

Content of PAHs, activities of γ -radionuclides and ecotoxicological assessment in biochars

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The aim of this research was to determine the effect of thermal conversion temperature and plant material addition to sewage sludge on the PAHs content and the activity of selected γ -radionuclides in biochars, and to conduct an ecotoxicological assessment. The pyrolysis of the mixtures of sewage sludge and plant materials at 300°C and such temperature caused an increase in the contents of 2- and 3-ring hydrocarbons. During the pyrolysis of organic materials at 600°C, the amount of the following compounds was reduced in biochars: benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, indeno[1,2,3c,d]pyrene, dibenzo[a,h]anthracene, and benzo[g,h,i]perylene. Among γ -radioisotopes of the elements, natural radiogenic isotopes were dominant. ¹³⁷Cs was the only artificial radioactive isotope. The pyrolysis of the mixtures of municipal sewage sludge and plant materials revealed that isotope ⁴⁰K had the highest radioactive activity. In the case of other analysed nuclides, activities of ²¹²Pb, ²¹⁴Pb, ²¹⁴Bi, and ¹³⁷Cs were determined after the sample pyrolysis. The extracts from the mixtures of sewage sludge and plant materials were non-toxic to *Vibrio fischeri*.

Keywords: biochar, sewage sludge, PAHs, γ -radionuclides, *Vibrio fischeri*.

INTRODUCTION

In recent years, significant changes in Europe have taken place in both the infrastructure related to water and sewage management, and the structure of municipal waste (including sewage sludge) management. The systematically growing amount of sewage sludge forces some changes in the administration of their resources, and, thereby, induces the search for alternative methods of their transformation, ensuring maximum safety to the environment^{1, 2}. Sewage sludge contains a lot of valuable substances which have a positive effect on the soil properties. However, it also contains various, and often, organic contaminants, which can significantly affect its management^{3, 4}. Polycyclic aromatic hydrocarbons (PAHs) are one of the most common groups of organic contaminants in sewage sludge. Most of them are mutagenic, toxic and carcinogenic substances which, due to their considerable stability in the environment, belong to the group of persistent organic pollutants (POPs). Introduction of sewage sludge with these pollutants into soil may pose a risk of their accumulation in plant biomass or their movement into the soil profile. This is dangerous to the underground water purity and, in consequence, may have a negative effect on living organisms, including humans⁵.

One of the methods to limit the leaching of contaminants from sewage sludge is thermal conversion through pyrolysis. This method entails multidirectional physical and chemical changes of these materials, which result in the formation of, inter alia, biochar⁶. The research

results published hitherto indicate that specific properties of biochar (such as microporosity, large specific surface area, and presence of functional groups) make the material prone to organic contaminants. Despite numerous advantages of biochar in limiting the bioavailability, toxicity, and mobility of many organic contaminants^{7, 8}, there are also hazards connected with its production process itself and the later use of the material. The possibility of introducing considerable amounts of PAHs into soil is one of the most frequently listed hazards related to the application of biochar. The amount of PAHs in biochar depends mainly on the pyrolysis parameters and the content of these compounds in feedstocks used in the process. One should bear in mind that thermal conversion of materials with greatly diversified chemical composition, such as sewage sludge, may produce extremely varied effects. On one hand, pyrolysis may lead to decomposition of organic compounds and, on the other, there is a synthesis of strongly condensed aromatic structures which may cause the formation of other compounds with aromatic structure, or lead to an increase in their content⁹. The natural use of the biochar was a determining factor when choosing the temperature of 300°C. It is assumed that the natural, including agricultural, use of biochar requires the pyrolysis temperature of 300°C, since biochar obtained in this way has a higher cation exchange capacity and higher stable carbon content¹⁰. On the other hand, the temperature of 600°C was selected with the aim to verify the hypothesis that higher temperatures have greater efficiency in removing PAHs. According to Oleszczuk et al.⁵, limited efficiency of lower

temperatures in removing PAHs is due to the fact that particles of these compounds are retained in the biochar pores. These authors argued that increase in the process temperature should result in a gradual release of water and volatile compounds and, in consequence, open the pores and release PAHs.

The available literature lacks information on changes in radioactivity, including γ -radionuclides which will affect living organisms after the introduction of biochar thus produced into the environment). Depending on its parameters and materials subjected to processing, thermal conversion may activate isotopes of some elements.

Given the possibility of natural use of organic materials, it is essential to evaluate the contents of polycyclic aromatic hydrocarbons and nuclides in these materials. Thermal conversion of sewage sludge may create an immediate risk of both the presence and creation of new organic compounds with hydrocarbon character, and radioactive elements^{10, 11}. Therefore, the presence of some contaminants calls the safe use of biochar into question, especially in the case of soils used for cultivation of plants for food purposes.

Compared to thermally converted sewage sludge alone, better environmental and technological effects can be achieved after mixing sewage sludge with plant waste materials, such as cereal straw, sawdust or bark. The research results proved that the addition of plant materials to sewage sludge leads to dilution of, e.g. the trace elements content. It also improves the conditions for thermal process by affecting the structure of the mass converted¹².

Based on the results of studies on the polycyclic aromatic hydrocarbon content in biochars of different origin, it must be stated that there are still only a few papers concerning the effect of feedstock or the pyrolysis method

on the PAHs content. However, there is no information on these factors' effect on radioactivity of γ -radionuclides in biochars. The aim of this research was to determine the effect of thermal conversion temperature and plant material addition to sewage sludge on the PAHs content and the activity of selected γ -radionuclides in biochars. This research also presents the evaluation of ecotoxicity of extracts from sewage sludge and mixtures of sewage sludge and plant materials to *Vibrio fischeri*.

