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Effect of Nitrogen Fertilization on the Dynamics of Concentration and Uptake of Selected Microelements in the Biomass of *Miscanthus x giganteus*

Izabela Gołąb-Bogacz [1], Waldemar Helios [2], Andrzej Kotecki [2], Marcin Kozak [2] and Anna Jama-Rodzeńska [2,*]

[1] Bugaj Sp. z o.o, Bugaj Zakrzewski 5, 97-512 Kodrąb, Poland; iza.golab@o2.pl
[2] Institute of Agroecology and Plant Production, Wroclaw University of Environmental and Life Sciences, Pl Grunwaldzki 24A, 50-363 Wrocław, Poland; waldemar.helios@upwr.edu.pl (W.H.); andrzej.kotecki@upwr.edu.pl (A.K.); marcin.kozak@upwr.edu.pl (M.K.)
* Correspondence: anna.jama@upwr.edu.pl; Tel.: +48-713-201-627

Abstract: This paper presents the effects of nitrogen (N) fertilization on the concentration of selected micronutrients as an important issue in reducing combustion-induced air pollution. We studied the effects of the dose of 60 kg ha^{-1} N in different terms of biomass sampling on the concentration and uptake of iron (Fe), manganese (Mn), zinc (Zn), and copper (Cu) in the dry matter of the underground and aerial parts of *Miscanthus x giganteus* in the years 2014–2016. The order of microelement concentrations (mg kg^{-1}) in rhizomes and the aboveground parts of plants was as follows: Fe > Mn > Zn > Cu. N fertilization had no significant effect on the concentrations of the selected microelements in the Mischanthus biomass (except for the Mn concentration in the stems and Cu in the leaves). The results indicated that the quality of the combustion biomass did not worsen under nitrogen fertilization. During the whole vegetation period, the iron concentration increased in the rhizomes and decreased for Zn and Cu. In the aboveground parts of the plant, the concentrations of all tested elements decreased. In turn, the uptake of Fe, Mn, Zn, and Cu (except for Fe in the stems) by rhizomes and the aboveground parts of Mischanthus depended significantly on the N fertilization.

Keywords: N application; translocation Fe; Mn; Zn; Cu; rhizomes; aboveground parts of plants; *Miscanthus*

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1. Introduction

Increasing concentrations of greenhouse gases, the global growth of energy demand, and rapidly decreasing reserves of fossil fuels cause renewable energy sources, including biomass, the center of interest for scientists and many sectors of industry [1,2]. The benefits of bioenergy crops, apart from the high yield potential, is cultivation on marginal land, which is characterized by low productivity of other field crops [3,4].

Due to the specific chemical structure and high calorific value, plant biomass can be destined for various conversion processes to produce liquid, gaseous, and solid fuels [5–7]. Plant biomass currently accounts for only 3% of basic energy consumption in highly developed countries [6,8]. Carbon dioxide (CO2) is also present during the biomass combustion process. However, since it comes from harvested or combustion plants that absorbed it from the atmosphere in the first place, these are not additional quantities [6,9]. Part of the carbon is stored in the soil [10].

Among perennial grasses, *Miscanthus x giganteus* is characterized by a high potential yield and low humidity of the harvested biomass [9]. *Miscanthus x giganteus* is also a tolerant plant for moderate concentrations of heavy metals and microelements in the soil [11]. The production of such plants depends on the climate conditions. In the current, fast-changing temperature conditions, *Miscanthus x giganteus* was shown to be 59% more productive compared to maize grain in field studies [12]. *Miscanthus x giganteus* is more efficient compared to other C4 cold resistant biomass crops, such as altered grass [12].

The C3 plants assimilate CO_2 directly in the Calvin cycle. The first durable product of this process is tricarbon 3-phosphoglyceric acid, whereas, in the case of C4, the four carbon oxaloacetic acid is formed. C4 plants are regarded as having the highest potential productivity compared to C3 plants. Detailed literature related to C4 plants is presented in Sage et al. (2011) [13].

Miscanthus biomass is presently used in the combustion process [14] as an ideal fuel plant, providing annual easy cultivation, harvesting, and high yields of dry matter [9]. The examination of the biomass chemical composition is necessary to test the suitability for bioenergy conversion [15], which is indispensable for the production of derived fuels and chemical substances [16]. The determination of the nutrient composition in plants is crucial, not only in the combustion process but also for examining the nutrient uptake by crops [17]. Fertilization has an impact on the concentration and the amount of nutrients in the biomass harvested. The choice of fertilizer type is one of the most important features in fertilizer management. Overly high concentrations of N and S can cause increased NO_x and SO_2 emissions [14,15].

Essential issues during combustion process include not only the macro- and microelement concentrations but also the humidity (internal and external), calorific value, and ash level. Many of the elements necessary for the normal growth of plants are also highly undesirable in the process of thermal conversion, as they can contribute to negative impacts on furnaces, heat exchangers, reactors, emission control equipment, turbines, or other boiler equipment [18,19].

Chemical composition is also dependent on the genotype, harvest time, location, and fertilization. Currently, delayed harvesting is effectively used as an element to improve the quality parameters of combustion, although this is carried out at the expense of the dry matter yield [20] due to the high leaf drop, as the stems are characterized with better combustion properties compared to the leaves [21].

Miscanthus, after three years of planting, is characterized by a stable composition of the biomass and provides an adequate biomass quantity for bioethanol production. Such an early harvest of biomass could play an essential role for the economics of young established plantations [22].

Our hypothesis assumes that fertilization in a dose of 60 kg ha^{-1} N will contribute to changes in the concentration and uptake of selected micronutrients. Research has estimated that particular parts of the plant (rhizomes, stems, and leaves) will be characterized with different Fe, Mn, Zn, and Cu accumulation. Additionally, fertilization at a dose of 60 kg ha^{-1} N caused an increase in the uptake of selected microelements.

