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The content of elements and quality parameters of winter rye grain as influenced by biochar-amended soil

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Abstract

A field experiment was conducted on a *Haplic Podzol* (PZha) during the period 2012–2014. The aim of this study was to evaluate the grain chemical composition of winter rye grown on a biochar-amended soil. The following grain quality traits were determined: content of N, P, K, Mg, Ca, Cu, Zn, Mn, Fe and B, total protein content (TPC), total starch content (TSC), reducing sugar content (RSC), phenolic content (PhC), flavonoid content (FC), chelating power (ChP) and reducing power (RP). Biochar was applied once before sowing winter rye in 2011 at three rates: 10 Mg ha⁻¹ (BC₁₀), 20 Mg ha⁻¹ (BC₂₀) and 30 Mg ha⁻¹ (BC₃₀). The biochar was pyrolyzed from wheat straw at 350–650°C. Rye grain was evaluated each year after harvest, i.e. 12, 24 and 36 months after biochar application to the soil.

At all assessment times, the N content in rye grain from the biochar-amended treatments was higher than in the control treatment (BC₀). In the first year of the study, the lowest P content (3.40 g kg⁻¹) was determined in grain from the treatment BC₃₀, while in the third year in the treatment BC₁₀ (3.31 g kg⁻¹). At the second and third assessment times, the content of K, Mg and Zn in grain from the biochar-amended treatments decreased with increasing biochar rate, whereas the Mn content increased. In the treatment BC₃₀, at the first assessment time the grain Ca content was found to be lowest (0.147 g kg⁻¹), while after 36 months it was highest (0.302 g kg⁻¹). In the first and third years of the study, the Cu content in the treatment BC₃₀ was higher than in the BC₁₀ (by 31.6% and 57.3%, respectively). At the second and third assessment times 24 and 36 months after biochar application, the Fe and B content in grain obtained from the treatments BC₁₀ and BC₂₀ was higher than in that harvested from the BC₃₀ plots. In grain harvested from the plots where 20 Mg ha⁻¹ biochar had been applied, the total protein content and total starch content were found to be higher than in grain from the plots where 30 Mg ha⁻¹ had been applied (by 10.7% and 17.1%, respectively). The highest reducing sugar content (9.02 mg g⁻¹ dry weight (DW)) and phenolic content (6.8 mg of gallic acid equivalent (GAE) g⁻¹ DW) were determined in rye grain from the BC₁₀ plots. Soil amendment at different biochar rates did not have an unambiguous effect on the antioxidant capacity parameters (chelating power and reducing power) of rye grains.

Key words: biochar, elemental composition, grain quality, *Secale cereale*.

Introduction

Biochar is a product of thermal decomposition of organic matter during the pyrolysis process with limited oxygen access in the temperature range of 300–1000°C (Gul et al., 2015). Today, biochar is actively researched all over the world, because it can be a viable option for sustainable agriculture due to its potential as a long-term sink for carbon in soil and on account of its benefits for crops (Alburquerque et al., 2013). Studies to date have confirmed the beneficial influence of biochar amendment

on soil physical, chemical and biological properties (Oleszczuk et al., 2014; Olmo et al., 2014; Prendergast-Miller et al., 2014; Cao et al., 2017; Pranagal et al., 2017). Biochar application results in an increase in pH in acidic soils and an increase in organic carbon content. As a result of that, an improvement in soil fertility and an increase in nutrient bioavailability occur (Lehmann et al., 2011; Alburquerque et al., 2013; Gul et al., 2015; Schimmelpfennig et al., 2015; Kraska et al., 2016).

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The use of biochar can reduce the consumption of mineral fertilizers (Zhang et al., 2012; Salim, 2016). It contains several nutrients necessary for plants, whose presence may contribute to increased crop yields (Farrell et al., 2014; Cao et al., 2017). In the opinion of Prendergast-Miller et al. (2014), biochar added to soil increases the availability of nutrients to plants – directly as a source of nutrients and indirectly by changing the soil nutrient content. In turn, Schulz et al. (2013) showed that biochar application did not have a direct impact on plant growth and soil fertility. However, many authors stress that the effect of biochar amendment on crop yields depends on many factors, such as the conditions of a given study or biochar type (Albuquerque et al., 2013; Kraska et al., 2016; Salim, 2016). The chemical composition of biochar is very variable and depends on the type of substrate and pyrolysis conditions. Biochar may contain harmful compounds such as heavy metals or polycyclic aromatic hydrocarbons (Kuśmierz et al., 2016).

In the available literature, there is little information on the effect of biochar on grain chemical composition and quality of crop plants. Some papers indicate that biochar application increases the content of macro- and microelements in cereal grain (Atkinson et al., 2010; Major et al., 2010; Salim, 2016). Due to the fact that most papers describe positive effect of biochar amendment on the concentration of available nutrients in the soil, we hypothesize that it can be a source of nutrients for plants during growth. The proposed experiment assumed that biochar application would result in an increase in the grain content of elements and contribute to an improvement in the quality of winter rye sown in a monoculture. The aim of this paper was to evaluate the chemical composition of winter rye grown on a biochar-amended soil. The study focused on the effect of biochar rates (10, 20 and 30 Mg ha⁻¹) on macro- and microelement content in grain as well as some quality parameters like total protein, total starch, reducing sugar, phenolic, flavonoid, chelating power and reducing power.

