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The Effect of Renewable Phosphorus Biofertilizers on Selected Wheat Grain Quality Parameters

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Abstract: Recycling and reusing phosphorus in agriculture can reduce the consumption of natural phosphorus resources, which are continuing to shrink. Phosphorus fertilizers made from renewable raw materials (sewage sludge ash, animal bones, dried animal blood) and activated with phosphorus solubilizing microorganisms (*Bacillus megaterium*, *Acidithiobacillus ferrooxidans*) offer an alternative to conventional fertilizers. These products should meet consumer and environmental safety standards. In this paper, based on field experiments conducted in northeast Poland, the effects of waste-derived biofertilizers on selected parameters of wheat yield quality are discussed. The study focuses on the technological properties of the grain (hectoliter weight, hardness index, Zeleny index, starch, wet gluten, and protein content), the content of proteogenic amino acids, macro- and micronutrients, and selected toxic elements in the grain. The quality parameters of wheat grain were not affected by the tested biofertilizers applied in P doses up to 35.2 kg ha^{-1}, nor by conventional fertilizers.

Keywords: nutrient recycling; secondary raw materials; waste management; microbial solubilization; *Triticum aestivum* L.

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1. Introduction

The urgent need to feed the world's growing population, coupled with increasing concerns about nutrient pollution of the environment and climate change, have made rational nutrient management one of the major challenges of this century [1,2]. Food production begins in the field, and crop productivity is highly dependent on nutrient availability [3]. The soil pool of many nutrients is usually insufficient for achieving satisfactory yields, and additional plant nutrition from external sources is required [2]. The most commonly used nutrient carriers are synthetic mineral fertilizers [1], although alternative nutrient sources are increasingly being adopted [4].

One of the six key elements in plant nutrition is phosphorus (P) [5]. It plays a vital role in all their major metabolic activities, including photosynthesis and respiration, as well as nucleic acid, protein, starch, and membrane phospholipid synthesis [6,7]. Phosphorus cannot be replaced by any other element, and its deficiency severely limits crop productivity [8,9]. Given the critical role of P in global crop production, demand for P fertilizers is expected to increase significantly by 2050 [1,10]. Regardless of this global trend, there are huge disparities between world regions in terms of the amount of P fertilizer applied [11] and thus the soil P budget. P fertilization is most commonly achieved through the application of chemical fertilizers derived from phosphate rock (PR) [1]. PR is a finite, non-renewable and geographically restricted resource [12]. In addition, the PR economy is currently predominantly linear, with significant P wastage and loss from mine to fork (currently about 90%) [13], due to inefficient use of P fertilizers and high P losses to the environment [14]. In Europe, PR reserves are almost non-existent [12], both PR and P are critical raw materials [15], and most European countries are dependent on imported

PR [16]. For the P economy in European countries, the significant increase in P prices since 2020 due to pandemic, geopolitical conflicts, trade wars and rising fuel prices, and the conflict between Russia and Ukraine, further disrupting the PR trade, is also of great importance [17]. Closing the P cycle and using P more efficiently, particularly in agricultural production, seems to be indispensable and inevitable not only in Europe but also at the global level [8,14].

Increasing the use of recycled P in the fertilizer industry, as an alternative or supplement to PR, is considered one of the key actions towards global P sustainability [18]. A goal for fertilizer products to contain a minimum of 20% recycled P by 2030 has been advocated [19]. In recent years, multiple strategies have been developed to reuse P from P-rich wastes, such as manures, abattoir residues, food processing and domestic wastes, sewage derived biosolids and wastewaters, and the ashes of incinerated residues [4]. Great potential for P recycling exists by applying P-rich organic wastes and manures to agricultural soils [20]. In some cases, however, there is a need to recover, detoxify and modify P from waste to make recycling safe and effective and to achieve higher levels of nutrient use efficiency [4]. According to Kabbe and Rinck-Pfeiffer [21], there are more than 30 different technologies available for recovering P from waste streams, and new ones are still emerging.

Numerous studies prove that recycling-derived P fertilizers match conventional P fertilizers in terms of performance [22–24]. However, making these products marketable requires legislative support [25,26]. National policies that optimize P recycling in some European countries (e.g., mandatory P recovery from sewage sludge and slaughterhouse waste [27]), as well as EU regulations [28] and strategies [29], can help recycled nutrient carriers become competitive in the market.