The aim of the experiment was to determine the concentration and uptake of microelements in unfertilized and fertilized (60 kg ha^{-1} N) *Miscanthus x giganteus* plants in the years 2014–2016. The chemical analysis included the rhizomes, leaves, and stems.

2. Materials and Methods

2.1. Field Experiment Site and Fertilization Treatments of Miscanthus x giganteus

The field experiment with Miscanthus and nitrogen fertilization (0 and 60 kg ha^{-1} N) was established in the years 2014–2016 at an experimental station belonging to the Wroclaw University of Environmental and Life Sciences, Pawlowice (geographical location 17°7′ E and 51°08′ N in the Lower Silesian Voivodship) (Figure 1).

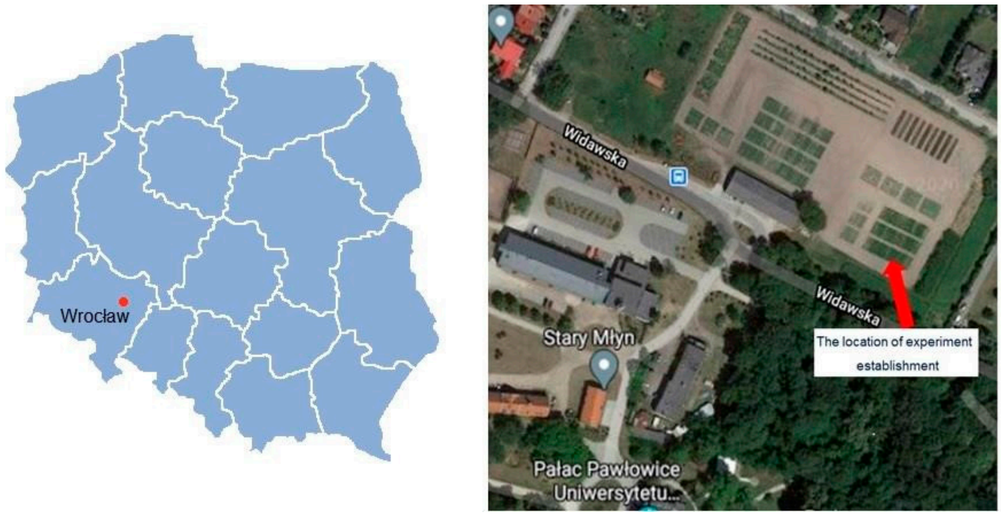

Figure 1. Location of experiment.

The soil was determined to be an alluvial, light, loose sand, and sandy gravel [23]. The chemical composition of the soil and fertilization treatment is presented in Table 1.

Table 1. Soil abundance and fertilization treatment.

Year	pH 1 NKCl	Chemical Composition of the Soil at the Depth of 0–20 cm (mg kg^{-1})							
		Macroelements				Microelements			
		N	P	K	Mg	Fe	Mn	Zn	Cu
2014	5.0	0.580	119.6	114.0	24.3	428	93.4	82.3	1.82
2015	5.0	0.600	119.6	115.3	27.3	461	97.1	79.4	1.69
2016	4.8	0.597	119.7	112.6	26.0	463	95.2	78.5	1.78

Fertilization			
Characteristics	**N**	**P**	**K**
Name	**Ammonium Nitrate**	**Enriched Superphosphate**	**Potassium Salt**
Amount of component (%)	32.0 N	17.4	49.8
Doses (kg ha^{-1})	0 or 60	17.5	50
method	Hand broadcast		
Term of use	March/April		

Miscanthus x giganteus rhizomes (length 10 cm long with the presence of three to six nodes) were planted with 75-cm row spacing and 48 cm spacing in a row (all together on 1 ha accruing 277,778 rhizomes). The size of single plots was 20 m^2. *Miscanthus x giganteus* sampling started from the 30th day of the vegetation period and took place every 30 days until the end of the vegetation period (June, July, August, September, October, November, and ending in December).

At each date of sampling, a plant sample of the aboveground part of the plant and rhizomes was sampled from an area of 0.25 m^2. Plant material was taken gently from an area of 0.25 m^2 by the extraction of the rhizomes from the soil with the whole stems.

The samples for chemical analysis were reduced according to the standard requirements of PN EN 96 ISO 14780:2017-07 [24] (defines methods for the appropriate decreasing in the combined samples to laboratory samples, laboratory samples to sub-samples, general analysis samples, and samples usable for solid biofuels). The dry mass for laboratory samples was examined by air-drying samples at 105 °C for three hours, according to the Polish standard [25].

2.2. Chemical Analysis of Plant Material

The concentration of micronutrients was determined in the laboratory belonging to the Institute of Agroecology and Plant Production and the Department of Plant Nutrition, Wroclaw University of Environmental and Life Sciences, Poland. The concentration of microelements in the aboveground part of the plants was calculated on the basis of the concentration of these elements in the leaves and stem according to the following formula (1).

$$CEA = ((CEL \times \%LS) + (CES \times \%SS))/100 \quad (1)$$

where CEA is the concentration (g kg^{-1}) of the element in the aboveground parts of plant (shoots),

CEL is the concentration (g kg^{-1}) of the element in the leaves,

%LS is the percentage of leaf dry matter (with leaf sheaths) in the shoot dry matter,

CES is the concentration (g kg^{-1}) of the element in the stems, and

%SS is the percentage of stem dry matter in the shoot dry matter.

The micronutrients in the examined parts of plants were determined in the following way: the dry mass and ground plant material in the amount of 5 g was used for the chemical analysis to determine the microelements. Then, plant samples were burnt in a muffle furnace at 450 °C. After obtaining homogeneous colored ash, concentrated nitric acid was added, and then vaporized to dryness on a heating plate. Afterward, the samples were transferred quantitatively to volumetric flasks, completed with 1 M HNO_3 up to a volume 25 mL, and then filtered.

