The potential of biochar for reducing the negative effects of soil contamination on the phytochemical properties and heavy metal accumulation in wheat grain

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The food industry faces the problem of soil contamination and consequently the deterioration of the quality of plant products. Here, we present a study on evaluation of the effect of fertilization with sewage sludge (SL) with varying, rates of biochar (BC 2.5, 5 and 10% of DW) on yield quality and the accumulation of heavy metals in wheat grains. The greatest grain yield with the highest content of protein and gluten as well as the highest total content of phenols and flavonoids, was obtained when SL+5%BC fertilization was applied. The addition of 5%BC and 10%BC to SL resulted in the greatest increase in the antioxidant capacity of grain. Among phenolic acids, syringic acid was found in the largest amount in grain in the SL+2.5%BC treatment. A significant decrease in Pb accumulation in wheat grain after application of SL+5%BC and a successive decrease in Al content with increasing BC addition were observed. To increase the quality of wheat grains and to reduce the bioaccumulation of harmful elements after the application of biochar to the soil is important in the context of food safety and health of humans especially in food production on acidic and/or contaminated soils.

Key words: antioxidant capacity, grain quality, heavy metals, flavonoids, nutritional value, phenolic acids

Introduction

There are plenty of studies about properties of biochar and its effect on soil parameters conducted already, but several processes are still understood poorly. Especially considering the varying properties of biochar and it's influence on plants. Biochar may significantly change both physico-chemical and biological properties of soil (Atkinson et al. 2010, Schimmelpfennig et al. 2015). The application of biochar to soil changes its structure as well as the distribution, size and density of pores; it changes soil aeration and water retention capacity. Due to its low susceptibility to biodegradation, porosity and large active surface, biochar added to soil changes its bulk density for several years and increases the net soil surface area and nutrient retention (Ding et al. 2010, Spokas et al. 2012, Clough et al. 2013, Ahmed and Schoenau 2015). This can lead to increased yield potential (with yield increases of 10–12%) (Biederman and Harpole 2013). Increased bioavailability of soil nutrients is mainly the result of direct or indirect change in soil pH after biochar application is made (Hossain et al. 2011, Lehmann et al. 2011, Schimmelpfennig et al. 2015).

Recently, several studies have been dealing with the effect of biochar on remediation/mediation of contaminants from the group of polycyclic aromatic hydrocarbons (PAHs) (Kołtowski et al. 2016, Kuśmierz et al. 2016), heavy metals and metalloids in soil (Beesley et al. 2013, Herath et al. 2015, Rajapaksha et al. 2015). The mobility and bioavailability of cationic metals and anionic metalloids in soils are affected by soil amendments in different ways. Several organic and inorganic soil amendments (biochar, coal fly ash, green waste compost, humic and fulvic acids, root exudates, microbial metabolites and nutrients) are described to reduce the mobility and bioavailability of metals/metalloids in contaminated soils (Violante et al. 2010, Tsang et al. 2014, Ahmad et al. 2016). However, depending on the environmental conditions, increased mobilization of metalloids in contaminated soils has been observed when they are treated with soil amendments (Uchimiya et al. 2012, Lim et al. 2013, Tsang et al. 2013).

Cereals, among which wheat has a dominant place, are one of the most popular sources of food for humans and animals. In 2013 global wheat production reached 715.9 million tons (according to FAOStat: http://faostat3.fao.org/home/E). Wheat grain has a high nutritional value and also contains many macro- and micronutrients as well as polyphenols (such as ferulic, vanillic, syringic, sinapinic, caffeic, and p-coumaric acids) with well-documented antioxidant activity (Okarter et al. 2010, Hung et al. 2011, Gawlik-Dziki et al. 2012). Food, apart from providing

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major nutritional components, is a source of biologically active substances regulating physiological processes in the human organisms. While applications of fertilizers and soil improvers aim the increase yield levels they can decrease yield quality. Therefore, it is important to identify and exclude any possible undesired effects, especially in the context of fertilizers reprocessed from waste materials.

Depending on the environmental conditions in which they grow, plants can 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). Chemical tests that determine contaminants, together with ecotoxicological assessments, are traditionally used in the evaluation of soil toxicity (Oleszczuk et al. 2012, Różyło et al. 2015). Although, such studies still provide no answer about biochemical changes in plants grown in contaminated soils.

