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Assessment of Migration of PAHs Contained in Soot of Solid Fuel Combustion into the Aquatic Environment

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Abstract: In the thermal processes of fossil fuel and biomass combustion, organic compounds are formed, belonging to the family of so-called Persistent Organic Pollutants. Among them, polycyclic aromatic hydrocarbons (PAHs) are of particular importance. Due to their toxic, mutagenic, and carcinogenic properties, these compounds seriously threaten living organisms. PAHs are formed during the incomplete combustion of organic matter in natural and anthropogenic processes. They are present in soot. Nevertheless, their combustion by-products from individual heat sources are not controlled. They are often used in horticulture or stored in arbitrary places. Collecting or using this waste without determining its toxicity carries the risk of contaminating the land and the water into which the pollutants enter. PAHs pose a severe threat to the aquatic environment as the pollutants contained in the soot are washed away. Therefore, it seemed advisable to conduct research on the combustion of solid fuels such as hard coal with particle sizes above 60 mm, hard coal with a granule size of 25–80 mm, hard coal with a granule size of 8–25 mm, pellets, and mixed firewood. Through the experiments, soot samples were obtained, and a leaching process was performed, thereby extracting eluents from the soot. The results of this work allowed for the determination and assessment of the degree of leachability of polycyclic aromatic hydrocarbons (PAHs) from by-products of combustion of solid fuels, such as soot. The influence of the type of solid fuel burned on the mobility of PAHs in the aquatic environment was determined. The carcinogenicity equivalents of carbon black extracts obtained from the combustion of solid fuels were also determined, as well as the acute toxicity of the tested media by performing biotests using the Microtox kit and *Aliivibrio fischeri* luminescent bacteria.

Keywords: PAHs migration; solid fuels; combustion; aquatic environment; toxicity

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1. Introduction

Combustion by-products are minerals produced by the combustion of various types of solid fuels. The combustion of fossil fuels, i.e., hard coal and lignite, and unconventional fuels, i.e., biomass, including pellets or firewood, causes the formation of solid waste, mainly in the form of chimney soot [1]. The quantity and quality of this waste depend primarily on the type of fuel used. They can be used as a secondary raw material in industrial or economic processes, as long as their chemical composition allows for such a use [1–3]. Apart from mineral substances, organic compounds are so-called persistent organic pollutants (Persistent Organic Pollutants) [2]. Among them, polycyclic aromatic hydrocarbons (PAHs) are of particular importance. These compounds pose a serious threat to living organisms due to their toxic, mutagenic, and carcinogenic properties. PAHs are formed during the incomplete combustion of organic matter in natural and anthropogenic processes [2–5]. They are present in soot [6–8]. Polycyclic aromatic hydrocarbons are a group of persistent pollutants that consist of two or more rings of aromatic carbon and hydrogen atoms, which are difficult to degrade and metabolise. PAHs are primarily by-products of industrial processes (such as oil production, coke production, and transportation) and the incomplete combustion or pyrolysis of organic materials (such as wood, fossil fuels, and other organic materials) [1]. As a result of emissions, PAHs can be widely dispersed in the environment, air, water, soil, and bottom sediment in water bodies. It has been documented that 90% of the total PAH load reaching the soil is retained in the surface layer. This can lead to an imbalance in the ecological balance of the soil system [2,9]. PAHs have been the subject of a great deal of scientific work due to their ubiquitous presence in the environment and potential mutagenicity, teratogenicity, and carcinogenicity [3,9].

PAHs present in surface waters are not solely dangerous to humans. They pose a threat to the entire ecosystem and indirectly affect its various parameters, including through metabolic transformation. PAHs have an ecotoxic effect on aquatic organisms and birds [10]. The toxicity of PAHs for aquatic organisms is influenced by metabolism and photooxidation, and their harmfulness increases with increasing ultraviolet radiation. PAHs show moderate to high acute toxicity in aquatic organisms and birds. Mammals are able to absorb PAHs in various ways, including ingestion, respiration, and skin absorption [11,12]. PAHs are moderately persistent in the environment and may bioaccumulate. The PAH concentrations in fish and crustaceans are expected to be much higher than in the environment from which they were collected [10]. The accumulation of PAHs in algae and lower marine animals (e.g., daphnia) results in higher trophic levels. Algae, as the main producers of coastal ecosystems, can play a significant role in the bioremediation of PAHs in aquatic ecosystems. Newsted and Giesy [13] classified individual PAHs according to their phototoxicity towards *Daphnia magna* crustaceans. Class 1, i.e., extremely toxic, includes NAP, ANT, FLT, PYR, B[a]A, B[k]F, B[a]P, B[ghi]P, and D[ah]A. Class 2, i.e., moderately toxic, includes CHR. On the other hand, class 3, i.e., non-toxic, includes PHE and FLU [14]. Several studies have reported the phototoxicity of PAHs to benthic species [14–16], thus emphasizing that the phototoxicity of PAHs is also species dependent. For example, although NAP was extremely phototoxic to *D. magna* [13], it was found to be non-phototoxic to the freshwater endobenthic species *C. riparius* [14,15]. The effective removal of PAHs via algae occurs through cellular biodegradation and/or bioaccumulation [17]. Some of these algae have the ability to metabolize both single and mixtures of PAHs. These algae include*: Selenastrum capricornutum*, *Chlorella vulgaris*, *Scenedesmus platydiscus*, and *Scenedesmus quadricauda* [18]. Due to the efficiency of the degradation of HMW PAHs, microalgae (cyanobacteria) have also received attention in the literature [17,19].

