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CHANGES IN THE CONTENT, UPTAKE AND BIOACCUMULATION OF MACRONUTRIENTS IN GENOTYPES OF *MISCANTHUS* GRASS. POSSIBILITIES OF USING ASH FROM *MISCANTHUS* BIOMASS

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ABSTRACT

The aim of this study was to determine the changes in the content, uptake and bioaccumulation of macronutrients in biomass of six genotypes of *Miscanthus* grass over a period of three years of cultivation. The possibilities of using ash from *Miscanthus* biomass were also assessed. The field experiment was conducted in the three years in central-western (Poznań) and in the central-northern (Elbląg) part of Poland. Six genotypes of *Miscanthus* grasses were grown: MG1 (M1) *Miscanthus giganteus* (as standard), MG2 (M2) (plant code M 116) - hybrid *M. sinensis* (2x) x *M. sacchariflorus* (4x), MS3 (M3) (code M 105)- hybrid as above, MS4 (M4) (code M 114)- hybrid as above, MS5 (M5)- *M. sinensis*, obtained from hybrids 92M012 2012, MS6 (M6)- *M. sinensis*, obtained from hybrids 92M017 2004. Deep ploughing and harrowing was performed and fertilisation at N-80; P-100 and K-120 kg ha^{-1}. Biomass of genotypes of *Miscanthus* grass grown in diverse soil and climatic conditions in Poland during three years contained the largest amount of carbon, hydrogen, silica, calcium, followed - in a decreasing order - by K, Mg, N, P, S and Na. The content of SiO_2, Ca, K and N was significantly differentiated in biomass of consecutive genotypes. The *Miscanthus* biomass yield has been found to be significantly negatively correlated with the total content of Ca, K, Mg and Na. High content of alkaline elements in crude ash obtained from biomass *Miscanthus* testifies to the possibility of its use in agriculture as liming agent.

KEYWORDS:
Yield, macronutrients, uptake, bioaccumulation, ash, *Miscanthus*

INTRODUCTION

Introduction of new energy plants into cultivation is stimulated by progressing depletion of sustainable resources of fossil fuels in Poland and world-wide [1, 2]. According to the assumptions of the EU Climate Package of 17/12/2008 member states by 2020 should reach a 20% share of energy from renewable sources. The waste product after burning biomass are ashes. On average, from the combustion of 1 ton of *Miscanthus's* biomass 55 kg of ash are obtained. In agriculture, plant ashes are frequently used as mineral fertilisers [3, 4]. The fertilisation value of ash depends on the type of combusted material, the type of plant and their fertilisation [5]. A study carried by [2, 6, 7, 8,] indicated an increase in alkalinity, and in the level of macro- and micronutrients in the soil. Indicated the possibility for using the ash from heat and power stations for composting sewage sludge and the obtained composts for soil reclamation [9]. An analysis of the consumption of nutrient uptake and biomass growth during the vegetation period will help to develop principles of fertilisation of the crop, since studies dealing with the issue have been scarce [10, 11]. Being an energy crop, *Miscanthus* is harvested during the stage of its technological ripeness, usually beginning in October [12]. It is recommended as the best period for studies of the uptake and accumulation of nutrients in the plant biomass. This is the time when there is a balance between the aboveground and the underground parts (mainly rhizomes), to which a certain amount of nutrient is supplied [13]. A delay in harvest can lead to its lodging caused by precipitation (rain, and especially snow) and in washing considerable amounts of potassium and magnesium from its leaves [14]. The findings of other studies [15, 16] have shown that more calcium is accumulated in *Miscanthus* biomass at the end of its growth, which probably prevents the plants from lodging. With shoots up to 2.5-3.0 m and with heavy leaves, *Miscanthus* stalks must be strong and flexible [17]. A high content of calcium in its biomass (like in other ash-forming components) increases the amount of ash, which is important in generating energy from this crop [2, 18, 19, 20]. In terms of its physiological activity, magnesium is similar to calcium, and - in many cases - antagonistic to potassium. According to [21], the content of potassium in biomass harvested in February or March was smaller by approx. 50% than in that harvested in October. The physical and chemical properties of ash from plants lead to the improvement of the structure of heavy soils and the fertilizer components contained in them are easily absorbed by plants [22].

