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Effect of *Bacillus megaterium* var. *phosphaticum* Bacteria and L-Alpha Proline Amino Acid on Iron Content in Soil and *Triticum aestivum* L. Plants in Sustainable Agriculture System

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Abstract: Research was conducted in Poland in 2017–2019 at Siedlce University of Natural Sciences and Humanities in Siedlce. It aimed at determining the effect of the bacteria *Bacillus megaterium* var. *phosphaticum*, the amino acid L-alpha proline, and the mineral nitrogen fertiliser regime on iron content in soil during the period of rapid growth of spring wheat plants, as well as in spring wheat grain and straw. The following two factors were examined: (I) biological products: untreated control, *Bacillus megaterium* var. *phosphaticum*, L-alpha proline, *Bacillus megaterium* var. *phosphaticum* + L-alpha proline; (II) mineral nitrogen fertiliser regime: nonfertilised control, 60 kg N·ha^{-1}, 90 kg N·ha^{-1}, 90 kg N·ha^{-1} + foliar fertilisation. The study demonstrated that, during the period of rapid spring wheat plant growth, Fe content was the highest in the soil following an application of *Bacillus megaterium* var. *phosphaticum* + L-alpha proline + mineral nitrogen fertiliser applied at the rate of 90 kg N·ha^{-1}. This combination resulted in the highest concentration and uptake of iron by spring wheat grain, whereas for straw, the same result was also achieved following mineral nitrogen fertiliser at a rate of 90 kg N·ha^{-1} + foliar fertilisation.

Keywords: spring wheat; *Bacillus megaterium* var. *phosphaticum*; L-alpha proline amino acid mineral fertilisation with nitrogen; iron

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1. Introduction

The use of bacteria of the genus *Bacillus* sp. in the cultivation of cereals not only releases phosphorus from forms inaccessible to plants, such as calcium, iron, and aluminium phosphates, turning them into phosphates available to plants, but also has a positive effect on the soil environment [1]. Bacteria improve the chemical, biological, and physical properties of the soil, especially when used in conjunction with amino acids. This enables the absorption of larger amounts of macro- and microelements from the soil environment, including iron, which is so important for plants. In turn, the direct effect of biological preparations is to support plant growth, including stronger development of the root system, which is associated with the supply of more nutrients from the soil, including bound nitrogen, phosphorus, and iron, with the synthesis of phytohormones stimulating plant development, as well as lowering the level of ethylene, which adversely affects the rooting of plants.

Iron plays a significant part in plant life. It is necessary for chlorophyll synthesis in green plants, which translates into the quantity of obtained yield. Thus, plant demand for iron is substantial, which usually coincides with sufficient stores of this element in the soil. In some cases, iron availability for plants is limited, which is due to the fact that in a soil environment where pH is higher than 6.9, Fe may be deficient due to its unavailability for plants. Therefore, solutions should be investigated that will make it possible for plants to take up iron from the soil, which can be achieved by improving soil chemical, biological, and physical properties [2]. With this in mind, the soil environment should be protected by combining conventional and biological agricultural methods. It is recommended to apply biological preparations that contain organic compounds and microorganisms, and create conditions encouraging their occurrence. An application of these preparations contributes to an induction of plant resistance against pests, pathogens, or other stress-inducing factors [3], and increases the availability of microelements, including iron. In turn, a direct influence includes the promotion of plant growth, including the development of a stronger rooting system, which is able to supply more nutrients taken up from the environment, such as bound nitrogen, phosphorus, and iron; synthesis of phytohormones stimulating plant development; and reduction of the level of ethylene, which negatively affect the development of plant roots [1,4]. Studies of Yadav and Sarkar [5] and Kumar [6] showed that all bacterial vaccines, especially mixed vaccines, significantly increase the uptake of micronutrients by wheat grain and straw. On the other hand, research by Buluts [7] showed that mixed bacterial vaccines can inhibit their diazotrophic activity or plant growth. Therefore, the literature reports in this area are ambiguous, which requires further research.

One way is to use biological preparations, especially in the developing system of sustainable agriculture. Bearing in mind the different effect of biological preparations on nutrient content in the environment (soil–plant), research was conducted to examine the influence of the *Bacillus megaterium* var. *phosphaticum* bacteria and the L-alpha proline amino acid on the soil content of Fe against the background of mineral fertilisation with nitrogen during the period of rapid growth of spring wheat plants on iron content in spring wheat grain and straw. The conducted research will show which of the tested combinations will have the most beneficial effect on the availability of iron in the soil and the content and uptake of Fe by grain and straw.