Many regulations have been proposed for PAH emissions. For example, the U.S. Environmental Protection Agency (U.S. EPA) has listed 16 unlisted PAHs as priority control pollutants [4]. Subsequently, many countries, including China, have placed several PAHs on the priority control pollutants' black or grey list [5,6]. Of the sixteen PAHs identified by the EPA, eight have been shown to have carcinogenic effects. However, it should be noted that other aromatic hydrocarbons that have not been confirmed to have toxic effects can react with other compounds in the environment to form derivatives that are often more toxic [20]. Thermal fuel conversion processes promote the formation of polycyclic aromatic hydrocarbons (PAHs), which, along with solid particles, can enter soils and groundwater or surface water, thereby polluting them [21]. It is common to test combustion residues for inorganic components such as heavy metals, chlorides, and sulphates. These tests are required by European and US legislation. Determinations of organic compounds in fly ash are rarely performed [22]. Most frequently, power companies transfer waste such as soot or fly ash for disposal or, after determining the exact composition, direct it to agricultural management [23]. However, the by-products of the combustion generated in individual heat sources are not controlled. Soot is typically found in random places. The accumulation of these wastes or their use without determining their toxicity carries the danger of contaminating the land and the waters into which the pollutants enter. Toxic and difficult-to-decompose compounds from aqueous solutions such as sewage can be removed by advanced disposal methods such as adding the Pd/ZrO_2 catalyst to the solution [24], by sorption on activated carbon (AC) and magnetically activated carbon

(MAC) [25], by sorption on activated carbon through photodegradation in an advanced stage of oxidation [26], using cheap and ecological sorbents such as sawdust [27], and employing sorbents with the addition of nanoparticles of Pd–Ni [28]. These compounds pose a severe threat to the aquatic environment, as the pollutants contained in soot leach out [29–31]. In addition, these compounds are included in the group of so-called refractory pollutants of a toxic nature, which can cause physiological disorders in plants and animals including humans, and in large doses, even death. Since these substances are not or only minimally biodegradable, PAHs exhibit one or more of the following properties: carcinogenicity, mutagenicity, teratogenicity, or bioaccumulation in the human and animal food chain [32,33].

Considering the above, it seemed expedient to conduct a study on the combustion of solid fuels such as hard coal with a size of more than 60 mm, hard coal with a grain size of 25–80 mm, hard coal with a grain size of 8–25 mm, pellets, and mixed firewood, from which soot samples were obtained. These samples were subjected to PAH leachability tests. The results of this work made it possible to determine and evaluate the degree of migration of polycyclic aromatic hydrocarbons (PAHs) from the by-products of solid fuel combustion, such as soot. The effect of the type of solid fuel burned on the migration of PAHs into the aquatic environment was determined. The carcinogenicity equivalents of the soot extracts obtained from solid fuel combustion were also determined. In addition, biotests were carried out for the samples from all the combusted fuels to determine their acute toxicity levels using the Microtox system.

2. Materials and Methods

This research was conducted under laboratory conditions. The experiment was conducted by burning the following fuels: hard coal with particle sizes above 60 mm, hard coal with a grain size of 25–80 mm, hard coal with a grain size of 8–25 mm, pellets with a grain size of 6 mm, and mixed firewood. The mixed firewood comprised hardwood mixed with trees such as beech, hornbeam, and alder. The wood pellet used in the experiment was a mixed hardwood pellet, the same as in the case of firewood. The combustion process was carried out on a laboratory bench [34] (Figure 1) consisting of a Moderator Unica Vento Eko 25 kW boiler (Moderator, Hajnówka, Poland) with an automatic feeder (Moderator, Hajnówka, Poland), equipped with a manual and retort grate (Moderator, Hajnówka, Poland). Three consecutive combustions of each of the 5 solid fuels selected for testing were carried out, collecting soot from the heat exchanger each day [35].

Figure 1. Schematic of solid fuel combustion experiment.

The research on the content of moisture, volatile matter, ash, and thermogravimetric decomposition of solid fuels was carried out on the LECO TGA-701 analyzer (LECO, Lakeview Ave, Richmond, VA, USA). The measurement of the above-mentioned quantities consisted of registering the loss of sample mass as a function of temperature, while the sample was in a controlled atmosphere of air, nitrogen, or oxygen. The results were carried out in 5 replications. The measuring station consisted of an analyzer and a computer. The obtained results of selected fuels are presented in Table 1.

Table 1. Analysis of the fuels used for the experiment.

Type of Fuel	Moisture [%]	Volatile Substances [%]	Ash Content [%]
Mixed firewood	7.82 ± 0.10	75.65 ± 0.33	0.44 ± 0.02
Pellet 6 mm	5.51 ± 0.16	77.37 ± 0.34	0.72 ± 0.04
Hard coal with a grain size of 8–25 mm	12.40 ± 0.13	39.07 ± 0.09	5.68 ± 0.02
Hard coal with a grain size of 25–80 mm	22.75 ± 0.20	40.36 ± 0.41	5.73 ± 0.65
Hard coal with particle sizes above 60 mm	21.56 ± 0.16	33.34 ± 0.20	6.93 ± 0.03

For each combustion test, 15 kg of each fuel was burned, maintaining the same combustion conditions, i.e., flue gas temperature and feed water temperature (at the boiler exit), which were about 70–80 °C, while upon return it was no less than 50 °C. After completion of the combustion process for a given fuel type, the collected soot samples of each cycle were mixed and subjected to aqueous extraction by pouring the weighed amount of soot into distilled water at a ratio of 1:10. They were then shaken for 24 h at room temperature. The solvent extraction of PAHs took place for all the aqueous extracts. The eluents made were neither centrifuged nor filtered. A suspension was later extracted with a solvent. PAHs were separated from the extracts during extraction to the solid phase under vacuum (SPE) conditions. Chromabond SPE columns, 5 mL and 500 mg with C-18 octadecyl filling, were used. The SPE columns were conditioned by washing them 2 times each with 3 cm^3 of methanol, and then 2 times each with 3 cm^3 of a water–methanol mixture (9:1, *v*:*v*). On the prepared column, 3 cm^3 of the sample was introduced without vacuum. The flow rate was set at 5 cm^3/min, the vacuum was turned on, and the remaining sample was passed through the column. The column was then washed with 3 cm^3 of the methanol–water mixture (1:1, *v*:*v*) and dried under vacuum for 20 min. PAHs were eluted from the column with 5 cm^3 of dichloromethane. The eluate was concentrated in an inert gas atmosphere (nitrogen) to a volume of 500 µL. Figure 2 shows the isolation scheme and analytical procedure leading to the quantification of PAHs in the samples.

