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Effect of Zinc along with Organic Fertilizers on Phosphorus Uptake and Use Efficiency by Cocksfoot (*Dactylis glomerata* L.)

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Abstract: Scarce findings on phosphorus (P) uptake and its utilization under increased zinc (Zn) levels in organic fertilizers amended soil led to conducting research. The aim of the study was to determine the effect of increasing the application of zinc (200, 400, and 600 mg·kg^{-1} of soil) together with different organic fertilizers (bovine manure, chicken manure, and spent mushroom substrate) on the content and uptake of phosphorus by cocksfoot and the phosphorus use efficiency from organic fertilizers. The application of different amounts of zinc did not affect phosphorus content in the grass, but it significantly influenced its accumulation ($p < 0.05$). The most phosphorus uptake was accumulated by plants following zinc application at 200 mg·kg^{-1} of soil and the least following application of 600 mg·kg^{-1} of soil. The phosphorus use efficiency from organic fertilizers was increased by zinc application of 400 mg·kg^{-1} of soil and reduced by 600 mg·kg^{-1} of soil. Organic fertilizers did not significantly affect the phosphorus content in the grass but did increase its uptake. The highest phosphorus use efficiency was obtained for bovine manure. The study showed no antagonistic relationships between zinc and phosphorus, but increasing zinc application affected the coefficient of phosphorus utilization from organic fertilizers.

Keywords: antagonism; heavy metals; bovine manure; chicken manure; spent mushroom substrate; phosphorus use efficiency

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1. Introduction

The yield of crop plants is limited not only by nutrient deficiencies but also by an imbalance between them, leading to synergistic or antagonistic effects [1]. These interactions take place when the presence of one nutrient affects the uptake, distribution or function of another [2–4]. When a plant's uptake by one nutrient increases with the content of another, the interaction is considered to be positive, and the nutrients are synergists. Conversely, when one nutrient limits the uptake of another, the interaction is considered negative, and the nutrients are antagonists [5–8]. Interdependencies between nutrients can be evaluated by analyzing the relationship between the soil application of one nutrient and the content of another nutrient in plants [1].

Phosphorus and zinc are essential nutrients for plants, but an increased level of zinc in the soil may affect the uptake and use of phosphorus from fertilizers [9,10]. The mobility of both elements is relatively low because they form compounds that are insoluble or poorly soluble in water [11–13]. They depend on soil properties, such as redox potential, pH value, and organic matter content. [14].

Phosphorus is involved in a number of processes taking place in plants: respiration, energy production, and regulation of enzymatic reactions and metabolic processes [15–18]. It also plays an important role in signal transduction and photosynthesis in plants and has a decisive influence on resistance to stress [19]. It is a component of nucleic acids, phospholipids, adenosine triphosphate (ATP), nicotinamide adenine dinucleotide phosphate (NADPH), and sugar phosphates [20–23]. Phosphorus, like nitrogen, is considered a nutrient whose deficiency most often reduces plant productivity [18,24–27]. An inadequate supply of phosphorus to plants disturbs chlorophyll production and causes leaf chlorosis and accumulation of anthocyanins, leading to purple discoloration on the surface of leaves [28]. Phosphorus deficiency negatively affects electron transport in plants [18]. Despite a large number of phosphates in the soil, its availability to plants is poor due to binding by soil minerals [29,30], and uptake by plants depends not only on the content of its available forms in soil but also on the antagonistic and synergistic effects of other elements [6,31].

Zinc is a catalytic and structural protein cofactor in numerous enzymes and performs key structural functions in protein domains that interact with other molecules [32,33]. Zinc deficiency limits growth, stress tolerance, and chlorophyll synthesis in plants [34–37].

Many researchers have assessed the effect of varied phosphorus content in soil on the content and accumulation of zinc in plants [6,9,10,13,38,39]. The most common zinc deficiency in plants is caused by an excess of phosphorus. Much less often, this relationship is reversed. This is due to the use of large amounts of phosphorus fertilizers and small amounts of zinc fertilizers. [40]. There are few findings in the literature regarding phosphorus uptake and its use efficiency under the influence of elevated zinc levels in the soil [39,41].

Grasses are plants with a high phosphorus demand [42]. Cocksfoot (*Dactylis glomerata* L.) grass is often grown in temperate climates. [43]. It grows well and adapts to various habitat conditions. It can be mowed or grazed and sown in mixtures with clovers or alfalfa [44]. Cocksfoot contains a lot of fiber; therefore, its nutritional value is sometimes lower than that of other grasses [45]. It is a commonly cultivated grass in Europe.

