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Yielding and Bioaccumulation of Zinc by Cocksfoot under Conditions of Different Doses of This Metal and Organic Fertilization

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Abstract: Zinc is essential for the growth and development of plants, but in excessive amounts in the soil it can be toxic for them. Its mobility depends in part on the organic matter content of the soil. The aim of the study was to investigate the effect of application of increasing amounts of zinc (200, 400 and 600 mg $Zn \cdot kg^{-1}$ of soil) together with various forms of organic fertilizer (cattle manure, chicken manure and spent mushroom substrate) on the yield of cocksfoot and the content and uptake of this metal, and to determine its bioaccumulation factor and tolerance indices. A minor effect of zinc on cocksfoot, expressed as a decrease in yield, was observed following the application of 400 mg $Zn \cdot kg^{-1}$ of soil. Increasing application to 600 mg $Zn \cdot kg^{-1}$ caused a significant decrease in yield. Application of 400 and 600 mg $Zn \cdot kg^{-1}$ of soil significantly reduced the value of the Zn/Org tolerance index. Increasing levels of zinc application increased its content and uptake by cocksfoot and reduced the bioaccumulation factor in the plants. All of the organic materials applied increased the yield of cocksfoot and its uptake of zinc. Spent mushroom substrate increased the Zn/Org tolerance index, while cattle manure and chicken manure increased the Org/Zn tolerance index, which confirms their protective action against high zinc content in the soil.

Keywords: bioaccumulation factor; cattle manure; chicken manure; *Dactylis glomerata* L.; mushroom substrate; tolerance index; zinc

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1. Introduction

Human activity is causing an increase in the content of heavy metals in the environment (soil, water and air), posing a threat to living organisms [1–7]. These elements are neither removed from the environment nor degraded, unlike other pollutants, which can be degraded by either chemical or biological means [8]. Excessive content of heavy metals in the soil, especially in bioavailable forms, increases their uptake and causes them to accumulate in plants. This negatively affects germination, root growth, the development of above-ground organs, biomass production, and transpiration. Heavy metals also disturb control mechanisms at the gene level, inhibit the activity of enzymatic proteins, impair the functioning of metabolic pathways, and contribute to apoptosis [9]. Zinc is a distinctive heavy metal that is essential for plants and animals in low concentrations, but becomes toxic for them above a critical concentration [10]. Its content in the soil depends mainly on its quantity in the bedrock and on anthropogenic factors [11]. Zinc is present in the soil solution, adsorbed on various minerals, e.g., clay minerals, oxides or carbonates, or bound to organic matter [12,13]. Soils with insufficient levels of zinc are also common throughout the world [14,15]. Zinc is an essential micronutrient for plants, in which it takes part in chlorophyll biosynthesis and gene expression and is a cofactor of many enzymes [16–20]. It is a component of carbonic anhydrase and a stimulator of aldolase, enzymes which take part in carbon metabolism [21]. It also influences the capacity for water uptake and transport, reduces the unfavourable effects of short periods of high temperatures and salt stress [22], and increases the resistance of plants to fungal diseases [23,24]. Stress associated with zinc deficiency in plants, caused by its low bioavailability, reduces productivity and the nutritional quality of food [25]. In the case of high content in the soil, however, zinc can be toxic for plants, and plants that have taken up excessive zinc exhibit symptoms similar to those occurring in the case of other toxic heavy metals, such as Cd or Pb [26]. In most cases, excessive Zn generates reactive oxygen species and displaces other metals from active sites in proteins. Its toxicity is manifested in part by impaired seed germination, limited growth, and chlorotic and necrotic changes on the leaves [27]. The mobility of zinc and thus its toxicity for plants can be reduced by increasing the content of organic matter in the soil. Its sources are organic fertilizers and organic waste materials used as fertilizers [28]. They then form mineral–organic complexes (chelates), thereby reducing the bioavailability of zinc for plants [3].

The aim of the study was to determine the effect of increasing levels of zinc application in combination with additional various organic fertilizers on the yield of cocksfoot and the content and uptake of the metal, as well as to determine the bioaccumulation factor and tolerance indices.

The research hypothesis was that the application of all organic substances to the soil would increase the tolerance of cocksfoot to increasing levels of zinc, reducing the accumulation of this heavy metal in the harvested biomass.