Challenges coming from enhanced recycling and efforts to increase agricultural productivity result in attempts to use various mineral and organic wastes to fertilize plants and to improve soil properties. Therefore increasingly, agriculture faces the problem of soil contamination and contamination of plant products. Especially dangerous is the use of low quality sewage sludge. This involves high risk of difficult reversible soil contamination (heavy metals or chemicals), disturbed ionic balance in the soil and consequently reduction of yield and nutritional value of plants. Growing plants under contaminated soil conditions requires the use of various treatments that would eliminate the negative effect of contaminants on the quality of food products. Recently, a significant attention has been focused on the properties of biochar, because biochar is a material with a large potential for sorption of soil contaminants and balancing of nutrient availability. The main aim of the present study was to evaluate the effect of different rates of biochar mixed to sewage sludge on the fertilizer properties and the effect of fertilization on spring wheat yield and yield quality. Special attention was also paid to the nutritional (protein and starch) and nutraceutical potential (phenols and antioxidant activity) of wheat grains. In addition, the study determined the effect of the studied factors on the degree of uptake and accumulation of heavy metals in wheat grains.

Materials and methods

Field experiment

A field experiment (N: 50°20’04.32”, E: 23°29’41.46) was carried out in the 2014 growing season at the Experimental Farm in Bezek, belonging to the University of Life Sciences in Lublin. The experiment was set up in a randomized block design in three replicates (5 blocks, 3 reps, and 15 plots each with an area 18.5m2) on podzolic soil (PS). The particle size distribution of PS was as follows: 72% of sand (2.0–0.05 mm), 14% of coarse silt (0.05–0.02 mm), 13% of fine silt (0.02–0.002 mm), and 1% of clay particle size (<0.002). The PS has a low content of total nitrogen and an average content of phosphorus, potassium and magnesium (Table 1).

The PS was amended with sewage sludge (SL) and biochar (BC) (characteristics SL and BC in Table 1). Used SL was hygienic stabilized (biologically with aerobic fermentation and chemically with treated with lime) and then dried in a glasshouse automatic dryer (solar dryer). The applied amount of sewage sludge was 20 t wet mass on ha (sewage sludge (SL) that contained 45% water and thus dry matter application was 11 t DW ha^{-1}). Based on the DW of SL calculated doses of SL were 2.5% =275; 5% =550; and 10% =1100 kg DW of SL ha^{-1}.”

Preparing the soil for spring wheat started with cultivating (cultivator with wing coulters) and harrowing of the field after harvesting the forecrop (winter oilseed rape). On 15 October 2013 ploughing was conducted to a 25±2cm depth. In spring (24 March, 2014), SL, BC and NPK were mixed with soil by the rotatory tiller (operating depth = 22±2cm, width = 185 cm). P (70 kg ha^{-1}), K (90 kg ha^{-1}) and first portion of N (40 kg ha^{-1}) fertilizers were applied during this period. Synthetic fertilizer (NPK) was used in the control (0) in order to offset a large deficit of these elements in the soil. After 12 days spring common wheat (*Triticum aestivum* L.) was sown. Due to the low fertility of the soil, the seeding rate was 450 seeds m^{-2}. The second dose of N (60 kg ha^{-1}) was introduced at the beginning of shooting (BBCH growth stages 30–33).

Table 1. Chemical properties of control soil (podzolic soil-PS), sewage sludge (SL) and biochar (BC) used in the experiment (mean, ± SD, n = 3)

Parameters and elements	PS	SL	BC
dry matter (%)	–	55	90
pH (in 1M KCl)	4.57±0.2	7.2±0.3	9.5±0.1
TOC (mg kg^{-1} DW)	8920±730	238410±5780	578900±15200
TN (mg kg^{-1} DW)	410±110	36180±886	680±96
C/N	23.8	6.5	96
mg kg^{-1} DW			
P	118.3±12.4	14702.8±58.3	121.8±5.1
K	160.2±3.5	2892.1±35.6	772±15.4
Mg	38.1±0.7	3170.2±28.3	32±1.3
Fe	3947.4±116.5	3506.7±120.1	853.38±30.1
B	1.8±0.04	21.5±0.2	3.4±0.1
Mn	216.90±12.3	74.18±2.8	146.95±4.3
Cu	2.44±0.8	75.80±0.6	9.98+0.2
Zn	19.42±0.1	624.13+1.2	81.39+0.5
Pb	13.745±0.3	23.12±0.1	5.05±0.2
Cr	13.46±0.4	18.67±2.6	6.9±0.1
Ni	4.12±0.1	12.84±0.1	4.84±0.1
Co	2.34±0.1	2.49±0.1	0.42±0.06
Cd	0.83±0.01	1.53±0.1	0.44±0.03
Ba	35.185±0.9	94.36±0.2	19.80±0.4
Sr	11.19±0.1	N/A	54.51±1.4
Al	904.62±1.7	854.59±1.8	633.16±8.2
Ag	1.20±0.09	0.10±0.04	5.65±0.4

