150KgP_2O_5 ha^{-1} of single superphosphate was used which was 10g plant^{-1} higher when the dosage was lowered to 50KgP_2O_5 ha^{-1} (TALIMAN et al., 2019). Finally, our plant dried mass improved by 1110,78% when the NSBMH was used in its high concentration versus the negative control which was better than the 11,94% registered by Kamran et al. (2018) who used mineral phosphorus.

Consequently, fresh and dried masses of root, shoot, and whole plant were improved as NSBMH concentration was increased too, being the high concentration of NSBMH which exhibited the best results.

During the different stages of soybean growth (e. g. vegetative, flowering, blooming, pod, and seed) water is vital because allows nutrient transportation and biochemical reactions (e. g. photosynthesis). Thus, as NSBMH concentration increased root, shoot, and total plant fresh masses did as well, meaning an increase on water demand as NSBMH concentration augmented too. So, the water added allowed the root system of soybean plants to absorb the nutrients from NSBMH present on the vermiculite. Consequently, the water contained on soybean plants (expressed as fresh weight) plus the nutrients from NSBMH allowed root and shoot growth. Especially in shoots, where water content and nutrients (N-P-S) allowed the maintenance and development of leaves where photosynthesis takes place, benefiting stem thickening too.

Regarding plant dry matter, NSBMH had enough nutrients (N-P-S) at the three different levels of concentration, which allowed soybean plants to metabolize them for the generation of new molecules (matter) by CO_2 fixation through photosynthesis. Nowadays, soybean dry matter is a vital agronomic characteristic because is the major driving force for pods (number of pods) and seeds (grain weight) formation which are productivity parameters of soybean cultivars. The increment of soybean productivity due to dry matter accumulation by phosphorus augmentation have been registered by Majumdar et al. (2001), Akter et al. (2013), Ao et al. (2013b), Singh et al. (2014), He et al. (2019), and Taliman et al. (2019).

3.4.2.3 Plant height

Plant height is an important morphological trait that shows plant growth and development during fertilizers evaluation and its utilization.

Our results showed that using diluted NSBMH affected positively total plant height when compared to the control (not fertilized), but no significant effect was obtained among the low, middle, and high concentrations of NSBMH for shoot, root, and total plant height (TABLE 2). A similar result was obtained by Devi et al. (2012) that registered no significant difference on soybean plant height after >120 DAS when using single super phosphate and di-ammonium phosphate as P nutrient source at four different levels 20 (40.25cm), 40 (41.50cm), 60 (44.58cm), and 80 (47.75cm) KgP_2O_5 ha^{-1}. Also, soybean plant height was not significantly affected by phosphorus treatment at 0.01 (160cm), 0.10 (161cm), and 0.50 (174cm) mM P after 110 DAS (SINGH et al., 2014). Moreover, Kamran et al. (2018) registered no significant effect among experiments and controls (no P added) on soybean plant height (≈40cm) after 42 days of growth when $(NH_4)_3PO_4$ (soluble) and $Ca(PO_4)_3(OH)$ (slowly soluble) were used as P source at low (100mg Kg^{-1}) and high (200mg Kg^{-1}) concentrations.

In contrast, among three different soybean cultivars plant height (≈115cm) of the Liaoning cultivar was significantly higher under a low phosphorus treatment (150Kg ha^{-1} di-ammonium phosphate) after >120 DAS (AO et al., 2013b). Also, after 104 DAS soybean plant height was significantly affected, registering heights of 64.46cm, 66.66cm, and 68.50cm as phosphorus dosage augmented by 15Kg P ha^{-1} (low), 30Kg P ha^{-1} (middle), and 50Kg P ha^{-1} (high), respectively (AKTER et al., 2013). Moreover, soybean plant height after 118 DAS was consistently lower (≈133cm) under P-deficient treatment (0.08mM triple superphosphate) versus P-sufficient treatment (0.5mM triple superphosphate) (≈147cm) (SINGH et al., 2018). Finally, our plant height improved by 41,75% when the NSBMH was diluted 10 times versus the negative control which is a better result than the 38,17% improvement obtained when mineral phosphorus was used versus the negative control (DEVI et al., 2012).

In order to phosphorus have a significant effect on soybean height, the dosage should be in a high rate before sowing (basal fertilization) (AKTER et al., 2013; AO et al., 2013b) and within the range of 30 to 90 DAS, where exponential growth takes place (SINGH et al., 2018), and also at the beginning of flowering stage (AO et al., 2013b). Even so, soybean height will improve when phosphorus dosage is coupled with a

standard plant density (e. g. 150 000 plants ha^{-1}) (AO et al., 2013b; SINGH et al., 2018), a suitable day/night temperature (e. g. 24/18 to 32-26° C), and/or an appropriate culture system (e. g. SPAR) (SINGH et al., 2018).

3.4.3 Chlorophyll pigments concentration

Chlorophyll *a* and *b* play an important role during the photosynthesis process because they are responsible for converting light energy into chemical energy (e. g. NADPH and ATP) through the electron transport chain system, which is then used for CO_2 fixation and the production of biomolecules (GITELSON et al., 2016). These two types of chlorophyll coexist together in plants, being chlorophyll *a* the most predominant form. During photosynthesis, chlorophyll *a* participates on light harvesting and electron transfer while chlorophyll *b* takes part only as an accessory pigment for light harvesting (CROCE; VAN AMERONGEN, 2014; HUMPHREY, 2006).

Our results showed that chlorophyll pigments concentration (*a*, *b*, and *a+b*) were positively enhanced as the concentration of the NSBMH solution increased, being the high concentration of NSBMH which presented the highest values of *Chl a*, *b*, and *a+b* (TABLE 2). The same results have been obtained in other studies. Ao et al. (2013b) registered an increment on chlorophyll content at different phosphorus concentrations and soybean stages for a high-P efficient cultivar: 2.37mg g^{-1} F.W. (1.0mmol L^{-1} KH_2PO_4, branching stage), 2.94mg g^{-1} F.W. (0.5mmol L^{-1} KH_2PO_4, blooming stage), and 3.50mg g^{-1} F.W. (0.5mmol L^{-1} KH_2PO_4, podding stage). Furthermore, Singh et al. (2014) registered a maximum of ≈100mg cm^{-2} total chlorophyll content when using 0.50 and 0.10mM P after 60 DAS. Moreover, Kamran et al. (2018) obtained an increment on chlorophyll content when using 200mg Kg^{-1} (high concentration) of $(NH_4)_3PO_4$ (soluble) and $Ca(PO_4)_3(OH)$ (slowly soluble) as P source after 42 days of growth. Finally, when the NSBMH was used in its high concentration improved by 229,3% chlorophyll pigment *a+b* versus the negative control a better result than Kamran et al. (2018) who obtained a 18,18% improvement when using mineral phosphorus.

In contrast, Vengavasi and Pandey (2018) registered that chlorophyll *a* (2.01±0.20mg g^{-1}F.W.) and total chlorophyll (2.64±0.21mg g^{-1}F.W.) concentrations were higher at low P concentration (2mgP Kg^{-1} soil) versus its high P concentration (25mgP Kg^{-1} soil) when using single super phosphate as P nutrient source.

Phosphorus is a vital nutrient for plant growth and development because participates on energy production (e. g. NADPH and ATP) and molecules biosynthesis (e. g. sugar phosphates, nucleic acids, phospholipids), and a deprivation of this element will have a negative effect on plant morphological (e. g. leaf number, foliar area, height, fresh and dried masses) and biochemical parameters (e. g. chlorophyll pigment concentration) (AKTER et al., 2013; AO et al., 2013a, 2013b; BULGARELLI et al., 2017; GITELSON et al., 2016; KAMRAN et al., 2018; PAN et al., 2008; RYCHTER; RAO, 2014; SINGH et al., 2014).

Here we see that as NSBMH dosage augmented chlorophyll pigments concentration (*a*, *b*, and *a+b*) also increased, especially the high concentration of NSBMH which had a well-balanced N-P-S that enhanced the production of chlorophyll pigments. Thus, the high nutrient content from the NSBMH (especially phosphorus) affected positively the leaf number, its chlorophyll pigments concentration, and the foliar area. These morphological and biochemical features together enhanced the photosynthesis process of soybean plants. As a result, soybean morphological features such as: leaf number, foliar area, height, and fresh and dried masses were improved as well. Being the last one, dried mass (dry matter), the parameter which reflects an increment on CO_2 fixation through the photosynthetic process. Investigations developed by Ao et al. (2009), Ao et al. (2013a), Singh et al. (2014), Bulgarelli et al. (2017), Kamran et al. (2018), Singh et al. (2018), Vengavasi and Pandey (2018) and Taliman et al. (2019) had demonstrated the positively and negatively effects of different doses and sources of phosphorus on the photosynthetic process (e. g. net photosynthesis, leaf gas exchange, chlorophyll pigment concentration) for different growth stages of soybean cultivars.

3.5 CONCLUSIONS

This study demonstrated the high potentiality that a neutralized sulfuric bone meal hydrolysate (NSBMH) has as an alternative phosphorus nutrient source on soybean morphological characteristics and chlorophyll pigment content. For instance, the high concentration of NSBMH improved significantly trifoliar leaves number, foliar area, plant fresh mass, plant dried mass, plant height, and chlorophyll pigment content *a+b* by 625%, 314%, 498%, 1110%, 42%, and 229%, respectively; which are vital agronomical traits that together will enhance soybean cultivars productivity.

Additionally, the use of alternative phosphorus nutrient sources (e. g. cattle bone meal) will promote it recycle from the different anthropogenic waste-streams reducing its dependency from mineral sources at the same time.

4 CHAPTER 2 – AGRONOMIC EVALUATION OF NEUTRALIZED SULFURIC BONE MEAL HYDROLYSATE AS AN ALTERNATIVE P SOURCE ON SOYBEAN PLANTS

ABSTRACT

Soybean is globally produced because a variety of products can be obtained from it (e. g. vegetable oil, protein meal, biodiesel) and its by-products (e. g. bioethanol, butanol, plant growth hormones, prebiotics). Usually, the phosphorus used for its growth comes from mineral ores which are highly contaminant and, in order to reduce its dependency, alternative phosphorus sources such as neutralized sulfuric bone meal hydrolysate (NSBMH) are being used. So, here we tested a NSBMH on soybean growth characteristics, pod yield, nitrogen, phosphorus, and chlorophyll pigment concentration under greenhouse conditions. From September to November 2021 soybean plants were grown and fertilized with NSBMH (3.28%N, 2.2%P_2O_5) and compared to a commercial fertilizer (2.38%N, 4.7%P_2O_5) and a negative control (unfertilized). Ninety days after sowing, soybean plants fertilized with NSBMH showed a significant ($p<0.05$) improvement versus the commercial fertilizer on foliar area, trifoliar leaves number, plant fresh mass, plant dried mass, plant height, pod number, pod fresh mass, and pod dried mass by 54.76%, 59.22%, 91.94%, 115.93%, 18.96%, 80.50%, 85.69%, and 78.34%, respectively. Also, nitrogen concentration (0.0405±0.003gN g^{-1}) and protein content (17.97%) in plants fertilized with NSBMH were greater when compared to the positive and negative control, while phosphorus concentration (0.00212±0.0003gP_2O_5 g^{-1}) and chlorophyll pigment concentration (Chl *a*+*b* = 41.037±2.55ug cm^{-2}) maintained suitable levels. Consequently, the NSBMH is an excellent alternative as phosphorus nutrient source because it improves soybean morphological characteristics, pod yield, nitrogen, phosphorus, and chlorophyll pigment concentration, as well decreases the utilization of mineral phosphorus sources and its dependence on main suppliers.

Key-words: Bone meal. Chlorophyll. Foliar area. Hydrolysate. Phosphorus. Soybean.

4.1 INTRODUCTION

Soybean production will reach 411 Mt by 2030, with Brazil being the world's largest producer with 149 Mt at that time (OECD-FAO Agricultural Outlook 2021-2030, 2021). Soybean is the most important oilseed cultivar around the world because a variety of products can be obtained from it, for example: vegetable oil, protein meal (for human and animal feed), oleochemicals, and biodiesel (AMARO BITTENCOURT et al., 2021; OECD-FAO Agricultural Outlook 2021-2030, 2021; SINGH et al., 2014). Also, soybean by-products such as: hulls, okara, okara flour, and soymilk can be used in biorefineries for the obtainment of high-added-value biomolecules, such as bioethanol, butanol, plant growth hormones, prebiotics, among others (AMARO BITTENCOURT et al., 2021; CANAAN et al., 2022).

