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Original Research

Effect of Three Years' Application of Biogas Digestate and Mineral Waste to Soil on Phytochemical Quality of Rapeseed

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Abstract

Using waste for plant fertilization requires identifying and reducing any possible undesired effects. The aim of this study was to investigate the effect of fertilization with biogas residue (BD) and mining waste (carboniferous mudstone and clay rock from a coal mine – MS) on oilseed rape (OSR) health quality of seeds in relation to conventional fertilization (NPK) and no fertilization. In the first and second years of the study the highest seed and fat yield was obtained from the NPK treatment. However, in the third year the highest yield was found using MS+BD. The lowest glucosinolate content was determined in OSR seeds fertilized with MS in the second year. The ratio of omega-6/omega-3 FA varied in a narrow range from 2.15 (NPK and MS) to 2.21 (BD and MS+BD). The most preferred form of fertilization in reducing heavy metal bioaccumulation (BAI) in seeds was MS+BD and NPK. NPK fertilization primarily reduced BAI of Zn, B, Mn, Cd, Co, and Fe, whereas MS+BD reduced BAI of Cu, Sr, Cr, Ba, and Pb. Results suggest that the wastes evaluated can be an interesting alternative for conventional fertilization in tested soil without the risk of a significant decrease in OSR quality.

Keywords: waste recycling, heavy metals, fatty acids, glucosinolate, clay rocks

Introduction

Environmental protection problems and efforts to increase agricultural productivity have resulted in attempts to use various mineral and organic wastes to fertilize plants and to improve soil properties [1-2]. The residues produced in methanogenesis in a biogas plant (biogas residue (BD), biogas digestate, biogas waste) are a relatively well-known material used for fertilization. The research on BD reveals that this waste improves soil physical and chemical properties, increases the organic carbon content, increases plant yield, and decreases the nitrate content in plants compared to mineral fertilization [3-5]. BD contains more ammonium nitrogen (NH_{4+}/NH_{3+}) than other types of organic biomass [6-8]. Depending on feedstocks used for biogas production, BD can significantly differ in the content and

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proportions of macro- and micronutrients [8-9]. A study conducted by Różyło et al. [10] shows that BD contains a disproportionately large amount of K (2.69 g kg^{-1} DM) and Na (0.29 g kg^{-1} DM) in relation to other elements.

Also, mining waste (MS) in the form of clay minerals has great potential to increase the agronomic efficiency of light soils. Mudstones and claystones, which constitute the major part of waste, exhibit high capabilities of organic and inorganic compound sorption. The sorption efficacy of claystones in relation to organic compounds may amount to up to 50% [11-12]. Thanks to these characters, MS can be used as stabilizers of fertilizer compound transformations in the soil and decrease the effect of leaching them out of the soil.

The modifications aimed at increasing production efficiency often cause negative changes of the obtained rapeseed oil properties. Studies conducted over the last several decades show that fertilization plays a huge role in the quality of plant production, modeling quantitative and qualitative changes in seed composition [13-16].

The change in the chemical composition and physical properties of soil after the incorporation of waste materials may disturb the ion balance of the soil and alter the properties of plants in an unpredictable way. In response to changing environmental conditions and adapting to existing conditions, plants modify their metabolism, and therefore the composition and quality of food of plant origins also changes [17-18]. While using waste materials for plant fertilization, it is important to identify and exclude or reduce any possible undesired effects. In the agronomic evaluation of the usability of waste, chemical analyses (chemical properties and the presence of organic and inorganic contaminants) as well as ecotoxicological evaluations are used [10, 19]. Nevertheless, such research does not answer the question concerning biochemical changes in plants.

Following palm and soybean oil, rapeseed oil is one of three most popular plant oil sources in the world. The majority of rapeseed oil is used by the food industry for direct or indirect consumption. Lipids are considered one of the most elemental nutrients for humans. The importance of food fats for the human organism indirectly stems from the fact that they provide high-energy respiratory substrates and essential fat-soluble vitamins (A, D, E, K). Lipid metabolism generates many bioactive lipid molecules, which are fundamental mediators of multiple signaling pathways, and they are also indispensable compounds of cell membranes. Lipids participate in lipoprotein metabolism, which is divided into two pathways: exogenous lipids and endogenous lipids. Lipids consist of fatty acids (FA), classified mostly according to the presence or absence of double bonds as saturated (SFA – without double bonds), monounsaturated (MUFA – with one double bond) and polyunsaturated fatty acids (PUFA – with two or up to six double bonds); further, as cis or trans based on the configuration of the double bonds and as n3 or n6 PUFA depending on the position of the first double bond from the fatty acid methyl-end. Exogenous FA for the human organisms are PUFA with the first double bond on C3 and C6 from the methyl-end. Since fatty acids from this group are indispensable for the normal functioning of metabolic processes, they have to be obtained from a diet [20-23]. Changes in lipid or lipoprotein metabolism can result in the modification of membrane composition and subsequently in changes in its permeability. It may also lead to disruption of signaling networks and could