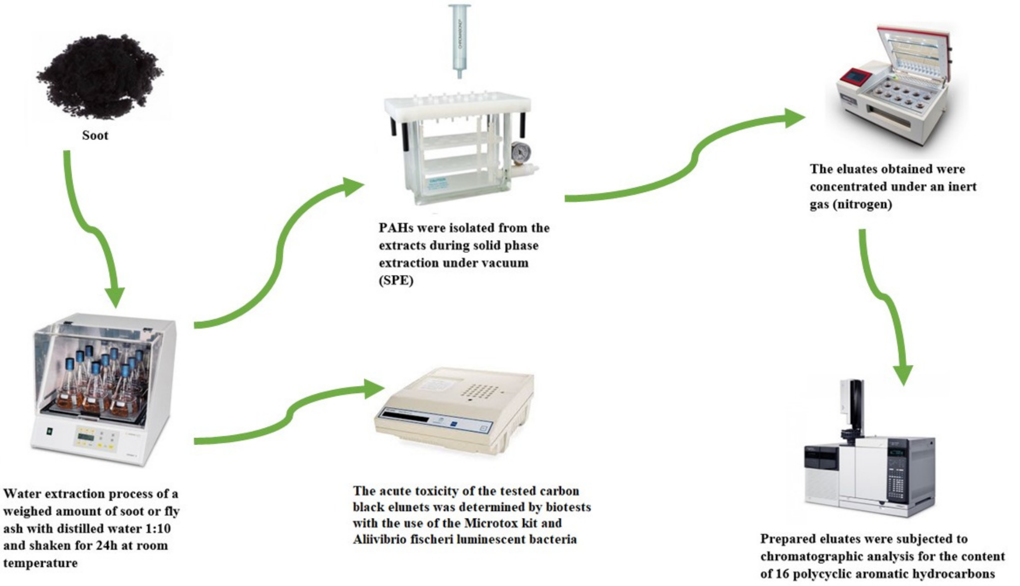

Figure 2. Isolation and analytical procedure for the determination of PAHs and Microtox test of eluents from soot samples.

The eluates prepared in this way were chromatographed for the content of 16 polycyclic aromatic hydrocarbons: naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[b]fluoranthene, benzo[a]pyrene, dibenzo[a,h]anthracene, indeno[1,2,3,c,d]pyrene, and benzo[g,h,i]perylene. The quantitative analysis of PAHs was performed using a set including a gas chromatograph (Agilent, Santa Clara, USA) with a mass spectrometer (Agilent GC/MS Triple Quad 7000C) (Agilent, Santa Clara, CA, USA), equipped with a split/splitless type dispenser (Agilent, Santa Clara, VA, USA) and an HP-5MS capillary column (Agilent, Santa Clara, CA, USA), with dimensions of 30 m × 0.25 mm and a film thickness of 0.25 µm. The operating conditions of the apparatus during the analysis were as follows: carrier gas—helium, purity 6.0; the flow rate of the carrier gas through the column—1 mL/min (splitless); injection volume—1 µL; dispenser temperature—260 °C; ion source temperature—230 °C; transfer line temperature—300 °C; quadrupole temperature—150 °C; scanning mode—single ion monitoring (SIM—selected ion monitoring). However, the temperature program was set as follows: initial temperature of the column oven 60 °C, isotherm 2 min; temperature increase of 30 °C/min to 120 °C; from 120 °C, increase temperature 5 °C/min to 300 °C; set final temperature of the column oven to 300 °C, isotherm 15 min. During the extraction, no internal standard was added, while the method was controlled by simultaneously carrying out the entire analytical procedure for a sample of certified reference material—Clean Soil Reference Material EDF-5183 (CERILLIANT Analytical Reference Standards). External calibration was used in the tests, employing calibration curves for individual compounds. Calibration solutions were prepared from a standard mixture of 16 PAHs (AccuStandard, Z-014G) [35].

To determine the harmfulness of PAHs contained in the eluents, the indicators (Formulas (1)–(4)) were used, which define the toxicity of individual PAHs and the whole group:

- The toxic equivalent RTBaP (TEQ—Toxic Equivalent) [36]:

$$\begin{gathered}\mathrm{RTBaP} = 0.001 \times [\mathrm{Nap}] + 0.001 \times [\mathrm{Acy}] + 0.001 \times [\mathrm{Ace}] + 0.001 \times [\mathrm{Fl}] \\ + 0.001 \times [\mathrm{Phe}] + 0.01 \times [\mathrm{Ant}] + 0.001 \times [\mathrm{Flu}] + 0.001 \times [\mathrm{Pyr}] + 0.1 \times [\mathrm{B(a)A}] \\ + 0.01 \times [\mathrm{Chr}] + 0.1 \times [\mathrm{B(b)F}] + 0.1 \times [\mathrm{B(k)F}] + 1 \times [\mathrm{B(a)P}] + 5 \times [\mathrm{D(ah)A}] + 0.1 \times \\ [\mathrm{B(ghi)P}] + 0.1 \times [\mathrm{I(cd)P}]\end{gathered} \tag{1}$$

- Mutagenic equivalent (MEQ) [37]:

$$\begin{gathered}\mathrm{MEQ} = 0.00056 \times [\mathrm{Acy}] + 0.082 \times [\mathrm{B(a)A}] + 0.017 \times [\mathrm{Chr}] + 0.25 \times [\mathrm{B(b)F}] + 0.11 \times [\mathrm{B(k)F}] + 1 \times [\mathrm{B(a)P}] + 0.31 \\ \times [\mathrm{I(cd)P}] + 0.29 \times [\mathrm{D(ah)A}] + 0.19 \times [\mathrm{B(ghi)P}]\end{gathered} \tag{2}$$

- Carcinogen equivalent (TCDD-TEQ) [36]:

$$\begin{gathered}\mathrm{TCDD\text{-}TEQ} = 0.000025 \times [\mathrm{B(a)A}] + 0.00020 \times [\mathrm{Chr}] + 0.000354 \times [\mathrm{B(a)P}] + \\ 0.00110 \times [\mathrm{I(cd)P}] + 0.00203 \times [\mathrm{D(ah)A}] + 0.00253 \times [\mathrm{B(b)F}] + 0.00487 \times [\mathrm{B(k)F}]\end{gathered} \tag{3}$$

- The ratio of PAHs considered to be carcinogenic to the sum of all measured PAHs [38]:

$$\begin{gathered}\Sigma\mathrm{WWAcarc}/\Sigma\mathrm{WWA} = ([\mathrm{B(a)A}] + [\mathrm{B(a)P}] + [\mathrm{B(b)F}] + [\mathrm{B(k)F}] + [\mathrm{Chr}] + [\mathrm{D(ah)A}] + \\ [\mathrm{I(cd)P}])/([\textstyle\sum \mathrm{WWA}])\end{gathered} \tag{4}$$

where:

Nap—naphthalene concentration;
Acy—acenaphthylene concentration;
Ace—acenaphthene concentration;
Fl—fluorene concentration;
Phe—phenanthrene concentration;
Ant—anthracene concentration;
Flu—fluoranthene concentration;
Pyr—pyrene concentration;
B(a)A—benzo[a]anthracene concentration;
Chr—chrysene concentration;

B(b)F—benzo[b]fluoranthene concentration;
B(k)F—benzo[k]fluoranthene concentration;
B(a)P—benzo[a]pyrene concentration;
D(ah)A—dibenz[a,h]anthracene concentration;
B(ghi)P—benzo[g,h,i]perylene concentration;
I(cd)P—indeno[1,2,3,c,d]pyrene concentration;
∑WWA—concentration of the sum of 16 PAHs.