2. Materials and Methods

2.1. Experimental Design

A three-year pot experiment was carried out in a greenhouse in 2014–2016. Pots with a capacity of 10 dm^3 were filled with 12 kg of Luvisol soil consisting of 71% sand, 24% silt, and 5% clay. The experiment was set up in a completely randomized design in triplicate, including two factors:

I—zinc application rate: control—no zinc application (0) and 200, 400 and 600 mg $Zn \cdot kg^{-1}$ of soil. Zinc was applied to the soil once, before sowing of the test plant, only in the first year of the study, in the form of an aqueous solution of $ZnSO_4 \cdot 5H_2O$. Zinc was not applied to the soil in subsequent years of the study.

II—organic fertilization: control—no application of organic materials (CO) and application of cattle manure (CM), chicken manure (ChM) and spent mushroom substrate (MS). Organic fertilizers were applied separately, once, in the first year of the study, two weeks before sowing of the test plant, at a rate of 2 g $Corg \cdot kg^{-1}$ of soil. In all years of the study the test plant was the Amera cultivar of cocksfoot (*Dactylis glomerata* L.), which was sown every year in the first ten days of May. The aerial parts of the plants were harvested four times each year at 30-day intervals.

2.2. Laboratory Analyses

Selected properties of the organic materials are presented in Table 1. The following were determined in samples of each of the organic materials: dry matter (DM at 105 °C), organic carbon by the Tyurin method, total nitrogen content by the CHNS method (CHN Autoanalyser with IDC detector, Series II 2400, Perkin-Elmer, Valencia, CA, USA), and the content of P, K, C, Mg, S and Zn by the ICP-AES method (Optima 3200 RL spectrometer, Perkin-Elmer, Waltham, MA, USA) after dry mineralization of the samples at 500 °C. The 6.65 pH soil on which the pot experiment was carried out contained 1.52 $g \cdot kg^{-1}$ total nitrogen, 16.40 $g \cdot kg^{-1}$ organic carbon, 176 $mg \cdot kg^{-1}$ P and 108 $mg \cdot kg^{-1}$ K in available forms for plants (determined by the Egner–Riehm method), and 56.6 $mg \cdot kg^{-1}$ total Zn. The following were determined in the soil: pH (potentiometrically in 1 $mol \cdot dm^3$ KCl), organic carbon by the Tyurin method, total nitrogen content by the CHNS method, and total Zn by ACP-AES, following wet mineralization of the material in a mixture of concentrated HCl and HNO_3 (3:1 radio).

Table 1. Chemical composition of organic materials used in pot experiment.

Organic Materials	Dry Matter DM (%)	C_{org}	N_{tot}	C:N	P	K	Ca	Mg	S	Zn	Cu	Pb	Cd	Cr	Ni
		$g \cdot kg^{-1}$ DM			$g \cdot kg^{-1}$ DM					$mg \cdot kg^{-1}$ DM					
Cattle manure	19.6	405.1	23.90	16.9:1	5.38	15.28	10.04	2.90	3.07	60.28	4.98	7.48	0.248	8.32	11.16
Chicken manure (layers)	27.8	167.3	13.50	12.4:1	8.44	9.32	13.72	2.68	3.12	190.80	41.60	11.65	0.412	4.96	32.14
Mushroom substrate	30.4	319.3	24.20	13.2:1	6.22	17.48	47.32	3.12	25.08	117.50	14.58	2.97	0.072	2.19	11.12

Dry matter yield was determined after drying at 75 °C. Zinc content in the plant material was determined by inductively coupled plasma atomic emission spectrometry (ICP-AES) following dry mineralization at 450 °C and dissolution in a 10% HCl solution.

2.3. Calculations

The results were used to calculate the zinc bioaccumulation factor [29,30] and tolerance indices [31], using the following mathematical formulas:

$$BA_{Zn} = Zn_{plant}/Zn_{soil} \tag{1}$$

where:

BA_{Zn}—zinc bioaccumulation factor
Zn_{plant}—zinc content in cocksfoot
Zn_{soil}—total zinc content in soil

$$TIo_{rg/Zn} = Y_{org}/Y_{0\_org} \tag{2}$$

where:

$TIo_{rg/Zn}$—tolerance index illustrating the effect of various organic fertilizers in combination with increasing levels of zinc
Y_{org}—weight (yield) of cocksfoot fertilized with a given organic material obtained following application of 0, 200, 400 and 600 mg $Zn \cdot kg^{-1}$ of soil
Y_{0_org}—weight (yield) of cocksfoot obtained without application of organic fertilizer following application of 0, 200, 400 and 600 mg $Zn \cdot kg^{-1}$ of soil
where:

$$TI_{Zn/Org} = Y_{Zn}/Y_{0\_Zn} \tag{3}$$

$TI_{Zn/Org}$—zinc tolerance index illustrating the effect of increasing levels of zinc in combination with application of various organic fertilizers
Y_{Zn}—weight (yield) of cocksfoot fertilized with a given amount of zinc obtained in the control treatment without organic fertilizers and following application of CM, ChM and MS
Y_{0_Zn}—weight (yield) of cocksfoot without zinc application obtained in the control treatment without organic fertilizers and following application of CM, ChM and MS

$$Zn_{up} = Y \times Zn_{plant} \tag{4}$$

where:

Zn_{up}—Zn accumulation in cocksfoot dry matter (uptake by cocksfoot)
Y—yield of cocksfoot
Zn_{plant}—Zn content in cocksfoot DM

2.4. Statistical Analyses

The results were analysed by analysis of variance using with the Fisher–Snedecor distribution, and LSD values at a significance level of $\alpha = 0.05$ were calculated by the Tukey test. Statistica 13 PL software (13.1 version, StatSoft, Tulsa, OK, USA) was used for the calculations. In addition, Pearson's linear correlation coefficient was calculated for some of the examined traits.

3. Results

Application of increasing amounts of zinc (0, 200, 400 and 600 mg $Zn \cdot kg^{-1}$ of soil) and various organic fertilizers (cattle manure, chicken manure and spent mushroom substrate) significantly affected the yield of cocksfoot, the tolerance indices, zinc content and uptake by cocksfoot, and the bioaccumulation factor of the metal (Tables 2–8).

Table 2. Cocksfoot yield (g·pot^{-1}).

Organic Fertilizer	Year	Zn Dose [mg·kg^{-1} of Soil]				Mean
		0	200	400	600	
Without Organic Fertilization	1st	15.20	18.09	12.94	11.56	14.49A
	2nd	12.44	11.11	9.05	12.65	11.31A
	3rd	9.15	8.36	7.46	8.08	8.26A
	mean	12.26BC	12.52C	9.82A	10.76AB	11.34a
Cattle manure	1st	25.40	29.40	26.55	21.10	25.61C
	2nd	14.50	15.28	12.84	12.56	13.80B
	3rd	12.36	11.98	10.56	11.65	11.64B
	mean	17.42BC	18.89C	16.65AB	15.10A	17.02c
Chicken manure	1st	26.78	28.60	26.54	22.92	26.21C
	2nd	16.25	16.82	15.10	14.78	15.74C
	3rd	10.15	11.64	8.65	9.48	9.98A
	mean	17.73BC	19.02C	16.76AB	15.73A	17.31c
Mushroom substrate	1st	21.50	25.23	21.88	17.56	21.54B
	2nd	13.06	11.33	16.06	14.38	13.71B
	3rd	10.49	11.17	9.96	10.22	10.46AB
	mean	15.02AB	15.91B	15.97B	14.05A	15.24b
Mean for Zn dose		15.61b	16.58c	14.80b	13.91a	15.23
Mean for years	1st	22.22B	25.33C	21.98B	18.29A	21.95c
	2nd	14.06A	13.64A	13.26A	13.59A	13.64b
	3rd	10.54AB	10.79B	9.16A	9.86AB	10.09a

a,b,c—means for investigated factors with different letters (in the columns for organics fertilization and for years but in the row for zinc doses) are significantly different. A,B,C—means for the interaction with different letters in the rows of the table are significantly different.

Table 3. Zinc tolerance index illustrating the effect of increasing levels of zinc in combi-nation with application of various organic fertilizers—Zn/Org.

Organic Fertilizer	Year	Zn Dose [mg·kg^{-1} of Soil]			Mean
		200	400	600	
Without organic fertilization	1st	1.19	0.85	0.76	0.93
	2nd	0.90	0.74	1.03	0.89
	3rd	0.92	0.82	0.90	0.88
	mean	1.00	0.81	0.90	0.90a
Cattle manure	1st	1.16	1.05	0.83	1.01
	2nd	1.07	0.90	0.89	0.95
	3rd	0.99	0.86	0.95	0.93
	mean	1.07	0.93	0.89	0.96ab
Chicken manure	1st	1.07	0.99	0.86	0.97
	2nd	1.04	0.94	0.91	0.96
	3rd	1.17	0.88	0.94	1.00
	mean	1.10	0.94	0.91	0.98ab
Mushroom substrate	1st	1.17	1.02	0.82	1.00
	2nd	0.88	1.25	1.11	1.08
	3rd	1.07	0.96	0.98	1.00
	mean	1.04	1.08	0.97	1.03b