The values are the mean of three analyzes; ± mean standard deviation (n=3); P, K, Mg–available; DW = dry weight

SL and BC were added according to the following experimental design:

A) 0 (control soil without amendments and fertilization)
B) SL (11 t DW ha^{-1})
C) SL (11 t DW ha^{-1}) + 2.5%BC (based on the DW of SL)
D) SL (11 t DW ha^{-1}) + 5%BC (based on the DW of SL)
E) SL (11 t DW ha^{-1}) + 10%BC (based on the DW of SL)

The total rainfall from March to July (Σ = 349.9 mm) was higher than the long-term total (1974–2010 = 273.4mm). Especially May was abundant in rainfall and the total rainfall (151.6mm) compared to the long-term average (57.7mm) was significantly higher. The sum of air temperature for 5 months relevant for wheat was 65.9 °C (from March to July 2014; average = 13.18 °C) and were significantly higher than the mean from the long-term period (56.6 °C; average = 11.32 °C). Only in June 2014 air temperature (15.8 °C) was lower than the long-term mean in June (16.5 °C).

Biochar applied to soil was produced from willow (*Salix viminalis*) and provided by Fluid S.A. (Sędziszów, Poland). Biochar was obtained by slow pyrolysis where the feedstock is thermochemically decomposed at a temperature range from 350 °C (start of combustion) to 700 °C (max. combustion temperature) in an oxygen-poor atmosphere (1–2% O_2). Temperature is maintained until the end of the degassing light hydrocarbons from biomass. The exact time depends on the amount of biomass. Particle size of biochar applied to the soil was 0.02–20 mm; CEC (the cation exchange capacity) = 148 mmol kg^{-1}; H/C (ratio of hydrogen to carbon) = 0.046 (Kołtowski et al. 2016). The specific surface area of biochar was determined according to the Brunauer, Emmett and Teller isotherm and amounted to 11.4 m^2 g^{-1} (Kołtowski et al. 2016).

Soil, sludge and biochar analysis

Soil samples were collected in 2014 at the stage of flag leaf just visible (37–39 vegetation code - Zadoks Scale) of spring wheat. Subsamples (10/plot) were taken from the depth of the plough layer (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 (Van Reeuwijk 1992): particle size distribution by the hydrometer method; pH in 1 M KCl solution potentiometrically (soil to solution ratio of 1:2.5); total nitrogen was determined by Kjeldahl's method (nitrate excluded).

The total organic carbon (TOC) content was determined by the gravimetric method (Van Reeuwijk 1992). The soil/sludge/biochar 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 –with atomic absorption spectrometry (AAS) method after extraction with 0.0125 mol l^{-1} $CaCl_2$ (PN-R-04020, 1994).

The metal and other elemental concentration 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) at a ratio of 4:1. Analysis of the Cr, Cu, Ni, Mn, Pb, Cd, Zn, Co, Fe, Ba, Al, Sr and Ag contents was 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 grain, the Accumulation Index (AI) was calculated according to the following formula: $AI_x=GC_x/SC_x$ (x–element; GC–total concentration in grain; SC–total concentration in soil).

Yield analysis

Growing period of spring wheat started on April 7 (sown) and ended on Juli 29 (harvest) thus lasting 112 days, When wheat grains were ready to be harvested (89–92 stages of BBCH scale [fully ripe: grain hard, difficult to divide with thumbnail - over-ripe: grain very hard, cannot be dented by thumbnail]), whole spring 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 (3 bundles from each plot x 15 plots = 35 bundles). 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, part (500 g) of samples were separated for qualitative evaluation.

Analysis of nutritional and health-promoting quality of wheat grain

Nutrients, gluten and starch

The grain samples were analyzed for their protein, wet gluten and starch contents, separately for each replicate/plot and the three sampling sites (1 m^2). Total protein content was determined for the whole milled grains (whole wheat flour). The protein content was calculated from the total nitrogen content (in the rate of N=5.7). Total nitrogen was determined by the Kjeldahl method (ISO/TS 16634–2:2009). Same total metal and element determinations were made from grains than from soil, sludge and biochar.

The amount of wet gluten was determined by mechanical means (ISO 21415–2:2015). The starch content was determined by the Clendenning method (ICC Standard no. 122/1). These data were the basis for calibration of the "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 enables repeatable results to be obtained for the tested grain parameters.

Phenolic content and antioxidant activities

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.

Phenolic compounds

Total phenols (TPC) were estimated according to the Folin-Ciocalteau method (Singleton and Rossi 1965). 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).