MATERIAL AND METHODOLOGY

Characteristics of sewage sludge and plant materials used in the research

The research material was anaerobically stabilised, dehydrated sewage sludge collected at the water treatment plant in Krakow. The plant utilises the mechanical and biological system for waste water treatment. Sludge digestion time in separate digesters was 19 days. The digested sludge was then dehydrated on a belt press.

The materials used in the preparation of mixtures with sewage sludge were selected based on their availability, possible management, as well as effectiveness in improving physical properties of sewage sludge. Wheat straw used in the research came from an agricultural holding. Sawdust and bark of conifers were obtained as waste products of industrial processing of timber. The plant materials used in the research came from the Małopolska Province (southern Poland).

Selected chemical properties of sewage sludge and plant materials are presented in Tables 1, 2 and 3.

Preparation of mixtures and their thermal conversion

In order to improve its physical properties, sewage sludge was mixed with plant materials. Before mixing

Table 1. The properties (dry matter and ash, C, N, S contents) of initial materials and mixtures before thermal treatment

Material	Dry matter	Ash	C, N, S		
	g kg ⁻¹ ±SD		g kg ⁻¹ DM ± SD		
Wheat straw (WS)	990 ±24	32 ±1	561 ±10	4.9 ±0.2	11.7 ±0.0
Sawdust (S)	989 ±24	7 ±1	576 ±11	1.2 ±0.2	4.3 ±0.1
Bark (B)	988 ±23	447 ±3	321 ±4	4.7 ±1.0	8.7 ±1.0
Sewage sludge (SS)	236 ±6	507 ±4	286 ±3	26.1 ±1.0	30.9 ±0.3
Sewage sludge + bark (SS+B)	351 ±6	406 ±12	344 ±10	20.3 ±1.9	17.8 ±3.5
Sewage sludge + wheat straw (SS+WS)	312 ±9	206 ±5	460 ±4	18.7 ±1.7	17.6 ±1.3
Sewage sludge + sawdust (SS+S)	302 ±7	225 ±9	450 ±8	20.6 ±2.4	20.8 ±2.7

± standard deviation, n = 3.

Table 2. The content of macroelements in sewage sludge and plant materials used in the experiment

Material	P	K	Ca	Mg	Na
	g kg ⁻¹ DM ± SD				
Wheat straw (WS)	0.75 ±0.01	5.25 ±0.06	2.51 ±0.08	0.41 ±0.01	0.11 ±0.01
Sawdust (S)	0.16 ±0.10	0.80 ±0.29	1.41 ±0.03	0.10 ±0.01	0.10 ±0.01
Bark (B)	0.75 ±0.21	1.31 ±0.04	6.57 ±0.13	0.40 ±0.01	0.17 ±0.02
Sewage sludge (SS)	35.1 ±1.8	3.12 ±0.06	33.1 ±1.1	4.72 ±0.19	0.87 ±0.03

± standard deviation, n = 3.

Table 3. The content of trace elements in sewage sludge and plant materials used in the experiment

Material	Cr	Ni	Cu	Zn	Cd	Pb
	mg kg ⁻¹ DM ± SD					
Wheat straw (WS)	1.85 ±0.10	1.16 ±0.08	2.83 ±0.10	36.0 ±0.2	0.53 ±0.01	1.41 ±0.04
Sawdust (S)	1.39 ±0.10	0.86 ±0.01	2.25 ±0.40	17.3 ±0.3	0.39 ±0.01	2.76 ±0.12
Bark (B)	7.99 ±0.27	3.92 ±0.06	7.19 ±1.00	49.0 ±3.8	0.48 ±0.00	10.60 ±0.3
Sewage sludge (SS)	420.00 ±16	64.70 ±0.6	330.00 ±5	1751.0 ±69	2.17 ±0.16	58.90 ±2.6

± standard deviation, n = 3.

with sewage sludge, wheat straw and bark of conifers were ground and 5 mm sieved. Sawdust of conifers was used in the form obtained from the manufacturer. The materials were mixed at 1:1 by weight calculated on the dry matter. The experimental design included: sewage sludge + wheat straw (SS + WS), sewage sludge + bark (SS + B), sewage sludge + sawdust (SS + S). The contents of dry matter, ash as well as N, C and S in unconverted mixtures are presented in Table 1.

Thermal conversion of mixtures of sewage sludge and various plant additions was conducted under laboratory conditions, at a station designed for thermal conversion of biomass under a limited supply of air (1–2%)⁶. The combustion chamber was closed, located under a fume cupboard for extracting fumes. Dimensions of the working chamber [mm]: (W × L × D) 290 × 220 × 350. The samples of mixtures were placed in the combustion chamber, and two temperature procedures were applied: 300°C and 600°C. The exposure time was 15 minutes. The rate of heating the combustion chamber was 10°C min⁻¹. The pyrolysis time and temperature were established on the basis of our preliminary examinations and results reported in the literature^{12, 13}.

