Phytochemical properties and heavy metal accumulation in wheat grain after three years' fertilization with biogas digestate and mineral waste

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Non-standard materials used for plant fertilization, require characterization to reduce any possible undesired effects. The aim of this study was to investigate the effect of fertilization with biogas digestate (BD) and mining waste (carboniferous mudstones (MS) from coal mine) on wheat yield quality and the bioaccumulation of heavy metals in wheat grains in relation to conventional fertilization (NPK) and no fertilization. Using the co-application of waste (MS+BD), the highest yield was obtained in the first and second year, but in the third year, a slight decrease in yield was observed compared to NPK. In all years, BD and MS+BD fertilization increased the content of protein, wet gluten, and phenols in wheat grain compared to NPK and no fertilization. Waste fertilization had a positive effect on the antioxidant capacity index of grain but only in the first year. The bioaccumulation index (BAI) demonstrated that NPK decreased BAI for Zn, B, Cd, and Mn, whereas MS+BD decreased BAI for Cu, Sr, Pb, Co, Ba, and Cr.

Key words: antioxidant capacity, grain quality, phenolics, flavonoids, nutritional value, protein, starch, gluten

Introduction

Environmental protection problems, especially challenges coming from enhanced recycling, result in attempts to use various organic and mineral wastes to fertilize plants and to improve soil properties. One of the methods used to increase efficiency in agriculture is the use of the residues produced in methanogenesis in biogas plants (biogas digestate - BD).

The research reveals that digestate improves the physical and chemical properties of the soil, increases yields, and decreases the nitrate content in plants compared to mineral fertilization. BD also increases the content of organic C in the soil and reduces the rate of its transformation in comparison to non-digested input organic materials (Chen et al. 2012, Johansen et al. 2013, Lopedota et al. 2013). BD contains more (60–70% of total N) mineral nitrogen ($NH4^+$ / $NH3^-$) compared to different types of organic fertilizers (e.g., compost, cattle manure contains 6–30% mineral N of total N) (Svensson et al. 2004, Alburquerque et al. 2012, Lopedota et al. 2013). Mineral nitrogen found in digestate occurs in readily available form to plants. When in available form, unused nitrogen can be leached or emitted from the soil and thus, it can be harmful to the environment. Nitrogen losses after fertilization with BD may be higher compared to other types of organic fertilizers, on the other side, these nitrogen losses may be similar to synthetic mineral fertilizers, especially in soils with low N retention (sorption). Efficient fertilization with BD should depend on its properties, and this means that it should be applied in small split doses adjusted to crop and soil requirements (Alburquerque et al. 2012, Lopedota et al. 2013). Depending on the feedstocks used for biogas production, BD can significantly differ in the content and proportions of macro- and micronutrients (Demirel et al. 2013, Różyło et al. 2015).

Clay rocks including mudstones represent another direction in the search for the increase in soil quality and agronomic yield. The basic problem in mining is the production of waste rocks; therefore, mines are interested in their dispersion into the environment of surrounding regions. Furthermore, a high dose addition of clay and mudstone (from different sources) to the soil is a procedure that can be undertaken only rarely. Not all waste rocks from the mining industry can be used for soil reclamation. For obvious reasons, waste that could have negative effects on the soil or plants cannot be used. Therefore, mining waste must be thoroughly tested before its addition to the soil. Clay rocks including mudstones possess a relatively high capacity for sorption of organic and inorganic compounds (Koutsopoulou et al. 2010, Jabłońska 2012). Their application to podzolic soil changes its structure as well as the distribution, size and density of pores; it changes soil aeration, water retention capacity, and increases the net soil surface area and nutrient retention. Depending on its composition, clay stone can increase the content of minerals and their bioavailability as a direct or indirect effect of pH change (Mader et al. 1997, Sudnitsyn 2015).

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An increase in the content and bioavailability of minerals for plants is of particular importance in reference to the issue of depleting the soil of micronutrients. This is translated into the overall soil yield potential. Clay minerals may also change the amount and the bioavailability of contaminants in the soil, including heavy metals. The mobility and bioavailability of cationic metals and anionic metalloids in soils are affected by soil amendments in different ways. To reduce the mobility and bioavailability of metals/metalloids in contaminated soils, several organic and inorganic soil amendments are described in literature. But, depending on the environmental conditions, increased mobilization of metalloids in these contaminated soils was often observed when they were treated with soil amendments (Violante et al. 2010, Lim et al. 2013).

Apart from providing major nutritional components, food should be a source of biologically active substances regulating physiological processes in the human organism (Zhao 2007, Świeca and Baraniak 2014). Wheat is one of the most popular sources of food and should have a high nutritional value. Wheat grown under appropriate conditions contains many macro- and micronutrients as well as phenolic acid compounds with well documented antioxidant activity (Okarter et al. 2010, Hung et al. 2011, Gawlik-Dziki et al. 2012). In response to changing environmental conditions and their adaptation to existing conditions, plants modify their metabolism and uptake of elements, including heavy metals and other contaminants. Therefore, the composition and quality of food of plant origin may also change (Zhao 2007, Świeca et al. 2012, Gawlik-Dziki et al. 2013).

Increasingly, agriculture faces the problem of soil degradation, reducing the quality of plant products. Growing plants under degraded soil conditions require the use of various treatments that may eliminate the negative effect on the quality of food products of disturbing the ion balance of the soil. Despite the increasing interest in the use of wastes for plant fertilization, there is a lack of comprehensive research on the effectiveness of this type of treatments and their effect on the nutritional and health-promoting value of cereals thus treated.