The microelements were determined by atomic absorption spectrophotometry in a flame on a Spectra AA 200 apparatus by Varian. To determine each of the examined microelements, the required conditions concerning wavelength, slot width, and flame height were used. The measurement principle is based on the phenomenon of absorption of radiation of a specific wavelength by free metal atoms. Standard procedures were applied, and the analytical wavelengths [nm] were as follows: Fe—372; Mn—403.1; Zn—140 213.9; and Cu—324.8 [26].

The uptake of selected microelements was calculated as a multiplication of the dry matter yield biomass and chemical concentration of the examined parts of the plant.

2.3. Statistical Analysis

The experiment was conducted with a randomized block design in four replications to test the effects of N fertilization on the concentration and uptake of the microelements of *Mischanthus*. The analysis of variance (ANOVA) and a linear mixed model with repeated measurements was used. Doses of nitrogen fertilizers were assumed to be the dependent variable factor, while the years were the independent variable factor. The nitrogen fertilization rate was selected (0 and 60 kg ha^{-1} N) as the dependent variable factor because it is often used in *Mischanthus* cultivation, whereas years and date of observation were chosen as the independent variable factor. The results of the chemical analysis of *Mischanthus* were analyzed by ANOVA in the Statistica 13.1 program (StatSoft, Poland) showing the variation over fertilization (0 and 60 kg ha^{-1} N) and the years (60 kg ha^{-1} N) of experiment.

3. Results

3.1. The Effect of Nitrogen Fertilization on the Concentration and Uptake of Selected Microelements

Nitrogen fertilization had no significant impact on the concentration of selected microelements in the rhizomes and aboveground parts of plants. Significant differences were found with respect to the concentration in the stems of Mn ($p = 0.0040$) and Cu in the leaves ($p = 0.0397$). The dose of 60 kg ha^{-1} N caused a decrease in the Mn level in the stems. In turn, factors under consideration contributed to increased Cu in the leaves (Table 2).

Table 2. The effects of nitrogen fertilization on the microelement concentration in dry matter (average for the years 2014–2016) (average of microelements concentration under nitrogen fertilization 0 and 60 kg ha^{-1} N in years 2014–2016).

Dose (kg ha^{-1} N)	Fe	Mn	Zn	Cu
	(mg kg^{-1})			
Rhizomes				
0	101	56.4	18.9	3.59
60	99	54.2	19.9	4.0
p value	0.6294	0.5220	0.5553	0.1484
Stems				
0	107	44.4	20.8	3.33
60	105	37.2	19.6	3.48
p value	0.4844	0.0040	0.6050	0.5302
Leaves				
0	89	57.6	18.1	3.56
60	78	62.3	19.1	3.84
p value	0.2691	0.2568	0.3213	0.0397
Aboveground part of plants				
0	113	55.4	21.6	4.01
60	109	53.6	21.5	4.20
p value	0.2575	0.6492	0.9895	0.5537

Data significance was assessed at p value ≤ 0.05 (Two Sample Comparison of Means).

In contrast, the concentration and uptake of microelements was dependent on nitrogen fertilization apart from the Fe uptake by stems. The tested dose of N contributed to an increase in the majority of examined microelements, except for Mn in the stems. The highest examined elements uptake percentage increase was observed in the leaves (Fe—14.8%; Mn—41.8%; Zn—45.8%; and Cu—49.3%), while the least was in the stems (M—15.3%; Zn—12.8; and Cu—12.3%) (Table 3).

Table 3. The effects of nitrogen fertilization on microelement uptake by Mischanthus (average of microelements uptake under nitrogen fertilization 0 and 60 kg ha^{-1} N in years 2014–2016).

Dose (kg ha^{-1} N)	Fe	Mn	Zn	Cu
	(mg m^{-2})			
Rhizomes				
0	126.7	66.6	19.8	3.50
60	138.2	70.9	22.6	4.46
p value	0.0000	0.0009	0.0000	0.0000
Stems				
0	171.6	71.4	30.5	5.29
60	180.6	60.5	34.4	5.94
p value	0.0538	0.0000	0.0000	0.0000
Leaves				
0	48.6	35.2	10.7	2.03
60	55.8	49.9	15.6	3.03
p value	0.0000	0.0000	0.0000	0.0000
Aboveground part of plants				
0	197.4	96.1	36.5	6.63
60	214.0	101.3	45.2	8.17
p value	0.0005	0.0000	0.0000	0.0000

Table 3. *Cont.*

Dose (kg ha^{-1} N)	Fe	Mn	Zn	Cu
	(mg m^{-2})			
Rhizomes and aboveground parts				
0	324.1	162.7	56.3	10.12
60	352.2	172.2	67.8	12.64
p value	0.0000	0.0004	0.0000	0.0000

Data significance was assessed at *p* value ≤ 0.05 (Two Sample Comparison of Means).

3.2. Microelement Concentration and Uptake in Different Parts of *Miscanthus x giganteus*

Significant changes of the microelement contents in rhizomes were observed. The lowest concentrations of selected elements in this part of the plant were found in the third year of the field experiment, apart from Mn. In turn, the lowest levels of micronutrient concentrations in stems were noted in the second year of research. Similarly, the minimum Mn concentration (p = 0.0049) in the leaves was determined in the third year (Table 4).

Table 4. The microelement concentrations in dry matter in different parts of *Miscanthus x giganteus* 2014–2016 (average of microelements concentration with dose of 60 kg ha^{-1} N in the years of experiment).