Textural parameters of organic materials

The specific surface area and porosity were determined from N₂ gas adsorption/desorption isotherms at 77 K using an ASAP 2420 apparatus (Micromeritics). The samples were outgassed for 24 h at 378 K. The BET equation was used for the specific surface area calculations (S_{BET})¹⁴. The total pore volume (V_{tot}^{0.99}) was calculated from the amount of N₂ adsorbed at a relative vapor pressure (P/P₀) ~0.99. The volume of micropores (V_{mic}^T) was calculated by applying the t-plot method. Mesopore volume (V_{mes}^{BJH}) was determined from the adsorption branch of the isotherms using the BJH method¹⁵ in the mesopore range proposed by Dubinin¹⁶.

The macropore volume (V_{mac}) was calculated using the following equation:

$$V_{\text{mac}} = V_{\text{tot}}^{0.99} - (V_{\text{mic}}^T + V_{\text{mes}}^{\text{BJH}})$$

Textural parameters of organic materials are presented in Table 4.

Chemical analyses and activity of γ -radionuclides

The initial materials (sewage sludge, wheat straw, sawdust, bark) and the unconverted mixtures of sewage sludge and plant materials were dried at 105°C for 12 hours in order to determine the dry matter content¹⁷. The research materials and mixtures before and after thermal conversion were ground in a laboratory mill and 1 mm sieved. The ash content in the prepared materials was determined after incinerating the sample in a chamber furnace at 600°C for 8 hours¹⁸. The carbon, nitrogen and sulphur contents were determined on the Vario MAX Cube analyser (manufactured by Elementar) equipped with a thermal conductivity detector (Vario MAX cube CNS, Elementar Analysensysteme GmbH, Hanau, Germany). Activity of γ -radionuclides in sewage sludge and plant materials used in the experiment are presented in Table 5.

The content of polycyclic aromatic hydrocarbons in thermally unconverted sewage sludge and converted mixtures

The procedure of PAH determination (16 compounds from US EPA list) was adopted from Maliszewska-Kordybach et al.¹⁹. Biochars (5–10 g) were spiked with mixture of five deuterated PAH standards (naphthalene-d₈, fluorene-d₁₀, phenanthrene-d₁₀, chrysene-d₁₂ and perylene-d₁₂) and then extracted with dichloromethane (two extraction cycles, temperature of 100°C and pressure of 1500 psi) in accelerated solvent extraction system (ASE 200, Dionex). Extracts were concentrated on the rotary evaporator (Büchi Rotavapor R-200) to a volume of 1 mL and cleaned-up on glass mini-columns filled with 1 g of silica gel and then evaporated to obtain 1 mL of

Table 4. Textural parameters of organic materials

Parameter	SS+B before	SS+B 300°C	SS+B 600°C	SS+WS before	SS+WS 300°C	SS+WS 600°C	SS+S before	SS+S 300°C	SS+S 600°C
S _{BET} [m ² g]	2.900	2.400	20.600	2.800	3.100	58.300	2.200	2.200	12.900
V _{tot} ^{0.99} [cm ³ g]	0.016	0.012	0.026	0.014	0.017	0.038	0.012	0.012	0.032
V _{mic} ^T [cm ³ g]	0.001	0.001	0.009	0.001	0.001	0.026	0.001	0.001	0.005
V _{mic} ^T V _{tot} ^{0.99}	0.062	0.083	0.346	0.071	0.059	0.684	0.083	0.083	0.156
V _{mes} ^{BJH} [cm ³ g]	0.011	0.007	0.012	0.009	0.010	0.011	0.008	0.006	0.011
V _{mes} ^{BJH} V _{tot} ^{0.99}	0.688	0.583	0.462	0.643	0.588	0.289	0.667	0.500	0.344
V _{mac} [cm ³ g]	0.004	0.004	0.005	0.004	0.006	0.001	0.003	0.005	0.016
V _{mac} ^{BJH} V _{tot} ^{0.99}	0.250	0.333	0.192	0.286	0.353	0.027	0.250	0.417	0.500

Table 5. Activity of γ -radionuclides in sewage sludge and plant materials used in the experiment

Nuklid	(SS)	(WS)	(B)	(S)
	Bq g ⁻¹ D.M. ± SD			
²³⁴ Th	<MDA	<MDA	<MDA	<MDA
²³⁵ U	<MDA	<MDA	<MDA	<MDA
²¹² Pb	0.15 ± 0.02	<MDA	<MDA	0.22 ± 0.04
²¹⁴ Pb	<MDA	<MDA	<MDA	0.13 ± 0.02
²⁰⁸ Tl	<MDA	<MDA	<MDA	<MDA
²¹⁴ Bi	<MDA	<MDA	<MDA	0.18 ± 0.02
¹³⁷ Cs	<MDA	<MDA	<MDA	<MDA
²²⁸ Ac	<MDA	<MDA	<MDA	<MDA
⁴⁰ K	0.47 ± 0.04	0.71 ± 0.07	<MDA	<MDA

± standard deviation, n = 3; <MDA – below the limit of determination.

extract in n-hexane. PAHs were determined on Agilent 6890N gas chromatograph equipped with Agilent 5973 Network mass spectrometer (70 eV) and 7683 B series autosampler. The resolution of PAH compounds was achieved with DB-5 MS+DG fused-silica capillary column (30 m x 0.25 mm I.D. with a film thickness of 0.25 μm), constant helium flow of 30 cm/sec^{-1} , the splitless injection system at a temperature of 250°C and the following GC oven programme: 60°C for 2 min, followed by 30°C/ min^{-1} ramp to 120°C and then with a ramp of 5°C/ min^{-1} to the final temperature of 290°C (10 min hold). Selected ion monitoring (SIM) system was applied for PAH molecular ion detection. The precision of the method for single PAH compounds was < 24%. The blank sample procedure was applied for all analytical series. PAH content in biochars was determined in three replications¹⁹. The generally accepted toxicity equivalency factors (TEF)²⁰ were used to calculate the TEF equivalent as the sum of products of the contents of individual PAHs and TEF coefficients. The content of polycyclic aromatic hydrocarbons in plant materials used in the experiment are presented in Table 6.

