Article

Effect of Foliar and Soil Fertilization with New Products Based on Calcinated Bones on Selected Physiological Parameters of Maize Plants

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Abstract: This manuscript presents the effect of foliar and soil fertilizer produced from thermally processed bone waste on the initial growth and development of maize plants. The developed fertilizers were tested in three different doses in a pot experiment. Because nutrient deficiency interferes with plant physiological processes, the impact of the developed fertilizers on gas exchange parameters, relative chlorophyll content, and chlorophyll fluorescence parameters were assessed. Based on the conducted research, it was found that fertilization with developed foliar and soil fertilizers increased the relative content of chlorophyll in maize leaves and increased the value of gas exchange parameters and chlorophyll fluorescence. All determined parameters of gas exchange and chlorophyll fluorescence showed a positive correlation depending on the dose of foliar fertilization used (average value $r = 0.8414$). In turn, the soil fertilization that was utilized during the experiment significantly correlated only with the content of chlorophyll ($r = 0.6965$). The tested fertilizers improvement of the physiological parameters of the plants, which indicates the fertilizing efficiency of the tested fertilizers.

Keywords: calcinated bones; foliar fertilizer; soil fertilizer; maize; photosynthesis efficiency

1. Introduction

Within the past century, a systematic growth in agricultural production has been observed, mainly due to fertilization, which results in an increased availability of plant nutrients [1]. The availability of macro- and micro-elements, such as Ca, S, Mg, K, N, P, and Fe, is crucial for the proper course of plant life processes that directly contribute to plant growth and yield [2]. Maize is a plant with high potential yield, which requires incurring the costs associated with fertilization, as well as suitable weather conditions during the growing season. A deficiency of nutrients in the early stages of maize growth is the main reason limiting its yield. As reported by Yanai et al. (1996) [3], maize during this period takes up minerals only if they occur in sufficiently high concentration in soil, due to the poorly developed root system. In turn, the rapid development of the root system is possible if the plant is

provided with an adequately high concentration of nitrogen and phosphorus in soil solution [4–6]. The relationship between the level of mineral fertilization and the amount of obtained crop yield is obvious, because no other factor shows such a high correlation with yield. From the total mass of nutrients introduced with the applied fertilization, only a part is effectively utilized by plants, and the efficiency of the nutrient uptake varies depending on the element. It is estimated that nitrogen utilization by plants is 50%, and that of phosphorus and potassium is 25% and 60%, respectively. One way to increase the availability of nutrients for plants is row fertilization located in close proximity to seeds, which when applied together with sowing is called initial. This approach of applying the fertilizer causes greater availability of nutrients and thus contributes to the stimulation of a plant's initial growth, but is also safer for the environment [7–9]. This applies especially to phosphorus, for which uptake can be significantly reduced due to various environmental conditions, causing inhibition of maize growth and development [7]. Another method is via utilized foliar fertilization, which results in a reduction of the overall dose of fertilizers and an increase in their use by providing plants nutrients in accordance with their needs in specific development phases [9,10]. This system allows for the precise supply of nutrients in accordance with the specific requirements of the plants in doses that are many times smaller than those supplied by the soil system (where they can be absorbed or blocked). Foliar fertilization can also be an effective way to correct deficiencies, especially of micronutrients, which sometimes results in an increase in yield and improvement of quality. Foliar fertilization, in addition to the positive effect documented in the literature, may also include adverse phenomena, such as withering of leaves as a result of the direct application of these fertilizers, which reduces the effective surface of the leaves and thus the efficiency of photosynthesis [11].

The proper growth and development of plants depends on the course of physiological processes, and mainly on the efficiency of photosynthesis. Measurements and testing of chlorophyll content in leaves (an accurate and convenient method for determining photosynthesis), among others tests, can be used to evaluate the effects of various factors on plants, including fertilizers applied to plant leaves [12–16]. Photosynthetic carbon assimilation is a key process for plant metabolism and is closely related to environmental conditions. Photosynthesis consists of two main parts: the photochemical processes running at the level of thylakoid membranes producing NADPH and ATP, as well as CO₂ reduction pathways (mainly the Calvin cycle) using ATP and NADPH for CO₂ assimilation [2]. A nutrient deficiency directly affects the photosynthetic apparatus, mainly through biosynthesis and the functioning of key photosynthetic components. The direct effect on the synthesis of protein complexes involved in photosynthetic reactions has been documented mainly in the case of nitrogen, sulfur, and iron deficiencies.

The aim of this study was to assess the impact of new foliar and soil fertilizer produced from calcinated bones on selected gas exchange parameters, relative chlorophyll content, and chlorophyll fluorescence of maize plants during pot experiment.