2. Materials and Methods

Field research was conducted in 2017–2019 on a family-owned farm located in Krzymosze near Siedlce, at the Siedlce University of Natural Sciences and Humanities, Poland. The experimental soil was Stagnic Luvisol. In the spring, before the experiment was set up, soil samples had been collected to determine soil contents: NH_4^+, 4.97 mg kg^{-1} soil; N-NO_3^-, 7.84 mg kg^{-1} soil; and contents of available P, K, and Fe forms (respectively, 8.2 mg·100 g^{-1} soil, 8.7 mg·100 g^{-1} soil, 845 mg ·kg^{-1} soil). The soil reaction was neutral (pH in KCl, 6.2), and C_{org} content was 1.09%. The experiment was a split-block arrangement with three replicates. The size of the plot was assumed to be 20 m^2, and for the harvest, it was 16 m^2. The following two factors were examined: (I) biological preparations (untreated control (no biological preparations applied), *Bacillus megaterium* var. *phosphaticum* bacteria, 1 l·ha^{-1}; L-alpha proline amino acid, 2 g·ha^{-1}; *Bacillus megaterium* var. *phosphaticum* bacteria, 1 l·ha^{-1}, + L-alpha proline amino acid, 2 g·ha^{-1}); (II) mineral nitrogen fertiliser regime (nonfertilised control (no mineral fertilisation with nitrogen), 60 kg N·ha^{-1} (preplant), 90 kg N·ha^{-1} (60 kg N·ha^{-1} preplant + 30 kg N·ha^{-1} at the stem elongation stage), 90 kg N·ha^{-1} + foliar fertilisation (60 kg N·ha^{-1} preplant + 30 kg N·ha^{-1} at the stem elongation stage + 30 kg N·ha^{-1} of foliar-applied 8% urea solution at the stage of initial ear formation)).

Spring wheat cv. Mandaryna followed maize. Potassium and phosphorus fertiliser rates depended on soil availability, which was as follows: P, 30.8 kg·ha^{-1}, and K, 99.6 kg·ha^{-1}. Mineral fertilisation with nitrogen followed the pattern described for factor II above. Spring wheat was seeded in early April at the rate of 500 grains per 1 m^2. The *Bacillus megaterium* var. *phosphaticum* bacteria were applied once, directly after sowing. The amino acid L-alpha proline was applied at the tillering stage of spring wheat plants. On the spring wheat plantation, weeds were controlled with the following herbicides: Sekator 1250D at a dose of 0.1 l ha^{-1} and Puma Uniwersal 069 at a dose of 1.0 l ha^{-1}. The insecticide Decis Mega 50 EW at a dose of 0.1 l ha^{-1} and fungicides were also used: the first treatment, Input 460 EC, at a dose of 1.0 ha^{-1}, and the second treatment, Fandango 200 EC, at a dose of 1 l ha^{-1}. During the period of rapid growth of spring wheat, that is, 30 days after the amino acid L-alpha proline was applied, soil samples were collected from the 0–30 cm layer in each plot in order to determine Fe content. After spring wheat harvest, grain and straw were sampled in each experimental unit to determine iron content and calculate iron uptake.

Each of the characteristics studied was analysed by means of ANOVA for the split-block arrangement. Comparison of means for significant sources of variation was achieved by means of Tukey's test at a significance level of $p \leq 0.05$. All the calculations were performed in Statistica, version 12.0, and MS Excel. The results for each characteristic were subjected to analysis of variance following the mathematical model yijl = n + ai + gi + eij(1) + bl + ejl(2) + abil + eijl(3), where a = 1, . . . 4; b = 1, 2, . . . 4; n = 1, 2, 3 (number of replicates); yijl—value of the examined characteristic; ai—effect of i-th level of factor A; gj—effect of replicates (blocks); eij(1)—error 1 resulting from the interaction: factor A × replicates; bl—effect of l-th level of factor B; ejl(2)—error 2 from the interaction: factor B × replicates; abil—effect of the interaction: factor A × factor B; eijl(3)—random error.