One of the approaches that could be applied to the recycled fertilizer industry is the use of microbial solubilization [30]. The mechanisms of this natural process in the P cycle are fairly well understood, and numerous microbial strains (phosphorus solubilizing microorganisms—PSMs) performing this process are known [31,32]. Studies have shown that PSMs can be activators of insoluble P compounds in soils [33] and fertilizer feedstocks from both primary and secondary sources [34–36]. PSMs also promote plant growth through other biological mechanisms [37], which is an additional benefit of their use as/in biofertilizers (definition according to Mącik et al. [38]).

The concept of incorporating living PSM cultures into waste-based formulations [39] has led to the development of several biofertilizers from sewage sludge ash (SSA), animal bones and blood. The substrate used was activated by *Bacillus megaterium* or *Acidithiobacillus ferrooxidans* strains. The agronomic usefulness of these biofertilizers was tested under field conditions. Promising results have already been reported on the yield and environmental performance of these products [40–44]. This paper deals with the influence of waste-derived biofertilizers on the selected quality parameters of wheat (test crop) yield, i.e., technological properties (hectoliter (test) weight, hardness index, Zeleny (sedimentation) index, starch, wet gluten, and protein content), proteinogenic amino acid contents, and the content of macro- and micronutrients and selected toxic elements. The listed technological characteristics of grain are among the properties that determine grain destination and suitability for processing [45,46], amino acid profile and other macro- and micronutrient compositions are elements of the nutritional value of grain [47], while the absence/presence of potentially toxic elements determines the safety of grain products for their consumers (humans or livestock) [48]. The study hypothesized that the tested bioproducts would not have a negative impact on the studied yield quality parameters when compared to conventional P fertilizers.

2. Materials and Methods

2.1. Fertilizers and Experiments

Six biofertilizers made from waste materials and activated with PSMs were evaluated agronomically based on field experiments. The raw materials used for fertilizer production were sewage sludge ash (SSA), animal (poultry) bones, and dried animal (porcine) blood. The PSMs used were *Bacillus megaterium* or *Acidithiobacillus ferrooxidans* strains. The fertilizers were formulated as suspensions or granules. Table 1 presents an overview of the biofertilizers under study, while Table S1 provides a detailed listing of their chemical composition.

Table 1. Biofertilizers tested in the field experiments.

Symbol	Raw Material	Bacteria
Suspension biofertilizers		
A_s*Bm*	Sewage sludge ash [1]	*Bacillus megaterium* [3]
B_s*Bm*	Animal bones [2]	*Bacillus megaterium*
Granular biofertilizers		
A_g*Af*	Sewage sludge ash	*Acidithiobacillus ferrooxidans* [4]
AB_g*Af*	Sewage sludge ash + animal bones	*Acidithiobacillus ferrooxidans*
AB_g*Bm*	Sewage sludge ash + animal bones	*Bacillus megaterium*
AH_g*Bm*	Sewage sludge ash + dried animal blood [2]	*Bacillus megaterium*

[1] from the 'Łyna' Municipal Wastewater Treatment Plant in Olsztyn, Poland; [2] from the meat industry; [3] from the Polish Collection of Microorganisms at the Institute of Immunology and Experimental Therapy of the Polish Academy of Sciences in Wrocław, Poland; [4] from Professor Zygmunt Sadowski, Wroclaw University of Science and Technology (strain isolated from the tailings impoundment "Iron Bridge", Poland) [49].

The study compared biofertilizers to conventional P fertilizers, including superphosphate Fosdar™40 (SP; Grupa Azoty FOSFORY Sp. z o.o. in Gdańsk, Poland), and phosphorite Syria (PR; Luvena in Luboń, Poland). Biofertilizer analogues without PSM, such as ash-water solution (A + H_2O), granular fertilizer from SSA and bones (AB_g), and granular fertilizer from SSA and blood (AH_g), were also included in the study. Additionally, a no-P treatment was used. The New Chemical Syntheses Institute in Puławy, Poland, produced biofertilizers and fertilizers from waste using a formula developed by the Department of Advanced Material Technologies at Wrocław University of Science and Technology, Poland.