Materials and methods

Biochar characteristics. Biochar applied to soil was obtained from a commercial manufacturer and was produced by pyrolysis where the feedstock is thermochemically decomposed at a temperature range from 350°C (start of combustion) to 650°C (max. combustion temperature) in an oxygen-poor atmosphere (1–2% O₂). Biochar was produced from wheat straw and was provided by Mostostal Sp. z o.o., Wrocław, Poland. Physico-chemical characteristics of biochar used in the experiment were as follows: pH_{KCl} 9.9; available forms of P – 235.6 mg kg⁻¹, K – 2344.6 mg kg⁻¹, Mg – 1163.2 mg kg⁻¹; C – 53.87%, N – 0.91%, H – 1.76%, ratio of hydrogen to carbon – 0.033, ash content – 41.2%; specific surface area – 26.3 m² g⁻¹ (Oleszczuk et al., 2014).

Weather conditions. In the 2011–2012 season, the total rainfall was only 70% of the long-term average (Fig.). The highest rainfall deficit occurred in the period from September to November and also from May to August. During these months, the total rainfall was lower than the long-term average for the relevant period by 77% and 27%, respectively. The 2012–2013 season was much

wetter, since during this season the total rainfall was higher than the long-term average by 22%. The months of May and June were most abundant in rainfall and the total rainfall in these months, compared to the long-term average was almost twice higher. The 2013–2014 season in which a particularly high amount of rainfall was recorded was also quite wet, especially in May.

The average air temperature during the field experiment was above the long-term average for most of the months. This was particularly the case in the periods from March to September 2012, from May to August 2013 as well as from February to May and in July 2014 (Fig.).

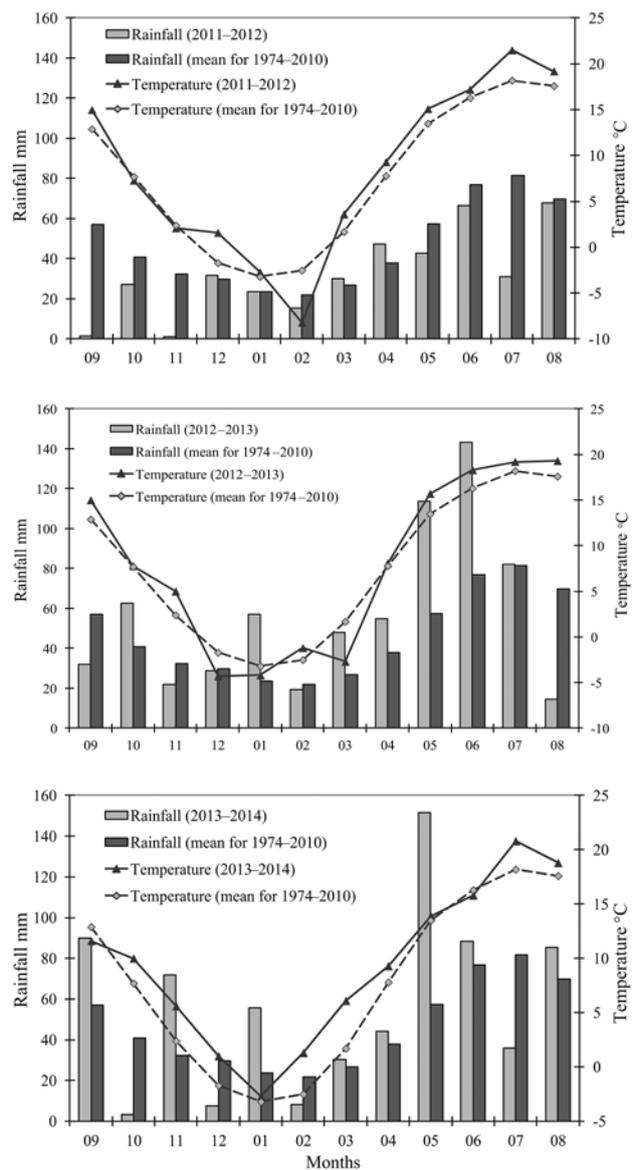


Figure. Rainfall and air temperature according to the Meteorological Station at Bezek, Poland in 2011–2014

Experimental site. The field study was conducted in the years 2012–2014 at the Experimental Farm in Bezek (51°19' N, 23°25' E), Poland. The experiment was established on a *Haplic Podzol (PZha)* originating from glaciofluvial fine-grained loamy sand (WRB, 2014). The particle size distribution of arable layer of this soil was as follows: 2.0–0.5 mm fraction – 9%, 0.5–0.25 mm fraction

– 24%, 0.25–0.05 mm fraction – 39%, 0.05–0.002 mm fraction – 26% and <0.002 mm fraction – 2%. In a three year study period the average amount of P in the soil was 59.42 mg kg⁻¹, K – 108.53 mg kg⁻¹, Mg – 12.79 mg kg⁻¹, Cu – 1.83 mg kg⁻¹, Zn – 5.39 mg kg⁻¹, Mn – 116.07 mg kg⁻¹, Fe – 481.28 mg kg⁻¹, B – 0.82 mg kg⁻¹, and pH in KCl was 5.32 (Kraska et al., 2016). The experiment, set up in a randomized block design in three replicates, compared the effect of three biochar rates in the cultivation of winter rye (*Secale cereale* L., 'Dańkowskie Diament') cultivated in a monoculture. The area of a single plot was 18 m². At the beginning of September 2011 biochar at rates of 10 Mg (BC₁₀), 20 Mg (BC₂₀) and 30 Mg (BC₃₀) per hectare was incorporated into the soil. Then the soil was ploughed and winter rye was sown in the fourth week of September 2011. The spacing between plots fertilized with the different rates of biochar was 2 m. Plots in which no biochar was applied were the control treatment (BC₀).