Analysis of γ -radionuclide activity in thermally unconverted sewage sludge and converted mixtures

The analysis of γ -radionuclide activity was conducted using high-purity germanium detectors (HPGe), as well as Ortec HPGe PopTop and Ortec HPGe P-Type detectors ensuring high energy resolution²¹. The measurement chain had electronic equipment manufactured by Canberra Packard (amplifier + multichannel amplitude analysers with Multiport II memory buffer); the software used to record the spectra was Genie 2000. Based on the analysis of the spectra obtained during the measurements, activities of individual elements were calculated:

$$A = \frac{N_S - N_B \frac{T_S}{T_B}}{T_S \cdot \text{eff} \cdot I}$$

where:

A – isotope activity in the sample [Bq],
 N_S – counts (peak area) of quanta of a given energy in the sample spectrum,

N_B – counts (peak area) of quanta of a given energy in the background spectrum,

T_S – time of sample measurement [s],

T_B – time of background measurement [s],

eff – efficiency of recording gamma quanta of a given energy,

I – percentage efficiency of the emission of gamma quanta of a given energy.

Detection limits, which then were calculated and expressed as Minimum Detectable Activity (MDA), were established using the Currie equation²²:

$$L_D = 2,706 + 4,653 \cdot \sqrt{(B + I)}$$

where:

L_D – counts corresponding to the detection limit,

B – background, background counts under the peak in the sample spectrum,

I – interference, counts (peak area corresponding to a given energy) in the background spectrum.

Ecotoxicity of sewage sludge and mixtures after thermal conversion

Toxicity of extracts from sewage sludge and of their mixtures after thermal conversion was determined using the Microtox bioassay. The extracts were prepared by mixing 1 volume of the material with 20 volumes of redistilled water and shaking mechanically for 24 hours. After that time, the samples were centrifuged for 10 minutes at 3000 rpm and filtered. In the Microtox[®] bioassay, the toxicity level of samples was determined by a decrease in *Vibrio fischeri* luminescence, which is an effect of metabolic inhibition in the bacteria after exposure to a toxic substance. The standard test procedure was applied for extract samples: 81.9% Screening Test. Luminescence was measured on a Microtox M500 analyser before and after 15-minute incubation of the bacterial suspension with the studied samples²³. Samples were tested in three replicates.

Statistical calculations

The materials were analysed in three replicates in order to exclude analytical errors. Such experimental arrangement prevented an objective analysis of variance for the results of chemical analyses; therefore, only the values of standard deviation were calculated.

Table 6. The content of individual PAHs in sewage sludge and plant materials used in the experiment

PAHs	(SS)	(WS)	(B)	(S)
	mg kg ⁻¹ DM \pm SD			
Naphthalene	0.094 \pm 0.004	0.216 \pm 0.005	0.290 \pm 0.006	0.287 \pm 0.004
Acenaphthylene	LoD	0.004 \pm 0.000	0.004 \pm 0.000	0.006 \pm 0.000
Acenaphthene	0.025 \pm 0.001	0.007 \pm 0.001	0.007 \pm 0.000	0.006 \pm 0.000
Fluorene	0.047 \pm 0.002	0.020 \pm 0.001	0.021 \pm 0.001	0.016 \pm 0.000
Phenanthrene	0.319 \pm 0.009	0.047 \pm 0.002	0.086 \pm 0.004	0.094 \pm 0.005
Anthracene	0.352 \pm 0.013	0.004 \pm 0.000	0.006 \pm 0.001	0.006 \pm 0.001
Fluoranthene	0.749 \pm 0.028	0.010 \pm 0.001	0.041 \pm 0.001	0.025 \pm 0.000
Pyrene	0.936 \pm 0.030	0.008 \pm 0.001	0.027 \pm 0.001	0.015 \pm 0.001
Benzo[a]anthracene	0.358 \pm 0.003	0.003 \pm 0.000	0.010 \pm 0.002	0.003 \pm 0.000
Chryzene	0.801 \pm 0.011	0.005 \pm 0.000	0.016 \pm 0.000	0.004 \pm 0.000
Benzo[b]fluoranthene	0.623 \pm 0.010	0.010 \pm 0.002	0.017 \pm 0.002	0.004 \pm 0.000
Benzo[k]fluoranthene	0.205 \pm 0.014	0.003 \pm 0.001	0.008 \pm 0.003	0.003 \pm 0.002
Benzo[a]pyrene	0.314 \pm 0.000	< LoD	0.007 \pm 0.001	< LoD
Indeno[1,2,3-cd]pyrene	0.342 \pm 0.002	< LoD	0.011 \pm 0.001	< LoD
Dibenz[a,h]anthracene	0.051 \pm 0.000	< LoD	0.001 \pm 0.000	< LoD
Benzo[g,h,i]perylene	0.327 \pm 0.002	< LoD	0.008 \pm 0.001	< LoD

\pm standard deviation, n = 3; < LoD – method quantification limit.