Dose (kg ha^{-1} N)	Fe	Mn	Zn	Cu
	(mg kg^{-1})			
Rhizomes				
2014	129	85.3	26.2	4.78
2015	118	32.7	21.2	4.79
2016	50	44.5	11.9	2.57
Mean	90	54.2	19.9	4.05
p value	0.0000	0.0000	0.0066	0.0104
Stems				
2014	175	37.9	15.5	4.20
2015	39	20.4	17.0	2.83
2016	100	53.2	26.4	3.40
Mean	105	37.2	19.6	3.48
p value	0.0000	0.0000	0.0083	0.0260
Leaves				
2014	87	78.5	14.2	3.19
2015	72	61.9	25.6	4.18
2016	75	46.5	17.7	4.15
Mean	78	62.3	19.1	3.84
p value	0.0679	0.0049	0.0030	0.0892
Aboveground part of plants				
2014	159	56.7	17.4	4.10
2015	62	46.9	23.0	4.39
2016	107	57.2	23.9	4.10
Mean	109	53.6	21.5	4.20
p value	0.0000	0.3224	0.0725	0.8939

Data significance was assessed at *p* value ≤ 0.05 (One-Way ANOVA).

On the other hand, the lowest Zn and Cu concentration in the leaves was stated in 2014. Significant changes in the Fe (except leaves) and Zn concentrations (aboveground parts of plants) were noted in the years of research. No significant differences were also noted in the case of Cu in the leaves and aboveground parts of plants. The microelement concentrations in the rhizomes, stems, leaves, and shoots in descending order were as follows: Fe > Mn > Zn > Cu. The proportion of the Fe:Mn:Zn:Cu levels in the rhizomes and the aboveground part of plants were similar. The average ratio for the Fe:Mn:Zn:Cu was as follows: 1.0:0.5:0.2:0.04. A higher Mn concentration in the leaves compared to the stems was found (Table 3).

On the basis of the research, significant differences were found in the microelement uptake in all examined parts of the plants in the experiment. Rhizomes accumulated the least amount of Fe, Zn, and Cu in the third year of research. In turn, the least uptake of microelements by the stems was observed in the second year of study, except for Cu. The highest uptake of Fe, Mn and Cu by the aboveground part of plants was found in the first year of research. Similar to the microelement concentrations, uptake by all examined part of plants in descending order was as follows: Fe > Mn > Zn > Cu (Table 5).

Table 5. Microelement uptake in dry matter in different parts of *Miscanthus x giganteus* in 2014–2016 (average of microelements uptake with dose of 60 kg ha^{-1} N in the years of experiment).

Dose (kg ha^{-1} N)	**Fe**	**Mn**	**Zn**	**Cu**
	(mg m^{-2})			
	Rhizomes			
2014	175	112.7	32.4	5.32
2015	161	37.7	22.4	4.96
2016	78	62.3	13.0	3.10
mean	138	70.9	22.6	4.46
p value	0.0000	0.0000	0.0000	0.0000
	Stems			
2014	320	74.9	31.6	8.03
2015	76	28.5	28.2	5.03
2016	146	79.0	43.5	4.75
mean	181	60.5	34.4	5.94
p value	0.0000	0.0000	0.0000	0.0000
	Leaves			
2014	61	62.1	10.7	2.34
2015	55	47.8	20.3	3.18
2016	51	39.8	15.7	3.56
mean	56	49.9	15.6	3.03
p value	0.0000	0.0000	0.0000	0.0000
	Aboveground part of plants			
2014	341	121.6	38.1	9.20
2015	121	74.0	44.7	7.75
2016	181	108.1	52.7	7.57
mean	214	101.3	45.2	8.17
p value	0.0000	0.0000	0.0000	0.0000
	Rhizomes and aboveground parts			
2014	516	234.4	70.5	14.5
2015	281	111.7	67.1	12.7
2016	259	170.6	65.7	10.7
mean	352	172.2	67.8	12.6
p value	0.0000	0.0000	0.0000	0.0000

Data significance was assessed at p value ≤ 0.05 (One-Way ANOVA).

3.3. Seasonal Variations in Micronutrients Content and Uptake

Analyzing the research results, decreasing concentrations of the examined micronutrients were found in the stems and aboveground parts of the plants during the vegetation period. A similar tendency was observed in the case of Fe and Cu in the leaves. A decrease in the Cu and Zn concentration in the rhizomes was found during the whole vegetation period and, in particular, in June. In turn, a clear increase in the Fe concentration in the rhizomes was found (Figures 2–5). Nitrogen fertilization contributed to a decrease in the zinc content in the rhizomes at the beginning of the vegetation period, and then the differences were insignificant (Figure 4).