The main aim of the present study was to evaluate the effect of fertilization with biogas digestate and mining waste on wheat yield and the level of major nutrients in grain. Special attention was also paid to the nutritional (protein and starch) and nutraceutical potential (phenols and antioxidant activity). In addition, the study determined the effect of the studied factors on the degree of uptake and bioaccumulation of heavy metals in wheat grain.

Materials and methods

Field experiment

A field experiment was performedin the growing seasons 2013/2014, 2014/2015, and 2015/2016 at the Experimental Farm in Bezek (N: 51.200696 E: 23.293073), which belongs to the University of Life Sciences in Lublin (Poland). In 2013, the research work comprised preparing the field, setting up a field experiment, collecting soil samples, and starting preliminary analysis. The experiment was set up in a randomized block design in three replicates (5 treatments x 3 replicates = 15 plots each with an area of 37.5 m^2 each) on podzolic soil (PS). The particle size distribution of PS was as follows: sand (2.0–0.05 mm) = 72%; coarse silt (0.05–0.02 mm) = 14%; fine silt (0.02–0.002 mm) = 13%, and clay particle size <0.002 = 1%. The PS had a low content of total N, P, K, and Mg (Table 1).

The podzolic soil was amended with BD and MS. Based on the following experimental design (Table 2), BD and MS were added (during autumn pre-sowing tillage operations on the depth 25±2cm).

Synthetic fertilizers in the conventional fertilization treatment and the waste materials were incorporated into the soil in the fall during the preparation of the field for sowing. The winter wheat cultivar ”Tonacja” was sown on September 18–21, 2013, 2014, and 2015. Nitrogen fertilizer (urea) was applied in amide form ($C\text{-}NH_2$) and was divided into two doses: one dose of 40 kg ha^{-1} was applied in the fall (immediately after sowing), while the other one (80 kg ha^{-1}) in the spring at the beginning of plant growth. The basis for determining the rate of tested materials was not mainly N, but also the high content of K, P, Mg, Na in BD and Al, Fe, Na, K in MS (Table 1). The amount of mining waste was based on the assumption that 5% (mass of MS to mass of soil) is the minimum to obtain noticeable changes in soil properties and their effects on plants (Różyło et al. 2016). The field experiment scheme from the first vegetative season (2013/2014) was repeated in the two subsequent seasons (2014/2015 and 2015/2016) to determine the influence of subsequent doses of wastes on the yield and quality of wheat (Table 2). The plots were at the same site (blocks) in the crop rotation: winter oilseed rape - winter wheat - oats. Annual NPK applications were: TN = 147; P = 29; K = 137 kg ha^{-1} from BD and TN = 558; P = 2.3; K = 51 kg ha^{-1} from MS. The very high C/N ratio for MS (77.4) indicates that despite high levels of TN, it was not available to plants.

Table 1. Chemical properties of control soil (podzolic soil - PS), biogas digestate (BD) and mining waste (mudstone - MS) used in the experiment (Różyło et al. 2015) (mean ± SD, n = 3)

Parameters and elements	PS	BD	MS
dry matter (%)	–	8–9	75–80
pH (in 1M KCl)	4.4 ± 0.23	9.9 ± 0.47	7.8 ± 0.31
C/N	23.8	22.0	77.4
EC (mS/cm)	1.20 ± 0.17	3.70 ± 0.25	0.84 ± 0.19
mg kg^{-1} DW			
TOC	9506 ± 781	633027 ± 1891	281195 ± 3180
TN	413 ± 89	28820 ± 118	3631 ± 177
P	49 ± 4.8	5580 ± 29.4	14 ± 27.1
K	45 ± 3.5	26906 ± 39.8	333 ± 11.9
Mg	10 ± 0.7	4420 ± 30.4	139 ± 5.3
Fe	393 ± 12.9	1445 ± 19.6	4200 ± 24.3
Ca	222 ± 18.5	311 ± 27.3	761 ± 37.1
Na	603 ± 29.4	2900 ± 38.9	1450 ± 40.6
$S\text{-}SO_4$	8 ± 0.8	225 ± 2.3	132 ± 1.5
B	0.5 ± 0.04	23.4 ± 0.15	10.1 ± 0.09
Mn	61 ± 4.5	246 ± 6.9	96 ± 5.1
Cu	0.5 ± 0.04	14.2 ± 0.12	14.6 ± 0.16
Zn	2.5 ± 0.2	145.1 ± 0.4	24.4 ± 0.7
Al	4505 ± 52.5	512 ± 45.2	20870 ± 87.3

EC = electrical conductivity; DW = dry weight; TOC = total organic carbon; TN = total nitrogen; C/N = ratio of carbon to nitrogen; P, K, Mg – (available P and K were determined by the Egner-Riehm method; and, available Mg – by the atomic absorption spectrometry (AAS) method after extraction with 0.0125 mol l^{-1} $CaCl_2$

Table 2. Experimental design

Experimental factors	Years (growing seasons) 2013/2014	2014/2015	2015/2016
C		0 (control soil)	
NPK	NPK	NPK	NPK
MS	MS (155 t DW ha^{-1})	MS (155 t DW ha^{-1})	MS (155 t DW ha^{-1})
BD	BD (5.1 t DW ha^{-1})	BD (5.1 t DW ha^{-1})	BD (5.1 t DW ha^{-1})
MS+BD	MS+BD (155 t DW ha^{-1} + 5.1 t DW ha^{-1})	MS+BD (155 t DW ha^{-1} + 5.1 t DW ha^{-1})	MS+BD (155 t DW ha^{-1} + 5.1 t DW ha^{-1})