The course of weather conditions in the years of the research was varied (Table 1). The most favourable year for the cultivation of spring wheat was 2017, when the highest amount of rainfall was recorded. Worse weather conditions were recorded in 2018, with a lower total of precipitation and an average air temperature higher than the long-term average. The strongest rainfall shortage was recorded in 2019. The average air temperature oscillated around the long-term average.

Table 1. Weather conditions in the growing season of spring wheat according to the Zawady Meteorological Station.

Years	Month					Average
	IV	V	VI	VII	VIII	
	Mean air temperature, °C					
2017	6.9	13.9	17.8	16.9	18.4	14.8
2018	13.1	17.0	18.3	20.4	20.6	17.9
2019	9.8	13.3	17.9	18.5	19.9	15.9
Long-term (15 yr) mean	8.2	14.2	17.6	19.7	19.1	15.8
	Rainfall sum, mm					Total
2017	59.6	49.5	57.9	23.6	54.7	245.3
2018	34.5	27.3	31.5	67.1	24.5	184.9
2019	5.9	59.8	35.9	29.7	43.9	175.2
Long-term (15 yr) mean	37.4	47.1	48.1	65.5	43.5	241.6

3. Results

The iron content in the arable layer of the soil during the period of intense spring wheat growth was significantly differentiated by the vegetation period of plants and their interaction with biological preparations (Table 2). The highest Fe content was recorded in the arable soil layer in 2017, with the highest total rainfall significantly lower in 2019 and the lowest in 2018, with a deficit of rainfall in May and June, during the period of intensive growth of spring wheat plants compared with the average sum from many years. An interaction was demonstrated, which shows that the highest iron concentration in the soil was recorded in 2017 after the use of *Bacillus megaterium* var. *phosphaticum* with the amino acid L-alpha proline, and the lowest in 2018–2019 at a control facility, without the use of biological preparations.

Table 2. Fe content in the arable layer of soil 30 days after the application of biological preparations in 2017–2019, mg·kg^{-1} of soil.

Biological Preparations (A)	**Years**		
	2017	**2018**	**2019**
Control	889a *	877a	880a
Bacillus megaterium var. *phosphaticum*	1065c	1050c	1053c
L-alpha proline	951b	941b	943b
Bacillus megaterium var. *phosphaticum* + L-alpha proline	1097d	1083d	1087d
Means	1001C	988A	991B
ANOVA		*p*-value	$HSD_{0.05}$
Years (Y)		0.001	2
Interaction: Y × A		0.001	4

* Values in columns followed by the same small letters and values in rows followed by the same capital letters do not differ significantly at $p < 0.05$.

Iron content in the topsoil layer during the period of rapid growth of spring wheat plants was significantly affected by the experimental factors and their interaction (Table 3).

Table 3. Fe content in topsoil 30 days after biological product application (means across 2017–2019), mg·kg^{-1} soil.

Biological Preparations (A)	**Mineral Nitrogen Fertilisation, kg N·ha^{-1} (B)**				**Means**
	Control	**60**	**90**	**90 + Foliar Fertilisation**	
Control	860a *	883a	897a	886a	882A
Bacillus megaterium var. *phosphaticum*	1030c	1056c	1073c	1062c	1056C
L-alpha proline	920b	941b	969b	950b	945B
Bacillus megaterium var. *phosphaticum* + L-alpha proline	1072d	1087d	1107d	1090d	1089D
Means	971A	992B	1011C	997B	-
ANOVA		*p*-value			$HSD_{0.05}$
Biological preparations (A)			0.001		7
Mineral nitrogen fertilisation (B)			0.001		6
Interaction: A × B			0.001		11

* Values in columns followed by the same small letters and values in rows followed by the same capital letters do not differ significantly at $p < 0.05$.

