

# The Effect of Low-Temperature Conversion of Plant Materials on the Chemical Composition and Ecotoxicity of Biochars

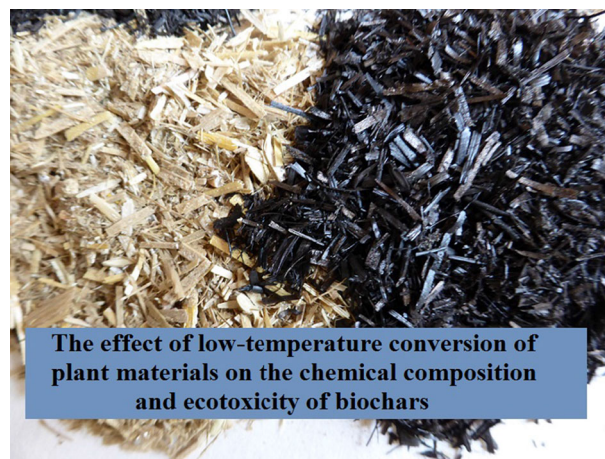
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Received: 12 February 2016 / Accepted: 20 June 2016 / Published online: 29 June 2016  
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**Abstract** The chemical composition of biochars varies considerably depending on the chemical composition of biomass used for its production and conditions of the thermal conversion process. In the context of chemical composition, biochars are different from other types of organic matter in that they contain many more aromatic carbon compounds. The aims of this study were as follows: (1) to investigate the effect of pyrolysis process of plant material on the concentrations of macroelements, trace elements and polycyclic aromatic hydrocarbons (PAHs) in the biochars; (2) to evaluate the ecotoxicity of the biochars; (3) to integrate chemical and toxicity properties of biochars in order to assess their safe utilisation as fertiliser materials. It was found that the elemental composition, the contents of macronutrients and trace elements in biochars were determined by the type of converted biomass. In the case of contents of volatile elements, such as nitrogen and sulphur, the process conditions were of great importance. Among the analysed trace elements, only the cadmium content exceeded the limit value for premium class biochars. The process of thermal conversion of organic materials did not cause mobilisation of available forms of most of the studied trace elements. In the course of the study, it was found that the extracts from wheat straw biochar (WSB) were low-toxic, while the extracts from biochars derived

from rape straw (RSB), Miscanthus straw (MSB), sawdust (SB), bark (BB) and leaves of trees (LTB) were highly toxic to *V. fischeri*. Based on the response of the test organism, the analysed biochars were arranged in the following order: WSB < BB < RSB < LTB < MSB < SB. The highest total content of PAHs ( $\sum$  16 PAHs) was determined in rape straw biochar, and the lowest, in sawdust biochar. The studies revealed a negative correlation between the content of most of PAHs and inhibition of *Vibrio fischeri* luminescence.

## Graphical Abstract



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**Keywords** Biochar · Plant materials · Macronutrients · Heavy metals · Ecotoxicity test

## Introduction

Biochar is one of the products obtained by thermal conversion of plant biomass and organic waste [1]. The possibility of using biochar in mitigating the effects of global warming and carbon sequestration resulted in a significant interest in this material. There are numerous studies evaluating the use of biochar to improve soil fertility or mitigate the effects of its contamination with pesticide residues, heavy metals, and polycyclic aromatic hydrocarbons (PAHs) [2, 3].

The chemical composition of biochars varies considerably depending on the chemical composition of biomass used for its production and conditions of the thermal conversion process [4]. In the context of chemical composition, biochars differ not only from each other, but also from other types of organic matter in that they contain many more aromatic carbon compounds. Biochar also includes the mineral fraction composed of macronutrients and trace elements, which is particularly important when considering this material as fertiliser [5]. Because of technical parameters of thermal conversion, temperature in particular, the resulting products differ not only in physical properties, especially specific surface area, but also in the contents of substances which are readily degradable under soil conditions [6–8].

The research results, which have been published so far, on the properties and chemical composition of biochars obtained from various feedstocks, indicate that there is still a limited number of studies on the effects of such materials on living organisms. Numerous concerns regarding the presence of organic and inorganic contaminants in biochar require testing of a wide range of feedstocks in order to confirm or disprove the present scientific findings. Knowledge of contaminants contained in biochar will allow the creation of appropriate standards enabling a safe use of this material in the environment. The research results to date indicated that the permissible level of 16 PAHs in biochars is from 6 to 20 mg/kg [9]. Unfortunately, these values are by no means linked with the actual toxic effect of biochar, which may due to synergy effects of several toxic agents.

A comprehensive assessment of the chemical composition of biochars, taking into account the heavy metal contents, including their readily available forms and the PAHs content, will allow the determination of the extent to which these elements can influence the toxicity of biochars. The relatively rapid verification of the impact of different materials, including biochars, on living organisms may be performed using biotests. Biotests constitute an important part of bioanalytics and can be used for environmental risk assessment concerning, inter alia, the application of various

types of substances. It is a common phenomenon that, as a result of the processes which occur during thermal conversion of organic materials, there is degradation of miscellaneous compounds as well as creation of new, or mobilisation of originally inaccessible, chemically bound substances or elements which may significantly affect the biological activity of the soil environment [10–12].