The crop was sown at a rate of 5 million seeds per hectare at 12 cm inter-row spacing. Mineral fertilizers were applied every year of the experiment at the following rates: 70 kg ha⁻¹ N (ammonium nitrate), 26 kg ha⁻¹ P (triple superphosphate), 66 kg ha⁻¹ K (muriate of potash, KCl). Phosphorus and potassium fertilizers as well as 20 kg ha⁻¹ N were applied before sowing. In spring, the remaining portion of the nitrogen (N) rate was applied before plant growth began (30 kg ha⁻¹) and at the stem elongation stage (20 kg ha⁻¹).

The winter rye crop protection program included the application of the herbicide Aminopielik Tercet 500 SL (a.i. 2.4 D 300 g l⁻¹, mecoprop 160 g l⁻¹ and dicamba 40 g l⁻¹) at a rate of 2 l ha⁻¹ (BBCH 23–29). Moreover, the fungicide Tango Star 334 SE (a.i. fenpropimorph 250 g l⁻¹ and epoxiconazole 84 g l⁻¹) was applied at a rate of 1 l ha⁻¹ (BBCH 51–56).

Grain analyses. The winter rye grain was sampled every year after harvest (12, 24 and 36 months after biochar application). In composite samples from each treatment, the content of the following elements was determined in three replicates: N – mineralization in sulfuric acid and oxygenated water, determination by the Kjeldahl distillation method, titration detection; P – mineralization in sulfuric acid and oxygenated water, determination by the vanadium-molybdate spectrophotometric method, spectrophotometer Spekol (Carl Zeiss AG, Germany); K and Ca – mineralization in sulfuric acid and oxygenated water, determination by flame photometry, photometer PFP7 (Jenway, UK); Mg – mineralization in sulfuric acid and oxygenated water, determination by atomic absorption spectrometry, spectrometer Perkin-Elmer (USA); Cu, Zn, Mn and Fe – mineralization in perchloric and nitric acid (1:4), determination by atomic absorption spectrometry, spectrometer Avanta (GBC Scientific Equipment, Australia); B – oven mineralization with Ca(OH)₂, determination by the spectrophotometric curcumin method, spectrophotometer Spekol (Carl Zeiss AG).

Phenolic content (PhC). Rye flour (0.5 g) was extracted three times with 4 ml of methanol/acetone/water 40:40:20, v/v/v. Extracts were centrifuged for 10 min at 6800 × g, and supernatants were collected. Finally, the fractions from each step of extraction were combined

and used for further analysis. The total phenolics were determined using Folin-Ciocalteu reagent (Singleton, Rossi, 1965). To 0.5 ml of the sample, 0.5 ml H₂O, 2 ml Folin-Ciocalteu reagent (1:5 H₂O) were added, and after 3 min, 10 ml of 10% Na₂CO₃ and the contents were mixed and allowed to stand for 30 min. Absorption at 725 nm was measured in a spectrophotometer UV-Vis (Shimadzu, Japan). The amount of total phenolics was calculated as a gallic acid equivalent (GAE) in mg per g of dry mass.

Total flavonoid content (FC) was determined according to the method described by Lamaison and Carnet (1990). One millilitre of extract was mixed with 1 mL of 2% AlCl₃ × 6H₂O solution and incubated at room temperature for 10 min. Thereafter, absorbance at 430 nm was measured. Total flavonoid content was calculated as a quercetin equivalent (QE) in mg per g of dry weight (DW).

Reducing power (RP) was determined by the method of Oyaizu (1986). Analysed sample (0.5 ml) was mixed with phosphate buffer (0.5 ml, 200 mM, pH 6.6) and potassium ferricyanide K₃[Fe(CN)₆] (0.5 ml, 1%). The mixture was incubated at 50°C for 20 min. Reactions were terminated by adding 0.5 ml 10% TCA (trichloroacetic acid) and centrifuging for 10 min at 8000 × g. The upper layer of solution (2.5 ml) was mixed with distilled water (0.5 ml) and 0.1 ml of 0.1% FeCl₃ and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increase in reducing power. Reducing power was expressed as trolox equivalent (TE) in mg per g of DW.

Metal chelating power (ChP) was determined by the method of Guo et al. (2001). The extract samples (1 ml) was added to 0.1 ml of 2 mM FeCl₂ solution and 0.2 ml 5 mM ferrozine and the mixture was shaken vigorously and left standing at room temperature for 10 min. Then, absorbance of the solution was measured spectrophotometrically at 562 nm. The percentage of inhibition of ferrozine-Fe²⁺ complex formation was calculated according to the formula:

$$\% \text{ inhibition} = [1 - (A_p/A_c)] \times 100, \text{ where } A_c \text{ is absorbance of control, } A_p \text{ – absorbance of sample.}$$

Chelating power was expressed as EDTA (ethylenediaminetetraacetic acid) equivalent in mg per g of DW.