C = control (without fertilization); NPK = PS + N, P, K, Mg, Ca, S (120 N, 100 P_2O_5, 120 K_2O, 40 MgO, 60 Ca, 30 SO_2 kg ha^{-1}, respectively)

Characteristics of waste materials

Biogas digestate (BD) was collected from a biogas plant operated byWikana Bioenergia Sp. z o.o. (Poland). The following feedstocks were used for energy production: corn silage (70%), sugar bagasse beet (15%), fruit pomace (5%), dairy wastes (5%), and manure (5%). The type of fermentation wasmesophilic (32–42 °C). This waste is a mixture of water and digested organic matter. The dry matter content in unprocessed BD used in the study was 8–9% (Różyło et al. 2015). In the subsequent years, BD originated from the same biogas plant, with the substrates for the biogas production from the same suppliers. Moreover, the proportions of substrates and fermentation conditions did not change significantly, which enabled the use of BD with similar parameters in the subsequent years.

The source of clay minerals was mudstones (MS) originating from carboniferous roof rocks, bottom rocks, or interlayers of exploited coal seams in a coal mine belonging to the coal company “Bogdanka” SA (Poland).

In petrographic terms, it is a mixture of mainly clays and mudstones. The mechanical composition of carboniferous mudstones (MS) is: fraction 120–200 mm = about 10%, fraction 120-20 mm = 30–40%, fraction 20–0.5 mm = 30–40%, and fraction <0.5 mm = about 20%. Stony fractions (200–1 mm) disintegrate under atmospheric conditions relatively quickly (few months); therefore, these wastes do not require mechanical treatment prior to application to the soil. These minerals complement organic matter concentrations. The mineral composition of this waste primarily consists of silica (SiO_2 = 470 g kg^{-1}) and aluminum oxide (Al_2O_3 = 220 g kg^{-1}) (Różyło et al. 2015). In all years, the same MS was used, which was shipped prior to the establishment of the field experiment, stored under cover, and each year, a suitable portion was transported to the field. Table 1 shows the properties of the waste materials used in the experiment.

Soil and waste analysis

First soil samples were collected in 2013 immediately before the start of the experiment (Table 1). Subsamples were collected from the entire length of the arable layer of the soil (27±2 cm) with a stainless steel corer (2 cm in diameter). Then, the subsamples from each plot were mixed to obtain a representative sample. The following soil properties were analyzed using Van Reeuwijk's standard laboratory procedures: particle size distribution by the hydrometer method; pH in 1 M KCl solution potentiometrically (a soil to solution ratio of 1:2.5); total nitrogen was determined by Kjeldahl's method without the application of Devarda's alloy (Cu–Al–Zn alloy reducer of nitrites and nitrates).

The total organic carbon (TOC) content was determined by the gravimetric method. The soil/BD/MS was dried at a temperature of 105 °C to constant weight and then incinerated at 550 °C and the weight loss was measured. The concentration of plant available P and K were determined by the Egner-Riehm method (KQ/PB-07); and, available Mg – by the atomic absorption spectrometry (AAS) method after extraction with 0.0125 mol l^{-1} $CaCl_2$ (PN-R-04020, 1994).

Waste and the majority of conventional fertilization were applied in the autumn of the preceding year prior to sowing of winter wheat. Due to the required homogenization time of waste with soil, the soil samples for the measurement of heavy metal content were collected in spring (2014 and 2016) prior to the commencement of winter wheat vegetation.

The metal and other elemental concentrations were determined using a START D microwave oven (Milestone, Italy) via a wet method in a mixture of nitric acid (8 ml) and hydrochloric acid (2 ml). Analysis of the Cr, Cu, Ni, Mn, Pb, Cd, Zn, Co, Fe, Ba, Al, Sr and Ag contents was performedusing ICP-OES (Thermo Scientific, ICAP 7000 Series, USA). Evaluation of the accuracy and precision of the analytical procedures used reference materials (Heavy Clay Soil, RTH 953. Promochem). Based on the total contents of elements in the soil and grain, the Bioaccumulation Index (BAI) was calculated according to the following formula: BAIx=GCx/SCx (x–element; GC–total concentration in grain; SC–total concentration in soil).

Yield analysis

Each year when wheat grains were ready to be harvested, whole winter wheat plants (stubble 10 cm left in the field) were sampled by hand from three randomly selected locations with an area of 1 m^2. Ears were separated from straw manually. Ear samples were threshed in a WINTERSTEIGER LD 180 laboratory thresher. Grain and crop residues were weighed separately, converting their yields to a per hectare basis and calculated harvest index (HI = grains / residues + grains). Next, 500 grams of the samples were separated for qualitative evaluation. Thousand grains weight was determined (counting 2 × 500 grains).

Analysis of nutritional and health-promoting quality of wheat grain

The grain samples were analyzed for their protein, wet gluten and starch contents, separately for each replicate/plot and the 3 sampling sites (1 m^2). Total protein (TP) was calculated from the total nitrogen content (in the rate of N - 5.7). The total nitrogen (TN) content was determined for the whole milled grains (whole wheat flour) by Kjeldahl method (ISO/TS 16634-2:2009). The amount of wet gluten was determined by mechanical means (ISO 21415-2:2015). The starch content was determined by Clendenning method (ICC Standard no. 122/1). These data were the basis for calibration "OmegAnalizer G" produced by Bruins Instruments NIR (near infrared) grain analyzers. Wavelength range is 730–1100 nm transmission with 5 nm scan increment. Automatic feed with multiple sub-sample measurements allowed to get repeatable results for the tested grain parameters.

Phenols and antioxidants were isolated from wheat flours (0.5 g) by extracting three times with 4 ml of acetone/water/hydrochloric acid (70:29:1, v/v/v). After centrifugation (10 min, 6800 × g) fractions were collected, combined, and used for further analysis.