Flavonoids

Total flavonoids (TFC) were estimated according to the method described by Bahorun et al. (2004). One milliliter of sample was mixed with 1 ml 2% $AlCl_3 \times 6H_2O$. After 10 min, absorbance at 430 nm was measured. The total flavonoid content was expressed as quercetin equivalent (QE) in milligrams per dry weight (DW).

Quantitative−qualitative analysis of free phenolics

Samples were analyzed with a Varian ProStar high-performance liquid chromatography (HPLC) system separation module (Varian, Palo Alto, CA, USA) equipped with a Varian ChromSpher C18 reverse phase column (250 mm × 4.6 mm) and a ProStar DAD detector. The column thermostat was set at 40 °C. The mobile phase consisted of 4.5% acetic acid (solvent A) and 50% acetonitrile (solvent B), and a flow rate of 0.8 ml min^{-1} was used. At the end of the gradient, the column was washed with 50% acetonitrile and equilibrated to the initial condition for 10 min. The gradient elution was used as follows: 0 min, 92% A; 30 min, 70% A; 45 min, 60% A; 80 min, 60% A; 82 min, 0% A; 85 min, 0% A; 86 min, 92% A; and 90 min, 92% A. Detection was carried out in the wavelength range from 270 to 370 nm. Spectrum analysis was used to compare their retention times with those of the standard compounds identified the phenolics in a sample. Quantitative determinations were carried out with the external standard calculation, using calibration curves of the standards. Phenolics were expressed in micrograms per gram of DW (Świeca and Baraniak 2014a).

Antiradical activity (ABTS)

Antiradical Activity (ABTS) was performed using an improved ABTS decolorization assay (Re et al. 1999). The ABTS radical cation (ABTS+.) was 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 before 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 % = $([A_C - A_A]/A_C) \times 100$, where:

A_C= absorbance of control and A_A= absorbance of sample.

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

Reducing power (RP)

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)

Chelating power 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 of 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 = $[1-(A_p/A_c)]\times100$, where:

A_c= absorbance of control, A_p= absorbance of sample. CP was expressed as ethylenediaminetetraacetic acid (EDTA) equivalent in micrograms per gram of DW.

Total antioxidant capacity index (ACI)

Three complementary antioxidant methods were integrated to obtain the ACI (Świeca and Baraniak 2014b). This index may be useful for evaluation of the total antioxidant potential of wheat from different fertilizations compared to control. 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 represented as mean ± standard deviation (SD) of three parallel replicates. One-way analysis of variance (ANOVA) and Tukey's post hoc test were used to compare groups within different elicitors ($HSD_{0.05}$= honestly significant difference). α values < 0.05 were regarded as significant. Obtained results were elaborated statistically using statistical program "ARStat" (developed in the Faculty of Applied Mathematics and Information Technology of the University of Life Sciences, Lublin, Poland). This program using the Tukey test (Tukey's confidence half-intervals with an error rate of 5%) calculates the significance of the difference, which are presented in the form of honestly significant differences (HSD). 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 the simple factors systems (equal sample sizes per group).

Results

Yield structure and nutrient content

The incorporation of sewage sludge (SL) and biochar (BC) into the soil (PS) significantly changed the spring wheat grain yield accumulation and above-ground biomass (grain + crop residues) accumulation. The biomass accumulation increased significantly (25.8%) after SL application compared to control (Table 2), but the highest yields were obtained when added SL+2.5%BC and SL+5%BC (53.3% and 57.4% higher than control respectively). Also SL+10%BC increased grain yield when compared to control (differences statistically insignificant), but a significant reduction in wheat yield and biomass of crop residues was observed compared to SL+2.5%BC and SL+5%BC . In treatment SL+5%BC wheat grain showed significantly highest total protein and nitrogen content (17.4% compared to control and 13.7% compored to SL) and wet gluten content compared to control 31.5% , while the lowest was in treatment SL+10%BC (significantly less than SL+5%BC and SL+2.5%BC). SL+10%BC resulted in the least favorable harvest index (HI) compared to SL which had the best HI (Table 2). The experimental factors did not have a statistically proven effect on the starch content in wheat grain. Despite the absence of statistically significant differences, it was observed that wheat grains harvested from the control plots contained the highest starch content. The lowest content of starch was found in wheat grain obtained from the treatment with SL+5%BC fertilization (Table 2).