The highest iron concentration was recorded in soil treated with *Bacillus megaterium* var. *phosphaticum* + L-alpha proline. When one or the other preparations had been applied, a significant decline in the soil content of iron was observed. However, in both of the latter cases, the concentration of this element was significantly higher compared with the control, where no biological products had been used. Mineral fertilisation with nitrogen had a significant influence on the soil content of iron. The highest concentration of this element was determined during the period of rapid growth of spring wheat after a rate of

90 kg N·ha^{-1} had been used. An application of the highest nitrogen rate (90 kg N·ha^{-1}) accompanied by foliar spraying and the lowest N rate (60 kg N·ha^{-1}) was followed by a significant decline in iron content in the soil. Still, the recorded values were higher compared with the control (no mineral nitrogen fertiliser). An interaction was observed which indicated that the highest concentration of iron during the period of rapid growth of spring wheat plants was in the soil treated with *Bacillus megaterium* var. *phosphaticum* + L-alpha proline + mineral nitrogen fertiliser applied at a rate of 90 kg N·ha^{-1}. By contrast, the lowest iron content was recorded in control soil, where neither biological preparations nor mineral nitrogen fertiliser had been applied.

The iron content in spring wheat grain was significantly differentiated by thermal and precipitation conditions during the vegetation period of plants and their interaction with biological preparations (Table 4).

Table 4. Fe content in spring wheat grain depending on the biological preparations used in 2017–2019, mg·kg^{-1} d.m.

Biological Preparations (A)	Years		
	2017	2018	2019
Control	34.90a *	35.32a	36.95a
Bacillus megaterium var. *phosphaticum*	72.15c	72.90c	75.03c
L-alpha proline	47.73b	48.53b	50.29b
Bacillus megaterium var. *phosphaticum* + L-alpha proline	83.11d	84.03d	86.18d
Means	59.47A	60.20B	62.11C
ANOVA		*p*-value	$HSD_{0.05}$
Years (Y)		0.001	0.68
Interaction: Y × A		0.001	0.97

* Values in columns followed by the same small letters and values in rows followed by the same capital letters do not differ significantly at $p < 0.05$.

The highest concentration of Fe in spring wheat zoos grain was recorded in dry 2019, significantly lower in 2018 and the lowest in 2017, with the highest total fumes. An interaction was demonstrated, which shows that the highest iron content was recorded in spring wheat grain in 2019 after the use of *Bacillus megaterium* var. *phosphaticum* with the amino acid L-alpha proline, and the lowest in 2017–2018 at a control facility, without the use of biological preparations. Statistical analysis demonstrated a significant impact of the experimental factors and their interaction on iron content in spring wheat grain (Table 5).

The highest concentration of iron in spring wheat grain was associated with an application of *Bacillus megaterium* var. *phosphaticum* + L-alpha proline. When applied on their own, these products contributed to a significant decline in iron content determined in spring wheat grain, this finding being particularly clear for the L-alpha proline, although even in this case, Fe content in spring wheat grain was significantly higher compared with that in control untreated with the biological products. Mineral fertilisation with nitrogen significantly affected the spring wheat grain content of iron. The highest concentration of this element in spring wheat grain was recorded in the unit fertilised with 90 kg N·ha^{-1}. For both the higher mineral fertiliser rate, that is, 90 kg N·ha^{-1} + foliar spraying, and the lower amount, which was 60 kg N·ha^{-1}, the response was a significant decline in the concentration of iron in spring wheat grain. The lowest iron content was recorded in the grain of spring wheat grown in the control unit, where no mineral nitrogen fertiliser had been applied. An interaction was confirmed, indicating that the highest Fe content was observed in the grain of spring wheat treated with the biological products *Bacillus* *megaterium* var. *phosphaticum* + L-alpha proline and fertilised with mineral nitrogen at a rate of 90 kg $\cdot$ha^{-1}. By contrast, the lowest Fe content was in the control unit, where neither biological products nor mineral nitrogen fertiliser had been applied.

Fe uptake with spring wheat grain, as a resultant product of grain yield and iron concentration in the grain, was significantly influenced by the experimental factors and their interaction (Table 6).

Table 5. Fe content in spring wheat grain (means across 2017–2019), mg$\cdot$kg^{-1} d.m.

Biological Preparations (A)	Mineral Nitrogen Fertilisation, kg N$\cdot$ha^{-1} (B)				Means
	Control	60	90	90 + Foliar Fertilisation	
Control	34.18a *	37.21a	36.18a	35.32a	35.72A
Bacillus megaterium var. phosphaticum	68.58c	72.47c	79.20c	73.17c	73.36C
L-alpha proline	41.03b	49.20b	54.12b	51.04b	48.85B
Bacillus megaterium var. phosphaticum + L-alpha proline	77.69d	84.12d	89.72d	86.24d	84.44D
Means	55.37A	60.75B	64.81C	61.44B	-
ANOVA	*p*-value				$HSD_{0.05}$
Biological preparations (A)			0.001		1.57
Mineral nitrogen fertilisation (B)			0.001		1.46
Interaction: A × B			0.001		2.01

* Values in columns followed by the same small letters and values in rows followed by the same capital letters do not differ significantly at $p < 0.05$.