In acute toxicity studies, extracts obtained from the combustion of tested fuels were biotested using the Microtox Model 500 luminometer (Strategic Diagnostics Inc., Newark, NJ, USA). The operation of the Microtox kit is based on the use of *Aliivibrio fischeri* luminescent bacteria, the characteristic feature of which is the ability to luminescence, i.e., to produce light under the influence of enzymatic reactions. As a result of the action of harmful substances, the luminescence of these microorganisms is inhibited, i.e., the light intensity decreases with the increase in toxic substances [39]. The natural habitat of these microorganisms are seas and oceans, but they are not present in sufficient numbers to allow them to shine directly in sea water. *Allivibrio fischeri* bacteria belong to a group of microorganisms that live in symbiosis with other marine organisms, e.g., squid. Their luminescence is, therefore, observed in those marine organisms where their concentration exceeds 1010 cells/cm^3 [40,41]. Tests with the Microtox kit and *Allivibrio fischeri* luminescent bacteria are frequently used ecotoxicological biological tests, the EC_{50} values of which have been correlated with the parameters of acute vertebrate toxicity, irritants, and cytotoxicity indices [42]. Although, according to the literature, the most commonly used tests for toxicity to aquatic organisms are tests using daphnia, e.g., *Daphnia magna*; the use of biotests with *Aliivibrio fischerii* gives comparable, reliable results [43–46]. The system used in the research consists of the Microtox Model 500 analyser (Modern Water Company, New Castle, United State), a PC, and software for collecting and processing data (Microtox Omni 4.1). Modern Water reagents dedicated to the Microtox system were used during the determinations (bacterial regeneration solution—reconstitution solution, osmotic solution—osmotic adjusting solution, and dilution water—diluent for the liquid and solid phases), and lyophilised *Aliivibrio fischeri* bacteria designated by the manufacturer as *Vibrio fischeri* NRRL B-11177 acting as a bioindicator. *Aliivibrio fischeri* bacteria in the lyophilised form can be stored for 1 year at −20 °C and used for tests at any time after suspending them in deionised water. The microorganisms placed in the dilution water (2% NaCl) glow with a constant intensity for 1–1.5 h, which is a sufficient amount of time to perform the determinations. The test reaction used during the determinations was to reduce the luminescence of bacteria after a specified incubation time of the tested samples of the combusted fuels. The test was performed according to the manufacturer's standard procedure (SDI). Statistical analysis was performed using the Omni 4.1 software, which is an integral part of the Microtox system. Each report for a specific sample provided: EC_{50} value expressed as % decrease in the luminescence of *Alivibrio fischeri* bacteria, 95 % confidence intervals, confidence coefficient, regression equation, correlation coefficient, and coefficient of determination (R^2). EC_{50} values obtained after 5 and 15 min were converted into toxic units, TUa, according to the following formula:

$$TU_a = \frac{1}{EC_{50}} * 100 \tag{5}$$

where:

TUa—Acute Toxicity Units;
EC_{50}—half maximal effective concentration.

Two systems were used to evaluate the obtained results and to determine the toxicity class of the tested samples. The first, proposed by Persoone [47], identified the following toxicity classes:

- Class 0: TUa = 0—non-toxic sample;
- Class 1: 0.4 < TUa < 1—no significant toxicity (low acute toxicity);

- Class 2: 1 < TUa < 10—significant toxicity;
- Class 3: 10 < TUa < 100—high acute toxicity;
- Class 4: TUa > 100—very high toxicity.

The second, presented by Sawicki et al. [48], assumed the following classification into toxicity classes:

- Class 1: TUa < 10—no significant toxic effect;
- Class 2: 10 < TUa < 25—significant toxic effect—low-toxic sample;
- Class 3: 25 < TUa < 100—significant toxic effect—toxic sample;
- Class 4: TUa > 100—significant toxic effect—highly toxic sample.

Statistical analyses were performed using Statistica (version 13.1) and Excel (version 2016). For all parameters, their compliance with the normal distribution was checked. The Shapiro–Wilk test was used to examine the distribution of the examined features. $p = 0.05$ was adopted as the critical level of significance. For the parameters, the arithmetic mean was calculated. The significance of differences in mean values in more than two populations for parameters with a normal distribution and with homogeneous variances was checked by the ANOVA test.

3. Results and Discussion

Many scientific studies have researched the migration of micropollutants such as PAHs from the solid to the liquid phase. Han and colleagues [49] studied the migration of PAHs from the air, where the source was the burning of oils and biomass, to tropical ponds and seawater. These studies showed that migration occurs over time and depends on the concentration of PAH emitted from the source. On the other hand, Sun et al.'s [50] research group proved that PAHs in the soils came mainly from the combustion of grass/wood/carbon, closely related to the essential fossil fuels used in the research area. Their research showed that the groundwater was heavily contaminated with PAHs in the karst areas of southwest China due to the vertical transfer of PAHs from surface soils, resulting in a need for urgent and adequate protection. PAHs can also pass from the solid to liquid phase under certain conditions. These hydrocarbons may be secondary contaminants of intermediates, residues, and waste after the combustion of solid fuels. Such by-products of combustion include, among others, soot [35].