It has been hypothesized that high doses of zinc would decrease the content, uptake and phosphorus use efficiency by the test plant. The aim of this study was to assess the effect of different amounts of zinc applied together with the spent mushroom substrate, chicken manure, and bovine manure on phosphorus content uptake and their use efficiency from applied fertilizers by cocksfoot.

2. Materials and Methods

2.1. Description of the Study Sites

A pot experiment was carried out in Siedlce, Poland (52°10′12″ N, 22°17′15″ E, 155 m above sea level) in 2014–2016 (three growing seasons). The study site was in a temperate, warm, transitional climate. Plant vegetation period—the number of days in a year with an average daily air temperature of at least 5 °C ranges from 200 to 210.

The experiment was conducted in a vegetation hall. Pots with a capacity of 10 L have been filled with 12 kg of soil. These Luvisols consisted of sand, silt and clay 71%, 24%, and 5%, respectively. Before filling the pots, the soil was sieved through a sieve with a mesh size of 1 cm. The experiment was set up in triplicate as a completely random system, with two factors:

I—zinc application rate: 0 (no zinc application) and zinc in doses 200, 400 and 600 $mg \cdot kg^{-1}$ of soil. This element was applied to pots in the form of an aqueous solution of $ZnSO_4 \cdot 5H_2O$. Its application took place before sowing cocksfoot seeds, only in the first year of the research.

II—organic fertilizer: no application of organic materials (CO) and application of spent mushroom substrate (MS), chicken manure (ChM) and bovine manure (CM). Organic fertilizers (Table 1) have been applied only once, as was zinc fertilization. Their application took place two weeks before the sowing of cocksfoot seeds, the dose of these fertilizers was 2 g of organic carbon per 1 kg of soil. Every year of the study, the test plant was the Amera cultivar of cocksfoot, which was sown every year in the first ten days of May in the amount of 0.3 g of seeds per pot. The aerial parts of the cocksfoot were sheared four times each year, and harvest intervals were 30 days. During the vegetation period, soil moisture was at the level of 60–70% of full water capacity by watering.

Table 1. Selected properties of organic fertilizers (mean ± SD, given on the dry matter, DM).

Organic Materials	Dry Matter (%)	Organic Carbon C_{org}	Total Nitrogen N_{tot}	C:N Ratio	Phosphorus P	Potassium K	Calcium Ca	Magnesium Mg	Sulphur S	Zinc Zn
		$g \cdot kg^{-1}$			$g \cdot kg^{-1}$					$mg \cdot kg^{-1}$
Bovine manure	19.6 ± 3.0	405.1 ± 9.0	23.90 ± 2.63	16.9:1	5.38 ± 0.29	15.28 ± 1.75	10.04 ± 2.32	2.90 ± 0.39	3.07 ± 0.36	60.28 ± 6.47
Chicken manure (layers)	27.8 ± 3.8	167.3 ± 6.2	13.50 ± 1.13	12.4:1	8.44 ± 1.09	9.32 ± 1.17	13.72 ± 1.33	2.68 ± 0.63	3.12 ± 0.43	190.8 ± 13.58
Mushroom substrate	30.4 ± 4.2	319.3 ± 14.6	24.20 ± 2.71	13.2:1	6.22 ± 0.98	17.48 ± 1.79	47.32 ± 5.57	3.12 ± 0.41	25.08 ± 1.78	117.5 ± 12.62

The chemical composition of the organic fertilizers used in the experiment was varied. The highest content of dry matter, total nitrogen, potassium, calcium, magnesium, and sulfur, was found in the mushroom substrate, and phosphorus and zinc in the chicken manure. Bovine manure contained the highest organic carbon and had the largest C:N ratio.

2.2. Laboratory Analyses

The selected properties of the organic materials used in our experiment are presented in Table 1. In organic fertilizers, the following have been determined: dry mass (DM, at 105 °C), carbon in organic compounds (Tyurin method), total nitrogen content (CHNS method on CHN Autoanalyzer, Perkin-Elmer, Santa Clara, CA, USA). In addition, the following were determined: total content of phosphorus, potassium, calcium, magnesium, and zinc by the ICP-AES method (Optima 3200 RL spectrometer, Perkin-Elmer, USA), determinations were made in the solutions obtained after samples dry mineralization at 500 °C. The soil on which the pot experiment was carried out had a pH value of 6.65 in 1 $mol \cdot dm^{-3}$ KCl. This soil contained: total nitrogen 1.52 $g \cdot kg^{-1}$, organic carbon 16.40 $g \cdot kg^{-1}$ organic carbon, phosphorus (P) 176 $mg \cdot kg^{-1}$ and potassium (K) 108 $mg \cdot kg^{-1}$ in available forms for plants (by Egner–Riehm method), and total zinc 56.6 $mg \cdot kg^{-1}$ zinc. Total zinc was determined by the ACP-AES method. Determinations were made in the solutions obtained after wet mineralization of fertilizers material samples in a mixture (3:1 ratio) of acids HCl and HNO_3.