Phosphorus is a vital nutrient for plant development and functionality because it plays an important role on macromolecules biosynthesis (e. g. nucleic acids, phospholipids, ATP), metabolic regulation, photosynthesis, and nutrient transport (BEHERA et al., 2014; KARUNANITHI et al., 2015; SHARMA et al., 2013; SOARES et al., 2016).

In the majority of industrial crop fields, such as soybean, mineral phosphorus is mostly used in its original form (highly insoluble) or solubilized with strong acids (e. g. HCl, H_2SO_4, HNO_3) in order to generate soluble phosphate products (e. g. mono-ammonium phosphate [MAP], di-ammonium phosphate [DAP], and triple superphosphate [TSP]) (RETA et al., 2018); however, in both cases they are added periodically and/or in a higher concentration in order to maintain high levels of soluble phosphorus on soil which can negatively affect the environment, producing erosion and/or eutrophication (AKTER et al., 2013; DEVI et al., 2012; HELYAR, 1998; SINGH et al., 2018; SOARES et al., 2016; TALIMAN et al., 2019; XUE et al., 2013). Additionally, Brazil soybean production will be severely affected by the reduction on fertilizers supply by Russia. A reaction taken by the Russian government due to the sanctions imposed over them by the global community because of its invasion over Ukraine this year (HOROWITZ, 2022). As a fact, Brazil imports around 85% of its fertilizers and one fifth of it come from Russia which is the main producer of ammonium nitrate, a chemical fertilizer that is mostly used as nitrogen source for crop production and as a raw material for the production of other chemical fertilizers such as mono-

ammonium phosphate and di-ammonium phosphate (MAGALHAES; PEARSON, 2022; RETA et al., 2018).

In order to reduce the environmental impact generated from the usage of mineral phosphorus and its derivatives, studies regarding the use of alternative phosphorus are still scarce. Meat bone is a waste with high phosphorus content (2.21-9.62%d.w.). For this reason, in the last decade investigations regarding the use of meat bone meals (MBM) have been developed on a variety of plants such as: barley, oat, maize, and wheat (CHEN et al., 2011; MOLLER, 2015; NOGALSKA, 2016; NOGALSKA; ZALEWSKA, 2013; SAEID et al., 2014). However, the correct use of MBM depends on soil pH, weather conditions, plant species, and method of application (NOGALSKA; ZALEWSKA, 2013).

Several studies have evaluated the effect of different mineral phosphorus sources and its dosage on soybean growth and yield parameters (AKTER et al., 2013; AO et al., 2013a; BULGARELLI et al., 2017; DEVI et al., 2012; HE et al., 2019; KAMRAN et al., 2018; SINGH et al., 2018; SOARES et al., 2016; TALIMAN et al., 2019; XUE et al., 2013), and investigations regarding the use of alternative phosphorus sources on soybean production are still scarce. So, aiming to reduce the gap of knowledge in this area, in Chapter 1 was registered the benefits of a neutralized sulfuric bone meal hydrolysate (NSBMH) on soybean morphological parameters and chlorophyll pigment content. Thus, here we compared the neutralized sulfuric bone meal hydrolysate versus a commercial fertilizer on soybean growth characteristics, pod yield, nitrogen, phosphorus, and chlorophyll pigment concentration under greenhouse conditions.

4.2 MATERIAL AND METHODS

4.2.1 Fertilizers

The neutralized sulfuric bone meal hydrolysate (NSBMH) containing 3.28%N (w/w) and 2.2%P_2O_5 (w/w) (obtained as described in Chapter 1) was compared against a commercial organomineral fertilizer (positive control) containing 2.38%N (w/w) and 4.7%P_2O_5 (w/w). Nitrogen and phosphorus content were performed by Kjeldahl and spectrophotometric molybdovanadate methods, respectively.

For fertilization, the NSBMH was diluted 10 times and the commercial fertilizer to 1% (according to product's label) both in deionized water.

4.2.2 Greenhouse test

Soybean seeds were germinated and fertilizers were tested in the spring of 2021 under greenhouse conditions during 90 days, from September to November.

4.2.2.1 Soybean germination

One-liter plastic cups were filled with 200g of dried vermiculite (substrate) which then was soaked with running water prior to sowing. Twenty-four hours later, four soybean seeds (*G. max*) per cup were sowed. Then, ten days after sowing (DAS) one germinated seed per cup was maintained.

4.2.2.2 Fertilization

Each fertilizer and the negative control (not fertilized) had three replicates with five cups each.

Fertilization was done on the 30^{th} and 60^{th} DAS by adding 10mL of each fertilizer to the vermiculite with a micropipette and nothing was added to the negative control. Watering was performed when needed.

4.2.3 Soybean growth measurements

4.2.3.1 Foliar area and leaf number

A week before each fertilization and before ending the experiment the perimeter of the last fully formed trifoliate leaf was drawn on a paper, then scanned, and the foliar area (cm^2) was obtained with the online version of Image J (RASBAND, 2012).

Leaf number was counted for both fertilizers and the negative control after 90 days of growth.

4.2.4 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 putted in a paper bag and air force dried at 65°C during 72h (SOARES et al., 2016), then the dried mass was obtained.

4.2.5 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.

4.2.6 Soybean pod yield

4.2.6.1 Pod number, fresh and dried masses

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

4.2.7 Nitrogen and phosphorus quantification

4.2.7.1 Sample processing

Leaves from the dried plants of each experiment 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.

4.2.7.2 Nitrogen

Nitrogen was quantified by the automated Kjeldahl method (method 920.87) to determine protein content on soya beans and lupins (AOAC, 1995; KAKIUCHI; KAMIJI, 2015). The nitrogen to protein conversion factor used was 4.43 (YEOH; WEE, 1994).

4.2.7.3 Phosphorus

Phosphorus as P_2O_5 (w/w) was quantified by the spectrophotometric molybdovanadate method according to Ministério da Agricultura; Pecuária e Abastecimento; Secretaria de Defensa Agropecuária (2014) (KAKIUCHI; KAMIJI, 2015).

4.2.8 Chlorophyll pigments

A week before each fertilization and a week before harvesting, three circular discs with 23mm diameter were cut from the middle of each leaf of the last fully expanded trifoliate. Pigments were extracted from these leaf discs with 8mL of cold methanol in a mortar and pestle, then the liquid was centrifuged at 2,500rpm during 10min. The absorbance of the supernatant was recorded in a range of 750 to 600nm in a Shimadzu UV-1601PC spectrophotometer, while methanol was used as blank (PORRA; THOMPSON; KRIEDEMANN, 1989). Finally, chlorophyll pigments *a*, *b*, and *a+b* by area (cm^2) were calculated with the equations proposed by Porra; Thompson; Kriedemann (1989) and Fan et al. (2018).

4.2.9 Statistical analysis

Three randomly selected samples from each replicate of each fertilizer and the negative control were taken for foliar area, trifoliar leaves number, pod number, and pod fresh and dried masses, and for chlorophyll pigments two randomly selected samples from each replicate of each fertilizer and the negative control were used. For nitrogen and phosphorus quantification in leaves, one randomly selected sample from each replicate of each fertilizer was used, while for the negative control a compose sample was obtained from three randomly selected samples of each replicate. Total plant fresh and dried masses, and plant height were obtained from all the replicates of each fertilizer and the negative control. Then the collected data was processed on Microsoft Office Excel 2019 and Tukey's test (95% confidence) was performed for foliar area, trifoliar leaf number, pod number, pod fresh and dried masses, plant fresh and dried masses, plant height, nitrogen, phosphorus and chlorophyll pigments

concentration in order to establish significant differences between experiments means using Minitab 19 Statistical Software.

4.3 RESULTS

4.3.1 Soybean growth characteristics

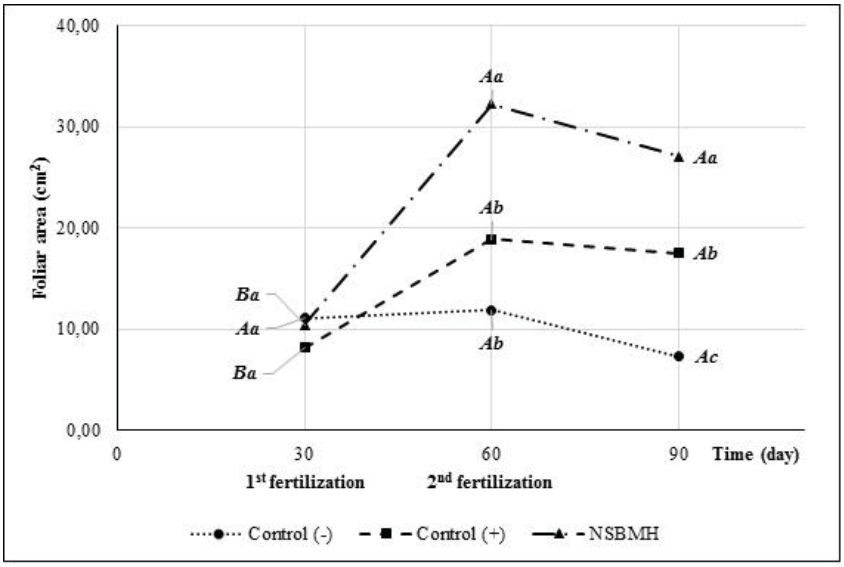
4.3.1.1 Foliar area

The plant groups (NSBMH, commercial fertilizer, and negative control) did not present significant difference ($p>0.05$) among them for foliar area thirty-days after sowing (Day 30), since the first fertilization was performed at day 30 (FIGURE 1, TABLE 1). At day 60, 30 days after the first fertilization, foliar area was significantly larger ($p<0.05$) for the NSBMH (32.24±4.27cm^2) when compared to the commercial fertilizer and the negative control (FIGURE 1, TABLE 1). On the other hand, this trait was not significantly different ($p>0.05$) between the commercial fertilizer (18.91±2.44cm^2) and the negative control (11.92±3.06cm^2) (FIGURE 1, TABLE 1). Then, at day 90, thirty days after the second fertilization, foliar area was significantly different ($p<0.05$) between the NSBMH (27.05±4.28cm^2), the commercial fertilizer (17.48±2.61cm^2), and the negative control (7.29±0.34cm^2) (FIGURE 1, TABLE 1). Finally, the NSBMH improved the foliar area by 70.47% (Day 60) and 54.75% (Day 90) versus the commercial fertilizer, and by 170.47% (Day 60) and 271.06% (Day 90) when compared to the negative control (TABLE 1).

Regarding to the effect of fertilization and no fertilization on foliar area during soybean growth within each group, the NSBMH registered the highest foliar area (32.24±4.27cm^2) 30 days after the first fertilization (Day 60) and after the second fertilization (Day 90) decreased to 27.05±4.28cm^2; however, this decrease was not significantly different (between Day 60 and Day 90), and both measurements at day 60 and 90 were significantly different ($p<0.05$) from the foliar area (10.43±3.56cm^2) registered on day 30 (when fertilizer was not applied) (FIGURE 1, TABLE 1). A similar effect was notice with the commercial fertilizer, after the first fertilization the foliar area was 18.91±2.44cm^2 (Day 60) and slightly decreased after the second fertilization to 17.48±2.61cm^2 (Day 90); nonetheless, this decrease was not significantly different (between Day 60 and Day 90), and both were significantly ($p<0.05$) different from the

foliar area (8.19±1.21cm^2) recorded on day 30 (when fertilizer was not applied) (FIGURE 1, TABLE 1). In contrast, the foliar area of the negative control (not fertilized) slightly increased from 11.05±1.88cm^2 (Day 30) to 11.92±3.06cm^2 (Day 60), and finally decreased to 7.29±0.34cm^2 (Day 90); no significant difference ($p>0.05$) was obtained within this group throughout the days (FIGURE 1, TABLE 1).

FIGURE 1 - COMPARISON OF FOLIAR AREA OF SOYBEAN PLANTS AFTER TWO FERTILIZATIONS WITH NSBMH AND A COMMERCIAL FERTILIZER DURING 90 DAYS.



Means that do not share a letter are significantly different according to Tukey's test ($p<0.05$), capital letters within the same group and low case letters among groups. Mean of three replicates.
SOURCE: The author (2022).

TABLE 1 - COMPARISON OF THE EFFECT OF TWO FERTILIZERS ON THE FOLIAR AREA (CM^2) OF SOYBEAN PLANTS AFTER 30, 60, AND 90 DAYS.