Table 1. Chemical properties of control soil (podzolic soil-PS), biogas residue (BD), and mining waste (MS) determined immediately before the start of the field experiment [9] (mean, n = 3).

Parameter	PS	BD	MS
dry matter (%)	-	8-9	75-80
pH (in 1M KCl)	4.4	9.9	7.8
C/N	23.8	22.0	77.4
EC (mS/cm)	1.20	3.70	0.84
Element	mg kg^{-1} DW		
TOC	9506	633027	281195
TN	413.7	28820	3631
P	49.4	5580.6	14.8
K	45.1	26907	333.8
Mg	10.7	4420.4	139.8
Fe	393.6	1445.4	4200.8
Ca	222.0	311.6	761.0
Na	603.3	2900.2	1450.3
$S-SO_4$	7.8	225.1	132.7
B	0.5	23.4	10.1
Mn	61.4	246.1	96.6
Cu	0.5	14.2	14.6
Zn	2.5	145.1	24.4
Al	4505.1	512.7	20870.1
Cr	10.45	<0.1	19.8
Pb	10.26	<0.1	13.7
Ni	11.55	<0.1	4.51
Hg	<0.1	<0.1	<0.1
Sr	9.91	<0.1	5.81
Ba	32.20	<0.1	0.63
Co	2.50	<0.1	<0.1
Cd	0.47	0.29	<0.1

EC = electrical conductivity; DW = dry weight; TOC = total organic carbon; TN = total nitrogen; C/N = ratio of carbon to nitrogen; P, K, Mg–available

Table 2. Experimental design.

Experimental factor	Year (growing season)		
	2013/2014	2014/2015	2015/2016
C	PS + 0 (control soil)		
NPK	PS + NPK	PS + NPK	PS + NPK
MS	PS + MS (155 t DW ha^{-1})	PS + MS (155 t DW ha^{-1})	PS + MS (155 t DW ha^{-1})
BD	PS + BD (5.1 t DW ha^{-1})	PS + BD (5.1 t DW ha^{-1})	PS + BD (5.1 t DW ha^{-1})
MS+BD	PS + MS+BD (155 t DW ha^{-1} + 5.1 t DW ha^{-1})	PS + MS+BD (155 t DW ha^{-1} + 5.1 t DW ha^{-1})	PS + MS+BD (155 t DW ha^{-1} + 5.1 t DW ha^{-1})

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

be associated with some pathological states such as cancer, cardiovascular, neurodegenerative, and metabolic diseases, and similarly with inflammatory complications [24-25].

Among commonly consumed plant oils and animal fats, rapeseed oil has the most favorable composition and proportions of fatty acids, adjusted for the metabolic requirements of the human organism. Moreover, rapeseed oil has the most favorable percentage contribution of energy of SFA, PUFA, omega-3 (n3) PUFA, and omega-6 (n6) PUFA of vegetable oils to recommended daily intakes for total fat (ERDI-37.7 kJ/g) [23].

The increasing interest in the use of wastes for plant fertilization is not always accompanied by comprehensive research on the effect of waste on the nutritional and health value of plants. The aim of this study was to determine the effect of three years of fertilization with biogas residue and mining waste on OSR yield and the quantitative and qualitative changes in the fatty acids composition. Additionaly, the study determined the effect of the investigated waste materials on the uptake and bioaccumulation of heavy metals in OSR seeds.

Material and Methods

Field Experiment

A field experiment was carried out in the growing seasons such as 2013/2014, 2014/2015, and 2015/2016 at the experimental farm in Bezek (N: 51.200696 E: 23.293073) belonging to the University of Life Sciences in Lublin. 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, 3 reps, and 15 plots 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 mm = 1%. The soil was characterized by a low content of total N and P as well as a very low content of K and Mg (Table 1).

