energies

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Analysis of Micro-Contaminants in Solid Particles from Direct Injection Gasoline Vehicles

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Abstract: Exhaust emissions from vehicles are the subject of numerous studies and legal acts. In the European Union, exhaust emissions are regulated by "Euro" emission standards, which limit emissions of gaseous pollutants such as CO, CO_2, HC, and NOx, as well as the particulate matter (PM) and particle number (PN). Solid particles consist of a number of micro-contaminants, inter alia polycyclic aromatic hydrocarbons (PAHs) and their nitrated and oxygenated derivatives. Despite their highly mutagenic and carcinogenic character, these micro-contaminants are not regulated in Euro emissions standards. This paper presents both a general discussion of the phenomenon of particulate formation in and emission from direct injection gasoline engines, as well as a wide range of results on the subject. The subject of the micro-contaminants in solid particles from modern gasoline vehicles is explored. The samples of solid particles were collected from 11 groups of vehicles according to the WLTP test methodology. Solid particles from gasoline vehicles were analyzed via various analytical techniques, including ion chromatography (IC) to measure selected anion concentrations, gas chromatography with mass spectroscopy (GC-MS) to study 16 PAHs and selected PAH derivatives, scanning electron microscopy with energy-dispersive spectroscopy (SEM/EDS) for images and elemental composition, and microwave plasma atomic emission spectroscopy (MP-AES) for qualitative screening analysis of 19 elements. The study of non-regulated compounds is crucial in efforts to establish the influence of solid particles on health and the environment. Furthermore, extended studies can provide a basis for further research on vehicle emissions or other fields, such as medicine or material engineering.

Keywords: micro-contaminants; solid particles; particulate matter; particle number; gasoline vehicles; WLTP; Euro 6

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Citation: Pacura, W.; Szramowiat-Sala, K.; Macherzyński, M.; Gołaś, J.; Bielaczyc, P. Analysis of Micro-Contaminants in Solid Particles from Direct Injection Gasoline Vehicles. *Energies* **2022**, *15*, 5732. https://doi.org/10.3390/en15155732

Academic Editor: Theodoros Zannis

Received: 15 July 2022
Accepted: 5 August 2022
Published: 7 August 2022

1. Introduction

One of the fastest-developing branches of industry is the automotive industry. Several years ago, the number of light-duty and passenger cars in the world exceeded 1 billion [1]. According to the European Automobile Manufacturers' Association [2], there are over 243 million road vehicles in the European Union, and 53% of them are powered by gasoline engines. About 18 million new cars are registered in the EU every year, while the mean age of all vehicles in use is 11.5 years. A typical car travels 12,000 km per year [3].

Vehicle emissions are counted as low-stack emissions because they are emitted at the human respiratory level. The World Health Organization warns about the negative influence of particulate matter (PM) on human health, life, and the environment [4]. The WHO estimates that PM is responsible for 3% of deaths from cardiovascular failure and 5% of deaths from lung cancer.

Currently, there are numerous legal acts in force that protect (or at least seek to maintain) air quality in the European Union. Examples are Best Available Techniques (BAT) or Fit for 55. The BAT forces the use of the best practicable means [5]. Fit for 55 is part of the 2030 Climate Target Plan and Green Deal, which, in the case of vehicular emissions, intends to achieve a 55% reduction in CO_2 emission in 2030, while in 2035 the greenhouse gas emission from cars will have to be completely eliminated [6]. The Polish air-quality laws, based on the European Union's guidance, limit the mean concentration of various pollutants such as particulate matter (PM), nickel, lead, and benzo(a)pyrene [7].

The subject of emissions of particulate emitted from road vehicles continues to be undergoing custody. and further stricter legislative requirements are expected in this area. While the European Union has been at the forefront of recent decades, other jurisdictions are making progress towards a more comprehensive control and restriction of emissions of particulate matter [8]. Vehicular exhaust emissions are restricted by European emissions standards that limit harmful pollutants such as carbon oxide, hydrocarbons (HCs), non-methane hydrocarbons (NMHCs), nitrogen oxides, particulate matter, and for diesel and gasoline direct injection (GDI) vehicles, also particle number (PN), as shown in Table 1 [9].

Table 1. Emission limits of the European Emission Standards for gasoline vehicles [9].

Compound	Year	CO	HC	NMHC	NO_x	PM $^{(1)}$	PN (GDI)
Unit				g/km			#/km
Euro 5	2011	1.0	0.1	0.068	0.060	0.0050	-
Euro 6b	2014	1.0	0.1	0.068	0.060	0.0045	$6 \times 10^{12\,(2)}$
Euro 6d	2022	1.0	0.1	0.068	0.060	0.0045	6×10^{11}
Euro 7 $^{(3)}$	?	0.4	?	0.025–0.045	0.020–0.030	0.0020	1×10^{11}

$^{(1)}$—for gasoline direct injection only; $^{(2)}$—for the first year of implementation; $^{(3)}$—proposed values; standard remains under deliberation.

Taking into account the mean annual mileage of a Euro 6-compliant vehicle, it can emit up to 12 kg of CO and 600 g of NO_x a year. Especially when considering the number of such vehicles in use, even seemingly insignificant emissions levels will add up to large quantities over time.

Direct injection gasoline engines with spark ignition (DISI) are becoming increasingly popular in passenger car applications, especially in the EU. It is known that particulate emissions are a specific disadvantage of DISI engines. Direct injection of gasoline may lead to a large amount of particles that can be produced, part of which survives and is emitted from the exhaust of the vehicle [10–12]. Particulate matter emission is limited by EU legislation. The number of particles is also limited, although the opt-out means that there are no immediate requirements for dedicated filters such as GPFs (for Euro 6b vehicles), but they are generally introduced for Euro 6d vehicles.

Spark ignition (SI) engines are susceptible to excess emissions at low ambient temperatures. Direct injection spark ignition (DISI) engines should show greater PM emissions at low ambient temperatures by mass and by number [13]. The highest particulate emissions are observed during engine cold start and warm-up [14].