Table 2. The effect of sewage sludge (SL) and biochar (BC) application to soil on yield structure and protein, nitrogen, gluten and starch contents of spring wheat grain (mean, ±SD, n = 3)

Parameters	Type of fertilization					$HSD_{0.05}$
	C	SL	SL+2.5%BC	SL+5%BC	SL+10%BC	
grain yield (t ha^{-1})	2.44^{a}±0.28	3.07^{b}±0.36	3.74^{c}±0.22	3.84^{c}±0.23	2.88^{ab}±0.39	0.53
crop residues (t ha^{-1})	6.06^{a}±0.30	6.68^{a}±0.31	8.77^{b}±0.28	9.81^{c}±0.35	7.97^{b}±0.40	0.91
harvest index (HI)	0.29	0.31	0.21	0.28	0.27	–
total N content (g kg^{-1})	19.9^{a}±1.1	20.5^{ab}±2.3	21.5^{bc}±1.7	23.4^{c}±2.0	19.6^{a} ±1.4	2.16
total protein content (g kg^{-1})	113.4^{a}±6.5	117.1^{ab}±13.2	122.9^{bc}±9.7	133.1^{c}±11.4	111.7^{a}±7.8	12.3
wet gluten content (g kg^{-1})	20.0^{a}±1.2	21.5^{ab}±4.3	22.8^{ab}±2.5	26.3^{b}±4.1	19.4^{a}±3.6	6.1
total starch content (g kg^{-1})	50.8±4.4	50.2±7.6	49.9±5.3	48.5±5.2	50.6±6.5	n.s.

C = podzolic soil without amendments (control); SL = 11 t dry weight (DW) of sewage sludge (SL) ha^{-1}; SL+2.5%BC = 11 t DW of SL ha^{-1} + 2.5% BC; SL+5%BC = 11 t DW of SL ha^{-1} + 5%BC; SL+10%BC = 11 t DW of SL ha^{-1} + 10% BC; $HSD_{0.05}$= honestly significant difference (Tukey's Test)

Phenolics, flavonoids and antioxidant capacity

Fertilization with SL and BC caused significant differences in the quality of studied grains, the total phenolics content (TPC) ranged from 4.69 to 5.50 mg g^{-1} DW and, importantly, significant differences were found between the control and treatments. After application of SL+5%BC significant increase was observed in TPC compared to all other fertilization treatments (from 8.5% to 14.7%). It is noteworthy that SL+10% BC significantly reduced TPC compared to the SL, SL+2.5%BC and SL+5%BC. While a significant increase in flavonoids was also found in grains obtained after SL+5%BC and SL+10%BC application (28.3% and 26.4% compared to the control) (Table 3). Due to the significant effect of the cultivation conditions studied on the total phenolics content, more accurate qualitative and quantitative data were provided using the HPLC technique. In all the studied samples, five main phenolics were found: catechin derivatives and syringic, p-coumaric, ferulic and sinapinic acids. Their content, depending on the experimental treatment, ranged from 300.4 µg g^{-1} DW to 422.4 µg g^{-1} DW in the control and SL treatment (without BC), respectively (Table 3). Syringic acid was dominant and its content was from 124.0 µg g^{-1} to 181.0 µg g^{-1} in the control and SL treatment (without BC), respectively). A similar trend was observed for catechin derivatives. Compared to the control, all the tested concentrations of BC decreased the ferulic acid content –the highest decrease (34.7%) was found for SL+2.5%BC (Table 3).

The analysis of the antioxidant capacity (ACI) of wheat grains showed that the application of SL+10%BC and SL+5%BC was most effective in causing an increase in the ability to chelate metal ions (CP) and to quench free radicals –an increase of 39.8% and 25.0%, respectively (compared to the control). The lowest CP (73.89 µg EDTA g^{-1} DW) and ABTS (0.82 µmol of TE g^{-1} DW) were found respectively for the control and wheat fertilized with SL+2.5%BC (Table 3). All studied fertilization treatments significantly increased the reducing capacity (RP). The highest increase was found for SL+5%BC –compared to the control an increase of about 60%. Based on the values of the Total Antioxidant Capacity Index (ACI), it was found that the highest results (an increase of 15.8% compared to the control) were obtained after application of SL+5%BC and SL+10%BC.