The PS was amended with BD and MS. Based on the following experimental design (Table 2), BD and MS were added (during summer 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 OSR cultivar Chagall was sown on August 20-25, 2013, 2014, and 2015. Due to the low class of the soil, the seeding rate was 60 seeds per m^2. Nitrogen fertilizer was applied in amide form (urea - $C\text{-}NH_2$) and was divided into two doses: one dose of 40 kg ha^{-1} was applied immediately after sowing (00 stage BBCH), while the other one (80 kg ha^{-1}) in the spring at the 29-30 stages BBCH). The basis for determining the rate of tested materials was not only mainly N, but also considered the high content of K, P, Mg, and Na in BD, as well as high content of Al, Fe, Na, and K in MS (Table 1). The content of the major fertilizer nutrients (NPK) in BD dry weight is many times higher than in MS dry weight. In order to avoid the effect of N overfertilization, the rates of BD were lower than the MS rates.

The field experiment scheme from the first vegetative season (2013/2014) was repeated in two subsequent seasons (2014/2015 and 2015/2016) in order to determine the influence of subsequent doses of wastes on the yield and quality of OSR (Table 2). The plots were at the same treatments (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, and 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

Characteristics of Waste Materials

BD was collected from a biogas plant of the company Wikana Bioenergia Sp. z o.o. in Poland. The following feedstocks were used for energy production: corn silage (70%), sugar bagasse beet (15%), pomace of fruit (5%), wastes from dairy (5%), and manure (5%). The type of fermentation is as follows: mesophilic (32-42ºC). 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% [10]. In 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 subsequent years (Table 1).

The source of clay minerals was MS originating from carboniferous roof rocks, bottom rocks, or interlayers of the exploited coal seams in a coal mine belonging to the coal company Bogdanka SA in Poland. In petrographic terms, it is a mixture of mainly clays and mudstones rapidly undergoing weathering. 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}) [9]. 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 the 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 (based) soil and waste samples were collected in 2013 immediately before the start of the experiment (Table 1). Soil subsamples were taken 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 (soil to solution ratio of 1:2.5); and 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), available Mg and Fe, Ca, NaS-SO_4, B, Mn, Cu, and Zn- with the atomic absorption spectrometry (AAS) method after extraction with 0.0125 mol/L $CaCl_2$ (PN-R-04020, 1994).

Evaluation Methods of Heavy Metal Bioaccumulation

Waste and the majority of conventional fertilization were applied in autumn of the preceding year prior to OSR being sown. Due to the required homogenization time of waste with soil, the soil samples for the measurement of heavy metals content were collected in spring 2016, prior to the commencement of OSR vegetation. The seed samples for measuring heavy metal content were collected in 2016, directly after OSR harvesting. The metal and other elemental concentrations in soil and seeds was 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) at a ratio of 4:1.

Analysis of the metals end metalloids (Cr, Cu, Ni, Mn, Pb, Cd, Zn, Co, Fe, Ba, Al, Sr were detected in most samples; other metals end metalloids, e.g., Hg, As, Se, Mo were not detected in most samples) contents were carried out using 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 seeds, the bioaccumulation index (BAI) was calculated according to the following formula: BAIx = SECx/SOCx (x – element, SEC – total concentration in seeds, SOC – total concentration in soil).

Yield Analysis

Each year when OSR were ready to be harvested, whole OSR plants (stubble 15 cm left in the field) were sampled by hand from three randomly selected locations of each plot with an area of 1 m^2. Siliques (pods) were separated from straw manually. Siliques samples were threshed in a WINTERSTEIGER LD 180 laboratory thresher. Seeds and crop residues were weighed separately, converting their yields to a per hectare basis and calculated harvest index (HI = seeds / residues + seeds). Thousand seeds weight was determined (counting 2 × 500 seeds).

Analysis of Nutritional Quality

The seed samples were subsequently analyzed for their fat and glucosinolate contents, separately for each replicate/plot and the 3 sampling sites (1m^2). Fat was determined by modified Soxhlet method (ISO 659:2009). The glucosinolates content was determined by method based on extraction by methanol, purification and enzymatic desulfatation, determination using reversed-phase chromatography (ISO 9167-1:1992). This data was 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 allow you to get repeatable results for the tested grain parameters.

Preparation of Fatty Acid Methyl Esters (FAME)

The seed samples for the measurement of fatty acids (FA) composition and heavy metal content were

Table 3. Effect of biogas residue (BD) and mining waste (MS) fertilization on OSR yields and seeds quality (mean, n = 3).