Scientists consider air quality protection to be one of the most important tasks in the field of environmental protection; vehicle exhaust is one of the significant polluting sources. To decrease overall exhaust pollution, actions can be taken in every step of combustion: i.e., before, during, and after [15,16]. In the first case, mainly to decrease fuel life-cycle CO_2 emissions, oxygenated compounds such as bioethanol are blended with gasoline [17]. The literature points to the fact that the addition of the oxygenated compounds is responsible for increases in certain non-regulated emissions [18]. The addition of ethanol to gasoline increases the emission of monocarboxylic acids [19], dicarboxylic acids [20], formaldehyde, and acetaldehyde [21]. However, the addition of up to 30% methanol could decrease the emission of CO_2 and NO_x by 47 g/km and 194 mg/km, respectively [22]. Liu et al. studied the influence of blending gasoline with ethanol, n-butanol, and 2,5-dimethylfuran (DMF) on the emission [23]. Overall, the additives have a positive influence on the emission, reducing the CO_2, CO, THC, and NOx emissions; for example, blending gasoline with

20% n-butanol decreased CO_2 emission by just under 6% and decreased fuel consumption compared to unmodified gasoline.

During combustion, the most common type of emission control is recirculation of exhaust gases or changing the engine timing and even the design of the engine architecture [24,25]. After combustion, exhaust treatment includes the use of a three-way catalyst (TWC) to decrease levels of nitrogen oxides, carbon monoxide, and hydrocarbons and the use of a gasoline particle filter to lower the particulate number [26]. Furthermore, researchers are directing their work towards the implementation of the more strict lower limit of particles below 23 nm, focusing on the measurement techniques and sampling [27–29].

In low-stack emissions studies, three of the most common particulate fractions are described: PM10, PM2.5, and PM1, which include, respectively, particles of aerodynamic diameters below 10 µm, 2.5 µm, and 1 µm. In air pollution studies, the division into size groups is most practical, because of their different physical properties. Depending on the size, solid particles can enter the human body in different ways. PM10 and PM2.5 enter the respiratory system, causing throat irritation and coughing, and settle in the lungs. Finer solid particles can also enter the body through pores in the skin, leading to them circulating within the bloodstream, leading to damage to the brain or other vital organs. PM is responsible for oxidative stress due to the reduction in the concentration of antioxidants such as glutathione and ascorbic acid [30–35].

Table 1 shows the PM and PN limits for vehicles. Both in the case of vehicles equipped with port fuel injection (PFI) and gasoline direct injection engines, the solid particles are limited by PM, currently for the EURO 6 emission standard 4.5 mg/km. The number of particles is limited only for direct injection engines. The current PN limit is set to 6×10^{11} #/km for particles of aerodynamic diameter greater than 23 nm [36]. Compared to PFI engines, units with direct injection emit more solid particles due to piston wetting [12], among other reasons.

The Particulate Measurement Programme (PMP), a subgroup of the United Nations Economic Commission for Europe (UNECE), World Forum of Emissions Rules Harmonisation (GRPE), is developing a new methodology for measuring PM/PN emissions and expanding the test methodology for the number of nanoparticles (PNs) at the current cut-off of 23 nm down to 10 nm, to evaluate the emissions of these particles that are not currently subject to restrictions, which is significant especially in the case of gasoline direct injection, gasoline port-fuel injection and gaseous fuelled engines [37–39]. The PMP team is also working on the introduction of testing and measuring methods for the emission of particles from brakes (discs and pads) used in road vehicles and planned work for new emission standards at the Euro 7/VII level [40].

Nonetheless, current studies released by the PMP group [41] point out that excess emissions measured in the sub-23 nm range (10–23 nm) for PFI vehicles can reach 140% and for some particular applications even 500% compared to the >23 nm result [42].

However, PM and PN results do not accurately describe the danger posed by solid particles. Table 2 shows the variety of contaminants that come from driving. This includes not only the combustion of gasoline (and engine oil) but also driving per se, which causes the emission of particles from the wheels (tires, brakes), which can be introduced to vehicles' engines via the intake air. In the case of the particles from the engine, literature divides them into two groups: primary and secondary [35,43]. Primary particles are formed during the combustion and reactions that occur in the exhaust stream before leaving the exhaust pipe. Secondary particles are formed due to the temperature gradient and reactions with other pollutants present in the atmospheric air. Raza et al. [35] describe the formation of polycyclic aromatic hydrocarbons (PAHs) and further PM from the volatile particles such as sulfates, nitrates, and organic carbon that originate from partial combustion. The formation is the result of various physical and chemical processes such as pyrolysis or nucleation. Considering chemical composition, PM can be divided into elemental carbon (EC), organic carbon (OC), and ash. EC contains mostly products of incomplete combustion; OC contains PAHs [15,44] and other harmful compounds such as hopanes, steranes, and organic phosphates [45]; and ash is composed of various metals and non-metals that originate from the additives and impurities [35].

Table 2. Characteristic chemical compounds and their source.

Source		Characteristic Elements/Compounds
Brakes pads and discs		Fe, Mg, S, Cl
Tires		Al, Si, Cu, Pb
Engine	Gasoline	Hydrocarbons, carbon oxide, PM, PN
	Engine oil	Steranes, hopanes, K, Mo
	Engine block	Metal particles

Despite the use of exhaust after-treatment systems such as TWC or GPF, exhaust gas still contains mentioned pollutants. All of these compounds can be classified as non-regulated contaminants or, due to their low concentrations, micro-contaminants (MCs).

Micro-contaminants, often referred to as micro-pollutants, are a group of harmful compounds from anthropogenic sources present in trace amounts in the soil, water, and air. MCs include pharmaceuticals, pesticides, agricultural chemicals, and PAHs. They can lead to drug resistance, mutations, cancer development, or other adverse effects on health and the environment. In most cases, MCs, such as pharmaceuticals, are not limited by any regulations [15,46–48].

PAHs are a group of more than 200 compounds; among them, 16 are remarkably harmful to human health and life. Raza et al. [35] describe the formation of PAHs as a result of the chemical and physical processes occurring during the combustion of hydrocarbons that make up the fuel. Furthermore, Keyte et al. [49] characterize the formation of PAH derivatives, that is, nitro-PAHs and oxy-PAHs, in reactions with free radicals or fuel impurities. To accurately establish the influence of solid particles containing micro-contaminants, the toxic equivalency factor (TEF) could be used to establish the toxic equivalent (TEQ) [50–52]. Examples of such compounds are benzo[a]pyrene, naphthalene, and chrysene. PAH and its nitrated derivatives such as 1-nitropyrene and 6-nitrochrysene or oxy-PAHs such as 1,4-naphthoquinone and acenaphthoquinone are highly carcinogenic and mutagenic [4,49,53]. Table 3 presents selected PAHs and their derivatives with their TEF values.