Table 1 shows the results with respect to the water extracts from the carbon blacks of 16 polycyclic aromatic hydrocarbons obtained by burning solid fuels. The tested hydrocarbons were divided into the following three groups: ΣLMW (Low Molecular Weight) (hydrocarbons containing two to four aromatic rings in their structure: naphthalene, acenaphthene, acenaphthylene, fluorene, phenanthrene, and anthracene); ΣHMW (High Molecular Weight) (5 and 6 ring compounds: fluoranthene, pyrene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, indene[1,2,3-c,d]pyrene, dibenzo[a,h]anthracene, and benzo[g,h,i]perylene), and the sum of 16 aromatic hydrocarbons—ΣPAH. Upon analysing the obtained results (Table 2) regarding the PAH content in the water extracts obtained from the soot samples from solid fuel combustion, it was found that the content of individual aromatic hydrocarbons, as well as their total sum, is high in water extracts from carbon blacks. Soot is a product of the incomplete combustion of fuels and other materials containing significant amounts of carbon in their chemical composition [51]. In addition, high-temperature and combustion conditions cause the formation of free radicals. The reducing conditions in the combustion process result in the formation of highly unsaturated bonds, from which simple aromatic products (BTX) are formed in other reactions. Further reactions lead to the synthesis of particles with several aromatic rings, called PAHs [51]. The highest content of total PAHs (Figure 3) was found in the water extract from soot from hard coal combustion with a granule size of 25–80 mm (4155.92 ng/g DM—*Dry Mass*), and the lowest from the soot from mixed firewood (225.58 ng/g DM). Upon analysing the content of ΣLMW in the tested soot water extracts (Figure 4), the highest amount was recorded in the extract of the soot obtained from the combustion of hard coal with a granule size of 25–80 mm (1093.12 ng/g DM), while the lowest was recorded in the water extracts from soot formed through mixed firewood combustion (108.65 ng/g DM). When analysing the soot water extracts in terms of the content of the sum of HMW aromatic hydrocarbons (Figure 4), i.e., PAHs with 5–6 rings, the same relationship was observed as in the case of the LMW sum. The highest content of Σ HMW was found in the water extract from the combustion of hard coal with a granule size of 25–80 mm (2681.19 ng/g DM), and the lowest was washed out of the soot from mixed firewood (99.35 ng/g DM).

Table 2. PAH in eluents from soot and fly ash samples and calculated indices.

ng/g DM (ng/g Dry Mass)	**Hard Coal with Dimensions over 60 mm**	**Hard Coal with a Grain Size of 8–25 mm**	**Mixed Firewood**	**Pellet**	**Hard Coal with a Grain Size of 25–80 mm**
Scheme for presenting the results					
Naphthalene [NAP]	33.99 ± 1.34 28.93–35.05	13.26 ± 0.46 9.57–16.30	10.70 ± 1.79 7.02–14.04	19.05 ± 3.02 16.07–24.04	11.94 ± 2.04 6.46–13.04
Acenaphthene [ACY]	204.09 ± 8.72 190.12–208.63	25.30 ± 1.35 22.34–29.05	30.24 ± 3.05 27.61–34.05	190.25 ± 11.13 178.35–195.35	65.87 ± 4.53 60.21–69.24
Acenaphthene [ACE]	48.81 ± 2.56 43.62–51.24	4.94 ± 0.81 3.57–6.03	5.26 ± 0,67 4.50–8.38	33.76 ± 2.04 27.35–37.35	9.07 ± 1.03 8.35–11.34
Fluorene [FLU]	83.61 ± 3.56 72.04–87.53	15.77 ± 1.45 11.23–17.03	14.68 ± 2.04 9.02–17.09	68.87 ± 3.96 62.95–78.34	54.82 ± 7.35 49.646–59.32
Phenanthrene [PHE]	323.48 ± 11.23 302.11–330.32	125.65 ± 9.63 119.35–130.56	47.76 ± 7.35 40.04–51.05	372.84 ± 26.75 356.24–384.46	951.42 ± 75.34 902.34–975.30
Anthracene [ANT]	189.19 ± 8.24 180.54–197.09	50.48 ± 4.50 45.24–54.26	17.58 ± 1,70 14.56–19.09	211.31 ± 16.57 197.45–226.68	381.61 ± 45.35 374.11–395.34
Fluoranthene [FLR]	34.98 ± 3.56 29.35–40.34	30.52 ± 6.24 26.73–34.63	5.56 ± 1,66 4.06–7.03	61.89 ± 5.78 53.84–67.45	277.51 ± 35.51 256.34–295.30
Pyrene [PYR]	162.86 ± 9.03 154.65–170.30	168.38 ± 8.56 159.85–173.45	26.81 ± 4.03 21.05–35.63	293.28 ± 16.45 282.56–297.10	1449.27 ± 124.4 1405.13–1535.03
Benzo[a]anthracene [B(a)A]	54.92 ± 2.67 48.11–59.61	56.32 ± 5.63 50.52–59.60	12.06 ± 9.34 10.01–17.51	52.31 ± 4.91 47.35–58.10	217.25 ± 23.45 204.54–234.51
Chrysene [CHR]	25.86 ± 2.56 22.45–32.45	41.18 ± 2.64 39.35–44.05	6.61 ± 1.64 3.40–8.03	29.22 ± 2.45 25.77–35.62	126.45 ± 15.51 110.43–134.21
Benzo[b]fluoranthene [B(b)F]	32.08 ± 1.67 28.90–36.34	60.99 ± 6.34 52.35–66.78	5.94 ± 1.05 4.06–7.03	20.46 ± 2.04 17.64–24.51	221.50 ± 26.20 194.32–230.53
Benzo[k]fluoranthene [B(k)F]	45.85 ± 1.89 38.13–47.35	104.90 ± 8.35 96.24–109.44	8.51 ± 1.04 6.03–10.01	44.20 ± 4.05 38.03–49.35	207.27 ± 26.45 191.02–218.34
Benzo[a]pyrene [B(a)P]	29.52 ± 2.01 25.35–32.56	53.38 ± 3.84 45.35–56.20	3.01 ± 0,23 2.04–4.04	24.43 ± 2.35 20.54–28.44	67.84 ± 16.24 61.34–74.35
Indeno[1,2,3,c,d]pyrene [I(cd)P]	38.03 ± 2.06 30.01–40.13	80.76 ± 7.56 71.20–85.60	7.64 ± 1.51 6.02–9.40	16.80 ± 1.56 14.56–19.45	40.19 ± 5.34 34.24–46.10
Dibenz[a,h]anthracene [D(ah)A]	27.74 ± 2.04 24.67–31.54	22.67 ± 2.01 19.90–26.70	4.54 ± 0.53 3.05–7.03	5.80 ± 1.56 4.03–6.89	29.62 ± 2,35 25.35–34.3
Benzo[g,h,i]perylene [B(ghi)P]	110.86 ± 7.45 104.22–115.60	123.81 ± 10.23 119.01–128.99	18.67 ± 3.02 15.06–20.01	19.05 ± 2.46 17.45–23.05	44.31 ± 4.35 35.13–46.24
SUMA	1445.86	978.32	225.58	1463.51	4155.92
∑LMW	693.98	184.92	108.65	684.77	1093.12
∑HMW	562.70	742.93	99.35	567.43	2681.19
TEQ = CEQ	523.59	337.23	80.11	445.63	1248.35
MEQ	88.54	140.64	13.78	49.81	195.48
TCDD-TEQ	0.60	1.23	0.11	0.48	2.52
ΣWWAcarc/ΣWWA	175.67	429.52	214.15	132.02	218.99