The total content of phosphorus and zinc in cocksfoot was also determined by the ICP-AES method. The grass samples were more often dry mineralized at 450 °C. The obtained ash was dissolved in a ten percent HCl solution. The content of the presented elements in the tested materials (organic fertilizers, soil, and cocksfoot) in the manuscript text was given in terms of dry weight (DM).

2.3. Calculations

Calculations of uptake and phosphorus use efficiency were made to the modified formulas provided by Sarkar et al. [46].

$$P_{UP} = Y \times P_{plant}/1000 \tag{1}$$

where:

P_{UP}—phosphorus uptake by cocksfoot (accumulation in cocksfoot dry matter), P $g \cdot pot^{-1}$
Y—yield of cocksfoot, $g \cdot pot^{-1}$
P_{plant}—phosphorus content in cocksfoot, P $g \cdot kg^{-1}$

$$P_{UC} = ((P_{U\_P} - P_{U\_0P})/P_{amt}) \times 100\% \tag{2}$$

where:

P_{UC}—phosphorus use efficiency, (%)

P_{U_P}—phosphorus uptake by cocksfoot fertilized separately with mushroom substrate, chicken manure and bovine manure, P g·pot^{-1}
P_{U_0P}—phosphorus uptake by cocksfoot from control treatments without organic fertilization, separately for all zinc doses), P g·pot^{-1}
P_{amt}—amount of phosphorus introduced into soil separately with mushroom substrate, chicken manure and bovine manure, P g·pot^{-1}.

2.4. Statistical Analyses

The obtained test results were subjected to variance analysis. The significance of the studied factors was concluded on the basis of the Fisher-Snedecor distribution. Tukey test was used to calculate the LSD value. Calculations were made at a significance level of $p = 0.05$. Calculations were made by using Statistica 13 PL software (StatSoft, Tulsa, OK, USA). The same program calculated Pearson's linear correlation coefficient.

3. Results

The phosphorus content in cocksfoot ranged from 3.48 to 3.97 g·kg^{-1} (on average, 3.79 g·kg^{-1} DM). This parameter was not dependent on increasing zinc application (200, 400, and 600 mg·kg^{-1} of soil) or different organic fertilizers (bovine manure, chicken manure, or spent mushroom substrate) (Table 2). There were also no significant differences in phosphorus content in cocksfoot in successive years of the study.

Table 2. Phosphorus content (P g·kg^{-1} DM) in cocksfoot under different zinc doses (Zn mg·kg^{-1} of soil) and organic fertilization (mean ± SD).