Table 3. TEF factors of selected micro-contaminants [50].

Compound	TEF	Compound	TEF
Benzo[a]pyrene	1	Dibenzo[a,h]anthracene	1
Naphthalene	0.001	Benzo[k]fluoranthene	0.1
Acenaphthene	0.001	5-nitroacenaphthene	0.03
Fluorene	0.001	2-nitrofluorene	0.01
Pyrene	0.001	1-Nitropyrene	0.1
Chrysene	0.01	6-Nitrochrysene	10

Benzo[a]pyrene is a reference compound with a TEF of 1, which means that it has a negative impact on health similar that of to dibenzo[a,h]anthracene and 10 times greater than that of benzo[k]fluoroanthene. Compared to their primary compounds, nitrated derivatives are more harmful, e.g., by three orders of magnitude for chrysene and nitrochrysene. The overall TEQ can be calculated using Equation (1), where C_i is the concentration of individual PAHs and TEF_i is an individual factor.

$$TEQ = TEF_i \bullet C_i \tag{1}$$

PAHs and their nitrated and oxygenated derivatives were detected in numerous sources, including diesel and gasoline vehicles [49,54,55]. McCaffery et al. studied gasoline vehicles in the USA, where PAH emission ranges from 0.05 µg/km to 0.75 µg/km for port fuel injection and GDI, respectively [56]. Solid particles from PFI engines contain mostly two- and three-ring PAHs, whereas GDI engines are responsible for a wide range of PAHs, with from three to six rings.

In-depth information about the impact of solid particles on health or the environment should also contain their specific surface area rather than their aerodynamic diameter [30,57]. In addition to the size or specific surface area, it is also necessary to determine the impact on health, toxicity, half-life in the lungs, and ability to circulate in the body (range); therefore, more studies should be conducted [58,59].

In addition to carbon-based components of exhaust gas, inorganic compounds or elements are also present [60]. They form part of the total mass and number of solid particles emitted from combustion processes. According to previous studies by the authors [27], inorganic matter originates from fuel, engine oil, the engine block, and ambient air. In the case of the fuel and engine oil, substances containing, for example, Na, Ca, K, or Zn are used as lubricating agents, to improve the quality of the combustion, or as a detergent to clean the surfaces of the engine. Inorganic micro-contaminants from the engine block, such as Al, Si, and Cu, result from engine wear. Finally, some of the MCs, e.g., those containing barium, might be present in the ambient air used for the combustion processes. They originate from road dust, tires, and brakes and travel through the air intake filter directly to the cylinder [61].

The aim of this paper is to study not only the most dangerous organic compounds such as PAHs and their nitrated and oxygenated derivatives, but also other presented chemical elements and compounds. As mentioned before, after leaving the exhaust pipe, solid particles and other exhaust components undergo further physical and chemical processes such as condensation (due to great temperature difference) or reactions with other pollutants present in the air.

2. Methods and Materials

To briefly summarize, the research was conducted at the BOSMAL Automotive Research and Development Institute Ltd. and AGH University of Science and Technology. BOSMAL was mainly responsible for the vehicle testing and PM collection, while the physical and chemical characteristics of the samples were studied at AGH University.

2.1. Solid Particle Sampling

Emissions tests were conducted on a pool of Euro 6 vehicles with SI DI fuelling systems in BOSMAL's climate-controlled exhaust emissions laboratory, which meets EN/ISO 17025 requirements, in accordance with the EU emissions legislative test procedure (WLTP) [62,63]. Specifically, a portion of the diluted exhaust gas was passed through foil-backed TX40 filters (one per test) to quantify PM, and an additional portion of the diluted exhaust gas was passed through a condensation particle counter to quantify solid particles in the exhaust gas. This study employed the legislative method for all test vehicles. Vehicles were tested in the climate chamber WEISS WK 643′ at 23 °C and a relative humidity of 5.5–12.2 g H_2O/kg dry air [62]. The air in the chamber is filtered with HEPA filters to exclude any pollutants from external sources, inter alia dust from nearby roads. Before the test, the vehicles were soaked in the same conditions for at least 8 h. All test subjects were tested according to the class 3 Worldwide Light-Duty Test Cycle on the AVL Zoellner 4WD dynamometer chassis. Note that the drivers conducting the tests were trained and experienced. The moment of the gearshift is presented in advance on the screen, which shows a visual driver's aid.

The 47 mm filters (Pallflex Emfab Air Monitoring Filters-TX40HI20WW, Pall Corporation, CA, USA) were conditioned in the air-conditioned weighting chamber under exactly the same conditions as in the case of the test vehicles, for 24 h. The filter was weighed before the test on a laboratory microbalance with a range of 0–2.7 g and accuracy of ±0.0001 mg (EU legislation requires a precision of at least ±2 µg, i.e., ±0.002 mg) and placed into the dedicated holder connected to the diluted exhaust gas stream. After the test, the test filter was again conditioned in the weighing chamber (for 8 h) and was then re-weighed after that. The difference in mass was used in the calculated final PM value in milligrams per km. The weighed filter was then placed in a PE Petri dish, sealed with Parafilm, and stored in a refrigerator at 4 °C prior to analysis. Such storage conditions are necessary to prevent air circulation that might influence the ion chromatography analysis or to protect organic compounds from photodegradation by ultraviolet (UV) light exposure, which could alter the gas chromatography–mass spectrometry analysis. Figure 1 shows the apportionment of the filter. While the diameter of the entire unit is 47 mm, solid particles are collected in the 37 mm circle, from which different sizes of aliquots are cut depending on the analysis requirements (ø 10–22 mm). The mean mass of the solid particles collected on the stain area of diameter 37 mm is 0.65 mg for group A (non-GPF), 0.48 mg for group B (GPF), and 0.17 mg for groups C and D (both with GPF).