Total phenols content (TPC) were estimated according to the Folin-Ciocalteau method. A 0.5 ml sample of the extract was mixed with 0.5 ml of H_2O, 2 ml of Folin reagent (1:5 H_2O), and after 3 minutes with 10 ml of 10% Na_2CO_3. After 30 minutes, the absorbance of mixed samples was measured at a wavelength of 720 nm. The amount of total phenolics was expressed as a gallic acid equivalent (GAE) per g of dry weight (DW).

Total flavonoids content (TFC) were estimated according to the method described by Bahorun et al. (2004). One milliliter of sample was mixed with 1 ml 2% $AlCl_3x6H_2O$. After 10 min absorbance at 430 nm was measured. The total flavonoid content was expressed as quercetin equivalent (QE) in milligrams per DW.

Antiradical activity was performed using an improved ABTS decolorization assay (Re et al. 1999). The ABTS radical cation as produced by reacting 7 mM of ABTS stock solution with 2.45 mM potassium persulfate (final concentration) and allowing the mixture to stand in the dark for at least 6 h at room temperature prior to use. The ABTS solution was diluted to an absorbance of 0.7 ± 0.05 at 734 nm (Lambda 40 UV–Vis spectrophotometer, Perkin Elmer). The affinity of the test material to quench the ABTS free radical was evaluated according to the following equation: scavenging % = $([A_C – A_A] / A_{C)})$× 100, where : A_C – absorbance of control, A_A – absorbance of sample.

Free radical scavenging ability was expressed as Trolox equivalent (TE) in micromoles per gram of DW.

The reducing power (RP) was determined by the method of Oyaizu (1986). The analyzed sample (2.5 ml) was mixed with phosphate buffer (2.5 ml, 200 mM, pH 6.6) and potassium ferricyanide $K_3(Fe[CN_6])$ (2.5 ml, 1%). The mixture was incubated at 50 °C for 20 min. Reactions were stopped with 0.5 ml 10% TCA and centrifuging for 10 min at 6500 g. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and 0.5 ml of 0.1% $FeCl_3$ and the absorbance was measured at 700 nm. RP was expressed as Trolox equivalent in micromoles per gram of DW.

Chelating power (CP) was determined by the method of Guo et al. (2001). The extract samples (5 ml) were added to 0.1 ml of 2 mM $FeCl_2$ solution and 0.2 ml 5 mM ferrozine. The mixture was shaken vigorously and left to stand at room temperature for 10 min. Then, the absorbance of the solution was measured spectrophotometrically at 562 nm. The percentage of inhibition of ferrozine–Fe^{2+} complex formation was calculated according to the following formula: % inhibition = $[1-(A_p/A_c)]$ ×100, where: Ac – absorbance of control, Ap – absorbance of sample. CP was expressed as ethylenediaminetetraacetic acid (EDTA) equivalent in micrograms per gram of DW.

Three complementary antioxidant methods were integrated to obtain the total antioxidant capacity index (ACI) (Świeca and Baraniak 2014). The index may be useful for evaluation of the total antioxidant potential of wheats from different fertilization with respect to control. The ACI was calculated as the sum of relative activities (RA) for each antioxidant chemical method divided by the number of methods (n). ACI = $\Sigma\ RA_{(n)}$ / n

RA was calculated as follows: RA = Ax/Ac, where: Ax = activity of modified sprouts for the method and Ac = activity of control sprouts determined for the method.

Statistical analysis

All experimental results were presented as means of three parallel replicates. One-way analysis of variance (ANOVA) and Tukey's post hoc test were used to compare groups within different elicitors. α values < 0.05 were regarded as significant. Tukey's HSD test (intermediate between LSD test and Scheffe's test) is an easy method of determining the critical significance of differences and is adequate in simple factor systems (equal sample sizes per group).

Results

Yield structure and nutrient content

The incorporation of wastes into the tested soil (PS) significantly changed the winter wheat yield accumulation or above-ground biomass (crop residues) accumulation. In all years the coapplication of wastes (MS+BD) increased the wheat grain yield compared to controls (significant differences) and in 2015 compared to NPK (Table 3). In the third year, the fertilization with MS+BD resulted in a non-significant decrease in wheat grain yield compared to NPK.

The highest grain yield was obtained in the second year on soil fertilized with MS+BD. The highest straw and other harvest residue weight was observed on those plots with NPK fertilization. Moreover, a significantly better harvest index (HI) was obtained by fertilizing with MS+BD and with MS alone when compared to the control (C), NPK, and BD. The subsequent doses of MS+BD in the following years led to a deterioration in the HI. In the first and third year, MS+BD significantly increased thousand grains weight (TGW) compared to other fertilization methods. In the second year, MS+BD significantly increased TGW only in comparison to C.