			Experiment			
Time	**Control (-)**		**Control (+)**		**NSBMH**	
Day	**+Mean ± S.D.**	****Improvement (%)**	**Mean ± S.D.**	**Improvement (%)**	**Mean ± S.D.**	**S.D. (±)**
30	11.05±1.88***Aa***	-	8.19±1.21***Ba***	-	10.43±3.56***Ba***	2.42
60	11.92±3.06***Ab***	170.55	18.91±2.44***Ab***	70.47	32.24±4.27***Aa***	3.35
90	7.29±0.34***Ac***	271.02	17.48±2.61***Ab***	54.76	27.05±4.28***Aa***	2.90
S.D. (±)	2.8		2.17		4.05	

**Means that do not share a letter are significantly different according to Tukey's test ($p<0.05$), capital letters within the same group and low case letters among groups. Mean=3±S.D.*
***Percentage of improvement of the NSBMH versus the negative control and positive control, respectively.*
SOURCE: The author (2022).

4.3.1.2 Leaf number

The number of trifoliar leaves was significantly different ($p<0.05$) among the fertilizers and the negative control 90 DAS (TABLE 2). The higher trifoliar leaves number was obtained with the NSBMH (14.33±2.03 leaves plant^{-1}) followed by the commercial fertilizer (9.00±1.20 leaves plant^{-1}), and the negative control (4.11±1.02 leaves plant^{-1}). The NSBMH improved the trifoliar leaves number by 59.22% versus the commercial fertilizer (TABLE 2).

4.3.1.3 Plant fresh and dried masses

Shoot fresh mass was significantly different ($p<0.05$) among the experiments 90 days after sowing (TABLE 2). The negative control (5.99±0.79 g plant^{-1}) presented the lowest shoot fresh mass followed by the commercial fertilizers (22.28±1.25g plant^{-1}), and the NSBMH (48.32±3.54g plant^{-1}). Moreover, root fresh mass of the NSBMH (37.48±9.07g plant^{-1}) was significantly different ($p<0.05$) from the commercial fertilizer (22.42±2.13g plant^{-1}) and negative control (10.49±0.59g plant^{-1}), and no significant difference ($p>0.05$) was observed between the commercial fertilizer and the negative control (TABLE 2). Also, total plant fresh mass was significantly different ($p<0.05$) among the three groups (TABLE 2). The NSBMH (85.81±7.38g plant^{-1}) presented the highest total plant fresh mass, followed by the commercial fertilizer (44.71±1.25g plant^{-1}), and negative control (16.48±1.05g plant^{-1}). Finally, the NSBMH improved shoot, root, and total plant fresh masses by 116.85%, 67.17%, and 91.94%, respectively, versus the commercial fertilizer (TABLE 2).

Shoot dried mass was significantly different ($p<0.05$) among the groups 90 DAS (TABLE 2). The highest shoot dried mass was obtained with the NSBMH (11.26±1.33g plant^{-1}) followed by the commercial fertilizer (5.19±0.42g plant^{-1}), and the negative control (1.36±0.19g plant^{-1}). Furthermore, root dried mass of the NSBMH (8.91±2.18g plant^{-1}) was significantly higher ($p<0.05$) than the commercial fertilizer (4.15±0.77g plant^{-1}) and the negative control (1.34±0.09g plant^{-1}), while no significant difference ($p>0.05$) was observed between the commercial fertilizer and the negative control (TABLE 2). Additionally, total plant dried mass was significantly different ($p<0.05$) among the groups (TABLE 2). The highest total plant dried mass was registered on the NSBMH (20.17±2.30g plant^{-1}) followed by the commercial fertilizer

(9.34±0.60g plant^{-1}), and the negative control (2.70±0.24g plant^{-1}). Finally, the NSBMH improved shoot, root, and total plant dried masses by 116.81%, 114.85%, and 115.93%, respectively, versus the commercial fertilizer (TABLE 2).

4.3.1.4 Plant height

Shoot height was significantly different ($p<0.05$) among the groups after 90 days (TABLE 2). The negative control (49.10±1.05cm plant^{-1}) presented the smallest shoot height followed by the commercial fertilizer (78.97±4.01cm plant^{-1}), and the NSBMH (106.73±3.13cm plant^{-1}). Moreover, no significant difference ($p>0.05$) was obtained for root length among the groups (TABLE 2). Also, total plant height was significantly different ($p<0.05$) among the three groups (TABLE 2). The highest plant height was achieved with the NSBMH (141.53±6.36cm plant^{-1}) followed by the commercial fertilizer (118.97±4.19cm plant^{-1}), and the negative control (83.90±2.29cm plant^{-1}). Finally, the NSBMH improved shoot and total plant height by 35.15% and 18.96%, respectively, versus the commercial fertilizer (TABLE 2).

4.3.2 Soybean pod yield

4.3.2.1 Pod number and fresh and dried masses

Pod number and fresh and dried masses were significantly different ($p<0.05$) among the groups 90 DAS (TABLE 2). The NSBMH presented the highest pod number (14.44±2.69pods plant^{-1}) followed by the commercial fertilizer (8.00±0.67pods plant^{-1}), and the negative control (1.89±0.51pods plant^{-1}). Also, the NSBMH treatment registered the highest pod fresh mass per plant (8.96±1.54g plant^{-1}), followed by the commercial fertilizer (4.82±0.53g plant^{-1}), and the negative control (0.86±0.41g plant^{-1}). Similarly, the highest pod dried mass was obtained with the NSBMH (1.77±0.46g plant^{-1}), followed by commercial fertilizer (0.99±0.18g plant^{-1}), and the negative control (0.16±0.08g plant^{-1}) (TABLE 2). Finally, the NSBMH improved pod number and pod fresh and dried masses by 80.50%, 85.69%, and 78.34%, respectively, versus the commercial fertilizer, and by 664.43%, 937.78%, and 1,021.75%, respectively, against the negative control (TABLE 2).

TABLE 2 - STATISTICAL DIFFERENCES AND IMPROVEMENT (%) OF TRIFOLIAR LEAVES NUMBER, POD NUMBER, POD FRESH AND DRIED MASSES, PLANT FRESH AND DRIED MASSES, AND PLANT HEIGHT OF SOYBEAN PLANTS AFTER 90 DAYS OF GROWTH UNDER GREENHOUSE CONDITIONS.

		Experiment				
		Control (-)		Control (+)		NSBMH
Parameter		*Mean ± S.D.	**Improvement (%)	Mean ± S.D.	Improvement (%)	Mean ± S.D.
Trifoliar leaves number (plant^{-1})		4.111±1.018***c***	248.58	9.000±1.202***b***	59.22	14.33±2.03***a***
Pod number (plant^{-1})		1.889±0.509***c***	664.43	8.000±0.667***b***	80.50	14.44±2.69***a***
Pods fresh mass (g plant^{-1})		0.863±0.406***c***	937.78	4.823±0.525***b***	85.69	8.956±1.54***a***
Pods dried mass (g plan^{-1})		0.1577±0.0814***c***	1,021.75	0.9919±0.1171***b***	78.34	1.769±0.462***a***
Fresh mass (g)	Shoot	5.992±0.798***c***	706.41	22.283±1.246***b***	116.85	48.32±3.54***a***
	Root	10.491±0.587***b***	257.26	22.42±2.13***b***	67.17	37.48±9.07***a***
	Plant	16.483±1.055***c***	420.60	44.706±1.251***b***	91.94	85.81±7.38***a***
Dry mass (g)	Shoot	1.364±0.187***c***	725.44	5.193±0.424***b***	116.81	11.259±1.332***a***
	Root	1.3387±0.0934***b***	565.57	4.147±0.766***b***	114.85	8.91±2.18***a***
	Plant	2.703±0.238***c***	646.21	9.341±0.603***b***	115.93	20.17±2.30***a***
Plant height (cm)	Shoot	49.100±1.054***c***	117.37	78.97±4.01***b***	35.15	106.73±3.13***a***
	Root	34.800±1.706***a***	-	40.00±2.46***a***	-	34.80±4.55***a***
	Plant	83.90±2.29***c***	68.69	118.97±4.19***b***	18.96	141.53±6.36***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 versus the negative control and positive control, respectively.*
SOURCE: The author (2022).

4.3.3 Nitrogen and phosphorus concentration

Nitrogen concentration was significantly different ($p<0.05$) between the NSBMH (0.0405±0.003gN g^{-1}) versus the commercial fertilizer (0.0307±0.003gN g^{-1}) and the negative control (0.0270±0.001gN g^{-1}), while there was not significant difference ($p>0.05$) between the commercial fertilizer and the negative control after 90 days of growth (TABLE 3). Consequently, the NSBMH registered the highest nitrogen percentage (4.056%N), followed by the commercial fertilizer (3.079%N), and the negative control (2.703%N). Also, the highest protein content was obtained with the NSBMH (17.97%), followed by the commercial fertilizer (13.64%), and the negative control (11.98%) (TABLE 3).

Phosphorus concentration (as P_2O_5) did not present significant difference ($p>0.05$) among the experiments after 90 days of growth (TABLE 3). Nonetheless, the highest phosphorus concentration was obtained with the NSBMH (0.00212±0.0003gP_2O_5 g^{-1}), followed by the commercial fertilizer (0.00170±0.0004gP_2O_5 g^{-1}), and the negative control (0.00145±0.0002gP_2O_5 g^{-1}) (TABLE 3).

TABLE 3 - NITROGEN CONCENTRATION, % N, % PROTEIN, AND PHOSPHORUS CONCENTRATION (AS P_2O_5) ON SOYBEAN LEAVES AFTER 90 DAYS OF GROWTH UNDER GREENHOUSE CONDITIONS.

Experiment	*Mean ± S. D. (gN g^{-1})	% N	% Protein (% Nx4.43)	Mean ± S. D. (gP_2O_5 g^{-1})	%P_2O_5
****Control (-)**	0.0270±0.001***b***	2.703	11.98	0.00145±0.0002***a***	0.15
*****Control (+)**	0.0307±0.003***b***	3.079	13.64	0.00170±0.0004***a***	0.17
NSBMH	0.0405±0.003***a***	4.056	17.97	0.00212±0.0003***a***	0.21

**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 replicate.*
SOURCE: The author (2022).

4.3.4 Chlorophyll pigments concentration

Chlorophyll pigments concentration *a*, *b*, and *a+b* (ug cm^{-2}) did not present significant difference ($p>0.05$) among the NSBMH, the commercial fertilizer, and the negative control after 30, 60, and 90 days (TABLE 4).

Regarding to the effect of fertilizer application on chlorophyll pigments concentration *a*, *b*, and *a+b* (ug cm^{-2}) during soybean growth, when no fertilization was due (negative control) the highest concentration of chlorophylls *a*, *b*, and *a+b* were

registered on the 30th day, then drastically decreased to the 60th day, and slightly decreased to the 90th day. Within this group significant difference ($p<0.05$) was observed for chlorophyll *a* and *a+b* between the 30th versus the 60th and 90th day (TABLE 4). Moreover, for the NSBMH the highest concentration of chlorophyll *a*, *b*, and *a+b* was registered when fertilization was not applied (Day 30), then considerably decreased even after the first fertilization (Day 60), and increased after the second fertilization (Day 90); however, no significant difference was observed ($p>0.05$) (TABLE 4). For the commercial fertilizer, chlorophyll concentrations *a*, *b*, and *a+b* were similar between the 30th day (with no fertilization) and the 60th day (after first fertilization), and increased for the 90th day (after second fertilization), but no significant difference was observed ($p>0.05$) within the group (TABLE 4).

TABLE 4 - COMPARISON OF THE EFFECT OF TWO FERTILIZERS ON CHLOROPHYLL PIGMENTS CONCENTRATION *A*, *B*, AND *A+B* (UG CM^{-2}) OF SOYBEAN PLANTS AFTER 30, 60, AND 90 DAYS OF GROWTH UNDER GREENHOUSE CONDITIONS.

		Chl *a*		
Time	**Control (-)**	**Control (+)**	**NSBMH**	
Days	***Mean ± S.D.**	**Mean ± S.D.**	**Mean ± S.D.**	**S.D. (±)**
30	34.597±1.32***Aa***	28.797±0.97***Aa***	37.470±7.55***Aa***	4.46
60	27.866±3.31***Ba***	28.492±2.90Aa	30.608±2.35***Aa***	2.88
90	26.404±1.30***Ba***	33.606±4.68Aa	32.320±2.26***Aa***	3.09
S.D. (±)	2.19	3.22	4.74	
		Chl *b*		
30	8.526±0.48***Aa***	7.234±0.08***Aa***	9.243±1.95***Aa***	1.16
60	7.294±0.92***Aa***	7.227±0.85***Aa***	8.272±0.78***Aa***	0.85
90	7.053±0.56***Aa***	9.091±1.57***Aa***	8.717±0.34***Aa***	0.98
S.D. (±)	0.68	1.03	1.23	
		Chl *a+b*		
30	43.123±1.77***Aa***	36.031±1.02***Aa***	46.713±9.50***Aa***	5.61
60	35.160±4.15***Ba***	35.719±3.68***Aa***	38.880±3.12***Aa***	3.67
90	33.458±1.80***Ba***	42.697±6.24***Aa***	41.037±2.55***Aa***	4.02
S.D. (±)	2.80	4.22	5.96	

**Means that do not share a letter are significantly different according to Tukey's test ($p<0.05$), capital letters within the same group and low case letters among groups. Mean=3±S.D.*
SOURCE: The author (2022).