Table 3. Effect of sewage sludge (SL) and biochar (BC) application to soil on the phenolics content and antioxidant capacity of spring wheat grain (mean, ±SD, n=3)

Parameters	Type of fertilization					$HSD_{0.05}$
	C	SL	SL+2.5%BC	SL+5%BC	SL+10%BC	
TPC (μg g^{-1} DW)	4690^{a}±140	4997^{b}±110	503^{b}±180	5498^{c}±209	4763^{a}±112	148
TFC (μg g^{-1} DW)	528^{a}±31	502^{a}±42	597^{b}±49	681^{c}±3	670^{c}±31	29
catechine derivete (μg g^{-1} DW)	31.5^{a}±26.8	100.9^{bc}±71.3	63.1^{ab}±17.8	88.3^{b}±53.5	50.5^{a}±0.0	31.2
syringic acid (μg g^{-1} DW)	124.0^{a}±3.5	181.0^{c}±14.0	159.9^{b}±12.3	162.1^{b}±5.3	162.4^{b}±8.8	9.5
p-coumaric acid (μg g^{-1} DW)	3.1^{ab}±2.2	1.9^{a}±0.5	22.1^{c}±18.8	0.8^{a}±0.0	4.6^{b}±0.0	3.6
ferulic acid (μg g^{-1} DW)	70.6^{c}±2.0	63.4^{b}±4.1	46.1^{a}±12.2	60.5^{b}±8.2	57.7^{b}±12.2	6.1
sinapinic acid (μg g^{-1} DW)	71.3^{cd}±0.0	75.2^{d}±5.6	55.4^{a}±11.2	67.3^{bc}±5.6	63.3^{b}±0.0	7.0
Σ phenolic acids (μg g^{-1} DW)	300.4	422.4	346.6	379.3	338.4	
chelating power (CP) (μg of EDTA g^{-1} DW)	73.9^{a}±8.7	88.2^{bc}±12.6	83.9^{b}±7.8	92.4^{c}±5.3	103.3^{d}±4.2	7.2
reducing power (RP) (μmol of TE g^{-1} DW)	1.36^{a}±0.15	1.70^{bc}±0.11	1.78^{c}±0.13	2.18^{d}±0.11	1.58^{b}±0.22	0.18
antiradical activity (ABTS) (μmol of TE g^{-1} DW)	0.94^{ab}±0.10	0.92^{ab}±0.18	0.82^{a}±0.16	1.04^{b}±0.11	1.32^{c}±0.10	0.15
Σ total antioxidant capacity index (ACI)	–	1.14	1.10	1.32	1.32	

C = podzolic soil without amendments (control); SL = 11 t dry weight (DW) of sewage sludge (SL) ha^{-1}; SL+2.5%BC = 11 t DW of SL ha^{-1} + 2.5% BC; SL+5%BC = 11 t DW of SL ha^{-1} + 5%BC; SL+10%BC = 11 t DW of SL ha^{-1} + 10% BC; $HSD_{0.05}$ = honestly significant difference (Tukey's Test); TPC = Total Phenolics Content; TFC = Total Flavonoids Content

Heavy metal contents and accumulation index (AI)

Table with heavy metal contents in soil (Table S1) shows that the lowest contents were in the soil fertilized with SL+5%BC (with the exception of Cu and Cd, that were lowest in the soil fertilized with SL+2.5%BC). The highest contents of Fe, Al, Pb, Cd and Co were in the control soil, Mn, Cr, Cu and Ba were in the soil fertilized with SL, and Zn, Sr and Ni were in the soil fertilized with SL+10%BC, respectively. The content of elements in grain of wheat had no clear differences related to the type of fertilization and were difficult to interpret (Table S2). A high AI value meanson increased uptake of metal in wheat grains from soil (Table 4). Zn and Cu, whose concentration in grain was almost twice as high as that in the soil, were the elements that were most accumulated. SL fertilization without BC increased the accumulations of Zn, Cd and Ba, but decreased the accumulations of Cu, Sr, Ni, Pb, Al, Cr and Fe compared to the control.

Table 4. Accumulation Index (AI) calculated according to the following formula: $AI_x = GC_x/SC_x$ (x–element; GC–total concentration in grain; SC–total concentration in soil

Elements	Type of fertilization					$HSD_{0.05}$
	C	SL	SL+2.5%BC	SL+5%BC	SL+10%BC	
Zn	1.62^{a}	1.77^{ab}	2.17^{c}	2.21^{c}	1.93^{b}	0.18
Cu	1.57^{b}	1.36^{a}	1.95^{c}	2.01^{d}	1.65^{b}	0.15
Sr	0.22^{a}	0.20^{a}	0.28^{b}	0.23^{a}	0.22^{a}	0.04
Mn	0.20^{a}	0.20^{a}	0.22^{ab}	0.25^{b}	0.22^{ab}	0.04
Cd	0.20^{b}	0.21^{b}	0.21^{b}	0.13^{a}	0.11^{a}	0.05
Ba	0.09^{a}	0.10^{ab}	0.13^{b}	0.11^{b}	0.09^{a}	0.02
Ni	0.13^{b}	0.12^{b}	0.12^{b}	0.06^{a}	0.04^{a}	0.03
Pb	0.09^{c}	0.07^{b}	0.07^{b}	0.02^{a}	0.09^{c}	0.01
Al	0.05^{d}	0.04^{c}	0.03^{b}	0.02^{a}	0.02^{a}	0.005
Cr	0.03^{ab}	0.02^{a}	0.03^{ab}	0.04^{bc}	0.05^{c}	0.015
Fe	0.02^{b}	0.01^{a}	0.02^{b}	0.02^{b}	0.02^{b}	0.005
Co	0.01^{b}	0.01^{b}	0.00^{a}	0.00^{a}	0.04^{c}	0.005

