agronomy

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Supplementation of Organic Amendments Improve Yield and Adaptability by Reducing the Toxic Effect of Copper in Cocksfoot Grass (*Dactylis glomerata* L. Cv Amera)

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Abstract: Copper is an element necessary for the proper growth and development of plants, but when taken in excess amounts, it can be toxic. Its availability for plant can be reduced by using organic fertilizers or soil liming. The aim of the study was to investigate the effect of increasing doses of copper (100, 200, and 300 mg $Cu \cdot kg^{-1}$ of soil) application in combination with various organic amendments (cattle manure, chicken manure, and spent mushroom substrate) on the yield of cocksfoot and its content and uptake of this metal, and to determine its coefficient of bioaccumulation and tolerance indices. The toxic effect of copper manifested by significant decrease in the yield of the test plant was after the application of 300 $mg \cdot kg^{-1}$ of soil. Increasing doses of copper application increased its content and uptake by the test plant, while observing the decreasing bioaccumulation factor. All the soil amendments reduced the toxic effect of copper on cocksfoot. The most effective organic amendment in terms of yield and protective effects against high levels of copper was cattle manure, in the case of which the Org/Cu and Cu/Org tolerance indices were highest.

Keywords: bioaccumulation coefficient; copper content; heavy metals; micronutrient; organic amendments; tolerance index; uptake; yield

Citation: Kuziemska, B.; Trębicka, J.; Wysokinski, A.; Jaremko, D. Supplementation of Organic Amendments Improve Yield and Adaptability by Reducing the Toxic Effect of Copper in Cocksfoot Grass (*Dactylis glomerata* L. Cv Amera). *Agronomy* **2021**, *11*, 791. https://doi.org/10.3390/agronomy11040791

Academic Editor: Radim Vácha

Received: 9 March 2021
Accepted: 14 April 2021
Published: 16 April 2021

1. Introduction

Accumulation of heavy metals in the soil can potentially limit its function, cause toxicity for plants, and pose a threat to humans and animals [1–6]. The mobility of heavy metals and their availability for plants depend on soil factors such as pH, content of organic matter, grain size composition, content of iron and manganese oxides, and sorption capacity, as well as on the type of metal [7]. Industrial emissions and intensification of agriculture, particularly the use of pesticides, as well as waste materials, lead to pollution of the natural environment, including soil [8–10]. A particular risk is posed by the increasing levels of heavy metals, which as elements do not undergo biodegradation and can remain in ecosystems for many years [11]. The toxic effects of heavy metals on plants can be limited through the application of organic, calcium, and calcium + magnesium fertilizers, which reduce their mobility [12–14].

The content of copper in the environment changes rapidly [15–17]. The content and form of this heavy metal in the soil are primarily influenced by geological and climatic conditions, but also by industrial pollution and other anthropogenic sources, especially those associated with agricultural activity [18]. Copper is an essential element for the growth and development of plants, but is also potentially toxic [19]. It takes part in physiological processes and is an essential cofactor for numerous metalloproteins, but in excessive amounts it disturbs important processes, including electron transport [9,20,21]. Excessive uptake of copper by plants causes serious damage to metabolic pathways and disturbs photosynthesis and biosynthesis of chlorophyll, thereby reducing plant productivity [22].

Copper phytotoxicity for plants can be reduced by applying organic fertilizers. The element is then sorbed interchangeably, and its complexation or chelation takes place. The susceptibility of copper to the formation of complex bonds and chelates with organic matter depends on the structure of organic compounds and the pH of the solution. Organic matter rich in high-molecular humic acids causes permanent immobilization of copper, while low-molecular compounds cause complexation or chelation [2,23,24].

According to Cuske and Karczewska [23], lignite, biochar, and compost are most commonly used to remediate copper-contaminated soils, but there may also be side effects with increased solubility of copper compounds and availability to plants. The literature has not clearly presented the effects of other organic amendments on toxicity copper to plants.

In recent years, there has been an increase in poultry farming and mushroom growing in Poland, so there is a need to develop large amounts of chicken manure and mushrooms substrate rich in calcium carbonates. These fertilizers can not only be a valuable source of nutrients for plants, but can also reduce the phytotoxicity of heavy metals, including copper.

The aim of the study was to investigate the effect of increasing doses of copper (100, 200, and 300 mg $Cu \cdot kg^{-1}$ of soil) application in combination with various organic fertilizers (cattle manure, chicken manure, and spent mushroom substrate) on the yield of cocksfoot and its content and uptake of this metal, and to determine its coefficient of bioaccumulation and tolerance indices. The research hypothesis assumed that the applied organic fertilizers would reduce the accumulation of copper by cocksfoot grass and limit the toxic effect of its high doses.