2. Materials and Methods

2.1. Materials

Bone material originated from a cattle slaughterhouse was thermally processed at temperatures in the range of 850–950 °C and ground in order to achieve particles not larger than 2 mm, before further processing to reclaim phosphorous. The composition of calcinated bones was determined by Balawejder et al. [17].

Lake chalk consisted mainly of calcium carbonate with a composition of 90 wt. CaCO₃, about 5 wt. carbonaceous matter, and less than 2 wt. terrigenous components, mainly quartz [18]. Whey protein isolate used was WPI Isolac Instant (Carbery, Ballineen, Country Cork, Ireland) with composition: 130 g kg⁻¹ N, 0.2 g kg⁻¹ lactose, 0.15 g kg⁻¹ fat, and moisture content 6%.

2.2. Procedure for Obtaining Foliar Fertilizer

For the solubilization procedure, a reactor equipped with a propeller stirrer with a volume of 1 m³ was utilized. A total of 480 kg of water was placed into the reactor and, next, 100 kg of calcinated bones was introduced. Then, 168 kg of 65% nitric acid was introduced into the reactor. The acid was dosed into the reactor for one hour. The obtained solution was then filtrated with a filter cloth with a pore size of 25 µm. The recovery of the mineral compounds present in the solution was at the level of 95%. In the next step, the solution was introduced into a fluidized bed granulator (Glatt GmbH, Binzen, Germany) that was filled with boric acid, which had a particle size of 0.2–0.5 mm, and glycine (1% w/w). The granulation procedure was conducted by the spraying of the solution on the surface of boric acid coupled with a drying process at a temperature of 45 °C.

2.3. Procedure for Obtaining Soil Fertilizer

Raw materials, i.e., thermally processed bone waste and lake chalk for fertilizer production, were subjected to grinding in a jet mill, reaching an average particle size below (<) 150 µm. Then, a mixture of 79% by weight bone waste, 19% by weight lake chalk and 2% by weight protein in the form of whey protein isolate was prepared. The mix was then transferred to a fluid bed granulator using water as a binder. A granule with a regular spherical shape was obtained with a fraction distribution: 14.7% by weight larger than 3 mm, 27.5% by weight in the range 3–2 mm, 53.2% by weight in the range 2–1 mm, 4.6% by weight smaller than 1 mm. The granules were then dried in an oven at 45 °C until a moisture content of 3%–4% by weight was obtained.

2.4. Pot Experimental Design

The experiments were conducted at University of Rzeszow (Poland). A one-factor pot experiment was carried out in the vegetation hall. In the vegetation hall the climate conditions were as follows: the average day/night temperature was 26/18 °C, day length was 16 h, relative humidity was around 50%–60%, and light intensity was 1400 mol·m⁻²·s⁻¹. External conditions, such as temperature and humidity, were controlled and a LED-based technology for lighting of plants during cultivation was used. Variable factors analyzed in the experiment were the effect of a dose of developed fertilizers (foliar and soil) on the growth and development of the Farm Gigant (FAO 250) maize plants variety-mass of one thousand seeds = 264 g. The pot experiment included the following objects: Control, without foliar and soil fertilization; Ff_1, Ff_2, Ff_3 on which the developed foliar fertilizer was applied in doses of 1, 2, and 4 kg ha⁻¹, respectively; and Sf_1, Sf_2, and Sf_3 on which the developed soil fertilizer was applied in doses of 30, 40, and 60 kg ha⁻¹, respectively. The pot experiment was carried out using 3 kg soil with loamy sand (USDA) and slightly acidic pH (pH H₂O-6.52). The utilized soil had an organic matter content of 10.78% w/w and included macro-elements such as P (P₂O₅, 11.2 mg), K (K₂O, 4.39 mg), and Mg (29.66 mg) per 100 g of soil. The pot experiment was carried out in 10 replicates for each variant in a completely random order. During the experiment, a constant soil moisture of 50% of the maximum water holding capacity (WHC) was maintained. The application of the developed foliar fertilizer was carried out at an air temperature of 22 °C in the 6-leaf phase (BBCH 16), while the micro-granulate for initial fertilization was applied once, before sowing the plants. Micro-granules with a diameter of 2–3 mm in the tested doses were applied at a depth of 4 cm below the maize grain. The solutions of the fertilizer doses tested were applied manually, dissolving the developed fertilizer in a volume of water corresponding to 200 dm³ ha⁻¹. Measurements of selected gas exchange parameters, chlorophyll fluorescence, and relative chlorophyll content were carried out on the 14th day after the fertilization treatment developed with a foliar product based on an alternative source of phosphorus. The measurements were conducted in the 9-leaf phase (BBCH19). Measurements of the parameters for plants fertilized with initial soil fertilizer were carried out at the same time.

