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Effects of Organic Amendments on the Morphology and Chemical Composition of Black Mustard (*Sinapis nigra* L.) Grown on Soil Contaminated with Copper

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Abstract: The present study aimed to determine the influence of organic amendments (OAs) on neutralizing the harmful effect of copper (Cu) on black mustard (*Sinapis nigra* L.). In a pot experiment, three levels of copper pollution were used: 200, 400, and 600 mg Cu kg^{-1}, against a control without Cu. The soil was amended with three types of OAs: pine bark (PB), peat moss (PM), and cattle manure (CM). Our research showed that plant condition depends on the Cu content in the soil. Increasing soil contamination significantly affected the plant yield, leaf greenness index, and dry matter content. The type of OA had no significant effect on the condition of black mustard (BM); however, each had a different effect on neutralizing the harmful effects of Cu. CM reduced Cu accumulation, PM showed no effect, while PB contributed to a significant increase in Cu content in BM plants. The chemical composition of BM depended on the Cu content in the soil. With increased soil contamination with Cu, the contents of N_{tot}, K, Mg, Ca, and Na in BM increased, while the content of P decreased. In terms of mitigating the harmful effects, CM was more beneficial than PM and PB. Among the analyzed OAs, CM, and PM contributed to Cu immobilization, while PB promoted Cu mobilization in contaminated soils.

Keywords: Cu soil contamination; organic amendments; cattle manure; peat moss; pine bark; plants

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1. Introduction

Nowadays, there is increasing concern for the state of the natural environment, which involves efforts to restore the original functions of sites that were previously exposed to strong anthropogenic impacts. These impacts were primarily related to the accumulation of heavy metals such as copper (Cu), cadmium (Cd), nickel (Ni), and others, primarily from the mining and processing industries, as well as from direct human impact [1]. These activities caused disturbances in the balance of metals in biogeochemical cycles and the functioning of ecosystems, one of the most important elements of which is soil [2]. Currently, places have been identified where excessive accumulation of xenobiotics occurred in the past, the effects of which are still felt today [3–7]. Among the xenobiotics mentioned, an important place is occupied by heavy metals, including Cu, the extraction and processing of which has left a mark for many decades, primarily in terrestrial ecosystems. This metal has been known for about 4000 years BC, but in the 20th century, it was widely used in energetics and electronics due to its very good electrical characteristics. Cu is also used to produce various alloys (brass, bronze) as well as in electroplating and other metallurgical processes. Cu is widely used in the rubber, pharmaceutical, textile, dyeing, and chemical industries [8]. The very high demand for Cu made its mining and processing a priority in the past, but at that time, little attention was paid to aspects related to reducing its emissions into the environment. According to the Chief Inspectorate of Environmental Protection [9], the average copper content in Polish soils in 2020 was 9.29 mg Cu kg^{-1}. As reported by Stanislawska-Glubiak and Korzeniowska [10], nearly 40% of the Polish soil surface is characterized by a deficiency of this element, because Cu is one of the most depletable soil microelements. Cu belongs to the so-called vital micronutrients for plants, animals, and humans. It performs many metabolic functions; in plants, it is responsible for the synthesis of chlorophyll and the course of the photosynthesis process, carbohydrate synthesis, and protein metabolism. Cu deficiency disrupts these functions, and its excess even causes toxicity [11]. Cu toxicity may manifest in the reduction and deformation of the plant root system [12,13], reduction of yield, including dry matter yield, disruption of ion balance due to changes in the uptake of phosphorus and potassium [13], leaf chlorosis and reduction of chlorophyll content [14,15]. There are many regions in the world where Cu occurs in excess, changing the physicochemical and biological properties of the soils, which poses a threat to plants, animals, and humans [16–18]. In Poland, examples of agricultural regions with high Cu content include soils around the Legnica Copper Smelter, which contain 265 mg Cu kg^{-1}; the vicinity of the city of Głogów, 90.8 mg Cu kg^{-1}; and the Rzgów commune, 109 mg Cu kg^{-1} [19]. However, soil contamination near the emitter, e.g., Głogów Copper Smelter, according to Kostecki et al. [3] ranges from 2163 to 3230 mg Cu kg^{-1}. In the world, soil contamination with copper occurs, among others, near copper mines and smelters. The literature describes cases of very high soil contamination with copper, even called ecological disasters, e.g., in the vicinity of the copper-nickel smelter in Sudbury, Canada, the recorded soil content ranged from 510 to 9700 mg Cu kg^{-1}; similarly, in Coniston, Canada, 1400–3700 mg Cu kg^{-1}. In the vicinity of the copper ore mine in Lubumbashi, DR-Congo 11,600–14,200 mg Cu kg^{-1} was recorded [5]. Cu contamination of soils was not always the result of industrial emissions. There are examples described in the literature where, as a result of unprofessional and long-term use of copper fungicides, combating, among others, downy mildew caused by *Plasmopora viticola* led to the accumulation of toxic amounts of Cu in the soil. In the vineyards of France, Brazil, Croatia, and Spain, 1030, 3216, 700, and 603 mg Cu kg^{-1} of soil were recorded, respectively [6,13,19]. In India, 320 mg Cu kg^{-1}, and in Australia, 320 mg Cu kg^{-1} were also found [20]. Most often, this was the result of the use of the so-called Bordeaux mixture fungicide produced on the basis of copper compounds ($CuSO_4$ $5H_2O$ + $Ca(OH)_2$) [6,13,19]. This preparation was also widely used to protect other crops, e.g., olives. Soils of Aetoliko, Greece contained 77–647 mg Cu kg^{-1} [21]. Soils significantly contaminated with Cu in the European Union are also found in Italy, Portugal, and Romania [22]. US-EPA data show that there are 201 places in the world where copper occurs in quantities that pose a threat to the environment [23].

In order to limit the effects of excess Cu in the soil, attempts are being made to recultivate contaminated areas by, on the one hand, immobilizing mobile forms of copper [12–14,23–26], and, on the other hand, through phytotreatments falling within the scope of the so-called phytoremediation [4,27,28]. Immobilization mainly involves the use of various additives designed to bind copper into forms that are less soluble or even inaccessible to plants and thus trap it in the soil sorption complex [23]. These additives include lime [12,25,29], clay minerals [12,13,25], zeolites [12,25,30], and minerals rich in iron (Fe) and phosphorus (P) [24,26,31].

Organic amendments (OAs), usually belonging to organic fertilizers, have long been a valuable source of nutrients for cultivated plants. Their use was primarily associated with improving soil abundance in basic macronutrients as well as micronutrients. Moreover, the organic matter introduced with organic fertilizers contributed to the improvement of soil structure parameters: bulk density, lichen, air-water relations, etc. [32,33]. OAs also directly increase the capacity of the sorption complex (CEC) [32], and in relation to soil, they are characterized by the potential to believe that they can create relatively stable metal-organic compounds [34]. OAs include primarily biochar and mussel shells [33], composts, animal manures, bone meal [35,36], and besides, maize straw and mushroom cultivation waste [32]. The essential features of OAs that influence the effectiveness of immobilization of metals, including Cu, are, on the one hand, high alkalinity and the presence of carbonates, and on the other, high content of organic matter (OM), which can bind Cu and thus reduce its toxicity [35–38]. OM contains phenolic and carboxyl groups, which can be important sorbents of heavy metals [39,40]. Regular use of OAs, e.g., in the form of manure or animal slurry, increases the content of the organic carbon (OC) fraction in the soil in the form of carbon of humic acids (C_{HA}), carbon of fulvic acids (C_{FA}), and humic fraction carbon (C_{HU}). The fraction of humic acids bound to calcium ($C_{HA\text{-}Ca}$) has a particularly important influence on the binding of heavy metals [41]. Cu of natural origin is not very labile, forming insoluble complexes with soil organic matter (SOM) and soil minerals; it does not pose a threat to the environment. However, the durability of these compounds depends on many factors such as soil pH, redox potential, amount and type of OM, soil structure, and humidity [10,39], on which organic amendments have a significant impact.