2. Materials and Methods

2.1. Field Experiment and Laboratory Analyses

A pot experiment was conducted in a greenhouse in the years 2014–2016. Pots with a volume of 10 dm^3 were filled with 12 kg of Luvisols soil containing 71% sand, 24% silt, and 5% clay. The experiment was set up in a completely randomized design including two factors. First factor were copper application rates: no copper application—control (0) and 100, 200, and 300 mg $Cu \cdot kg^{-1}$ of soil. The element was applied to the soil a single time in the first year, prior to sowing of the test plant, in the form of an aqueous solution of $CuSO_4 \cdot 5H_2O$. Second factor were organic fertilizers: control—no application of organic amendment (CO), cattle manure (BM), chicken manure (ChM), and spent mushroom substrate (MS), each applied separately, a single time in the first year, two weeks before sowing of the test plant, at a rate of 2 g $Corg. \cdot kg^{-1}$ of soil. Selected parameters of the organic amendments are given in Table 1. The following were determined in each sample of organic amendments: 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, California, USA), and content of P, K, C, Mg, S, and Cu by the inductively coupled plasma atomic emission spectrometry (ICP-AES) method (Optima 3200 RL, Perkin-Elmer, Waltham, USA) following dry mineralization of samples at 500 °C. The soil used for the study, with pH 6.7, contained 1.48 $g \cdot kg^{-1}$ total nitrogen; $16.10 \cdot kg^{-1}$ organic carbon; 189 $mg \cdot kg^{-1}$ available phosphorus and 110 $mg \cdot kg^{-1}$ available potassium determined by the Egner-Riehm method; and 12.93 $mg \cdot kg^{-1}$ Cu total copper. 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 Cu by the ICP-AES method following prior wet mineralization of the material in a mixture of HCl and HNO_3 (in a 3:1 ratio).

In each year of the study, the 'Amera' variety of cocksfoot (*Dactylis glomerata* L.) was grown in all treatments. Seeds of the grass were sowed each year in the first 10 days of May. The aerial parts were harvested four times a year (four cuts), at 30-day intervals. The dry matter yield was determined after drying at 75 °C. Copper content in the plant material was determined by ICP-AES after prior dry mineralization in a muffle furnace at 450 °C and dissolution of the ash in 10% HCl solution.

Table 1. Some properties of organic amendments used in experiment.

Organic Amendments	Dry Matter, DM (%)	C_{org}	N_{tot}	C:N	P	K	Ca	Mg	S	Cu $mg \cdot kg^{-1}$ DM
		$g \cdot kg^{-1}$ DM			$g \cdot kg^{-1}$ DM					
Cattle manure	20.0	394.5	23.70	16.6:1	6.50	17.02	11.28	3.24	3.68	5.97
Chicken manure (layers)	29.0	160.3	14.10	11.4:1	8.74	9.10	13.59	2.43	3.07	42.98
Mushroom substrate	31.0	315.7	24.50	12.9:1	6.14	17.20	45.18	3.12	26.20	15.61

2.2. Calculations

In addition, the results were used to calculate the coefficient of bioaccumulation of copper [25] and the tolerance indices [26], according to the following formulas:

$$BC_{Cu} = Cu_{plant}/Cu_{soil} \quad (1)$$

where:

BC_{Cu}—bioaccumulation coefficient of copper
Cu_{plant}—copper content in plant
Cu_{soil}—total copper content in soil

$$TI_{Cu} = Y_{Cu} - Y_{0Cu} \quad (2)$$

where:

TI_{Cu}—tolerance index illustrating the effect of different copper doses on the tolerance of this heavy metal by cocksfoot
Y_{Cu}—weight (yield) of plants fertilized with different doses of copper, obtained in the control without organic amendment and following application with BM, ChM and MS
Y_{0Cu}—weight (yield) of plants without copper application, obtained in the control without organic amendment and following application with BM, ChM, and MS, respectively

$$TI_{SA} = Y_{SA} - Y_{0SA} \quad (3)$$

where:

TI_{SA}—tolerance index illustrating the effect of each soil amendment type on the tolerance of copper by cocksfoot
Y_{SA}—weight (yield) of plants on objects with different organic amendments, obtained in the control and following application of 100, 200, and 300 mg $Cu \cdot kg^{-1}$ of soil
Y_{0SA}—weight (yield) of plants on object without soil amendment, obtained in the control and following application of 100, 200, and 300 mg $Cu \cdot kg^{-1}$ of soil, respectively

2.3. Statistical Analyses

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

3. Results

Various levels of copper application and the application of organic amendments—cattle manure, chicken (layer hen) manure, and spent mushroom substrate—significantly influenced the yield of cocksfoot and its content and uptake of copper (Tables 2–5). Copper application in the amount of 300 $mg \cdot kg^{-1}$ of soil reduced yield by 6.1% relative to the control and by 8.8% and 6.7% in comparison to the treatments with application of 100 and

200 mg Cu·kg^{-1} of soil, respectively. All of the organic amendments applied increased the yield of cocksfoot.