Organic Fertilizers	Years	Zn Doses				Mean
		0	200	400	600	
Without organic fertilization	1st	3.59 ± 0.74	3.73 ± 0.53	3.83 ± 0.14	3.78 ± 0.59	3.73 ± 0.55
	2nd	3.50 ± 0.28	3.52 ± 0.17	3.60 ± 0.39	3.58 ± 0.39	3.55 ± 0.32
	3rd	3.72 ± 0.23	3.88 ± 0.24	3.77 ± 0.24	3.88 ± 0.53	3.81 ± 0.34
	mean	3.60 ± 0.48	3.71 ± 0.38	3.73 ± 0.29	3.75 ± 0.52	3.70 ± 0.43
Bovine manure	1st	3.76 ± 0.21	3.84 ± 0.70	3.90 ± 0.18	3.80 ± 0.28	3.83 ± 0.40
	2nd	3.74 ± 0.20	3.82 ± 0.35	3.76 ± 0.15	3.48 ± 0.17	3.70 ± 0.27
	3rd	3.76 ± 0.40	3.80 ± 0.29	3.86 ± 0.14	3.70 ± 0.17	3.78 ± 0.28
	mean	3.75 ± 0.28	3.82 ± 0.48	3.84 ± 0.17	3.66 ± 0.25	3.77 ± 0.33
Chicken manure	1st	3.90 ± 0.39	3.77 ± 0.31	3.95 ± 0.28	3.86 ± 0.09	3.87 ± 0.30
	2nd	3.80 ± 0.26	3.92 ± 0.28	3.84 ± 0.31	3.90 ± 0.16	3.87 ± 0.25
	3rd	3.74 ± 0.24	3.82 ± 0.22	3.76 ± 0.21	3.65 ± 0.28	3.74 ± 0.25
	mean	3.81 ± 0.31	3.84 ± 0.26	3.85 ± 0.28	3.80 ± 0.22	3.83 ± 0.27
Mushroom substrate	1st	3.67 ± 0.40	3.85 ± 0.29	3.90 ± 0.44	3.90 ± 0.32	3.83 ± 0.38
	2nd	3.76 ± 0.30	3.80 ± 0.25	3.82 ± 0.46	3.89 ± 0.30	3.82 ± 0.34
	3rd	3.80 ± 0.22	3.90 ± 0.21	3.90 ± 0.31	3.97 ± 0.25	3.89 ± 0.26
	mean	3.74 ± 0.32	3.85 ± 0.26	3.87 ± 0.41	3.92 ± 0.29	3.85 ± 0.33
Mean for zinc doses		3.73 ± 0.37	3.80 ± 0.36	3.82 ± 0.30	3.78 ± 0.36	3.79 ± 0.35
Mean for years	1st	3.73 ± 0.49	3.80 ± 0.49	3.90 ± 0.29	3.84 ± 0.37	3.81 ± 0.42
	2nd	3.70 ± 0.29	3.77 ±0.30	3.77 ± 0.36	3.71 ± 0.33	3.73 ± 0.32
	3rd	3.76 ± 0.28	3.85 ± 0.25	3.82 ± 0.24	3.80 ± 0.36	3.81 ± 0.29

Means for all investigated factors (in the columns for organics fertilization and for years but in the row for zinc doses) are not significantly different, $p < 0.05$.

Phosphorus uptake by the tested plant, calculated as the average from the three years of the study (Table 3) and as the total uptake for the three years (Table 4), was significantly dependent on all studied factors: zinc doses, applied organic materials, and the years of the research. In the first year of the research, cocksfoot accumulated the most phosphorus uptake following zinc application of 200 mg·kg^{-1} of soil and the least following application of 600 mg·kg^{-1} of soil. In subsequent years of the study, different amounts of zinc in the soil did not significantly influence zinc uptake by the grass.

Table 3. Phosphorus uptake (P g·pot^{-1}) by cocksfoot under different zinc doses (Zn mg·kg$^{-1:}$ of soil) and organic fertilization (mean ± SD).