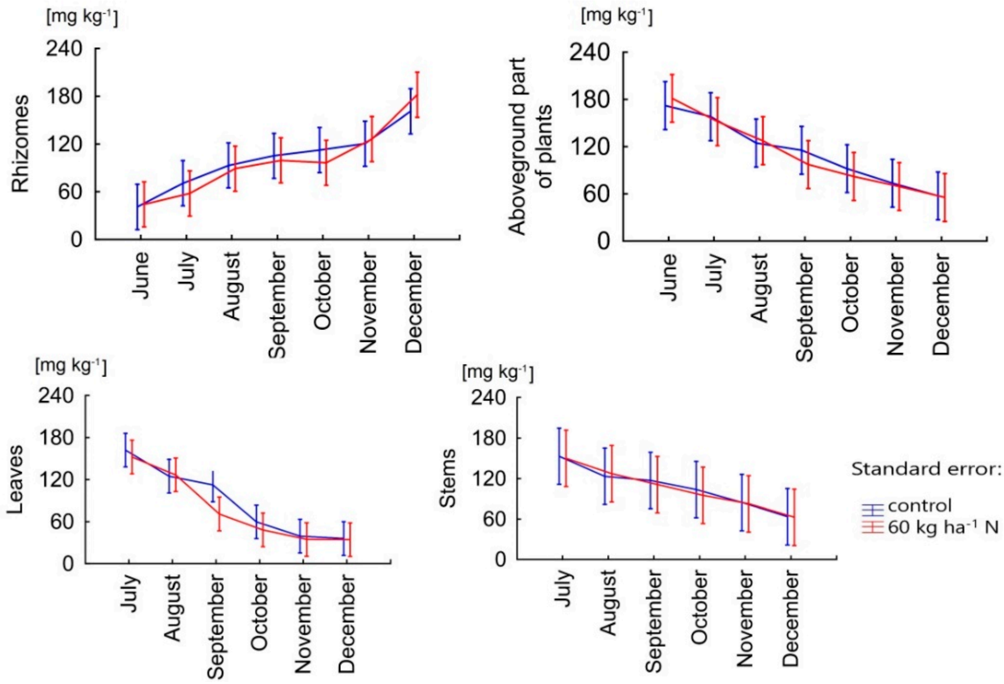

Figure 2. The iron concentration in the examined parts of *Miscanthus x giganteus* (average for 2014–2016).

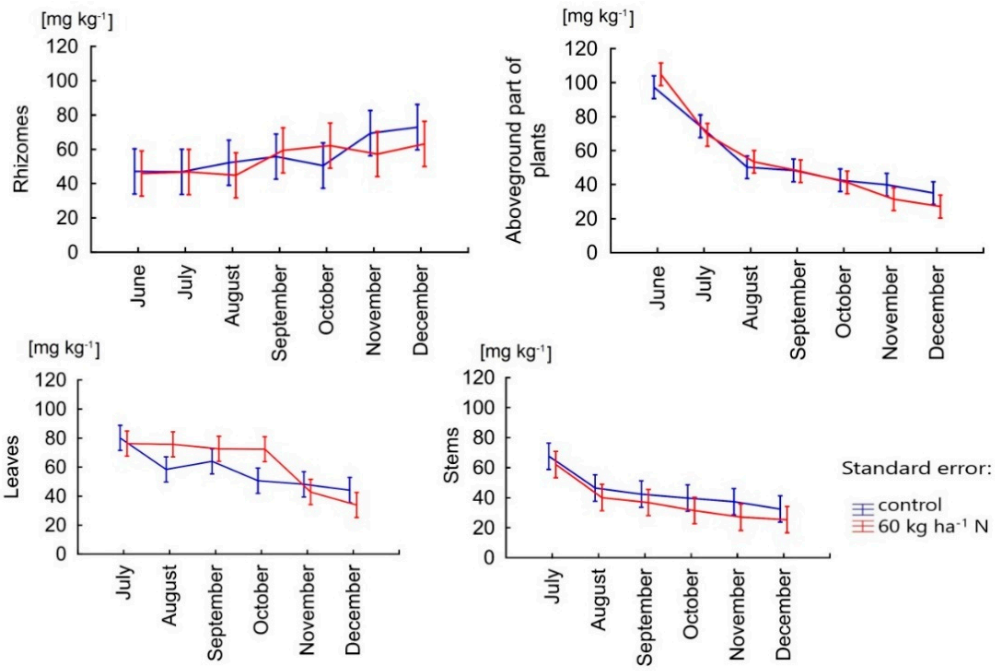

Figure 3. The manganese concentration in the examined parts of *Miscanthus x giganteus* (average for 2014–2016).

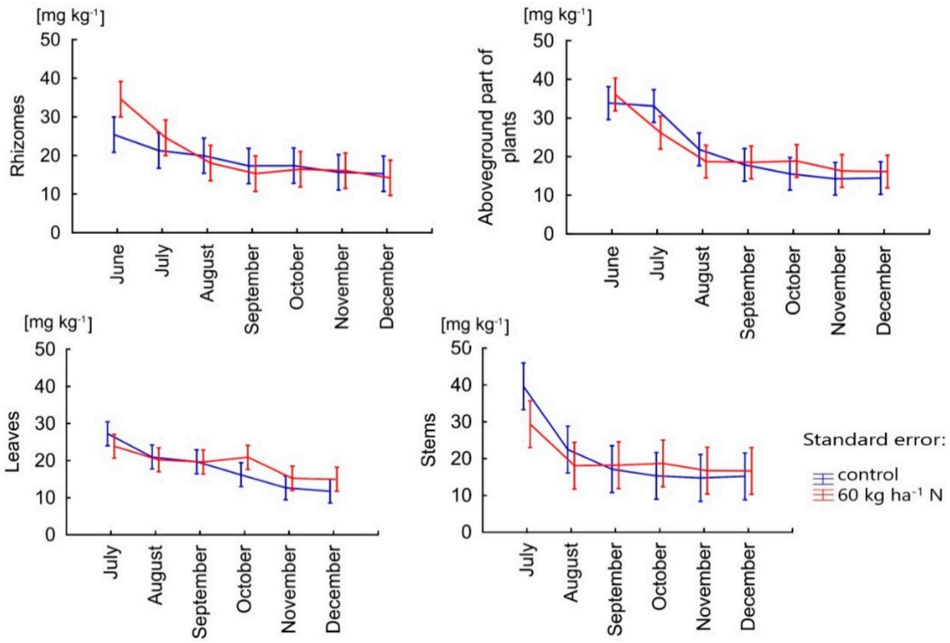

Figure 4. The zinc concentration in the examined parts of *Mischanthus* (average for 2014–2016).