The presented research aimed to investigate the hypothesis about the beneficial effect of OAs—dried cattle manure, peat moss, and ground pine bark—have a beneficial effect on reducing Cu toxicity in plants growing in Cu-contaminated soils. The model plant analyzed was black mustard (BM) (*Sinapis nigra* L.). During the research, changes in morphological and biological parameters were monitored, and the chemical composition and ionic balance of plants were assessed. The achieved results were compared to the null hypothesis that there is no influence of the above-mentioned factors on the examined features.

2. Materials and Methods

2.1. Experimental Design

The presented study was based on a pot experiment carried out in a greenhouse of the Faculty of Agriculture and Forestry of the University of Warmia and Mazury in Olsztyn (Olsztyn, Poland). The randomized block method was used in the research. The brown soil used in the experiment was collected from the Ap arable layer (0–30 cm) from the field of the Didactic and Experimental Center of the University of Warmia and Mazury in Olsztyn, located in Tomaszkowo village, near Olsztyn (53°42′35″ N, 20°26′01″ E). Soil properties are presented in Section 2.2.

The experiment was conducted according to a factorial experimental design with two factors: contamination with Cu (4 levels: 0, 200, 400, and 600 mg Cu kg^{-1} soil) and organic amendments (OAs): without OAs, dried cattle manure (CM), peat moss (PM), and ground pine bark (PB). The characteristics of the OAs used are presented in Section 2.3.

A Cu solution (100 mg Cu cm^{-3}) was prepared by dissolving 393 g of $CuSO_4 \cdot 5H_2O$ (copper sulfate pentahydrate) (POCh, Gliwice, Poland) in deionized water and made up to a volume of 1000 cm^3. The soil was artificially enriched with 16, 32, and 48 cm^3 of the Cu pot^{-1} solution. All pots were fertilized with NPK–pure chemical reagents (POCh, Gliwice, Poland) in the doses: 2.17 g N (urea $CO(NH_2)_2$), 0.60 g P (monopotassium phosphate KH_2PO_4), and 1.25 g K (monopotassium phosphate KH_2PO_4 (0.75 g K) + potassium sulfate K_2SO_4 (0.50 g K)) pot^{-1} before starting the experiment. Each treatment was carried out in triplicate. The soil moisture during the plants' growth was kept at 60% of full water capacity. Black mustard (*Brassica nigra* L.) (Piast Agro, Dębno, Poland) was grown for green mass and harvested in the BBCH 71–79 phase (pod formation phase).

2.2. Soil Properties

For the experiment, we used polyethylene pots filled with 8 kg of soil. The soil was previously sifted through a sieve with a mesh size of 1 cm. The particle size distribution of the soil was 75.1% sand, 24.3% silt, and 0.6% clay. This soil belongs to the group of loamy fine sands according to the US Department of Agriculture (USDA) Soil Texture Calculator [42], and was classified as Cambisols–Brown Soils [43]. Basic soil properties are presented in Table 1.

Table 1. Soil properties.

Parameter	Unit	Value
Particle size distribution: Sand 0.05–2 mm	%	75.1
Silt 0.002–0.05 mm	%	24.3
Clay ≤ 0.002 mm	%	0.60
Soil texture	–	loamy fine sand
Soil reaction (pH_{KCl})	$–log_{10}[H^+]$	6.44
Hydrolytic acidity (HAC)	cmol(+) kg^{-1}	2.46
Total exchangeable bases (TEB)	cmol(+) kg^{-1}	6.57
Total nitrogen (N_{tot})	g kg^{-1}	0.58
Total carbon (C_{tot})	g kg^{-1}	4.79
C/N	ratio	8.26
Cu	mg kg^{-1}	18.2

2.3. Organic Amendments (OAs)

The three OAs used in the experiment were: (1) pine bark–waste from the wood industry. There are two paper production plants located relatively close to Olsztyn: Kwidzyn (Mayr-Melnhof–formerly International Paper) and Świecie-Przechowo (Mondi S.A.); (2) cattle manure—waste (organic fertilizer) generated on cattle farms. Northern/eastern Poland is a typically agricultural area focused on cattle breeding and milk production. In the case of farms with limited soil surface, there is a danger of introducing excessive doses of N to the environment. Using manure in this way seems to be beneficial for the environment; (3) peat moss—this is the only component that is not a waste. This material was introduced to the soil in the presented research because we have appropriate resources of peat moss in the Warmia and Mazury Province and because of scientific reports about Cu deficiencies in peat soils. We found that if such a situation occurs, the addition of peat could effectively immobilize copper in Cu-contaminated areas.

OAs origin and doses used in the experiment: dried cattle manure (CM) (PPHU CDN, Janków Przygodzki, Poland) in a dose of 3% of soil (7.5 g kg^{-1} of dried manure containing 75% dry matter (DM), which corresponds to a dose of 30 g kg^{-1} of fresh manure containing 25% DM on average); peat moss (PM) (Athena Bio-Produkty, Szczecin, Poland) 3% of soil (30 g kg^{-1}); and ground pine bark (PB) fraction ø 2–5 mm (Hollas, Pasłęk, Poland) in a dose of 3% of soil (30 g kg^{-1}). Basic OA properties are presented in Table 2.

Table 2. Characteristics of the organic amendments (OAs) used in the experiment.

Parameter	Unit	Manure (CM) Dried Form	Peat Moss (PM) Fresh Form	Pine Bark (PB) Fresh Form
Reaction (pH_{KCl})	−log10 [H+]	6.98	4.12	3.28
Dry mass (DM)	g kg^{-1}	922	750	678
Total nitrogen (N_{tot})	g kg^{-1} (DM)	46.5	12.1	1.18
Phosphorus (P)	g kg^{-1} (DM)	32.4	0.62	0.34
Potassium (K)	g kg^{-1} (DM)	22.5	8.45	0.24
Calcium (Ca)	g kg^{-1} (DM)	48.2	1.35	6.23
Magnesium (Mg)	g kg^{-1}	21.2	0.56	0.33
Sodium (Na)	g kg^{-1} (DM)	0.74	0.47	0.07
Total carbon (C_{tot})	g kg^{-1} (DM)	210	379	490
C/N	ratio	4.52	31.39	415
Copper (Cu)	mg kg^{-1} (DM)	26.3	42.0	3.60

2.4. Analytical Methods

The chemical analyses of soil, OAs, and plant material samples were carried out in the laboratory of the Department of Agricultural and Environmental Chemistry of the University of Warmia and Mazury in Olsztyn, Poland.

2.4.1. Soil Analysis

The soil particle size distribution was measured with a Mastersizer 3000 equipped with a Hydro EV module (Malvern Instruments, Worcestershire, UK). The soil reaction (pH_{KCl}) was determined in the suspension soil/1 mol KCl solution at a ratio of 1:2.5 (w/v) with a 538 WTW pH Meter and pH SenTix61 electrode (WTW, Weilheim, Germany) [44]. Hydrolytic acidity (HAC) was measured after soil extraction with 0.5 M $Ca(OAc)_2$ (calcium acetate solution) in the soil/$Ca(OAc)_2$ suspension at a ratio of 1:2.5 (w/v) according to Kappen's method [45]. The total exchangeable bases (TEB) were determined after soil extraction with 1 M NH_4OAc (ammonium acetate) at pH 7 and calculated as the sum of individual base cations [44]. Total nitrogen (N_{tot}) was determined according to Kjeldahl's distillation method [46]. Wet digestion of samples was performed using the speed digester K-439 equipped with a vapor absorber K-415 (BÜCHI Labortechnik AG, Flawil, Switzerland). Nitrogen distillation was performed using the K-355 distillation unit (BÜCHI Labortechnik AG, Flawil, Switzerland). Total carbon content (C_{tot}) was determined by non-dispersive infrared spectroscopy (ND-IR) with the Shimadzu TOC–L analyzer coupled with an SSM–5000A solid samples analyzer (Shimadzu Corporation, Kyoto, Japan). The C/N ratio was calculated based on the C_{tot} and N_{tot} content. The contents of copper (Cu) in OAs were determined after wet mineralization of the samples using a MARS 5 microwave oven (CEM Corporation, Matthews, NC, USA) in Teflon vessels according to the US-EPA 3051 protocol [47]. Acid solutions (65% HNO_3 and 38% HCl) were used in a ratio of 4:1 (v/v) for OAs mineralization.