The aims of this study were as follows: (1) to investigate the effect of pyrolysis process of plant material on the concentrations of macroelements, trace elements and polycyclic aromatic hydrocarbons (PAHs) in biochars; (2) to evaluate the ecotoxicity of biochars; (3) to integrate chemical and toxicity properties of biochars in order to assess their safe utilisation as fertiliser materials.

## Materials and Methods

### Characteristics of Organic Materials Used in the Study

The feedstocks for the study were wheat straw (WSB), rape straw (RSB), Miscanthus straw (MSB), sawdust from coniferous trees (SB), bark from coniferous trees (BB), and leaves of trees (LTB).

The main criterion for selection of materials used as feedstocks for the production of biochar was the ease of their access. Wheat straw, rape straw, and Miscanthus straw used in the study came from an agricultural holding. Sawdust and bark from coniferous trees were obtained as materials resulting from the processing of lumber, whereas leaves of trees came from the tending of tree stands in a park. The materials used in the study came from Malopolska Province (southern Poland).

### Thermal Conversion of Biomass

The collected organic materials were dried at 65 °C, ground in the laboratory mill and passed through a sieve with apertures of 5 mm. Thermal conversion of the prepared materials was carried out in laboratory conditions using test stand for thermal conversion of biomass under a limited supply of air (1–2 %) [9]. The samples weighting 100 g were placed in a closed combustion chamber. The temperature in the combustion chamber was  $300 \pm 10$  °C, and exposure time, 15 min. The rate of heating the combustion chamber was  $10$  °C  $\text{min}^{-1}$ . The pyrolysis time and temperature were established on the basis of own preliminary examinations and results reported in literature [11, 13]. Weight loss (WL) of the materials was calculated on the basis of material weight introduced into the process (MM1) and its residues after thermal conversion (MM2).

$$WL = MM1 - MM2$$

## Chemical Analyses of Biochar

The following parameters were determined in the thermally converted materials: dry matter content at 105 °C for 12 h [8], ash [14], and pH by potentiometric method [15]. The elemental composition (C, H, N, S) of biochars was determined using CHNS analyser (Vario EL Cube manufactured by Elementar) [16]. Total O was derived by subtraction according to DIN 51733 [17] method as follows:

$$O(\%w/w) = 100 - \text{ash}(\%w/w) - C(\%w/w) - N(\%w/w) - H(\%w/w) - S(\%w/w)$$

The total contents of the analysed incineration elements, including P, K, Ca, Mg, Na, Cu, Cd, Cr, Zn, Pb, Ni, Mn, Fe, were determined after incinerating the sample in chamber furnace at 450 °C for 12 h and mineralising its residues in a mixture of concentrated nitric and perchloric acids (3:2 v/v) [18]. The contents of the elements in the obtained solutions were determined by ICP-OES using the Perkin Elmer Optima 7300 DV instrument [19].

The available forms of trace elements were extracted from biochars with redistilled water (biochar:solution = 1:10) for 2 h and 0.01 M solution of CaCl<sub>2</sub> (biochar = solution 1:10) for 2 h [20, 21]. The contents of trace elements were determined by ICP-OES using Perkin Elmer Optima 7300 DV instrument [19].

The contents of polycyclic aromatic hydrocarbons (PAHs) (16 compounds from US EPA list) were determined in line with PN-ISO-18287 [22] using Varian 450-GC gas chromatograph coupled to Varian 240-MS mass spectrometer by calibration curve method.

## Ecotoxicity Test of Biochars

The acute toxicity assessment of biochar was performed using the following Microtox<sup>®</sup>. The Microtox<sup>®</sup> test was used to assess the inhibition of luminescence in the bacteria *Vibrio fischeri* [23]. The toxicity of the extracts (1:4), prepared from the studied materials after their thermal transformation, was evaluated for one test organisms—*V. fischeri*. The analysis of measurement of the change in luminescence was performed on a Microtox M500 Analyzer. The standard test protocol was applied for water samples: 81.9 % Basic Test (basic test, MicrotoxOmni software was used). Measurement of the luminescence was carried out before and after 15 min incubation of the bacterial suspension with the studied sample. Three replicate samples were tested. Toxicity results were expressed as Effective Concentration (EC50 value) defined as the

sample concentration that would produce a 50 % reduction in luminescence *V. fischeri*. The EC50 values were converted to Toxic Units (TU) according to the formula  $TU = 1/EC50 \times 100$ . The system of risk assessment developed by Persoone et al. [24] was used to estimate toxicity. Finally, the results were classified: class I  $TU \leq 0.4$  no significantly toxic effect; no acute hazard, class II:  $0.4 < TU \leq 1$  slight acute toxic effect; low acute hazard, class III:  $1 < TU \leq 10$  significantly toxic effect; acute hazard, class IV:  $10 < TU \leq 100$  high acute hazard, class V:  $TU > 100$  very high acute hazard.