Table 3. *Cont.*

Organic Fertilizer	Year	Zn Dose [mg·kg^{-1} of Soil]			Mean
		200	400	600	
Mean for Zn dose		1.05b	0.94a	0.92a	0.97
Mean for years	1st	1.15C	0.98B	0.82A	0.98a
	2nd	0.97A	0.96A	0.99A	0.97a
	3rd	1.04B	0.88A	0.94AB	0.95a

a,b—means for investigated factors with different letters (in the columns for organics fertilization and for years but in the row for zinc doses) are significantly different. A,B,C—means for the interaction with different letters in the rows of the table are significantly different.

Table 4. Tolerance index illustrating the effect of various organic fertilizers in combination with increasing levels of zinc—Org/Zn.

Organic Fertilizer	Year	Zn Dose [mg·kg^{-1} of Soil]				Mean
		0	200	400	600	
Cattle manure	1st	1.67	1.63	2.06	1.82	1.80B
	2nd	1.17	1.39	1.44	0.99	1.25A
	3rd	1.35	1.48	1.42	1.46	1.43B
	mean	1.40	1.50	1.64	1.43	1.49b
Chicken manure	1st	1.77	1.59	2.06	1.99	1.85B
	2nd	1.33	1.52	1.68	1.17	1.42A
	3rd	1.13	1.42	1.16	1.18	1.22A
	mean	1.41	1.51	1.63	1.44	1.50b
Mushroom substrate	1st	1.42	1.40	1.69	1.52	1.51A
	2nd	1.07	1.02	1.81	1.15	1.26A
	3rd	1.15	1.36	1.35	1.28	1.29A
	mean	1.21	1.26	1.62	1.32	1.35a
Mean for Zn dose		1.34a	1.42a	1.63b	1.40a	1.45
Mean for years	1st	1.62A	1.54A	1.94B	1.78AB	1.72b
	2nd	1.19A	1.31A	1.64B	1.10A	1.31a
	3rd	1.21A	1.42A	1.31A	1.31A	1.31a

a,b—means for investigated factors with different letters (in the columns for organics fertilization and for years but in the row for zinc doses) are significantly different. A,B—means for the interaction with different letters in the rows of the table are significantly different.

Table 5. The content of zinc in cocksfoot (mg Zn kg^{-1} DM).

Organic Fertilizer	Year	Zn Dose Introduced into the Soil [mg·kg^{-1} of Soil]				Mean
		0	200	400	600	
Without Organic Fertilization	1st	35.26	117.85	140.85	190.88	121.21
	2nd	38.63	95.80	120.60	155.10	102.53
	3rd	30.35	48.16	63.90	81.04	55.86
	mean	34.75	87.27	108.45	142.34	93.20c
Cattle Manure	1st	35.98	92.14	110.86	146.20	96.30
	2nd	39.55	82.60	94.80	112.40	82.34
	3rd	30.86	39.82	51.46	60.48	45.66
	mean	35.46	71.52	85.71	106.36	74.76a

Table 5. *Cont.*

Organic Fertilizer	Year	Zn Dose Introduced into the Soil [mg·kg^{-1} of Soil]				Mean
		0	200	400	600	
Chicken Manure	1st	38.14	106.20	128.14	162.50	108.75
	2nd	41.50	91.46	106.42	132.51	92.97
	3rd	33.40	46.98	58.40	76.84	53.91
	mean	37.68	81.55	97.65	123.95	85.21b
Mushroom Substrate	1st	37.74	95.89	118.17	154.55	101.59
	2nd	46.31	86.43	98.35	119.61	87.68
	3rd	31.25	43.31	52.08	65.33	47.99
	mean	38.43	75.21	89.53	113.16	79.09a
Mean for Zn Dose		36.58a	78.89b	95.34c	121.45d	83.06
Mean for Years	1st	36.78	103.02	124.51	163.53	106.96c
	2nd	41.50	89.07	105.04	129.90	91.38b
	3rd	31.47	44.57	56.40	70.92	50.85a

a,b,c—means for investigated factors with different letters (in the columns for organics fertilization and for years but in the row for zinc doses) are significantly different.

Table 6. Zn uptake by cocksfoot (mg Zn·pot^{-1}).