Parameter	Year	Type of fertilization				
		C	NPK	MS	BD	MS+BD
OSR seeds yield (t ha^{-1})	2014	1.64^{a}	2.76^{d}	2.12^{b}	2.21^{bc}	2.59^{cd}
	2015	1.78^{a}	2.95^{c}	2.31^{b}	2.38^{b}	2.92^{c}
	2016	1.69^{a}	2.88^{c}	2.40^{b}	2.27^{b}	3.05^{c}
Harvest residues (t ha^{-1})	2014	2.94^{a}	6.39^{c}	4.15^{b}	4.30^{b}	5.41^{c}
	2015	3.18^{a}	6.65^{c}	4.58^{b}	4.72^{b}	6.10^{c}
	2016	2.90^{a}	6.40^{c}	4.41^{b}	4.71^{b}	5.86^{c}
Harvest index – HI	2014	0.36^{c}	0.30^{a}	0.34^{bc}	0.34^{bc}	0.32^{ab}
	2015	0.36^{c}	0.31^{a}	0.34^{bc}	0.34^{bc}	0.32^{ab}
	2016	0.37^{c}	0.31^{a}	0.35^{b}	0.33^{ab}	0.34^{abc}
Thousand seeds weight (TSW) (g)	2014	5.43^{b}	4.85^{a}	4.87^{a}	5.37^{b}	5.08^{ab}
	2015	5.28^{a}	5.01^{a}	4.95^{a}	5.35^{a}	5.20^{a}
	2016	5.31^{b}	4.96^{a}	5.14^{ab}	5.09^{ab}	5.32^{b}
Fat content (%)	2014	44.1^{a}	43.9^{a}	43.7^{a}	44.1^{a}	44.0^{a}
	2015	44.9^{b}	44.4^{b}	44.6^{b}	43.1^{a}	44.2^{b}
	2016	44.7^{bc}	43.0^{a}	45.1^{cd}	45.8^{d}	43.9^{ab}
Fat yield (t ha^{-1})	2014	0.72^{a}	1.21^{c}	0.93^{b}	0.98^{b}	1.14^{c}
	2015	0.80^{a}	1.31^{c}	1.03^{b}	1.03^{b}	1.29^{c}
	2016	0.76^{a}	1.24^{cd}	1.08^{bc}	1.04^{b}	1.34^{d}
Glucosinolates (μmol g^{-1})	2014	13.3^{ab}	14.7^{c}	12.9^{a}	14.4^{bc}	13.5^{abc}
	2015	12.7^{ab}	11.5^{a}	12.7^{ab}	15.7^{d}	14.3^{c}
	2016	12.4^{a}	12.8^{a}	14.0^{ab}	14.9^{b}	14.6^{b}

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

collected in 2016, directly after OSR harvesting. Fatty acids (FA) composition of the fats was determined as their corresponding methyl esters. Preparation of FAME was carried out according to the PN-ISO5509 method. Per 100 mg of fat we added 4 ml of methanolic potassium hydroxide solution. Then the sample was heated and stirred at 85-95ºC. After cooling, 4 ml of methanol was added to a solution of boron trifluoride, heated again at 85-95ºC for 10 min, and cooled rapidly. One ml of hexane was added to the cooled ampoule. After stirring, 2 ml of saturated sodium chloride solution was added and mixed again. Using a pipette, we collected the organic layer (hexane) to an Eppendorf tube and dried it by adding anhydrous sodium sulfate. 100 µl from an Eppendorf tube was transferred to a vial containing 900 µl of hexane and placed in the autosampler. Analysis of FAME was performed by gas chromatography (PN-EN ISO12966-1:2015-01) with a Varian 450-GC gas chromatograph. Dispenser temperature – 250ºC, split – 1:50, column temperature – 200ºC for 10 minutes and 3 ºC/min to 240ºC. Total analysis time was 28 min. Type of carrier gas – Hel, Carrier gas flow – 2.5 ml/min, detector temperature (FID) – 300ºC, injection volume – 1 µl.

Statistical Analysis

All experimental results were represented as mean 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 for determining the critical significance of differences and is adequate in the simple factors systems (equal sample sizes per group).