C = podzolic soil without amendments (control); SL = 11 t dry weight (DW) of sewage sludge (SL) ha^{-1}; SL+2.5%BC = 11 t DW of SL ha^{-1} + 2.5% BC; SL+5%BC = 11 t DW of SL ha^{-1} + 5%BC; SL+10%BC = 11 t DW of SL ha^{-1} + 10% BC; $HSD_{0.05}$–honestly significant difference (Tukey's Test)

SL fertilization without BC addition proved to be most beneficial for decreasing accumulation of heavy metals in spring wheat grain. Compared to the control, SL+5%BC significantly increased AI of Zn, Cu and Mn (by 36.4%, 40.8% and 25.0% respectively) and significantly decreased AI of Pb and Al (by 77.8% and 60.0% respectively). The highest rate of BC (SL+10%BC) significantly decreased the accumulation of Cu (compared to SL+2.5%BC and SL+5%BC), Cd and Ni (compared to control, SL, SL+2.5%BC) (Table 4).

Discussion

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

Increased crop yields after biochar application may result both from an improvement in the soil structure and from reduced nutrient leaching. Moreover, biochar application increases water retention in the soil, which may in turn affect favourably the growth and development of plants during water deficit periods. In experiment of Kraska et al. (2016), an increase of grain yield was due to the fact that application of biochar enriched the soil with high amount of magnesium and potassium. In their studies, as in our study, the highest dose of biochar (30 t ha^{-1}) significantly reduced yield (compared to 20 t BC ha^{-1}). Also Karer et al. (2013) demonstrated a decrease in spring barley, maize and winter wheat yields after highest dose (72 t ha^{-1}) of biochar application. This is probably the result of strong sorption properties of biochar, which in high doses dominate the positive effect (e.g. increasing pH) of biochar and/or disturb the ionic balance in the soil.

The form and availability of N are considered to be the factors that most affect yield as well as protein, nitrogen and gluten content of plants. In this study, grain N uptake increased from 49 kg ha^{-1} in control, to 63 kg ha^{-1} in sludge and to 90 kg ha^{-1} in SL+5%BC. Although the nitrogen content of crop residues is not known, we can state that biochar additions clearly increased N uptake, except in the highest biochar rate.

Increased intensity of the reversible sorption mechanisms after biochar application to soil may be the reason for reduced losses of ammonia, which retains nitrogen for plants (Taghizadeh-Toos et al. 2012). Maru et al. (2015) showed that in an acidic soil (pH 5.3) co-application of biochar (5 t ha^{-1}) with 100% and 75% urea recommendation rates significantly increased nutrient availability (especially P and K) and significantly increased rice growth variables and grain yield. In the study by Ahmed and Schoenau (2015), biochar (1–2 t ha^{-1}) did not alter the availability of N and P, and its effects on soil pH, organic carbon and electrical conductivity were minor; it increased the yield of the crops studied only in some treatments. In the study by Schimmelpfennig et al. (2015) interactions between the carbon amendments (uncarbonized feedstock, hydrochar, biochar from *Miscanthus giganteus*) with slurry did not occur, neither improving nor worsening the efficiency of nutrient use. The reasons for the changes in the yield structure can be sought not only in the properties of BC but also in the properties of macro- and micronutrients, because complexation is an important mechanism controlling the mobility of cationic metals, whereas competition for available sites on soil minerals between metalloids and soil amendments could be a dominant factor involved in sorption/ desorption of metalloids (Violante et al. 2010). In studies of Guimarães et al. (2016) oxidized charcoal was more effective for prolonging NH_4^+ and N availability in soil. This means that biochar may inhibit (or stimulate –depending on the dose of biochar) enzymatic activity of soil microorganisms (e.g., urease) and thus change the intensity of the urea hydrolysis. But the biggest amendment (in studies of these autors) was Zn, which significantly increased total N uptake and the efficiency of urea N fertilization. In our studies SL and BC contained a large amount of Zn which also may have contributed to changes in the availability of elements for plants. Additionally high rainfall in May during our field experiment probably lead to N leaching which was smaller in biochar treatments.