Table 2. Cocksfoot yield, g·pot^{-1} (mean ± SD).

Organic Amendments	Year	Cu Dose [mg·kg^{-1} of Soil]				Mean
		0	100	200	300	
Without organic amendments	1st	15.67 ± 0.49	12.57 ± 0.31	10.90 ± 0.91	12.63 ± 0.19	12.97 ± 1.80 B
	2nd	12.92 ± 1.16	14.42 ± 0.70	13.14 ± 0.79	11.38 ± 0.50	13.04 ± 1.37 B
	3rd	9.05 ± 0.47	9.03 ± 0.23	8.42 ± 0.48	7.57 ± 0.33	8.52 ± 0.72 A
	mean	12.55 ± 2.82 B	12.01 ± 2.28 B	10.92 ± 2.18 A	10.53 ± 2.19 A	11.50 ± 2.52 a
Cattle manure	1st	24.97 ± 1.00	27.93 ± 2.29	24.43 ± 0.39	20.30 ± 0.96	24.41 ± 3.04 C
	2nd	13.46 ± 0.10	14.81 ± 0.32	14.94 ± 0.22	13.84 ± 0.73	14.26 ± 0.76 B
	3rd	11.88 ± 0.65	11.70 ± 0.64	14.26 ± 0.73	15.40 ± 0.25	13.31 ± 1.68 A
	mean	16.77 ± 5.88 A	18.15 ± 7.17 B	17.88 ± 4.67 B	16.51 ± 2.84 A	17.33 ± 5.43 c
Chicken manure	1st	27.00 ± 0.43	26.20 ± 1.06	27.83 ± 1.09	24.33 ± 0.63	26.34 ± 1.55 C
	2nd	14.94 ± 0.42	14.37 ± 0.48	14.20 ± 0.44	14.51 ± 0.38	14.50 ± 0.51 B
	3rd	10.33 ± 0.46	11.60 ± 0.57	10.23 ± 0.76	10.67 ± 0.81	10.71 ± 0.86 A
	mean	17.42 ± 7.04 A	17.39 ± 6.38 A	17.42 ± 7.58 A	16.50 ± 5.79 A	17.18 ± 6.74 c
Mushroom substrate	1st	22.57 ± 0.74	23.23 ± 0.54	23.93 ± 0.66	21.80 ± 1.16	22.88 ± 1.13 C
	2nd	14.45 ± 0.89	15.68 ± 0.21	15.04 ± 0.74	13.12 ± 0.58	14.60 ± 1.50 B
	3rd	10.72 ± 0.20	11.99 ± 0.92	11.44 ± 0.20	10.92 ± 0.49	11.27 ± 0.73 A
	mean	15.94 ± 4.98 A	16.97 ± 4.72 B	16.81 ± 5.28 B	15.28 ± 4.76 A	16.25 ± 4.99 b
Mean for Cu dose		15.67 ± 5.72 b	16.13 ± 5.98 b	15.76 ± 5.99 b	14.71 ± 4.83 a	15.56 ± 5.68
Mean for years	1st	22.55 ± 4.33 B	22.48 ± 6.11 B	21.78 ± 6.51 B	19.77 ± 4.44 A	21.64 ± 5.55 a
	2nd	13.96 ± 1.11 A	14.82 ± 0.70 B	14.41 ± 0.88 B	13.21 ± 1.30 A	14.10 ± 1.18 b
	3rd	10.49 ± 1.12 A	11.08 ± 1.35 A	11.09 ± 2.20 A	11.14 ± 2.84 A	10.95 ± 2.02 c

a,b,c—means for investigated factors with different letters (in the columns for organics amendments and for ears but in the row for cooper doses) are significantly different. A,B,C—means for the interaction of the studied factors with uppercase different letters in the rows and in the column 'Mean' of the table are significantly different.

Table 3. Copper content in cocksfoot, mg Cu·kg^{-1} DM (mean ± SD).