Table 6. Fe uptake by spring wheat grain (means across 2017–2019), g$\cdot$ha^{-1}.

Biological Preparations (A)	Mineral Nitrogen Fertilisation, kg N$\cdot$ha^{-1} (B)				Means
	Control	60	90	90 + Foliar Fertilisation	
Control	7.45a *	15.14a	17.33a	18.47a	14.60A
Bacillus megaterium var. *phosphaticum*	23.80c	32.76c	50.77c	43.76c	37.77C
L-alpha proline	14.93b	23.57b	38.53b	34.40b	27.86B
Bacillus megaterium var. *phosphaticum* + L-alpha proline	41.33d	52.49d	71.33d	64.94d	57.52D
Means	21.88A	30.99B	44.49D	40.39C	-
ANOVA	*p*-value				$HSD_{0.05}$
Biological preparations (A)			0.001		3.21
Mineral nitrogen fertilisation (B)			0.001		2.84
Interaction: A × B			0.001		3.77

* Values in columns followed by the same small letters and values in rows followed by the same capital letters do not differ significantly at $p < 0.05$.

The highest iron accumulation was recorded in the grain of spring wheat harvested in plots treated with either *Bacillus megaterium* var. *phosphaticum* or L-alpha proline. When applied without the other, the products contributed to a lower Fe uptake by spring wheat grain. Still, the amounts were higher compared with those in control untreated with bioproducts. Mineral fertilisation with nitrogen had a significant effect on iron uptake by spring wheat grain. An application of a rate of 90 kg N·ha^{-1} was followed by the highest iron uptake. When either the higher or the lower nitrogen rate had been used, the amount of iron taken up by spring wheat grain was significantly lower. Still, the quantities were higher compared with those in the grain of spring wheat grown in the control unit, where no mineral nitrogen fertiliser had been applied. An interaction between the experimental factors was confirmed: the highest Fe accumulation in spring wheat grain was observed following an application of the *Bacillus megaterium* var. *phosphaticum* and L-alpha proline as well as nitrogen fertiliser at a rate of 90 kg N·ha^{-1}, it being the lowest in the control. The Fe content in spring wheat straw was significantly differentiated by weather conditions during the vegetation period of plants and their interaction with biological preparations (Table 7).

Table 7. Fe content in spring wheat straw, depending on the biological preparations used in 2017–2019, mg kg^{-1} d.m.

Biological Preparations (A)	Years		
	2017	2018	2019
Control	57.60a *	58.41a	60.81a
Bacillus megaterium var. *phosphaticum*	86.58c	87.37c	89.96c
L-alpha proline	72.43b	73.29b	75.74b
Bacillus megaterium var. *phosphaticum* + L-alpha proline	95.87d	96.73d	99.32d
Means	78.15A	78.95B	81.46C
ANOVA		*p*-value	$HSD_{0.05}$
Years (Y)		0.001	0.72
Interaction: Y × A		0.001	0.98

* Values in columns followed by the same small letters and values in rows followed by the same capital letters do not differ significantly at $p < 0.05$.

The highest iron concentration was recorded in spring wheat straw in dry 2019, significantly lower in 2018 and the lowest in 2017, with the highest total rainfall. An interaction was demonstrated, which shows that the highest Fe content was recorded in spring wheat straw in 2019 from the facility where *Bacillus megaterium* var. *phosphaticum* bacteria and the amino acid L-alpha proline were used, and the lowest in 2017–2018 at a control facility, without the use of biological preparations. Statistical analysis demonstrated a significant effect of the experimental factors and their interaction on iron content in spring wheat straw (Table 8).