According to the literature data, ferulic acid is the dominant phenolic compound of wheat grains –its content ranged from 286.5 to 787.3 µg g^{-1} DW (Okarter et al. 2010, Gawlik-Dziki et al. 2012). The difference in the content of phenolic compared to our results may result from the use of another variety of wheat or other methods of determination of phenolic compounds.

In our study, syringic acid was dominant. Similar observations were made by Mazzoncini et al. (2015), who found that syringic acid was dominant in winter wheat and its content was significantly increased by organic fertilization (compared to conventional cultivation). Also, Hung et al. (2011) found syringic acid to be the main compound in the free phenolic alcoholic extracts of the wheat meal representing 77.0% of the total amount of detected free phenolic compounds. These differences come from the evidence that only free phenolics were studied and it is a well-known fact that in cereals most phenolics are found in bound form.

The antioxidant capacity of wheat grains usually correlates with the content of total phenolics (Okarter et al. 2010, Hung et al. 2011). According to the literature, accessibility of nitrogen to plants is important due to effects on the antioxidant potential. The results of Ma et al. (2015) showed that fertilization with nitrogen (180 and 240 kg N ha^{-1}; 150 kg P_2O_5 ha^{-1}; 150 kg K_2O ha^{-1}) decreased the antioxidant activity of winter wheat when compared to non-fertilized cultivation. The results obtained in the present study are in opposition to the one cited above. However, in our research, control was N deficient, while in research of Ma et al. (2015) they are most likely in optimum and higher than in optimum. It also may be speculated that the increased biosynthesis (accumulation) of phenolics (cross-talk response to stress conditions) was caused by the induction of the plants' natural resistance by some components present in SL, especially Cd^{2+} and Pb^{2+} (Michalak 2006, Lin et al. 2007). However, phenolic composition and antioxidant capacity of plant products are highly interactive mechanisms that occur in the plant in response to soil conditions and they are still not well understood by researchers. Therefore, the effect of changes in soil processes on the quality of the crop yield is not yet possible to explain.

The impact of the 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 factors affecting the accumulation of elements in the plant (apart from its species). This study evaluated the effect of sewage sludge (SL) fertilization and different rates of BC added to SL on the uptake of heavy metals by spring wheat.

Certain heavy metals, like Cd, Cr, Ni and Pb, are can be found in high contents in fertilizer products processed from waste materials. On the other hand, an abundant element in soil, Al increases the risk of Alzheimer's disease (Wang et al. 2016). 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 (Melo et al. 2016).

Similar to our study, Suksabye et al. (2016) noted a reduction in Cd concentration in grains of rice grown as a result of biochar application (1% for DW of cadmium-contaminated [650 mg Cd kg^{-1}]). In their research, sawdust fly ash biochar was the best biochar for reducing cadmium accumulation in rice grain when compared to bagasse fly ash and rice husk ash under the same conditions. Xu et al. (2016) also found a significant reduction in

Cd concentration in maize and ryegrass stems after biochar application. In the case of Pb, they obtained better effects in reducing accumulation when rice and wheat straw were used than after biochar application. Increased soil sorption or precipitation after biochar application may be the main mechanisms causing reduced accumulation of Cd and other metals. However, these processes are reversible under buffer acidic conditions (Wang et al. 2016). On the other hand, in the study by Schimmelpfennig et al. (2015) the use of various forms of carbon (uncarbonized feedstock, hydrochar, biochar from *Miscanthus giganteus*) did not significantly change the concentration of heavy metals (Cu, Zn, Cl) in grass biomass.

As reported by Ahmad et al. (2016), biochar reduces the mobility of Pb, Cu and Zn in alkaline soil due to carboxylation, while in acidic soil due to a biochar-induced pH increase. However, biochar also increases the mobility of Sb and As. According to these authors, this is possibly due to enhanced electrostatic repulsion and competition with phosphate.

Conclusion

The results show that the application of sewage sludge together with biochar at the appropriate dosage (2.5% and 5% BC added to the 11 t DW of SL) generally increases the biochemical quality and nutritional value of wheat grain. However, too high dose of BC (10% BC added to the 11 t DW of SL) reduces grain yield and in most cases grain quality. The application of biochar is not equally effective in immobilizing metals in different soils and thus, due to the different requirements of plants, its effectiveness may also depend on the plant species. The effects of biochar use are not unambiguous and, therefore, decisions on its application should take into account the factors that change its action and also the concentration of heavy metals in the soil.

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