2.5. Gas Exchange

A Portable Photosynthesis Measurement System LCpro-SD (ADC BioScientific Ltd., Hoddesdon, UK) was used to determine the net photosynthetic rate (P_N), transpiration rate (E), stomatal conductance (g_s), and intercellular CO_2 concentration (C_i) on fully expanded leaves. In the determination process, the light intensity was $1400 \text{ mol m}^{-2} \text{ s}^{-1}$ and the temperature was 26°C . The determination of light intensity was done between 9:00 and 11:00 a.m. Two leaves were analyzed for each pot.

2.6. Relative Chlorophyll Content (CCI)

In the 5-leaf phase of development (BBCH 15) a measurement of the relative amount of chlorophyll within the flag leaf was conducted using a Chlorophyll Content Meter CCM-200plus (Opti-Sciences, Hudson, NH, USA). Five leaves were analyzed for each pot.

2.7. Chlorophyll Fluorescence

The chlorophyll fluorescence measurements were performed by using an analyzer fluorimeter (Pocket PEA, Hansatech Instruments, King's Lynn, Norfolk, UK). The fluorescence signal was collected in the red actinic light with peak wavelength of 627 nm light diode source and applied for 1 s at the maximal available intensity of $3500 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1}$. Fluorescence measurements were assessed in dark-adapted (30 min) leaves, using the leaf-clips which were put on the adaxial leaf blades away from the leaf vein. Two measurements were made on each pot. The following parameters were recorded during the study: the maximal quantum yield of PSII photochemistry (F_v/F_m), the maximum quantum yield of primary photochemistry (F_v/F_0), and the performance index (PI).

2.8. Statistical Analysis

Obtained results were analyzed using STATISTICA 13.1 software (StatSoft, Palo Alto, California USA). One way analysis of variance (ANOVA) followed by the post-hoc Tukey HSD test were applied ($\alpha = 0.05$) to show the differences between the impact of developed foliar and soil fertilizer on selected gas exchange parameters, relative chlorophyll content (CCI), and chlorophyll fluorescence.

3. Results and Discussion

3.1. Description of the Fertilizer's Functionality

Calcinated bones mainly consisted of phosphorus, calcium and magnesium compounds [17]. The forms in which these elements are found are mainly insoluble, which limits their absorption by plants. One way to increase the mobility of these ingredients is to convert them into soluble forms. This can be achieved using strong mineral acid solutions. The presence of calcium compounds in the raw material eliminates the possibility of using the popular sulfuric acid, due to the formation of gypsum. Nitric acid as a strong acid can be used to solubilize thermally processed waste components. However, its use is problematic due to the possibility of NO_x emission [19]. This problem was solved by using appropriate nitric acid solutions [20]. In this way, 95% of the components of thermally processed bone waste were recovered. A fraction rich in phosphorus, nitrogen, calcium, and magnesium compounds was obtained. Boric acid and the addition of glycine were used in the granulation process, which allowed the attainment of granules with appropriate mechanical properties. The addition of these two components not only enabled the granulation process, but also enriched the fertilizer with plant nutrients supplied by foliar fertilization. The developed fertilizer mainly contained: 48.8 g kg^{-1} P, 12.5 g kg^{-1} N, 80.3 g kg^{-1} Ca, 4.4 g kg^{-1} Mg, and 70 g kg^{-1} B [17,20]. It should be noted that the composition of the foliar fertilizer obtained is unique because it allows the coexistence of calcium and phosphorus compounds remaining in a soluble form, which are thus available to plants in one fertilizing product.

Microgranules for initial fertilizing were mainly produced from thermally processed bone waste (79%), lake chalk (19%), and whey protein isolate (2%). The mineral composition of soil fertilizer was: 136.81 g kg⁻¹ P, 3.93 g kg⁻¹ Na, 29.96 g kg⁻¹ K, 13.20 g kg⁻¹ Mg, 347.81 g kg⁻¹ Ca, 3.2 N g kg⁻¹, 1 mg kg⁻¹ Fe, 6.5 mg kg⁻¹ Mn, 0.8 mg kg⁻¹ Zn, and 3.2 mg kg⁻¹ Cu (Patent 2019). It has been shown that the components of micro-granules, especially calcinated bones, despite their low solubility, show a strongly alkaline character. Alkaline compounds break down organic matter rich in nitrogen compounds, including protein that is part of the fertilizer, and one of the products of this decomposition is nitrogen in the form of ammonium [21]. This decomposition is controlled and spread over time. As shown [21], the maximum concentration of ammonium nitrogen occurs after 5 days from the application of fertilizer to the soil, with appropriate humidity conditions. If the soil moisture level allows maize seeds to germinate, the process of protein degradation and the release of ammonium nitrogen also begins. These decomposition products are available in a relatively high concentration in the immediate vicinity of the growing root system of plants, which correlates with the nutritional requirements of plants in the early stages of their development. Only the right P:N ratio ensures proper plant growth and development. The rate of phosphorus absorption depends on the form of nitrogen. Ammonium nitrogen acidifies the soil solution, which usually increases the phosphorus concentration and the rate of absorption of this element by plants. In turn, the nitrate form of nitrogen causes alkalization of the soil solution, which reduces phosphorus absorption [7]. Other mineral components are available to plants at a later stage after the fertilizer is being broken down by the soil microbiome [22,23].