The aim of this study was to determine the changes of the content, uptake and bioaccumulation macronutrients in biomass of six genotypes of *Miscanthus* grass over a period of three years of cultivation. The possibilities of using ash from *Miscanthus* biomass were also assessed.

MATERIALS AND METHODS

The field experiment was conducted in the three years in central-western (Poznań) and in the central-northern (Elbląg) part of Poland. Six genotypes of *Miscanthus* grasses were grown [17]; most of them were obtained from TINPLANT Gmbtl Klein Wanz (Germany): MG1 (M1) *Miscanthus giganteus* (as standard), MG2 (M2) (plant code M 116) - hybrid *M. sinensis* (2x) x *M. sacchariflorus* (4x), MS3 (M3) (code M 105)- hybrid as above, MS4 (M4) (code M 114)- hybrid as above, MS5 (M5)- *M. sinensis*, obtained from hybrids 92M012 2012, MS6 (M6)- *M. sinensis*, obtained from hybrids 92M017 2004.

Rhizomes of the genotypes were planted in May 2007 (10.000 plants per 1 ha), in two cities: Poznań (16°55' E, 25°25' N) on experimental fields of IGR-PAN, on sandy lessive, formed from light sandy loam [23]. According to the [24] *ochric luvisol*. It was a quality class IVa soil, with pH_{KCl} 6.82, total carbon content 9.81 g kg^{-1} and nitrogen content of 0.80 g kg^{-1} and medium abundance of available forms of phosphorus, potassium and magnesium; Elbląg (19°23'E, 53°47' N) – the experiment was carried out on typical brown eutrophic soil formed from medium loam [23]. According to the [24] *ochric cambic*. It was a quality class IVa soil, with pH_{KCl} 5.12, total carbon content 8.54 g kg^{-1} and nitrogen content of 0.71 g kg^{-1} and medium abundance of available forms of phosphorus, potassium and magnesium.

Deep ploughing and harrowing was performed and fertilisation at N-80; P-100 and K-120 kg ha^{-1} as ammonium nitrate (34% N), triple superphosphate (20% P) and potassium salt (50% K) were applied before the plantation was set up.

The three-year experiment was carried out with no plant protection measures and the growth and development of the plants was observed each year. The biomass was harvested during the phase of technological ripeness, in March of each consecutive year.

Samples for tests were taken from the soil and plant material, air-dried and then dried at 105°C in order to determine dry weight. Total carbon, hydrogen and nitrogen by dry combustion using auto analyser series II CHNS/O 2400, Perkin Elmer. Plant samples were ground and analysed at a chemical laboratory. The organic mass was dry-oxidised (in crucibles made of semi-vitreous chinaware material) at 450°C for 6 hours with a gradient increase in the temperature to the desired level. The ash was cooled down and poured over with 5 cm^3 HCl in order to decompose the carbonates formed in the oxidation process and to form chlorides of the elements present in the ash. Excess HCl was evaporated on a sand bath. Silica precipitated in the process. HCl (10 cm^3 10%) was added to the crucible again and the contents was transferred through a hard filter (to separate the silica) to a volumetric flask. The solution was made up to mark and the flask contents were used as the working analytic solution, in which the total content of Ca, Mg, K and Na was determined by ICP-AES, on a Perkin Elmer 3200RL apparatus. The assay accuracy was compared with the company's reference standard solutions. The bioaccumulation factors were calculated as the quotient of the content of an element in the plant to its content in the soil.

The experimental data were analysed with uni- and multivariate statistical methods. In the first step a three factor analysis of variance was applied to examine differences between genotypes of *Miscanthus* grass with respect to the yield and the presence of macronutrients in the biomass of six clones. Much attention has been devoted to comparing genotypes due to their main effects, stability and adaptability to environments. Methods of statistical analysis of a series of experiments with genotypes, conducted at places over successive years. The analysis is based on the mixed linear model, which is constructed under assumption that the choice of the experimental site within each environment is random. This model allows to test the general hypothesis of no genotype x environment interaction HGE hypothesis of no genotype by environment interaction within places (Poznań, Elblag) and years (1^{st}, 2^{nd}, 3^{rd}) and specially interesting hypotheses concerning main effects of individual genotypes, their interaction with environments and regression of interaction for genotypes on environment. Important information about sensitiveness of genotypes to environment were obtained by the regression analyses. In addition figures illustrating main effects and regression lines of interaction for all studying genotypes on environmental deviations have been made for the yield and for each of the chemical elements C, N, H, SiO_2, P, K, Ca, Mg, S and Na. Finally, in order to investigate the relationship between K, Ca, Mg and Na accumulation by