Organic Amendments	Year	Cu Dose [mg·kg^{-1} of Soil]				Mean
		0	100	200	300	
Without organic amendments	1st	3.58 ± 0.46	6.66 ± 0.58	8.82 ± 0.64	10.92 ± 0.68	7.50 ± 2.78 A
	2nd	3.34 ± 0.46	5.45 ± 0.72	8.08 ± 0.70	9.21 ± 1.03	6.52 ± 2.41 A
	3rd	2.42 ± 0.13	4.01 ± 0.32	5.20 ± 0.68	5.85 ± 0.48	4.37 ± 1.38 A
	mean	3.11 ± 0.63 A	5.37 ± 1.22 B	7.37 ± 1.70 C	8.66 ± 2.24 D	6.13 ± 2.62 b
Cattle manure	1st	3.98 ± 0.34	5.82 ± 0.50	6.98 ± 0.77	8.12 ± 0.23	6.23 ± 1.61 A
	2nd	4.26 ± 0.49	5.43 ± 0.29	5.95 ± 0.71	7.63 ± 0.49	5.82 ± 1.32 A
	3rd	2.74 ± 0.28	2.98 ± 0.36	4.45 ± 0.18	5.12 ± 0.37	3.82 ± 1.04 A
	mean	3.66 ± 0.76 A	4.74 ± 1.32 B	5.79 ± 1.21 C	6.96 ± 1.37 D	5.29 ± 1.71 a
Chicken manure	1st	5.42 ± 0.35	6.38 ± 0.85	7.54 ± 0.64	9.12 ± 0.66	7.12 ± 1.53 A
	2nd	4.94 ± 0.44	5.70 ± 0.40	6.51 ± 0.49	8.86 ± 0.97	6.50 ± 1.59 A
	3rd	3.52 ± 0.34	4.14 ± 0.18	5.06 ± 0.24	5.45 ± 0.45	4.54 ± 0.82 A
	mean	4.63 ± 0.89 A	5.41 ± 1.09 A	6.37 ± 1.13 B	7.81 ± 1.82 C	6.05 ± 1.75 b
Mushroom substrate	1st	3.89 ± 0.24	5.78 ± 0.51	7.26 ± 0.87	8.98 ± 0.75	6.48 ± 1.98 A
	2nd	3.90 ± 0.48	4.74 ± 0.44	6.22 ± 0.44	7.82 ± 0.26	5.67 ± 1.55 A
	3rd	2.91 ± 0.41	3.27 ± 0.47	4.56 ± 0.44	5.48 ± 0.40	4.06 ± 1.11 A
	mean	3.57 ± 0.61 A	4.60 ± 1.13 B	6.01 ± 1.27 C	7.43 ± 1.54 D	5.40 ± 1.88 a
Mean for Cu dose		3.74 ± 0.92 a	5.03 ± 1.25 b	6.39 ± 1.47 c	7.71 ± 1.88 d	5.72 ± 2.06
Mean for years	1st	4.22 ± 0.79 A	6.16 ± 0.74 B	7.65 ± 1.02 C	9.29 ± 1.19 D	6.83 ± 2.10 c
	2nd	4.11 ± 0.75 A	5.33 ± 0.60 B	6.69 ± 1.02 C	8.38 ± 1.01 D	6.13 ± 1.81 b
	3rd	2.90 ± 0.51 A	3.60 ± 0.60 B	4.82 ± 0.54 C	5.48 ± 0.50 C	4.20 ± 1.14 a

a,b,c,d—means for investigated factors with different letters (in the columns for organics amendments and for ears but in the row for cooper doses) are significantly different. A,B,C,D—means for the interaction of the studied factors with uppercase different letters in the rows and in the column 'Mean' of the table are significantly different.

Table 4. Amount of copper uptake by cocksfoot, mg Cu·pot^{-1} (mean ± SD).