It should be noted that micro-granules with a moisture level below 5% are stable, which allows them to be transported and stored without loss of product quality.

3.2. Pot Experiments

Chlorophyll plays a key role in biosynthesis processes in the green parts of plants, enabling the conversion of light energy into the energy of chemical bonds in the process of photosynthesis. The indicator of relative chlorophyll content (CCI) in the leaves of maize fertilized with developed foliar fertilizer was the lowest in the control and increased relative to the applied doses of the developed fertilizer (Figure 1). The relative content of chlorophyll in fertilized objects with developed foliar fertilizer was higher by 14%, 24%, and 34%, respectively, according to dose in comparison with the control. The significance of differences was confirmed only in the case of the highest dose of foliar fertilizer. Another relationship was found for soil fertilization, where the relative chlorophyll content was significantly higher in all cases where soil fertilization was applied compared to the control. However, the object with the lowest dose of soil fertilizer had the highest relative content of chlorophyll. According to Möhr and Dickinson [24], the initial fertilization method should be implemented primarily under conditions of application of limited doses of fertilizers. As the applied doses increase, the effectiveness of the initial fertilization decreases.

The availability of nitrogen is the main factor limiting the growth and yield of plants. Nitrogen compounds are one of the main building blocks of the plant organism and the basic component of many elements responsible for the proper course of photosynthesis [25]. Nitrogen deficiency significantly reduces the relative content of chlorophyll in leaves, similar to P or Mg. The increase of relative content of chlorophyll in the leaves of plants fertilized with foliar and soil fertilizers compared to the control may indicate improved bioavailability of nutrition from utilized fertilizers.

Plant growth and final yields are the result of many processes, among which the intensity of photosynthesis is one of the most important. As shown by the results from the conducted tests, plants fertilized with utilized foliar fertilizer were characterized by higher values of the analyzed gas exchange parameters compared to the control, and these values increased relative to the applied doses of fertilizers (Table 1). In the case of soil fertilization, the values of the analyzed gas exchange parameters were higher compared to the control, however, the highest values of these parameters were observed in plants on which the lowest doses of fertilizers were used (Table 2). Plants fertilized with

utilized foliar fertilizer were characterized by higher values of gas exchange parameters in comparison with plants fertilized with soil fertilizers. The increase in photosynthesis intensity under the influence of foliar fertilization is widely documented in the literature [9,14,15,26].

Figure 1. The relative content of chlorophyll in the leaves of maize (CCI) fertilized with utilized foliar (a) and soil fertilizer (b). Ff_1, Ff_2, Ff_3—object with foliar fertilizer applied in doses of 1, 2, and 4 kg ha⁻¹, respectively; Sf_1, Sf_2 and Sf_3—object with soil fertilizer applied in doses of 30, 40, and 60 kg ha⁻¹, respectively.

Table 1. Mean values of gas exchange parameters in maize leaves, depending on the foliar dose (n = 10).

Object	Intensity of Photosynthesis Net (PN)	Transpiration Rate (E)	Stomatal Conductance (g _s)	Intercellular CO ₂ Concentration (C _i)
	μmol(CO ₂)m ⁻² s ⁻¹	mmol(H ₂ O)m ⁻² s ⁻¹	mmol m ⁻² s ⁻¹	mmol L ⁻¹
Control	14.4 ^a ± 0.1	2.11 ^a ± 0.05	0.07 ^a ± 0.002	45 ^a ± 0.5
Ff_1	17.5 ^b ± 0.1	2.36 ^a ± 0.02	0.10 ^b ± 0.001	65 ^b ± 0.5
Ff_2	18.3 ^{bc} ± 0.1	2.51 ^a ± 0.23	0.11b ^c ± 0.002	67 ^b ± 0.5
Ff_3	19.4 ^c ± 0.1	2.64 ^a ± 0.02	0.13 ^c ± 0.002	81 ^c ± 0.7

Means values with the same lower case are not statistically significant according to the T-Tukey test ($\alpha = 0.05$). Ff_1, Ff_2, and Ff_3—object with foliar fertilizer applied in doses of 1, 2, and 4 kg ha⁻¹, respectively. Sf_1, Sf_2, and Sf_3—object with soil fertilizer applied in doses of 30, 40, and 60 kg ha⁻¹, respectively.