Between 2014 and 2017, seven field experiments were conducted to test renewable fertilizers on winter or spring common wheat (*Triticum aestivum* ssp. *vulgare* Mac Key). Table 2 presents basic information on the experiments. More details on the experiment designs and other agricultural data can be found in Table S2. All agricultural practices, except for P treatments, were consistent within each experiment and followed the principles of good agricultural practice.

Table 2. Basic data on the experiments performed.

Experiment	Biofertilizers Tested	Reference Treatments	P Doses, kg ha^{-1}	P-Treatment Number (*n*)	Test Crop	Growing Season
I	A_s*Bm*, B_s*Bm*	no P, SP, PR, A + H_2O	0, 21	6 (24)	Spring wheat	2014
II	A_s*Bm*	no P, SP, PR	0, 17.6, 26.4, 35.2	10 (80)	Spring wheat	2015
III	A_g*Af*, AB_g*Af*	no P, SP	0, 17.6, 26.4, 35.2	10 (40)	Winter wheat	2014/2015
IV	AB_g*Bm*	no P, SP, AB_g	0, 17.6, 26.4, 35.2	10 (40)	Winter wheat	2015/2016
V	AH_g*Bm*	no P, SP, AH_g	0, 17.6, 26.4, 35.2	10 (40)	Spring wheat	2016
VI	AH_g*Bm*	no P, SP, AH_g	0, 17.6, 26.4, 35.2	10 (40)	Winter wheat	2016/2017
VII	AH_g*Bm*	no P, SP, AH_g	0, 17.6, 26.4, 35.2	10 (40)	Spring wheat	2017

2.2. Experimental Site Description

The experiments were conducted at the Production and Experimental Station "Bałcyny" Sp. z o.o. in Bałcyny, located in the Warmińsko-Mazurskie province of northeastern Poland. The region has a temperate climate and glacial landforms, with the most common soil type being Luvisols [50]. Soils meeting the requirements of the test crop were used in the trials. The pH_{KCl} values of the 0–30 cm soil layer ranged from 4.98 to 6.28. The total contents of C, N, P, K, and Mg were 6.48 to 8.90 g kg^{-1}, 1.01 to 1.42 g kg^{-1}, 0.43 to 0.61 g kg^{-1}, 2.90 to 3.30 g kg^{-1}, and 1.88 to 2.25 g kg^{-1}, respectively (see Table S3 for detailed basic soil characteristics before the start of the individual experiments). The precipitation and thermal regimes during the experimental growing seasons differed from those typical of the region. Seasons in experiments I–III were too dry for wheat, while seasons in experiments IV–VII were rather too wet for this species (see Table S4 for detailed data).

2.3. Grain Sampling and Analyses

Samples of wheat grain weighing approximately 1 kg were taken from each plot after combine harvesting. From these samples, approximately 200 g portions of grain were weighed, cleaned of impurities and weed seeds, and forwarded for further analyses. The technological properties of the grain were analyzed, including hectoliter (test) weight, hardness index, Zeleny (sedimentation) index, starch, wet gluten, and protein content (in Experiments II–VII). Additionally, proteinogenic amino acid contents (in Experiments II and V), and contents of macronutrients, micronutrients, and selected toxic elements (in Experiments I–V) were determined.

The technological properties of wheat grain were analyzed using a near-infrared (NIR) grain analyzer (Infratec 1241, FOSS, Hillerød, Denmark) following the manufacturer's instructions.

The analysis of amino acids in grain was performed by Eurofins Steins Laboratorium (Vejen, Denmark; accredited according to DS-EN ISO/IEC 17025 [51], the Danish Accreditation Fund DANAK Reg. No. 222) according to the standard methods [52] and regulation [53]. Three different methods were used to hydrolyze the plant material for amino acid analysis: alkaline hydrolysis for tryptophan, acid hydrolysis preceded by oxidation for cysteine and methionine, and acid hydrolysis for the remaining amino acids. The hydrolyzed amino acids were quantified via ion exchange chromatography with ultraviolet detection (IC-UV).