Organic Amendments	Year	Cu Dose [mg·kg^{-1} of Soil]				Mean
		0	100	200	300	
Without organic amendments	1st	0.056 ± 0.006	0.084 ± 0.007	0.096 ± 0.009	0.138 ± 0.010	0.093 ± 0.031 B
	2nd	0.043 ± 0.002	0.079 ± 0.013	0.109 ± 0.012	0.105 ± 0.010	0.084 ± 0.028 B
	3rd	0.022 ± 0.002	0.036 ± 0.004	0.044 ± 0.006	0.044 ± 0.003	0.037 ± 0.010 A
	mean	0.040 ± 0.015 A	0.066 ± 0.023 A	0.083 ± 0.030 A	0.096 ± 0.040 A	0.071 ± 0.035 a
Cattle manure	1st	0.099 ± 0.008	0.163 ± 0.023	0.171 ± 0.021	0.165 ± 0.009	0.149 ± 0.034 C
	2nd	0.057 ± 0.006	0.080 ± 0.006	0.089 ± 0.012	0.106 ± 0.012	0.083 ± 0.020 B
	3rd	0.033 ± 0.005	0.035 ± 0.006	0.063 ± 0.005	0.079 ± 0.005	0.053 ± 0.020 A
	mean	0.063 ± 0.46 A	0.093 ± 0.46 A	0.108 ± 0.46 A	0.117 ± 0.46 A	0.095 ± 0.46 b
Chicken manure	1st	0.146 ± 0.007	0.168 ± 0.029	0.209 ± 0.011	0.222 ± 0.012	0.186 ± 0.035 C
	2nd	0.074 ± 0.005	0.082 ± 0.003	0.092 ± 0.007	0.129 ± 0.016	0.094 ± 0.023 B
	3rd	0.036 ± 0.002	0.048 ± 0.003	0.052 ± 0.006	0.058 ± 0.009	0.049 ± 0.010 A
	mean	0.085 ± 0.046 A	0.099 ± 0.053 A	0.118 ± 0.067 A	0.136 ± 0.068 A	0.110 ± 0.062 c
Mushroom substrate	1st	0.088 ± 0.006	0.135 ± 0.015	0.174 ± 0.022	0.196 ± 0.022	0.148 ± 0.045 C
	2nd	0.057 ± 0.010	0.074 ± 0.006	0.094 ± 0.010	0.103 ± 0.007	0.082 ± 0.020 B
	3rd	0.031 ± 0.004	0.039 ± 0.003	0.052 ± 0.005	0.060 ± 0.007	0.046 ± 0.012 A
	mean	0.059 ± 0.024 A	0.083 ± 0.041 A	0.107 ± 0.052 A	0.120 ± 0.058 A	0.092 ± 0.051 b
Mean for Cu dose		0.062 ± 0.034 a	0.085 ± 0.047 b	0.104 ± 0.053 c	0.117 ± 0.054 d	0.092 ± 0.052
Mean for years	1st	0.097 ± 0.033 A	0.137 ± 0.039 B	0.162 ± 0.045 C	0.180 ± 0.034 D	0.144 ± 0.049 c
	2nd	0.058 ± 0.013 A	0.079 ± 0.008 B	0.096 ± 0.013 C	0.110 ± 0.016 D	0.086 ± 0.023 b
	3rd	0.031 ± 0.006 A	0.040 ± 0.007 AB	0.053 ± 0.009 BC	0.060 ± 0.014 C	0.046 ± 0.015 a

a,b,c—means for investigated factors with different letters (in the columns for organics amendments and for ears but in the row for cooper doses) are significantly different. A,B,C,D—means for the interaction of the studied factors with uppercase different letters in the rows and in the column 'Mean' of the table are significantly different.

Table 5. Total copper uptake by cocksfoot at the sum for three years, mg Cu·pot^{-1} (mean ± SD).

Organic Amendments	Cu Dose [mg·kg^{-1} of Soil]				Mean
	0	100	200	300	
Without organic amendments	0.121 ± 0.006	0.199 ± 0.021	0.248 ± 0.009	0.287 ± 0.004	0.214 ± 0.063 a
Cattle manure	0.189 ± 0.020	0.279 ± 0.028	0.323 ± 0.021	0.350 ± 0.006	0.285 ± 0.064 b
Chicken manure	0.256 ± 0.005	0.298 ± 0.025	0.354 ± 0.020	0.409 ± 0.021	0.329 ± 0.061 c
Mushroom substrate	0.176 ± 0.001	0.248 ± 0.013	0.320 ± 0.016	0.359 ± 0.028	0.275 ± 0.072 b
Mean	0.185 ± 0.049 a	0.25 ± 0.044 b	0.311 ± 0.042 c	0.351 ± 0.047 d	0.276 ± 0.077

a,b,c,d—means for investigated factors with different letters (in the columns for organics amendments and for ears but in the row for cooper doses) are significantly different. All interactions are not important.

Significantly the highest yield was obtained following application of cattle manure and chicken manure—50.7% and 49.4% higher than the yield from the control treatment and 6.7% and 5.7% higher than the yield obtained following application of spent mushroom substrate. The yield of the test plant decreased significantly in successive years of the study. The experiment also showed that the application of organic amendments, irrespective of their origin, limited the negative effect of copper on the yield of cocksfoot in all years of experiment.

The significant effect of application of increasing amounts of copper and varied organic fertilizers on the yield of cocksfoot was confirmed by the tolerance indices—the effect of increasing levels of copper following application of varied organic fertilizers (Cu/Org) (Table 6) and the effect of organic amendments following application of increasing levels of copper (Org/Cu) (Table 7). The tolerance index can have values less than, equal to, or more than 1. A value less than 1 indicates inhibition of plant growth; a value of 1 indicates that the factor has no influence; and a value greater than 1 indicates that the factor has a positive effect on the growth and development of the plants. Application of copper at rates

200 and 300 mg·kg^{-1} of soil decreased the Cu/Org tolerance index, while all the organic amendments increased it. The highest value for this index was noted for the plants from the treatments where cattle manure was applied. It increased in successive years of the study, which is indicative of the decreasing negative effect of copper on cocksfoot yield. It should also be noted that in the treatments without organic fertilizer and those with chicken manure and spent mushroom substrate, increasing levels of copper application caused a slight decrease in the Cu/Org tolerance index. Different relationships were observed in the case of cattle manure, where the index remained constant and greater than 1 irrespective of the level of copper application. The values obtained for the Org/Cu tolerance index show that it was increased by copper applied in the amount of 200 and 300 mg·kg^{-1} of soil. The plants fertilized with chicken manure and spent mushroom substrate had the same Org/Cu tolerance index, close to 1, while cattle manure significantly increased it, irrespective of the level of copper application. The highest value for the index was noted in the first year of research and the lowest in the last year, but in all years of the study it was greater than 1, which indicates that the organic fertilizers had a positive effect on cocksfoot yield.