Table 2. Mean values of gas exchange parameters in maize leaves, depending on the dose of soil fertilization (n = 10).

Object	Intensity of Photosynthesis Net (PN)	Transpiration Rate (E)	Stomatal Conductance (g _s)	Intercellular CO ₂ Concentration (C _i)
	$\mu\text{mol}(\text{CO}_2)\text{m}^{-2}\text{s}^{-1}$	$\text{mmol}(\text{H}_2\text{O})\text{m}^{-2}\text{s}^{-1}$	$\text{mmol m}^{-2}\text{s}^{-1}$	mmol L^{-1}
Control	14.4 ^a ± 0.1	2.11 ^a ± 0.05	0.07 ^a ± 0.002	45 ^a ± 0.5
Sf_1	17.9 ^c ± 0.2	2.61 ^c ± 0.01	0.12 ^c ± 0.003	71 ^c ± 0.5
Sf_2	17.2 ^{bc} ± 0.1	2.49 ^{bc} ± 0.05	0.09 ^b ± 0.002	62 ^{bc} ± 0.5
Sf_3	16.1 ^b ± 0.1	2.34 ^{ab} ± 0.03	0.08 ^b ± 0.001	58 ^b ± 0.7

Means values with the same lower case are not statistically significant according to the T-Tukey test ($\alpha = 0.05$). Ff_1, Ff_2, and Ff_3—object with foliar fertilizer applied in doses of 1, 2, and 4 kg ha⁻¹, respectively, Sf_1, Sf_2, and Sf_3—object with soil fertilizer applied in doses of 30, 40, and 60 kg ha⁻¹, respectively.

Deficiency of plant nutrients, in particular N, P, Mg, and K, not only leads to a reduction in photosynthesis efficiency, but also contributes to a decrease in the level of transpiration, stomata conductivity, content of chlorophyll, and carotenoids. Under the condition of insufficient nitrogen supply of plants, the number of electron acceptors in PSII decreases. As a result of this process, the activity of RuBisCo and phosphoenolpyruvate carboxylase-PEP carboxylase decreases [27–29].

Phosphorus is the main component of many important molecules involved in plant growth and the control of key enzymatic reactions. Even a slight lack of phosphorus in plants causes an increase in the chlorophyll content, especially in the leaves. Moreover, a greater deficit of this element causes negative changes in the structure of chloroplasts and, in chlorophyll-protein complexes, causes a decrease in PSII activity [30].

In plant physiology research, non-invasive measurement of fluorescence allows the determination not only of photosynthetic activity, but of also plant responses to adverse environmental conditions, such as shading, the presence of contaminants, and deficiencies of macro- and micro-nutrients [31]. Measurement of chlorophyll fluorescence allows an overall assessment of the physiological state of plants, because it is associated with all metabolic and physiological processes occurring in the plant cell; hence any change in the environment causing changes in these processes will affect the process of photosynthesis [32,33].

The F_v/F_m ratio is a measure of the light efficiency in primary photosynthesis reactions, and its value is proportional to the quantum efficiency of PSII photochemical reactions [34]. This parameter is considered a reliable measure of the photochemical activity of the photosynthetic apparatus. For leaves under normal conditions in most vascular species, the F_v/F_m ratio is around 0.832 relative units. A decrease of this value (in proportion to the quantum efficiency) indicates a reduction of PSII efficiency [34,35]. Phosphorus deficiency could be one of the factors contributing to the reduction of PSII maximum quantum efficiency. The recorded values of the F_v/F_m ratio in the maize leaves of the control objects were 0.753 and below the optimal value (Tables 3 and 4). In foliar fertilized plants, the values of the F_v/F_m ratio increased compared to the control. However, the dependents were not statistically significant relative to the applied dose of foliar fertilizer. A different relationship was observed in the case of soil fertilization, where F_v/F_m values in fertilized facilities were higher than in the control, but the values of this parameters decreased with the increase of the applied fertilization dose (Tables 3 and 4).

A much more sensitive ratio is F_v/F_0 . This ratio contains the same basic information but exhibits higher values and a higher dynamic range than F_v/F_m . The ratio F_v/F_0 shows higher amplitude under stress conditions, since it immediately reflects all changes of F_v and/or F_0 [36]. As shown in Tables 3 and 4, the values of the F_v/F_0 ratio increased significantly with the increase in the dose of foliar and soil fertilizer applied in comparison with the control. However, as is the case of the F_v/F_m ratio, the F_v/F_0 ratio increased proportionally to the dose of foliar fertilizer. A different relationship was observed in a case of soil fertilization.