Organic Fertilizer	Year	Zn Dose [mg·kg^{-1} of Soil]				Mean
		0	200	400	600	
Without Organic Fertilization	1st	0.535	2.137	1.818	2.211	1.675A
	2nd	0.482	1.062	1.098	1.972	1.154A
	3rd	0.281	0.402	0.478	0.653	0.454A
	mean	0.432A	1.200B	1.131B	1.612C	1.094a
Cattle Manure	1st	0.915	2.717	2.946	3.067	2.411C
	2nd	0.570	1.257	1.224	1.436	1.122A
	3rd	0.383	0.479	0.546	0.709	0.529A
	mean	0.623A	1.484B	1.572BC	1.737C	1.354b
Chicken manure	1st	1.024	3.028	3.403	3.719	2.793D
	2nd	0.679	1.539	1.596	1.956	1.442B
	3rd	0.337	0.548	0.507	0.728	0.530A
	mean	0.680A	1.705B	1.835B	2.134C	1.588c
Mushroom substrate	1st	0.810	2.415	2.577	2.708	2.127B
	2nd	0.606	0.976	1.575	1.715	1.218A
	3rd	0.327	0.483	0.517	0.668	0.498A
	mean	0.581A	1.291B	1.556C	1.697C	1.281b
Mean for Zn dose		0.579a	1.420b	1.524b	1.785c	1.329
Mean for years	1st	0.821A	2.574B	2.686B	2.926C	2.252c
	2nd	0.584A	1.209B	1.373B	1.770C	1.234a
	3rd	0.332A	0.478A	0.512AB	0.689B	0.530b

a,b,c—means for investigated factors with different letters (in the columns for organics fertilization and for years but in the row for zinc doses) are significantly different. A,B,C,D—means for the interaction with different letters in the rows of the table are significantly different.

Table 7. Total Zn uptake by cocksfoot at the sum for three years (mg Zn·pot^{-1}).

Organic Fertilizer	Zn Dose [mg·kg^{-1} of Soil]				Mean
	0	200	400	600	
Without organic fertilization	1.297A	3.601B	3.391B	4.837C	3.282a
Cattle manure	1.863A	4.453B	4.716BC	5.211C	4.062b
Chicken manure	2.040A	5.115B	5.505B	6.402C	4.765c
Mushroom substrate	1.742A	3.874B	4.669C	5.091C	3.844b
Mean	1.737a	4.263b	4.571b	5.385c	3.988

a,b,c—means for investigated factors with different letters (in the columns for organics fertilization and for years but in the row for zinc doses) are significantly different. A,B,C—means for the interaction with different letters in the rows of the table are significantly different.

Table 8. Zinc bioaccumulation factor in cocksfoot.

Organic Fertilizer	Year	Zn Dose [mg·kg^{-1} of Soil]				Mean
		0	200	400	600	
Without Organic Fertilization	1st	0.623	0.459	0.308	0.291	0.420
	2nd	0.683	0.373	0.364	0.236	0.389
	3rd	0.536	0.188	0.140	0.123	0.247
	mean	0.614	0.340	0.238	0.217	0.352b
Cattle Manure	1st	0.632	0.359	0.243	0.223	0.364
	2nd	0.695	0.322	0.207	0.171	0.349
	3rd	0.542	0.155	0.113	0.092	0.226
	mean	0.623	0.278	0.188	0.162	0.313a
Chicken Manure	1st	0.648	0.410	0.279	0.247	0.396
	2nd	0.705	0.353	0.232	0.201	0.373
	3rd	0.567	0.121	0.127	0.117	0.248
	mean	0.640	0.315	0.213	0.188	0.339b
Mushroom Substrate	1st	0.658	0.373	0.258	0.235	0.381
	2nd	0.808	0.336	0.215	0.182	0.385
	3rd	0.545	0.168	0.114	0.099	0.232
	mean	0.670	0.292	0.196	0.172	0.333ab
Mean for Zn Dose		0.637d	0.306c	0.208b	0.185a	0.334
Mean for Years	1st	0.640	0.400	0.272	0.249	0.390b
	2nd	0.723	0.346	0.230	0.198	0.374b
	3rd	0.548	0.173	0.123	0.108	0.238a

a,b,c,d—means for investigated factors with different letters (in the columns for organics fertilization and for years but in the row for zinc doses) are significantly different.

Significantly the highest yield of cocksfoot was harvested following application of 200 mg Zn·kg^{-1} of soil. It was greater than the yield of plants that were not fertilized with zinc and those fertilized with 400 and 600 mg Zn·kg^{-1} of soil, by 6.2%, 12.0% and 19.2%, respectively.