RESULTS AND DISCUSSION

Chemical composition of the materials used

Materials used in the research differed not only in their origin but also in chemical parameters (Table 1–4). Sewage sludge had the lowest contents of dry matter and carbon, and the highest ash content of all the materials used in the research. Wheat straw, sawdust, and bark used in the preparation of mixtures with sewage sludge, had a small water content, which was an important factor improving physical properties of the mixtures obtained²⁴. This was reflected in the dry matter content in these mixtures. Plant materials used to prepare the mixtures had a large carbon content – 486 g kg⁻¹ DM on average (Table 1). A relatively lower ash content was determined in those materials. Sewage sludge had higher nitrogen and sulphur contents than other materials used to form the mixtures (Table 1). It manifested itself in the content of these elements in the mixtures. The contents of the analysed components determined in the plant materials used in the preparation of mixtures did not differ from the values recorded by other authors²⁵.

Textural parameters of organic materials

The specific surface area (S_{BET}) of SS+B was 2.9 m² g⁻¹ and the total pore volume ($V_{\text{tot}}^{0.99}$) was 0.016 cm³ g⁻¹ (Table 4). The thermal conversion of the mixtures at 300°C had no influence on the textural parameters. Until thermal conversion the mixtures at 600°C had significant influence on the specific surface area and total pore volume. The S_{BET} and $V_{\text{tot}}^{0.99}$ were 20.6 m² g⁻¹ and 0.026 cm³ g⁻¹, respectively. The specific surface area (S_{BET}) of SS+WS was 2.8 m² g⁻¹ and the total pore volume ($V_{\text{tot}}^{0.99}$) was 0.014 cm³ g⁻¹ (Table 4). The thermal conversion of the mixtures at 300°C, resulted in an insignificant increasing of the textural parameters ($S_{\text{BET}} = 3.1$ m² g⁻¹, $V_{\text{tot}}^{0.99} = 0.017$). A significant increase was observed after thermal conversion of the mixtures at 600°C ($S_{\text{BET}} = 58.3$ m² g⁻¹, $V_{\text{tot}}^{0.99} = 0.038$). The lowest value was noted for the SS+S. The specific surface area and the total pore volume of initial material was 2.2 m² g⁻¹ and 0.012 cm³ g⁻¹, respectively. The thermal conversion of the mixtures at 300°C had no influence on the textural parameters. The thermal conversion of the mixtures at 600°C caused an increase S_{BET} to 12.9 m² g⁻¹ and $V_{\text{tot}}^{0.99}$

to 0.032. In almost all samples, unmodified and modified, mesopore volumes prevails over the macropore volume and micropore volume. The thermal conversion of the mixtures caused a decrease the value $V_{\text{mes}}/V_{\text{tot}}$ and $V_{\text{mac}}/V_{\text{tot}}$ and an increase the value $V_{\text{mic}}/V_{\text{tot}}$.

The PAH content in the mixtures of sewage sludge and plant materials, and in the biochar obtained

The content of polycyclic aromatic hydrocarbons in biochars varies depending on the feedstock used, as well as on the pyrolysis conditions^{26,27}. It is possible that high concentrations of these compounds will cause the introduction of high amounts of organic contaminants into soil, even at low doses of biochar. This poses a risk of toxic effect of polycyclic aromatic hydrocarbons on soil microorganisms, e.g. inhibition of biochemical processes related to changes in nutrients^{28,29}.

When comparing the content of polycyclic aromatic hydrocarbons in the studied biochars with their content in unconverted plant materials (Table 6), sewage sludge and mixtures with sewage sludge, a decrease in the contents of naphthalene, acenaphthene, fluorene, and phenanthrene, as well as of other compounds was observed (Table 7). The addition of plant materials (straw, bark, sawdust) to municipal sewage sludge diluted the contents of most of the studied compounds. Increase in the contents of these four PAHs in the mixtures prepared for pyrolysis resulted from the load of these substances introduced with plant materials. It should be noted that the PAH content in plants is conditioned by the chemical properties of a compound (the number of aromatic rings), plant species, and soil properties³⁰. The above compounds are 2- and 3-ring PAHs, which suggests that plant materials added to sludge contained these contaminants.

The pyrolysis of mixtures of sewage sludge and plant materials changed the studied compound contents depending on the plant material added (wheat straw, bark, sawdust) and the temperature applied (300°C, 600°C). Regardless of the type of plant material added, thermal conversion of the mixtures at 300°C increased the contents of naphthalene, acenaphthene, and fluorene (Table 8) compared to their contents determined in unconverted municipal sewage sludge (Table 7). Increase in the conversion temperature to 600°C decreased the contents of: benzo[b]fluoranthene, benzo[k]fluoranthene,