Table 3. Effect of biogas digestate (BD) and mining waste (mudstones – MS) on the yield of winter wheat (mean, n = 3)

Parameters	Years	Type of fertilization				
		C	NPK	MS	BD	MS+BD
grain yield ($t\ ha^{-1}$)	2014	2.17^{a}	3.43^{c}	2.73^{b}	2.97^{b}	3.85^{c}
	2015	2.53^{a}	3.76^{b}	3.25^{b}	3.12^{ab}	4.17^{c}
	2016	2.94^{a}	3.98^{b}	3.08^{a}	3.35^{a}	3.82^{b}
harvest residues ($t\ ha^{-1}$)	2014	4.16^{ab}	6.28^{d}	3.90^{a}	5.45^{c}	4.91^{bc}
	2015	4.78^{a}	6.32^{c}	4.78^{a}	5.62^{b}	5.96^{bc}
	2016	4.97^{a}	6.67^{c}	4.28^{a}	6.00^{b}	5.89^{b}
harvest index – HI	2014	0.34^{b}	0.35^{b}	0.41^{a}	0.35^{b}	0.43^{a}
	2015	0.35^{b}	0.37^{ab}	0.40^{a}	0.36^{b}	0.41^{a}
	2016	0.37^{ab}	0.37^{ab}	0.42^{a}	0.36^{b}	0.39^{ab}
thousand grain weight (TGW) (g)	2014	35.1^{a}	36.6^{a}	35.7^{a}	35.9^{a}	39.8^{b}
	2015	37.8^{a}	39.3^{b}	40.1^{b}	39.7^{b}	40.5^{b}
	2016	39.1^{a}	40.6^{a}	39.8^{a}	40.2^{a}	42.7^{b}

C = control (podzolic soil – PS without fertilization); NPK = PS + N, P, K, Mg, Ca, S (120 N, 100 P_2O_5, 120 K_2O, 40 MgO, 60 Ca, 30 SO_2 kg ha^{-1}, respectively); MS = PS + 155 t DW of MS ha^{-1}; BD = PS + 5.1 t DW of BD ha^{-1}; MS+BD = PS + 155t DW of MS ha^{-1} + 5.1 t DW of BD ha^{-1}; a, b, c. - data marked with the same letters do not differ significantly

The most efficient in terms of the total protein and wet gluten content in the wheat grain were BD (in the first year) and MS+BD (in the second and third year) fertilizations. Digestate significantly increased the content of protein and gluten compared to C, NPK, and MS. In the second and third year, the MS+BD fertilization significantly increased the protein and gluten content compared to C and MS. Moreover, the use of MS+BD fertilization efficiently increased the protein and wet gluten content in the subsequent years (Fig. 1).

The experimental factors in the first and third year didn't have a statistically proven effect on the starch content in the studied wheat grain. In the second year, a significant decrease in the starch content was recorded in the wheat grain fertilized conventionally (NPK) compared to that with no fertilization (C) or waste fertilization (MS, BD, MS+BD) (Fig. 1).

Phenolics, flavonoids and antioxidant potential

In the first year, a significant increase in the phenolic content (PC) in the wheat grain was obtained on those plots fertilized with digestate (4.89 mg g^{-1} DW) and with MS+BD (4.71 mg g^{-1} DW) compared to C, NPK, and MS (Table 4). Similar relationships occurred in the second year, yet the differences were not statistically significant. In the third year, all combinations of waste fertilization significantly increased the PC compared to C and NPK. The experimental factors determined the flavonoid content (FC) in the wheat grain differently than PC, as in the first season, a significantly higher FC was obtained using NPK and MS compared to C, BD, and MS+BD. In the second and third year, the statistically highest FC characterized grain from BD fertilized plots (compared to C, MS+BD in the second year and compared to C, MS and BD+MS in the third year) (Table 4).

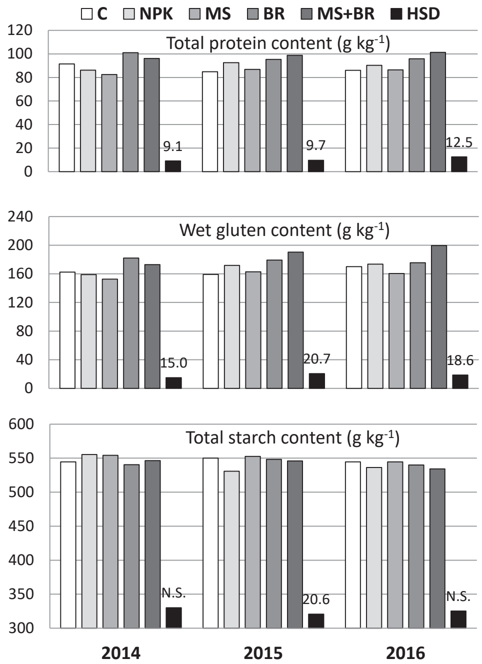

Fig. 1. Effect of biogas digestate (BD) and mining waste (mudstones -MS) on the protein, gluten and starch contents of winter wheat grain (n = 3); C = control (podzolic soil – PS without fertilization); NPK = PS + N, P, K, Mg, Ca, S (120 N, 100 P_2O_5, 120 K_2O, 40 MgO, 60 Ca, 30 SO_2 kg ha^{-1}, respectively); MS = PS + 155 t DW of MS ha^{-1}; BD = PS + 5.1 t DW of BD ha^{-1}; MS+BD = PS + 155t DW of MS ha^{-1} + 5.1 t DW of BD ha^{-1}; $HSD_{0.05}$ – honestly significant difference (Tukey's Test)

Table 4. Effect of biogas digestate (BD) and mining waste (MS) on the phenolics content and on the antioxidant capacity of winter wheat grain (mean, n = 3)