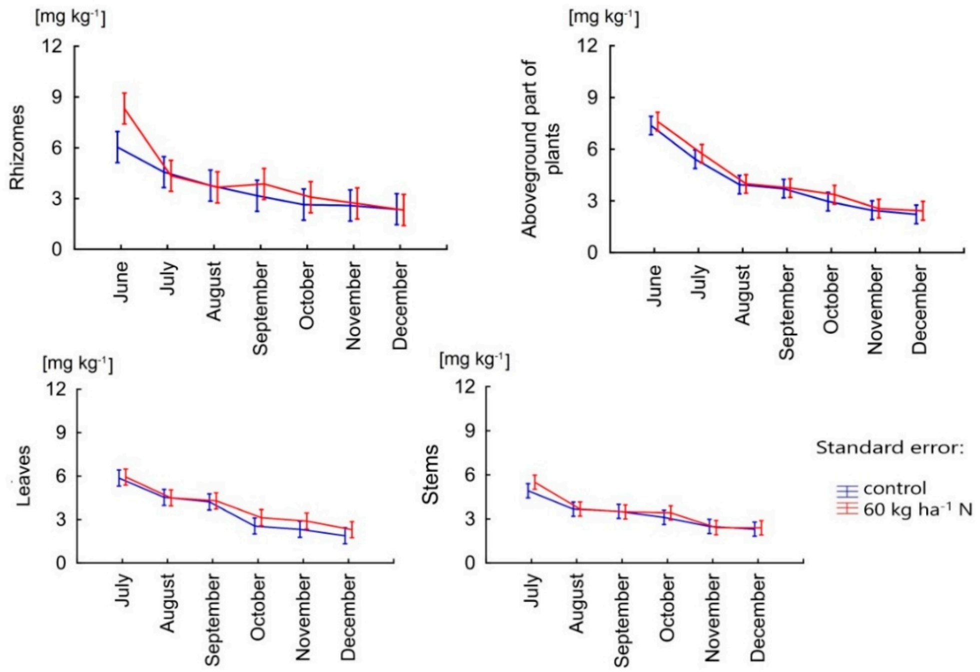

Figure 5. The copper concentration in the examined parts of *Miscanthus x giganteus* average for 2014–2016).

The results demonstrated an increase in Fe, Mn, and Zn on the whole in unfertilized plants (Figures 6–8). Increase uptake of all microelements by the aboveground parts of plants and stems was found until October. Figures 8 and 9 show the significant values of the differences in the Zn and Cu accumulation in the subsequent months of observation for both variants of fertilization: control and the dose of 60 kg ha^{-1} N in the leaves. In turn, we found that Fe and Mn increased in uptake in the rhizomes during the vegetation period (Figures 6 and 7). Nitrogen fertilization caused an increase in the Zn and Cu uptake by rhizomes at the beginning of the vegetation period.

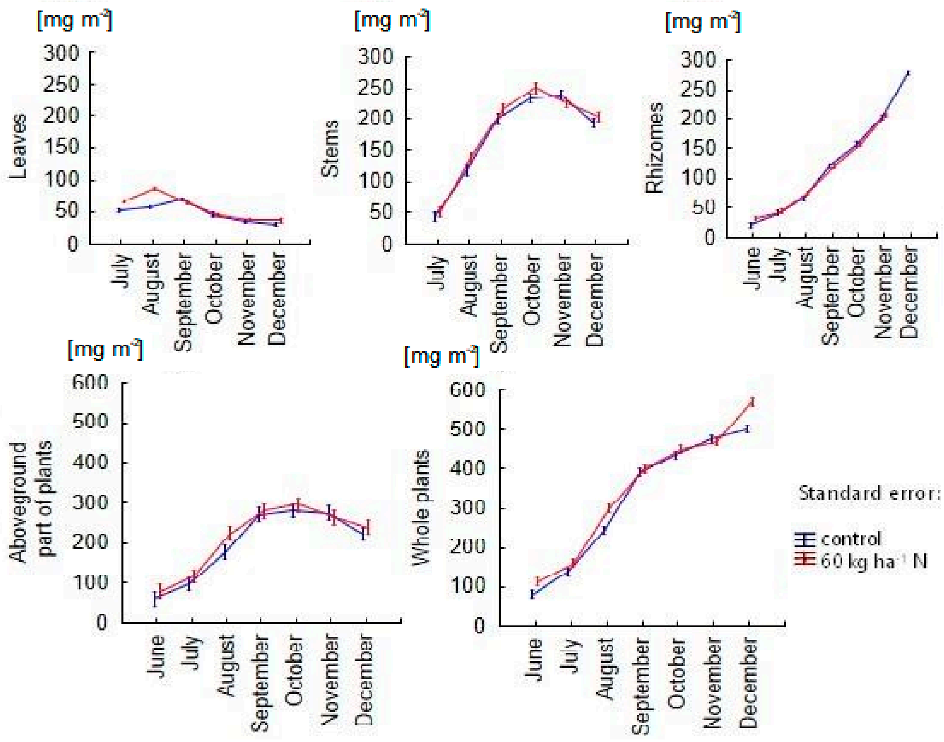

Figure 6. The iron uptake in the examined parts of *Miscanthus x giganteus* (average for 2014–2016).