4.4 DISCUSSION

4.4.1 Soybean growth characteristics

4.4.1.1 Foliar area and leave number

The NSBMH significantly improved the foliar area of soybean plants after 60 and 90 days of growth versus the commercial fertilizer and the negative control (FIGURE 1, TABLE 1). Our findings are in agreement with the ones described on Chapter 1 where foliar area increased to 26.73±2.19cm^2 when using a NSBMH after 120 DAS. Moreover, the same tendency (foliar area increase during soybean growth) has been seen by other studies but with bigger foliar areas when mineral fertilizers such as ammonium dihydrogen phosphate (HE et al., 2019) and diammonium phosphate (XUE et al., 2013) were used. Finally, our foliar area improvement versus the negative control was better than the 50% improvement obtained by He et al. (2019) when using mineral phosphorus.

The leaf number of soybean plants was enhanced significantly after 90 days of growth when using the NSBMH versus the commercial fertilizer and the negative control (TABLE 2). Our results were better than those obtained in Chapter 1 (8.12±1.34 leaves plant^{-1}) after 120 DAS with consecutive fertilizations of NSBMH. Also, our findings are better than those obtained by Akter et al. (2013) (11.38 leaves plant^{-1}) and Singh et al. (2014) (10.1 leaves plant^{-1}) when mineral phosphorus was used. Finally, we registered a better leaf number improvement versus the negative control when compared to those of 51% (KAMRAN et al., 2018) and 10,48% (AKTER et al., 2013) registered when mineral phosphorus was used.

These results are important since foliar area and leaf number are both relevant morphological features because together, they improve carbon assimilation and light interception (HUSSAIN et al., 2019).

4.4.1.2 Fresh and dried masses

A suitable fertilization during plant growth can be observed on its fresh and dried masses.

After 90 days of growth and two fertilizations with NSBMH shoot, root, and total plant fresh and dried masses were significantly different to the commercial fertilizer and the negative control (TABLE 2).

Regarding to fresh weights, our results were better than registered on Chapter 1 for shoot (18.30±1.01g), root (23.60±5.31g), and total plant (41.90±5.87g) after consecutive fertilizations with NSBMH during 120 days of growth. However, our total plant fresh weight was lower compared to 93.57g registered by Akter et al. (2013) when using mineral phosphorus. It is worth noticing that the researchers took the measurement after 104 days of growth, while the result presented here is for 90 days. Finally, our total plant fresh weight improvement versus the negative control was better than the 4,62% improvement obtained by Akter et al. (2013) when using mineral phosphorus.

For dried matter, in this study higher values were registered than those described on Chapter 1 for shoot (5.49±0.32g), root (6.85±2.06g), and total plant (12.35±2.14g) after consecutive fertilizations with NSBMH during 120 days of growth. Also, we registered a higher shoot dried weight than the one obtained by Milton; Eiswerth; Ager (1991) for shoot dried weight (7.19±0.38g) but our root dried weight value was lower than the one registered for the same author (22.2±0.12g). In this case Milton; Eiswerth; Ager (1991) used mineral phosphorus in a Hoagland's solution (hydroponic nutrient solution) and collected data after 60 DAS. Moreover, Xiang-Wen et al. (2008) obtained lower values than ours for shoot dried weight (1.81±0.22g $plant^{-1}$) and root dried weight (0.63±0.06 g $plant^{-1}$) when KH_2PO_4 was used as phosphorus nutrient source after 45 days of germination.

Furthermore, our total plant dried weight was higher than the obtained by Taliman et al. (2019) (30g $plant^{-1}$) when single superphosphate was used after 120 DAS. Nonetheless, better results regarding to this trait were registered by Xue et al. (2013) (50g $plant^{-1}$) on a field experiment under conventional plant density (150 000plants ha^{-1}) and phosphorus concentration (150kg ha^{-1} of diammonium phosphate). Also, Singh et al. (2018) registered 76.2g $plant^{-1}$ mean dried weight when using 0.08mM of triple superphosphate in a Soil-Plant-Atmosphere-Research (SPAR) chamber. Finally, our total plant dried weight improvement versus the negative control was better than the 11,94% observed by Kamran et al. (2018) when using mineral phosphorus.

4.4.1.3 Plant height

During fertilizers evaluation plant height is a vital morphological characteristic because represents plant growth and development.

After 90 days of growth and two fertilizations with NSBMH shoot and total plant height were significantly different to the commercial fertilizer and the negative control (TABLE 2).

We registered higher results than those obtained in Chapter 1 for shoot height (66.40±5.40cm), root length (20.92±1.54cm), and total plant height (87.32±6.31cm) after consecutive fertilizations with NSBMH during 120 DAS. Moreover, our results for total plant height were better than those registered by Devi et al. (2012) when using single super phosphate (35.00cm) and di-ammonium phosphate (36.58cm) after >120 DAS. Also, our total plant height was better than the obtained by Kamran et al. (2018) (≈40cm) after 42 days of growth when $(NH_4)_3PO_4$ (soluble) and $Ca(PO_4)_3(OH)$ (slowly soluble) were used. Additionally, Xue et al. (2013) and Akter et al. (2013) registered smaller plant heights ≈115cm and 68.50cm when di-ammonium phosphate and mineral phosphate were used after 104 DAS and >120 DAS, respectively. On the other hand, our results on total plant height were lower than those obtained by Singh et al. (2014) (174cm) after 110 DAS and by Singh et al. (2018) (≈147cm) after 118 DAS when mineral phosphorus and triple superphosphate were used, respectively. Finally, our total plant height improvement versus the negative control was better than those of 6,93% and 44,41% registered by Akter et al. (2013) and Devi et al. (2012) when using mineral phosphorus.

4.4.2 Soybean pod yield

Soybean yield parameters such as pod number (AKTER et al., 2013; HE et al., 2019; MAJUMDAR et al., 2001; SINGH et al., 2014; TALIMAN et al., 2019) and pod weight (AO et al., 2014; BULGARELLI et al., 2017; SINGH et al., 2014) have been registered mostly after long periods of time (>110DAS), during the evaluation of a variety of mineral phosphorus sources, and under controlled environments or field trials.

Nine-days after growth and two fertilizations with NSBMH pod number increased significantly by 664.43% and 80.50% versus the negative control and the

commercial fertilizer, respectively (TABLE 2). This improvement is very significant when comparing it to other investigations developed on field trials [except by Singh et al. (2014) and Taliman et al. (2019)], after long periods of time (>110DAS) and when using a variety of mineral phosphorus sources. These studies registered an increase on pod number by 36.78% (MAJUMDAR et al., 2001), 90.32% (AKTER et al., 2013), 200.00% (SINGH et al., 2014), 200.00% (HE et al., 2019), and 130.19% (TALIMAN et al., 2019) when comparing the phosphorus fertilized group versus the negative control or the low phosphorus fertilized group.

Furthermore, when the NSBMH was used pod fresh mass increased significantly by 937.78% (vs the negative control) and by 85.69% (vs the commercial fertilizer), also pod dried mass augmented significantly by 1,021.75% (vs the negative control) and by 78.34% (vs the commercial fertilizer) (TABLE 2). Our pod dried mass improvement was better than Singh et al., (2014) who registered a 265.02% improvement when comparing the high phosphorus concentration group versus the low phosphorus concentration group, after 110 DAS and in a controlled environmental growth chamber. Also, we obtained a better pod dried mass improvement than Bulgarelli et al. (2017) who recorded a 100.00% improvement when comparing the sufficient P supplied group versus the low P supplied group, within the period of September to November of 2015, and under greenhouse conditions.

4.4.3 Nitrogen and phosphorus concentration

Nitrogen and phosphorus are vital nutrients for plant growth and development, because they are part of important biomolecules such as: amino acids, proteins, phospholipids, coenzymes, and chlorophylls. Additionally, they play an important role in metabolic regulation, photosynthesis, and nutrient transport (BEHERA et al., 2014; GAO et al., 2022; KARUNANITHI et al., 2015; KUBAR et al., 2021; NARASIMHAN et al., 2013; SHARMA et al., 2013; SOARES et al., 2016).

Our results showed that the plants fertilized with NSBMH presented a greater nitrogen concentration (0.0405±0.003gN g^{-1}) versus the commercial fertilizer and the negative control. Consequently, nitrogen percentage (4.056%N) and protein content (17.97%) increased as well (TABLE 3). Singh et al. (2014) registered lower concentrations of nitrogen 0.0310gN g^{-1} and 0.0324gN g^{-1} in soybean leaves after 110 DAS when using high (0.5mM) and middle (0.1mM) concentrations of inorganic

phosphate in a modified Hoagland's nutrient solution, whereas the same author reported a higher nitrogen concentration (0.0497gN g^{-1}) in soybean leaves under low (0.01mM) concentration of inorganic phosphate.

Regarding to phosphorus concentration, no significant difference was registered among the experiments after 90 days of growth (TABLE 3). However, phosphorus concentration was higher with the NSBMH (0.00212±0.0003gP_2O_5 g^{-1}), followed by the commercial fertilizer (0.00170±0.0004gP_2O_5 g^{-1}), and the negative control (0.00145±0.0002gP_2O_5 g^{-1}) (TABLE 3). The phosphorus concentration (0.00212±0.0003gP_2O_5 g^{-1} or 0.9273mgP g^{-1}) obtained in this study was slightly higher to that obtained by SINGH et al. (2014) (0.90mgP g^{-1}) in soybean leaves when using low (0.01mM) concentration of inorganic phosphate in a modified Hoagland's nutrient solution after 110 DAS, whereas the same author registered higher phosphorus concentrations 5.21mgP g^{-1} and 1.76mgP g^{-1} under high (0.5mM) and middle (0.1mM) concentration of inorganic phosphate, respectively.

The insignificant difference on phosphorus concentration registered in this study could be an effect of plant age and nutrient (N and P) movement, where nutrients move from leaves to pods and seeds (HANWAY; WEBER, 1971; SINGH et al., 2014). As an example, Singh et al. (2014) registered higher phosphorus concentration in pods and seeds than in leaves after 110 DAS, even under different phosphorus concentrations used for growth. Additionally, under phosphorus deprivation (for this study the negative control) legumes such as soybean activate its regulatory systems and adaptability characteristics such as phosphorus accumulation in nodules and/or phosphorus re-cycling from organic phosphorus (present in different plant organs like leaves) in order to maintain phosphorus homeostasis within the plant (BULGARELLI et al., 2017; SULIEMAN; TRAN, 2015).

4.4.4 Chlorophyll pigments concentration

During the photosynthesis process chlorophyll pigments *a* and *b* are in-charge of transforming light into chemical energy (ATP), which is then utilized for the biosynthesis of molecules through carbon dioxide fixation (GITELSON et al., 2016). In plants, chlorophyll *a* and *b* coexist together in a ratio 3:1 (Chl *a*: Chl *b*) being chlorophyll *a* mostly responsible for light harvesting and electron transfer while chlorophyll *b* just

participates on light harvesting (CROCE; VAN AMERONGEN, 2014; HUMPHREY, 2006).

Our results showed that chlorophyll pigments concentration *a*, *b*, and *a+b* did not differ significantly among the NSBMH, the commercial fertilizer, and the negative control after 30, 60, and 90 days of growth (TABLE 4). However, when fertilization was not performed (negative control) chlorophyll pigments concentration *a* and *a+b* decreased significantly after 60 and 90 days of growth, whereas when fertilization was performed with the NSBMH and the commercial fertilizer after 90 days of growth chlorophyll pigments concentration *a* and *a+b* presented an increment which was not significantly different between them (TABLE 4).