Elemental analysis of grain samples was performed by the Chemical Laboratory of Multielemental Analysis at Wrocław University of Science and Technology (Wrocław, Poland; accredited according to PN-EN ISO/IEC 17025, Polish Center for Accreditation Certificate No. AB 696). The Vario Macro Cube Elementar (C,H,N) analyzer (Elementar Analysensysteme, Langenselbold, Germany) was used to analyze the C and N contents of the grain samples, with D-phenylalanine as the standard solution. The contents of other elements were determined using an inductively coupled plasma-optical emission spectrometer (ICP-OES) with a pneumatic nebulizer and axial view (iCAP Duo, Thermo Scientific, Waltham, MA, USA) [54]. The levels of detection (LoD) were 1.0, 2.5, 0.5, 0.025, 1.0, 0.5, 0.04, 0.04, 0.025, 0.025, 0.002, 0.013, 0.05, 0.015, 0.001, 0.005, and 0.01 mg kg^{-1} for P, K, Ca, Mg, S, B, Cu, Fe, Mn, Mo, Ni, Zn, As, Al, Cd, Cr, and Pb, respectively.

2.4. Statistical Analysis

The effect of P fertilization on the studied grain quality traits was tested for significance using the analysis of variance (ANOVA) or the Kruskal-Wallis test when the assumptions of ANOVA were not met. Statistical analysis was performed for each experiment separately. The normality of variable distribution and homogeneity of variance were verified by applying the Shapiro-Wilk W test and Levene's test, respectively. For statistical calculations, values of element content below the level of detection (LoD) were replaced by the LoD. Statistical analysis was performed with Statistica 13.3 [55]. When there were no significant differences between fertilizer treatments, only the means, medians and standard errors (SEs) of the variables from the entire experiment are shown in the tables.

3. Results

There was no significant effect of P fertilization ($p > 0.05$), whether in the form of conventional fertilizers, recycled fertilizers, or biofertilizers, applied at different rates, on the technological traits of wheat grain, i.e., hectoliter weight, hardness index, Zeleny index, starch, wet gluten, and protein content, in any of the experiments under study (II–VII) (Table 3).

Table 3. Technological traits of wheat grain; means, medians, standard errors (SE), and *p*-values for ANOVA or Kruskal-Wallis tests for all phosphorus treatments in the individual experiments [1].

Traits	Statistics	Experiments					
		II	III	IV	V	VI	VII
Hectoliter (test) weight, kg/hL	mean	79.6	83.0	76.9	76.7	79.4	75.1
	median	79.7	83.1	76.9	76.7	79.4	75.1
	SE	0.08	0.06	0.05	0.08	0.08	0.12
	p	0.771	0.816	0.851	0.842	0.991	0.719
Hardness index	mean	87.4	95.0	50.9	59.0	67.4	54.0
	median	81.5	96.6	51.2	58.1	67.7	54.2
	SE	2.89	1.16	0.48	0.56	0.47	0.36
	p	0.471	0.188	0.693	0.133	0.397	0.849
Zeleny (sedimentation) index	mean	50.0	33.3	32.7	44.5	23.8	38.3
	median	45.5	32.6	32.4	44.6	23.9	38.3
	SE	0.48	0.49	0.36	0.34	0.25	0.42
	p	0.860	0.969	0.855	0.841	0.842	0.817
Starch content, %	mean	67.3	70.8	69.9	68.1	69.4	68.3
	median	65.8	70.9	69.9	68.1	69.4	68.4
	SE	0.32	0.09	0.08	0.06	0.06	0.06
	p	0.845	0.222	0.867	0.794	0.987	0.509
Wet gluten content, %	mean	33.2	26.4	24.3	28.2	21.3	26.3
	median	33.4	26.3	24.2	28.3	21.4	26.3
	SE	0.28	0.18	0.15	0.12	0.10	0.13
	p	0.877	0.940	0.922	0.887	0.982	0.872
Protein content, %	mean	14.3	11.8	11.9	13.3	10.3	12.6
	median	14.2	11.7	11.9	13.3	10.3	12.6
	SE	0.06	0.06	0.05	0.04	0.04	0.05
	p	0.996	0.876	0.782	0.987	0.980	0.883

[1] no significant differences between phosphorus treatments in the individual experiments ($p > 0.05$).

The content of essential and non-essential amino acids in wheat grain was not affected by the tested phosphorus fertilization treatments in Experiments II and V ($p > 0.05$) (Table 4).