Application of 600 mg Zn·kg^{-1} significantly reduced yield by 10.9% in comparison with the control treatment and by 16.1% and 6.0% compared to the yield following application of 200 and 400 mg Zn·kg^{-1} of soil. Yield was not significantly affected by application of 400 mg Zn·kg^{-1} of soil, but it was somewhat higher than in the control treatment.

All of the organic materials increased the yield of cocksfoot. Significantly the highest yields were obtained following application of chicken manure and cattle manure. They were

52.6% and 50.0% higher, respectively, than the yield obtained from the control treatment and 13.6% and 11.7% greater than the yield following application of spent mushroom substrate.

The cocksfoot yield decreased in successive years of the study. In the second and third year it was 62.1% and 46.0% of the yield in the first year.

Application of organic fertilizers, irrespective of their origin, did not reduce the negative impact of application of 600 mg Zn·kg^{-1} of soil on the yield of the grass. The negative effect of this amount of zinc on the yield of the plant was also shown to be significant only in the first year after its application.

The effect of various amounts of zinc and organic materials in the soil on the yield of cocksfoot was confirmed by the tolerance indices—the effect of increasing application of zinc in combination with various organic fertilizers (Zn/Org) and the effect of various organic fertilizers in combination with increasing application of zinc (Org/Zn) (Tables 3 and 4). Values of these indices smaller or greater than 1 indicate that the effect of the factor on plants is negative or positive, respectively, while values close to 1 indicate a lack of effect.

Application of 400 and 600 mg Zn·kg^{-1} of soil significantly reduced the Zn/Org tolerance index in the first and third year of cocksfoot cultivation. This index was increased by spent mushroom substrate. The Zn/Org tolerance index was not found to be significantly influenced by the application of cattle manure or chicken manure, nor did it differ in different years of the study.

The Org/Zn tolerance index was higher following application of 400 mg Zn·kg^{-1} of soil than in the control treatment and after application of 200 and 600 mg Zn·kg^{-1} of soil. It was highest following the application of cattle manure and chicken manure, which confirms the positive effect of these fertilizers on cocksfoot, expressed as its yield. The Org/Zn tolerance index was the same in the second and third years of the study, but significantly lower than in the first year.

Application of increasing amounts of zinc increased its content in the cocksfoot biomass (Table 5). Significantly the highest content of the metal was found in the plants harvested following application of 600 mg Zn·kg^{-1} of soil. It was 232.0%, 53.9% and 27.4% higher, respectively, than the content in plants from the control treatment and those fertilized with 200 and 400 mg Zn·kg^{-1} of soil. Application of all organic materials decreased the content of zinc in the test plant. Significantly the lowest content of zinc was noted following application of cattle manure and spent mushroom substrate. In successive years of the study, the zinc content in the biomass of cocksfoot decreased. In the second and third year it was 14.6% and 52.5% lower, respectively, than in the first year. All application rates of zinc increased its uptake by cocksfoot, calculated as the average from the three years of the study 3 (Table 6), as well as the total uptake in the three-year cycle (Table 7). On average in the three-year cycle, following application of 200, 400 and 600 mg Zn·kg^{-1} of soil, the plants accumulated 145.3%, 163.2% and 208.3% more of this metal than plants from the control treatment. Total zinc uptake during the three years of the study was highest following application of 600 mg Zn·kg^{-1} of soil. Following application of cattle manure, chicken manure and spent mushroom substrate, zinc uptake by cocksfoot was higher than in the control treatment. The most zinc was accumulated by plants fertilized with chicken manure. Zinc uptake decreased in successive years of the study, and in the second and third year of the study it was only 54.8% and 23.5% as high as in the first year.

In addition, the effect of increasing application of zinc and application of organic fertilizers on zinc accumulation in the biomass of the grass was shown to vary in successive years of the study. In the third year of the study, zinc uptake by plants was similar following application of 400 and 600 mg Zn·kg^{-1} of soil. In this year of the study, the uptake of zinc after the application of 600 mg of Zn was higher than in the control object and after the application of 200 mg of Zn. In the third year of the study, application of various organic fertilizers had no effect on the accumulation of zinc in cocksfoot.

Application of increasing amounts of zinc decreased its bioaccumulation factor in the test plant (Table 8). It was significantly the lowest for the plants grown following application of 600 mg Zn·kg^{-1} of soil. The bioaccumulation factor of zinc in cocksfoot was not affected by chicken manure or spent mushroom substrate, but was reduced by the application of cattle manure.