TABLE 1
The total content of nutrients (g kg^{-1}) in soil depending on the applied experimental factors

	Treatment	C	N	P	K	Ca	Mg	S	Na
Genotypes	M1	9.04	0.76	0.51	0.58^a	4.53^b	0.61	0.38	0.12
	M2	9.58	0.73	0.62	0.72^b	4.42^b	0.52	0.42	0.10
	M3	9.72	0.84	0.75	0.87^b	3.28^a	0.48	0.40	0.09
	M4	9.67	0.79	0.80	0.84^b	3.11^a	0.52	0.38	0.11
	M5	9.79	0.77	0.68	0.78^b	3.04^a	0.46	0.45	0.10
	M6	9.80	0.82	0.72	0.82^b	3.24^a	0.47	0.40	0.13
	p	n.s.	n.s.	n.s.	*	*	n.s.	n.s.	n.s.
Place of research	Poznań	9.81	0.82	0.72^b	0.38^a	5.22^b	0.67^b	0.45	0.11
	Elbląg	8.54	0.71	0.45^a	0.91^b	2.92^a	0.45^a	0.42	0.13
	p	n.s.	n.s.	*	*	*	*	n.s.	n.s.
Genotypes x place of research		n.s.	n.s.	n.s.	*	*	n.s.	n.s.	n.s.

a, b - values marked with the same letter do not differ significantly at $p \leq 0.05$, * - the level of significance for $p \leq 0.05$, n.s.- non significant

Miscanthus genotypes and the *Miscanthus* biomass yield, the multiple regression analyses were applied.

The following was the rainfall in Poznań during the growing season (April-October) in the study years (1st, 2nd, 3rd): 350 mm, 320 mm and 409 mm; the rainfall in Elbląg was as follows: 530 mm, 400 mm and 548 mm. The average temperature in the months of the growing season was: in Poznań 15.2^{0}C, 14.8^{0}C and 15.3^{0}C; in Elbląg 15^{0}C, 14.3^{0}C and 14.8^{0}C.

RESULTS AND DISCUSSION

The genotypes, experiment sites as well as interaction between them significantly differentiated the total content of potassium and calcium in the soil (Table 1). Experiment sites significantly differentiated in the content of potassium, phosphorus, calcium and magnesium. The total content of the analysed elements in the soil was at a medium level. Their contents can be presented in the following series of decreasing values: C > Ca > N > P > K > Mg > S > Na.