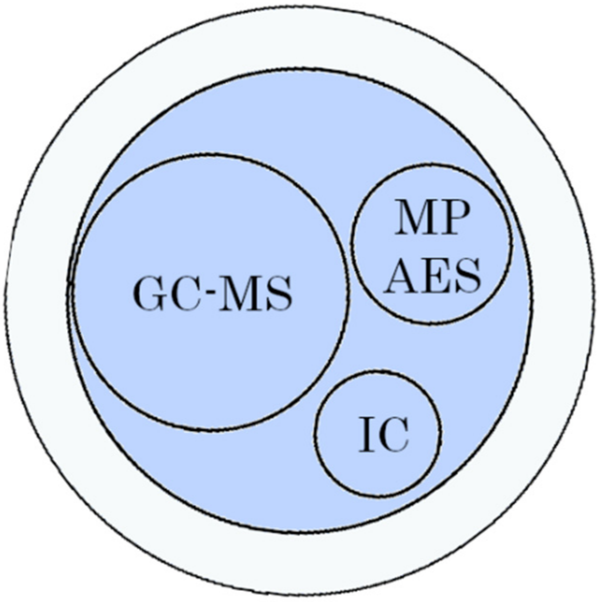

Figure 1. The 47 mm filter with 37 mm stain area and filter area apportionment scheme for GC-MS, MP-AES, and IC analysis.

To provide a quantitative control, the blank filters were conditioned in exactly the same way. The results of the analysis were compared with other parameters such as engine type, gearbox, PM and PN emission, and fuel consumption. Table 4 shows the basic parameters of the vehicles tested, divided into four main groups based on the manufacturer and model (A, B, C, and D). Groups C and D were further divided based on the gearbox and engine type.

Table 4. Tested vehicles, assigned groups, number of tests, and basic parameters.

Group	WLTC Tests in a Group	Inertia (kg)	Gearbox	Engine Displacement (cc)	Power (kW)	GPF	Fuel [1]	Emission Standard
				2017 Small Family Car				
A	36	1260	M	1200	90	No	E5	Euro 6b
B	29	1440	M	1400	80	Yes		
				2019 Crossover SUV				
C_1	3	1460	A	1000	80	Yes	E10	Euro 6d-TEMP
C_2	9	1420	M	1000	80			
C_3	2	1470	A	1400	100			
C_4	2	1460	M	1400	100			
D_1	4	1450	A	1000	80			
D_2	2	1440	M	1000	80			
D_3	1	1490	A	1400	100			
D_4	2	1470	M	1400	100			
D_5	2	1490	M	1400	95			

(1)—See supplementary material for E5 fuel specification and E10 gasoline fuel certificate.

The vehicles selected for the test were from two main producers, representing two types of vehicle bodies: small family car and crossover sport utility vehicle. The numbers of the same car in a group varied from 1 to 9 units, and the test WLTC numbers varied from 1 to 17. The inertia setting of the chassis dynamometer ranged from 1260 to 1490 kg, while the displacement of the engines varied from 1.0 to 1.4 L. The power of the engines ranged from 80 kW to 100 kW. The 80 kW engines in groups C and D were from the same manufacturer, and the 80 kW engine in group B was from a different producer. Depending on the vehicle model year, the vehicles were powered by gasoline containing 5% or 10% ethanol. Both manual and automatic gearboxes were fitted to the test vehicles. Vehicle mileage was not considered as a critical factor for emissions. According to EU regulations, PM emission has a deterioration factor of 1.0 [64], which means that PM emission should remain constant during the first 5 years or 160,000 km (whichever occurs first). The vehicles were tested after meeting the break-in requirements.

2.2. Gas Chromatography–Mass Spectrometry

Gas chromatography with mass spectrometry allows the study of the chemical composition of the particles. Polycyclic aromatic hydrocarbons and their nitric and oxygen derivatives can be quantitatively and qualitatively identified. Examples of such compounds are benzo[a]pyrene, 1.4-naphthoquinone, and 6-nitrochyrsene, which are products of incomplete combustion. In this study, 16 PAHs, 1 nitro-PAH, and 1 oxy-PAH listed in Table 5 were analyzed.

Table 5. Physical and chemical properties of the studied PAHs and PAH derivatives with their retention time, and ions used for quantitative GC-MS analysis.

Name	Molecular Formula	MW (g/mol)	No. of Rings	BP [(1)] (°C)	RT (min)	Quantitation Ion	Confirmation Ions [(2)]
Reference compound							
Benzo[a]pyrene-d12	$C_{20}D_{12}$	264	5	495	30.55	264	209; 253
PAHs							
Naphthalene	$C_{10}H_8$	128	2	218	8.02	128	129; 127; 102
Acenaphthylene	$C_{12}H_8$	152	3	280	10.22	152	151; 150; 153
Acenaphthene	$C_{12}H_{10}$	154	3	279	10.53	153	154; 152; 151
Fluorene	$C_{13}H_{10}$	166	3	295	11.48	165	166; 163; 164
Phenanthrene	$C_{14}H_{10}$	178	3	340	13.91	178	176; 179; 177
Anthracene	$C_{14}H_{10}$	178	3	340	14.04	178	176; 179; 177
Fluoranthene	$C_{16}H_{10}$	202	4	375	18.06	202	200; 203; 201
Pyrene	$C_{16}H_{10}$	202	4	404	18.91	202	200; 203; 201
Benz[a]anthracene	$C_{18}H_{12}$	228	4	438	24.13	228	226; 229
Chrysene	$C_{18}H_{12}$	228	4	448	24.30	228	226; 229
Benzo[b]fluoranthene [(3)]	$C_{20}H_{12}$	252	5	480	28.75	252	250; 253
Benzo[a]pyrene	$C_{20}H_{12}$	252	5	495	29.98	252	250; 253
Indeno[1,2,3-c,d]pyrene	$C_{22}H_{12}$	276	6	536	34.07	276	274; 277; 275
Dibenz[a,h]anthracene	$C_{22}H_{14}$	278	5	524	34.22	278	276; 279
Benzo[g,h,i]perylene	$C_{22}H_{12}$	276	6	500	34.89	276	274; 277; 275
2-Bromonaphthalene	$C_{10}H_7Br$	207	2	281	10.42	206	208; 207
Oxy-PAH							
1,4-Naphthoquinone	$C_{10}H_6O_2$	158	2	212	9.88	158	130; 102; 159
Nitro-PAH							
6-Nitrochrysene	$C_{18}H_{11}NO_2$	273	4	416	30.55	215	226; 243; 273

[(1)]—Boiling point (BP) data from PubChem [65]; [(2)]—in order of decreasing intensity; [(3)]—including benzo[j]fluoroanthene and benzo[k]fluoroanthene.