The bioaccumulation factor of zinc in cocksfoot was similar in the first and second years of the study and did not exceed 1. In the third year, it was significantly lower.

Correlation analysis revealed significant relationships between the zinc application rate and its content in and uptake by cocksfoot. A significant correlation between zinc content in the plant and its accumulation was noted as well (Table 9).

Table 9. Linear correlation coefficients between selected properties of cocksfoot.

Specification	Cocksfood Yield	Zn Content	Zn Uptake
Zn Dose	−0.29	0.95 *	0.85 *
Cocksfood Yield	–	−0.39	0.14
Zn Content	–	–	0.84 *

* the value of correlation coefficient are important.

4. Discussion

The yield and chemical composition of plants depend not only on the soil content of macroelements but on that of microelements and trace elements as well [32–34]. Microelements perform very important physiological functions in plants, taking part in metabolism of proteins, carbohydrates, and sugars. They are also activators of enzymatic reactions. Important microelements for plant nutrition include zinc [35,36]. Deficiencies of this element are a major problem around the world, but in soil contaminated by mining and metallurgical activity, soil fertilized with wastewater sludge, and urban and suburban soils anthropogenically enriched with zinc, it can have toxic effects on plants [37–39]. In the present study, cocksfoot responded with a significant increase in yield to the application of 200 mg Zn·kg^{-1} of soil and with a small decrease following the application of 400 mg Zn·kg^{-1} of soil. A significant decrease in yield followed the application of 600 mg Zn·kg^{-1} of soil. The Zn/Org tolerance index was also reduced following the application of 400 and 600 mg Zn·kg^{-1} of soil. A stimulating effect of soil application of zinc in the form of $ZnSO_4 \times 7H_2O$ at rates of 15, 30 and 45 kg of fertilizer per ha^{-1} on maize yield was reported by Liu et al. [40]. However, these levels of application were much lower per 1 kg of soil than in the present study. Ryegrass yield reduction following zinc application was demonstrated by Zalewska [41]. In a pot experiment, the author tested zinc application rates from 25 to 400 mg Zn·kg^{-1} of soil and found that even the smallest dose decreased the yield of the grass when it was grown on sand, but in the case of cultivation on sandy clay only the highest application rate caused decreased yield. Baran [42] demonstrated that zinc had a negative effect on maize. In a pot experiment, the author applied zinc in the form of an aqueous solution of $ZnSO_4 \times 7H_2O$ in the amount of 0, 50, 250 and 750 mg Zn·kg^{-1} of soil and observed a decrease in maize yield following application of just 50 mg Zn·kg^{-1} of soil. Chaney [43] notes that zinc toxicity thresholds marked in leaves depend on the species and even the variety of the plant. No information describing the toxicity level of zinc in soil for grasses has been found in the scientific literature. In an experiment by Long et al. [44] the threshold of toxicity of this metal was 413 mg Zn·kg^{-1} of soil for Chinese cabbage (*Brassica chinensis* L.), 224 mg Zn·kg^{-1} of soil for pok choi (*Brassica chinensis* L.), and 272 mg Zn·kg^{-1} of soil for celery (*Apium graveolens* L.).

The bioavailability of zinc for plants depends in part on its total content in the soil, the amount and type of organic matter. High content of organic matter can limit zinc bioavailability due to adsorption by organic ligands [11]. At the same time, application of organic fertilizers increases soil fertility, which increases crop yield [45]. In the present study, all of the organic materials increased the yield of cocksfoot. The best effect on yield and the highest Org/Zn tolerance index were obtained following application of chicken manure and cattle manure, which may be linked to their chemical composition and the

C:N ratio. Varied effects of organic fertilizers depending on their chemical composition were obtained in fertilization of grassland by Štýbnarová et al. [46] and by Tong et al. [47].

Application of spent mushroom substrate increased the Zn/Org tolerance index, which may indicate a minor protective effect counteracting the negative effect of high levels of zinc on cocksfoot.

The content of zinc in grasses is an important indicator of the fodder value of hay. Both a deficiency and a surplus of zinc in feed adversely affect the health of animals, especially ruminants, which are among the most sensitive to this metal [48,49]. Zinc content above 100 mg $Zn \cdot kg^{-1}$ DW in feed can be harmful to animals due to various interactions.