The highest iron concentration was recorded in the straw of spring wheat harvested in units treated with *Bacillus megaterium* var. *phosphaticum* and L-alpha proline. When applied on their own, the products contributed to a significant decline in iron concentration in spring wheat straw, it still being significantly higher compared with the control unit without an application of biological products. Mineral fertilisation with nitrogen had a significant impact on iron content in spring wheat straw. The highest Fe concentration was determined in the straw of spring wheat fertilised with 90 kg N·ha^{-1}. An application of either the higher or the lower nitrogen rate contributed to a significant drop in Fe content determined in spring wheat straw. The lowest concentration of iron was recorded in spring wheat straw harvested in the control unit, where no mineral nitrogen had been used. An interaction was confirmed that indicated that the highest iron concentration was found in spring wheat straw grown in plots treated with *Bacillus megaterium* var. *phosphaticum* + L-alpha proline and fertilised with either 90 kg N·ha^{-1} or 90 kg N·ha^{-1} + foliar application, it being the lowest in the control unit where neither bioproducts nor mineral nitrogen fertiliser had been applied. Iron uptake by spring wheat straw was significantly influenced by the experimental factors and their interaction (Table 9).

Table 8. Fe content in spring wheat straw (means across 2017–2019), mg·kg^{-1} d.m.

Biological Preparations (A)	Mineral Nitrogen Fertilisation, kg N·ha^{-1} (B)				Means
	Control	60	90	90 + Foliar Fertilisation	
Control	57.32a *	61.12a	59.23a	58.07a	58.94A
Bacillus megaterium var. *phosphaticum*	81.26c	88.34c	92.15c	90.14c	87.97C
L-alpha proline	68.14b	74.21b	77.10b	75.81b	73.82B
Bacillus megaterium var. *phosphaticum* + L-alpha proline	94.30d	97.23d	99.56d	98.27d	97.34D
Means	75.26A	80.23B	82.01B	80.57B	-
ANOVA	*p*-value				$HSD_{0.05}$
Biological preparations (A)	0.001				1.89
Mineral nitrogen fertilisation (B)	0.001				1.84
Interaction: A × B	0.001				2.06

* Values in columns followed by the same small letters and values in rows followed by the same capital letters do not differ significantly at $p < 0.05$.

Table 9. Fe uptake by spring wheat straw (means across 2017–2019), g·ha^{-1}.

Biological Product (A)	Mineral Nitrogen Fertilisation, kg N·ha^{-1} (B)				Means
	Control	60	90	90 + Foliar Fertilisation	
Control	13.41a*	26.40a	29.38a	33.62a	25.70A
Bacillus megaterium var. *phosphaticum*	29.98b	43.29c	63.77b	56.97b	48.50C
L-alpha proline	27.05b	36.96b	60.83b	53.98b	44.71B
Bacillus megaterium var. *phosphaticum* + L-alpha proline	55.83c	66.80d	83.03c	78.92c	71.15D
Control	31.57A	43.36B	59.25D	55.87C	-
ANOVA	*p*-value				$HSD_{0.05}$
Biological product (A)	0.001				3.24
Mineral nitrogen fertilisation (B)	0.001				3.21
Interaction: A × B	0.001				3.42

* Values in columns followed by the same small letters and values in rows followed by the same capital letters do not differ significantly at $p < 0.05$.

The highest amount of iron was accumulated in the straw of spring wheat following an application of both *Bacillus megaterium* var. *phosphaticum* and L-alpha proline. When used on their own, the bioproducts contributed to a decline in the quantity of iron accumulated in spring wheat straw. Still, the Fe amount was higher compared with that in control untreated with biological products. Mineral fertilisation with nitrogen had a significant effect on iron uptake by spring wheat straw. The highest amount of this element was recorded in the straw of spring wheat fertilised with 90 kg N·ha^{-1}. Fe amount in spring wheat straw was lower following an application of either the highest nitrogen rate or a rate of 60 kg N·ha^{-1}, it being the lowest in the nonfertilised control straw. An interaction of the experimental factors was found to be significant: the highest amount of iron was recorded in the straw of spring wheat harvested from units treated with *Bacillus megaterium* var. *phosphaticum* + L-alpha proline + 90 kg N·ha^{-1}, it being the lowest in the straw harvested in the control unit, where neither bioproducts nor mineral nitrogen fertiliser had been applied.