Results and Discussion

Yield and Nutrient Content

The adding of wastes to the tested soil (PS) significantly affected yield and the structure of OSR yield. In all years of the study the mining waste (MS) and biogas digestate (BD) significantly increased the seed and biomass yield compared to the control treatment (C - without fertilization) (Table 3). In 2014

Table 4. Effect of three-year fertilization of biogas residue (BD) and mining waste (MS) on the fatty acids (FA) composition of OSR seeds (content of FA in % of total FAME (FA Methyl Esters).

Fatty acid	Type of fertilization				
	C	NPK	MS	BD	MS+BD
Palmitic C16:0	4.34[a]	4.53[b]	4.51[b]	4.42[ab]	4.30[a]
Palmitooleic C16:1	0.26[a]	0.29[b]	0.28[ab]	0.27[ab]	0.25[a]
Stearic C18:0	1.54[ab]	1.51[a]	1.62[b]	1.59[b]	1.56[ab]
Oleic C18:1 (+ Elaidic)	62.65[b]	60.33[a]	60.40[a]	61.61[ab]	62.53[b]
Linoleic C18:2 (+ Linolenelaidic)	20.05[a]	21.26[b]	21.16[b]	20.59[ab]	20.01[a]
α - Linoleic C18:3n3	9.11[a]	9.90[b]	9.82[b]	9.30[a]	9.05[a]
Arachidic C20:0	0.46[a]	0.48[ab]	0.49[ab]	0.49[ab]	0.50[b]
Arachidoic C20:4	1.01[a]	1.04[ab]	1.08[b]	1.08[b]	1.13[c]
Behenic C22:0	0.24[a]	0.25[a]	0.25[a]	0.26[ab]	0.28[b]
Σ SFA (% FA)	6.71[a]	6.93[ab]	7.03[b]	6.91[ab]	6.84[ab]
Σ MUFA (% FA)	64.01[c]	61.75[a]	61.85[ab]	63.04[bc]	63.99[c]
Σ PUFA (% FA)	29.16[a]	31.22[b]	31.04[b]	29.95[a]	29.12[a]
UFA/SFA	13.89[c]	13.42[ab]	13.21[a]	13.46[ab]	13.61[bc]
n3 (% FA)	9.11[a]	9.90[b]	9.82[b]	9.30[a]	9.05[a]
n6 (% FA)	20.05[a]	21.26[b]	21.16[b]	20.59[ab]	20.01[a]
n6 / n3	2.20	2.15	2. 15	2.21	2.21

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

and 2015, the NPK increased the OSR seed yield compared to C, MS, BD (significant differences), and MS+BD (insignificant differences). In 2016 the highest seed yield was obtained after coapplication of wastes (MS+BD) (significant compared to C, MS, BD, and insignificant compared to NPK). The significant highest straw and other harvest residue weight was observed on the plots with NPK and MS+BD fertilization compared to C, MS, and BD. Moreover, a significantly lower harvest index (HI) was obtained by fertilizing with NPK compared to C, MS, and BD (excepting BD in 2016). The subsequent doses of MS and MS+BD in the following years deteriorated the HI (Table 3).

The least favorable HI in the NPK treatment is probably caused by too high nitrogen availability. Nitrogen is used by the plants for vegetative development. The majority of nutrient resources are depleted in the early developmental stages and their deficiency occurs in the silique filling phases. Similar relationships were observed in the parallel experiment with winter wheat [10].

In the first year, NPK and MS significantly increased thousand seed weight (TSW) compared to C. In the second year differences were similar to the first year but were statistically insignificant. In the third year a significant reduction of TSW occurred only after NPK fertilization (Table 3).

In 2014 the content of fat in OSR seeds slightly depended on the type of fertilization. In 2015 fertilization with BD significantly reduced the fat content compared to other plots, whereas in 2016 the OSR seeds fat content in MS and BD was significantly higher than in NPK and MS+BD treatments. The conversion of the fat content to its amount obtained from one hectare demonstrates that statistically the most efficient in terms of the total fat yield was NPK and MS+BD in each year (Table 3).