Table 4. Contents of amino acids in wheat grain (g kg^{-1} DM); means, medians, standard errors (SE), and *p*-values for ANOVA or Kruskal-Wallis tests for all phosphorus treatments in the individual experiments [1].

Essential Amino Acids	Statistics	Experiments		Non-Essential Amino Acids	Statistics	Experiments	
		II	V			II	V
Histidine	mean	2.99	2.74	Alanine	mean	4.63	4.20
	median	2.99	2.75		median	4.63	4.21
	SE	0.02	0.02		SE	0.03	0.03
	p	0.989	0.593		*p*	0.982	0.667
Isoleucine	mean	4.47	3.97	Arginine	mean	6.21	5.69
	median	4.44	6.96		median	6.20	5.72
	SE	0.03	0.03		SE	0.05	0.06
	p	0.990	0.921		*p*	0.946	0.379

Table 4. *Cont.*

Essential Amino Acids	Statistics	Experiments		Non-Essential Amino Acids	Statistics	Experiments	
		II	V			II	V
Leucine	mean	9.01	8.06	Aspartic acid	mean	6.78	5.91
	median	8.93	8.05		median	6.78	5.88
	SE	0.06	0.07		SE	0.05	0.06
	p	0.983	0.585		*p*	0.748	0.396
Lysine	mean	3.52	3.32	Cysteine	mean	2.81	2.55
	median	3.52	3.28		median	2.83	2.57
	SE	0.02	0.05		SE	0.02	0.02
	p	0.991	0.689		*p*	0.796	0.352
Methionine	mean	2.08	1.85	Glutamic acid	mean	43.8	37.1
	median	2.10	1.88		median	43.6	37.0
	SE	0.02	0.02		SE	0.42	0.35
	p	0.497	0.161		*p*	0.915	0.752
Phenylalanine	mean	6.45	5.60	Glycine	mean	5.63	5.09
	median	6.41	5.62		median	5.60	5.10
	SE	0.05	0.05		SE	0.04	0.04
	p	0.985	0.377		*p*	0.985	0.602
Threonine	mean	3.91	3.51	Proline	mean	14.2	12.2
	median	3.92	3.51		median	14.1	12.3
	SE	0.03	0.03		SE	0.14	0.11
	p	0.918	0.576		*p*	0.998	0.692
Tryptophan	mean	1.53	1.41	Serine	mean	6.61	5.77
	median	1.54	1.42		median	6.59	5.77
	SE	0.01	0.01		SE	0.06	0.07
	p	0.437	0.617		*p*	0.992	0.523
Valine	mean	5.62	5.14	Tyrosine	mean	3.61	3.23
	median	5.59	5.14		median	3.60	3.22
	SE	0.03	0.04		SE	0.03	0.05
	p	0.992	0.903		*p*	0.988	0.697

[1] no significant differences between phosphorus treatments in the individual experiments ($p > 0.05$).

No significant changes ($p > 0.05$) were observed in the content of macronutrients, micronutrients and potentially toxic elements in wheat grain under the applied P fertilization treatments in the experiments studied (I–V) (Tables 5 and 6).

Table 5. Contents of macroelements in wheat grain (g kg^{-1} DM); means, medians, standard errors (SE), and *p*-values for ANOVA or Kruskal-Wallis tests for all phosphorus treatments in the individual experiments [1].

Elements	Statistics	Experiments				
		I	II	III	IV	V
P [2]	mean	3.63	3.51	1.97	2.84	3.84
	median	3.62	3.49	1.98	2.84	3.83
	SE	0.024	0.027	0.025	0.014	0.018
	p	0.291	0.141	0.359	0.795	0.995
C	mean	406	409	341	416	413
	median	405	406	341	416	413
	SE	0.46	1.52	1.85	0.25	0.28
	p	0.895	0.340	0.910	0.482	0.934