The indicator PI (performance index) describes the effective energy amount processed by PSII [37]. In the conducted experiment, a significant difference of PI value was observed with relation to applied fertilizer dose. In the case of other parameters, PI values decreased with an increased dose of applied fertilizer. It should be noted that this value is still significantly higher compared to the control (Tables 3 and 4). Deficiencies in particular elements disrupt the normal functioning of the photosynthetic apparatus. In the results, a decrease of PSII photochemical efficiency and changes in chlorophyll fluorescence parameters were observed [38].

Table 3. Average values of chlorophyll fluorescence parameters in maize leaves depending on the dose of foliar fertilization (n = 10).

Object	Maximal Photochemical Efficiency of PSII (F_v/F_m)	Maximum Quantum Yield of Primary Photochemistry (F_v/F_0)	Performance Index (PI)
Control	0.753 ^a ± 0.001	3.06 ^a ± 0.1	2.72 ^a ± 0.1
Ff_1	0.769 ^a ± 0.001	3.44 ^b ± 0.1	2.84 ^a ± 0.1
Ff_2	0.777 ^a ± 0.001	3.59 ^b ± 0.1	3.15 ^b ± 0.2
Ff_3	0.798 ^a ± 0.002	3.72 ^b ± 0.1	3.33 ^b ± 0.1

Means values with the same lower case are not statistically significant according to the T-Tukey test ($\alpha = 0.05$). Ff_1, Ff_2, and Ff_3—object with foliar fertilizer applied in doses of 1, 2, and 4 kg ha⁻¹, respectively; Sf_1, Sf_2, and Sf_3—object with soil fertilizer applied in doses of 30, 40, and 60 kg ha⁻¹, respectively.

Table 4. Average values of chlorophyll fluorescence parameters in maize leaves depending on the dose of soil fertilization (n = 10).

Object	Maximal Photochemical Efficiency of PSII (F_v/F_m)	Maximum Quantum Yield of Primary Photochemistry (F_v/F_0)	Performance Index (PI)
Control	0.753 ^a ± 0.001	3.06 ^a ± 0.1	2.72 ^a ± 0.1
Sf_1	0.787 ^b ± 0.002	3.56 ^b ± 0.3	3.39 ^a ± 0.1
Sf_2	0.771 ^{ab} ± 0.004	3.41 ^b ± 0.1	3.14 ^b ± 0.2
Sf_3	0.762 ^{ab} ± 0.001	3.19 ^a ± 0.1	2.99 ^b ± 0.1

Means values with the same lower case are not statistically significant according to the T-Tukey test ($\alpha = 0.05$). Ff_1, Ff_2, and Ff_3—object with foliar fertilizer applied in doses of 1, 2, and 4 kg ha⁻¹, respectively; Sf_1, Sf_2, and Sf_3—object with soil fertilizer applied in doses of 30, 40, and 60 kg ha⁻¹, respectively.

All determined parameters of gas exchange and chlorophyll fluorescence showed a positive correlation depending on the dose of foliar fertilization used (average value $r = 0.8414$). In turn, the soil fertilization utilized during the experiment was significantly correlated only with the content of chlorophyll ($r = 0.6965$).

4. Conclusions

The effects of foliar and soil fertilization produced from calcinated bones on the relative chlorophyll content, gas exchange, and fluorescence parameters of maize were described. The conducted research under pot conditions showed that fertilization with utilized foliar and soil fertilizers increased the relative content of chlorophyll in maize leaves and increased the value of gas exchange parameters and chlorophyll fluorescence. The tested fertilizers improved the physiological parameters of the plants, indicating their fertilizing efficiency. The efficacy of the tested fertilizers should be verified at the field scale.