Total starch content (TSC) was determined after dispersion of the starch granules in 2 M KOH (50 mg sample, 6 ml KOH) at room temperature (30 min at constant shaking) and hydrolysis of the solubilized starch with 80 μl (1 mg ml⁻¹) amyloglucosidase (14 U mg⁻¹, EC 3.2.1.3) at 60°C for 45 min (Goni et al., 1997). Glucose content was determined by using the standard dinitrosalicylic acid (DNSA) method (Miller, 1959). Total starch content was calculated as glucose × 0.9.

The free **reducing sugar content (RSC)** of the samples was determined in order to correct the obtained total starch values obtained.

Extraction of proteins. Total proteins were extracted according to procedure described by Hurkman and Tanaka (2007). For this method, 50 mg of flour was suspended in 1 ml of urea buffer: 2 M urea, 10% glycerol, 65 mM DTT (dithiothreitol) and 20 mM Tris (2-amino-2-hydroxymethyl-1,3-propanediol, pH 8.0).

The suspension was incubated at room temperature for 1 h, and insoluble material was removed by centrifugation for 10 min at $16\,000 \times g$. Protein content in samples was determined with the Bradford reagent (Sigma-Aldrich) according to the method described by Bradford (1976). Protein content was expressed as gluten equivalent in mg per g of dry weight.

Statistical analysis. The obtained results were statistically processed by the analysis of variance using statistical program *ARStat* (developed in the Faculty of Applied Mathematics and Information Technology of the University of Life Sciences, Lublin, Poland). The means were compared using the least significant differences based on the Tukey's test ($P \leq 0.05$).

Results and discussion

Application of biochar changes soil properties depending on its type as well as biochar type and rate (Vaccari et al., 2011; Rogovska et al., 2014; Tammeorg et al., 2014). Therefore, interpretation of study results, seeking relationships and discussion with studies of other authors concerning the indirect effects of biochar on plants is a difficult task. In the present study, at the first assessment time (12 months after biochar application), the nitrogen (N) content in winter rye grain from all biochar treatments was significantly higher than in the BC_0 treatment (Table 1).

Moreover, the N content in grain harvested from the BC_{20} and BC_{30} plots was higher than in the other experimental treatments. Taking into account the

fact that N content in plant material is affected by many factors, it is difficult to suggest the main reason why N content increased in the biochar amended treatments. Our previous study (Kraska et al., 2016) found that application of biochar positively affected the soil reaction and caused the significant increase of soil pH. It is well known that soil pH plays an important role in the N nutrition of plants. Under conditions of higher pH, the nitrification process is more rapid, and crops generally take up more nitrate. However, there are other factors, including soil moisture, temperature, texture, cation exchange capacity that can affect N content in grain of winter rye, but they were not studied in our experiment. In the second year of the study, a significantly higher grain N content, relative to the control (BC_0), was found in the treatment where 10 and 30 Mg biochar had been applied. At the third assessment date, grain harvested from the control plots had lower N content than that from the biochar-amended treatments. Salim (2016) also found the N content in wheat grain to increase with increasing biochar amendment levels. Vaccari et al. (2011), in turn, found the N content in durum wheat grain not to be dependent on biochar application rate. According to Alburquerque et al. (2013) and Tammeorg et al. (2014), adding biochar to soil in the long term prevents leaching of nutrients beyond the agroecosystem and in consequence increases the amount of elements available to plants. In turn, the indirect effects of biochar involve its sorption capacity (retention of nutrients) and changes in soil physicochemical and biological properties, which leads to better nutrition of plants (Xu et al., 2013; Olmo et al., 2014).

Table 1. The content ($g\ kg^{-1}$) of macroelements in winter rye grain harvested 12, 24 and 36 months after biochar addition to the soil

Assessment date / year	Treatment	N	P	K	Mg	Ca
After 12 months	BC_0	10.40 a	3.50 ab	4.70 a	0.800 a	0.293 a
	BC_{10}	10.90 b	3.53 a	4.70 a	0.814 ab	0.213 b
	BC_{20}	12.00 c	3.57 a	4.77 a	0.824 bc	0.227 b
	BC_{30}	11.80 c	3.40 b	4.60 a	0.835 c	0.147 c
	LSD _{0.05}	0.232	0.107	0.171	0.0176	0.0604
After 24 months	BC_0	11.83 a	3.35 a	3.16 a	1.387 a	0.310 a
	BC_{10}	12.21 b	3.26 a	7.98 b	2.090 b	0.510 b
	BC_{20}	11.95 a	3.39 a	5.86 c	0.639 c	0.525 b
	BC_{30}	13.14 c	3.32 a	5.27 d	1.237 d	0.403 c
	LSD _{0.05}	0.228	0.132	0.328	0.0589	0.0375
After 36 months	BC_0	13.70 a	3.58 a	4.66 a	0.65 a	0.218 a
	BC_{10}	16.80 b	3.31 b	4.28 b	0.450 b	0.202 b
	BC_{20}	17.20 c	3.53 a	3.61 c	0.383 c	0.178 c
	BC_{30}	15.10 d	3.55 a	3.22 d	0.320 d	0.302 d
	LSD _{0.05}	0.262	0.129	0.048	0.0155	0.0096
2012	Independent of biochar rates	11.28 a	3.50 a	4.60 a	0.818 a	0.220 a
2013		12.28 b	3.33 a	5.57 b	1.338 b	0.437 b
2014		15.70 c	3.49 a	3.94 c	0.442 c	0.225 a
LSD _{0.05}		0.094	0.174	0.082	0.0139	0.0160

Note. BC_0 – control treatment (without biochar), biochar rates: BC_{10} – 10 Mg ha^{-1} , BC_{20} – 20 Mg ha^{-1} , BC_{30} – 30 Mg ha^{-1} ; values marked with different letters differ significantly at $P \leq 0.05$.