Table 5. *Cont.*

Elements	Statistics	Experiments				
		I	II	III	IV	V
N	mean	22.4	22.8	19.0	18.9	21.5
	median	22.3	22.6	18.7	18.7	21.5
	SE	0.12	0.09	0.10	0.12	0.06
	p	0.836	0.431	0.910	0.826	0.358
K	mean	4.03	4.62	3.86	3.88	4.20
	median	4.00	4.29	3.82	3.87	4.219
	SE	0.030	0.024	0.031	0.020	0.019
	p	0.395	0.791	0.386	0.985	0.988
Ca	mean	0.49	0.33	0.29	0.32	0.33
	median	0.49	0.32	0.27	0.31	0.33
	SE	0.005	0.007	0.017	0.006	0.004
	p	0.855	0.516	0.991	0.847	0.608
Mg	mean	1.40	1.40	1.01	1.06	1.34
	median	1.39	1.39	1.00	1.06	1.34
	SE	0.011	0.009	0.008	0.004	0.006
	p	0.529	0.200	0.364	0.695	0.995
S	mean	1.35	1.38	1.18	1.16	1.34
	median	1.34	1.38	1.16	1.16	1.35
	SE	0.010	0.010	0.010	0.007	0.007
	p	0.276	0.544	0.388	0.909	0.511

[1] no significant differences between phosphorus treatments in the individual experiments ($p > 0.05$); [2] detailed data are a part of a separate paper [42].

Table 6. Contents of microelements and potentially toxic elements in wheat grain (mg kg^{-1} DM); means, medians, standard errors (SE), and *p*-values for ANOVA or Kruskal-Wallis tests for all phosphorus treatments in the individual experiments [1].

Elements	Statistics	Experiments				
		I	II	III	IV	V
B	mean	<LoD	<LoD	0.54	0.53	0.57
	median	<LoD	<LoD	<LoD	<LoD	<LoD
	SE			0.024	0.014	0.022
	p			0.571	0.720	0.749
Cu [2]	mean	2.84	3.81	2.29	3.81	4.37
	median	2.79	3.80	2.29	3.81	4.12
	SE	0.067	0.051	0.051	0.113	0.276
	p	0.536	0.525	0.608	0.966	0.994
Fe [3]	mean	39.7	56.3	59.6	32.7	40.8
	median	39.2	55.2	56.9	31.7	39.4
	SE	0.51	1.91	2.09	0.72	1.03
	p	0.917	0.882	0.523	0.348	0.385
Mn	mean	26.5	20.7	21.6	24.5	23.5
	median	26.9	20.4	22.2	24.9	23.3
	SE	0.51	0.26	0.36	0.25	0.18
	p	0.133	0.376	0.217	0.668	0.985

Table 6. *Cont.*

Elements	Statistics	Experiments				
		I	II	III	IV	V
Mo	mean	1.01	1.99	1.84	1.21	1.70
	median	0.98	2.11	1.76	0.95	1.16
	SE	0.127	0.138	0.180	0.217	0.244
	p	0.053	0.122	0.585	0.732	0.512
Ni [2]	mean	0.107	0.038	0.197	0.155	0.187
	median	0.096	0.021	0.164	0.126	0.059
	SE	0.024	0.005	0.022	0.014	0.043
	p	0.131	0.258	0.922	0.421	0.868
Zn [2]	mean	22.2	40.5	25.6	24.9	26.3
	median	22.1	40.3	25.4	24.8	26.
	SE	0.42	0.52	0.47	0.38	0.27
	p	0.597	0.226	0.576	0.405	0.823
As [2]	mean	<LoD	0.058	0.061	0.056	0.100
	median	<LoD	<LoD	<LoD	<LoD	0.076
	SE		0.003	0.004	0.002	0.009
	p		0.252	0.776	0.468	0.713
Al [3]	mean	8.98	<LoD	<LoD	2.69	2.51
	median	8.95	<LoD	<LoD	2.11	2.05
	SE	0.353			0.354	0.324
	p	0.721			0.170	0.590
Cd [4]	mean	0.086	0.039	0.012	0.016	0.036
	median	0.082	0.040	0.011	0.016	0.038
	SE	0.004	0.001	0.001	0.002	0.002
	p	0.489	0.452	0.638	0.085	0.463
Cr [2]	mean	0.193	0.087	0.082	0.253	0.432
	median	0.141	<LoD	<LoD	0.168	0.292
	SE	0.047	0.015	0.025	0.043	0.071
	p	0.477	0.268	0.754	0.284	0.984
Pb [4]	mean	0.014	0.046	0.078	0.019	0.038
	median	0.012	0.027	0.041	<LoD	<LoD
	SE	0.001	0.005	0.010	0.004	0.007
	p	0.590	0.686	0.888	0.725	0.869

[1] no significant differences between phosphorus treatments in the individual experiments ($p > 0.05$); [2] a part of the data is published in [43]; [3] a part of the data is published in [56]; [4] a part of the data is published in [41].