In the tested samples of the water extracts from carbon blacks, the presence of all the analysed hydrocarbons from the analysed group of 16 PAHs was found. Phenanthrene and pyrene belonged to the group of polycyclic aromatic hydrocarbons most often found in carbon blacks and were determined at the highest concentrations. The highest concentrations of these compounds were recorded in a sample of water extract from soot produced via coal combustion, with a granule size of 25–80 mm. The phenanthrene concentration for this extract was 951.42 ng/g DM, and the pyrene concentration was 1449.27 ng/g DM. In the remaining extracts of soot from the combustion of hard coal and other fuels, these values ranged from 26.81 to 372.84 ng/g DM of the sample. The least frequent hydrocarbons from the PAH group in the samples of water extracts from carbon blacks were naphthalene and acenaphthylene. Ali (2019) [52] determined that the most toxic of all PAHs is benzo[a]pyrene (five rings per molecule), which is an indicator compound identifying PAH contamination in the environment. Its presence was found in all the analysed water extracts from soot. Its highest concentration was recorded in a sample of the water extract of soot from the combustion of hard coal with a grain size of 25–80 mm. The research group of Kusmierz [53] also studied the release of PAHs but regarding biocarbon deposited into clay sand in a field experiment lasting 2.5 years. According to the results obtained by this research group, the addition of biochar to soils increased the PAH content from 0.239 µg/g in the control soil to 0.526 µg/g and 1.310 µg/g in 30 and 45 t/ha of biocarbon-enriched soil, respectively. On the other hand, Enell's [54] research group investigated the leaching of PAHs from fly ash obtained from the combustion of firewood. It was found that due to the number of PAHs introduced into forest soils through the addition of stabilized fly ash, recyclable ash products can be determined primarily by the ash particles' weathering rate and the total concentration of pollutants [54].

Ali (2019) [52] and the research group of Mon (2019) [55] report that individual PAHs show a very different degree of carcinogenicity. Due to the above, the toxicity equivalent value of TEQ was determined following Equation (1), characterising the total toxicity of the tested compounds against the indicator benzo(a)pyrene. The results of the calculations are presented in Table 2. The value of the TEQ toxicity equivalent depends mainly on the content of PAHs with high toxicity factors TEF [56]. Based on the performed calculations, it was found that the highest TEQ was found in the extract of soot from the combustion of hard coal with a granule size of 25–80 mm and in the extract of soot produced after the combustion of coal with a granule size greater than >60 mm, which were 1248.35 ng/g DM and 523.59 ng/g DM, respectively. On the other hand, the water extract from soot produced via mixed firewood combustion had the lowest toxicity equivalent value, which was 80.11 g/g DM. The MEQ mutagenicity equivalent was calculated based on Equation (2). The results of the calculations are presented in Table 1. The highest MEQ value, similarly to TEQ, was also obtained for the water extract from the soot from hard coal combustion with a granule size of 25–80 mm (195.48 ng/g DM) and hard coal with a 9–25 mm granule size (140.64 ng/g DM). The lowest MEQ value was noticed in the soot extract obtained from the combustion of mixed firewood (13.78 ng/g DM). The Toxic Effect Equivalent (TCDD-TEQ) was calculated using Equation (3) for the same aqueous extracts as the previous equivalents. The obtained results of the calculations are presented in Table 1. It was found that the highest value was achieved in the water leached from soot from hard coal combustion, with a granule size of 25–80 mm (2.52 ng/g DM). A slightly lower value of TCDD-TEQ was calculated for the 8–25 mm hard coal combustion water extracts (1.23 ng/g DM). The smallest TCDD-TEQ was obtained for the samples from mixed firewood combustion, and it was 0.11 g/g DM.

The conducted research also aimed at analysing the ratio of PAHs considered carcinogenic to the sum of all marked PAHs (ΣPAHcarc/ΣPAH) (Equation (4), Table 2). Based on calculations, it was found that the water extract of the soot from hard coal combustion with a granule size of 8–25 mm (429.52 ng/g DM) has the highest ratio. A slightly lower value of ΣPAHcarc/ΣPAH had a water extract of soot from hard coal combustion with a granule size of 25–80 (218.99 ng/g DM). The lowest ΣPAHcarc/ΣPAH ratio was obtained for the water extracts from soot from pellet combustion (132.02 ng/g DM).

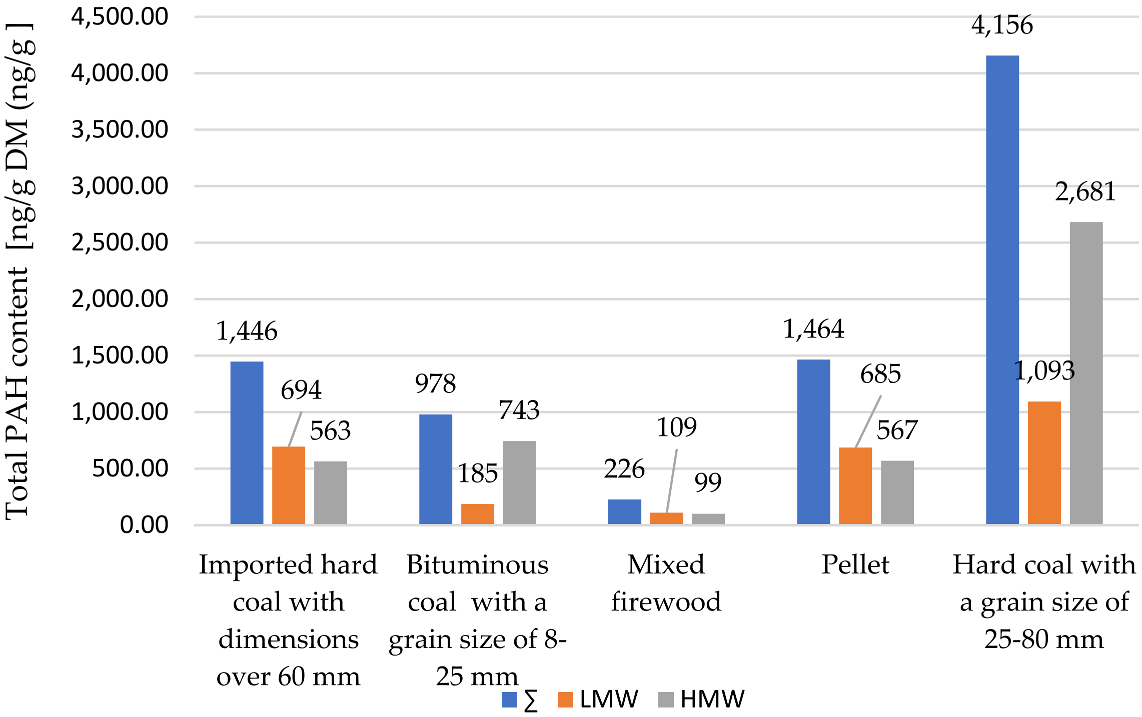

Figure 3. Comparison of the total PAHs, ΣLMW, and ΣHMW concentration in water extracts from soot generated by solid fuels combustion.