PAH concentration was determined by a Thermo Scientific Trace 1310 gas chromatograph (GC) coupled with an ITQ 900 mass spectrometer (MS) equipped with a Triplus RSH autosampler. A single circular aliquot (ø 10–22 mm) was punched from the filter with a circular cutter. Filter aliquots were placed in a glass tube (20 mL) and spiked with 40 µL (10 µg·mL^{-1}) solution of benzo[a]pyrene-d12 (BaP-d12; Merck). Then, 0.5 mL of cyclohexane (99.5%, Avantor) and 1 mL of dichloromethane (99.8%, Avantor) were added. The analytes were then ultrasonically extracted for 20 min. The solution was transferred to a vial (4 mL), while the aliquots were ultrasonically extracted again for 20 min with the addition of 0.5 mL of cyclohexane and 0.5 mL of dichloromethane. After ultrasonic extraction, the solution was combined with the extract from the previous step. The extract was evaporated in a laboratory evaporator using nitrogen 5.0 (AirLiquide) and filled with 250 μL of cyclohexane. The concentrate was transferred to a 1.5 mL chromatographic vial equipped with a 300 μL conical glass insert for analysis by GC-MS. This extraction method was developed to prevent any decomposition of the organic micro-contaminants. The procedure was repeated for all samples.

The chromatographic separation of target analytes was performed with a TG-5MS (0.25 μm × 0.25 mm × 30 m) capillary column with 1.2 $\text{mL}\cdot\text{min}^{-1}$ helium 6.0 as carrier gas. The oven temperature program for all methods was as follows: isothermal hold at 65 °C for 4 min, temperature ramp of 20 $^{\circ}\text{C}\cdot\text{min}^{-1}$ to 180 °C and then 5 $^{\circ}\text{C}\cdot\text{min}^{-1}$ to 300 °C, isothermal hold at 300 °C for 5 min. The injection was performed with a split/splitless injector at a temperature of 310 °C. The splitless time was set at 1 min and the injection volume to 1 μL. The GC-MS transfer line temperature and the ion source were maintained at 310 °C and 250 °C, respectively. Detection was performed with an ion trap mass spectrometer operated in positive-ion polarity mode. Mass spectra were obtained in the full scan mode over a mass range (m/z) of 50 to 300 amu and in the single ion monitoring (SIM) mode. The identification of target compounds in the analyzed samples was based on the retention time, quantitative ion, and confirmation ion. Each GC analysis was repeated at least twice. Calibration was performed with the use of the external standard solutions diluted from a certified reference material (CRM). The calibration of PAHs was performed using QTM PAH Mix (Supleco), while nitro- and oxy-PAHs were calibrated against a mixture of single-compound CRMs (Merck).

2.3. Anion Analysis by Ion Chromatography

Ions mostly originate from gasoline or engine oil additives that improve their properties, impurities in gasoline, or polluted intake air. Ion chromatography allows the concentration of cations and ions in the particles to be examined; however, this study focused on anion analysis. The research included measurement of the ions listed in Table 6.

Table 6. Anions reported in this study and their formulas, retention times for IC analysis, and origins.

Anion	Formula	RT (min)	Origin
Fluorides	F^-	3.021	Additives, impurities
Formate	$COOH^-$	3.447	-
Chlorides	Cl^-	4.209	Additives, impurities
Nitrites	${NO_2}^-$	4.999	Nitrogen in the combustion air
Bromides	Br^-	5.912	-
Nitrates	${NO_3}^-$	6.691	Nitrogen in the combustion air
Phosphates	${PO_4}^{3-}$	8.747	Engine oil
Sulfates	${SO_4}^{2-}$	10.003	Sulfur in the gasoline

A single circular aliquot (ø 10–22 mm) was punched from the filter with a circular cutter. Filter aliquots were placed in Eppendorf vials, and then 1.5 cm^3 of deionized water was added. The sample was ultrasonically extracted for 20 min. This extraction was designated for the extraction of any water-soluble compounds, to simulate real-life conditions, *inter alia* rain. An anion chromatography analysis was performed using an ICS-1100 instrument (Thermo Scientific) equipped with an autosampler and ion exchange column. For the analysis of anions, the instrument was equipped with Ion Pac AS22 (4 × 250 mm) with 4.5 mM $Na_2CO_3^+$ and 1.4 mM $NaHCO_3$ as mobile phase. Electrochemical suppression was achieved with the AERS 500 4mm suppressor, while quantification was performed with a conductivity detector. The injection volume was set to 25 μL with an eluent flow rate of 1.0 $\text{mL}\cdot\text{min}^{-1}$. Calibration was performed by means of the external standard solution diluted from a manufacturer’s stock solution (Thermo Scientific).

2.4. Scanning Electron Microscopy

As in the authors' previous studies [61], scanning microscopy was performed using a field emission scanning electron microscope (SEM, FEI Versa 3D), while the chemical analysis of the solid particles was performed using an energy-dispersive X-ray spectrometer (EDS, Oxford Instruments Ultim Max, High Wycombe, UK). Three groups were chosen for the SEM/EDS analysis: A with 44 analytical points, B with 48 analytical points, and D_5 with 15 points.

2.5. Microwave Plasma Atomic Emission Spectrometry

Microwave plasma atomic emission spectrometry (MP-AES) permits qualitative and quantitative trace analysis of the elements by their atomization, excitation in a high-temperature nitrogen plasma, and finally a spectral intensity measurement. The MP-AES method allows the analysis of several dozen elements; however, in this work, 19 metals were initially (semi-qualitatively) analyzed, and then, on the basis of the obtained results, three quantitative sub-applications were elaborated and used for distinguished metals. The MP-AES 4200 (Agilent Tech, Santa Clara, CA, USA) apparatus, equipped with a double-pass quartz chamber and a OneNeb Series 2 nebulizer, was used in the measurements. For plasma generation, nitrogen gas of 3.7 purity was supplied by an air-separating nitrogen generator (Agilent Tech 4107). Calibration was performed using external standard solutions obtained from 1000 ppm stock solutions (InorganicVentures/MSSpektrum SPEX CertiPrep).

Filter aliquots (ø 10–19 mm) were placed in PE vials. Then, 4 cm^3 of 10% HNO_3 (Merck) solutions were added. The extraction was performed in an ultrasonic bath at 60 °C for 4 h. The samples were separated in an MPW-352 centrifuge (rotor No. 11459) at 15,000 rpm for 15 min. The purpose of the extraction was to simulate the substance leaching that might occur in real-life conditions, i.e., during prolonged rainfall at low pH.