2.4.2. OAs Analysis

The reaction (pH_{KCl}), the content of N_{tot}, C_{tot}, and Cu, and the C/N ratio were determined according to the procedure described in Section 2.4.1. Dry matter (DM) of OAs was determined after 24 h of drying at 65 °C with the Binder FED–720 drier (Binder GmbH, Tuttlingen, Germany). The total form of macronutrients was determined in samples mineralized in concentrated sulfuric acid (according to the procedure described in Section 2.4.1. Soil Analysis). The total form of P was determined with the colorimetric vanadate–molybdate method [45]; Mg with atomic absorption spectrometry (AAS); and K, Ca, and Na with flame atomic emission spectrometry (FAES) using the fast sequential atomic absorption spectrometer VARIAN SpectrAA–FS240 (Varian Inc., Mulgrave, Australia).

2.4.3. Plant Analysis

Leaf greenness (SPAD index) was determined as the indicator of the nutritional status of plants [14,48–51]. The measurement was performed with the chlorophyll meter SPAD–502Plus (Konica–Minolta, Osaka, Japan) [52]. The harvest of plants was carried out 58 days after sowing with the simultaneous determination of the yield of the aboveground plant mass. The content of macronutrients and copper was determined using the same methods that were used to determine the chemical composition of the OAs.

2.4.4. Statistical Analysis

The results obtained were statistically analyzed at a significance level of $\alpha \leq 0.05$ by performing a two-way mixed ANOVA model where the main effects of factors and their interaction were fixed, and the replications were random effects. The significance of the differences between the mean values was determined by Duncan's test with $p < 0.05$. Normality was assessed using the Shapiro–Wilk test and homogeneity of variance using Levene's test. These analyses were performed in the Statistica 13.3 software package (Tibco Software Inc., Palo Alto, CA, USA) [53]. Standard deviation (SD) was calculated with Microsoft Excel® (version 2206) for Microsoft 365 MSO (Microsoft Corporation, Albuquerque, NM, USA) [54]. The simple Pearson coefficient (r) was used to determine the relationship between the tested features. The significance of the obtained values of the correlation coefficient (r) was determined based on statistical tables [55].

3. Results

3.1. Leaf Greenness

Experimental treatments significantly modified the leaf greenness index (Table 3). Soil contamination with Cu caused a significant, linear increase in the greenness index of BM leaves, which was particularly visible in the series without the addition of OAs. The OAs used in the experiment influenced the development of this parameter in various ways. Analyzing the mean values of the SPAD index for the tested treatments, it should be stated that CM was the only additive that caused a decrease in the analyzed index in relation to the control series without OAs, which was not demonstrated in relation to PM and PB. In this respect, they constituted a homogeneous group with the control series (without OAs). It should be added that in relation to the SPAD index, in the presented experiment, there was an interaction between soil pollution and OAs. In the treatments without OAs, the greenness increased significantly under the influence of increasing Cu doses, which is confirmed by a significant correlation coefficient (*r* = 0.73 **), while in the series with PM and PB, a tendency to lower the SPAD index was observed (*r* = −0.68 *). In the series with CM, there was no effect of this amendment on the changes in the SPAD index.

Table 3. Effect of increasing Cu contamination and OA supply on the greenness index of black mustard (*Sinapis nigra* L.).

Soil Pollution with Cu (mg kg^{-1})	Without OAs	OAs			Mean
		CM	PM	PB	
Greenness Index (SPAD Units)					
0	30.2 ± 0.8 a–d	31.9 ± 1.8 b–f	35.3 ± 1.9 f–g	35.0 ± 1.3 e–g	33.1 ± 2.6 B
200	33.0 ± 1.1 c–g	33.5 ± 0.7 c–g	36.6 ± 3.0 g	31.4 ± 1.3 b–f	33.7 ± 2.5 B
400	34.1 ± 1.7 c–g	26.3 ± 1.0 a	29.5 ± 3.9 a–c	29. 7 ± 1.7 a–c	29.9 ± 3.5 A
600	34.5 ± 2.4 d–g	30.2 ± 1.1 a–d	27.5 ± 6.1 ab	30.5 ± 2.6 a–e	30.7 ± 4.0 A
Mean:	33.0 ± 2.2 *B*	30.5 ± 3.0 *A*	32.2 ± 5.3 *AB*	31.7 ± 2.6 *AB*	31.8 ± 3.5
r	0.73 **	−0.48 ^{ns}	−0.68 *	−0.68 *	−0.36 ^{ns}

Means followed by different letters are significantly different by the Duncan$_{\alpha \leq 0.05}$ test; ± standard deviation of means; *—correlation coefficient *r* significant for $\alpha \leq 0.05$; **—correlation coefficient *r* significant for $\alpha \leq 0.05$; ns—non-significant; (uppercase regular—the differences between soil pollution used; uppercase italics—differences between OAs used; lowercase—the interaction of soil pollution vs. OAs); *n* = 12.

3.2. Plant Height, Number of Branches, and Pod Number

The height of corn plants was significantly dependent on the degree of soil contamination with Cu (Table 4). In the control series without OAs, in the treatment polluted with 600 mg Cu kg^{-1}, a significant linear reduction in plant height in relation to the treatment without Cu by an average of over 70% was demonstrated. The observed changes in plant height under the influence of copper occurred in all series of the experiment, which can be observed in Figure 1.

Analyzing the effect of the OAs used, it can be concluded that the only additive that increased the height of plants was PB. With this amendment, the plants were higher than the others by an average of 7.17 cm, 6.48, and 4.31 cm for CM, PM, and without OAs, respectively.

Copper toxicity also affected plant shape, which resulted in a significant reduction in the number of branches. As in the case of height, an almost linear effect of Cu on the number of branches was demonstrated, documented by highly significant correlation coefficients ranging from *r* = −0.90 ** to *r* = −0.94 **. The addition of PB, compared to CM and PM, had a beneficial effect and was the only one that increased the number of branches. In addition to plant height and the number of branches, the number of pods on the plant is a structural element of the canopy that influences yield. In the analyzed case, the highest number of pods was observed in objects not contaminated with Cu. The number of pods in these objects for individual series ranged from 33.07 to 44.13 pieces in the object with the addition of PM. Soil contamination with copper was manifested by a significant reduction in the number of pods and, consequently, a serious reduction in the yield potential of BM. The type of organic amendment did not influence the average number of pods on BM plants.

Table 4. Effect of increasing Cu contamination and OA supply plant height, number of branches, and pod number of black mustard (*Sinapis nigra* L.).

Soil Pollution with Cu (mg kg^{-1})	Without OAs	OAs			Mean
		CM	PM	PB	
Plants' Height (cm)					
0	83.0 ± 1.7 fg	72.0 ± 1.7 e	76.3 ± 5.9 ef	85.3 ± 7.5 g	79.2 ± 6.9 D
200	52.1 ± 5.8 d	60.3 ± 5.1 d	53.2 ± 5.4 d	54.2 ± 2.2 d	55.0 ± 5.3 C
400	37.6 ± 7.0 b	26.4 ± 3.7 a–c	35.2 ± 8.6 bc	39.7 ± 1.6 b	34.7 ± 7.3 B
600	24.5 ± 3.6 a	24.9 ± 4.4 a	23.8 ± 5.2 a	35.1 ± 4.5 b	27.1 ± 6.2 A
Mean:	49.3 ± 23.2 *A*	45.9 ± 21.8 *A*	47.1 ± 21.4 *A*	53.6 ± 20.8 *B*	49.0 ± 21.3
r	−0.96 **	−0.94 **	−0.96 **	−0.92 **	−0.93 **
Number of Branches (pcs)					
0	3.77 ± 0.39 e	3.04 ± 0.15 d	3.89 ± 0.87 e	3.84 ± 0.65 e	3.63 ± 0.61 C
200	2.02 ± 0.33 bc	2.53 ± 0.54 cd	2.34 ± 0.25 c	2.46 ± 0.10 cd	2.34 ± 0.36 B
400	1.49 ± 0.50 ab	1.11 ± 0.19 a	1.33 ± 0.26 a	1.60 ± 0.18 ab	1.38 ± 0.33 A
600	1.13 ± 0.13 a	1.00 ± 0.00 a	1.12 ± 0.12 a	1.19 ± 0.08 a	1.11 ± 0.11 A
Mean:	2.10 ± 1.10 *AB*	1.92 ± 0.96 *A*	2.17 ± 1.21 *AB*	2.28 ± 1.10 *B*	2.12 ± 1.07
r	−0.90 **	−0.92 **	−0.90 **	−0.94 **	−0.90 **
Number of Pods (pcs)					
0	33.1 ± 4.6 d	36.9 ± 4.6 de	44.1 ± 11.2 e	40.1 ± 5.9 de	38.5 ± 7.4 C
200	17.5 ± 3.3 bc	21.9 ± 4.8 c	19.0 ± 2.7 c	20.8 ± 3.6 c	19.8 ± 3.6 B
400	10.1 ± 3.5 ab	5.18 ± 1.5 a	8.46 ± 4.3 a	10.7 ± 1.3 ab	8.59 ± 3.4 A
600	5.94 ± 2.3 a	4.89 ± 1.5 a	4.78 ± 3.4 a	8.07 ± 0.9 a	5.92 ± 2.4 A
Mean:	16.7 ± 11.2 *A*	17.2 ± 14.2 *A*	19.1 ± 16.9 *A*	19.9 ± 13.5 *A*	18.2 ± 13.7
r	−0.92 **	−0.93 **	−0.89 **	−0.92 **	−0.90 **