The dry weight of the *Miscanthus* biomass (Table 2a and 3) was highly differentiated by the genotypes under study, the experiment site, study years and the interaction of these factors. The yield of *Miscanthus* grass in Elbląg was higher by 18% than in Poznań. Regardless of the experiment site, the highest yield was obtained for the genotype marked as M1 and M2, and the lowest was for the genotype marked as M5 and M6. The study years also had a great effect on the yield of the clones under study expressed as the biomass weight. A similar yields of *Miscanthus* aboveground biomass was observed [25] on gleysol. In the three years of the experiment, a relationship was observed between the yield in the experiment years and the dry weight yield of the genotypes under study (r = - 0.66) and between the yield obtained in the experiment years and the experiment sites (r = 0.99). The content of nitrogen and silica of the *Miscanthus* grass under study (Table 2a) was significant differentiated depending on the genotypes, experiment site and study year (Table 3). Significantly more nitrogen was found in *Miscanthus* grass grown in Elbląg (mean 7.90 g kg^{-1} D.M.). The content of silica in biomass harvested in Poznań was significantly higher (by 60%) than in that harvested in Elbląg. The content of the remaing elements in the *Miscanthus* biomass presented in Table 2a did not differ significantly from the influence on the studied factors. The mean potassium content (Table 2b) in *Miscanthus* grass biomass at all sites was 15.66 g kg^{-1} D.M. ranging from 5.16 to 40.14 g kg^{-1} D.M. The genotypes, experiment sites and study years as well as interaction between them significantly differentiated the content of potassium in *Miscanthus* biomass. An analysis of the potassium content in biomass of the clones under study revealed the highest level (20.88 g kg^{-1} D.M.) in biomass of the M1 clone, and the lowest level (12.56 g kg^{-1} D.M.) in biomass of M6. The content of potassium in *Miscanthus* biomass harvested in Elbląg was twice as high as in Poznań (20.55 and 10.76 g kg^{-1} D.M., respectively) – the opposite to the content of calcium (Elbląg: 26.08, Poznań: 35.47 g kg^{-1} D.M.). Such a high content of this element was caused by a high content of calcium in soil in Poznań, which restricts potassium uptake, because these two elements are antagonists in uptake by a plant. Potassium does not form strong bonds in a plant like calcium and it can move with water within it. Potassium was transferred from the over ground parts to rhizomes at the end of the growing season and it was washed out by rain, mainly from leaves, which is a consequence of increase in their weight with extending cultivation period. The content of potassium in *Miscanthus* biomass was consistently balanced by the interaction of the experiment site and study years. The calcium content (Table 2b) in biomass of the six *Miscanthus* genotypes under study varied and ranged from 17.36 to 47.83 g kg^{-1} D.M., mean 30.78 g kg^{-1} D.M. The content of this element was affected significantly by the experimental factors: genotypes, conditions at the experiment site, study years and their interaction. The significantly highest content of calcium was determined in biomass of M4 (34.24 g kg^{-1} D.M.) and M6 (33.52 g kg^{-1} D.M.), less in M1, M2 and M5 (32.02, 29.91, 29.63 g kg^{-1} D.M., respectively) and the least in M3 (25.34 g kg^{-1} D.M.). Significantly more calcium was found in *Miscanthus* grass grown in Poznań (mean 35.47 g kg^{-1} D.M.), and in Elbląg (26.08 g kg^{-1} D.M.), where the soil pH was lower. The statistical calculations revealed a significant correlation between the *Miscanthus* yield and the calcium content in the plant in consecutive experiment years (r = -0,99). The content of magnesium in biomass of the *Miscanthus* genotypes under study (Table 2b) was highly differentiated depending on the experiment site and study years (Table 3). Significantly more magnesium was found in *Miscanthus* biomass harvested in Elbląg (mean 6.28 g kg^{-1} D.M.). The largest amounts of magnesium were found in biomass of M1 and M2 (6.31 and 5.96 g kg^{-1} D.M.) and the least in M5 (4.66 g kg^{-1} D.M.). Aerial parts of grasses *Miscanthus sacchariflorus, Miscanthus sinensis and Miscanthus giganteus* accumulated mostly calcium, potassium and magnesium [26]. The content of sodium in biomass harvested in Elbląg was significantly higher (by 71%) than in that harvested in Poznań (Table 2b). Significant differences in the sodium content in *Miscanthus* biomass for the experiment site and study years were observed in Elbląg. The content of sodium in *Miscanthus* biomass was differentiated significantly by the experiment site, study years and their interaction (Table 3).

TABLE 2a
The yields of biomass (t ha^{-1} D.M.) and macronutrients content in biomass genotypes of *Miscanthus* grass (g kg^{-1} D.M.)

	Treatment	Yield	C	N	H	SiO_2
Genotypes	M1	16.59[a]	429.9	6.60[b]	62.90	45.25[b]
	M2	18.94[a]	434.0	6.85[b]	63.15	39.80[a]
	M3	12.64[b]	431.7	5.25[a]	61.75	36.05[a]
	M4	10.74[b]	430.6	5.30[a]	62.05	38.50[a]
	M5	8.24[c]	430.0	6.55[b]	62.00	45.30[b]
	M6	9.19[c]	428.7	6.65[b]	61.90	41.50[b]
	p	*	n.s.	*	n.s.	*
Place of research	Poznań	11.71[a]	429.6	4.50[a]	62.4	50.50[b]
	Elbląg	13.76[b]	432.0	7.90[b]	62.1	31.50[a]
	p	*	n.s.	*	n.s.	*
Genotypes x place of research		*	n.s.	*	n.s.	*

a, b, c - values marked with the same letter do not differ significantly at p ≤ 0.05, * - the level of significance for p ≤ 0.05, n.s.- non significant

TABLE 2b
Macronutrients content in biomass genotypes of *Miscanthus* grass (g kg^{-1} D.M.)