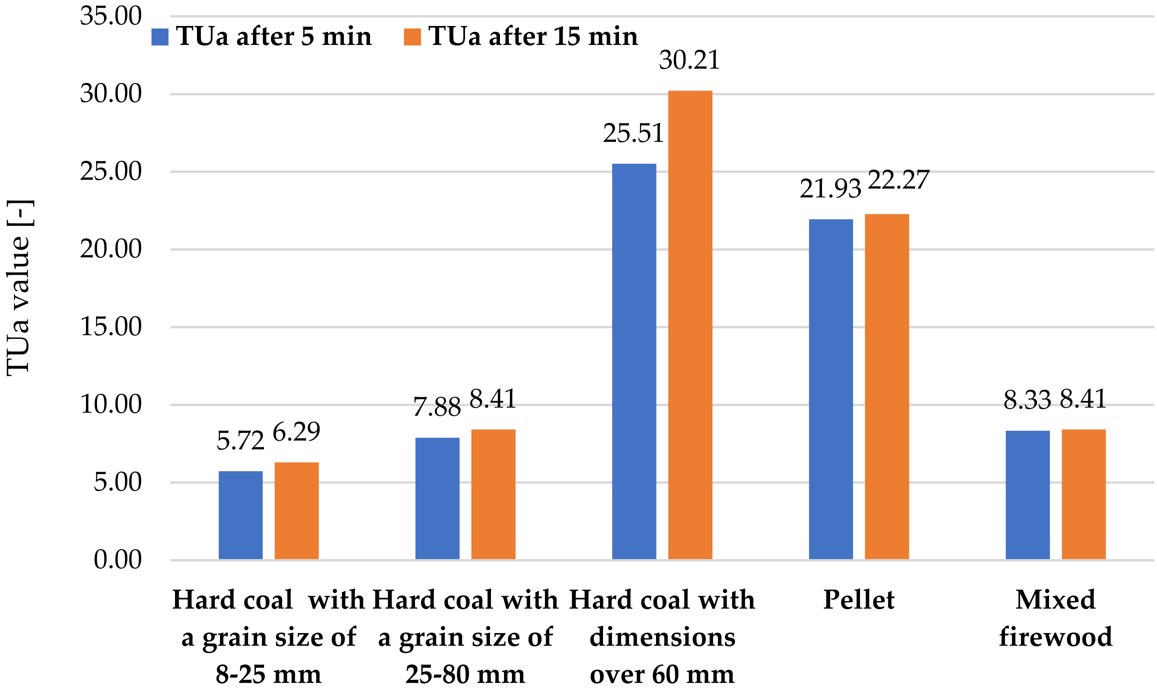

Figure 4. Acute toxicity (TUa) after 5 and 15 min of exposure to toxic substances contained in water extracts from samples of soot from the combustion of solid fuels.

Table 3 shows the biotoxicity results with respect to the aqueous carbon black extracts tested with the Microtox kit and *Aliivibrio fischeri* luminescent bacteria. The conducted research was divided into two groups: the acute toxicity (TUa) of the extracts obtained from combusted fuels concerning 5 and 15 min of their exposure. Upon analysing the obtained results (Table 2) of the acute toxicity of the water extracts from the soot samples from solid fuel combustion, it was found that in both the case of the 5 min and 15 min exposure to toxic substances contained in the tested material, an activity showing harmfulness to organisms was noted. The highest values of acute toxicity TUa (Figure 4) in relation to both the tested reaction times were obtained for the combustion of hard coal with dimensions over 60 mm (TUa 25.51 after 5 min and 30.21 after 15 min of exposure to toxic substances), while the lowest values were obtained for the samples of soot from hard coal with a grain size of 25–80 mm (7.88 after 5 min, 8.41 after 15 min). The obtained results (Table 2) were compared to the systems used to determine the toxicity class of the tested samples, proposed by Persoone [47] and Sawicki et al. [48]. Following the cited qualification systems, the highest values were obtained for the samples from the combustion of hard coal with dimensions over 60 mm (Class 3—high acute toxicity [47]/ significant toxic effect—toxic sample [48]) at both reaction times tested. The water extract from the pellet combustion according to TUa after 5 and 15 min was assigned to the 3rd toxicity class according to Persoone [47] (high acute toxicity) and to the 2nd class according to Sawicki et al. [48] (significant toxic effect—low-toxicity sample). In the case of the other fuels burned, i.e., hard coal with a grain size of 8–25 mm and 25–80 mm, as well as mixed firewood, both after 5 and 15 min of exposure to toxicants, the samples were classified within the 2nd toxicity class following Persoone [47] (significant toxicity) and the 1st class in relation to Sawicki et al.'s classification [48] (no significant toxic effect).

Table 3. Acute toxicity of extracts obtained from the combustion of tested fuels.

	EC_{50} after 5 min	**TU_a after 5 min**	**Toxicity According Persoone [47]**	**Toxicity According Sawicki et al. [48]**	**EC_{50} after 15 min**	**TU_a after 15 min**	**Toxicity According Persoone [47]**	**Toxicity According Sawicki et al. [48]**
Hard coal with a grain size of 8–25 mm	17.49	5.72	Class 2	Class 1	15.91	6.29	Class 2	Class 1
Hard coal with a grain size of 25–80 mm	12.69	7.88	Class 2	Class 1	11.89	8.41	Class 2	Class 1
Hard coal with dimensions over 60 mm	3.52	25.51	Class 3	Class 3	3.85	30.21	Class 3	Class 3
Pellet	4.56	21.93	Class 3	Class 2	4.49	22.27	Class 3	Class 2
Mixed firewood	12.01	8.33	Class 2	Class 1	11.89	8.41	Class 2	Class 1

Research on the leachability and migration of pollutants—especially trace elements that threaten the environment, such as Cr, Pb, Ni, Ba, Sr, V, and Zn in UPS, mainly in fly ash—has been conducted by many scientists [57–61]. Recent studies have shown that Sr and Se are released as a result of the combustion of hard coal and are deposited on high molecular organic compounds in the by-products of solid fuel combustion [62].