Atkinson et al. (2010) and Cao et al. (2017) have reported that in biochar-amended soils a change in soil pH occurs as well as soil microbial activity and the amount of available phosphorus increases. Likewise, Albuquerque et al. (2013) and Xu et al. (2013) found biochar to be a valuable source of plant available phosphorus. In the present study, at the first assessment time the P content in grain harvested from the BC₃₀ treatment was significantly lower than in grain from the BC₁₀ and BC₂₀ treatments. In the second year after biochar application, the grain P content did not vary significantly. In the third year of the study, in turn, the lowest P content was found in grain harvested from the BC₁₀ treatment. As part of the same experiment, Kraska et al. (2016) obtained a higher winter rye grain yield from the biochar-amended plots, which was probably attributable to greater nutrient uptake. Hence, the slightly lower P content determined in rye grain harvested from some biochar-amended plots was probably associated with the increase in grain yield and the nutrient dilution effect. Similarly, Olmo et al. (2014) have noted greater P uptake by wheat grown in biochar-amended plots, with a higher yield and a slightly lower P content in grain, compared to the control treatment without biochar amendment.

The K content in rye grain did not vary significantly in the first year of the study. Such results could have been affected by the low level of rainfall in 2012 in the period from May to July (Fig.). Under water deficit conditions, plants take up and accumulate less nutrients. In the second year of the study, the lowest grain K content was determined in the control treatment without biochar. At this assessment time, the K content in grain from the biochar-amended treatments decreased with increasing biochar rate. In the third year of the study, a similar relationship was found in the grain harvested from the biochar-amended plots. However, the K content was highest in the grain from the control treatment. Similarly as in the case of P, this was probably due to the fact that in the treatments with the highest BC application rate the rye produced a higher grain yield within the available pool of nutrients and hence the grain K content decreased (Kraska et al., 2016).

In the first year of the study, the highest amount of Mg was determined in grain harvested from the BC₂₀ and BC₃₀ treatments (Table 1). At the second assessment time, grain from the plots where 10 Mg biochar had been applied was characterized by the highest Mg content, while the lowest one was found in the treatment BC₂₀. In the third year of the study, the highest Mg content was determined in grain from the control treatments. Albuquerque et al. (2013) reported that wheat straw biochar incorporated into the soil reduced Mg uptake by plants. A certain confirmation of this finding was provided 36 months after biochar addition when the grain Mg content was found to decrease with increasing biochar application rate. Likewise, Olmo et al. (2014) determined the grain Mg content in wheat grown in biochar-amended plots at a level of $0.99 \pm 0.04 \text{ g kg}^{-1}$, whereas in the control treatment without biochar amendment it was $1.08 \pm 0.04 \text{ g kg}^{-1}$.

In the first year after biochar application, the highest amount of Ca was found in the grain from the control plots, whereas the lowest one in the treatment with the rate of 30 Mg biochar (Table 1). In the second year of the study, in turn, its highest content was determined in the grain from the BC₁₀ and BC₂₀ treatments. In the third year after biochar application, the highest Ca content was found in grain harvested from the BC₃₀ plots. Salim (2016) found the content of P, K and Mg in wheat grain to increase under the influence of higher biochar rates. He observed a reverse relationship for Ca content. Albuquerque et al. (2013) also revealed decreased Ca uptake by wheat grown in wheat straw biochar-amended plots. Olmo et al. (2014), on the other hand, determined a slightly higher Ca content ($5.52 \pm 0.12 \text{ g kg}^{-1}$) in wheat grain in the control treatment without biochar amendment, compared to the treatment where biochar had been applied at a rate of 40 Mg per hectare ($5.38 \pm 0.11 \text{ g kg}^{-1}$).

Meteorological conditions during field experiment significantly influenced the content of macrolelements in winter rye grain, except for P. The highest amount of N was achieved in the third year of the study, while in 2013, which was wet and warm, the grain contained the highest K, Mg and Ca.

Twelve months after biochar addition, the lowest Cu content was determined in the grain obtained from the BC₁₀ and BC₂₀ plots. Similar trends were observed for the Cu content in the soil fertilized with biochar at rates of 10 and 20 Mg ha⁻¹ (Kraska et al., 2016). At the second assessment time, the grain copper content was not significantly dependent on the biochar application rate, while three years after biochar application, in turn, the highest amount of copper was found in the grain from the BC₃₀ treatment (Table 2).