	Treatment	P	K	Ca	Mg	S	Na
Genotypes	M1	0.46	20.88[b]	32.02[b]	6.31	0.66	1.10
	M2	0.50	17.15[b]	29.91[b]	5.96	0.63	0.99
	M3	0.42	13.69[a]	25.34[a]	4.84	0.52	0.86
	M4	0.53	14.64[a]	34.24[b]	5.10	0.48	0.73
	M5	0.48	15.03[a]	29.63[b]	4.66	0.51	1.22
	M6	0.45	12.56[a]	33.52[b]	4.91	0.60	0.82
	p	n.s.	*	*	n.s.	n.s.	n.s.
Place of research	Poznań	0.42	10.76[a]	35.47[b]	4.31[a]	0.54	0.75[a]
	Elbląg	0.52	20.55[b]	26.08[a]	6.28[b]	0.60	1.16[b]
	p	n.s.	*	*	*	n.s.	*
Genotypes x place of research		n.s.	*	*	n.s.	n.s.	n.s.

a, b – values marked with the same letter do not differ significantly at p ≤ 0.05, * - the level of significance for p ≤ 0.05, n.s.- non significant

TABLE 3
Factors significantly affect the yield and content indicated elements *Miscanthus* grass

Components	Investigated factors		
	years of research	interaction genotypes x years	interaction place of research x years
Yield	*	*	*
C	*	n.s.	n.s.
N	*	*	*
H	n.s.	n.s.	n.s.
SiO_2	*	*	*
P	*	n.s.	n.s.
K	*	n.s,	*
Ca	*	*	*
Mg	*	n.s.	n.s.
S	*	n.s.	*
Na	*	n.s.	*

* - the level of significance for p ≤ 0.05, n.s.- non significant

TABLE 4
Uptake of macronutrients in the yield of biomass genotypes of *Miscanthus* grass, kg ha^{-1}

	Treatment	N	P	K	Ca	Mg	S	Na
Genotypes	M1	109.5^{b}	7.6^{c}	346.4^{c}	531.2^{c}	104.7^{c}	10.9^{b}	18.2^{b}
	M2	129.7^{b}	9.5^{c}	324.8^{c}	566.5^{c}	112.9^{c}	11.9^{b}	18.7^{b}
	M3	66.4^{a}	5.3^{b}	173.0^{b}	320.3^{b}	61.2^{b}	6.6^{a}	10.9^{a}
	M4	56.9^{a}	5.7^{b}	157.2^{b}	367.7^{b}	54.8^{a}	5.1^{a}	7.8^{a}
	M5	54.0^{a}	3.9^{a}	123.8^{a}	244.1^{a}	38.4^{a}	4.2^{a}	10.0^{a}
	M6	61.1^{a}	4.1^{a}	115.4^{a}	308.0^{b}	45.2^{a}	5.5^{a}	7.5^{a}
	p	*	*	*	*	*	*	*
Place of research	Poznań	52.7^{a}	4.9^{a}	126.0^{a}	415.3^{b}	50.5^{a}	6.3^{a}	8.8^{a}
	Elbląg	108.7^{b}	7.1^{b}	282.8^{b}	358.9^{a}	86.4^{b}	8.2^{b}	16.0^{b}
	p	*	*	*	*	*	*	*
Genotypes x place of research		*	*	*	*	*	*	*

a, b, c - values marked with the same letter do not differ significantly at $p \leq 0.05$, * - the level of significance for $p \leq 0.05$