Table 6. Cocksfoot tolerance index to copper depending on Cu doses (Cu/Org), calculated according to equation number 2.

Organic Amendments	**Year**	**Cu Dose [mg·kg^{-1} of Soil]**			**Mean**
		100	**200**	**300**	
Without organic amendments	1st	0.80	0.87	0.81	0.83 A
	2nd	1.12	0.93	0.89	0.98 B
	3rd	1.00	0.93	0.84	0.92 AB
	mean	0.98 A	0.91 A	0.85 A	0.91 a
Cattle manure	1st	1.12	0.98	0.81	0.94 A
	2nd	1.10	1.01	1.03	1.05 B
	3rd	0.99	1.22	1.30	1.17 C
	mean	1.07 A	1.04 A	1.05 A	1.05 c
Chicken manure	1st	0.97	1.06	0.90	0.98 A
	2nd	0.96	0.99	0.97	0.98 A
	3rd	1.12	0.89	1.04	1.02 A
	mean	1.02 A	0.98 A	0.97 A	0.99 b
Mushroom substrate	1st	1.03	1.03	0.97	1.01 A
	2nd	1.08	0.96	0.90	0.98 A
	3rd	1.12	0.96	1.02	1.03 A
	mean	1.08 A	0.98 A	0.96 A	1.01 bc
Mean for Cu dose		1.04b	0.98 a	0.96 a	0.99
Mean for years	1st	0.98 B	0.96 B	0.87 A	0.94 a
	2nd	1.07 B	0.97 A	0.95 A	1.00 b
	3rd	1.06 A	1.00 A	1.05 A	1.04 b

a,b,c—means for investigated factors with different letters (in the columns for organics amendments and for ears but in the row for cooper doses) are significantly different. A,B,C—means for the interaction of the studied factors with uppercase different letters in the rows and in the column 'Mean' of the table are significantly different.

Table 7. Cocksfoot tolerance index to copper depending on the soil amendment type (Org/Cu), calculated according to equation number 3.

Organic Amendments	Year	Cu Dose [mg·kg^{-1} of Soil]				Mean
		0	100	200	300	
Cattle manure	1st	1.60	2.22	2.26	1.61	1.92 C
	2nd	1.05	1.03	1.12	1.22	1.10 A
	3rd	1.31	1.30	1.70	2.04	1.59 B
	mean	1.32 A	1.52 B	1.69 C	1.62 BC	1.54 b
Chicken manure	1st	1.08	0.95	1.14	1.20	1.09 B
	2nd	1.11	0.97	0.95	1.05	1.02 B
	3rd	0.87	0.99	0.72	0.69	0.82 A
	mean	1.02 A	0.97 A	0.94 A	0.98 A	0.98 a
Mushroom substrate	1st	0.84	0.89	0.86	0.90	0.87 A
	2nd	0.98	1.09	1.06	0.90	1.01 B
	3rd	1.04	1.04	1.12	1.03	1.06 B
	mean	0.95 A	1.01 A	1.02 A	0.94 A	0.98 a
Mean for Cu dose		1.10a	1.16 ab	1.21 b	1.18 b	1.16
Mean for years	1st	1.17 A	1.35 B	1.42 B	1.24 A	1.30 c
	2nd	1.05 A	1.03 A	1.04 A	1.06 A	1.04 a
	3rd	1.08 A	1.11 A	1.18 AB	1.25 B	1.15 b

a,b,c—means for investigated factors with different letters (in the columns for organics amendments and for ears but in the row for cooper doses) are significantly different. A,B,C—means for the interaction of the studied factors with uppercase different letters in the rows and in the column 'Mean' of the table are significantly different.

Increasing levels of copper application caused an increase in its content in the biomass of cocksfoot (Table 3), as confirmed by the linear correlation coefficient (Table 8). Significantly the highest content of this metal was noted in the plants harvested following application of 300 mg Cu·kg^{-1} of soil. It was 106.2%, 53.3%, and 20.7% higher than the concentration in plants not fertilized with copper and following application of 100 and 200 mg Cu·kg^{-1} of soil, respectively. Application of cattle manure and spent mushroom substrate significantly decreased the copper content in the biomass of the test plant relative to the control. The content of the metal in the biomass of the grass decreased in successive years of the study.