Organic Fertilizers	Years	Zn Doses				Mean
		0	200	400	600	
Without organic fertilization	1st	0.055 ± 0.012	0.068 ± 0.012	0.050 ± 0.004	0.044 ± 0.006	0.054 ± 0.013 ^{A}
	2nd	0.044 ± 0.007	0.039 ± 0.004	0.032 ± 0.002	0.045 ± 0.003	0.040 ± 0.007 ^{A}
	3rd	0.034 ± 0.002	0.032 ± 0.003	0.028 ± 0.001	0.032 ± 0.007	0.031 ± 0.004 ^{A}
	mean	0.044 ± 0.012	0.046 ± 0.017	0.037 ± 0.010	0.040 ± 0.008	0.042 ± 0.013 ^{a}
Bovine manure	1st	0.095 ± 0.003	0.113 ± 0.024	0.104 ± 0.006	0.080 ± 0.009	0.098 ± 0.018 ^{C}
	2nd	0.054 ± 0.008	0.058 ± 0.008	0.048 ± 0.006	0.044 ± 0.004	0.051 ± 0.009 ^{B}
	3rd	0.046 ± 0.002	0.045 ± 0.005	0.041 ± 0.003	0.043 ± 0.002	0.044 ± 0.004 ^{B}
	mean	0.065 ± 0.022	0.072 ± 0.033	0.064 ± 0.028	0.056 ± 0.018	0.064 ± 0.027 ^{bc}
Chicken manure	1st	0.105 ± 0.013	0.108 ± 0.012	0.105 ± 0.009	0.089 ± 0.004	0.102 ± 0.013 ^{C}
	2nd	0.062 ± 0.009	0.066 ± 0.007	0.057 ± 0.005	0.057 ± 0.004	0.061 ± 0.007 ^{C}
	3rd	0.038 ± 0.008	0.044 ± 0.001	0.032 ± 0.002	0.035 ± 0.005	0.037 ± 0.007 ^{AB}
	mean	0.068 ± 0.029	0.073 ± 0.028	0.065 ± 0.031	0.060 ± 0.022	0.067 ± 0.028 ^{c}
Mushroom substrate	1st	0.079 ± 0.010	0.097 ± 0.010	0.086 ± 0.015	0.069 ± 0.008	0.083 ± 0.015 ^{B}
	2nd	0.049 ± 0.002	0.043 ± 0.005	0.062 ± 0.010	0.056 ± 0.005	0.052 ± 0.009 ^{B}
	3rd	0.040 ± 0.003	0.043 ± 0.001	0.039 ± 0.003	0.041 ± 0.003	0.041 ± 0.003 ^{B}
	mean	0.056 ± 0.018	0.061 ± 0.026	0.062 ± 0.022	0.055 ± 0.013	0.059 ± 0.021 ^{b}
Mean for zinc doses		0.058 ± 0.023 ^{ab}	0.063 ± 0.029 ^{b}	0.057 ± 0.027 ^{ab}	0.053 ± 0.018 ^{a}	0.058 ± 0.025
Mean for years	1st	0.083 ± 0.022 ^{B}	0.097 ± 0.023 ^{C}	0.086 ± 0.024 ^{B}	0.070 ± 0.018 ^{A}	0.084 ± 0.024 ^{c}
	2nd	0.052 ± 0.010 ^{A}	0.052 ± 0.013 ^{A}	0.050 ± 0.013 ^{A}	0.050 ± 0.008 ^{A}	0.051 ± 0.011 ^{b}
	3rd	0.039 ± 0.006 ^{A}	0.041 ± 0.006 ^{A}	0.035 ± 0.006 ^{A}	0.037 ± 0.006 ^{A}	0.038 ± 0.007 ^{a}

$^{a, b, c}$—averages for studied factors marked with different small letters (for organics fertilization and for years in the columns but for zinc doses in the row) are significantly different, $p < 0.05$. $^{A, B, C}$—significant differences between the means for the interactions are marked with uppercase in the lines, $p < 0.05$.

Table 4. Total phosphorus uptake (P g·pot^{-1}) by cocksfoot in three years under different zinc doses (Zn mg·kg^{-1} of soil) and organic fertilization (mean ± SD).

Organic Fertilizers	Zn Doses				Mean
	0	200	400	600	
Without organic fertilization	0.132 ± 0.020	0.139 ± 0.016	0.110 ± 0.006	0.120 ± 0.017	0.125 ± 0.018 ^{a}
Bovine manure	0.196 ± 0.013	0.217 ± 0.037	0.193 ± 0.002	0.167 ± 0.004	0.191 ± 0.025 ^{bc}
Chicken manure	0.205 ± 0.019	0.218 ± 0.006	0.195 ± 0.018	0.181 ± 0.011	0.200 ± 0.019 ^{c}
Mushroom substrate	0.167 ± 0.015	0.184 ± 0.019	0.186 ± 0.033	0.165 ± 0.012	0.176 ± 0.021 ^{b}
Mean	0.175 ± 0.033 ^{ab}	0.190 ± 0.039 ^{b}	0.171 ± 0.040 ^{ab}	0.158 ± 0.026 ^{a}	0.173 ± 0.036

$^{a, b, c}$—averages for studied factors marked with different small letters (for organics fertilization and for years in the columns but for zinc doses in the row) are significantly different, $p < 0.05$.

In the three-year cycle, following zinc application of 200 mg·kg^{-1} of soil, the plants accumulated 8.6% more of this element than the plants from the control object and 11.1% and 20.2% more than following application of 400 and 600 mg·kg^{-1} of soil. All organic fertilizers increased phosphorus uptake by cocksfoot. Following the application of bovine manure, chicken manure and spent mushroom substrate, the plants accumulated 52.8%, 60.0% and 40.0% more phosphorus, respectively, than the plants in the control treatment. Phosphorus uptake by cocksfoot significantly decreased in successive years of the experiment. In the second and third years, it was 60.7% and 45.2% of the amount accumulated in the first year. The correlation coefficients (Table 5) showed no significant relationship between phosphorus content (Table 2) and zinc content in the test plant, which was reported in a previous paper [47]. At the same time, they showed a significant relationship between the content and uptake of phosphorus (Tables 3 and 4) and the yield reported in the study cited.