TABLE 5
Bioaccumulation factors (BF) of macronutrients

	Treatment	N	P	K	Ca	Mg	S	Na
Genotypes	M1	8.68^{b}	0.90^{b}	36.00^{c}	7.07^{a}	10.34	1.74	9.17^{b}
	M2	9.38^{b}	0.81^{b}	23.82^{b}	6.77^{a}	11.46	1.50	9.90^{b}
	M3	6.25^{a}	0.56^{a}	15.73^{a}	7.72^{a}	10.08	1.30	9.55^{b}
	M4	6.71^{a}	0.66^{a}	17.43^{a}	11.01^{b}	9.81	1.26	6.64^{a}
	M5	8.51^{b}	0.70^{a}	19.27^{b}	9.75^{b}	10.13	1,13	12.20^{c}
	M6	8.11^{b}	0.62^{a}	15.32^{a}	10.34^{b}	10.45	1,50	6.31^{a}
	p	*	*	*	*	n.s.	n.s.	*
Place of research	Poznań	5.49^{a}	0.58^{a}	28.31^{b}	6.43^{a}	6.43^{a}	1.20	6.82^{a}
	Elbląg	11.13^{b}	1.15^{b}	22.58^{a}	13.95^{b}	13.95^{b}	1.43	8.92^{b}
	p	*	*	*	*	*	n.s.	*
Genotypes x place of research		*	*	*	*	n.s.	n.s.	*

a, b, c - values marked with the same letter do not differ significantly at $p \leq 0.05$, * - the level of significance for $p \leq 0.05$, n.s.- non significant

TABLE 6
Chemical composition of crude ash from biomass genotypes Miscanthus
(in percent of ash = 100%, the mean 55 kg ash from 1 t burned biomass

	Treatment	P	K	Ca	Mg	S	Na
Genotypes	M1	0.84	37.96	58.22	11.47	1.20	2.00
	M2	0.91	31.18	54.38	10.84	1.14	1.80
	M3	0.76	24.89	46.07	8.80	0.94	1.56
	M4	0.96	26.62	62.25	9.27	0.87	1.33
	M5	0.87	27.33	53.87	8.47	0.93	2.22
	M6	0.82	22.84	60.94	8.93	1.09	1.49
Place of research	Poznań	0.76	19.56	64.49	7.84	0.98	1.36
	Elbląg	0.94	37.36	47.42	11.42	1.09	2.11

All the experimental factors under study significantly affected the uptake of individual macronutrients under study (Table 4). Significantly more nitrogen, phosphorus, calcium, magnesium, sulphur and sodium was uptake in biomass of *Miscanthus* genotype M2. A much higher uptake of the analysed elements was recorded in biomass *Miscanthus* grass grown in Elbląg. A similar uptake nitrogen, potassium and magnesium was observed by [27, 28].

In the Table 5 show factors of nitrogen, phosphorus, potassium, calcium, magnesium, sulphur and sodium bioaccumulation. The genotypes, experiment sites as well as interaction between them significantly differentiated the bioaccumulation factor N, P, K, Ca and Na. The largest bioaccumulation coefficients calculated for potassium. Bioaccumulation factors for calcium, magnesium, nitrogen and sodium on remained average a similar level. The variation in the richness of grasses in macronutrients and other organic components (hemicellulose, cellulose, et.) can be caused by the plants varied capacity for absorbing mineral nutrients from soil and their biological and genotype properties [29].

In the Table 6 shows the percentage share macronutrients in ashes obtained after the combustion of *Miscanthus* biomass. The genetic features, soil, rainfall, fertilization, date of harvest have a significant influence on the chemical composition of ash [19, 28, 30, 31]. Analysed ashes were characterized by high percentage of alkaline elements (mean Ca (53.09, K (27.30), Mg (9.63), Na (1.73), S (1.03) and P (0.86). In agricultural practice for fertilization ash from *Miscanthus* seems to be the most useful [19].

The results of individual analysis of GE interaction of genotypes are given in Table 1 for the yield and in Table 2-5 for K, Ca, Mg, and Na. The individual analysis for genotypes that follows gives, for each genotype: the main effect estimate, the F-statistic for it and the F-statistic for the interaction. Additionally, coefficients of regression and determination and F-statistic for regression are also given in this table. By comparing the F-values with the corresponding critical values given at the lower of the table, for levels 0.05 and 0.01, it is possible to evaluate the significance of the genotypes with regard to their main effects and interactions with environments. Finally, in order to investigate the relationship between the yield level of the *Miscanthus* genotypes and the chemical elements in *Miscanthus* biomass, multiple regression analyzes were performed separately for Poznań and Elbląg, and for both cities combined. Below, the multiple regression equations for yield (Y) for Poznań Y = 11.015 - 0.539K + 0.552Mg + 5.490Na (Ca - non-significant influence on yield), correlation coefficient R = 0.576, coefficient of determination (R^2) = 33.13%. For Elbląg Y = 33.729 - 0.486K - 1.108Ca + 3.021Mg (Na - non-significant influence on yield), correlation coefficient R = 0.844, coefficient of determination R^2 = 71.24%. For Poznań and Elbląg together correlation coefficient R = 0.523, coefficient of determination R^2= 27.32%, Y = 25,310 - 0.409Ca (K, Mg and Na - non-significant).