3. Results and Discussion

3.1. General Analysis of the Samples

General results are shown in Table 7, by vehicle group. The mean PM and PN values are from the WLTP tests performed on the dynamometer chassis. The mass concentration of the PAHs in the PM is from the analysis performed with the use of the GC-MS. The mean anion concentration in PM is a result of the IC analysis. Table 7 also shows information about additional methods used in the elemental analysis: EDS and MP-AES.

Table 7. General results of the analysis conducted in this study. Mean PM and PM values from the WLTC tests, concentrations of studied PAHs and anions in the solid particles, and information about additional analysis with the use of SEM/EDS or MP-AES.

	PM	**PN × 10^{11}**	**PAHs in PM**	**Anions in PM**	**Advanced Analysis**	
Group	(mg/km)	(#/km)	(%)	(%)	SEM/EDS	MP-AES
A	4.010	92.0	n/a	7.00	+	+
B	2.235	30.2	0.58	5.70	+	+
C_1	1.260	6.3	0.37	48.10		+
C_2	1.161	4.2	0.36	45.70		
C_3	1.260	12.2	0.43	31.00		
C_4	1.436	14.3	0.36	30.50		
D_1	1.267	6.4	0.37	34.70		+
D_2	0.835	5.8	0.77	46.20		
D_3	1.009	8.7	0.51	36.70		
D_4	0.813	8.9	0.40	57.50		
D_5	1.244	0.9	0.55	37.10	+	

Every group of vehicles tested met the requirements of the Euro 6b PM limit. Group A shows the highest value, at more than 4 mg/km. The crossover SUVs in groups C and D have relatively lower PM emission values, from 0.8 to 1.4 mg/km. In the case of the PN, vehicles from group A exceed the Euro 6b limit of 6×10^{12} particles/km. The rest of the vehicles meet the PN emission standard set during the first three years of the emission standard's implementation. Vehicles from the 2019 model year all have a GPF, which limits the PN compared to the 2017 model year vehicles. The concentration of PAHs studied varies from 0.33% to 0.77%.

The anion concentration differs between small family cars and crossover SUVs. Firstly, there are around 6.4% of the ions studied, which suggests a low share of inorganic matter in the total PM mass. The last group varies from 30 to 57% of anions in PM. The main difference between these two groups is the fuel. The 2017 model year vehicles were powered by gasoline containing up to 5% bioethanol, while the 2019 model year vehicles were powered by E10 gasoline. Detailed results are presented in the following sections of this paper.

3.2. GC-MS Measurements

At least two random samples from groups B–D were analyzed. Table 8 shows the results of the analysis. Note that the PM values listed in Table 8 are means only from the vehicles that were analyzed with the use of the GC-MS technique. The vehicles in group B show the highest concentration of PAHs, around 12.8 μg/km, compared to the other groups that emit 4–7 μg/km. There is no consistent difference between results from vehicles with manual and automatic gearboxes, which, as mentioned above, might be explained by the driver's aid and test driver's level of experience. The most abundant PAH in group B is fluoranthene, at almost 2.2 μg/km. In groups C and D, the emission of benzo[a]anthracene is the highest, at around 1 μg/km.

Table 8. Results of the GC-MS analysis of the solid particles from vehicle groups B–D. The emission of studied PAHs is a mean from at least two samples from each group. The PM shows a mean mass of solid particles from vehicles used in this part of the study.

Group		B	C_1	C_2	C_3	C_4	D_1	D_2	D_3	D_4	D_5
PM *	(μg/km)	2202	1096	1215	1354	1334	1355	835	1189	1100	1244
PAHs											
Acenaphthene	(μg/km)	nd	nd	nd	nd	nd	nd	0.29	0.12	nd	nd
Fluorene		nd	nd	0.23	nd	nd	0.24	0.45	0.54	nd	nd
Anthracene		1.56	0.35	0.17	0.28	0.27	0.31	0.31	0.14	0.14	nd
Fluoranthene		2.18	0.55	0.54	0.53	0.49	0.57	0.67	0.63	0.53	0.55
Pyrene		1.67	0.56	0.45	0.43	0.37	0.51	0.43	0.41	0.47	0.50
Benz[a]anthracene		nd	0.94	0.91	0.94	0.91	0.92	0.95	0.94	0.89	0.93
Chrysene		1.65	0.75	0.70	0.89	0.45	0.69	0.64	0.53	0.59	0.65
Benzo[b]fluoranthene(2)		1.59	nd	0.97	0.91	1.05	0.99	0.98	0.98	0.94	0.95
Indeno[1,2,3-c,d]pyrene		0.73	nd	nd	nd	nd	nd	0.52	0.52	nd	1.04
Dibenz[a,h]anthracene		0.88	nd	nd	1.01	1.02	0.51	0.50	0.99	nd	0.99
Benzo[g,h,i]perylene		1.18	nd	nd	0.46	nd	nd	0.46	nd	0.46	0.94
2-Bromonaphthalene		1.33	0.89	0.41	0.35	0.27	0.31	0.26	0.24	0.35	0.35
Sum of PAHs	(μg/km)	12.77	4.04	4.38	5.80	4.83	5.05	6.46	6.04	4.37	6.90
Share in PM	(%)	0.58	0.37	0.36	0.43	0.36	0.37	0.77	0.51	0.40	0.55

*—PM values only from the samples used in the GC-MS analysis; nd—not detected.

The emission of benzo[b]fluoroanthene was studied; however, the chromatographic column was unable to sufficiently separate peaks from other benzofluoroanthenes that coeluted. Therefore, this value is in fact the total concentration of the benzo[b]fluoroanthene, benzo[j]fluoroanthene, and benzo[k]fluoroanthene.

The most common PAHs among studied groups are fluoranthene, pyrene, chrysene, and 2-bromonaphthalene, which are present in every studied group.

Naphthalene, acenaphthylene, phenanthrene, and benzo[a]pyrene were not detected. Naphthalene, due to the relatively low boiling point and low complexity of the structure (only two rings), might be completely combusted in the engine or catalytically oxygenated in the TWC. Benzo[a]pyrene might not be detected due to the applied methodology and the use of the benzo[a]pyrene-d12 as a reference compound.