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References

1. Ludwig, B.; Geisseler, D.; Michel, K.; Joergensen, R.G.; Schulz, E.; Merbach, I.; Raupp, R.; Rauber, R.; Hu, L.; Liu, X.; et al. Effects of fertilization and soil management on crop yields and carbon stabilization in soils. A review. *Agron. Sust. Dev.* **2011**, *31*, 361–372. [\[CrossRef\]](#)
2. Kalaji, H.M.; Oukarroum, A.; Alexandrov, V.; Kouzmanova, M.; Brestic, M.; Zivcak, M.; Samborska, I.A.; Cetner, M.D.; Allakhverdiev, S.I.; Goltsev, V.; et al. Identification of nutrient deficiency in maize and tomato plants by in vivo chlorophyll a fluorescence measurements. *Plant Physiol. Biochem.* **2014**, *81*, 16–25. [\[CrossRef\]](#) [\[PubMed\]](#)
3. Yanai, J.; Linehan, D.J.; Robinson, D.; Young, I.M.; Hackett, C.A.; Kyuma, K.; Kosaki, T. Effects of inorganic nitrogen application on the dynamics of the soil solution composition in the root zone of maize. *Plant Soil* **1996**, *180*, 1–9. [\[CrossRef\]](#)
4. Mollier, A.; Pellerin, S. Maize root system growth and development as influenced by phosphorus deficiency. *J. Exp. Bot.* **1999**, *50*, 487–497. [\[CrossRef\]](#)
5. Grzebisz, W.; Szczepaniak, W. Systems of fertilization (in Polish). *J. Elem.* **2003**, *8*, 95–107.
6. Wolkowski, R.P. Row-placed fertilizer for maize grown with an in-row crop residue management system in southern Wisconsin. *Soil Tillage Res.* **2000**, *54*, 55–62. [\[CrossRef\]](#)
7. Kruczek, A. Phosphorus utilization from fertilizer and accumulation of mineral components in the initial stage of maize development. *Pol. J. Environ. Stud.* **2005**, *14*, 467–475.
8. Kruczek, A.; Szulc, P. Effect of fertilization method on the uptake and accumulation of mineral components in the initial period of maize development. *Int. Agrophysics* **2006**, *20*, 11–22.
9. Ling, F.; Silberbush, M. Response of Maize to foliar vs. soil application of nitrogen-phosphorus-potassium fertilizers. *J. Plant Nutr.* **2002**, *25*, 2333–2342. [\[CrossRef\]](#)
10. Thavaprakash, N.; Velayudham, K.; Panneerselvam, S. Foliar nutrition of baby corn (*Zea mays* L.). *Arch. Agron. Soil Sci.* **2006**, *52*, 419–425. [\[CrossRef\]](#)
11. Kaur, G.; Nelson, K.A. Effect of foliar boron fertilization of fine textured soils on corn yields. *Agronomy* **2015**, *5*, 1–18. [\[CrossRef\]](#)
12. Boote, K.J.; Gallaher, R.N.; Robertson, W.K.; Hinson, K.; Hammond, L.C. Effect of foliar fertilization on photosynthesis, leaf nutrition and yield of soybeans. *Agron. J.* **1978**, *70*, 787–791. [\[CrossRef\]](#)
13. Gontarczyk, M. Photosynthesis and transpiration of C₄ plants under drought condition. *Plant Physiol. Biochem.* **1996**, 328–329.
14. Górny, A.G. Photosynthetic activity of flag leaves in diallel crosses of spring barley under varied nutrition and soil moisture. *Cereal Res. Commun.* **2001**, *29*, 159–166. [\[CrossRef\]](#)
15. Michałek, S. Response of some soybean cultivars to foliar fertilization with Insol W under herbicide stress condition. In *Biostimulators in Modern Agriculture—Field Crops*; Dąbrowski, Z., Ed.; Editorial House Wieś Jutra: Warszawa, Poland, 2008; pp. 92–99.
16. Borkowski, E.; Michałek, S. The effect of placement and light conditions during foliar application of Insol U fertilizer on gas exchange, yield and quality of spinach (*Spinacia oleracea* L.). *Folia Hortic.* **2009**, *21*, 61–71. [\[CrossRef\]](#)
17. Balawejder, M.; Matłok, N.; Gorzelany, J.; Pieniążek, M.; Antos, P.; Witek, G.; Szostek, M. Foliar Fertilizer Based on Calcined Bones, Boron and Molybdenum—A Study on the Development and Potential Effects on Maize Grain Production. *Sustainability* **2019**, *11*, 5287. [\[CrossRef\]](#)
18. Hycnar, E.; Ratajczak, T.; Jończyk, M. Kreda jeziorna z Bełchatowa jako sorbent SO₂ w paleniskach fluidalnych. In *Sorbenty Mineralne. Surowce, Energetyka, Ochrona Środowiska, Nowoczesne Technologie*; AGH: Kraków, Poland, 2013; pp. 153–168.
19. Nielsson, F.T.; Yates, L.D.; Roy, L.F.; Heil, F.G. Nitric Phosphates from Phosphate Rock, Nitric Acid, Ammonia, and Carbon Dioxide. *J. Agric. Food Chem.* **1953**, *1*, 1050–1054. [\[CrossRef\]](#)