Parameters	Year	Type of fertilization				
		C	NPK	MS	BD	MS+BD
phenolics content (PC) (mg GAE g^{-1} DW)	2014	4.35^{b}	4.20^{a}	4.22^{ab}	4.89^{d}	4.71^{c}
	2015	4.11^{a}	4.29^{a}	4.30^{a}	4.57^{a}	4.50^{a}
	2016	3.82^{a}	4.05^{a}	4.81^{c}	4.44^{b}	4.57^{bc}
flavonoids content (FC) (mg QE g^{-1} DW)	2014	0.53^{b}	0.59^{c}	0.57^{bc}	0.53^{b}	0.47^{a}
	2015	0.50^{a}	0.58^{ab}	0.59^{b}	0.60^{b}	0.51^{a}
	2016	0.47^{ab}	0.49^{bc}	0.43^{ab}	0.57^{c}	0.40^{a}
chelating power (ChP) (µg of EDTA g^{-1} DW)*	2014	88.5^{b}	74.7^{a}	84.3^{b}	85.4^{b}	85.0^{b}
	2015	90.6^{a}	86.4^{a}	98.9^{b}	97.6^{b}	89.8^{a}
	2016	76.2^{ab}	103.7^{c}	91.0^{bc}	83.2^{ab}	69.7^{a}
reducing power (RP) (µmol of TE g^{-1} DW)	2014	1.68^{a}	1.89^{b}	1.86^{b}	2.01^{c}	1.82^{b}
	2015	1.70^{a}	1.97^{b}	1.94^{ab}	2.18^{b}	2.13^{b}
	2016	1.52^{a}	1.58^{a}	1.81^{a}	1.68^{a}	2.34^{b}
antiradical activity (ABTS) (µmol of TE g^{-1} DW)	2014	0.93^{a}	0.97^{a}	1.19^{a}	2.63^{c}	1.95^{b}
	2015	1.31^{a}	1.40^{a}	1.40^{a}	1.94^{b}	1.82^{b}
	2016	1.45^{a}	1.24^{a}	1.87^{b}	1.62^{ab}	1.54^{ab}
Σ total antioxidant capacity index (ACI)	2014	–	1.01	1.11	1.66	1.38
	2015	–	1.06	1.10	1.28	1.21
	2016	–	1.08	1.20	1.11	1.17

C = control (podzolic soil – PS without fertilization); NPK = PS + N, P, K, Mg, Ca, S (120 N, 100 P_2O_5, 120 K_2O, 40 MgO, 60 Ca, 30 SO_2 kg ha^{-1}, respectively); MS = PS + 155 t DW of MS ha^{-1}; BD = PS + 5.1 t DW of BD ha^{-1}; MS+BD = PS + 155t DW of MS ha^{-1} + 5.1 t DW of BD ha^{-1}; QE = quercetin equivalent; GAE = gallic acid equivalent; TE = trolox equivalent; EDTA = ethylenediaminetetraacetic acid; DW = dry weight; a, b, c - data marked with the same letters do not differ significantly

Table 4 demonstrates that experimental factors significantly influenced the parameters linked to the antioxidant characters of the grain (chelating power, reducing power, antiradical activity), yet they did not possess a directional character in any of the seasons. Thus, the total antioxidant capacity index (ACI) was calculated based on these parameters. The ACI calculation demonstrated that the best fertilization method was BD in the first and second year. In the third year, the highest ACI was noticed for MS.

Heavy metal contents and bioaccumulation index (BAI)

Tables S1 and S2 demonstrate that the content of toxic heavy metals in wheat grain as well as in the soil did not exceed the international permissible limits for heavy metals in soil and plants (in reference to WHO [1996]), following Ogundele et al. (2015) and Nazir et al. (2015) as well as with reference to EU Commission regulation No. 1275/2013 of 6—FAOLex) (EU 2013), even after the use of the third dose of waste in the third year of wheat cultivation. The content of elements in soil and grain of wheat had no clear differences related to the type of fertilization (2014 and 2016) and these results were difficult to interpret (Table S2). Therefore, the BAI was calculated by dividing the total content of heavy metals in wheat grain by the total content of heavy metals in the soil. A higher BAI value means a greater accumulation of metals in wheat grains (Table 5). Zn and Cu, whose concentrations in grain were significantly higher thanthose in the soil, were the elements that were most accumulated. In 2014, the highest BAI for Zn occurred on those plots without fertilization and with fertilization with MS+BD, whereas in 2016, it was for those plots fertilized with MS and BD. In both 2014 and 2016, the highest BAI for Cu occurred on plots without fertilization and with fertilization with BD. In both years, conventional fertilization in comparison to no fertilization or waste fertilization decreased the BAI for Zn, B, Cd, and Mn. Compared to the remaining fertilization methods, the first dose of MS+BD (in 2014) caused a decrease in BAI for Cu, Sr, Pb, Co, Ba, Al, and Cr. In 2016, similar relationships were observed (after three years of using MS+BD) when the elements with the lowest BAI in reference to 2014 included B and excluded Al.

The mean BAI for all elements demonstrates that in 2014, the lowest accumulation of elements occurred on the conventionally fertilized plots, while it was the highest on the plots without fertilization. In 2016, the lowest accumulation of elements occurred on the plots fertilized with MS+BD and the highest on the plots fertilized with BD (Table 5).

Table 5. Microelements Bioaccumulation Index (BAI) calculated according to the following formula: $BAI_x = GC_x / SC_x$ (x–element; GC–total concentration in grain; SC–total concentration in soil) (n = 3)