In studies of nutrient uptake by plants, it is not only their absolute content that is important, but also their weight and molar ratios. Such ratios are derivatives of their content in biomass. They provide grounds for conclusions concerning the availability of determined elements to plants, their antagonism or synergism. It must be stressed that the data only partially explain the relations between the soil and a plant, because the content of nutrients in a plant is - to a certain extent - determined by its genetic features. The weight components of K, Na, Ca and Mg in *Miscanthus* biomass are presented in Table 7. The monovalent-to-divalent cation ratio (K+Na) : (Ca+Mg) in biomass of the clones harvested in Poznań was highly diverse. It was much lower in biomass of M1 and M6 than in the others, which indicates a higher content of divalent cations. This indicates that M1 and M6 took up more divalent cations than monovalent ones. This may result from genetic factors and from larger amounts of these elements in soil. It increases the resistance to lodging, which facilitates the biomass harvest considerably. The K:Ca ratio in the genotypes under study was similar. This indicates that contrasting the ratio of monovalent-to-divalent cations was affected mainly by potassium and calcium cations. Considerable changes in the cation ratios were found in *Miscanthus* biomass harvested in Elbląg. The ratios were twice higher (0.68) than in Poznań (0.29). They are indicative of greater accumulation of potassium and sodium in *Miscanthus* biomass harvested in Elbląg. The total content of potassium and sodium in biomass harvested in Poznań was 11.51 g kg^{-1} D.M. whereas in Elbląg it was 21.71 g kg^{-1}, with a much smaller difference in the total content of Ca+Mg of 39.77 and 32.37 g kg^{-1} D.M. respectively. The differences may have been caused by the properties of soil in Poznań and Elbląg, mainly its pH of 6.82 and 5.12, respectively. A varying (K+Na) : (Ca+Mg) ratio was also observed in the *Miscanthus* biomass harvested in Elbląg, where the highest value was calculated for the biomass of M1 and the lowest was for M4. In studies with eastern galega in which the seeds had been inculated with the blue-green *Nostoc* algae calculated ratios (K+Na) : (Ca+Mg) and K : Ca were level 0.33 and 0.35 [32]. The (K+Na) : (Ca+Mg) ratio in *Miscanthus* in the consecutive study years in Poznań decreased, whereas in Elbląg it was the greatest in the second year, and the same in the first and the third year (Table 8). The mean value of the ratio was more than twice as high in the biomass harvested in Elbląg as in Poznań. The values for the consecutive years made the following sequence: 2nd year > 1st year > 3rd year. The study years significantly differentiated K : Mg ratios in seeds, straw and pea pods [33].

TABLE 7
Value of the (K + Na):(Ca + Mg) and K:Ca ratio in *Miscanthus* grass, depending on the place of research and genotypes (mean for years of cultivation)

Element	Genotypes						Mean
	M1	M2	M3	M4	M5	M6	
Poznań							
(K+Na): (Ca+Mg)	0.24	0.33	0.31	0.32	0.35	0.20	0.29
K:Ca	0.25	0.35	0.33	0.34	0.37	0.21	0.31
Elbląg							
(K+Na): (Ca+Mg)	1.08	0.73	0.68	0.47	0.60	0.53	0.68
K:Ca	1.39	0.92	0.81	0.53	0.66	0.59	0.82

TABLE 8
Value of the (K + Na):(Ca + Mg) ratio in *Miscanthus* grass, depending on the place of research and the years of cultivation (mean for genotypes)

Place of research	Years of cultivation			Mean
	1st	2nd	3rd	
Poznań	0.42	0.23	0.19	0.28
Elbląg	0.58	0.86	0.59	0.68
Mean	0.50	0.55	0.39	0.48

TABLE 9
Value of the K:Na and Ca:Mg ratio in *Miscanthus* grass depending on the genotypes and place of research