The results presented here are slightly lower to those registered in Chapter 1 for chlorophyll pigments concentration *a* (35.20±6.03ug cm^{-2}), *b* (10.30±1.34ug cm^{-2}), and *a+b* (45.50±7.36ug cm^{-2}) after 120 DAS. These differences could be an effect of leaf sample time (120 DAS vs 90 DAS), leaf placement on the plant (3^{th} fully formed trifoliate from top to bottom [older leaf] vs last fully formed trifoliate [younger leaf]), and phosphorus and other nutrients availability because fertilization rate (4 vs 2 fertilizations before sampling). Furthermore, other investigations have registered positive effects of mineral phosphorus fertilization on chlorophyll pigment content, for example: Ao et al. (2013b) registered an increment on chlorophyll content (3.50-2.57mg g^{-1} F.W.) at the podding stage when monopotassium phosphate (KH_2PO_4) was used in different concentrations (0.5-1.5mmol L^{-1}), Singh et al. (2014) registered a total chlorophyll content of ≈100mg cm^{-2} when inorganic phosphate was used after 60 DAS, and Kamran et al. (2018) obtained an increment on chlorophyll content when applying $(NH_4)_3PO_4$ (soluble) and $Ca(PO_4)_3(OH)$ (slowly soluble) as phosphorus source after 42 days of growth. Finally, our chlorophyll pigment *a+b* improvement versus the negative control was better than the 18,18% obtained by Kamran et al. (2018) when using mineral phosphorus.

On the other hand, Vengavasi and Pandey (2018) registered lower concentrations of chlorophyll *a* (1.64±0.04mg g^{-1}F.W.) and total chlorophyll (2.25±0.01mg g^{-1}F.W.) when single super phosphate was applied at a rate of 25mgP Kg^{-1} soil whereas when phosphorus was not applied (low phosphorus concentration of 2mgP Kg^{-1} soil) chlorophyll *a* (2.01±0.20mg g^{-1}F.W.) and total chlorophyll (2.64±0.21mg g^{-1}F.W.) were higher.

4.4.5 Final remarks

Industrialized crops, such as soybean, must be provided with a suitable amount of nutrients in order for them to grow, develop, and produce satisfactorily. Within those nutrients, phosphorus is a vital element because of its involvement in energy and molecules production, and when phosphorus is not present it can affect negatively soybean morphological features (AKTER et al., 2013; DEVI et al., 2012; HE et al., 2019; MILTON; EISWERTH; AGER, 1991; SINGH et al., 2014; TALIMAN et al., 2019; XIANG-WEN et al., 2008; XUE et al., 2013), biochemical parameters (e. g. chlorophyll content) (AO et al., 2013a; KAMRAN et al., 2018; RYCHTER; RAO; RAO, 2005; SINGH et al., 2014; VENGAVASI; PANDEY, 2018; XUE et al., 2013), and yield (e. g. pod number, pod weight, seed weight) (AKTER et al., 2013; AO et al., 2014; BULGARELLI et al., 2017; HE et al., 2019; MAJUMDAR et al., 2001; SINGH et al., 2014; TALIMAN et al., 2019; XUE et al., 2013).

The neutralized sulfuric bone meal hydrolysate (NSMBH) utilized in this study demonstrated to be an excellent alternative as phosphorus source versus the commercial phosphoric fertilizer (used in this study) and other mineral phosphate fertilizers (described above). Moreover, the NSBMH had a suitable amount of nitrogen, a well-balanced quantity of nitrogen and phosphorus, and an optimum pH. Furthermore, the quantity and the rate of application of the NSBMH enhanced soybean growth and development.

After the addition of the NSBMH to the substrate, soybean plants were able to absorb (through the root system) and translocate the nutrients (N and P) to the areal parts for its usage, such as leaves where nitrogen concentration was 0.0405±0.003gN g^{-1} and phosphorus concentration was 0.00212±0.0003gP_2O_5 g^{-1} (TABLE 3). This allowed to increase the foliar area (FIGURE 1, TABLE 1) and maintain an adequate photosynthetic process (TABLE 4) through the 90 days of growth. These characteristics plus the fertilization with the NSBMH and the adequate water regimen utilized improved the light absorption and CO_2 fixation which enhanced protein content (17.97%) in leaves (TABLE 3). Also allowing leave enlargement (FIGURE 1, TABLE 1), leave development and maintenance (TABLE 2), plant elongation (especially shoot enlargement, TABLE 2), and the augmentation of the total plant fresh and dried masses (TABLE 2). These morphological characteristics (especially dried matter), nutrient concentration (e. g. N and P), and biochemical parameters (e. g. protein

percentage and chlorophyll pigment concentration) significantly benefited soybean yield, throughout the formation and augmentation of pods and the increment of its fresh and dried masses as well (TABLE 2).

4.5 CONCLUSIONS

The utilization of a neutralized sulfuric bone meal hydrolysate (NSBMH) as an alternative phosphorus nutrient source improved significantly, after 90 days of growth, soybean foliar area (54.76%), trifoliar leaves number (59.22%), plant fresh mass (91.94%), plant dried mass (115.93%), plant height (18.96%), pod number (80.50%), pod fresh mass (85.69%), pod dried mas (78.34%), nitrogen concentration (0.0405±0.003gN g^{-1}), and protein content (17.97%), while maintaining an adequate phosphorus concentration (0.00212±0.0003gP_2O_5 g^{-1}) and chlorophyll pigments concentration (Chl *a*+*b* = 41.037±2.55ug cm^{-2}) versus a commercial phosphoric fertilizer. Based on these results, NSBMH is an alternative phosphorus source that can be recycled from anthropogenic waste-streams in order to decrease the dependency on main suppliers (e. g. Russia) of mineral phosphoric fertilizers and reduce its environmental impact.

5 CHAPTER 3 – THE POTENTIAL USE OF NEUTRALIZED SULFURIC BONE MEAL HYDROLYSATE AS FOLIAR FERTILIZER

ABSTRACT

Plants can just absorb 30 to 40% of nutrients from the soil through the root system because depends on plant species, soil properties, and wheatear conditions. Therefore, foliar fertilization of macro and micro nutrients have proven to be an excellent alternative. Thus, here we tested the foliar application of a neutralized sulfuric bone meal hydrolysate (NSBMH) on soybean growth parameters and chlorophyll pigment concentration under greenhouse conditions. From September to November 2021 soybean plants were grown and foliar fertilized with 1% of NSBMH (3.28%N, 2.2%P_2O_5), other group with 1% of commercial fertilizer (2.38%N, 4.7%P_2O_5), and a negative control group (unfertilized). After 90 days of growth, the group fertilized with the NSBMH improved significantly ($p<0.05$) soybean foliar area (52,53%), plant fresh mass (103,88%), plant dried mass (182,96%), plant height (36,83%), and chlorophyll pigment concentration *a+b* (27,93%) when compared to the negative control group, and presented higher values versus the commercial fertilizer group but were no significantly different ($p>0.05$), except for shoot dried mass. Consequently, foliar fertilization with neutralized sulfuric bone meal hydrolysate demonstrates that nutrient recycling such as phosphorus from anthropogenic waste-streams (e. g. cattle bone) decreases the environmental impact generated from it and from the use of soil fertilizers, and at the same time reduces the dependency on main fertilizers suppliers.

Key-words: Bone meal. Foliar. Fertilizer. Hydrolysate. Phosphorus. Soybean.

5.1 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, mineral content), and/or weather conditions (BERNAL et al., 2007; HELYAR, 1998). So, in order to overcome these drawbacks, 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 (ADHIKARI et al., 2020; BERNAL et al., 2007; GÖRLACH et al., 2021; LIZARAZO; LAMPI; MÄKELÄ, 2021; MAHAPATRA; SATAPATHY; PANDA, 2022; SHAHENA et al., 2021).

Soybean is an important oil seed cultivar because a variety of products can be obtained from it, such as: oleochemicals, vegetable oil, and protein meal for human and animal feed (AMARO BITTENCOURT et al., 2021; OECD-FAO Agricultural Outlook 2021-2030, 2021; SINGH et al., 2014). Additionally, soybean by-products like: hulls, okara, okara flour, and soymilk can be used for the production of plant growth hormones, prebiotics, and bioethanol in biorefineries (AMARO BITTENCOURT et al., 2021; CANAAN et al., 2022). Moreover, industrialized crops like 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 (KUMAR et al., 2022).

Phosphorus is a vital nutrient for plant growth and development because participates in cellular metabolism, for example: cell division, photosynthesis, nutrient transport, among others; and cellular structure, such as: nucleic acids, adenosine triphosphate (ATP), and phospholipids (BEHERA et al., 2014; KARUNANITHI et al., 2015; SHARMA et al., 2013). Currently, most of the phosphorus used for the production of soybean come from solid granulated phosphate-based products, such as: mono-ammonium phosphate (MAP), di-ammonium phosphate (DAP), and triple superphosphate (TSP) (AKTER et al., 2013; AO et al., 2013a; REETZ, 2016; BULGARELLI et al., 2017; DEVI et al., 2012; HE et al., 2019; KAMRAN et al., 2018; SINGH et al., 2018; SOARES et al., 2016; TALIMAN et al., 2019; XUE et al., 2013), which are added periodically in order to maintain soluble phosphorus on soils;

however, its extensive use can have negative effects on the environment by causing erosion and eutrophication (HELYAR, 1998).

According to Jayathilakan et al. (2012) cattle bones represent from 10 to 15 % of the live animal which after the slaughtering process can be further processed for the obtainment of cattle bone meal (MOLLER, 2015). Bone meals contain high phosphorus content (2.21-9.62%d.w.) (MOLLER, 2015) and have been used as an alternative phosphorus source for the production of barley, oat, maize, and wheat (CHEN et al., 2011; NOGALSKA, 2016; NOGALSKA; ZALEWSKA, 2013; SAEID et al., 2014). But, the correct use of bone meals depends on soil pH, weather conditions, plant species, and method of application (NOGALSKA; ZALEWSKA, 2013). Thus, in order to overcome the disadvantages presented by bone meals and liberate the phosphorus present on it, a chemical hydrolysis using a strong acid can be applied to release the fixed phosphorus on the bone meal to the liquid (hydrolysate), resulting in a renewable phosphorus fertilizer (LEINWEBER et al. 2018; TARAYRE et al. 2016).

In Chapter 1 and Chapter 2 we proved the benefits of a neutralized sulfuric bone meal hydrolysate (NSMBH) as an alternative phosphorus source for soybean plants, however it was applied on high concentration to the substrate (vermiculite) which can result on negative effects and disadvantages as described above. Thus, here we evaluated the foliar application of the neutralized sulfuric bone meal hydrolysate on soybean growth parameters and chlorophyll pigment concentration under greenhouse conditions.

5.2 MATERIALS AND METHODS

5.2.1 Fertilizers

Ten milliliters of the neutralized sulfuric bone meal hydrolysate (NSBMH) containing 3.28% N (w/w) and 2.2% P_2O_5 (w/w) (obtained as described in Chapter 1) was mixed with 20 mL of glycerin in deionized water, then the solution was brought up to 1 L with deionized water.

The commercial organo-mineral fertilizer (positive control) containing 2.38%N (w/w) and 4.7% P_2O_5 (w/w) was prepared by mixing 10 mL of it in deionized water and brought up to 1 L (according to manufacture).

Nitrogen and phosphorus content were performed by Kjeldahl and spectrophotometric molybdovanadate methods, respectively.

5.2.2 Greenhouse test

Soybean seeds were germinated and fertilizers were tested in the spring of 2021 under greenhouse conditions during 90 days, from September to November.

5.2.2.1 Soybean germination

One-liter plastic cups were filled with 200g of dried vermiculite (substrate) which then was soaked with running water prior to sowing. Twenty-four hours later, four soybean seeds (*G. max*) per cup were sowed. Then, ten days after sowing (DAS) one germinated seed per cup was maintained.

5.2.2.2 Fertilization

Each fertilizer and the negative control (not fertilized) had three replicates with five cups each.

Fertilization was done on the 30^{th} and 60^{th} DAS by spraying three times each fertilizer to the leaves and nothing was sprayed in the negative control. Watering was performed with running water when needed.

5.2.3 Soybean growth measurements

5.2.3.1 Foliar area

A week before each fertilization and before ending the experiment the perimeter of the last fully formed trifoliate leaf was drawn on a paper, then scanned, and the foliar area (cm^2) was obtained with the online version of Image J (RASBAND, 2012).

5.2.4 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 putted in a paper bag and air force dried at 65°C during 72h (SOARES et al., 2016), then the dried mass was obtained.

5.2.5 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.

5.2.6 Chlorophyll pigments

A week before each fertilization and a week before harvesting, three circular discs with 23mm diameter were cut from the middle of each leaf of the last fully expanded trifoliate. Pigments were extracted from these leaf discs with 8mL of cold methanol in a mortar and pestle, then the liquid was centrifuged at 2,500 rpm during 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 (PORRA; THOMPSON; KRIEDEMANN, 1989). Finally, chlorophyll pigments *a*, *b*, and *a+b* by area (cm^2) were calculated with the equations proposed by Porra; Thompson; Kriedemann (1989) and Fan et al. (2018).