4. Discussion

The undertaken research unequivocally demonstrated that modern agriculture should combine conventional and biological methods. Such approach protects the soil environment and creates favourable conditions for the uptake of nutrients, including iron, which is such a valuable microelement as it stimulates spring wheat plant growth and development. In the present study, it was demonstrated that an application of biological products, that is, the *Bacillus megaterium* var. *phosphaticum* bacteria + L-alpha proline amino acid, contributed to a high concentration of iron in the soil during the period of rapid spring wheat plant growth. Additionally, Jankiewicz [8] and Glick [9] claim that bioproducts guarantee a higher supply of nutrients from the soil environment, including nitrogen, iron, and phosphorus associated with synthesis of phytohormones stimulating plant development, and they reduce the level of ethylene, which influences plant root development. In the experiment reported here, after one bioproduct was applied without the other, poorer conditions were established and the soil content of iron was lower. It was due to the fact that less organic matter entered the soil and a lower number of soil microorganisms were present, as a result of which the positive effect of the bioproducts due to an increased access of plant poorly available elements, such as iron, was lower.

Nitrogen is one of the most important yield-forming elements, but effective fertilisation with this nutrient is efficient when the soil is rich in the remaining elements, including iron [10]. An application of excessive rates of mineral fertilisation with nitrogen leads to contamination of the soil environment and limits iron uptake from the soil [11], which was confirmed in the study reported here. The highest iron concentration in soil was recorded following an application of 90 kg N·ha^{-1}.

Mona et al. [12] found that an application of biological preparations stimulated iron content in wheat plants. Additionally, in the present study, an application of the *Bacillus megaterium* var. *phosphaticum* bacteria and L-alpha proline amino acid increased iron concentration in spring wheat grain and straw, the effect being more pronounced when the bioproducts were combined. Additionally, in studies by Popko et al. [13], the use of Amino-Prim and Amino-Hort biostimulators increased the accumulation of nutrients in winter wheat. Biostimulants work by increasing the uptake of minerals by plants, including Fe, and improve the efficiency of nutrient use [14–16]. Kocira et al. [17] and Kołodziejczyk [18] demonstrated that biofertilisers used together with biostimulants and reduced mineral nitrogen fertiliser rates improved soil properties, which is associated with an increase in nutrients, including iron, available for crop plants. This, in turn, translates into their concentration and accumulation in cereals [19]. Additionally, Adesemoye et al. [20], Sharma et al. [21], and Kumar et al. [6] claim that an application of biological products in cereal cultivation reduces chemical fertiliser rates. According to Emilsson et al. [22], Wu et al. [23], Granta et al. [24], and Kumar et al. [25], an application of *Bacillus megaterium* var. *phosphaticum* bacteria and L-alpha proline amino acid contributes to lower losses of nutrients, including iron, in the soil environment, and guarantees a steadfast supply of nutrients for plants throughout the growing season, which ensures a high accumulation of the nutrients, including iron, in cereals. These findings were confirmed in the study reported here as the highest iron concentration and accumulation in spring wheat grain and straw were recorded in the unit treated with *Bacillus megaterium* var. *phosphaticum* bacteria + L-alpha proline amino acid and fertilised with 90 kg N·ha^{-1}. This is very important as iron plays an important role in plant life. It is necessary for the synthesis of chlorophyll in green wheat plants, which translates into the quantity of the yield obtained. Such a pattern of fertilisation alleviates environmental pollution and is recommended for application in the system of sustainable agriculture [26].

An application of bioproducts and lower rates of mineral fertilisation protects the soil environment, is profitable, and enriches the soil in necessary macro- and microelements, which are easily available for plants. Biological products stimulate plant growth and development. In the present study, an application of *Bacillus megaterium* var. *phosphaticum* bacteria + L-alpha proline amino acid and a mineral nitrogen rate of up to 90 kg ·ha^{-1} contributed to a high soil concentration of iron, which is such an important element for plants. Spring wheat cultivated under such conditions grows a more developed rooting system as a result of which the crop takes up more iron from the soil, the element being necessary for chlorophyll synthesis in green plants, which translates into higher grain and straw yields of spring wheat and a higher iron content in plants.

5. Conclusions

During the period of rapid spring wheat plant growth, the highest soil, grain, and straw content of iron was recorded after an application of *Bacillus megaterium* var. *phosphaticum* bacteria + L-alpha proline amino acid + mineral nitrogen fertiliser at a rate of 90 kg N·ha^{-1}.

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