The literature shows the concentration of the PAH derivatives to be a couple of orders of magnitude lower in the air samples from urban sites [33,34,50,56]. The analyzed nitro- and oxy-PAHs were not detected, which might suggest very low concentrations in the exhaust. This might suggest that the formation of the derivative compounds mainly occurs following emission (after leaving the exhaust system). Exhaust gases and particles further react with pollutants present in the air. Additionally, due to a significant temperature gradient, the condensation and formation of secondary particles also occur.

3.3. Ion Chromatography

Table 9 shows the results of the analysis of the vehicles of groups A–D. The results presented are the means of all WLTC tests in a group. The percentage shares of the anions in the total mass of the PM fraction are also shown and presented as mean values. As mentioned in the general results section, the share of anions in the PM differs greatly between small family cars and crossover SUVs.

Table 9. The emission of the studied anions from the A–D vehicle groups. The mean emission and standard deviation are based on several analyses with the use of IC.

ID	PM	F^-	Cl^-	NO_2^-	NO_3^-	PO_4^{3-}	SO_4^{2-}	Sum	Share in PM
Unit				(µg/km)					(%)
A	4010	42.0	46.6	22.7	60.6	7.7	77.6	257.0	7.0
SD	1257	33.7	28.8	19.5	41.9	10.9	59.5	96.1	3.1
B	2235	21.4	21.5	9.8	24.6	8.4	43.4	129.0	5.7
SD	309	18.4	17.3	12.0	15.5	9.5	24.0	63.3	2.8
C_1	1260	40.9	11.9	141.3	94.6	nd	313.4	602.1	48.1
SD	240	12.9	8.4	3.6	2.7		114.5	92.7	1.8
C_2	1161	39.5	9.4	134.9	96.8	nd	151.5	432.1	45.7
SD	537	7.0	7.5	12.8	37.7		158.9	172.9	24.5
C_3	1260	24.0	2.2	112.7	123.8	nd	128.3	391.1	31.0
SD	3	5.8	2.2	7.1	22.8		80.2	118.1	9.3
C_4	1436	18.6	1.3	121.8	100.6	nd	95.7	338.0	30.5
SD	669	8.6	1.3	11.0	8.3		22.2	9.5	14.8
D_1	1267	71.7	15.0	141.8	106.6	nd	100.3	435.3	34.7
SD	127	10.1	10.9	24.3	15.6		37.7	26.0	3.7
D_2	835	43.8	0.6	124.3	95.1	nd	65.7	329.5	46.2
SD	316	6.4	0.6	17.1	0.8		26.6	3.3	17.9
D_3	1009	35.4	nd	134.3	85.1	nd	115.5	370.3	36.7
SD					n/a				
D_4	813	30.5	4.4	122.4	89.1	nd	220.8	467.2	57.5
SD	35	5.1	4.4	3.2	4.4		12.8	19.7	0.0
D_5	1244	40.8	17.3	125.5	118.6	nd	158.2	460.4	37.1
SD	139	1.3	7.4	5.4	3.1		62.4	47.9	0.3

SD—standard deviation; nd—not detected; n/a—data not available.

Vehicles in group A with 1.2 L engines emit on average around 260 µg/km of anions. The most abundant sulfates originate mostly from the sulfur present in the gasoline, while nitrates originate mostly from the combustion process itself. The PM concentration is the highest of all groups; however, it is still under the Euro 6b limit. In the case of group B, despite a more powerful 1.4 L engine, the overall emission of the anions is lower. The PM composition of the samples from groups A and B also contains trace amounts of phosphates, around 8 µg/km, that might originate from engine oil.

The most important difference between vehicles from groups C_1 and C_2 is the gearbox. Both groups have similar mean PM emissions of around 1200 µg/km, similar anion emissions except for sulfur, and a total share of the anions in the PM of around 47%. The three most abundant ions are nitrates, nitrites, and sulfates. In the case of the sulfates, group C_1 shows over 2 times greater emission than the C_2 group cars with a manual gearbox. The difference is most likely due to the variation of the sulfur content in the gasoline. Despite the presence of a larger and more powerful 1.4 L engine, the PM emission from the C_3 group of 1260 µg/km is similar to the 1.0 L units (groups C_1 and C_2). C_4 vehicles have a higher PM emission. This might be caused by driver influence during gearshifts. The overall share of the anions is lower, at around 31%.

The vehicles from groups D_1 and D_2 show a similar tendency in emission to those from group C. The most abundant ions contain nitrogen and sulfur. However, group D_2 emits lower levels of PM than almost every other group, except for D_4. The emission of sulfates is also the lowest. Group D_4 contains only one vehicle; therefore, the SD is not available for this group. D_4 has the lowest PM emissions, although the ions in the solid particles are at a similar concentration, around 460 µg/km, half of them being the sulfides. Group D_5 contains vehicles with newer generation engines compared to other vehicles in groups C and D. The emission of PM and ions is similar to that in group C, and the share of the anions is about 37%.

There are few significant differences between cars. The 2017 units have 2 to 3 times greater PM emission, around 4000 µg/km in group A and more than 2200 µg/km in group B, and much lower anion concentrations, from 4.6–8.3%. This means that most of the PM from groups A and B is made of organic compounds. The phosphates are present only in groups A and B, while they were below the detection limit in groups C and D. The difference might originate from different compositions of engine oil. Groups A and B have an overall lower concentration of nitrogen-based ions, and the nitrates have a higher concentration than nitrites, compared to groups C and D, where the nitrites have a higher concentration than NO^{3-} ions. This might mean that ions with nitrogen reacted with organic matter in side reactions, leading to the emergence of the various nitric derivatives.

The ion chromatography setup allows for the analysis of the two additional ions, formate and bromides, during the main analysis. However, in all groups, the concentration of the ions mentioned was below the detection limit.

3.4. SEM/EDS Analysis

Three samples, one from each of groups A, B, and D, were selected for the analysis. Figure 2 shows the SEM images, while Table 10 presents the results from the EDS analysis. Blue circles show exemplary analytical points, including particles from PM10, PM2.5, and PM1 sizes. In the case of agglomerates larger than 10 um aerodynamic diameter, multiple points were analyzed. Figure 2A–C show filters from groups A, B, and D_5 respectively. Figure 2D shows the blank filter mesh.