4. Discussion

Wheat grain quality is influenced by genetics, environment, and management practices, including fertilization [57]. After nitrogen (N), phosphorus (P) is the second limiting element for plant growth, and is usually supplemented with fertilizers. The forms of P in the applied sources and soil characteristics influence the amount of P in the soil solution [58]. The availability of P in the soil solution can lead to improved nutrient uptake by wheat plants, especially N, which ultimately affects the levels of protein, wet gluten, starch, macronutrients and micronutrients in the wheat grain. In addition, it can have a positive impact on hectoliter (test) weight, wheat grain hardness, and Zeleny (sedimentation) index. Hectoliter weight is a measure of the bulk density and soundness of grain [59], wheat grain hardness refers to the endosperm texture and resistance to deformation that affects grinding and milling processes [60], and Zeleny (sedimentation) index is a measure of gluten strength and protein quality [61].

The present study found no significant differences in the effects of recycled and conventional fertilizers on wheat grain technological properties, amino acid and nutrient/element contents, which is a satisfactory result. This finding indicates that neither the form of P carrier nor the dose of P played a significant role in the development of these grain quality characteristics. The studies by Gaj et al. [62] and Boukhalfa-Deraoui et al. [63] found no significant difference in grain protein content as a function of P source used. Similarly, Wołoszyk et al. [64] observed no effect of different waste-derived soil amendments on test weight, protein content, and Zeleny test. In contrast, Jiao et al. [65] observed no variation in N content in durum wheat grain depending on the type of P source (different commercial fertilizers), while the type of P source differentiated P and K content in grain. Although the lower solubility of P compounds in the waste feedstock has been reported elsewhere [23], this potential drawback did not alter grain quality characteristics in the present study.

The lack of response of technological properties of the grain, particularly protein, gluten, and starch content, to an increase in P dosage may be explained by the findings of Agapie and Bostan [66], which suggest that unilaterally applied P does not significantly affect the studied qualitative parameters, but is used as a support for N. Furthermore, the results of the present study are consistent with the findings of Eppendorfer [67] that P affects the amino acid composition of wheat grain only indirectly through its effects on N concentration. Boukhalfa-Deraoui et al. [63] reported that the P application rates (30, 60, 90 and 120 kg P ha^{-1}) were not significant for the protein content of the wheat grain when the N fertilization level was fixed. In the present study, the N rate was the same for all plots within each experiment, including those with no P treatments.

The present study found no significant differences in grain quality between the control (no P) and P-treated plots, regardless of the dose. The results indicate that plants from the control (no P) plots did not experience a P deficit that would contribute to yield quality deterioration, however, P supplementation appeared to help maintain the level of quality traits while increasing yield (Table S5). This could be attributed to the fact that P application stimulates root development, more intensive plant uptake of other nutrients as well as their translocation, assimilation and accumulation of assimilates in the grain [6,7,9], leading to the observed stability in yield quality. According to current knowledge, plants use a variety of molecular, physiological, and ecological mechanisms to maintain nutrient homeostasis [68,69].

Other authors [66,70] have also reported no response of the protein, starch, and wet gluten content in wheat grains, as well as the Zeleny and hardness indices, to P application and increased rates (0–120 kg P_2O_5 ha^{-1}). No significant effect of P fertilization on protein and amino acid contents was observed by Zheng et al. [71]. Jordan-Meille et al. [72] reported non-significant effects of P treatments (no P; 25–100 kg P $ha^{-1}y^{-1}$) on the concentration of some macronutrients, micronutrients, and trace elements in wheat grain (long-term experiment). On the other hand, positive responses of protein content [63,73–75], hectoliter (test) weight [75,76], gluten content [75] to P application compared to no P treatments have been reported by other authors, with no differences between P rates in some cases [73,74]. In other studies conducted under different environmental conditions, the application of external P was observed to have varying effects on the amino acid [77–80] and elemental content [81,82] of wheat grain.