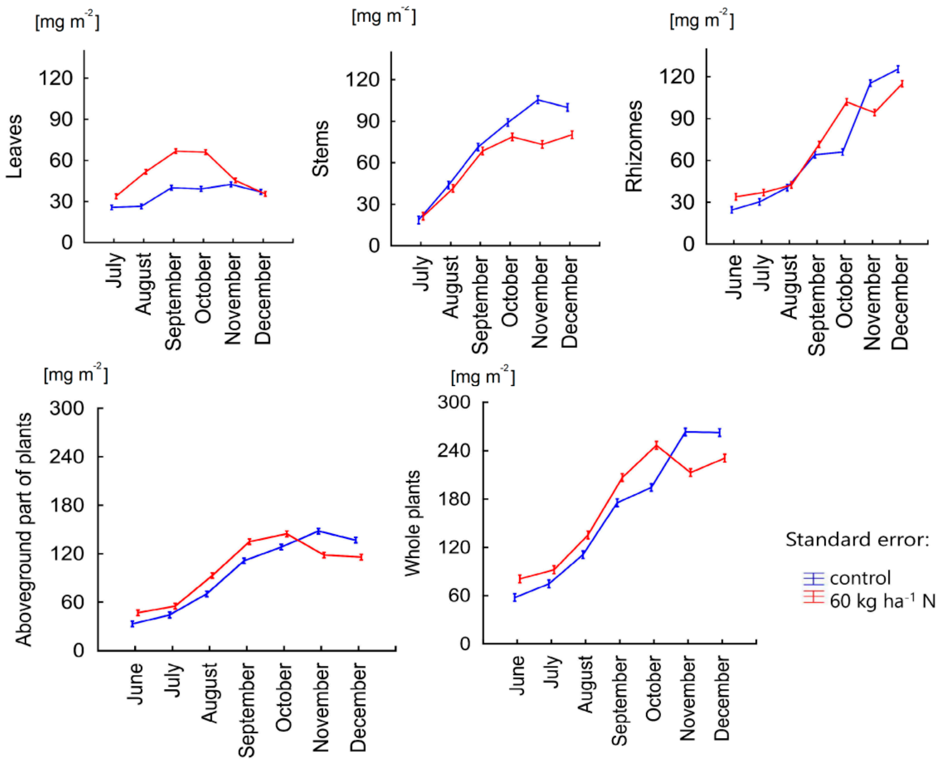

Figure 7. The manganese uptake in the examined parts of *Miscanthus x giganteus* (average for 2014–2016).

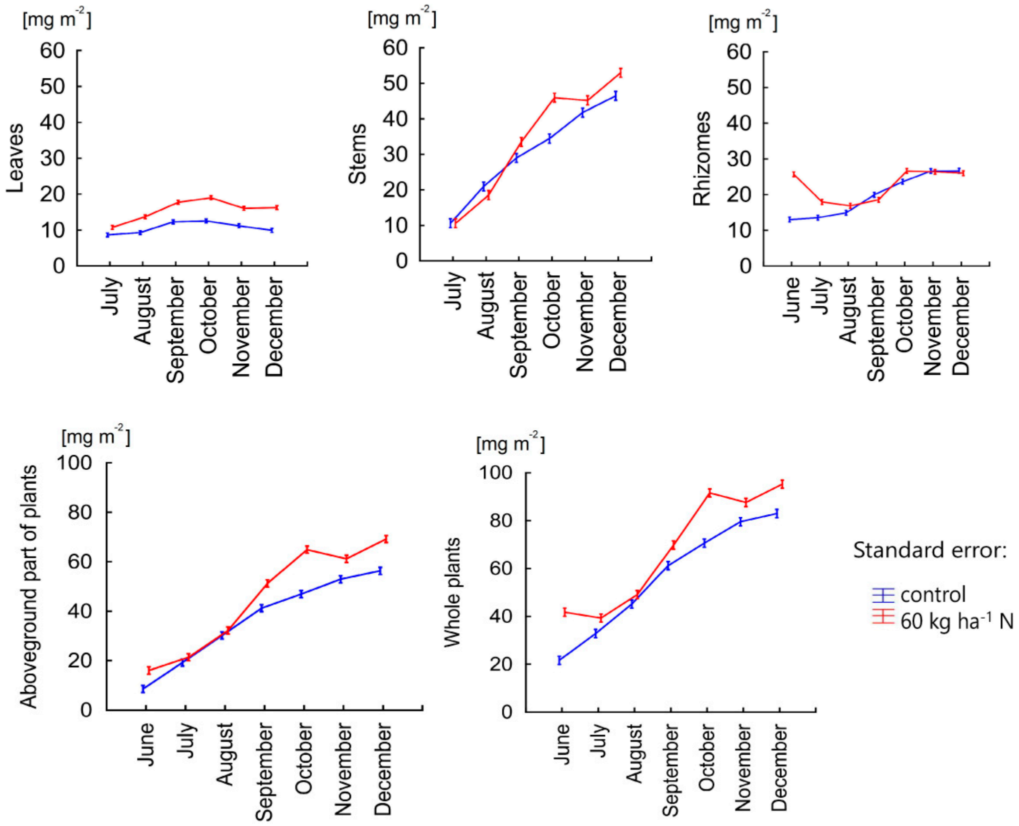

Figure 8. The zinc uptake in the examined parts of *Miscanthus x giganteus*) (average for 2014–2016).