Table 7. The content of individual PAHs in mixtures before thermal treatment

PAH	(SS + WS)	(SS + B)	(SS + S)
	mg kg ⁻¹ DM ± SD		
Naphthalene	0.466 ± 0.006	0.271 ± 0.026	0.306 ± 0.090
Acenaphthylene	<LoD	<LoD	<LoD
Acenaphthene	0.127 ± 0.012	0.089 ± 0.006	0.083 ± 0.014
Fluorene	0.110 ± 0.005	0.087 ± 0.002	0.079 ± 0.014
Phenanthrene	0.471 ± 0.034	0.488 ± 0.004	0.479 ± 0.001
Anthracene	0.029 ± 0.002	0.023 ± 0.002	0.029 ± 0.001
Fluoranthene	0.361 ± 0.061	0.450 ± 0.072	0.436 ± 0.017
Pyrene	0.350 ± 0.076	0.379 ± 0.076	0.427 ± 0.000
Benzo[a]anthracene	0.132 ± 0.000	0.147 ± 0.011	0.133 ± 0.012
Chrysene	0.375 ± 0.114	0.272 ± 0.021	0.373 ± 0.038
Benzo[b] fluoranthene	0.241 ± 0.004	0.259 ± 0.003	0.238 ± 0.015
Benzo[k] fluoranthene	0.080 ± 0.021	0.097 ± 0.005	0.090 ± 0.007
Benzo[a] pyrene	0.092 ± 0.009	0.115 ± 0.015	0.099 ± 0.002
Indeno[1,2,3-cd]pyrene	0.116 ± 0.020	0.116 ± 0.001	0.127 ± 0.002
Dibenz[a,h]anthracene	<LoD	<LoD	<LoD
Benzo[g,h,i]perylene	0.130 ± 0.016	0.139 ± 0.005	0.141 ± 0.005

± standard deviation, n = 3; < LoD – method quantification limit.

Table 8. The content of individual PAHs in biochars after thermal treatment

PAHs	(SS + WS)		(SS + B)		(SS + S)	
	300°C	600°C	300°C	600°C	300°C	600°C
	mg kg ⁻¹ DM ± SD					
Naphthalene	0.448±0.037	0.064±0.029	0.305±0.018	0.093±0.000	0.347±0.015	0.056±0.026
Acenaphthylene	< LoD	< LoD	< LoD	< LoD	< LoD	< LoD
Acenaphthene	0.068±0.019	0.014±0.007	0.042±0.005	0.028±0.000	0.050±0.003	0.013±0.006
Fluorene	0.071±0.017	0.016±0.006	0.032±0.003	0.025±0.001	0.050±0.002	0.016±0.002
Phenanthrene	0.245±0.042	0.052±0.025	0.140±0.006	0.105±0.000	0.175±0.012	0.044±0.008
Anthracene	0.014±0.000	0.143±0.024	0.006±0.002	0.004±0.000	0.010±0.001	0.005±0.001
Fluoranthene	0.046±0.001	0.093±0.042	0.040±0.000	0.281±0.003	0.035±0.001	0.033±0.022
Pyrene	0.064±0.004	0.154±0.084	0.051±0.033	0.263±0.001	0.053±0.005	0.104±0.026
Benzo[a]anthracene	0.047±0.000	0.026±0.003	0.032±0.002	0.156±0.002	0.044±0.000	0.003±0.001
Benzo[a]anthracene	0.177±0.087	0.165±0.004	0.109±0.061	0.276±0.003	0.085±0.002	0.205±0.026
Benzo[b]fluoranthene	0.150±0.008	< LoD	0.081±0.004	0.374±0.005	0.109±0.005	< LoD
Benzo[k]fluoranthene	0.052±0.004	< LoD	0.028±0.001	0.159±0.011	0.040±0.002	< LoD
Benzo[a]pyrene	0.063±0.005	< LoD	0.030±0.001	0.211±0.008	0.049±0.000	< LoD
Indeno[1,2,3-cd]pyrene	0.077±0.009	< LoD	0.033±0.006	0.184±0.009	0.049±0.003	< LoD
Dibenz[a,h]anthracene	0.012±0.007	< LoD	0.008±0.000	0.024±0.002	0.012±0.001	< LoD
Benzo[g,h,i]perylene	0.070±0.004	< LoD	0.041±0.003	0.180±0.002	0.055±0.002	< LoD
Equivalent TEF	0.159	0.007	0.089	0.423	0.135	0.006

± standard deviation, n = 3; <LoD method quantification limit.

benzo[a]pyrene, indeno[1,2,3c,d]pyrene, dibenzo[a,h]anthracene, and benzo[g,h,i]perylene in the mixtures of municipal sewage sludge and wheat straw (SS + WS) and sawdust (SS + S) below the method quantification limit. Compared to the initial materials, an increase in the compound content was observed for the mixture of municipal sewage sludge and bark (SS + B). In the course of the analysis it was found that, regardless of the plant material added, the total content of the sixteen determined compounds decreased between over 2-fold and over 5-fold compared to their total content in unconverted municipal sewage sludge (5.55 ± 0.04 mg kg⁻¹ dry matter) (Table 7). It should be highlighted that, in general, the temperature of 300°C was more efficient in reducing the contents of the studied PAHs. The results published by Kołtowski and Oleszczuk²⁷ indicate that the temperature of biomass conversion is of great importance for thermodesorption and decrease in the PAH content. The literature lacks information on the thermodesorption of polycyclic aromatic hydrocarbons. According to Mastro et al.³¹, the formation and release of PAHs depend on the conditions of the process, and the type of material converted. These compounds can be released in the gaseous phase, emitted or adsorbed as a solid residue of the process when the temperature drops. The results of our study showed that, among the sixteen compounds determined, naphthalene was dominant in the mixtures processed at 300°C. This is reflected in the results of Mastro et al.³¹ who proved that PAHs with a small molecular mass, mainly naphthalene, were dominant in the ash obtained. Van den Heuvel and Van Noort³² also suggested that the process temperature is a decisive factor for the reduction of PAHs. Our results do not fully confirm this observation. Although a reduction in contents of benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, indeno[1,2,3-cd]pyrene, dibenzo[a,h]anthracene, and benzo[g,h,i]perylene was obtained at 600°C in the mixtures of municipal sewage sludge and wheat straw (SS + WS), and municipal sewage sludge and sawdust (SS + S), these compound contents increased in the mixture of sewage sludge and bark (SS + B) compared to the material processed at