Panayotova et al. [83] and Stefanova-Dobreva et al. [75] observed that the over-application of P fertilizer (160 kg P_2O_5 ha^{-1}) led to a decrease in test weight, protein, and gluten content, particularly when N was not supplied simultaneously [83]. A reduction in grain protein content and zinc (Zn) bioavailability in wheat due to excessive P fertilizer application was reported by Zhang et al. [84]. In the present study, no changes in grain quality were observed when the highest, already yield-ineffective dose of P was applied. With constant N and K fertilization, the excess P could be deposited in the straw, taken up by weeds, immobilized in the soil or leached into the soil profile [42].

Given the role of PSMs in increasing P availability for plant uptake [33,37], this increased P availability can be expected to increase the uptake of other nutrients, particularly

N, by the wheat plant, ultimately affecting the quality of the traits studied. However, the present study did not find any evident effect of the PSM strains used in waste-based biofertilizers on grain quality. It is noteworthy that the experiments revealed also a rather weak response of wheat yield to these bioactivators, with more promising results only under poorer habitat conditions (lower soil P content, worse previous crop; Tables S2, S3 and S5). There are many reports in the literature of significant responses of certain crop/cereal quality traits to PSMs when used alone [85,86] or in combination with a P substrate [73,79,87,88]. Most reports relate to increases in seed/grain protein content under PSMs [73,79,86–91], but increases in hectoliter weight [92,93], sedimentation value [89], starch content [90], and some amino acids [90] and elements [88,92] have also been demonstrated. The results of the present study, however, are consistent with those that found no effects of PSMs on yield quality: protein content [73,92,94], gluten content [92], hectoliter weight [85,89,91,95], grain hardness [96], and some element contents [89,92]. The effects may also depend on the microbial strain used [73]. The limited effectiveness of applied PSMs as plant growth promoters may be due to their low abundance when introduced with biofertilizers, poor competitiveness with other soil microorganisms [97], and susceptibility to uncontrolled environmental factors under varying field conditions [98].

Primary and secondary raw materials for P fertilizer production often contain potentially toxic elements, and the possibility of these elements accumulating in consumable plant parts, including grain, is a concern [99]. In the present study, the predominant feedstock for recycled fertilizers and the potential source of toxic elements was SSA (Table S1). However, the application of P fertilizers, including products based on SSA, did not change the PTE content in the wheat grain and the content remained below the permitted or recommended limits for plant material intended for human and animal consumption (Table S6). This can be attributed to the low concentration of toxic elements in the fertilizers, reasonable fertilizer application rates and, consequently, negligible PTE input to soil and poor translocation to wheat grain. The PSMs are claimed to affect the levels of toxic elements in wheat grain by influencing their availability, uptake and distribution in the plant. These microbes can immobilize heavy metals and prevent their redistribution in plants through precipitation, binding affinity, and sorption [100–102]. Moreover, they can help reduce the translocation and accumulation of toxic elements in wheat grain by improving overall plant health and vigor, nutrient uptake and plant growth [103]. Such phenomena were not observed in the present study. In previous articles by the authors [41,43,56], the issue of PTE levels in soils and plants under the influence of certain recycled P fertilizers was discussed in more detail, and caution was recommended for their repeated application due to the chemical heterogeneity of secondary nutrient sources [104] and the complexity of toxic element fate along the source-pathway-sink/receptor chain [105].

5. Conclusions

Phosphorus biofertilizers made from renewable raw materials, i.e., sewage sludge ash, animal bones, dried animal blood and activated with *Bacillus megaterium* or *Acidithiobacillus ferrooxidans* bacteria, similarly to conventional fertilizers, did not affect the technological properties of wheat grain, the content of proteogenic amino acids, macro and micronutrients or selected toxic elements in wheat grain when applied at P doses up to 35.2 kg ha^{-1}.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture14050727/s1, Table S1: Elemental composition of P-fertilizers used in the field experiments; Table S2: Field experiments conducted; experiment details and basic agricultural data; Table S3: Soil characteristics before the start of the experiment; Table S4: Precipitation and air temperature during the study period according to the Meteorological Station in Bałcyny, Poland; Table S5: Wheat yields (t ha^{-1}) under the influence of P treatments in the experiments; Table S6: Reference values for potentially toxic elements (mg kg^{-1}) in plants, according to various sources; references [106–114] are used in the Supplementary Materials section.

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