As the amount of zinc applied to the soil was increased, its amount in the biomass of cocksfoot increased as well. In the first year of the study, the average content of the metal in the grass following its application, irrespective of the amount applied, was greater than 100 mg $Zn \cdot kg^{-1}$ DW. In the second year of the study, this was the case only following application of 400 and 600 mg $Zn \cdot kg^{-1}$ of soil. In the cocksfoot harvested in the third year of the study, the content of zinc did not exceed 100 mg $Zn \cdot kg^{-1}$ DW. An increase in zinc content in plants grown on soil contaminated with this metal was obtained by Mishra et al. [50]. Chaney [43] reports that symptoms of zinc toxicity most often appear at concentrations of 300 mg $Zn \cdot kg^{-1}$ DW in the aerial parts of plants, although some plants show symptoms of toxicity at concentrations of more than 100 $Zn \cdot kg^{-1}$ DW. According to Marschner [23], the zinc content in leaves exceeding the value of 300–600 mg $Zn \cdot kg^{-1}$ DM is a toxic amount for plants. Research by Broadley et al. [37] shows that toxicity symptoms often make themselves evident at leaf Zn concentrations higher than 300 $mg \cdot kg^{-1}$ DM. The physiology of Zn phytotoxicity in leaves is complicated, resulting from Zn interference in chlorophyll biosynthesis, and other biochemical reactions.

While none of the organic fertilizers used in the experiment mitigated the negative effect of 600 mg $Zn \cdot kg^{-1}$ of soil on the yield of cocksfoot, they did reduce its content of zinc. Its lowest content was noted following the application of cattle manure and spent mushroom substrate. This indicates potential binding and immobilization of zinc by organic matter applied to the soil together with the metal. Binding of zinc by organic matter has been reported by Fan et al. [51], who showed that soil organic matter is the main factor determining sequestration of heavy metals in the soil.

The efficiency of accumulation of heavy metals in plants can be assessed by means of the bioaccumulation factor [52]. The bioaccumulation factor (BAF) can be used to describe active transport of metals from the environment to plants and animals via metabolism [53]. According to Netty al. [54], a bioaccumulation factor of 1–10 indicates a hyperaccumulator plant, a value of 0.1–1 indicates a moderate accumulator plant, a value of 0.01–0.1 indicates a low accumulator plant, and a value of <0.01 indicates a non-accumulator plant. In the present study, the bioaccumulation factor of zinc in cocksfoot ranged from 0.092 to 0.808, which indicates moderate accumulation. In an experiment by Łukowski et al. [30], the bioaccumulation factor of zinc in fodder grasses ranged from 0.07 to 1.55. Baran and Wieczorek [55] obtained values for the factor ranging from 0.26 to 1.01 in monocotyledonous plants (on average 0.63) and from 0.41 to 0.89 in dicotyledonous plants (on average 0.83). In a study by Aladesanmi et al. [56], the bioaccumulation factor of zinc in maize grown on soil contaminated with this metal ranged from 0.011 to 0.99. Klatka et al. [29] found that the value of this index also depends on the species of plant. As the level of zinc application increased, its bioaccumulation factor in cocksfoot significantly decreased. This may indicate that plants have mechanisms counteracting excessive uptake of zinc. According to Emamverdian et al. [57], a key element of these mechanisms is chelation of zinc through the formation of a metal complex of phytochelatins or metallothionein at the intra- and intercellular levels. This is followed by the removal of zinc ions from susceptible sites or sequestration of the ligand-zinc complex in the vacuoles.

Cattle manure showed a protective effect against accumulation of zinc, decreasing its bioaccumulation in the grass. This confirms the hypothesis that the organic matter contained in it binds the metal [51].

5. Conclusions

Following soil application of zinc at 200, 400 and 600 mg Zn·kg^{-1} of soil, the lowest amount increased the yield of cocksfoot, while the highest level reduced it. The small reduction in yield following the application of 400 mg Zn·kg^{-1} of soil indicates a threshold level of zinc toxicity for cocksfoot. The minor and major negative effect of application of 400 and 600 mg Zn·kg^{-1} of soil, respectively, are confirmed by the Zn/Org tolerance indices. Spent mushroom substrate increased the Zn/Org tolerance index, while cattle manure and chicken manure increased the Org/Zn tolerance index, confirming both the positive effect of these fertilizers on yield and their protective effect against high levels of zinc.

Increasing zinc application rates increased its content in and uptake by the grass, while reducing the bioaccumulation factor of this heavy metal. All of the organic materials reduced the content of zinc in the biomass of the test plant and increased the total uptake. Cattle manure also reduced the bioaccumulation factor of zinc in the plants, which is indicative of its protective effect.

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