In the first year of the study, the highest Zn content was determined in the grain from the BC₃₀ plots, whereas in the second year in that from the BC₁₀ and BC₂₀ treatments. Thirty six months after biochar addition, grain harvested from the BC₁₀ plot contained the largest amount of Zn. In the study by Albuquerque et al. (2013), wheat straw biochar was found to promote Zn and Cu uptake by the crop plant. Olmo et al. (2014) found a lower Zn content in wheat grain harvested from biochar-amended plots ($24.4 \pm 1.1 \text{ mg kg}^{-1}$) than in the control treatment ($26.2 \pm 1.4 \text{ mg kg}^{-1}$). They noted a similar relationship with respect to Cu – $3.5 \pm 0.2 \text{ mg kg}^{-1}$ (control treatment) and $3.2 \pm 0.2 \text{ mg kg}^{-1}$ (biochar treatment).

At the first assessment time, the grain Mn content in rye obtained from the biochar-amended treatments was higher than in the control plots, but grain from the BC₃₀ treatment contained most manganese. Twenty four months after biochar addition to the soil, the highest Mn content was found in the grain from the control treatment. At the same time, it was demonstrated that the grain Mn content significantly increased with increasing biochar rate. The same relationship was found in the soil as affected by biochar amendment (Kraska et al., 2016). At the third assessment time, grain harvested from the BC₁₀

Table 2. The content (mg kg⁻¹) of microelements in winter rye grain harvested 12, 24 and 36 months after biochar addition to the soil

Assessment date / year	Treatment	Cu	Zn	Mn	Fe	B
After 12 months	BC ₀	3.33 a	22.53 a	29.27 a	33.33 a	1.73 a
	BC ₁₀	2.53 b	21.73 b	34.40 b	29.67 b	1.37 b
	BC ₂₀	2.80 ab	21.60 b	34.00 b	28.33 b	1.07 c
	BC ₃₀	3.33 a	26.00 c	43.73 c	29.00 b	1.37 b
	LSD _{0.05}	0.641	0.427	0.770	3.460	0.151
After 24 months	BC ₀	3.17 a	22.90 ac	53.33 a	20.46 a	4.07 a
	BC ₁₀	4.20 a	26.33 b	21.96 b	40.38 b	3.40 b
	BC ₂₀	3.41 a	24.00 a	38.76 c	27.40 c	4.60 c
	BC ₃₀	3.21 a	21.76 c	47.16 d	37.30 d	1.18 d
	LSD _{0.05}	1.049	1.388	1.725	2.338	0.321
After 36 months	BC ₀	3.87 ab	30.20 a	33.00 a	37.40 a	3.78 a
	BC ₁₀	3.47 a	32.01 b	40.40 b	39.10 b	2.20 b
	BC ₂₀	4.01 b	29.20 c	32.70 c	38.90 b	2.11 b
	BC ₃₀	5.46 c	29.40 c	33.70 d	32.70 c	1.39 c
	LSD _{0.05}	0.471	0.329	0.223	0.212	0.178
2012	Independent on biochar rates	3.00 a	22.97 a	35.35 a	30.08 a	1.38 a
2013		3.50 b	23.75 b	40.30 b	31.39 b	3.31 b
2014		4.20 c	30.20 c	34.95 a	37.02 c	2.37 c
LSD _{0.05}		0.316	0.327	0.425	0.939	0.080

Explanations under Table 1

treatment was characterized by the highest Mn content, while the lowest one was found in that from the control plots without biochar. In the study by Albuquerque et al. (2013), Fe and Mn uptake by wheat decreased under the influence of biochar application. In turn, Atkinson et al. (2010), Major et al. (2010) and Olmo et al. (2014) found that the bioavailability and uptake of P, K, Ca, Zn and Cu by plants in biochar-amended soils increased, whereas N leaching from the soil decreased. Tammeorg et al. (2014) and Gul et al. (2015) suggest that changes occurring in the soil under the influence of biochar amendment are dynamic, so it is difficult to conclude on the direct effects of biochar on plants.

In the first year of the study, in all biochar-amended treatments a lower Fe content was found compared to the control treatment (Table 2). A similar relationship was revealed by Olmo et al. (2014) for wheat grain. At the second assessment time, a reverse situation was found – grain from the control plots contained least Fe. In turn, in the biochar-amended treatments grain from the BC₁₀ plots was characterized by the highest Fe content. At the third assessment time, the highest Fe content was determined in rye grain from the BC₁₀ plots, while the lowest one for the treatment where 30 Mg of biochar had been applied. In the study by Salim (2016), an increased level of biochar amendment caused an increase in Mn and Zn content in wheat grain and at the same time a decrease in Cu content. In turn, Fe content varied depending on the growing season.

At the first and third assessment times, the B content in grain harvested from the control plots was significantly

higher, compared to that harvested from the biochar-amended plots (Table 2). This could have been attributable to the fact that biochar application resulted in an increase in soil pH, which in consequence reduces the availability of B to plants (Kraska et al., 2016). Hu and Brown (1997) found a similar relationship; according to these authors, boron availability to plants decreases with increasing soil pH and therefore soil boron content increases.

In the first year of the experiment, the weather conditions were the least favourable for growing of winter rye (the lowest amount of rainfall). As a result, the lowest content of Cu, Zn, Fe and B in grain was found compared to the years 2013 and 2014. In turn, the highest amount of Cu, Zn and Fe was achieved in 2014.