Table 5. Linear correlation coefficients between selected properties of test plant.

Specification	Yields	P uptake	Zn Content	Zn Dose
P content	0.551 *	0.630 *	0.213	0.267
P uptake	0.995 *	-	−0.333	−0.240

*—is important, $p < 0.05$.

One of the most important parameters used to determine the efficiency of mineral and organic fertilizers is the nutrient use efficiency coefficient. The question of the efficiency of phosphorus utilization from fertilizers cannot be limited to a single year because, in the case of this element, the effect in successive years is important. The phosphorus use efficiency from bovine manure, chicken manure, and spent mushroom substrate (Tables 6 and 7) depended on the level of zinc application. The total phosphorus efficiency for the three-year study was highest following zinc introduction of 400 mg·kg^{-1} of soil, lower following the introduction of 200 mg·kg^{-1} of soil, and the lowest without zinc application and following zinc introduction of 600 mg·kg^{-1} of soil.

Table 6. Phosphorus use efficiency (%) from organic fertilizers under different zinc doses (Zn mg·kg^{-1} of soil) and organic fertilization (mean ± SD).

Organic Fertilizers	Years	Zn Doses				Mean
		0	200	400	600	
Bovine manure	1st	12.6 ± 3.8	14.1 ± 6.3	16.6 ± 0.5	11.3 ± 1.7	13.6 ± 4.3 ^{C}
	2nd	3.3 ± 0.3	5.9 ± 3.4	5.0 ± 1.9	−0.4 ± 1.9	3.5 ± 3.3 ^{A}
	3rd	3.8 ± 0.8	4.0 ± 2.3	3.9 ± 0.7	3.5 ± 2.7	3.8 ± 1.8 ^{B}
	mean	6.5 ± 4.8	8.0 ± 6.1	8.5 ± 5.9	4.8 ± 5.3	7.0 ± 5.7 ^{c}
Chicken manure	1st	4.2 ± 2.1	3.3 ± 0.6	4.6 ± 1.1	3.8 ± 0.1	4.0 ± 1.3 ^{A}
	2nd	1.5 ± 1.3	2.2 ± 0.9	2.1 ± 0.3	1.0 ± 0.2	1.7 ± 0.9 ^{A}
	3rd	0.4 ± 0.7	1.0 ± 0.2	0.4 ± 0.2	0.3 ± 0.2	0.5 ± 0.5 ^{A}
	mean	2.0 ± 2.2	2 2 ± 1.1	2.4 ± 1.9	1.7 ± 1.5	2.1 ± 1.7 ^{a}
Mushroom substrate	1st	5.2 ± 1.2	6.3 ± 1.3	7.6 ± 2.3	5.4 ± 1.2	6.2 ± 1.8 ^{B}
	2nd	1.0 ± 1.6	0.8 ± 0.5	6.3 ± 2.2	2.3 ± 1.0	2.6 ± 2.5 ^{A}
	3rd	1.3 ± 0.2	2.4 ± 0.7	2.3 ± 0.6	1.9 ± 2.0	2.0 ± 1.2 ^{AB}
	mean	2.5 ± 2.2	3.2 ± 2.5	5.4 ± 3.0	3.2 ± 2.2	3.6 ± 2.7 ^{b}
Mean for zinc doses		3.7 ± 3.9 ^{a}	4.5 ± 4.6 ^{ab}	5.4 ± 4.7 ^{b}	3.2 ± 3.7 ^{a}	4.2 ± 4.3
Mean for years	1st	7.3 ± 4.5	7.9 ± 5.9	10.0 ± 5.3	6.8 ± 3.5	7.9 ± 5.0 ^{b}
	2nd	2.0 ± 1.5	3.0 ± 2.9	4.5 ± 2.4	1.0 ± 1.7	2.6 ± 2.6 ^{a}
	3rd	1.8 ± 1.5	2.5 ± 1.8	2.2 ± 1.6	1.9 ± 2.4	2.1 ± 1.9 ^{a}

$^{a, b, c}$—averages for studied factors marked with different small letters (for organics fertilization and for years in the columns but for zinc doses in the row) are significantly different, $p < 0.05$. $^{A, B, C}$—averages for the interaction with uppercase in the lines of the table are significantly different, $p < 0.05$.

Table 7. Total phosphorus use efficiency (%) from applied organic materials in three years times under different zinc doses (Zn mg·kg^{-1} of soil), (mean ± SD).