Means followed by different letters are significantly different by the Duncan$_{\alpha \leq 0.05}$ test; ± standard deviation of means; **—correlation coefficient *r* significant for $\alpha \leq 0.05$; ns—non-significant; (uppercase regular—the differences between soil pollution used; uppercase italics—differences between OAs used; lowercase—the interaction of soil pollution vs. OAs); *n* = 12.

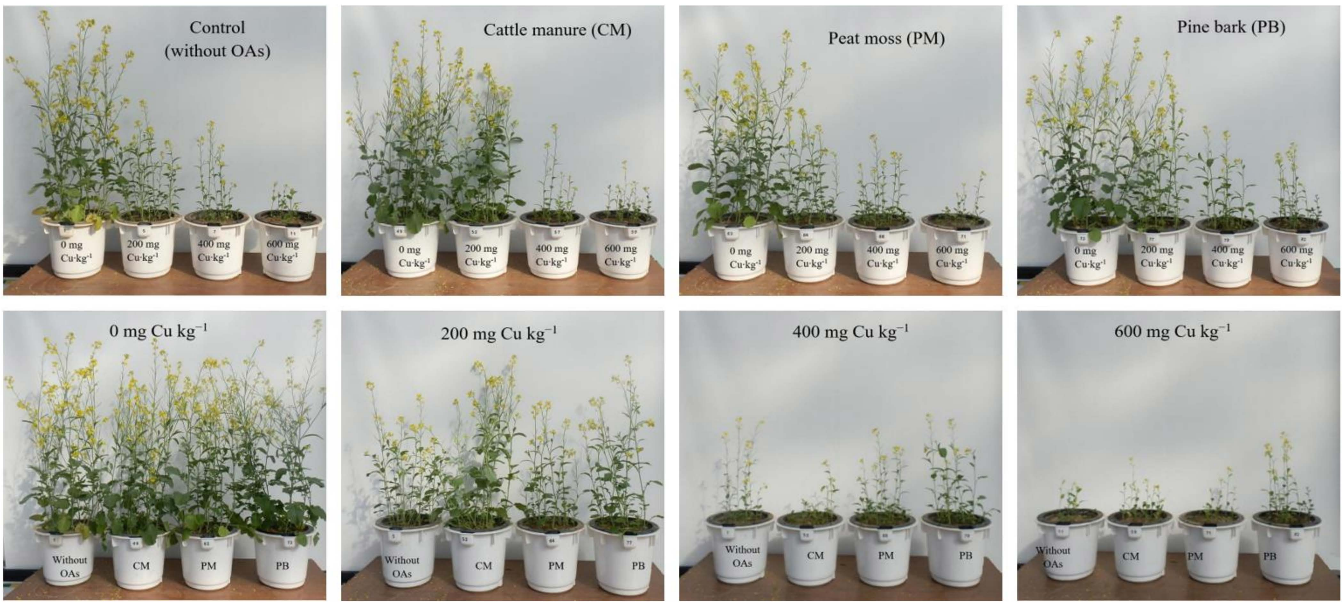

Figure 1. The impact of soil contamination with copper against the background of the OAs on black mustard (*Sinapis nigra* L.).

3.3. Plant Yield and Dry Matter Content

The previously demonstrated harmful effect of Cu on plant height, the number of branches, and the number of pods in connection with the synthesis and content of chlorophyll was reflected in the amount of yield obtained (Table 5). Each level of soil Cu contamination resulted in a significant reduction in plant yield, regardless of the research series, which, as in the case of the previously discussed features, is documented by very high correlation coefficients (-0.89 ** $\leq r \leq -0.98$ **). Against the background of this unfavorable effect of Cu, a positive interaction of the additives used appeared. This effect concerned the level of 200 mg Cu, at which all OAs had a positive effect; however, CM and PM increased the yield of BM compared to the series without OAs by approximately 48%, and PB by approximately 24%. This beneficial effect was not observed at higher Cu contents. In addition to the harmful effect of Cu on BM biomass yield, it was shown that Cu contributed to the decrease in DM content regardless of the OAs used. Additionally, the use of CM and PM further deepened the tendency to reduce the DM content. In this system, the addition of PB had the most beneficial effect on the DM content.

Table 5. Effect of increasing Cu contamination and OA supply on the yield and dry matter content in the aboveground mass of black mustard (*Sinapis nigra* L.).

Soil Pollution with Cu (mg kg^{-1})	Without OAs	OAs			Mean
		CM	PM	PB	
		Yield (g of DM pot $^{-1}$)			
0	116 ± 19.5 f	158 ± 4.2 g	156 ± 4.7 g	143 ± 2.1 g	143 ± 21.0 D
200	76.8 ± 11.1 de	148 ± 6.7 g	148 ± 0.0 ef	113 ± 5.3 f	109 ± 34.1 C
400	28.4 ± 11.6 a–c	21.4 ± 14.0 ab	21.4 ± 1.7 bc	53.6 ± 3.5 cd	37.6 ± 17.5 B
600	8.84 ± 2.0 a	11.3 ± 12.2 a	11.3 ± 8.0 a	19.2 ± 5.9 ab	13.3 ± 5.9 A
Mean:	57.5 ± 45.0 *A*	84.7 ± 27.1 *B*	84.2 ± 45.6 *B*	82.2 ± 40.2 *B*	75.7 ± 57.1
r	−0.96 **	−0.89 **	−0.97 **	−0.98 **	−0.91 **
		Dry Matter Content (DM%)			
0	24.6 ± 1.9 j	19.8 ± 0.3 i	20.0 ± 0.31 i	22.0 ± 0.3 i	21.6 ± 2.2 D
200	15.8 ± 0.3 gh	16.8 ± 2.1 h	13.2 ± 0.77 fg	13.9 ± 0.3 fg	14.9 ± 1.8 C
400	12.2 ± 0.7 ef	8.75 ± 0.9 b–d	10.4 ± 1.11 de	12.6 ± 0.4 ef	11.0 ± 1.7 B
600	5.74 ± 0.4 a	6.20 ± 0.7 ab	6.77 ± 3.99 a–c	9.41 ± 2.9 cd	7.03 ± 2.6 A
Mean:	14.6 ± 7.2 *B*	12.9 ± 5.9 *A*	12.6 ± 5.36 *A*	14.5 ± 5.0 *B*	13.6 ± 5.8
r	−0.98 **	−0.96 **	−0.92 **	−0.91 **	−0.93 **

Means followed by different letters are significantly different by the Duncan$_{\alpha \leq 0.05}$ test; ± standard deviation of means; **—correlation coefficient *r* significant for $\alpha \leq 0.05$; ns—non-significant; (uppercase regular—the differences between soil pollution used; uppercase italics—differences between OAs used; lowercase—the interaction of soil pollution vs. OAs); *n* = 12.