Table 8. Linear correlation coefficient between Cu doses, cocksfoot yield, Cu content, and Cu uptake by cocksfoot.

Specification	Cocksfoot Yields	Cu Content	Cu Uptake
Cu dose	−0.065	0.747 *	0.405
Cocksfoot yield	-	0.260	0.819 *
Cu content	-	-	0.738 *

* the value of correlation coefficient are important, $p < 0.05$.

As the level of copper application increased, its uptake by cocksfoot, calculated as the mean from the three years of the study (Table 4), as well as the total uptake in the three-year cycle (Table 5), increased significantly. On average during the three-year cycle, following application of 100, 200, and 300 mg Cu·kg^{-1} of soil, the plants accumulated 38.4%, 68.1%, and 89.7% more of this metal, respectively, than the control plants. Total uptake of copper during the three years of the study was highest following application of 300 mg Cu·kg^{-1} of soil. Application of all organic amendments caused a significant increase in copper uptake by the grass. The values for the linear correlation coefficients indicate that this relationship is linked to both the yield obtained and the content of this metal in the biomass of the plants (Table 8). Significantly the most copper was accumulated in plants following application of chicken manure. The plants fertilized with cattle manure and spent mushroom substrate took up similar amounts of the metal. The amount was significantly greater than in the control plants, but significantly smaller than in the plants fertilized with chicken manure.

Uptake of copper by the grass significantly decreased in successive years of the study. In the second and third year it was 59.7% and 31.9%, respectively, of the value obtained in the first year.

The value for the coefficient of bioaccumulation of copper in the test plant depended on the copper application rate, organic fertilization, and the year of the study (Table 9). Higher copper application rates reduced the value of the coefficient, which is indicative of defense mechanisms against excessive uptake of this metal in the test plant. The value of this coefficient for plants following application of copper in the amount of 100 mg $Cu \cdot kg^{-1}$ of soil was significantly lower than for plants from the control and at the same time significantly higher than for plants fertilized with 200 and 300 mg $Cu \cdot kg^{-1}$ of soil. Cattle manure and spent mushroom substrate were not found to affect the coefficient of bioaccumulation, but application of chicken manure significantly increased it. In the first two years of the study, the average coefficient of bioaccumulation of copper remained constant, but in the third year it fell by 36.4%.

Table 9. Copper bioaccumulation factor in cocksfoot.

Organic Amendments	Year	Cu Dose [mg·kg^{-1} of Soil]				Mean
		0	100	200	300	
Without organic amendments	1st	0.28	0.06	0.04	0.04	0.10 A
	2nd	0.26	0.05	0.04	0.03	0.09 A
	3rd	0.19	0.04	0.02	0.02	0.07 A
	mean	0.24 C	0.05 B	0.03 A	0.03 A	0.09 a
Cattle manure	1st	0.31	0.05	0.03	0.03	0.10 A
	2nd	0.33	0.05	0.03	0.02	0.11 A
	3rd	0.21	0.03	0.02	0.02	0.07 A
	mean	0.28 C	0.04 B	0.03 AB	0.02 A	0.09 a
Chicken manure	1st	0.40	0.06	0.04	0.03	0.13 A
	2nd	0.37	0.05	0.03	0.03	0.12 A
	3rd	0.26	0.04	0.02	0.02	0.09 A
	mean	0.34 C	0.05 B	0.03 A	0.03 A	0.11 b
Mushroom substrate	1st	0.30	0.05	0.03	0.03	0.10 A
	2nd	0.30	0.04	0.03	0.03	0.10 A
	3rd	0.22	0.03	0.02	0.02	0.07 A
	mean	0.27 C	0.04 B	0.03 A	0.03 A	0.09 a
Mean for Cu dose		0.29 c	0.04 b	0.03 a	0.03 a	0.10
Mean for years	1st	0.32 C	0.05 B	0.04 AB	0.03 A	0.11 b
	2nd	0.31 C	0.05 B	0.03 A	0.03 A	0.11 b
	3rd	0.22 B	0.03 A	0.02 A	0.02 A	0.07 a

a,b,c—means for investigated factors with different letters (in the columns for organics amendments and for ears but in the row for cooper doses) are significantly different. A,B,C—means for the interaction of the studied factors with uppercase different letters in the rows and in the column 'Mean' of the table are significantly different.