Organic Fertilizers	Zn Doses				Mean
	0	200	400	600	
Bovine manure	19.7 ± 3.8	24.0 ± 13.3	25.5 ± 2.4	14.4 ± 4.5	20.9 ± 7.8 ^{b}
Chicken manure	6.1 ± 3.1	6.5 ± 0.8	7.1 ± 1.9	5.1 ± 0.8	6.2 ± 1.8 ^{a}
Mushroom substrate	7.5 ± 3.7	9.6 ± 0.7	16.2 ± 6.0	9.6 ± 1.0	10.8 ± 4.6 ^{a}
Mean	11.1 ± 7.1 ^{a}	13.4 ± 10.5 ^{ab}	16.3 ± 8.7 ^{b}	9.7 ± 4.7 ^{a}	12.6 ± 8.1

$^{a, b}$—averages for studied factors with different small letters (for organics fertilization and for zinc doses in the columns but for zinc doses in the row) are significantly different, $p < 0.05$.

The phosphorus use efficiency (total for the entire experiment) was significantly the highest in the case of bovine manure. It was three times as high as chicken manure and nearly twice as high as spent mushroom substrate. The highest efficiency of phosphorus from organic fertilizers was noted in the first year of the study. In the second and third years, it significantly decreased (nearly threefold in the second year and nearly fourfold in the third year).

Different amounts of phosphorus introduced into the soil with organic fertilizers (Table 8) did not affect its content in cocksfoot (Table 2) but increased its uptake (Tables 3 and 4). The use efficiency of phosphorus from the chicken manure (Tables 5 and 6), in which the greatest amount of this element was introduced into the soil, was least. Thus, after the application of chicken manure, the amount of phosphorus that remained in the soil after three years of cultivating cocksfoot was the highest.

Table 8. Phosphorus balance introduced into soil with organic fertilizers in three years under different zinc doses (P remained in the soil in g).

Organic Fertilizers	Amount of P Introduced into Soil, g·pot^{-1}	Years of Study	Zn Doses								Mean	
			0		200		400		600			
			Uptake	Residual	Uptake	Residual	Uptake	Residual	Uptake	Residual	Uptake	Residual
Bovine manure	0.324	1st	0.095	0.229	0.113	0.211	0.104	0.220	0.080	0.244	0.098	0.226
		2nd	0.054	0.174	0.058	0.152	0.0148	0.172	0.044	0.200	0.043	0.175
		3rd	0.046	0.128	0.045	0.107	0.041	0.131	0.043	0.157	0.044	0.131
Chicken manure	1.200	1st	0.105	1.095	0.108	1.092	0.105	1.095	0.089	1.111	0.102	1.098
		2nd	0.062	1.033	0.066	1.026	0.057	1.038	0.057	1.054	0.061	1.038
		3rd	0.038	0.995	0.044	0.982	0.032	1.005	0.035	1.019	0.037	1.000
Mushroom substrate	0.468	1st	0.079	0.389	0.097	0.371	0.086	0.382	0.069	0.399	0.083	0.385
		2nd	0.049	0.340	0.043	0.328	0.062	0.320	0.056	0.343	0.053	0.333
		3rd	0.040	0.301	0.043	0.284	0.039	0.282	0.041	0.303	0.041	0.293
Mean		1st	0.093	0.571	0.106	0.558	0.098	0.566	0.079	0.585	0.094	0.570
		2nd	0.055	0.516	0.056	0.502	0.045	0.510	0.052	0.532	0.052	0.515
		3rd	0.041	0.475	0.044	0.458	0.037	0.473	0.040	0.493	0.041	0.475

4. Discussion

The present study found no significant effect of different levels of zinc application on phosphorus content in cocksfoot (Table 2) or relationships between its content of phosphorus and zinc (Table 7). Aboyeji et al. [6] carried out a field experiment to test the effect of different application rates of zinc (0, 4, and 8 kg·ha^{-1} of soil) and phosphorus (0, 40, 80, and 120 kg·ha^{-1} of soil) on the yield and chemical composition of peanuts. They showed that zinc application, irrespective of the amount, had no effect on phosphorus content in the biomass of the test plant. Similar relationships were reported by Soltangheisi et al. [48], who conducted a pot experiment using maize to test the effect of different levels of zinc