5.2.7 Statistical analysis

Three randomly selected samples from each replicate of each fertilizer and the negative control were taken for foliar area and for chlorophyll pigments two randomly selected samples from each replicate of each fertilizer and the negative control were used. Total plant fresh and dried masses, and plant height were obtained from all the replicates of each fertilizer and the negative control. Then the collected data was processed on Microsoft Office Excel 2019 and Tukey's test (95% confidence) was performed for foliar area, plant fresh and dried masses, plant height, and chlorophyll

pigments concentration in order to establish significant differences between experiments means using Minitab 19 Statistical Software.

5.3 RESULTS

5.3.1 Soybean growth characteristics

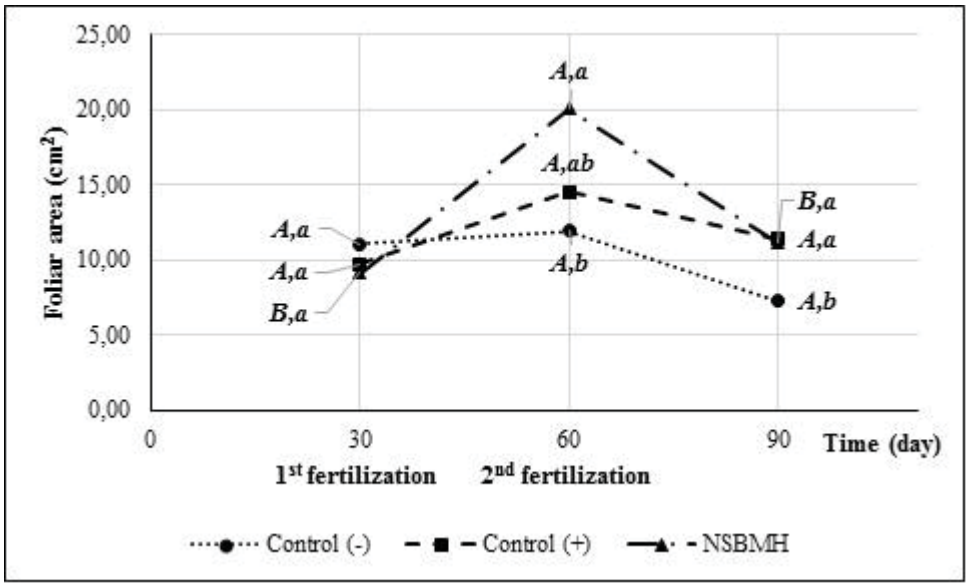
5.3.1.1 Foliar area

FIGURE 1 and TABLE 1 compares the effect of the foliar fertilization done with the NSBMH versus a commercial fertilizer (positive control) on foliar area of soybean plants after 90 days of growth under greenhouse conditions.

The group fertilized with the NSMBH registered a significant improvement ($p<0.05$) on foliar area versus the negative control group for the 60^{th} day (30 days after the first fertilization) and 90^{th} day (30 days after the second fertilization), whereas no significant difference ($p>0.05$) was registered for this trait between the NSMBH and the positive control during the time of growth (FIGURE 1, TABLE 1).

In addition, the group fertilized with the NSBMH registered the higher foliar area at the 60^{th} day (30 days after the first fertilization) which was significantly different ($p<0.05$) from the 30^{th} and 90^{th} day within this group, while no significant difference was registered for this characteristic within the negative control and positive control groups (FIGURE 1, TABLE 1).

FIGURE 1 - COMPARISON OF FOLIAR AREA OF SOYBEAN PLANTS AFTER TWO FOLIAR FERTILIZATIONS WITH NSBMH AND A COMMERCIAL FERTILIZER [CONTROL (+)] DURING 90 DAYS.



Means that do not share a letter are significantly different according to Tukey's test (p<0.05), capital letters within the same group and low case letters among groups. Mean of three replicates.
SOURCE: The author (2022).

TABLE 1 - COMPARISON OF THE EFFECT OF TWO FOLIAR FERTILIZERS ON THE FOLIAR AREA (CM^{-2}) OF SOYBEAN PLANTS AFTER 30, 60, AND 90 DAYS OF GROWTH UNDER GREENHOUSE CONDITIONS.

			Experiment			
Time	**Control (-)**		**Control (+)**		**NSBMH**	
Day	***Mean ± S. D.**	****Improvement (%)**	**Mean ± S. D.**	**Improvement (%)**	**Mean ± S. D.**	**D.P. (±)**
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
D.P. (±)	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 within the same group and low case letters among groups. Mean = 3 ± S. D.*
***Percentage of improvement of the NSBMH versus the negative control and positive control, respectively.*
SOURCE: The author (2022).

5.3.1.2 Plant fresh and dried masses

After 90 days of growth, the group foliar fertilized with the NSBMH improved significantly ($p<0.05$) plant fresh and dried masses (except for root fresh mass) versus the negative control (TABLE 2). Also, a significant difference ($p<0.05$) was registered just for shoot dried mass when comparing the NSBMH versus the positive control (TABLE 2).

5.3.1.3 Plant height

Ninety days after growth, the group foliar fertilized with the NSBMH improved significantly ($p<0.05$) shoot and plant height versus the negative control, whereas no significant difference ($p>0.05$) was registered for shoot, root, and plant height when compared to the positive control (TABLE 2).

TABLE 2 – STATISTICAL DIFFERENCE AND IMPROVEMENT (%) OF SOYBEAN PLANTS FRESH AND DRIED MASSES, AND HEIGHT AFTER 90 DAYS GROWTH UNDER GREENHOUSE CONDITIONS.

			Experiment				
			Control (-)		Control (+)		NSBMH
Parameter		**n**	***Mean ± S. D.**	****Improvement (%)**	**Mean ± S. D.**	**Improvement (%)**	**Mean ± S. D.**
Fresh mass (g)	Shoot	3	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	3	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***
Height (cm)	Shoot	3	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***

**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 versus the negative control and positive control, respectively.*
SOURCE: The author (2022).

5.3.2 Chlorophyll pigments

TABLE 3 compares the significant difference found for the concentration of chlorophyll pigments *a*, *b*, and *a+b* obtained after 30, 60, and 90 days of growth for the experiments that were foliar fertilized with the NSBMH and the commercial fertilizer (positive control), and the negative control group under greenhouse conditions.

The experiment fertilized with the NSBMH increased significantly ($p<0.05$) the concentration of chlorophyll pigments *a* and *a+b* when compared to the negative control group just for the 90th day (30 days after the second fertilization), whereas no significant difference ($p>0.05$) was found for chlorophyll pigments concentration *a*, *b*, and *a+b* versus the positive control group during the time of growth (TABLE 3).

In addition, chlorophyll pigment concentration *a*, *b*, and *a+b* changed over the time of growth within each group (TABLE 3). The negative control group presented a significant decrease ($p<0.05$) on chlorophyll pigments concentration *a* and *a+b* for the 60th and 90th day when compared to the 30th day. Also, a similar trend was registered for the positive control group 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 group 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. On the contrary, no significant difference ($p>0.05$) on chlorophyll pigments concentration was registered within the NSBMH group.

TABLE 3 - COMPARISON OF THE EFFECT OF TWO FOLIAR FERTILIZERS ON CHLOROPHYLL PIGMENTS CONCENTRATION *A*, *B*, AND *A+B* (UG CM^{-2}) OF SOYBEAN PLANTS AFTER 30, 60, AND 90 DAYS OF GROWTH UNDER GREENHOUSE CONDITIONS.

		Chl *a*		
Time	**Control (-)**	**Control (+)**	**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 within the same group and low case letters among groups. Mean = 3 ± S.D.*
SOURCE: The author (2022).

5.4 DISCUSSION

5.4.1 Soybean growth characteristics

5.4.1.1 Foliar area

Foliar area is a relevant morphological feature because improves carbon assimilation and light interception (HUSSAIN et al., 2019).

Foliar fertilization with NSBMH significantly improved soybean foliar area when compared to the negative control group during the 90 days of growth. This result is in accordance with Boote et al. (1978) who observed a leaf area extension after foliar spraying potassium polyphosphate, a mineral phosphorus source, 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. (1978) just lasted one-day and ours lasted sixty days.

On the contrary, Haq (1998) sprayed during the vegetative stage of soybean plants a commercial fertilized (N-P-K:3-8-15) composed by H_3PO_4, liquid ammonia,

and KOH in two different years and registered a decrease on foliar area of the sprayed group between 2,21 to 3,79% when compared to its negative control group.

The short period extension on leaf area observed by Boote et al. (1978) and the decrease on foliar area registered by Haq (1998) can be explained by burning and necrotic spots caused from the fertilizer salts and the application rate used as suggested by Boote et al. (1978). These negative effects were not present in our experiment that was foliar sprayed with the NSBMH. Firstly, because the NSBMH foliar fertilizer was correctly applied during the growth of soybean plants, for example: twice within a period of time of 30 days between sprays, whereas Boote et al. (1978) did five fertilizations during 5 week period (1 fertilization each week) and Haq (1998) just did one fertilization. Secondly, due to the well-balanced and dissolved amount of nutrients (1% of each nutrient from the original NSBMH, where cattle bone meal was the only phosphorus source), its optimum pH (pH=6.5), and the amount of glycerin (2%) present on it. According to Barèl (1975), when glycerin is present on foliar fertilizers it prevents sprays to dry completely from the leaf surface of plants which at the same time diminishes the appearance of burning and necrotic spots on leaves caused from foliar fertilization. Additionally, when glycerin is part of foliar fertilizers containing N-P-K nutrients, it can increase by 7^{th} fold the absorption of phosphorus by leaves which allows them to enlarge (BARÈL, 1975).

5.4.1.2 Plant fresh and dried masses

Plant fresh and dried masses are vital morphological traits because represents a suitable fertilization during plant growth.

The total plant fresh mass improved significantly when the NSBMH was foliar sprayed when compared to the negative control group after ninety days of growth. Our percentage of improvement was higher than Akter et al. (2013) who registered a 4,62% improvement versus its negative control group after 110 days of growth when using mineral phosphorus as soil fertilizer. Here we confirm that soil fertilization can reduce nutrients (e. g. phosphorus) availability for plants due to soil properties and environmental factors (BERNAL et al., 2007; HELYAR, 1998).

The utilization of the NSBMH as foliar fertilizer significantly improved the total plant dried mass after 90 days growth versus the negative control group. Our result is in accordance with Mannan (2014), however Mannan (2014) obtained after

approximately 120 DAS (reproductive stage) a total dry weight improvement on soybean plants of just 32,64% and 19,93% when compared to their negative and positive control groups, respectively; even after using a N-P-K-S-Mg mineral foliar fertilizer which contained sodium dihydrogen orthophosphate as phosphorus source. On the other hand, our total plant dried mass improvement was better than Haq (1998) who did not register dry weight improvement on the group sprayed with a commercial fertilized (N-P-K:3-8-15) composed by H_3PO_4, liquid ammonia, and KOH during the growth of soybean plants.

The higher improvement on total fresh and dried masses obtained for the group foliar fertilized with the NSBMH was due, first of all, by the correct procedure used for the application of the NSBMH during the growth of soybean plants, for example: two foliar applications each one separated by a 30 days span, which was similar to the procedure used by Mannan (2014) and differed from Haq (1998), who just did one foliar application. Second, the NSBMH foliar fertilizer had a well-balanced and dissolved amount of nutrients (1% of each nutrient from the original NSBMH, where cattle bone meal was the only phosphorus source), had an optimum pH (pH=6.5), and had a good amount of glycerin (2%). Where glycerin when is present in foliar fertilizers containing N-P-K nutrients it can enhance phosphorus absorption by leaves in up to a 7^{th} fold (BARÈL, 1975). This allowed soybean plants foliar fertilized with the NSBMH to absorb, transport (within the plant), and utilize the nutrients (e. g. nitrogen and phosphorus) for its growth (e. g. mass production).

5.4.1.3 Plant height

Plant height is an important morphological characteristic because represents plant growth and development.

The total plant height improved significantly when the NSBMH was foliar sprayed when compared to the negative control group after 90 days of growth, which is in agreement with other studies, however they registered lower improvement on total plant height than ours. For example, Mandic et al. (2015) found that the foliar fertilization during the reproductive stage of soybean with two different commercial fertilizers Waxal and Ferticare I improved total plant height just by 15,68% and 17,61%, respectively, when compared to its negative control group. Also, Mannan (2014) registered after approximately 120 DAS (reproductive stage) a total plant height

improvement on soybean plants of just 7,16% when compared to its negative control group, even after using a N-P-K-S-Mg mineral foliar fertilizer which contained sodium dihydrogen orthophosphate as phosphorus source. In contrast, Milanez de Rezende et al. (2005) did not found improvement on total plant height versus its negative control group when using the foliar commercial fertilizer Quimifol P30 (as phosphorus source), and even after applying it more than once during the growth of soybean plants.