4. Discussion

Copper is one of the micronutrients necessary for the growth and development of plants, but its excessive amount can be toxic. The total soil content of copper is affected by anthropogenic factors and by its content in the parent material of the soil. The availability of copper and other heavy metals for plants is determined by soil properties, including organic matter content, pH, sorption capacity, redox potential, and microbial activity [27,28]. In order to limit their mobility and stabilization in the soil environment, the soil is fertilized with calcium and calcium + magnesium fertilizers as well as with organic amendments, which promote sorption and immobilization [29]. Organic fertilizers play a special role as they exhibit the ability to permanently bind metals, and to complex and chelate them, and the direction of changes depends primarily on the quality of the organic matter contained in them. A common response of plants to stress associated with high copper content in the soil is inhibition of growth and a reduction in yield, and in extreme cases a complete loss of the crop [30–33]. In the present study, cocksfoot responded to application of this metal in the amount of 300 mg·kg^{-1} of soil with a significant reduction in yield. A decrease in the Cu/Org tolerance index was also noted following application of this metal at 200 and 300 mg Cu·kg^{-1} of soil. A toxic effect of copper on the growth and morphology of grass was reported by Sheldon and Menzies [34]. Ghani and Neheed [35]—in a pot experiment testing copper application rates from 0 to 50 mg·kg^{-1} of soil—showed that rates up to 30 mg Cu·kg^{-1} of soil significantly increased the yield of spring wheat, while higher rates of 40 and 50 mg Cu·kg^{-1} reduced it. In an experiment by Yang et al. [31], the threshold of copper toxicity depended on the species of plant and was 430 mg Cu·kg^{-1} of soil for pak choi, 608 mg Cu·kg^{-1} for celery, and 835 mg Cu·kg^{-1} for Chinese cabbage. Studies have confirmed that the tolerance of plants to increased copper content in the soil depends on the species and is species specific. For *Dactylis glomerata* copper toxicity threshold is 300 mg Cu·kg^{-1} of soil.

The varied mechanisms of tolerance of different plants to copper and other heavy metals is associated with processes limiting their transport and with detoxification processes on cell membranes and within the cell. [7] In the present study, application of organic amendments, irrespective of their origin, increased the yield of cocksfoot and the value of the Cu/Org tolerance index. The most beneficial effect on yield was obtained following application of cattle manure and chicken manure, which may be linked to their chemical composition and transformations in the soil. Moreover, cattle manure also caused an increase in the Org/Cu tolerance index, which confirms its beneficial effect on the test plant. Similar results were reported by Wiśniewska-Kadżajan and Jankowski [36], who tested the effect of cattle manure and spent mushroom substrate on cocksfoot yield and obtained better effects following the use of manure. Kalembasa et al. [37] also recorded an increase in the yield of *Dactylis glomerata* after the application of organic amendments with varied chemical composition and varied C:N ratio levels.

All the organic amendments used, especially cattle manure, reduced the toxic effects of copper on cocksfoot yield. As the level of copper introduced to the soil was increased, its amount in the grass increased as well. An increase in the content of copper in the biomass of test plants following various levels of soil application of this metal was noted by Sherrell and Rawnsley [38] and by Amin et al. [39]. Application of cattle manure and spent mushroom substrate significantly reduced the content of copper in the test plant, which may be associated with its immobilization in the soil. Matijevic et al. [40]), examining the impact of soil contamination with copper (250 and 500 mg Cu·kg^{-1} soil) on its accumulation in horse bean (*Vicia faba* L.), found that the complexation of this element by organic matter is an effective mechanism of its retention in soil, limiting bioavailability and accumulation in plants.

The mean coefficient of bioaccumulation of copper in the grass was 0.10. It was much lower than that obtained by Łukowski et al. [25], who reported a mean level of 0.80 in fodder grasses. This suggests that among the two mechanisms of copper uptake by plants, i.e., passive diffusion and active uptake influenced by the gradient of concentrations and powered by metabolic energy, the latter mechanism dominates in the case of cocksfoot. The reduction in the value of this coefficient in the third year of the study indicates a reduction in the mobility of copper.

5. Conclusions

Following soil application of copper in the amount of 100, 200, and 300 mg·kg^{-1} of soil, only the highest dose decreased the yield of cocksfoot, so this can be regarded as the threshold of toxicity. The negative effect of 300 mg Cu·kg^{-1} of soil on the test plant was confirmed by the fact that the tolerance index was lowest in the case of this level. All of the organic amendments increased the yield of the test plant, reducing the toxic effects of copper on cocksfoot. The most effective fertilizer in terms of yield and protective effects against high levels of copper was cattle manure, in the case of which the tolerance indices were highest. A somewhat smaller positive effect was obtained in the case of chicken manure, but smallest in case of mushroom substrate, which provided less protection than cattle manure.

Author Contributions: Conceptualization, B.K. and J.T.; Methodology, B.K. and J.T.; Resources, B.K., J.T., and D.J.; Writing—original draft preparation, B.K. and A.W.; Writing—review and editing, B.K. and A.W.; Visualization, A.W. and B.K. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Polish Ministry of Science and Higher Education, grants number 314/12/S, 106/14/MN, and 33/20/B.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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