In the first year of the experiment, NPK and BD increased glucosinolate content the most in comparison to C. However, in the case of BD, the difference was statistically insignificant. In the two next years, a significant increase in glucosinolate content occurred under BD and MS+BD fertilization. This was probably caused by high $S\text{-}SO_4$ content in BD and accumulation of this compound in the soil in subsequent years of waste use. The studies of Malhi et al. [26] and Ma et al. [27] indicate that the most yielding factors are N and S combined in a 10:2 ratio in comparison to other forms of mineral fertilization, organic fertilization, and their combinations. One of the most important elements for OSR fertilization is sulfur and also B and Cu. A dose of

S of 40-60 kg ha^{-1} may increase OSR yield by 12%, whereas B or B + Cu by approximately 10%. What is more, these microelements in higher doses can increase oil content in OSR. Additionally, S fertilization increases glucosinolate content in seeds [28]. In the work of Wang et al. [29], the use of Zn in the standard OSR fertilization increased seed yield by 5 to 16% and oil yield by 6 to 19%. The application of Zn fertilizer increased the concentrations of total oil, oleic acid, and linoleic acid in rapeseed, but reduced stearic acid concentration.

FA Composition

OSR fat contained mostly oleic acid, including its trans isomer form (the trans isomer of oleic acid), i.e., elaidic acid classified as a n9 MUFA. The highest oleic acid content was observed in seeds from control fields. The significant decrease of its content in relation to C occurred under NPK and MS. The lowest decrease in comparison to C occurred under MS+BD fertilization (Table 4). At the same time, we noticed a significant increase of α-Linoleic, Linoleic + Linolenelaidic, Palmitic, and Palmitooleic acid content in variants NPK and MS in comparison to C, BD, and MS+BD. NPK fertilization significantly reduced the content of stearic acid in comparison to MS and BD. Wastes fertilization (in particular MS+BD) increased the percentage content of arachidic, arachidonic, and behenic acids in relation to C, and sometimes in relation to NPK.

An important parameter within the context of evolutionary aspects of the human diet is the FA n6/n3 ratio. The origin ratio of n6/n3 was 1. In recent years, the increase of n6 PUFA in relation to n3 in the diet is considered to be an adverse civilization phenomenon [22, 30]. In our study BD and MS+BD decreased the content of n3 and n6 in the total FA compared to NPK and MS. The n6/n3 relationship varied in a narrow range (2.15–2.21). The most preferred n6/n3 ratio was obtained after applying NPK and MS, but the differences between fertilization methods were not significant (Table 4). Usually, the ratio of n6/n3 for OSR ranges from 0.5 to 2.6 [31-32] but it may drop to 16.3. Despite this fact, the oil obtained from OSR is characterized by one of the best FA compositions among plant oils [23]. The content of n6 and n3 PUFA and other FA is primarily genetically determined [32-33]. However, our studies show the significant effect of fertilization on n3 and n6 percentages. BD and MS+BD decreased the share of n3 and n6 in the total content of FAs compared to C, NPK, and MS. Onemli [31] provides that along with the increase of the amount of organic matter in the soil, the oleic acid content increases.

The unsaturated (UFA)/saturated (SFA) ratio of cold-pressed oil from pomagranate seeds in a Khoddami et al. [34] study varied between 12.43 and 13.07. In our study, this ratio amounted to 13.21 (MS) – 13.89 (C). Furthermore, NPK and MS increased the PUFA and decreased MUFA in relation to C, BD, and MS+BD (Table 4). Salama et al. [35], who studied the content and composition of oil in black sesame seeds (BS), determined that biofertilizers and micronutrients can increase oil content in BS by up to 20% compared with control treatment. Furthermore, the fertilization used in their experiment increased the oleic acid content by 27% and linoleic acid by up to 33% as compared to control. In the work of Wang et al. [29], the use of Zn for standard OSR fertilization increased seed yield by 5.0 to 16.0% and oil yield by 6.2 to 19.5%. The application of Zn fertilizer increased the concentrations of total oil, oleic acid, and linoleic acid in rapeseed, but reduced stearic acid concentration.

Table S1. Heavy metal contents (mg kg^{-1}) in OSR seeds after three-year fertilization with biogas residue (BD) and mining waste (MS) (n = 3).