Elements	Type of fertilization									
	2014					2016				
	C	NPK	MS	BD	MS+BD	C	NPK	MS	BD	MS+BD
Zn	1.26^{c}	0.80^{a}	1.02^{b}	0.95^{ab}	1.25^{c}	1.21^{b}	0.86^{a}	1.61^{c}	1.47^{c}	0.97^{a}
Cu	1.01^{c}	0.64^{a}	0.55^{a}	0.89^{b}	0.54^{a}	0.81^{c}	0.52^{b}	0.44^{b}	0.98^{c}	0.27^{a}
B	0.26^{b}	0.17^{a}	0.27^{b}	0.30^{b}	0.25^{b}	0.20^{b}	0.14^{a}	0.18^{ab}	0.20^{b}	0.14^{a}
Sr	0.24^{c}	0.18^{b}	0.16^{b}	0.29^{c}	0.08^{a}	0.28^{c}	0.27^{c}	0.20^{b}	0.27^{c}	0.10^{a}
Cd	0.24^{c}	0.14^{a}	0.22^{bc}	0.24^{c}	0.21^{b}	0.24^{b}	0.09^{a}	0.21^{b}	0.24^{b}	0.22^{b}
Mn	0.15^{a}	0.13^{a}	0.16^{b}	0.15^{ab}	0.18^{b}	0.17^{b}	0.13^{a}	0.25^{c}	0.19^{b}	0.18^{b}
Al	0.14^{b}	0.16^{b}	0.14^{b}	0.15^{b}	0.11^{a}	0.16^{b}	0.14^{a}	0.13^{a}	0.16^{b}	0.16^{b}
Pb	0.16^{c}	0.14^{c}	0.11^{ab}	0.13^{bc}	0.09^{a}	0.15^{c}	0.13^{bc}	0.10^{ab}	0.14^{c}	0.08^{a}
Ba	0.16^{d}	0.10^{b}	0.13^{c}	0.14^{cd}	0.07^{a}	0.10^{b}	0.06^{a}	0.05^{a}	0.10^{b}	0.04^{a}
Ni	0.09^{a}	0.09^{a}	0.08^{a}	0.14^{b}	0.09^{a}	0.03^{a}	0.05^{ab}	0.06^{b}	0.05^{ab}	0.04^{ab}
Co	0.08^{c}	0.07^{bc}	0.06^{b}	0.11^{d}	0.04^{a}	0.08^{b}	0.08^{b}	0.08^{b}	0.15^{c}	0.05^{a}
Cr	0.07^{c}	0.05^{b}	0.05^{b}	0.08^{c}	0.02^{a}	0.03^{c}	0.02^{ab}	0.02^{ab}	0.02^{ab}	0.01^{a}
Fe	0.009^{c}	0.005^{a}	0.008^{bc}	0.008^{bc}	0.006^{ab}	0.008^{ab}	0.005^{a}	0.006^{ab}	0.009^{b}	0.005^{a}
Average	0.30	0.21	0.23	0.27	0.23	0.27	0.19	0.26	0.31	0.17

C = control (podzolic soil – PS without fertilization); NPK = PS + N, P, K, Mg, Ca, S (120 N, 100 P_2O_5, 120 K_2O, 40 MgO, 60 Ca, 30 SO_2 kg ha^{-1}, respectively); MS = PS + 155 t DW of MS ha^{-1}; BD = PS + 5.1 t DW of BD ha^{-1}; MS+BD = PS + 155t DW of MS ha^{-1} + 5.1 t DW of BD ha^{-1}; a, b, c, d - data marked with the same letters do not differ significantly

Discussion

The impact of the factors on the yield, quality and nutritional value of wheat grain

The increase in grain yield in the subsequent years was independent of the experimental conditions. This was caused by the plant rotation system (winter rapeseed–winter wheat–oats), in which following the harvest of all plants, their crop residues were left on the plot and plowed. It is likely that the nutrient content increased in the soil, and thus an increase in wheat yield was observed in the subsequent vegetative seasons.

The reasons for this pattern of yield structure can be seen in the sorption properties of MS. Clayey materials, to which MS belongs, have a relatively high capacity to sorb organic and inorganic compounds (Koutsopoulou et al. 2010, Jabłońska 2012). The application of MS or MS+BD may result in an improvement in the soil structure and reduce the leaching of minerals from the soil. Due to such characteristics, clay material content in MS may stabilize the processes of transformation of the compounds of fertilizers in the soil (especially NH_4^+ / NH_3 derived from digestate). Moreover, application of MS increases water retention in the soil, which may in turn favorably affect the growth and development of plants during water deficit periods. This may explain the highest wheat yield after using MS+BD in 2014 and 2015.

The decrease in grain yield in 2016, compared to previous years, when fertilized by MS+BD, suggests the optimum time period in the use of tested materials is two years. The use of a third dose of MS+BD in the last study year probably disturbed the ion balance in the soil (excess of P and K) and decreased the wheat yield.

The form and availability of N are considered to be the factors that most affect yield as well as protein and gluten content (López-Bellido et al. 2001, Lin et al. 2015). Mayer et al. (2015) found a significantly higher crude protein content (180 g kg^{-1} DM) in wheat fertilized with mineral fertilizers or mineral fertilizers in combination with organic fertilizers than in wheat fertilized with organic fertilizers, even at double rates, and in non-fertilized wheat (about 150 g kg^{-1} DM). In their research, wheat without fertilization was characterized by a significantly higher content of dry gluten (more than 120 g kg^{-1} DW) compared to the other fertilization treatments (about 100 g kg^{-1} DW). The differences in gluten content between the individual fertilization treatments were negiligble in the current study.

The yield of wheat also depend on the distribution of nitrogen doses over time and their amounts. According to López-Bellido et al. (2001), the rate of 150 kg N ha^{-1} significant increased the protein content but did not significant increase the grain yield compared to the rate of 100 kg N ha^{-1}. The authors found that there were no significant differences in grain protein content between the three N rates studied, which they explained by the effect of variable weather conditions during cultivation in the individual years of the experiment. A greater effect of weather conditions on the content of major nutrients than the effect of the type and rate of fertilizers is also confirmed by the study of Mayer et al. (2015).