Research of elements	Genotypes						Mean
	M1	M2	M3	M4	M5	M6	
Poznań							
K:Na	13.43	15.44	11.35	17.99	15.35	13.29	14.48
Elbląg							
K:Na	22.09	18.62	20.21	21.95	10.98	16.61	18.41
Poznań							
Ca:Mg	8.32	8.10	6.89	8.60	8.20	9.26	8.23
Elblag							
Ca:Mg	2.94	3.18	4.03	5.33	5.06	5.08	4.27

TABLE 10
Relationship between the yield of *Miscanthus* grass (t ha^{-1}D.M.) and the sum of the content elements in years of cultivation

Year of cultivation	1st	2nd	3rd
Yield (t ha^{-1} D.M.)	7.84	12.39	17.93
Sum of the K, Ca, Mg and Na content (g kg^{-1} D.M.)	63.02	53.03	42.02
r = - 0.99 y = 7.94 - 2.09x (for year cultivation)			

The ratios for monovalent (K:Na) and divalent (Ca:Mg) elements also made an interesting pattern (Table 9). An analysis of the K:Na ratio revealed its different values for different clones and experiment sites. The greatest value of the ratio was calculated for biomass of M4 clone grown at both sites, which indicates an increased capability for taking up potassium, and the lowest was for the M3 clone in Poznań and M5 in Elbląg. Moreover, higher levels of sodium were found in biomass harvested in Poznań than in Elbląg. The pattern for the Ca:Mg ratio was the opposite to that of K:Na. The values were nearly twice as high for *Miscanthus* grass harvested in Poznań than that harvested in Elbląg (8.23 and 4.27, respectively). This indicates that more calcium than magnesium was taken up by the plants grown in Poznań. An analysis of this ratio within the clones revealed that it was the highest for M6 and the lowest for M3 in the biomass harvested in Poznań; the respective values for Elbląg: M4- 5.23 and M1-2.94. These ratios for *Miscanthus* grass are important in regard to the chemical composition of the ash produced. Biomass with a high content of potassium has a negative effect on ash, which tends to form slag in the combustion process [19, 34] and is a deciding factor in using ash in the soil-plant circulation. Determination of the demand for nutrients in potential years of biomass growth and harvest is an important element of studies on the content of minerals in biomass of perennial plants and its changes. *Miscanthus* grass is of particular importance in this regard because studies [35, 36] have shown that while producing large amounts of biomass, these plants respond poorly to fertilisation, whose effectiveness was low. The low effectiveness of fertilisation may be a consequence of high photosynthetic capability of *Miscanthus,* which follows the C4 photosynthesis pathway, the function of rhizomes which accumulate large amounts of nutrients and effective use of light and water [37]. An important role in growth and development is played by rhizomes, which accumulate nutrients; report that the accumulated nutrients are transferred from rhizomes to the over ground parts during the growth period and then back to rhizomes at the end of the growing season [13, 38]. This may suggest that nutrients must be replenished to meet the *Miscanthus* needs in terms of fertilisation.

The relationships between the amount of biomass and the total content of Ca, K, Mg and Na in biomass are also interesting (Table 10) and negatively significant. A year-by-year increase in the yield of *Miscanthus r*esults in an increase in its biomass with a simultaneous decrease in the total content of the elements under study in the biomass during the phase of technological usability as a source of energy. Similar relationships have also been reported by [39] for *Miscanthus* as a perennial crop.

CONCLUSIONS

1. The average yield of *Miscanthus* biomass amounted 12.72 t ha^{-1}.
2. Biomass of genotypes of *Miscanthus* grass grown in diverse soil and climatic conditions in Poland during three years contained the largest amount of carbon, hydrogen, silica, calcium, followed - in a decreasing order - by K, Mg, N, Na, S and P.
3. Uptake macronutrients by the biomass yields of *Miscanthus* was higher for calcium, potassium, magnesium and nitrogen.
4. The *Miscanthus* biomass yield has been found to be significantly negatively correlated with the total content of Ca, K, Mg and Na.
5. The content of the elements under study in biomass differentiated the weight ratios of monovalent and divalent elements, which indicated the nutritional demands of the genotypes under study and the chemical composition of the ash produced.
6. Ashes from burned biomass of *Miscanthus* can be suitable for use in agriculture as a liming agent to be applied on medium and heave soils.

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