20. Balawejder, M.; Matłok, N.; Gorzelany, J.; Kania, K.; Witek, G. Sposób Wytwarzania Rozpuszczalnego Nawozu. PL Patent PL424243A1, 10 January 2018.
21. Balawejder, M.; Matłok, N.; Gorzelany, J.; Antos, D.; Piątkowski, W.; Bochenek, R.; Przywara, M.; Olbrycht, M.; Kołodziej, M.; Antos, P.; et al. Sposób Wytwarzania Nawozu Wieloskładnikowego o Kontrolowanym Uwalnianiu Składników. PL Patent 429318, 11 May 2019.
22. Mulinns, G.L. *Phosphorus, Agriculture & the Environment*. Virginia Cooperative Extension; Virginia State University: Petersburg, Russia, 2009.
23. Jones, D.L.; Oburger, E. Solubilization of Phosphorus by Soil Microorganisms. In *Phosphorus in Action*; Springer: Berlin/Heidelberg, Germany, 2011; pp. 169–198.
24. Möhr, P.J.; Dickinson, E.B. Mineral nutrition in maize. *Tech. Monogr.* **1978**, 26–32.
25. Masclaux-Daubresse, C.; Daniel-Vedele, F.; Dechorgnat, J.; Chardon, F.; Gaufichon, L.; Suzuki, A. Nitrogen uptake, assimilation and remobilization in plants: Challenges for sustainable and productive agriculture. *Ann. Bot.* **2010**, *105*, 1141–1157. [[CrossRef](#)]
26. Michałek, S.; Chwil, S.; Pranagal, J.; Ligeza, S. Maize early growth under conditions of foliar nutrition with VIFLO fertilizers (in Polish). *Zesz. Probl. Postępów Nauk Rol.* **2009**, *542*, 333–339.
27. Correia, C.M.; Moutinho Pereira, J.M.; Coutinho, J.F.; Björn, L.O.; Torres-Pereira, J.M.G. Ultraviolet-B radiation and nitrogen affect the photosynthesis of maize: A Mediterranean field study. *Eur. J. Agron.* **2005**, *22*, 337–347. [[CrossRef](#)]
28. Huang, Z.A.; Jiang, D.A.; Yang, Y.; Sun, J.W.; Jin, S.H. Effects of nitrogen deficiency on gas exchange, chlorophyll fluorescence, and antioxidant enzymes in leaves of rice plants. *Photosynthetica* **2004**, *42*, 357–364. [[CrossRef](#)]
29. Foyer, C.; Spencer, C. The relationship between phosphate status and photosynthesis in leaves. *Planta* **1986**, *167*, 369–373. [[CrossRef](#)] [[PubMed](#)]
30. Tuffers, A.; Naidoo, G.; Von Willert, D.J. Low salinities adversely affect photosynthetic performance of the mangrove, *Avicennia marina*. *Wetl. Ecol. Manag.* **2001**, *9*, 225–232. [[CrossRef](#)]
31. Kalaji, H.M. Oddziaływanie abiotycznych czynników stresowych na fluorescencję chlorofilu w roślinach wybranych odmian jęczmienia *Hordeum vulgare* L. *Rozpr. Nauk. Monogr.* **2011**.
32. Kalaji, H.M.; Guo, P. Chlorophyll fluorescence: A usefull tool in barley plant breeding programs. In *Photochemistry Research Progress*; Sánchez, A., Gutierrez, S.J., Eds.; Nova Science Publishers: New York, NY, USA, 2008; pp. 439–463.
33. Kalaji, H.M.; Cetner, M.D.; Dąbrowski, P.; Samborska, I.A.; Łukasik, I.; Swoczyna, T.; Pietkiewicz, S.; Bąba, W. Chlorophyll fluorescence measurements in environmental studies. *Kosmos* **2016**, *65*, 197–205.
34. Furmańczyk, A. Response of the photosynthetic apparatus of string-bean to cobalt excess in the substrate (in Polish). *Proc. Ecopole* **2013**, *7*, 207–213.
35. Angelini, G.; Ragni, P.; Esposito, D.; Giardi, M.T. A device to study the effect of space radiation on photosynthesis organisms. *Phys. Med.* **2001**, *17* (Suppl. 1), 267–268.
36. Lichtenthaler, H.K.; Buschmann, C.; Knapp, M. How to correctly determine the different chlorophyll fluorescence parameters and the chlorophyll fluorescence decrease ratio RfD of leaves with the PAM fluorometer. *Photosynthetica* **2005**, *43*, 379–393. [[CrossRef](#)]
37. Strasser, R.J.; Srivastava, A.; Tsimilli-Michael, M. Screening the vitality and photo-synthetic activity of plants by fluorescent transient. *Crop Improv. Food Secur.* **1999**, *79*, 72–115.
38. Smethurst, C.F.; Garnett, T.; Shabala, S. Nutritional and chlorophyll fluorescence responses of lucerne (*Medicago sativa*) to waterlogging and subsequent recovery. *Plant Soil* **2005**, *270*, 31–45. [[CrossRef](#)]