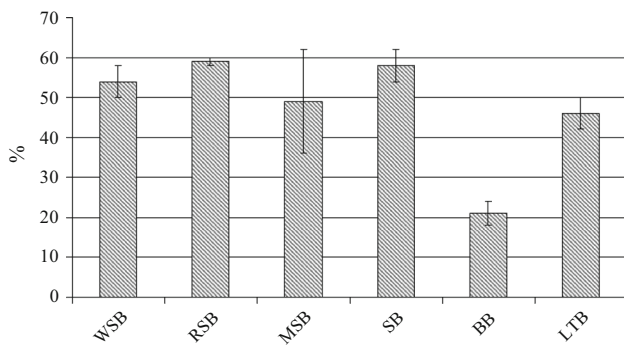
## Statistical Analysis

The value of standard deviation (SD) was calculated for the obtained results. Furthermore, in order to determine the diversity within the analysed population, the coefficient of variations (CV%) was calculated as a share of standard deviation (SD) in the arithmetic mean of the analysed parameters. All statistical analyses were performed using Statistica 12 software package (Statsoft Inc.).

## Results and Discussion

### Weight Loss of Organic Materials After Thermal Conversion

Following the analysis of weight loss of organic materials subjected to thermal conversion, the highest process efficiency was noted for bark and leaves (Fig. 1). In the case of other materials, the weight loss due to pyrolysis was much larger, but did not exceed 60 %. Hossain et al. [25] and Sun et al. [26] demonstrated in their studies that, next to qualitative or quantitative composition, a decisive factor in the weight loss of materials subjected to pyrolysis is temperature of the process. Regardless of the type of pyrolysed material, less residue is observed at higher temperatures as a result of increased losses of oxidisable substances, including inorganic compounds [4, 8]. As noted by Song and Guo [27] and Mukherjee and Zimmermana [7], the recommended temperature of material pyrolysis is approximately 300 °C. According to these authors, biochars obtained during the low-temperature pyrolysis have a high content of compounds which easily undergo degradation in soil, and hence, nutrients assimilable by plants. In addition, biochars produced at temperatures between 300 and 400 °C contain the highest amount of readily degradable carbon compounds and exhibit a wide signal range for alkyl groups and carbohydrates, which is undetectable at temperatures above 400 °C [28]. On the other hand, Al-Wabel et al. [13] argued that biochars obtained at



**Fig. 1** Weight loss of organic materials after thermal conversion. *WSB* wheat straw, *RSB* rape straw, *MSB* Miscanthus straw, *SB* sawdust, *BB* bark, *LTB* leaves of trees

temperatures above 300 °C include much fewer aliphatic carbon compounds and functional groups, which substantially reduces the effectiveness of these materials in improving the soil quality. Also, Guo and Chen [29] stated that thermal conversion of plant biomass at temperatures above 400 °C favours the creation of the Si–C bonds that increase the amount of aromatic biochar components, thus hindering the material’s degradability.

### Chemical Composition of Biochars

Biochars obtained in the studies differed in dry matter and ash contents, and the pH values (Table 1). All of the biochars produced contained over 900 g kg<sup>-1</sup> dry matter, with little variety among them. Much greater differentiation was observed for the ash content (CV% = 94). The highest amount of ash was discovered in bark biochar (BB) 488 g kg<sup>-1</sup>, which was due to contamination of the material during transport and in the industrial processing of wood. The least ash was determined in sawdust biochar (SB) 9 g kg<sup>-1</sup> D.M. The measured pH values were not significantly different (CV% = 13); however, sawdust and bark biochars featured the lowest values of the parameter:

4.88 and 5.38 respectively. Wang et al. [30] discovered higher pH values in biochars derived from wood and green waste biomass.

### Elemental Composition of Biochars

The analytical methods applied for biochar are difficult and expensive. However, determination of the detailed characteristics of the biochar chemical properties enables a full assessment of the material’s natural, and also, agricultural applications. Elemental composition of biochar and values of O/C and H/C ratios are recognised as one of the most important indicators of the biochar quality [31]. The results obtained in the course of our own studies indicated that the content of elementary components was dependant on the type of material used for the production of biochar (Table 2). The lowest contents of C and H were determined in bark biochar (respectively, 342 g kg<sup>-1</sup> D.M. and 24.8 g kg<sup>-1</sup> D.M.). The carbon and hydrogen contents showed no significant variation in other biochars.

In the case of nitrogen, the highest content was determined in RSB, LTB and WSB, and amounted to: 14.2 g, 11.2 g and 10.0 g kg<sup>-1</sup> D.M. respectively. In other biochars, the element content did not exceed 10 g kg<sup>-1</sup> D.M. (Table 2). Another crucial element in terms of fertilisation and improvement of the biomass quality is sulphur. The highest sulphur content was determined in rape straw biochar (RSB) and wheat straw biochar (WSB) in which its amounts were: 3.6 g and 0.6 g kg<sup>-1</sup> D.M. respectively (Table 2). Concluding from the research by Cheah et al. [32], the total sulphur content in biochars is mostly influenced by the material used, while the sulphur speciation in these materials is mainly dependent on the temperature and method of thermochemical conversion. However, it is to be noted that in the course of thermal conversion of biomass, the element content will be reduced [13]. Additionally, as stated by Yu et al. [33] and Knudsen et al. [34], significant diversification in N and S contents in biochars results from