Element	Type of fertilization				
	C	NPK	MS	BD	MS+BD
Al	104.38	83.29	70.85	85.45	98.82
Mn	47.24	35.31	43.68	75.84	41.58
Fe	53.90	46.49	44.43	47.51	48.43
Zn	31.39	26.08	29.08	35.31	29.92
Sr	16.59	13.38	17.26	15.73	19.23
B	26.54	8.06	12.51	9.26	5.69
Ba	5.83	5.77	4.93	11.09	6.05
Cu	6.26	6.67	6.17	5.48	6.76
Ni	1.26	1.16	3.42	1.72	2.30
Cr	2.09	2.17	1.97	1.92	1.51
Pb	0.21	0.18	0.17	0.20	0.14
Co	0.06	0.02	0.12	0.09	0.11
Cd	0.05	0.05	0.06	0.06	0.06
∑	295,80	228,63	234,64	289,66	260,59

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

Heavy Metal Contents and Bioaccumulation Index (BAI)

After three years of conventional fertilization or waste fertilization the content of heavy metals in OSR seeds as well as in the soil did not (in most cases) exceed the international permissible limits of heavy metals concentration in soil and plants (in reference to WHO (1996), as well as with reference to the EU Commission regulation No. 1275/2013 of 6-FAOLex [34]. The exception was Al, Fe, and Cr (NPK slightly increased Cr content compared to control) and Cd in seeds, whose

Table S2. Heavy metal contents (mg kg^{-1}) in soil after three-year fertilization with biogas residue (BD) and mining waste (MS) (n = 3).

Element	Type of fertilization				
	C	NPK	MS	BD	MS+BD
Al	4665.82	4466.97	5174.67	4172.95	5184.63
Mn	231.70	191.18	204.19	212.87	227.53
Fe	3572.55	4206.13	3690.67	3088.86	4224.57
Zn	14.65	18.83	16.73	15.20	19.68
Sr	9.91	11.53	17.75	7.85	27.66
B	12.16	11.84	10.09	7.46	11.89
Ba	32.20	32.89	35.27	30.36	51.42
Cu	2.85	4.20	6.61	3.13	7.07
Ni	11.55	8.76	9.10	8.60	12.09
Cr	10.45	13.54	12.99	9.35	18.55
Pb	10.26	10.68	11.27	9.61	14.18
Co	2.50	2.26	2.90	1.97	4.09
Cd	0.47	0.57	0.42	0.39	0.48
∑	8577,07	8979,37	9192,66	7568,59	9803,84

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

Table 5. Heavy metal bioaccumulation index (BAI) calculated according to the following formula: BAIx= SECx/SOCx (x–element; SEC–total concentration in seeds; SOC–total concentration in soil) (n = 3).

Element	Type of fertilization				
	C	NPK	MS	BD	MS+BD
Cu	2.19^{c}	1.59^{b}	0.93^{a}	1.75^{b}	0.96^{a}
Zn	2.14^{c}	1.39^{a}	1.74^{b}	2.32^{c}	1.52^{ab}
B	2.18^{a}	0.68^{a}	1.24^{b}	1.24^{b}	0.48^{a}
Sr	1.67^{c}	1.16^{b}	0.97^{ab}	2.00^{d}	0.70^{a}
Mn	0.20^{a}	0.18^{a}	0.21^{b}	0.36^{c}	0.18^{a}
Cr	0.20^{c}	0.16^{b}	0.15^{b}	0.20^{c}	0.08^{a}
Ba	0.18^{b}	0.18^{b}	0.14^{a}	0.37^{c}	0.12^{a}
Ni	0.11^{a}	0.13^{a}	0.38^{c}	0.20^{b}	0.19^{b}
Cd	0.10^{ab}	0.08^{a}	0.14^{c}	0.15^{c}	0.12^{bc}
Co	0.025^{b}	0.011^{a}	0.040^{c}	0.046^{c}	0.026^{b}
Al	0.022^{b}	0.019^{b}	0.014^{a}	0.021^{b}	0.019^{b}
Pb	0.020^{b}	0.017^{b}	0.015^{ab}	0.021^{b}	0.010^{a}
Fe	0.015^{b}	0.011^{a}	0.012^{ab}	0.015^{b}	0.012^{ab}
Avarage	0.70	0.43	0.46	0.67	0.34

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

concentration was recorded above the permissible limit set by WHO. However, this had no connection with the use of waste to fertilize OSR, because conventional fertilization and waste decreased the content of these elements in seeds compared to control (Tables S1, S2, and 5). NPK and BD decreased Al content in soil compared to C (Table S2).