3.4. Chemical Composition of Plants

Experimental treatments modified the copper content in plant tissue (Table 6). The Cu content in BM increased significantly with increasing levels of copper soil contamination, especially in the treatment with 200 mg Cu kg^{-1}. The increase in the copper content in plants took place up to a dose of 400 mg Cu kg^{-1}, while in the most polluted object, there was a decrease in Cu content by approximately 88 mg Cu kg^{-1} compared to 400 mg Cu kg^{-1}. The OAs used significantly reduced the Cu content in plants, especially in the control treatment (with natural Cu content). At increased Cu level (200 mg Cu kg^{-1}), CM and PM had an immobilizing effect on the Cu content. In turn, PB caused a significant increase in Cu content, both in the case of 200, 400, and 600 mg Cu kg^{-1}. Analyzing the mean values, it can be concluded that the addition of CM and PM had the most beneficial effect on reducing Cu uptake by plants by 18.02 and 23.96 mg Cu kg^{-1}, respectively, while the addition of PB increased the Cu content in the plant by as much as 380.09 mg Cu kg^{-1}.

Table 6. Effect of increasing Cu contamination and OA supply on the Cu content in black mustard (*Sinapis nigra* L.).

Soil Pollution with Cu (mg kg^{-1})	Without OAs	Organic Amendments (OAs)			Mean
		Cattle Manure (CM)	Peat Moss (PM)	Pine Bark (PB)	
		Cu (mg kg^{-1} DM)			
0	77.0 ± 33.8 b	17.6 ± 5.5 a	28.7 ± 1.1 a	25.4 ± 11.10 a	37.1 ± 28.8 A
200	371 ± 26.9 fg	293 ± 2.4 c	348 ± 1.2 ef	892 ± 26.31 j	476 ± 253 B
400	398 ± 9.8 gh	348 ± 6.1 ef	372 ± 8.6 fg	791 ± 14.91 i	477 ± 190 B
600	310 ± 16.9 cd	402 ± 18.6 h	336 ± 8.5 de	969 ± 2.81 k	504 ± 283 C
Mean:	289 ± 133 *B*	265 ± 154 *A*	271 ± 147 *A*	669 ± 394 *C*	374 ± 285
r	0.63 *	0.91 **	0.75 **	0.81 **	0.56 ^{ns}

Means followed by different letters are significantly different by the Duncan$_{\alpha \le 0.05}$ test; ± standard deviation of means; *—correlation coefficient *r* significant for $\alpha \le 0.05$; **—correlation coefficient *r* significant for $\alpha \le 0.05$; ns—non-significant; (uppercase regular—the differences between soil pollution used; uppercase italics—differences between OAs used; lowercase—the interaction of soil pollution vs. OAs); *n* = 12.

The increasing soil contamination with Cu had a diverse impact on the content of macronutrients in the tested BM plants (Tables 7 and 8). The increase in Cu content in the soil generally increased the content of N_{tot}, K, Ca, Mg, and Na, which is confirmed by highly significant correlation coefficients (*r*), respectively: 0.92 **, 0.84 **, 0.80 **, and 0.84 ** in the series without organic additives. The only component negatively correlated with the level of soil Cu contamination was phosphorus (P) (*r* = −0.96 **). However, no significant effect of Cu contamination on the Ca content in plants was demonstrated.

Table 7. Chemical composition (N, P, and K content) of black mustard (*Sinapis nigra* L.).

Soil Pollution with Cu (mg kg^{-1})	Without OAs	OAs			Mean
		CM	PM	PB	
		N_{tot} (g kg^{-1} DM)			
0	9.88 ± 0.98 a	24.64 ± 0.00 c	24.92 ± 0.00 c	22.68 ± 0.28 b	20.53 ± 6.50 A
200	28.56 ± 0.00 d	28.70 ± 0.14 d	42.00 ± 0.00 i	38.22 ± 0.14 h	34.37 ± 6.16 B
400	36.26 ± 0.14 f	36.68 ± 0.28 f	46.76 ± 0.56 k	35.00 ± 0.28 e	38.68 ± 4.93 C
600	37.38 ± 0.14 g	38.50 ± 0.14 h	43.82 ± 0.14 j	35.00 ± 0.28 e	38.68 ± 3.38 C
Mean:	28.02 ± 11.51 *A*	32.13 ± 5.94 *B*	39.38 ± 8.90 *D*	32.73 ± 6.21 *C*	33.06 ± 9.15
r	0.92 **	0.97 **	0.81 **	0.63 *	0.73 **
		P (g kg^{-1} DM)			
0	3.89 ± 0.09 fg	4.02 ± 0.00 h	4.19 ± 0.02 i	4.19 ± 0.17 i	4.07 ± 0.16 A
200	3.01 ± 0.17 d	3.79 ± 0.00 f	3.95 ± 0.02 gh	4.85 ± 0.00 j	3.90 ± 0.69 B
400	2.38 ± 0.02 b	2.70 ± 0.06 c	3.50 ± 0.02 e	2.98 ± 0.10 d	2.89 ± 0.43 C
600	2.15 ± 0.06 a	2.21 ± 0.00 a	2.46 ± 0.02 b	2.70 ± 0.02 c	2.38 ± 0.23 D
Mean:	2.86 ± 0.71 *A*	3.18 ± 0.79 *B*	3.53 ± 0.69 *C*	3.68 ± 0.92 *D*	3.31 ± 0.82
r	−0.96 **	−0.97 **	−0.95 **	−0.81 **	−0.84 **
		K (g kg^{-1} DM)			
0	17.14 ± 0.26 b	21.48 ± 0.85 bc	20.63 ± 0.56 bc	16.65 ± 1.27 b	18.98 ± 2.31 A
200	22.91 ± 0.58 cd	29.73 ± 0.66 e	26.65 ± 3.74 de	27.82 ± 1.91 e	26.78 ± 3.18 B
400	42.25 ± 2.70 g	41.44 ± 0.38 g	31.13 ± 3.34 e	17.58 ± 5.92 b	33.10 ± 10.88 C
600	36.29 ± 2.51 f	47.77 ± 1.22 h	22.60 ± 5.19 cd	6.52 ± 1.06 a	28.29 ± 16.29 B
Mean:	29.65 ± 10.62 *C*	35.10 ± 10.66 *D*	25.25 ± 5.22 *B*	17.14 ± 8.34 *A*	26.79 ± 10.94
r	0.84 **	0.99 **	0.23 ^{ns}	−0.57 *	0.35 ^{ns}

Means followed by different letters are significantly different by the Duncan$_{\alpha \le 0.05}$ test; ± standard deviation of means; *—correlation coefficient *r* significant for $\alpha \le 0.05$; **—correlation coefficient *r* significant for $\alpha \le 0.05$; ns—non-significant; (uppercase regular—the differences between soil pollution used; uppercase italics—differences between OAs used; lowercase—the interaction of soil pollution vs. OAs); *n* = 12.

Table 8. Chemical composition (Ca, Mg, and Na content) of black mustard (*Sinapis nigra* L.).