(0, 5, and 10 mg·kg^{-1} of soil) and phosphorus (0, 50, 100, and 200 mg·kg^{-1} of soil) on yield and on the content and uptake of these elements by the plants. The researchers found that none of the levels of zinc applied differentiated the phosphorus content in the biomass of the plants, whereas phosphorus application decreased their zinc content. Zhu et al. [39] showed that zinc applied in the form of $ZnSO_4 \cdot 5H_2O$ at 0.22 and 2.2 mg·kg^{-1} of soil had no effect on phosphorus content in spring wheat (*Triticum aestivum* L.). Contrasting results were reported by Das et al. [41], who studied the interactions between zinc and phosphorus in a greenhouse experiment in which the test plant was stevia (*Stevia rebaudiana*). They noted a reduction in phosphorus content in the biomass of the test plant as the zinc content increased, which indicates antagonistic relationships between these elements. Antagonistic relationships between zinc and phosphorus were also described by Barben et al. [49], who showed that zinc application caused a decrease in phosphorus content in the aerial parts of potatoes and an increase in its content in the roots. The authors suggest that phosphorus/zinc complexes are formed in the roots, preventing the transport of phosphorus at high concentrations of zinc.

The conflicting scientific reports regarding the relationships between zinc and phosphorus suggest the need for further research using different plant species.

The present study found that bovine manure, chicken manure, and spent mushroom substrate had no effect on phosphorus content in cocksfoot (Table 2), despite the introduction of different amounts of this element into the soil (Table 8).

An important element in assessing the efficiency of applied organic materials is their effect on the accumulation of nutrients. Our study showed that phosphorus uptake by test plants significantly depended on the amount of zinc applied to the soil and on organic fertilizer (Tables 3 and 4). Total phosphorus uptake over the three years of the study was highest following zinc application of 200 mg·kg^{-1} of soil. All of the organic fertilizers caused an increase in phosphorus uptake by cocksfoot. It was accumulated in the greatest amounts following the application of chicken manure or bovine manure. Chicken manure has been described as a potential source of phosphorus for plants by Materechera et al. [50] and Dróżdż et al. [51], while according to Kwiatkowski et al. [52], bovine manure and spent mushroom substrate can also be important sources of phosphorus. The present study also showed that phosphorus uptake by test plants was significantly correlated with the yield and content of this element (Table 7).

The efficiency of nutrient utilization from fertilizers is a measure of their value as fertilizers. Nutrient utilization from fertilizers usually does not exceed 40% of their total content, which is a serious economic and environmental problem. Increasing the application of fertilizer entails additional costs and also the risk of contaminating the environment [53,54]. The efficiency of phosphorus utilization is usually low because plants can take up only about 20% of the amount applied to the soil in fertilizer [15,55]. According to Etesami [56], 75–90% of phosphorus applied as fertilizer is bound by ions of calcium, magnesium, iron, and aluminum into insoluble compounds that are not available for plants. In our own studies, phosphorus use efficiency values higher than 20% were obtained only after the use of bovine manure in combination with zinc was administered in doses of 200 and 400 mg kg^{-1} (Table 6). Generally, after the introduction of 600 mg kg^{-1} of zinc along with all organic fertilizers, lower phosphorus use efficiency was obtained than after the application of 200 and 400 mg kg^{-1} of zinc.

Due to the lack of a significant influence of different doses of phosphorus on its content in cocksfoot, relatively small differences in its uptake after the application of various organic fertilizers (the difference between the highest and the lowest uptake equal to 12%), and different amounts of phosphorus applied to the soil, resulted in a significant diversification of its use efficiency.

5. Conclusions

Zinc application at 200, 400, and 600 mg·kg^{-1} of soil did not differentiate phosphorus content in the biomass of cocksfoot, while at the same time, it influenced its uptake. The most phosphorus uptake by plants was noted following zinc application of 200 mg·kg^{-1} of soil, slightly less following application of 400 mg·kg^{-1}, and the least following application of 600 mg ·kg^{-1} of soil. The absence of significant relationships between the content of zinc and phosphorus in the plants indicates no interactions between these elements. The organic fertilizers did not influence phosphorus content in the grass, but they did increase its uptake. The most phosphorus was accumulated following the application of chicken manure and bovine manure, but least after mushroom substrate application. The highest coefficient of phosphorus utilization from organic fertilizers was noted following the application of zinc at 400 mg·kg^{-1} of soil, and the lowest following application of 600 mg·kg^{-1} of soil and in the control treatment. Among the organic fertilizers tested, the phosphorus utilization coefficient was highest for bovine manure and much smaller after mushroom substrate and chicken manure application. In the three-year experiment as a whole, the more phosphorus content introduced into the soil with organic fertilizers, the lower its use efficiency.

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