On the other hand, soot is a product formed during the incomplete combustion of fossil fuels (peat; coal; lignite; crude oil; natural gas; biomass (wood and agricultural waste); biofuels (ethanol and methanol) and waste; carbon with a content of fine graphite particles; small amounts of fullerenes; ashes, heavy metals, and their compounds; and organic matter containing polycyclic aromatic hydrocarbons, paraffins, phenols, etc.). The mentioned groups of compounds occur in soot in a wide range of concentrations, depending on the chemical composition of the fuel as well as the conditions and the course of the fuel combustion process [63,64]. The chemical composition of carbon black shows significant qualitative and quantitative differentiation regarding its organic matter. Due to the presence of PAHs, nitro and amine groups [63], dioxins, and heavy metals [65], carbon black is a potentially hazardous waste. This waste's quantitative and qualitative composition depends on the fuel's chemical composition, the combustion process' thermodynamic conditions, the type of furnace used, and the technical conditions of the installation [63].

Considering the above, assessing the leachability of pollutants, especially PAHs, is currently essential. There are few studies on the leachability of PAHs from carbon black in the available literature. This article's authors wanted to obtain new data and carried out a laboratory combustion process, from which they obtained soot from various solid fuels. Then, the degree of leaching of the PAHs from the collected samples was assessed. PAHs were found in all the tested water extracts.

Due to the number of UPS products being manufactured, finding ways to develop them further is essential. However, their safe use requires extensive knowledge based on the results of experimental research, considering environmental and technical aspects as well as changes in legal regulations. The origin of the coal and biomass, the share of biomass in the fuel mixture, and the combustion parameters are some of the factors that determine the chemical and mineral composition of soot, and thus the degree to which this material can contaminate the environment through the leaching process [22].

On the other hand, assessing the content and leachability of PAHs from boiler soot is particularly important because the soot stream and the smoke from the chimneys is released into the atmosphere. In such cases, residents may be exposed, by inhaling soot particles containing PAHs, to absorption that remains in the air. The International Agency for Research on Cancer IARC [5] showed that 7 of the 16 recommended PAHs, namely, benzo[a]pyrene, benzo[a]anthracene, dibenzo[a,h]anthracene, chrysene, benzo[b]fluoranthene, benzo[j]fluoranthene, benzo[k]fluoranthene, and indeno[1,2,3-c,d]pyrene, have a carcinogenic effect on animals including humans, showing systemic toxicity and causing damage to the adrenal glands and lymphatic, hematopoietic, and respiratory systems. At the same time, attention should be paid to the fact that these compounds do not occur individually, but as a group of compounds. The best-known hydrocarbon from the PAH group is benzo (a) pyrene. Due to its common occurrence in the environment, and above all, due to its carcinogenic potency, this compound has been recognised as an indicator of carcinogenicity among the entire group of PAHs. The carcinogenicity of PAHs depends on the exposure time to the PAH and on the concentration. The higher the concentration, the shorter the onset of carcinogenic symptoms. For example, mice that were administered benzo[a]pyrene in their diet at a concentration of 33.3 mg/kg forday immediately developed pre-gastric tumours. However, those given the lower dose of 13.3 mg/kg for day for up to seven days showed no effect. The hamsters also had an increased incidence of tumours compared to the control animals after a single dose of 100 mg/kgB[a]P [66].

Regarding benzo[a]pyrene, the carcinogenicity coefficients of individual polycyclic aromatic hydrocarbons have been determined. For benzo[a]pyrene, a carcinogenicity factor of 1 has been assigned. Of the popular PAHs, only dibenzo[a,h]anthracene has a carcinogenicity factor of 5, which is significantly higher than that of benzo[a]pyrene. Other compounds classified as PAHs have carcinogenicity coefficients significantly lower than 1. Given the above information, environmental monitoring in terms of the content and leachability of PAHs from combustion by-products plays a considerable role in the classification of UPS in terms of environmental management.

An additional aspect that the authors of this study want to draw attention to is the biotoxicity of the compounds present in water extracts from boiler soot, which may confirm the research of the International Agency for Research on Cancer IARC [5]. The mere detection and determination of potentially toxic compounds, which are undoubtedly PAHs, does not provide information on how a given compound can interact with living organisms present in the environment, for which methods based on bioindication are successful. Bioindication, as a technique that involves living organisms, allows for the determination of the toxicity of all harmful substances and acts synergistically—an example of which is the use of the Microtox kit with the *Aliivibrio ficheri* luminescent bacteria [39,67,68]. This method is widely known and used by many researchers [45,69–73]. The results obtained in this study confirm the toxicity of the compounds detected in the tested water extracts of boiler-based carbon blacks, showing the differentiation of harmfulness depending on the type of fuel used.

4. Conclusions

1. Among the soot aqueous extracts tested from the combustion of five solid fuels, the highest concentration of total PAHs was obtained in the soot aqueous extracts from the combustion of 25–80 mm hard coal (4155.92 ng/g DM), while the lowest was acquired from the combustion of mixed wood fuel (225.58 ng/g DM), which was more than 20 times lower.
2. The analysis of the compactness of the light fraction of LMW showed that the smallest share of this fraction in the total concentration was recorded in the samples of soot water extracts from the combustion of 6 mm pellets; this indicates the low contribution of the more biodegradable compounds from the PAH group.
3. Considering the calculated TEQ = CEQ, MEQ, and TCDD-TEQ, it was found that the most toxic and carcinogenic soot extract from the combustion of 25–80 mm hard coal was the most toxic and carcinogenic, while the least impactful on living organisms and the environment were samples of soot water extracts from the combustion of mixed firewood.
4. The highest ΣWWAcarc/ΣWWA ratio was obtained for the samples of aqueous extracts from coal combustion soot with a granule size of 8–25 mm.
5. The acute toxicity analysis based on biotests using *Aliivibrio fischeri* luminescent bacteria indicated the toxic effect of the tested carbon black samples, among which the water extract characterised the highest value of the toxicity index TUa from hard coal with dimensions over 60 mm.

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