Soil Pollution with Cu (mg kg^{-1})	Without OAs	OAs			Mean
		CM	PM	PB	
Ca (g kg^{-1} DM)					
0	14.7 ± 0.37 c	11.5 ± 0.18 a	13.5 ± 0.16 b	11.7 ± 0.65 a	12.9 ± 1.44 A
200	23.8 ± 0.03 i	17.6 ± 0.18 e	21.5 ± 0.33 g	19.7 ± 0.12 f	20.6 ± 2.39 C
400	25.5 ± 0.35 j	23.6 ± 0.03 hi	21.3 ± 0.09 g	23.6 ± 0.15 hi	23.5 ± 1.57 D
600	19.4 ± 0.49 f	21.5 ± 0.13 g	17.1 ± 0.22 h	23.2 ± 0.07 d	20.3 ± 2.39 B
Mean:	20.9 ± 4.38 *C*	18.5 ± 4.80 *A*	18.3 ± 3.45 *A*	19.5 ± 4.99 *B*	19.3 ± 4.42
r	0.41 ^{ns}	0.87 **	0.36 ^{ns}	0.90 **	0.64 *
Mg (g kg^{-1} DM)					
0	1.70 ± 0.10 a	2.11 ± 0.01 b	1.91 ± 0.05 ab	1.78 ± 0.05 ab	1.87 ± 0.17 B
200	5.74 ± 0.11 e	4.03 ± 0.06 c	4.76 ± 0.03 d	4.00 ± 0.09 c	4.63 ± 0.74 C
400	8.39 ± 0.04 h	7.59 ± 0.07 g	5.76 ± 0.07 e	7.00 ± 0.03 f	7.19 ± 1.00 A
600	6.75 ± 0.46 f	8.62 ± 0.05 h	5.58 ± 0.64 e	7.39 ± 0.01 g	7.09 ± 1.19 A
Mean:	5.64 ± 2.59 *A*	5.59 ± 2.75 *A*	4.50 ± 1.64 *B*	5.04 ± 2.40 *C*	5.19 ± 2.35
r	0.80 **	0.98 **	0.86 **	0.97 **	0.87**
Na (g kg^{-1} DM)					
0	1.00 ± 0.03 c	0.52 ± 0.01 b	0.22 ± 0.00 a	0.28 ± 0.01 a	0.51 ± 0.32 B
200	2.51 ± 0.06 f	1.83 ± 0.02 d	1.99 ± 0.03 e	1.10 ± 0.00 c	1.86 ± 0.53 C
400	3.90 ± 0.10 i	6.58 ± 0.09 l	3.01 ± 0.01 g	4.23 ± 0.00 j	4.43 ± 1.38 A
600	3.27 ± 0.09 h	6.66 ± 0.20 l	3.16 ± 0.05 h	4.51 ± 0.01 k	4.40 ± 1.48 A
Mean:	2.67 ± 1.13 *C*	3.90 ± 2.89 *D*	2.09 ± 1.23 *A*	2.53 ± 1.95 *B*	2.80 ± 1.99
r	0.84 **	0.94 **	0.94 **	0.95 **	0.81 **

Means followed by different letters are significantly different by the Duncan$_{\alpha \le 0.05}$ test; ± standard deviation of means; *—correlation coefficient *r* significant for $\alpha \le 0.05$; **—correlation coefficient *r* significant for $\alpha \le 0.05$; ns—non-significant; (uppercase regular—the differences between soil pollution used; uppercase italics—differences between OAs used; lowercase—the interaction of soil pollution vs. OAs); *n* = 12.

The tendency to increase the content of macronutrients in BM plants occurred mainly up to the level of 400 mg Cu kg^{-1}. This fact often depended on the type of OAs used. For example, in the case of N_{tot}, its content in the CM-added series systematically increased across all Cu contamination levels. However, after the application of PM and PB, an increase in N_{tot} content was observed only up to a dose of 400 mg Cu kg^{-1}. In the series with PM and PB, the dose of Cu (600 mg Cu kg^{-1}) did not increase the N_{tot} content; in the case of PM, a significant reduction in the content of this macroelement was observed. All OAs contributed to increasing the N_{tot} content in plants compared to the treatment without OAs. The addition of PM had the greatest effect on increasing the N_{tot} content, followed by PB and CM.

In addition to the above-mentioned negative effect of Cu on the P content in BM plants, it should be noted that the applied OAs significantly increased the P content compared to the treatment without OAs. In this respect, the addition of PB had the most beneficial effect, followed by PM, and to a slightly lesser extent by CM.

With regard to the K content, the level causing an increase in the Cu content in BM plants in the series without OAs was 400 mg Cu kg^{-1}, while a dose of 600 mg Cu kg^{-1} resulted in a decrease in the content of K in the plants. In the CM series, the K content increased linearly at all doses. In the series with PM, a significant increase in K to the level of 400 mg Cu kg^{-1} was recorded, and in the series with PB, the K content, compared to the object uncontaminated with Cu, increased significantly under the influence of 200 mg Cu kg^{-1}; under the influence of 400 and 600 mg Cu kg^{-1}, there was a decrease in the K content in BM plants in relation to the object with 200 mg Cu kg^{-1}. Comparing the effect of the OAs used, it should be concluded that the addition of CM increased the K content, while PM and PB decreased the average K content the conditions of copper contamination of the soil.

In the case of Ca, the content increased, regardless of the OAs used, to the level of 400 mg Cu kg^{-1}. A higher level of copper contamination in the soil contributed to a lower

Ca content in the plants. However, it should be added that each of the OAs significantly reduced the Ca content in the tested plants.

Similarly, Cu contamination influenced the Mg content; however, the use of CM and PB in this case increased the Mg content in plants, even in objects contaminated with copper to the greatest extent. The beneficial effect of CM and PB on the accumulation of Mg (also in objects with 600 mg Cu kg^{-1}) is also confirmed by the high correlation coefficients, *r* = 0.98 ** and *r* = 0.97 **, respectively. Concerning the average Mg content, it can be indicated that the addition of CM did not significantly change the Mg content, while PM and PB caused its reduction compared to the series without OAs.

Cu contamination caused a linear increase in the Na content in each series with the addition of OAs. Generally, the addition of CM contributed to a significant increase, while PM and PB reduced the Na content in the BM plants.

3.5. Ion Balance

The ionic balance of the analyzed plants was significantly dependent on the Cu content in the soil (Table 9). The Ca:P molar ratio increased almost linearly as a result of increasing soil Cu pollution. These changes were counteracted to some extent by the introduction of OAs. In this case, PM had the most beneficial effect, followed by PB and CM. With regard to the Ca:Mg ratio, an opposite tendency was demonstrated. Increasing levels of copper contamination in each series, regardless of the additive used, resulted in its significant narrowing in the analyzed plants. The OAs used resulted in a further narrowing of the Ca:Mg ratio. The K:(Ca+Mg) ratio also gradually narrowed under the influence of increasing soil Cu contamination. The changes were not as striking as in the case of Ca:P, but they were most visible in the series with PB (*r* = −0.95), where the K:(Ca+Mg) value was the narrowest and amounted to 0.37. The addition of CM increased the value of this ratio to 0.66, while the addition of PM did not significantly affect this feature.

Table 9. Ca:P, Ca:Mg, and K:(Ca+Mg) ratios in the tissue of black mustard (*Sinapis nigra* L.).

Soil Pollution with Cu (mg kg^{-1})	Without OAs	OAs			Mean
		CM	PM	PB	
Ca:P (molar ratio)					
0	2.94 ± 0.14 c	2.21 ± 0.03 a	2.49 ± 0.01 b	2.16 ± 0.03 a	2.45 ± 0.33 A
200	6.14 ± 0.33 h	3.59 ± 0.04 d	4.19 ± 0.09 e	3.13 ± 0.02 c	4.26 ± 1.21 B
400	8.28 ± 0.05 l	6.76 ± 0.16 ij	4.70 ± 0.01 f	6.12 ± 0.17 h	6.46 ± 1.35 C
600	6.94 ± 0.01 j	7.50 ± 0.05 k	5.37 ± 0.11 g	6.63 ± 0.03 i	6.61 ± 0.82 D
Mean:	6.08 ± 2.06 *D*	5.02 ± 2.29 *C*	4.19 ± 1.12 *A*	4.51 ± 1.99 *B*	4.95 ± 1.99
r	0.80 **	0.97 **	0.96 **	0.96 **	0.83 **
Ca:Mg (meq(+) ratio)					
0	5.29 ± 0.19 k	3.30 ± 0.03 h	4.28 ± 0.05 j	3.98 ± 0.11 i	4.21 ± 0.75 D
200	2.52 ± 0.04 e	2.65 ± 0.06 ef	2.74 ± 0.06 f	2.98 ± 0.09 g	2.72 ± 0.18 C
400	1.84 ± 0.02 b	1.88 ± 0.02 b	2.24 ± 0.02 d	2.04 ± 0.00 c	2.00 ± 0.16 B
600	1.74 ± 0.08 b	1.51 ± 0.00 a	1.88 ± 0.24 bc	1.90 ± 0.01 bc	1.76 ± 0.20 A
Mean:	2.85 ± 1.51 *B*	2.34 ± 0.73 *C*	2.79 ± 0.96 *AB*	2.73 ± 0.87 *A*	2.67 ± 1.04
r	−0.88 **	−0.99 **	−0.93 **	−0.96 **	−0.87 **
K:(Ca+Mg) (meq(+) ratio)					
0	0.50 ± 0.02 d–f	0.73 ± 0.02 k	0.64 ± 0.01 ij	0.58 ± 0.02 ghi	0.61 ± 0.09 C
200	0.35 ± 0.01 c	0.63 ± 0.02 ij	0.47 ± 0.07 de	0.54 ± 0.04 fgh	0.50 ± 0.11 B
400	0.55 ± 0.04 f–h	0.59 ± 0.00 g–j	0.52 ± 0.05 e–g	0.26 ± 0.09 b	0.48 ± 0.14 AB
600	0.61 ± 0.02 hi	0.69 ± 0.01 jk	0.44 ± 0.09 d	0.09 ± 0.02 a	0.46 ± 0.24 A
Mean:	0.50 ± 0.10 *A*	0.66 ± 0.06 *C*	0.51 ± 0.09 *A*	0.37 ± 0.22 *B*	0.51 ± 0.16
r	0.60 *	−0.37 ^{ns}	−0.67 **	−0.95 **	−0.34 ^{ns}