**Table 1** The content of dry matter and ash in organic materials before thermal conversion

Material	Dry matter g kg <sup>-1</sup> ± SD	Ash g kg <sup>-1</sup> D.M. ± SD	pH H <sub>2</sub> O
Wheat straw (WSB)	949 ± 2	134 ± 5	6.52 ± 0.60
Rape straw (RSB)	934 ± 5	205 ± 5	7.32 ± 0.51
Miscanthus straw (MSB)	969 ± 10	87 ± 3	6.18 ± 0.43
Sawdust (SB)	955 ± 3	11 ± 1	4.88 ± 0.34
Bark (BB)	969 ± 2	488 ± 10	5.38 ± 0.32
Leaves of trees (LTB)	946 ± 11	131 ± 2	6.34 ± 0.57
CV %	1	94	13

± SD standard deviation, CV% coefficient of variation, *WSB* wheat straw, *RSB* rape straw, *MSB* Miscanthus straw, *SB* sawdust, *BB* bark, *LTB* leaves of trees



**Table 2** Elemental composition of the biochars derived from different feedstocks

Biochar	C g kg <sup>-1</sup> D.M. ± SD	H	N	S	O
WSB	629 ± 18	45.8 ± 0.9	10.0 ± 0.5	0.6 ± 0.1	180 ± 18.8
RSB	538 ± 19	34.6 ± 0.3	14.2 ± 0.8	3.6 ± 0.2	205 ± 19.5
MSB	677 ± 33	46.7 ± 1.9	5.0 ± 0.6	0.1 ± 0.0	154 ± 34.5
SB	671 ± 7	47.2 ± 0.7	1.0 ± 0.2	0.1 ± 0.0	272 ± 7.2
BB	342 ± 33	24.8 ± 2.6	5.7 ± 0.6	0.3 ± 0.1	140 ± 36.0
LTB	551 ± 2	42.8 ± 1.8	11.2 ± 0.2	0.3 ± 0.1	264 ± 2.2
CV%	25	25	64	179	26

**Table 3** The content of macroelements in biochars

Biochar	P g kg <sup>-1</sup> D.M. ± SD	K	Ca	Mg	Na
WSB	2.2 ± 0.1	11.5 ± 1.0	6.8 ± 0.4	1.5 ± 0.11	0.17 ± 0.04
RSB	5.6 ± 0.2	38.7 ± 2.7	28.3 ± 1.2	2.2 ± 0.15	0.49 ± 0.03
MSB	3.3 ± 0.2	15.4 ± 0.5	8.4 ± 0.5	2.3 ± 0.09	0.20 ± 0.11
SB	0.2 ± 0.1	1.1 ± 0.3	2.3 ± 0.3	0.3 ± 0.05	0.15 ± 0.05
BB	0.9 ± 0.1	2.8 ± 0.2	8.9 ± 0.9	1.2 ± 0.28	0.27 ± 0.06
LTB	2.8 ± 0.2	8.7 ± 0.6	32.7 ± 2.9	1.7 ± 0.15	0.18 ± 0.02
CV%	69	96	79	43	48

± SD standard deviation, CV% coefficient of variation, WSB wheat straw, RSB rape straw, MSB Miscanthus straw, SB sawdust, BB bark, LTB leaves of trees

the retention of N in heterocyclic compounds, such as pyridones or pyrrols, and complexation of inorganic S forms by biochar.

The contents of other studied macronutrients in the obtained biochars were conditioned by the type of biomass used as feedstock, as well as the type of element (Table 3).

The contents of phosphorus and potassium in the analysed biochars were similar to the one obtained for nitrogen, but they were more varied among the materials (CV% = 69). When comparing the phosphorus and potassium contents determined in biochars with these observed in manure or compost from the plant waste, it should be concluded that the first mentioned were much lower [35]. When considering biochar as fertiliser, smaller contents of phosphorus and potassium determined in bark and sawdust biochars demonstrate the need to supplement the two elements in the form of mineral fertilisers.

According to Gaskin et al. [36] and Endersa et al. [4], the mineral composition of biochar depends on the type of feedstock used in the process. The above authors confirmed the increase in the contents of elements, such as potassium, calcium, and magnesium, and simultaneously, and put forward the thesis that the application of higher temperature increases the biochar mineral content. This is due to the loss of organic substances. By analysing changes in the nitrogen content in materials subjected to thermal conversion, Gaskin et al. [36] demonstrated that the element

losses are very diversified and substantially determined by the type of organic matter converted. The quoted authors also observed higher nitrogen losses for thermally converted poultry litter, which were probably caused by the oxidation of ammonia nitrogen and readily degradable compounds, such as uric acid. The research results obtained by Gaskin et al. [36] and Endersa et al. [4] have also shown that significantly smaller nitrogen loss is achieved for thermally converted pine sawdust. This can be attributed not only to lower N content in this material, but also the presence of nitrogen in complex structures that were not easily transformed into volatile compounds.