300°C. According to Guilloteau et al.³³, the smaller efficiency of lower temperatures in the reduction of PAHs results from the retention of the compound particles in biochar pores. As stated by these authors, increase in the process temperature should cause a gradual release of water and volatile components and, in consequence, opening of pores and release of PAHs. Kołtowski and Oleszczuk²⁷ argued that differences between the contents of the studied PAHs in individual biochars may result from the type and strength of the interaction between these compounds and biochar. These authors emphasised that lower temperatures lead to the release of PAHs from biochar surface, whereas higher temperatures result in the release of these compounds from pores. This theory was not fully confirmed in our research.

Evaluation of the PAH toxicity in the biochar obtained

Epidemiological studies were conducted with the aim to assess the relationship between PAHs and frequency of carcinogenic symptoms²⁰. A dose-response relationship was found between the concentration of B(a)P administered in food and the frequency of stomach cancers in male and female mice. Our research attempted to determine the level of toxicity of materials subjected to pyrolysis at the proposed temperatures, using relative carcinogenic potencies TEF (toxic equivalence factor²⁰). Being aware of the elaboration of TEFs for conditions of direct exposure, absolute values were multiplied with an attempt to estimate (based on the sum of products) the potential level of risk in materials that could possibly enter the environment during their natural use, without taking into account the effect of soil-biochar dilution. Values calculated in this way are presented in Table 8 with TEF as an equivalent. These values confirm the previous observations. For sewage sludge mixed with straw and sawdust, there was a 23.5-fold reduction in the sum of relative toxicity products when the pyrolysis temperature was increased from 300°C to 600°C. The mixture of bark and sewage sludge (SS + B) showed a reverse relationship under the same thermal conditions, since the above factor increased 4.7 times. These observations suggest great differences in the internal

structure of plant materials, which may pose risks during their conversion.

Activity of γ -radionuclides in the mixtures of sewage sludge and plant materials, and in the biochar obtained

The available literature on biochar contains no reports on the activity of γ -radionuclides. Thermal conversion of biomass entails a series of changes in the properties of the solid product of the process – biochar^{12, 27, 29, 31}. Thermal conversion of organic materials can reduce the self-absorption of gamma radiation, which, in turn, may lower the limits of detection of the activity of the studied radioactive isotopes. Out of the nine studied radionuclides, only low contents of natural radioactive isotopes ²¹²Pb (0.15 ± 0.02 Bq g⁻¹ DM) and ⁴⁰K (0.47 ± 0.04 Bq g⁻¹ DM) were determined in municipal sewage sludge used to prepare the mixtures (Table 9). Only the mixture of municipal sewage sludge and wheat straw (SS + WS) before pyrolysis showed low activity of isotopes ²³⁴Th, ¹³⁷Cs, and ⁴⁰K. The artificial isotope ¹³⁷Cs, as a fission product, is present in the environment due to releases from nuclear facilities and nuclear weapon testing (global fallout). Given its half-time of 31.17 y and high bioavailability caused by chemical analogy to potassium, it can accumulate in plant biomass. Interactions with potassium are an important issue which must be addressed when it comes to caesium accumulation in plant biomass³⁴. Isotope ⁴⁰K is the dominant radionuclide in tissues of living organisms, and its occurrence is not related to biological changes³⁵. Since its abundance reaches 0.0117% (half-life period equal to approx. $1.3 \cdot 10^9$ y), it is present in living organisms as a result of physiological uptake of potassium from soils or food. According to Rosik-Dulewska and Dulewski³⁶, ⁴⁰K constitutes 98% of the total activity of natural radionuclides in plants. The content of isotope ²³⁴Th in the mixture of sewage sludge and straw

amounted to 0.18 ± 0.02 Bq g⁻¹ DM, which could also result from the amount of this nuclide added to sludge together with plant materials. Thermal conversion of the mixture of sewage sludge and wheat straw (SS + WS) increased the concentration of isotope ⁴⁰K (Table 9). In the case of mixtures of sewage sludge and bark (SS + B), and sewage sludge and sawdust (SS + S), the activity of isotope ⁴⁰K was also observed, at a level comparable to the one determined for unconverted sewage sludge. It should be noted that in both unconverted mixtures, the results obtained were below the detection limit. In addition to isotope ⁴⁰K, activities of isotopes of lead, ²¹²Pb and ²¹⁴Pb, were noted in the mixtures subjected to pyrolysis. The activity of both isotopes was recorded in the mixture of sewage sludge and wheat straw (SS + WS), after converting the material at 600°C. In the case of mixtures of sewage sludge and bark (SS + B), and sewage sludge and sawdust (SS + S), ²¹²Pb was detectable after pyrolysis at 300°C (Table 9). In addition, there were observed the activity of isotope ¹³⁷Cs in thermally converted mixture of sewage sludge and bark (SS + B), and the activity of isotope ²¹⁴Bi in the mixture of sewage sludge and wheat straw (SS + WS) (Table 9). Neither of these activities was noted before pyrolysis. The apparent lack of activity of the studied radionuclides in sewage sludge, except for ²¹²Pb and ⁴⁰K, suggests that they can be used in the production of biochar without the risk of contaminating the final product³⁷.