In the present study, the significantly higher protein and wet gluten content with digestate fertilization (2014) and MS+BD (2015 and 2016) may be due to the better timing of uptake of N by plants. Nitrogen from mineral fertilizer in the podzolic soil is freely available and probably is too quickly taken up to enhance vegetative growth of wheat. Increased intensity of the reversible sorption mechanisms (Taghizadeh-Toos et al. 2012) may explain the reduced losses and balanced consumption of N in all phases of plant development. This effect is confirmed by the higher harvest index after MS+BD application.

ChP of wheat grain ranged from 69.7 to 103.7 μg of EDTA g^{-1} DW. For comparison, ChP of the grain of six spelt wheat cultivars can have a value ranging from 29.8 to 91.4 extract EC_{50} mg DW ml^{-1} (bound) (Gawlik-Dziki et al. 2012). In the study by Mazzoncini et al. (2015), the fertilization system (organic/conventional) did not significantly affect the antioxidant power (DPPH and ABTS) of white flour, whereas the antioxidant power in bran showed higher values under the organic system relative to the conventional system. The rate of fertilizer also has a significant influence on the antioxidant power. The results of Ma et al.'s (2015) study reveal that N fertilization at the rates of 180 and 240 kg ha^{-1} (150 kg P_2O_5 ha^{-1}; 150 kg K_2O ha^{-1}) reduced the antioxidant activity of winter wheat grain compared to no fertilization. They observed higher antioxidant activity only after the application of 300 kg N ha^{-1}. Hung et al. (2011), as well as Okarter et al. (2010) analyzed the antioxidant activity of sprouted wheat grains and their phenolic content, and positive correlations between these parameters were found when whole wheat grains were investigated. The results of the present study also confirmed this, since the ACI calculated for each fertilization treatment in most cases coincides with the total phenolic content.

A significant increase in the activity of antioxidant enzymes in the plant may also result from the defense mechanism induced in response to heavy metal stress (Belhaj et al. 2016) or disturbance of the ion balance in the soil. Indeed, the used variants of fertilization diversify phenolic content making it difficult to state clearly the mechanism of this phenomenon without additional tests concerning the level of oxidative stress markers as well as the activities of enzymes involved in phenolic synthesis and metabolism. But, from the nutritional point of view, these issues are important and lead to the conclusion that the most important factor is the final effect that can be generated by an improvement of antioxidant potential of wheat obtained in cultivation fertilized with BD and MS+BD (2014 and 2015) in relation to NPK and C. The successive decrease in ACI in the subsequent years after fertilization with BD and MS+BD was probably influenced by either the introduction of excessive K, P, Mg, and Na to the soil or a disturbance of the ionic balance in the soil.

The impact of various factors on the accumulation of heavy metals in wheat grain

The content of elements, including heavy metals, in a plant depends on many factors and their interactions. However, soil properties, which determine the bioavailability of these elements, are the main factor modeling the accumulation of elements in the plant (apart from its species). This study evaluated the effect of organic and mineral waste (MS, BD, and MS+BD) fertilization on the rate of uptake of heavy metals by winter wheat compared to control (without fertilization) and conventional fertilization (NPK).

Certain heavy metals, e.g. Cd, Cr, Ni and Pb, can be found in high contents in fertilizer products processed from waste materials. On the other hand, Al, which is an abundant element in soil, increases the risk of Alzheimer's disease (Wang et al. 2016). The solubility of heavy metals can become problematic under conditions of acidic soils and low Ca and Mg content. Under such conditions, the heavy metal retention mechanisms in which the exchange of heavy metals with Ca and Mg occur, primarily surface precipitation, are disturbed (Melo et al. 2016).

The basic parameter regulating soil's ability to buffer is its pH, and this is correlated to the redox potential (pE) and the content of Ca and Mn in the soil (Eshel et al. 2015). An increase in the accumulation of heavy metals is further linked to the mechanisms of plant physiology facilitating the uptake of elements under conditions of their deficiency in the soil (phosphorus and manganese in particular). A deficiency of macro- and micronutrients in soil results in the exudation of carboxylates and phenols by plant roots. These compounds increase the biological assimilation of nutrient elements, unfortunately also increasing the bioavailability of heavy metals (Clemens et al. 2002, Gherardi and Rengel 2004). Another mechanism of bioavailability changes of heavy metals is the activity of soil microorganisms related to soil parameters (e.g. pH, organic matter content) (Clemens et al. 2002, Jiang et al. 2008).

Conclusions

The results indicate that digestate and mudstone on a podzolic soil are not a significantly weaker nutrient sources to increase wheat yield compared to conventional mineral fertilization. In all years the co-application of wastes (MS+BD) increased the wheat grain yield compared to controls (significant differences) and in 2015 compared to NPK. In the third year, fertilization with MS+BD resulted in a non-significant decrease in wheat grain yield compared to NPK.In all years, BD and MS+BD fertilization increased the content of total protein, wet gluten, and phenols in wheat grain compared to NPK. Waste fertilization also had a positive effect on the total antioxidant capacity index (ACI) of grain, but only in the first year. Three years' use of waste did not result in exceeding international norms for the heavy metal content in soil and grain. It is interesting to see that the concentration of the total content of heavy metals in soil as well as in the grains are lowest (with a few exceptions) in the digestate treatments. The calculation of the bioaccumulation index (BAI) demonstrated that NPK decreased BAI for Zn, B, Cd, and Mn, whereas MS+BD decreased BAI for Cu, Sr, Pb, Co, Ba, and Cr. These results suggest that digestate and mudstone can be an alternative to conventional fertilization, especially when used in combination (MS+BD). But, they can be used for up to two consecutive years. To confirm the test results and exclude the impact of other factors, research in this area should be continued.

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