In the present study, biochar addition to the soil had a statistically proven effect on the biochemical parameters of rye grain (Table 3). A slight decrease in total protein content in rye grain was found in the BC₁₀ and BC₃₀ treatments compared to the control treatment, whereas in the BC₂₀ treatment an increase was found, but the statistical verification did not confirm these differences to be significant. However, significant differences were shown between the BC₂₀ and BC₃₀ treatments. In the first year of their study, Tammeorg et al. (2014) obtained the highest protein content in spring wheat grain by applying a rate of 10 Mg biochar per ha (a 3% higher protein content compared to the control without biochar), whereas the lowest one in the treatment with a rate of 30 Mg ha⁻¹ (a 2% lower content compared to BC₀). In the second year of the study, on the other hand, these authors found higher grain protein content in

Table 3. Comparison of nutraceutical potential of winter rye

Factor	Treatment	TPC	TSC	RSC	PhC	FC	ChP	RP
Biochar rate	BC ₀	119.5 ab	524.2 a	8.86 a	3.96 a	0.06 ab	6.37 a	0.69 a
	BC ₁₀	118.5 ab	517.6 a	9.02 b	6.8 b	0.05 a	5.26 b	0.71 a
	BC ₂₀	127.1 a	570.9 b	5.76 c	3.12 c	0.06 ab	6.56 a	0.51 b
	BC ₃₀	114.8 b	487.7 a	6.12 d	3.58 ac	0.07 b	6.86 a	0.56 b
	LSD _{0.05}	9.42	46.23	0.045	0.616	0.018	0.80	0.07
Year (independent of biochar rates)	2012	121.0 ab	537.8 a	7.30 a	4.39 a	0.06 ab	6.23 a	0.61 a
	2013	114.7 a	519.9 a	7.60 a	4.15 b	0.07 a	6.06 a	0.62 a
	2014	124.3 b	517.6 a	7.42 a	4.56 a	0.05 b	6.50 a	0.63 a
	LSD _{0.05}	6.78	34.79	0.336	0.230	0.012	0.51	0.05

TPC – total protein content, mg g⁻¹ DW (dry weight); TSC – total starch content, mg g⁻¹ DW; RSC – reducing sugar content, mg g⁻¹ DW; PhC – phenolic content, mg of GAE g⁻¹ DW; FC – flavonoid content, mg of QE g⁻¹ DW; ChP – chelating power, mg of EDTA g⁻¹ DW; RP – reducing power, mg of TE g⁻¹ DW; BC₀ – control treatment (without biochar), biochar rates: BC₁₀ – 10 Mg ha⁻¹, BC₂₀ – 20 Mg ha⁻¹, BC₃₀ – 30 Mg ha⁻¹

the BC₃₀ treatment relative to the control (BC₀) and the other biochar-amended treatments (BC₅, BC₁₀ and BC₂₀). But these differences were statistically insignificant.

In the treatment with the biochar rate of 20 Mg ha⁻¹, a significantly higher total starch content was found, compared to the other experimental treatments, whereas the lowest total starch content value was determined for the grain from the plots where the biochar rate 30 Mg ha⁻¹ had been applied. In the first year of the study, Tammearg et al. (2014) showed the highest starch content in spring wheat grain from the BC₃₀ treatment, whereas in the second year of the study the grain starch content was similar in all experimental treatments. In the study by Olmo et al. (2014), biochar addition had a beneficial influence on the biology of durum wheat growth and in consequence caused an increase in grain yield. Nevertheless, no significant influence of biochar on grain quality was demonstrated. The lack of significant changes in grain chemical composition with a simultaneous increase in crop yield can be considered to be a positive effect of biochar application for soil amendment.

Phenolic acids are the main antioxidants in cereal grains. They seem to have the greatest potential to be beneficial to our health as a result of free radical scavenging activity, inhibition of lipid peroxidation, and thus anticancer activity (Anson et al., 2011). The highest reducing sugar and phenolic contents were determined in rye grain from the plots where the lowest (BC₁₀) biochar rate had been applied. Moreover, in grain from the BC₂₀ and BC₃₀ treatments the content of reducing sugars and phenolics was lower than in the control treatment (BC₀).

Flavonoids play an essential role in the creation of the nutraceutical potential of cereals (Świeca et al., 2014). In the present study, the flavonoid content determined in grain from the plots where 10 Mg of biochar had been applied was slightly lower than in the BC₀ and BC₂₀ treatments. On the other hand, a significantly higher value of flavonoid content was found in grain from the BC₃₀ plots compared to BC₁₀.

Soil amendment at different biochar rates did not have an unambiguous and clear effect on the antioxidant capacity parameters (chelating and reducing power) of rye grains. The lowest value of chelating power was determined in rye grain obtained from the BC₁₀ treatment. In turn, the highest value of reducing power was found in the case of grain from the BC₁₀ and BC₀ treatments. Hung et al. (2011) reported that cereal grain, above all wheat grain, contains large amounts of polyphenols with well-documented antioxidant activity. Analysing the antioxidant activity and phenolic content of wheat grain, Falcioni et al. (2002), Okarter et al. (2010) and Hung et al. (2011) found proportional relationships between these parameters. However, phenolic composition and antioxidant capacity of plant products are interaction mechanisms that occur in the plant in response to soil conditions and these mechanisms are still studied by researchers. Therefore, it is not always possible to explain the effect of changes in soil processes on crop yield quality (Różyło et al., 2015).

Weather conditions significantly affected the grain quality parameters. In the year 2013, winter rye grain was characterized by the highest flavonoid content, whereas the largest amount of total protein and phenolics was found in the year 2014 which was characterized by higher air temperature and rainfall compared to the long-term means.