The calcium content in the obtained biochars ranged between 2.3 g and 32.7 g kg<sup>-1</sup> D.M. (Table 3). With the exception of biochars produced from rape straw (RSB) and leaves of trees (LTB), the calcium content did not exceed 10 g kg<sup>-1</sup> D.M. The least calcium was determined in sawdust biochar (SB).

The magnesium and sodium contents in the produced biochars showed similar differentiation patterns (Table 2). The highest magnesium content was determined in biochars derived from rape straw (RSB) and Miscanthus straw (MSB), while, in the case of sodium, equally high element content was also determined in bark biochar (BB).

According to Chan et al. [37], biochar is a more stable source of nutrients for plants compared to manure and compost. The results of studies conducted by Beesely

**Table 4** The content of trace elements in biochars

Biochar	Cu mg kg <sup>-1</sup> D.M. ± SD	Zn mg kg <sup>-1</sup> D.M. ± SD	Fe	Mn	Pb	Cr	Cd	Ni
WSB	4.9 ± 0.4	115 ± 8	797 ± 53	149 ± 9	3.6 ± 0.3	15.5 ± 0.2	1.50 ± 0.12	7.6 ± 0.2
RSB	5.5 ± 0.1	83 ± 2	593 ± 49	69 ± 4	4.2 ± 0.5	11.2 ± 0.1	1.60 ± 0.03	5.5 ± 0.2
MSB	6.5 ± 0.5	143 ± 8	279 ± 15	100 ± 5	4.0 ± 0.1	8.8 ± 0.65	1.70 ± 0.03	5.2 ± 0.5
SB	2.9 ± 0.7	32 ± 4	241 ± 52	305 ± 36	1.7 ± 0.2	4.5 ± 1.2	0.81 ± 0.08	2.5 ± 0.7
BB	9.0 ± 0.4	60 ± 4	3278 ± 42	308 ± 14	14.1 ± 0.7	9.9 ± 1.1	0.62 ± 0.04	5.9 ± 0.1
LTB	10.5 ± 0.8	57 ± 3	786 ± 136	162 ± 14	4.9 ± 0.7	8.2 ± 2.2	0.17 ± 0.02	5.7 ± 0.7
CV%	39	46	105	51	74	34	46	28
EBC basic <sup>a</sup> grade	<100	<400	–	–	<150	<90	<1.5	<50
EBC premium grade <sup>a</sup>	<100	<400	–	–	<120	<80	<1.0	<30
IBI <sup>b</sup>	63–1500	200–7000	–	–	70–500	64–1200	1.4–39	47–600

± SD standard deviation, CV% coefficient of variation, WSB wheat straw, RSB rape straw, MSB Miscanthus straw, SB sawdust, BB bark, LTB leaves of trees

<sup>a</sup> The maximum content of heavy metals referred to EBC (2012)

<sup>b</sup> IBI (2012) standards

et al. [2] and Nigussie et al. [38] showed that biochar introduced into poor and degraded soils may improve their fertility and productivity. In this aspect, it should also be noted that biochar can be a valuable feedstock for recreating the humus layer of soils devastated by human industrial activity.

The total content of studied trace elements in the obtained biochars varied depending on the feedstock subjected to thermal conversion, and the type of element (Table 4). Except for cadmium, the contents of other trace elements were much lower than the values proposed for premium class biochars (<1 mg Cd kg<sup>-1</sup> D.M.) [17]. The cadmium content exceeding 1 mg kg<sup>-1</sup> D.M. was determined in biochars produced from wheat straw (1.50 mg kg<sup>-1</sup> D.M.), rape straw (1.60 mg kg<sup>-1</sup> D.M.), and Miscanthus straw (1.70 mg kg<sup>-1</sup> D.M.). However, given the criterion of trace element contents for biochars, which was proposed by IBI [9], the permitted content was not exceeded by any trace element. As concluded by Hossain et al. [25], the contents of trace elements, corresponding to the weight loss, show an increase depending on the temperature at which the pyrolysis is conducted. According to Song and Guo [27], the recommended temperature of material pyrolysis for agricultural purposes is approximately 300 °C. In the studies conducted by Yachigo and Sato [39], higher pyrolysis temperature resulted in a considerable increase in the zinc and copper contents in the resulting material, but it did not change the cadmium content. In the view of He et al. [40], the pyrolysis temperature exceeding 350 °C results in a greater stability of such trace element as Cd, Pb, Zn and Cu. This phenomenon occurs due to the loss of unstable aliphatic connections and creation of more stable aromatic

connections [41]. According to Hossain et al. [25], despite the increase in the total content of trace elements by the pyrolysis of sewage sludge, no significant changes in the content of bioavailable forms were noted.

Regardless of the extractant used, only the available forms of iron, manganese, and cadmium were determined in biochars produced from wheat, rape, and Miscanthus straws (Table 5) among the studied trace elements. In the case of iron, irrespective of the biochar type, more available forms of this element was determined after extraction with water, while for manganese, a higher amount of its available forms was extracted with the CaCl<sub>2</sub> solution. It should be noted that a significant content of available forms of manganese extracted with CaCl<sub>2</sub> was determined in bark biochar. Based on our study results, we found that the process of thermal conversion of organic materials did not cause mobilisation of available forms of most of the studied trace elements. Research results published by He et al. [40] indicate that thermal conversion of organic materials results in a greater stability of such trace elements as Cd, Pb, Zn, and Cu.