Means followed by different letters are significantly different by the Duncan$_{\alpha \leq 0.05}$ test; ± standard deviation of means; *—correlation coefficient *r* significant for α ≤ 0.05; **—correlation coefficient *r* significant for α ≤ 0.05; ns—non-significant; (uppercase regular—the differences between soil pollution levels used; uppercase italics—differences between OAs used; lowercase—the interaction of soil pollution vs. OAs); *n* = 12.

A very similar situation was demonstrated with respect to the K:Mg ratio (Table 10). In the case of this indicator, a significant narrowing was also observed due to Cu contamination. The analysis of mean values shows that the value of this ratio decreased by more than half due to the increasing soil contamination with Cu. The addition of CM had a very positive effect on mitigating these changes, with the K:Mg value being 2.22, while the addition of PB had an unfavorable effect.

Table 10. K:Mg, K:Ca, and K:Na ratios in the tissue of black mustard (*Sinapis nigra* L.).

Soil Pollution with Cu (mg kg^{-1})	Without OAs	OAs			Mean
		CM	PM	PB	
K:Mg (meq(+) ratio)					
0	3.16 ± 0.24 g	3.16 ± 0.11 g	3.35 ± 0.01 g	2.90 ± 0.14 f	3.14 ± 0.21 D
200	1.24 ± 0.01 c	2.29 ± 0.02 e	1.74 ± 0.23 d	2.16 ± 0.10 e	1.86 ± 0.44 C
400	1.56 ± 0.11 d	1.70 ± 0.00 d	1.68 ± 0.16 d	0.78 ± 0.27 b	1.43 ± 0.42 B
600	1.67 ± 0.00 d	1.72 ± 0.03 d	1.24 ± 0.15 c	0.27 ± 0.04 a	1.23 ± 0.61 A
Mean:	1.91 ± 0.78 *A*	2.22 ± 0.62 *C*	2.00 ± 0.85 *A*	1.53 ± 1.11 *B*	1.91 ± 0.87
r	−0.62 **	−0.92 **	−0.88 **	−0.98 **	−0.81 **
K:Ca (meq(+) ratio)					
0	0.60 ± 0.02 cd	0.96 ± 0.02 j	0.78 ± 0.01 f–i	0.73 ± 0.02 e–g	0.77 ± 0.14 B
200	0.49 ± 0.01 bc	0.87 ± 0.03 h–j	0.64 ± 0.10 de	0.73 ± 0.05 e–g	0.68 ± 0.15 A
400	0.85 ± 0.07 g–j	0.90 ± 0.01 ij	0.75 ± 0.08 e–h	0.38 ± 0.13 b	0.72 ± 0.22 AB
600	0.96 ± 0.04 j	1.14 ± 0.02 k	0.68 ± 0.16 d–f	0.14 ± 0.02 a	0.73 ± 0.40 AB
Mean:	0.72 ± 0.20 *A*	0.97 ± 0.11 *C*	0.71 ± 0.11 *A*	0.50 ± 0.26 *B*	0.72 ± 0.24
r	0.85 **	0.61 *	−0.2 ^{ns}	−0.92 **	−0.03 ^{ns}
K:Na (meq(+) ratio)					
0	10.08 ± 0.20 g	24.25 ± 0.67 i	55.33 ± 0.58 k	34.91 ± 1.93 j	31.14 ± 17.27 D
200	5.37 ± 0.00 de	9.54 ± 0.31 g	7.87 ± 0.97 f	14.83 ± 0.95 h	9.40 ± 3.67 C
400	6.36 ± 0.25 e	3.70 ± 0.02 c	6.08 ± 0.67 e	2.44 ± 0.82 b	4.65 ± 1.77 B
600	6.55 ± 0.64 e	4.22 ± 0.23 cd	4.19 ± 0.90 cd	0.85 ± 0.14 a	3.95 ± 2.18 A
Mean:	7.09 ± 1.89 *A*	10.43 ± 8.67 *B*	18.37 ± 22.34 *D*	13.26 ± 14.26 *C*	12.29 ± 14.15
r	−0.59 *	−0.89 **	−0.81 **	−0.94 **	−0.69 **

Means followed by different letters are significantly different by the Duncan$_{\alpha \leq 0 \cdot 05}$ test; ± standard deviation of means; *—correlation coefficient *r* significant for $\alpha \leq 0.05$; **—correlation coefficient *r* significant for $\alpha \leq 0.05$; ns—non-significant; (uppercase regular—the differences between soil pollution levels used; uppercase italics—differences between OAs used; lowercase—the interaction of soil pollution vs. OAs); *n* = 12.

Also in the case of the K:Ca ratio, the least beneficial was the addition of PB, which reduced the value of this ratio to 0.50. For the series without OAs, this value was 0.72. The most beneficial effect in this case was the addition of CM, where the K:Ca ratio was 0.97.

4. Discussion

Many different organic materials, often waste, are widely used to immobilize heavy metals in soils from contaminated areas [12,32,33,38]. Their diversity means that they also have a different impact on the transformation of heavy metals in soils. The main goal, which is the effectiveness of immobilization of heavy metals, depends not only on the properties of the OAs themselves but also on the metal to be remedied [32]. In the presented work, we focused on three different OAs, i.e., cattle manure, peat moss, and pine bark, which were used in conditions of increasing soil Cu contamination. The OAs differed significantly in their chemical and physical properties (Table 2), so we also expected different effects related to their impact on the test plant, black mustard (*Sinapis nigra* L.). Essentially, plant yield is influenced by many agrotechnical factors, among which the most important role is played by the supply of nutrients to plants. The decisive environmental factors include soil conditions and weather conditions, i.e., temperatures, sunlight, and precipitation. These factors directly affect the growth and development of plants. Through appropriate soil moisture, they determine the availability of soluble forms of macro- and micronutrients, and through appropriate sunlight, they determine the course of assimilate synthesis processes—organic components of the cells. The amount of this synthesis depends on the presence of chlorophyll in the leaves [51], which in the presented work was expressed as the SPAD leaf greenness index. The SPAD index is currently considered an excellent indicator expressing the nutritional status of plants because it is closely correlated with plant yield. In precision agriculture, it is used to determine the demand of plants for nitrogen fertilization [25,48–51]. In the presented research, increasing soil Cu contamination contributed to an increase in leaf greenness. The obtained results do not correspond to the results of other authors. As a rule, under the influence of excessive copper content in the soil, plants develop leaf chlorosis [56] due to ultrastructural changes in chloroplasts [57]. It should be added, however, that among plants there are groups that respond with different sensitivities to Cu contained in the soil. For example, wheat is highly sensitive to Cu deficiency or excess, while the sensitivity of corn, sugar beet, or cotton to Cu is relatively low [58]. Changes in the chloroplasts are the result of oxidative stress manifested by damage to cell membranes due to the accelerated production of reactive oxygen species (ROS) [59]. In the presented case, as a result of Cu toxicity, there was probably a "rescue" synthesis of chlorophyll, which, as inactive, accumulated in the leaf tissue. According to Rehmann [58], plants affected by Cu toxicity may even have a bluish tint, which in our case could have resulted in overestimated greenness readings. As a result, the leaves of plants exposed to excess Cu turn yellow or brown [58].