The content of other elements in soil and seeds of OSR had no clear differences related to the type of fertilization and were difficult to interpret (Table S1 and S2), therefore the BAI was calculated (Table 5). Among the analyzed heavy metals, the highest BAI (regardless of fertilization) was found in the case of Cu, Zn, B, and Sr. The most preferred form of fertilization in reducing heavy metal accumulation was MS+BD and NPK. Fertilization of NPK in the highest degree reduced BAI of Zn, B, Mn, Cd, Co, and Fe compared to control and waste fertilization. Fertilization of MS+BD reduced BAI of Cu, Sr, Cr, Ba, and Pb compared to C and NPK, and B, Mn, compared to C, MS and BD. Differences were statistically significant but not in all cases. The mean BAI for all elements demonstrates that the lowest accumulation of elements occurred on the plots fertilized with MS+BD and the highest on the plots without fertilization (Table 5).

The basic parameter regulating the soil's ability to buffer is its pH, which is correlated to the redox potential pe and the content of Ca and Mn in the soil [37]. 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 occurs, primarily surface precipitation, are disturbed [38].

An increase in the accumulation of heavy metals is further linked to the mechanisms of plant physiology facilitating the uptake of elements under their deficiency in the soil (P and Mg in particular). The deficiency of macro- and micronutrients in the soil results in the exudation of carboxylates and phenols by plant roots. These compounds increase the biological assimilation of nutrient elements, unfortunately increasing the bioavailability of heavy metals [37-38]. Another mechanism of bioavailability changes of heavy metals is the activity of soil microorganisms related to the soil parameters (pH, organic matter content, etc.) [39, 41]. Moreover, the use of suitable combinations of rhizobacteria strains (*Bacteroidetes bacterium*, *Pseudomonas fluorescens*, and *Variovorax* sp.) can control the metal uptake of rapeseed, selectively increasing either metal extraction or metal stabilization in the rhizosphere [42].

In studies by Gisbert et al. [43], concentrations of Pb and Zn in *Brassica oleracea* were related more closely to total soil concentration than to DTPA-extractable concentrations of these elements. In addition, their control soil (pH = 6.51) had a higher bioaccumulation index of Cu and Mn than contaminated soil (pH = 7.34). As a result, *B. oleracea* growing on soil with a lower concentration of Cu and Mn (pH = 6.51) contained more Cu and Mn than *B. oleracea* growing on soil with a higher concentration of Cu and Mn, but higher pH (7.34).

Particularly noteworthy is the Al content that was high in OSR seeds (from 70.85- MS to 104.38 mg kg^{-1} D.W. – C) and in soil (from 4172.95 – BD to 5184.63 mg kg^{-1} D.W. – MS+BD) (Tables S1 and S2). In 2007, Joint FAO/WHO Expert Committee on Food Additives (JECFA) developed a Provisional Tolerable Weekly Intake (PTWI) for Al from all sources of 1 mg kg^{-1} of body weight (FAO/WHO, 2007). However, there remain uncertainties as to the extent of aluminium absorption from drinking water, which depends on a number of parameters, such as the Al salt administered, pH (for Al speciation and solubility), bioavailability, and dietary factors. Therefore, the committee applied an uncertainty factor of 100 to the lower end of this range of LOELs (50 mg kg^{-1} b.w. per day expressed as Al) to allow for inter- and intraspecies differences. In the natural environment, Al can be found mainly in the form of sparingly soluble silicates and aluminosilicates that are not harmful to humans. Some forms of aluminum in the right amount may be necessary for the proper functioning of living organisms. An increase in the most toxic form of aluminum ($[Al(H_2O)_6]^{+3}$ – in simplification: Al^{3+}) in the soil is associated with an increase in soil acidification. Its harmfulness is the chemical sorption of phosphates and Al^{3+} antagonism in relation to Ca^{2+} and Mg^{2+} [44].

Conclusions

The results obtained after three years of organic waste (biogas digestate) and mineral waste (carboniferous mudstones and clay rocks from coal mine) use as fertilization for rapeseed suggest that the wastes evaluated can be an interesting alternative for conventional fertilization. The biochemical quality parameters, nutritional value, and yield of oilseed rape were similar to conventional fertilization. In some cases (especially coaplication of wastes) these parameters were better than in conventional fertilization and in no fertilization. The content of heavy metals in rapeseeds as well as in the soil did not exceed the international permissible limits of heavy metals concentrations in soil and plants. The exception was Al, Fe, Cr, and Cd in seeds, whose concentration was noticed above the restrictive limit set by WHO. However, this had no connection with the use of wastes to fertilization, because wastes and conventional fertilization decreased the content of these elements in seeds compared to control (without fertilization). Nevertheless, to recognize tested wastes as a full-value fertilizer, studies in this area should be continued.

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Conflict of Interest

The authors declare no conflict of interest.

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