Despite the many advantages of biochar in reducing the bioavailability, toxicity and mobility of many organic contaminants [26, 42], there are also hazards associated with the production process itself and the subsequent use of the material. The most common risk encountered when using biochar is the possibility of introducing substantial amounts of PAHs into the soil. The amount of PAHs in biochar depends primarily on the parameters at which the pyrolysis is conducted, as well as the PAHs content in feedstocks used in the process [43, 44]. It is likely that high levels of these compounds can stimulate the introduction of large amounts of organic contaminants into the soil, even at

**Table 5** The content of available forms of trace elements in biochars

Biochar	Cu mg·kg <sup>-1</sup>	Zn D.M. ± SD	Fe	Mn	Pb	Cr	Cd	Ni
Extraction with water								
WSB	nd	nd	3.59 ± 0.17	1.92 ± 3.34	nd	nd	0.12 ± 0.08	nd
RSB	nd	nd	2.28 ± 0.08	0.18 ± 0.00	nd	nd	0.06 ± 0.00	nd
MSB	nd	nd	3.53 ± 0.42	0.93 ± 0.04	nd	nd	0.06 ± 0.00	nd
SB	nd	nd	3.41 ± 0.42	0.93 ± 0.13	nd	nd	nd	nd
BB	nd	nd	3.08 ± 0.04	1.98 ± 0.08	nd	nd	nd	nd
LTB	nd	nd	3.47 ± 0.25	1.14 ± 0.17	nd	nd	nd	nd
CV%	–	–	15	57	–	–	–	–
Extraction with 0.01 M CaCl <sub>2</sub>								
WSB	nd	nd	0.63 ± 0.21	4.55 ± 0.42	nd	nd	nd	nd
RSB	nd	nd	0.75 ± 0.04	0.12 ± 0.00	nd	nd	nd	nd
MSB	nd	nd	0.45 ± 0.04	2.16 ± 1.08	nd	nd	nd	nd
SB	nd	nd	1.20 ± 0.08	6.95 ± 1.27	nd	nd	nd	nd
BB	nd	nd	0.45 ± 0.04	25.4 ± 3.2	nd	nd	nd	nd
LTB	nd	nd	0.81 ± 0.13	2.84 ± 0.30	nd	nd	nd	nd
CV%	–	–	39	132	–	–	–	–

± SD standard deviation, CV% coefficient of variation, WSB wheat straw, RSB rape straw, MSB Miscanthus straw, SB sawdust, BB bark, LTB leaves of trees, nd not determined

low doses of biochar. This entails a risk of toxic effects of polycyclic aromatic hydrocarbons on soil microorganisms, including inhibition of biochemical processes related to the metabolism of nutrients [45].

After comparing the results, we found a high diversity in the content of polycyclic aromatic hydrocarbons in the studied biochars, depending on the feedstock used and the type of determined compound (Table 6). Generally, the most commonly determined PAHs were bicyclic, tricyclic, and tetracyclic compounds. Among the analysed bicyclic, tricyclic, and tetracyclic PAHs, their highest amount was determined in biochar produced from rape straw (RSB). This confirmed the observations of Vacha et al. [46] that the PAHs content in the plant biomass used for the production of biochar is determined by the chemical properties of the compound (the number of aromatic rings). The lowest content of the studied PAHs ( $\sum$  16 PAHs) was determined in sawdust biochar (SB), and the highest, in rape straw biochar (RSB) (Table 6). According to the results obtained by Kołtowski and Oleszczuk [44], the temperature at which the biomass is converted is of great importance for thermodesorption and reduction of the PAHs content. Only several studies on the thermodesorption of polycyclic aromatic hydrocarbons have been published so far. As stated by Masto et al. [47], the creation and release of PAHs depend on the conditions under which the process is conducted, as well as the type of material converted. These compounds can be released and emitted in the gaseous phase or adsorbed as a solid residue of the process when the temperature decreases. In the studies,

naphthalene dominated among the sixteen determined compounds. This is reflected in the findings of Masto et al. [47], who discovered high amounts of low molecular weight PAHs, mainly naphthalene, in the resulting ash. According to Kołtowski and Oleszczuk [44], differences between the contents of the studied PAHs in individual biochars may result from the type and strength of interactions between these compounds and biochar. These authors assumed that PAHs are released from the biochar surface at lower temperatures, while higher temperatures make PAHs to be released from pores.

### Ecotoxicity of Biochars

The studies conducted so far have indicated that biochar may have a negative effect on living organisms [48, 49]. Toxicity may be conditioned by the soil reaction (pH), electrical conductivity, biochar elemental composition, ash content, presence of ammonium and calcium ions, trace elements, and organic contaminants, such as PAHs and dioxins. Unfortunately, despite numerous studies, scientists failed to clearly identify the share of these sources in the biochar toxicity [43, 50]. This is due to the diversity of biomass used for the production of biochar, as well as pyrolysis conditions which serve as significant determinants of biochar physical and chemical properties.