The higher improvement on total plant height obtained for the group foliar sprayed with the NSBMH was due, initially, by the correct practice developed for the application of the NSBMH during the growth of soybean plants, for example: first foliar application at 30 DAS and second foliar application at 60 DAS (with a 30 days span between each fertilization), which was similar to the procedure used by Mannan (2014). However, our procedure differed from the studies of Mandic et al. (2015) and Milanez de Rezende et al. (2005), where the first did two fertilizations with a 10 days span between each fertilization at the reproductive stage of soybean, and the second fertilized once and more than twice at different stages of soybean. Additionally, the NSBMH foliar fertilizer had a well-balanced and dissolved amount of nitrogen and phosphorus (1% of each nutrient from the original NSBMH, where cattle bone meal was the only phosphorus source), whereas the commercial fertilizers used by Mannan (2014) and Mandic et al. (2015) mainly contain high pure content of mineral phosphorus plus other macronutrients (e. g. nitrogen and potassium) and micronutrients (e. g. magnesium, iron, zinc, copper, among others). Also, the NSBMH foliar fertilizer had an optimum pH (pH=6.5) and a good amount of glycerin (2%). The last one, according to Barèl (1975), can enhance phosphorus absorption by leaves in up to a 7^{th} fold when is present in foliar fertilizers containing N-P-K nutrients. The chemical characteristics of the NSBMH foliar fertilizer allowed soybean plants sprayed with it to absorb, transport (within the plant), and utilize the nutrients (e. g. nitrogen and phosphorus) for its development (e. g. plant enlargement).

5.4.2 Chlorophyll pigments

Chlorophyll pigments *a* and *b* are important biochemical traits because they are in-charge of transforming light into chemical energy which is then used for the production of biomolecules through CO_2 fixation, during the process known as photosynthesis (GITELSON et al., 2016).

Ninety days after growth the group foliar sprayed with the NSBMH improved significantly the chlorophyll pigments *a* and *a+b* by 26,85% and 27,93%, respectively, when compared to the negative control group. Our results are better than the registered by Boote et al. (1978) who observed a slight enhance on gross photosynthesis after spraying potassium polyphosphate, a mineral phosphorus source, during the seed-filling period of soybean when compared to its non-foliar sprayed group. Moreover, we noted a better chlorophyll *a+b* improvement than Haq (1998) who found a slight decrease on chlorophyll of the soybean group sprayed with the commercial fertilized (N-P-K:3-8-15) composed by H_3PO_4, liquid ammonia, and KOH when compared to its negative control group.

Moreover, after 90 days of growth within each group, the negative control group of our experiment was the only one to present a significant decrease on chlorophyll pigments *a* and *a+b* by 23,68% and 22,41%, respectively. Boote et al. (1978) registered the same trend on the gross photosynthesis of the non-foliar fertilized group but with a higher decrease, approximately 94,28%, which was slightly similar to group foliar fertilized with potassium polyphosphate, a mineral phosphorus source, during the seed-filling period of soybean.

The no improvement on gross photosynthesis (BOOTE et al., 1978) and chlorophyll (HAQ 1998) could be an effect of burning and necrotic spots caused from the fertilizer salts and the application rate used as suggested by Boote et al. (1978), effects that were not present in the group that was foliar fertilized with the NSBMH in our experiment. Initially, because the NSBMH foliar fertilizer was sprayed correctly during the growth of soybean plants, for example: two times within a period of time of 30 days between sprays, whereas Boote et al. (1978) did five fertilizations during 5 weeks period (1 fertilization each week) and Haq (1998) just did one fertilization. Secondly, due to the well-balanced and dissolved amount of nutrients (1% of each nutrient from the original NSBMH, where cattle bone meal was the only phosphorus source), the optimum pH (pH=6.5), and the amount of glycerin (2%) present on the NSBMH foliar fertilizer. According to Barèl (1975), when glycerin is present on foliar fertilizers it prevents sprays to dry completely from the leaf surface of plants which at the same time diminishes the appearance of burning and necrotic spots on leaves caused from foliar fertilization. Additionally, when glycerin is part of foliar fertilizers containing N-P-K nutrients, it can increase by 7^{th} fold the absorption of phosphorus by leaves (BARÈL, 1975). These chemical characteristics present on the NSBMH foliar

fertilizer allowed the soybean plants sprayed with it to absorb, transport (within the plant), and utilize the nutrients (e. g. nitrogen and phosphorus) for the production of chlorophyll pigments, which as suggested by Gitelson et al. (2016) are vital for the photosynthesis process.

5.4.3 Final remarks

The foliar fertilizer produced in this study which was composed by 1% of neutralized sulfuric bone meal hydrolysate [pH=6.5, nitrogen, and phosphorus (cattle bone meal as the only phosphorus source)] and 2% of glycerin, and when it was sprayed twice (first foliar application at 30 DAS and second foliar application at 60 DAS, with a 30 days span between each spray) during the ninety days of growth of soybean plants improved significantly its morphological characteristics such as: foliar area, plant fresh and dried masses, plant height, and chlorophyll pigments concentration when compared to the negative control group (unfertilized), whereas attained similar values when compared to the group sprayed with a commercial foliar fertilizer (positive control, containing mineral phosphorus).

Therefore, in order to foliar fertilizers containing phosphorus from anthropogenic waste-streams (e. g. cattle bones) have positive effects on plants it must meet the following requirements: 1) have available phosphorus at pH nearly to neutrality for example pH=6.5, 2) have phosphorus in low concentrations such as 1% when its original form has 2.2% P_2O_5 (w/w), 3) contain nitrogen, 4) have a humectant like glycerin, and 5) be applied twice for example 1st application at 30 DAS and 2nd application at 60 DAS, with a 30 days span between each application. These characteristics will enhance the translocation of nutrients (e. g. nitrogen and phosphorus) from soybean leaf surfaces into the plant for its utilization. Once in the plant, nutrients will be used immediately for leaf enlargement which will increase foliar area too (TABLE 1). At the same time, in leaves, nutrients will be utilized for the production of chlorophyll pigments as well (TABLE 3). Thus, coupling a bigger foliar with a higher concentration of chlorophyll pigments plus a suitable amount of nutrients will guaranteed the production of energy and formation of biomolecules through the photosynthesis process, which will result in plant mass augmentation (TABLE 2) and in plant height increase (TABLE 2).

5.5 CONCLUSIONS

The use and rate of application of the neutralized sulfuric bone meal hydrolysate (NSBMH) as foliar fertilizer improved significantly, after 90 days of growth, soybean foliar area (52,53%), plant fresh mass (103,88%), plant dried mass (182,96%), plant height (36,83%), and chlorophyll pigment concentration *a*+*b* (27,93%) versus the negative control group. Therefore, the use of NSBMH as foliar fertilizer proves that anthropogenic waste-streams (e. g. cattle bones) can be recycled for the obtainment of nutrients (e. g. phosphorus) which can be used for the production of foliar fertilizers that can be utilized in agriculture. Decreasing, at the same time, the dependency on mineral phosphoric soil and foliar fertilizers, and in its main suppliers too. Also, reduces the environmental impact generated from soil fertilization and human waste-stream.

6 CONCLUSIONS

Phosphorus is a vital nutrient for plant growth and development, and most of it comes from mines ores which are highly contaminant. Also, the wrongly use of chemical phosphorus fertilizers causes negative effects to the environment such as soil erosion and eutrophication of waters. Moreover, cattle bones which are usually consider a contaminant are an excellent source of phosphorus if they are further processed. Furthermore, Brazil will become after 2030 the first producer of cattle meat and soybean, as an effect cattle bone and phosphoric fertilizers demand will increase. Thus, the research performed here demonstrated that cattle bone meal is an excellent alternative as phosphorus nutrient source, and when is used as soil fertilizer improved soybean morphological characteristics such as: foliar area, trifoliar leaves number, plant fresh mass, plant dried mass, and plant height. Also, enhanced its pod yield, pod number, pod fresh and pod dried masses. Moreover, increased nitrogen concentration, protein content, phosphorus concentration, and maintained a suitable concentration of chlorophylls *a*, *b*, and *a*+*b*. Additionally, a foliar fertilizer derived from cattle bone demonstrated to enhance soybean: foliar area, plant fresh mass, plant dried mass, plant height, and chlorophylls *a*, *b*, and *a*+*b*. So, the neutralized sulfuric bone meal hydrolysate developed in this study is an excellent alternative as phosphorus nutrient

source allowing Brazil to reduce its dependency on foreign phosphoric fertilizer and become the first sustainable agroindustry.

REFERENCES

ADHIKARI, B. et al. Effect of foliar application of potassium fertilizers on soybean plants under salinity stress. **Journal of the Saudi Society of Agricultural Sciences**, v. 19, n. 4, p. 261–269, 1 maio 2020.

AKTER, F. et al. Effect of phosphorus and sulphur on growth and yield of soybean (*Glycine Max*. L). **International Journal of Bio-resources and Stress Management**, v. 4, n. 4, p. 556–561, 2013.

AMARO BITTENCOURT, G. et al. Soybean hulls as carbohydrate feedstock for medium to high-value biomolecule production in biorefineries: A review. **Bioresource Technology**, v. 339, 2021.

AOAC. **Official methods of analysis.** Washington, D.C.: [s.n.].

AO, X. et al. Comparison of Photosynthetic Characteristics in Soybean Cultivars with Different Phosphorus Efficiencies. **Acta Agronomica Sinica**, v. 35, n. 3, p. 522–529, 6 maio 2009.

AO, X. et al. Study on Plant Morphological Traits and Production Characteristics of Super High-Yielding Soybean. **Journal of Integrative Agriculture**, v. 12, n. 7, p. 1173–1182, 2013a.

AO, X. et al. Physiological and Biochemical Characteristics of Soybean Genotypes with Different Phosphorus Efficiencies under Various Phosphorus Concentrations. **Communication in Soil Science and Plant Analysis**, v. 44, n. 19, p. 2796–2808, out. 2013b.

AO, X. et al. Effect of Phosphorus Fertilization to P Uptake and Dry Matter Accumulation in Soybean with Different P Efficiencies. **Journal of Integrative Agriculture**, v. 13, n. 2, p. 326–334, fev. 2014.

BARÈL, D. **Foliar application of phosphorus compounds**. PhD dissertation—Ames: Iowa State University, 1975.

BEHERA, B. C. et al. Diversity, mechanism and biotechnology of phosphate solubilising microorganism in mangrove—A review. **Biocatalysis and Agricultural Biotechnology**, v. 3, n. 2, p. 97–110, 2014.

BERNAL, M. et al. Foliar and root Cu supply affect differently Fe- and Zn-uptake and photosynthetic activity in soybean plants. **Environmental and Experimental Botany**, v. 60, n. 2, p. 145–150, jun. 2007.

BOOTE, K. J. et al. Effect of Foliar Fertilization on Photosynthesis, Leaf Nutrition, and Yield of Soybeans. **Agronomy Journal**, v. 70, p. 787–791, 1978.

BULGARELLI, R. G. et al. Mycorrhizae enhance nitrogen fixation and photosynthesis in phosphorus-starved soybean (*Glycine max* L. Merrill). **Environmental and Experimental Botany**, v. 140, p. 26–33, 2017.

CANAAN, J. M. M. et al. Soybean processing wastes and their potential in the generation of high value added products. **Food Chemistry**, v. 373, p. 131476, 2022.

CHEN, L. et al. Meat bone meal as fertiliser for barley and oat. **Agricultural and Food Science**, v. 20, p. 235–244, 2011.

CROCE, R.; VAN AMERONGEN, H. Natural strategies for photosynthetic light harvesting. **Nature Chemical Biology**, v. 10, n. 7, p. 492–501, 2014.

DEVI, K. N. et al. Response of Soybean [*Glycine max* (L.) Merrill] to Sources and Levels of Phosphorus. **Journal of Agricultural Science**, v. 4, n. 6, p. 44–53, 2012.

ENVIRONMENTAL PROTECTION AGENCY. **Enforcement and Compliance History Online**. Disponível em: <https://www.epa.gov/>. Acesso em: 17 mar. 2022.

EUROPEAN PHOSPHATE FERTILIZER ALLIANCE. **European Phosphate Fertilizer Alliance**. Disponível em: <http://aeep.eu/about-us/>. Acesso em: 17 mar. 2019.

FAN, Y. et al. Effect of shading and light recovery on the growth, leaf structure, and photosynthetic performance of soybean in a maize-soybean relay-strip intercropping system. **PLoS ONE**, v. 13, n. 5, 2018.