Ecotoxicity of sewage sludge and mixtures after thermal conversion

Bioassays were demonstrated to be useful for detecting potentially ecotoxicological effects of biochar, not captured by the physicochemical limit values set in different, currently available biochar quality standards which do not provide guidance for application rates specific to soil or crop types. That is why ecotoxicological tests are proposed as important criteria to develop management recommendations. The stimulation of *Vibrio fischeri* luminescence was found (Fig. 1). The extract samples showed negative mean values of toxicity. The inhibition of *V. fischeri* luminescence was –12 and –18% (SS + B); –25 and –22% (SS + WS), and –21 and –20% (SS + S). This means that the intensity of *V. fischeri* luminescence increased after exposure to the extracts, which can be attributed to their lower toxicity compared to the reference medium. The extracts from the mixtures of sewage sludge and plant materials will be non-toxic to *Vibrio fischeri*. In the literature, the stimulatory effect of sublethal or low concentrations of toxic chemicals

Table 9. Activity of γ -radionuclides in the mixtures of sewage sludge and plant materials prior to thermal processing

Nuklid	(SS + WS)	(SS + B)	(SS + S)
	Bq g ⁻¹ DM \pm SD		
²³⁴ Th	0.18 \pm 0.02	<MDA	<MDA
²³⁵ U	<MDA	<MDA	<MDA
²¹² Pb	<MDA	<MDA	<MDA
²¹⁴ Pb	<MDA	<MDA	<MDA
²⁰⁸ Tl	<MDA	<MDA	<MDA
²¹⁴ Bi	<MDA	<MDA	<MDA
¹³⁷ Cs	0.015 \pm 0.003	<MDA	<MDA
²²⁸ Ac	<MDA	<MDA	<MDA
⁴⁰ K	0.42 \pm 0.0400	<MDA	<MDA

\pm standard deviation, n = 3; <MDA Minimum Detectable Activity.

Table 10. Activities of γ -radionuclides in biochars

Nuklid	(SS + WS)		(SS + B)		(SS + S)	
	300°C	600°C	300°C	600°C	300°C	600°C
	Bq g ⁻¹ DM \pm SD					
²³⁴ Th	<MDA	<MDA	<MDA	<MDA	<MDA	<MDA
²³⁵ U	<MDA	<MDA	<MDA	<MDA	<MDA	<MDA
²¹² Pb	<MDA	0.085 \pm 0.013	0.11 \pm 0.01	<MDA	0.064 \pm 0.009	<MDA
²¹⁴ Pb	<MDA	0.061 \pm 0.009	<MDA	<MDA	<MDA	<MDA
²⁰⁸ Tl	<MDA	<MDA	<MDA	<MDA	<MDA	<MDA
²¹⁴ Bi	<MDA	0.067 \pm 0.008	<MDA	<MDA	<MDA	<MDA
¹³⁷ Cs	<MDA	<MDA	0.039 \pm 0.009	<MDA	<MDA	<MDA
²²⁸ Ac	<MDA	<MDA	<MDA	<MDA	<MDA	<MDA
⁴⁰ K	1.14 \pm 0.12	0.49 \pm 0.05	<MDA	0.36 \pm 0.03	0.36 \pm 0.03	0.35 \pm 0.03

\pm standard deviation, n = 3; <MDA Minimum Detectable Activity.

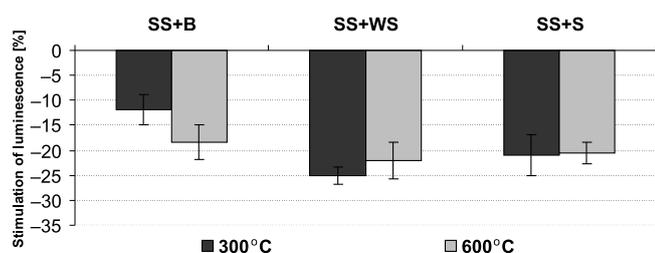


Figure 1. Luminescence stimulation of *Vibrio fischeri* – notoxicity of extracts from sewage sludge and mixtures sewage sludge and plant materials

on organism metabolism is referred to as hormesis³⁸. Hormesis was found to be common in the widely used *Vibrio fischeri* luminescence bioassay^{39, 40}.

CONCLUSIONS

The results of our research indicate that during the pyrolysis of organic materials at 600°C, the contents of the following compounds were reduced in biochars: benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, indeno[1,2,3-cd]pyrene, dibenzo[a,h]anthracene, and benzo[g,h,i]perylene. Among γ -radioisotopes of the elements, natural radiogenic isotopes were dominant. ¹³⁷Cs was the only artificial radioactive isotope. The pyrolysis of the mixtures of municipal sewage sludge and plant materials revealed that isotope ⁴⁰K had the highest radioactive activity. In the case of other analysed nuclides, activities of ²¹²Pb, ²¹⁴Pb, ²¹⁴Bi, and ¹³⁷Cs were determined after the sample pyrolysis. The extracts from the mixtures of sewage sludge and plant materials will be non-toxic to *Vibrio fischeri*.

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