The results of toxicity of water extracts from biochars are shown in Table 7. A percent effect toxicity (PE 15 min) for the studied biochars was 34–95 %. The research results of Persoone et al. [24] proved that toxicity percent effect

**Table 6** The content of PAHs in biochars

PAH	WSB mg kg <sup>-1</sup> D.M. ± SD	RSB	MSB	SB	BB	LTB	CV%
Naphthalene	0.261 ± 0.039	1.291 ± 0.138	0.340 ± 0.016	0.064 ± 0.005	0.098 ± 0.012	0.178 ± 0.007	124
Acenaphthylene	0.007 ± 0.000	0.124 ± 0.012	0.014 ± 0.001	0.004 ± 0.000	0.007 ± 0.000	0.012 ± 0.001	169
Acenaphthene	0.008 ± 0.000	0.262 ± 0.018	0.028 ± 0.003	0.003 ± 0.000	0.004 ± 0.000	0.060 ± 0.003	166
Fluorene	0.028 ± 0.002	1.169 ± 0.066	0.249 ± 0.012	0.005 ± 0.000	0.038 ± 0.007	0.196 ± 0.002	159
Phenanthrene	0.026 ± 0.001	0.300 ± 0.029	0.109 ± 0.012	0.009 ± 0.005	0.028 ± 0.005	0.047 ± 0.004	127
Anthracene	0.004 ± 0.000	0.252 ± 0.037	0.016 ± 0.004	0.001 ± 0.000	0.005 ± 0.000	0.005 ± 0.000	204
Fluoranthene	0.007 ± 0.000	0.059 ± 0.004	0.018 ± 0.001	0.007 ± 0.000	0.038 ± 0.007	0.024 ± 0.002	78
Pyrene	0.006 ± 0.000	0.050 ± 0.006	0.018 ± 0.002	0.004 ± 0.000	0.011 ± 0.001	0.026 ± 0.001	90
Benzo[ <i>a</i> ]anthracene	0.001 ± 0.000	0.010 ± 0.001	0.004 ± 0.001	0.001 ± 0.000	0.008 ± 0.002	0.012 ± 0.002	75
Chrysene	0.003 ± 0.000	0.015 ± 0.001	0.008 ± 0.001	0.003 ± 0.000	0.013 ± 0.003	0.026 ± 0.006	74
Benzo[ <i>b</i> ] fluoranthene	0.002 ± 0.000	0.012 ± 0.002	0.049 ± 0.009	0.001 ± 0.000	0.040 ± 0.007	<MQL	123
Benzo[ <i>k</i> ] fluoranthene	0.003 ± 0.000	<MQL	0.010 ± 0.001	0.001 ± 0.000	0.032 ± 0.034	<MQL	165
Benzo[ <i>a</i> ] pyrene	0.004 ± 0.000	0.013 ± 0.000	0.005 ± 0.003	0.001 ± 0.000	0.009 ± 0.003	<MQL	98
Indeno[1,2,3- <i>cd</i> ] pyrene	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	–
Dibenz[ <i>a,h</i> ] anthracene	<MQL	<MQL	<MQL	<MQL	<MQL	0.107 ± 0.008	–
Benzo[ <i>ghi</i> ] perylene	0.838 ± 0.040	<MQL	<MQL	<MQL	<MQL	0.592 ± 0.026	158
∑ 16 PAHs	1.199	3.564	0.878	0.105	0.332	1.286	

<MQL—below the limit of determination

WSB wheat straw, RSB rape straw, MSB Miscanthus straw, SB sawdust, BB bark, LTB leaves of trees

PE < 20 % indicates no significant toxic effect; however, toxicity percent effect in the range of 20 % < PE < 50 % means that the sample is slightly toxic. On the other hand, samples with toxicity percent effect in the range of 50 % < PE < 100 % are considered to be toxic. Among the six studied biochars, low toxicity (20 % < PE < 50 %) was determined only for wheat straw biochar (PE = 34 %). The PE value for other biochars was >50 % which means that these materials were highly toxic (Table 7).

The EC50 values for *V. fischeri* ranged between 14 and 112 %. The lowest EC50 value was determined for biochars SB (EC50 = 14 %) and MSB (EC50 = 17 %). The highest EC50 value was determined for biochar WSB (EC50 = 112 %). According to TU values obtained for *V. fischeri* biochar WSB should be classified as II class toxic compounds (slight acute toxicity; low acute hazard) and biochars: RSB, MSB, SB, BB and LTB as class III toxic compounds (significantly toxic effect; acute hazard). The toxicity of the water extracts from biochars was increased in the following sequence: WSB < BB < RSB < LTB < MSB < SB. Research conducted by Koltowski and Oleszczuk [44] for Miscanthus straw, willow, and wheat straw biochars at 100–300 °C proved that the TU value increased with the increase of the pyrolysis temperature. The above authors indicated that this may be attributable to changes in the structure and chemical composition of the pyrolysed materials, and in particular, changes in the carbon content. The

reduced content of this element reduces the force between the soil matrix and contaminants (PAHs, heavy metals), thus increasing their bioavailability and toxicity.