Conclusions

1. In the experiment, significant differences in the content of the elements in grain of winter rye in the successive growing seasons were found. This, in turn, largely depends on the weather conditions and processes occurring in the soil. Moreover, plant growing conditions directly affect the possibility of uptake of nutrients from the soil solution and in consequence their accumulation in grain. The high content of elements in winter rye grain was obtained in the last year of the study in which the warm and wet weather positively affected the content

of N, Cu, Zn and Fe. In turn, the highest amounts of K, Mg, Ca, Mn and B in winter rye grain were achieved in 2013. Unfavourable weather conditions probably were the primary cause of decrease of N, Ca, Cu, Zn, Fe and B in 2012 compared to 2013 and 2014.

2. The biochar application rates used in growing winter rye did not have an unambiguous and clear effect on the chemical composition of grain and its quality. It was only in the case of N that its higher content was determined in grain harvested from the biochar-amended plots at all assessment times (except for BC₂₀ treatment in the second year of the study). Additionally, 12 months after biochar incorporation to the soil increase of Mg (except for BC₁₀) and Mn and decrease of Ca, Fe and B was found compared to the treatment without biochar. In the second assessment date, in the biochar treatments the content of K, Ca and Fe in grain was significantly higher, and the content of Mn was significantly lower than in the control treatment, whereas in the third year of the experiment biochar caused significant decrease of K, Mg and B.

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Elementų kiekis žieminių rugių grūduose ir grūdų kokybės parametrai, priklausomai nuo dirvožemio papildymo medžio anglimi

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Santrauka

Lauko eksperimentas 2012–2014 m. buvo atliktas paprastajame jaurazemyje (*Haplic Podzol, PZha*). Tyrimo tikslas – įvertinti medžio anglimi papildytame dirvožemyje augintų žieminių rugių grūdų cheminę sudėtį. Nustatyti šie grūdų kokybės parametrai: N, P, K, Mg, Ca, Cu, Zn, Mn, Fe ir B kiekis, suminiai baltymų ir krakmolo kiekiai, redukuojančio cukraus, fenolinių junginių bei flavonoidų kiekiai ir chelatinė bei redukuojamoji geba. 2011 m. prieš žieminių rugių sėją medžio anglis buvo panaudota vieną kartą trimis normomis: 10 Mg ha⁻¹ (MA10), 20 Mg ha⁻¹ (MA20) ir 30 Mg ha⁻¹ (MA30). Medžio anglis buvo pirolizuota iš kviečių šiaudų 350–650 °C temperatūroje. Rugių gūdų kokybės rodikliai buvo tirti kiekvienais metais po derliaus nuėmimo, t. y. po medžio anglies įterpimo į dirvožemį praėjus 12, 24 ir 36 mėnesiams.

Visais vertinimo laikotarpiais rugių grūduose esantis N kiekis buvo didesnis iš medžio anglimi papildytų laukelių nei iš kontrolinio (MA0). Pirmaisiais tyrimo metais mažiausias P kiekis grūduose buvo nustatytas MA30 varianto (3,40 g kg⁻¹), trečiaisiais – MA10 varianto (3,31 g kg⁻¹) laukeliuose. Antrojo ir trečiojo vertinimo metu didinant medžio anglies normą K, Mg ir Zn kiekis grūduose iš medžio anglimi papildytų variantų mažėjo, o Mn kiekis didėjo. MA30 varianto laukeliuose mažiausias Ca kiekis (0,147 g kg⁻¹) grūduose buvo nustatytas pirmojo vertinimo metu, o po 36 mėnesių jis buvo didžiausias (0,302 g kg⁻¹). Pirmaisiais ir trečiaisiais tyrimo metais Cu kiekis buvo didesnis grūduose iš MA30 varianto laukelių nei iš MA10 (atitinkamai 31,6 ir 57,3 %). Antrojo ir trečiojo vertinimo metu, po medžio anglies panaudojimo praėjus 24 ir 36 mėnesiams, Fe ir B kiekis grūduose, gautuose iš MA10 ir MA20 variantų laukelių, buvo didesnis nei MA30 varianto.

Antrojo ir trečiojo vertinimo metu, po medžio anglies panaudojimo praėjus 24 ir 36 mėnesiams, Fe ir B kiekis grūduose, gautuose iš MA10 ir MA20 variantų laukelių, buvo didesnis nei MA30 varianto. Grūduose, užaugintuose laukeliuose, tręštuose 20 Mg ha⁻¹ medžio anglies, buvo nustatyti didesni bendri baltymų ir krakmolo kiekiai, palyginus su grūdais, augintais panaudojus 30 Mg ha⁻¹ (atitinkamai 10,7 ir 17,1 %). Didžiausi redukuojančio cukraus (9,02 mg g⁻¹ sausos masės (SM)) ir fenolinių junginių (6,8 mg galo rūgšties ekvivalento (GAE) g⁻¹ SM) kiekiai rugių grūduose buvo nustatyti MA10 variante. Dirvožemio pagerinimas įvairiomis normomis medžio anglies neturėjo vienareikšmės įtakos rugių grūdų antioksidacinės gebos parametrams (chelatų ir redukuojančiai gebai).

Reikšminiai žodžiai: elementų sudėtis, grūdų kokybė, medžio anglis, *Secale cereale*.