Disturbances in metabolism caused by heavy metals described in the literature in relation to chlorophyll content [56–59] had a direct impact on plant morphology. Increasing Cu pollution caused a significant decrease in plant height and changes in their habit, manifested by a significant reduction in branching and the number of pods. The obtained results are consistent with information in the literature that confirms the occurrence of plant dwarfism [58]. The dwarfing effect is caused mainly by limiting the activity of meristems, which results not only in a change in the shape of the aboveground part but also in limited development of the root system [60]. Cellulose is very often synthesized and deposited in the roots as a natural protective barrier of plants, in which harmful Cu ions are sequestered [12,61]. The obtained results confirm previous studies in which the roots of corn grown in Cu-contaminated soil were characterized by significant shortening and the presence of a larger number of root hairs and contained an average of 1350 mg Cu kg^{-1}, which was six times higher than the content recorded in the aboveground part [12]. The organic amendment in the form of PB increased the height of the plants, while the other OAs did not affect plant size, number of branches, or number of pods.

Nevertheless, the mentioned OAs, despite having no effect on the above-mentioned parameters, significantly increased the yield of the aboveground matter of plants, compared to the series without OAs. This beneficial effect particularly concerned objects with a contamination level of 200 mg Cu kg^{-1}, and the plants from the series treated with CM and PM were characterized by higher hydration than those treated with PB. The toxicity of Cu to plants, in addition to parameters such as pH, CEC, redox potential, particle size distribution, and type of clay minerals, is influenced by the content of OM. It determines the mobilization or immobilization of potentially toxic elements [62]. In the case of OAs, both mechanisms can be taken into account, and the selection of the appropriate amendment may be crucial in the case of immobilization or mobilization of metals to improve phytoextraction while aiming to shorten the phytoextraction time [34,35]. In the present research, the OAs used in the form of CM and PM reduced the uptake of Cu by plants, but it should be explained that this effect was noticeable up to the level of 400 mg Cu kg^{-1}. At higher Cu contents, under the influence of CM and PM, the copper content increased compared to the series without additives. PB, however, significantly influenced the mobilization of Cu ions at each pollution level. It can be assumed that the increased mobility of Cu could result from the highly acidic reaction of PB (pH = 3.28) (Table 2), which contributed to the acidification of the soil and the increase in the mobility of copper ions, resulting in an increase in Cu uptake by plants. Parameters such as pH and the content of organic carbon forms significantly influence the mobility of metals in the soil [12,36,63,64]. The second reason for the increased mobility of Cu could be the result of the carbon (TOC) contained in PB (490 g of TOC kg^{-1}) (Table 2) and the dissolved organic carbon fraction (DOC), which, by forming complexes with Cu, thus increased the mobility of this metal in the soil [65]. In Filipović's research [66], the concentration of mobile Cu in leachates from vineyard soil depended on the complexation of Cu by DOC, as it was shown that 99.9% of the total Cu pool was Cu-DOC. In the present study, the addition of PB caused at least a twofold increase in Cu uptake by plants, which could also be related to the mobile forms of Cu-DOC. Comparing the effect of PB mentioned here with the effect obtained in relation to the yield and dry matter content, it can be assumed that PB may be a recommended additive in phytoextraction, as it caused an increase in the yield of the aboveground matter of black mustard with a simultaneous increase in Cu uptake by the crop.

Soil Cu contamination generally resulted in increased macronutrient content in plants. The contents of N_{tot}, K, Mg, and Na increased, except for P, the content of which decreased significantly under the influence of soil contamination with copper. The antagonistic effect of Cu on the P content is also confirmed in other works on the effect of Cu on plants such as cucumber, clover, wheat, and rye [8,67,68]. The Ca content increased only to the contamination level of 400 mg Cu kg^{-1}. Higher copper contents resulted in a decrease in Ca content in the aboveground parts of plants. The literature confirms the obtained results regarding synergism between Cu and N_{tot} [69]. Confirmation of this can be found in relation to cocksfoot grass biomass [69], subterranean clover [70], and broad bean [71]. There are also reports of the lack of synergistic effect between Cu and N in plants such as maize, barley, wheat, oilseed rape, and oat [72,73]. Possible synergism or lack of synergism between Cu and minerals in plants depends on the already mentioned sensitivity of plants to deficient or excess amounts of Cu in the soil [58]. All OAs used resulted in an increase in the content of N_{tot} and P compared to the series without OAs. In relation to the remaining components, the impact of OAs was varied. Under the influence of PB, a significant decrease in the K content and an increase in the Ca content were observed compared to the series with PM and CM. The addition of CM, in turn, had a positive effect on the Mg and Na content. The diversity of the chemical composition of the analyzed plants results from impaired efficiency of ion uptake and transport from the roots to the aboveground part. Under the toxic influence of Cu, root cell membranes [60,68] and transport proteins responsible for the transport of assimilates are damaged [73].

Among the indicators informing about the quality of plants are the ionic ratios between individual macronutrients. The previously demonstrated antagonistic or synergistic interaction between Cu and macronutrients may interfere with their proper amounts. The values of the most important ratios according to the literature data should be as follows: K:(Ca+Mg) = 1.6–2.2; K:Mg = 6; K:Ca = 2; K:Na = 5–10; Ca:Mg = 2–3; K:Ca = 2; and Ca:P = 1.5–2 (molar ratio) [74,75]. The harmful effects of copper were manifested by a gradual narrowing of the K:Mg, K:(Ca+Mg), Ca:Mg, and K:Na ratios and an expansion of the Ca:P ratio. This effect was the result of increased accumulation of Mg, Na, and Ca rather than K by plants under stress caused by the presence of Cu. In the literature, the effect of narrowing the K:Ca and K:Mg ratios under the influence of copper was also described in spring barley [76], although, in the case of white mustard, oats, barley, and oats, the opposite effect was obtained.

The above reports indicate a significant diversity of plants in terms of their response to Cu. An additional element that changes these responses is the soil, its chemical and physical properties, and fertilization. In our case, the relevant OAs influenced the value of individual ratios differently. The addition of CM improved the average values of the considered ratios, except for the K:Na ratio, which was within the upper limit of the norm. The addition of PM improved the Ca:P and Ca:Mg values but significantly increased the K:Na ratio. In turn, PB narrowed the values of Ca:P, Ca:Mg, K:(Ca+Mg), K:Mg, and K:Ca but significantly widened the K:Na ratio.

5. Conclusions

Soil contamination with copper significantly influenced the yield and plant nutritional status. Cu, as a stress factor, had a significant impact on the chlorophyll content in leaves. As a result of heavy metal stress, plants synthesized "rescue" chlorophyll, which increased the SPAD index. In parallel with the synthesis of chlorophyll, the destruction of chloroplasts responsible for the synthesis of assimilates probably occurred. The effect of this phenomenon was a decrease in the yield of aboveground mass of plants. Among the organic amendments used, cattle manure, and peat moss turned out to be additives that alleviated the harmful effects of Cu, increasing the yield while limiting Cu uptake by plants. It should be added that effective Cu immobilization was recorded only in the treatment with Cu at a level of 200 mg Cu kg^{-1}. In the case of pine bark, its mobilizing nature was demonstrated. This addition also mitigated the harmfulness of Cu ions because, in this case, the highest yield of the aboveground part of black mustard was obtained, with the highest dry matter content and the highest Cu content. Generally, the effect of Cu was manifested by an increase in the content of N_{tot}, K, Ca, Mg, and Na and a decrease in the content of P in plants. In the case of the tested black mustard, the critical dose in terms of the chemical composition of the crop seems to be 400 mg Cu kg^{-1}, up to which the above-mentioned macronutrients accumulated. At the highest Cu content of 600 mg Cu kg^{-1}, a decrease in their content was recorded. Such effects were obtained in the series without organic amendments and with peat moss and pine bark. The use of CM resulted in the accumulation of N_{tot}, K, Mg, and Na also at a pollution level of 600 mg Cu kg^{-1}. The organic amendments used also modified the ratios between macronutrients in plants, and their beneficial effect was essentially visible on objects contaminated with Cu up to the level of 200 mg Cu kg^{-1}. To sum up, it can be indicated that among the analyzed organic amendments, the first two, i.e., cattle manure and peat moss, may contribute to immobilization, while pine bark is an additive promoting copper mobilization in contaminated soils. Their use should therefore be consistent with the adopted soil remediation strategy.

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