The studies also included an analysis of the correlation between the chemical composition of biochars and the results of toxicity to *V. fischeri* (Table 8). The calculated negative correlation coefficients indicate that the higher the concentration of the compound, the more toxic is the sample. This means that the EC values are decreasing with an increase in the compound concentration. The positive correlation coefficients indicate that the increase of the compound concentration has no effect on the sample toxicity. The studies revealed a negative correlation between the content of most of PAHs and inhibition of *V. fischeri* luminescence. However, these relationships were statistically insignificant (Table 8). Generally, positive correlation coefficients were determined for trace elements; however, these relationships were statistically significant for Fe and Cd contents extracted with H<sub>2</sub>O and for the total contents of Cr and Ni (Table 7). Negative correlations were found between the total contents of Mn and Cu as well as Mn extracted with 0.01 M CaCl<sub>2</sub> and the reaction of the test organism. In the light of this, one may assume that PAHs are responsible for the demonstrated toxicity of the studied biochars. However, the presented results do not constitute an unequivocal evidence of a direct relationship between the toxicity of biochar to *V. fischeri* and contents of the analysed compounds in biochars. This is a quite



**Table 7** Toxicity of biochars to *V. fischeri*

Biochar	PE%	EC50%	TU
WSB	34	112	0.9
RSB	73	37	2.7
MSB	93	17	5.9
SB	95	14	7.1
BB	80	49	2.0
LTB	72	25	4.0
CV%	30	86	63

CV% coefficient of variation, WSB wheat straw, RSB rape straw, MSB Miscanthus straw, SB sawdust, BB bark, LTB leaves of trees, PE percent effect (PE 15 min)

**Table 8** Relationships between toxicity of biochars and PAHs and heavy metals content

PAH	<i>V. fischeri</i>	Parameters	<i>V. fischeri</i>
Naphthalene	−0.02	Fe H <sub>2</sub> O	0.61*
Acenaphthylene	−0.09	Mn H <sub>2</sub> O	0.14
Acenaphthene	−0.14	Cd H <sub>2</sub> O	0.73*
Fluoranthene	−0.17	Fe CaCl <sub>2</sub>	0.11
Phenanthrene	−0.17	Mn CaCl <sub>2</sub>	−0.32
Anthracene	−0.10	Cd CaCl <sub>2</sub>	−
Fluorene	−0.18	Cu <sub>total</sub>	−0.11
Pyrene	−0.25	Zn <sub>total</sub>	0.33
Benzo[a]anthracene	−0.31	Fe <sub>total</sub>	0.22
Chrysene	−0.33	Mn <sub>total</sub>	−0.10
Benzo[b]fluoranthene	−0.23	Pb <sub>total</sub>	0.11
Benzo[k]fluoranthene	0.06	Cr <sub>total</sub>	0.89***
Benzo[a]pyrene	0.13	Cd <sub>total</sub>	0.27
∑PAHs	0.09	Ni <sub>total</sub>	0.79**

Significant at \*\*\*  $p \leq 0.001$ , \*\*  $p \leq 0.01$ , \*  $p \leq 0.05$

common situation in studies on the toxicity of different materials. Many authors point to a lack of correlation between the contents of hazardous substances in samples and their toxicity to test organisms [11, 44]. This can be attributed to the fact that bioassays use a living organism as an indicator, whose response is the basis for the assessment of the total toxicity of all substances present in the analysed sample, and which often acts synergistically or antagonistically.

To sum up, the water extracts from biochars should be assessed. For the assessment of toxicity leachates in the environment, the ecotoxicological tests can be used as a useful, sensitive and quick tool. Moreover, using biotests working on living organisms it will be possible to evaluate the potential risk connected with the application of different materials for fertilising purposes.

## Conclusions

1. The elemental composition, the contents of macronutrients and trace elements in biochars are determined by the type of converted biomass. In the case of contents of volatile elements, such as nitrogen and sulphur, the process conditions are of great importance.
2. Among the analysed trace elements, only the cadmium content exceeded the limit value for premium class biochars ( $>1.5 \text{ mg kg}^{-1}$  D.M.) defined by the European Biochar Certificate.
3. The process of thermal conversion of organic materials did not cause mobilisation of available forms of most of the studied trace elements.
4. In the study it was found that the water extracts from WSB had low toxicity, while water extracts from RSB, MSB, SB, BB and LTB had high toxicity towards *V. fischeri*. Based on organism response the tested biochars were in the following order: WSB < BB < RSB < LTB < MSB < SB.
5. The highest total content of PAHs ( $\sum 16$  PAHs) was determined in rape straw biochar, and the lowest, in sawdust biochar.
6. The studies revealed a negative correlation between the content of most of PAHs and inhibition of *V. fischeri* luminescence. However, the presented results do not constitute an unequivocal, significant evidence of a direct relationship between the toxicity of biochar to *V. fischeri* and contents of the analysed compounds in biochars.

**Acknowledgments** The Research was financed by the Ministry of Science and Higher Education of the Republic of Poland.

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