FAO. **Food and Agriculture Organization of the United Nations: Management of Waste from Animal Product Processing.** [s.l: s.n.].

FAO. **World Food and Agriculture - Statistical Yearbook 2020**. [s.l.] FAO, 2020.

FLORIDA INDUSTRIAL AND PHOSPHATE RESEARCH INSTITUTE. **Florida Industrial and Phosphate Research Institute**. Disponível em: <https://fipr.floridapoly.edu/>. Acesso em: 17 mar. 2019.

GAO, S. et al. Comparative differences in photosynthetic characteristics, ion balance, and nitrogen metabolism between young and old wild soybean leaves under nitrogen deficiency. **Plant Stress**, v. 4, 1 abr. 2022.

GITELSON, A. A. et al. Efficiency of chlorophyll in gross primary productivity: A proof of concept and application in crops. **Journal of Plant Physiology**, v. 201, p. 101–110, 2016.

GÖRLACH, B. M. et al. Uptake, subcellular distribution, and translocation of foliar-applied phosphorus: Short-term effects on ion relations in deficient young maize plants. **Plant Physiology and Biochemistry**, v. 166, p. 677–688, 1 set. 2021.

GREEN LEAF AQUARIUMS. **Green Leaf Aquariums**. Disponível em: <https://greenleafaquariums.com/products/bloom-grow-phosphorus-p-liquid-fertilizer-for-aquarium-plants.html>. Acesso em: 17 mar. 2022.

HANWAY, J. J.; WEBER, C. R. Accumulation of N, P, and K by Soybean (*Glycine max* (L.) Merrill) Plants 1. **Agronomy Journal**, v. 63, n. 3, p. 406–408, 1971.

HAQ, M. U. **Foliar fertilization of soybeans at early vegetative stages**. Ph D dissertation—Ames: Iowa State University, 1998.

HE, J. et al. Phosphorus application increases root growth, improves daily water use during the reproductive stage, and increases grain yield in soybean subjected to water shortage. **Environmental and Experimental Botany**, v. 166, p. 103816, 1 out. 2019.

HELYAR, K. R. Efficiency of nutrient utilization and sustaining soil fertility with particular reference to phosphorus. **Field Crops Research**, v. 56, p. 187–195, 1998.

HOROWITZ, J. **War has brought the world to the brink of a food crisis**. Disponível em: <https://edition.cnn.com/2022/03/12/business/food-crisis-ukraine-russia/index.html>. Acesso em: 17 mar. 2022.

HUMPHREY, A. M. Chlorophyll as a color and functional ingredient. **Journal of Food Science**, v. 69, n. 5, p. C422–C425, 2006.

HUSSAIN, F. et al. Waste Animal Bones as Catalysts for Biodiesel Production; A Mini Review. **Catalysts**, v. 11, n. 5, p. 630, 2021.

HUSSAIN, S. et al. Changes in morphology, chlorophyll fluorescence performance and Rubisco activity of soybean in response to foliar application of ionic titanium under normal light and shade environment. **Science of the Total Environment**, v. 658, p. 626–637, 2019.

JAYATHILAKAN, K. et al. Utilization of byproducts and waste materials from meat, poultry and fish processing industries: A review. **Journal of Food Science and Technology**, jun. 2012.

KAKIUCHI, J.; KAMIJI, Y. Relationship between phosphorus accumulation and dry matter production in soybeans. **Plant Production Science**, v. 18, n. 3, p. 344–355, 7 jul. 2015.

KAMRAN, M. A. et al. Effect of different phosphorus sources on soybean growth and arsenic uptake under arsenic stress conditions in an acidic ultisol. **Ecotoxicology and Environmental Safety**, v. 165, p. 11–18, 2018.

KARUNANITHI, R. et al. Phosphorus Recovery and Reuse from Waste Streams. **Advances in Agronomy**, v. 131, p. 1–78, 2015.

KUBAR, M. S. et al. Optimizing nitrogen supply promotes biomass, physiological characteristics and yield components of soybean (*Glycine max* L. Merr.). **Saudi Journal of Biological Sciences**, v. 28, n. 11, p. 6209–6217, 1 nov. 2021.

KUMAR, S. et al. Biofertilizers: An ecofriendly technology for nutrient recycling and environmental sustainability. **Current Research in Microbial Sciences.** Elsevier Ltd,1 jan. 2022.

LEINWEBER, P. et al. Handling the phosphorus paradox in agriculture and natural ecosystems: Scarcity, necessity, and burden of P. 2018.
LIZARAZO, C. I.; LAMPI, A. M.; MÄKELÄ, P. S. A. Can foliar-applied nutrients improve caraway (*Carum carvi* L.) seed oil composition? **Industrial Crops and Products**, v. 170, 15 out. 2021.

MAGALHAES, L.; PEARSON, S. **Ukraine War Hits Farmers as Russia Cuts Fertilizer Supplies, Hurting Brazil**. Disponível em: <https://www.wsj.com/articles/ukraine-war-hits-worlds-farmers-as-russia-cuts-fertilizer-supplies-hurting-brazil-11646487815#:~:text=Soybeans%20are%20harvested%20on%20a%20farm%20near%20Bras%C3%ADlia.&text=S%C3%83O%20PAULO%E2%80%94Brazil%20is%20searching,already%20high%20global%20food%20inflation.>. Acesso em: 17 mar. 2022.

MAHAPATRA, D. M.; SATAPATHY, K. C.; PANDA, B. Biofertilizers and nanofertilizers for sustainable agriculture: Phycoprospects and challenges. **Science of the Total Environment,** Elsevier B.V., 10 jan. 2022.

MAJUMDAR, B. et al. Response of soybean (Glycine max) to phosphorus and sulphur in Acid alfisol of Meghalaya. **Indian Journal of Agronomy**, v. 46, n. 3, p. 500–505, 2001.

MANDIC, V. et al. Effect of foliar fertilization on soybean grain yield. **Biotechnology in Animal Husbandry**, v. 31, n. 1, p. 133–143, 2015.

MANNAN, M. A. FOLIAR AND SOIL FERTILIZATION EFFECT ON SEED YIELD AND PROTEIN CONTENT OF SOYBEAN. **Bangladesh Agron. J**, v. 17, n. 1, p. 67–72, 2014.

MILANEZ DE REZENDE, P. et al. ADUBAÇÃO FOLIAR. I. ÉPOCAS DE APLICAÇÃO DE FÓSFORO NA CULTURA DA SOJA. **Ciênc. agrotec., Lavras.**, v. 29, n. 6, p. 1105–1111, 2005.

MILTON, N. M.; EISWERTH, B. A.; AGER, C. M. Effect of phosphorus deficiency on spectral reflectance and morphology of soybean plants. **Remote Sense. Environ**, v. 36, p. 121–127, 1991.

MINISTÉRIO DA AGRICULTURA; PECUÁRIA E ABASTECIMENTO; SECRETARIA DE DEFENSA AGROPECUÁRIA. **Manual de métodos analíticos oficiais para fertilizantes minerais, orgânicos, organominerais e corretivos**. Brasil: [s.n.].

MÖLLER, K. Assessment of Alternative Phosphorus Fertilizers for Organic Farming: Meat and Bone Meal. 25 nov. 2015.

NARASIMHAN, R. et al. Differential changes in galactolipid and phospholipid species in soybean leaves and roots under nitrogen deficiency and after nodulation. **Phytochemistry**, v. 96, p. 81–91, 2013.

NOGALSKA, A. Meat and bone meal as fertilizer for spring barley. **Plant, Soil and Environment**, v. 62, n. 8, p. 373–378, 2016.
NOGALSKA, A.; ZALEWSKA, M. The effect of meat and bone meal on phosphorus concentration in soil and crop plants. **Plant Soil Environ**, v. 59, n. 12, p. 575–580, 2013.

OECD-FAO Agricultural Outlook 2021-2030. Paris: OECD Publishing, 2021.

PAN, X. WEN et al. Assessment on Phosphorus Efficiency Characteristics of Soybean Genotypes in Phosphorus-Deficient Soils. **Agricultural Sciences in China**, v. 7, n. 8, p. 958–969, 1 ago. 2008.

PORRA, R. J.; THOMPSON, W. A.; KRIEDEMANN, P. E. Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls a and b extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. **Biochimica et Biophysica Acta**, v. 975, p. 384–394, 1989.

RASBAND, W. S. **ImageJ**, Maryland, 2012. Disponível em: <http://imagej.nih.gov/ij>. Acesso em: 24 fev. 2022.

REETZ, H. F.; IMPR. POINT 44). **Fertilizers and their efficient use**. [s.l.] International Fertilizer industry Association, IFA, 2016.

RETA, G. et al. Environmental impact of phosphate mining and beneficiation: review. **International Journal of Hydrology**, v. 2, n. 4, 2018.

RYCHTER, A.; RAO, I. Role of phosphorus in photosynthetic and carbon metabolism. In: **Handbook of Photosynthesis**. 2nd editio ed. [s.l.] Taylor and Francis Group, LLC, 2014.

SAEID, A. et al. Valorization of bones to liquid phosphorus fertilizer by microbial solubilization. **Waste and Biomass Valorization**, v. 5, n. 2, p. 265–272, 2014.

SHAHENA, S. et al. Conventional methods of fertilizer release. In: **Controlled Release Fertilizers for Sustainable Agriculture**. [s.l.] Elsevier, 2021. p. 1–24.

SHARMA, S. B. et al. Phosphate solubilizing microbes: Sustainable approach for managing phosphorus deficiency in agricultural soils. **SpringerPlus**, v. 2, n. 1, 2013.

SINGH, S. K. et al. Growth, nutrient dynamics, and efficiency responses to carbon dioxide and phosphorus nutrition in soybean. **Journal of Plant Interactions**, v. 9, n. 1, p. 838–849, 2014.

SINGH, S. K. et al. Phosphorus nutrition affects temperature response of soybean growth and canopy photosynthesis. **Frontiers in Plant Science**, v. 9, 2018.

SOARES, M. M. et al. Nodulação, crescimento e rendimento de plantas de soja em resposta ao recobrimento das sementes e parcelamento da adubação fosfatada. **Journal of Seed Science**, v. 38, n. 1, p. 30–40, 2016.

SULIEMAN, S.; TRAN, L. S. P. Phosphorus homeostasis in legume nodules as an adaptive strategy to phosphorus deficiency. **Plant Science**, v. 239, p. 36–43, 25 jun. 2015.

SUN, D. et al. Phosphorus recovery and reuse by pyrolysis: Applications for agriculture and environment. **Chemosphere**, v. 194, p. 682–691, 1 mar. 2018.

TALIMAN, N. A. et al. Effect of phosphorus fertilization on the growth, photosynthesis, nitrogen fixation, mineral accumulation, seed yield, and seed quality of a soybean low-phytate line. **Plants**, v. 8, n. 5, 2019.

TARAYRE, C. et al. New perspectives for the design of sustainable bioprocesses for phosphorus recovery from waste. **Bioresource Technology**, v. 206, p. 264–274, 1 abr. 2016.

UNITED NATIONS, D. OF E. AND S. A. P. D. **World Population Prospects 2019, Volume II: Demographics Profiles**. [s.l: s.n.].

VENGAVASI, K.; PANDEY, R. Root exudation potential in contrasting soybean genotypes in response to low soil phosphorus availability is determined by photo-biochemical processes. **Plant Physiology and Biochemistry**, v. 124, p. 1–9, 2018a.

VERDE MANIA. **Verde Mania**. Disponível em: < https://verdemania.com.br/produto/farinha-de-osso-dimy-adubo-organico-5kg/>. Acesso em: 24 fev. 2022.

World Food and Agriculture - Statistical Yearbook 2020. [s.l.] FAO, 2020.

XIANG-WEN, P. et al. Assessment on Phosphorus Efficiency Characteristics of Soybean Genotypes in Phosphorus-Deficient Soils. **Agricultural Sciences in China**, v. 7, n. 8, p. 958–969, 2008.

XIA, W. T.; REN, Z. DE; GAO, Y. F. Removal of Phosphorus From High Phosphorus Iron Ores by Selective HCl Leaching Method. **Journal of Iron and Steel Research, International**, v. 18, n. 5, p. 1–4, 1 maio 2011.

XUE, A. et al. Physiological and Biochemical Characteristics of Soybean Genotypes with Different Phosphorus Efficiencies under Various Phosphorus Concentrations. **Communications in Soil Science and Plant Analysis**, v. 44, n. 19, p. 2796–2808, 2013.

YEOH, H.-H.; WEE, Y.-C. Leaf protein contents and nitrogen-to-protein conversion factors for 90 plant species. **Food Chemistry**, v. 49, p. 245–250, 1994.