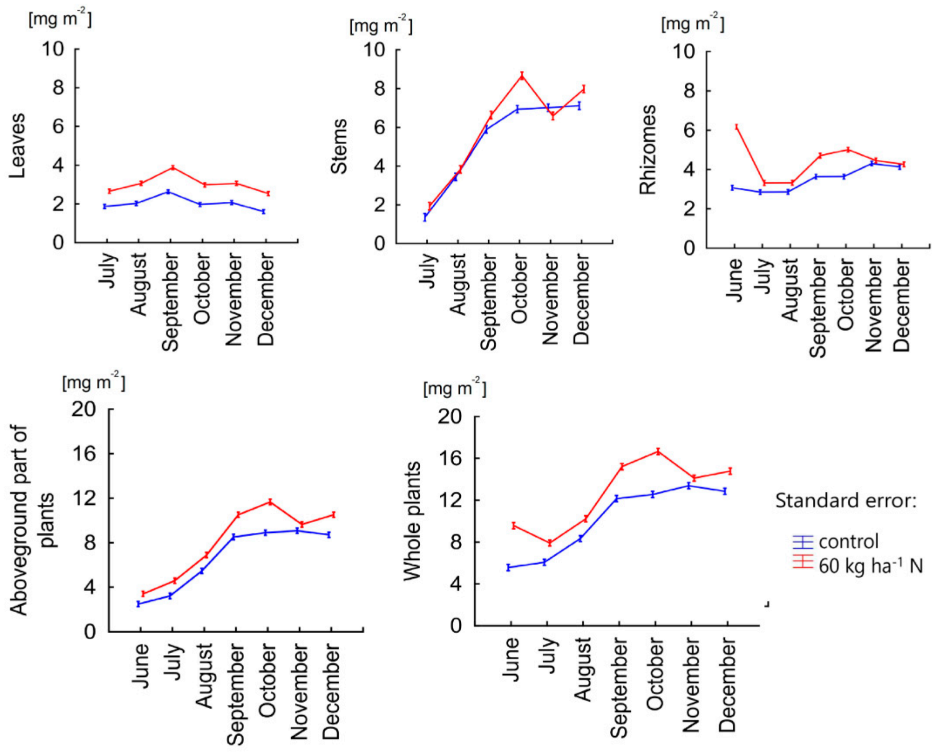

Figure 9. The copper uptake in the examined parts of *Miscanthus x giganteus* (average for 2014–2016).

4. Discussion

Nutrients are accumulated in different parts of plants and the concentrations within the plant vary depending on many external factors, such as the genotype [27], date of harvest, year of cultivation [28–30], and fertilization [28,30]. On the basis of Kalembasa and Malinowska's (2009) [31] research, we concluded that the fertilization (NPK) and harvest date could influence the concentration of heavy metals and microelements, such as cadmium, lead, nickel, and zinc, in the biomass.

On the basis of Smith and Slater's research, we found a greater Cu concentration under inorganic N fertilization (0.9–1.8 ppm) compared to the control (0.7–1.1) in the aerial parts of plants [32]. In our research, nitrogen fertilization had no effect on the Cu concentration in the aboveground parts of plants (p = 0.5537). The impact of this factor was stated in rhizomes only at the beginning of vegetation (Figure 5).

However, in certain experiments, *Miscanthus x giganteus* fertilized with sludge was characterized by two- or three-times higher Fe and Mn uptake compared to the control [31]. In our research, the trace element uptake was significantly dependent on the nitrogen fertilization (except Fe in the stems), and this was differentiated from the weather conditions in the years of experiment.

The concentrations of iron, manganese (except aboveground parts of plants), zinc, c and copper (except the stems and shoots) depended on the years of our experiment (Table 4). In the studies of Kocoń and Jurga (2017) [11], the highest microelement concentrations in plant tissues were observed in the first year of research and gradually decreased in the next years. Similar results were recorded in other studies [7].

There were no significant differences in the mineral composition (Fe, Mn, and Zn) in the aerial plants of *Miscanthus x giganteus,* similar to the Smith and Slater study [32] (Tables 3 and 4). In their research, the concentration of mineral elements was as follows: Fe (36.6–502); Mn (86.9–162); Zn (10.9–30.1); and Cu (0.7–2.2) ppm, and, in our research, respectively, the average values in mg kg^{-1} were: Fe—111; Mn—54.5; Zn—21.5; and Cu—4.11 (Tables 3 and 4).

In our study, the Mn concentration was lower and comparable with the Zn value based on the experiments conducted by the above authors. In turn, in the rhizomes, the Mn concentration was similar in both studies.

Different results were obtained in the case of Fe in the rhizomes. In our research, the Fe value ranged from 52 to 135 mg kg^{-1}, while in Smith and Slater (2010), they ranged from 198 to 1808 ppm [32] (Table 4). A great range of Fe concentrations in the above-mentioned studies may have resulted from different types of fertilizers (organic and inorganic) and doses (50, 100, and 150 kg ha^{-1} N). A similarity was found in the case of the Cu concentration. Dželetović and Glamočlija [15] applied 0.325 g pot^{-1} N and obtained similar Zn concentrations (18.48 mg kg^{-1}) in rhizomes as in our research.

Greater levels of Fe, Mn, Cu were found in their research in comparison to our study (Table 4). It may have resulted from a low and medium abundance of these elements in the soil in our research. Similar Cu concentrations in the rhizomes were found in our research (2.61–4.78 mg kg^{-1}) and Stypczyńska et al.'s [33] study (3.6 mg kg^{-1}). In contrast, the Cu concentration in the leaves and stems was higher in their research. In turn, the Zn concentration in all organs was greater in our study (Table 4).

According to Saletnik et al. (2018) [7], the average total concentration of the examined elements in the biomass of *Miscanthus x giganteus* in the first year of cultivation was determined as a chain of decreasing concentration value Fe > Mn > Zn. Kalembasa (2009) [31] also confirmed higher Fe compared with the Mn concentration in the biomass of *Miscanthus sacchariflorus*. In our research, the following decreasing value concentration was observed: Fe > Mn > Zn > Cu. There is little information connected to the microelement flow through *Miscanthus x giganteus* plants. In Liu et al. (2014) [34], the Fe concentration in rhizomes was higher in August than in June as in our research. In turn, the lowest concentration of Mn in their study was found in June. Our research did not prove the above statement. All examined microelements concentrations in the aboveground parts of *Miscanthus x* *giganteus* declined through the whole growth season. It might be explained by the dilution effect. During the fast growth of plants, more carbon, oxygen and hydrogen molecules are present, relative to the mineral elements. Detailed information is provided by the Jarrel and Beverly (1981) research [35].

5. Conclusions

Nitrogen fertilization had minor effects on the concentrations of the microelements in *Miscanthus x giganteus* dry matter yield. The highest concentrations of microelements in the aboveground parts of *Miscanthus x giganteus* were found in the third (Mn, Zn, and Cu) and the first year of research (Fe).

In the experiment, *Miscanthus x giganteus* accumulated the highest concentrations in the order Fe > Mn > Zn > Cu. Most heavy metals were accumulated in the aboveground parts with a predominance of the stems.

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