The blank Pallflex filter contains only carbon, oxygen, fluorine, and silicon. The analysis of the singular fibers of the filters shows that despite the clean appearance of the SEM images, they are in fact covered with a thin layer of ultrafine particles. In addition to the expected elements, the fiber from group A also contains 3.7% aluminum; over 1% sodium; and less than 0.6% zinc, barium, potassium, and calcium. A fiber from the B series shows over 0.5% sulfur, in addition to the elements that can be found in group A. The fibers from the D_5 series consist of less carbon than samples from A and B, which is consistent with the anion analysis, indicating a lower share of the organic compounds in the total mass of the particles. The D_5 series consists of almost 12% aluminum, which might originate from the engine itself due to thermal and physical degradation of its internal surfaces.

In the case of the EDS analysis of the PM specimens, groups A and B have similar concentrations of most of the elements, except for Al, S, Cr, and Ti. The most abundant element is carbon at over 62% and 67%, respectively, mostly from gasoline combustion. The second is oxygen at around 17%. The concentrations of fluorine (over 7%) and silicon (4%) originate from the filter itself. Elements such as sodium, potassium, calcium, and magnesium originate from the additives used in the fuel and engine oil. Metals such as chromium, nickel, copper, and zinc originate from the engine block. The sulfur content is probably related to the sulfur concentration in the gasoline. The Al, Cr, and Ti might originate from the engine. The group D_5 has a lower carbon concentration, around 36% compared to around 65% in other groups, which similarly to the fibers is also consistent with the IC analysis. Only group A contains trace amounts of Sb and Co. Manganese is present in trace amounts in A and at over 0.3% in D_5. Additionally, the latter group contains about 0.5% Nb and more than 1% Bi that might originate from the impurities in gasoline or engine oil. Phosphorus is present only in groups A and B, which is also consistent with the IC analysis. Note that despite SEM/EDS being a non-destructive method, the elongated influence of the ambient air and humidity, as well as UV light, might influence sample composition and should not be used in further analysis.

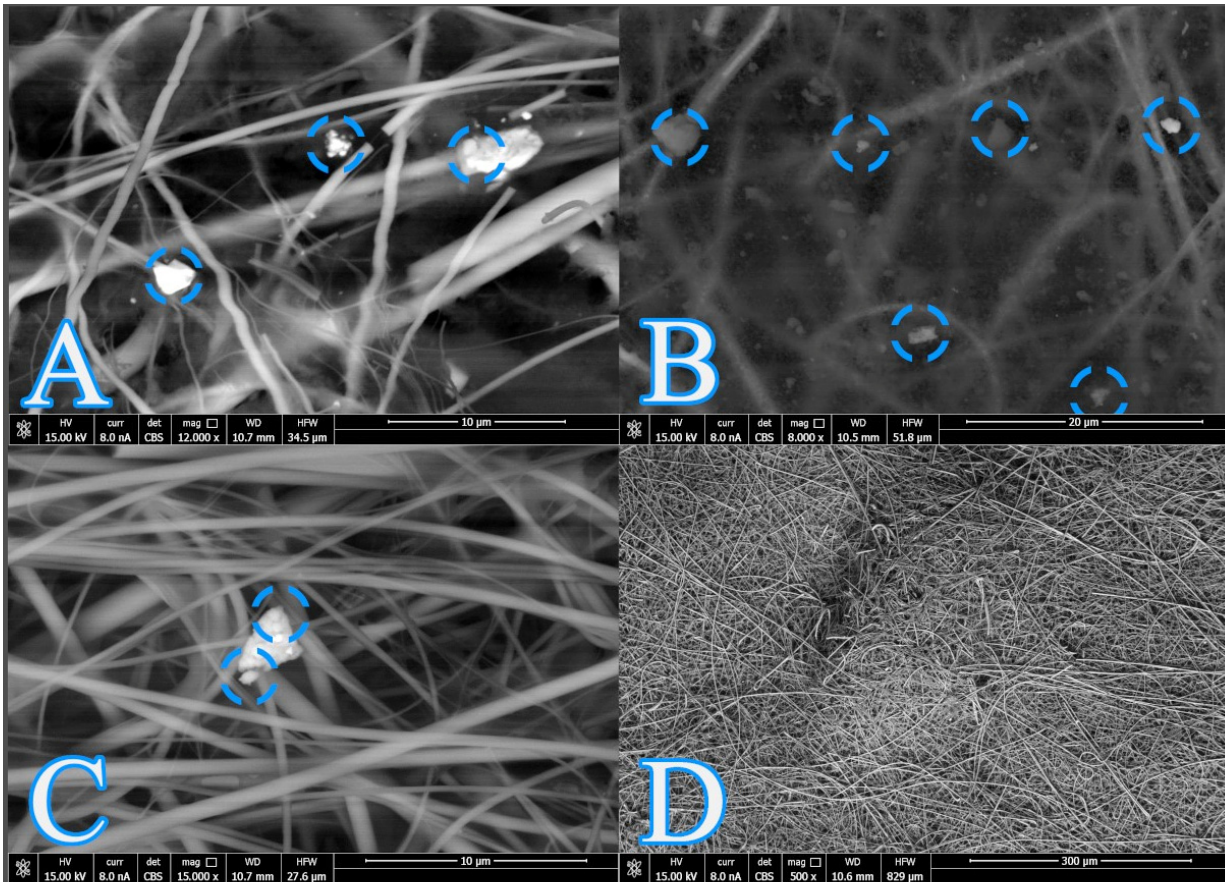

Figure 2. The SEM images of the PM samples and the blank filter. The blue circles mark the analytical points. (**A**) group A; (**B**) group B; (**C**) group D_5; (**D**) blank filter.

3.5. MP-AES Analysis

The qualitative and semi-quantitative 19-element "screening analyses" were performed on the randomly chosen samples from groups A, B, C_1, and D_1.

Table 11 shows the results of the qualitative analysis of the selected samples. In the study, MP-AES was used as a method to provide fast information about the mean concentration of the selected elements.

The origin of the elements was described in detail in the SEM/EDS section of this paper.

Elements were grouped based on their signal strength compared to the background and possible interferences. Group I consists of elements that were easy to detect with strong signals not subject to interference. There were also no signals with interference detected from all group II elements, but these elements were present at significantly lower amounts. In conclusion, group II requires larger filter aliquots than group I. Group III represents trace elements that require a bigger filter sample diameter if a successful and reliable analysis is required. Copper and nickel are present in all groups. Titanium was detected only in the particulate matter from small family cars. Manganese was present in groups A and D_1, which is consistent with the EDS analyses. Bi and Co were specific in groups D_1 and A, respectively, which might indicate impurities in the gasoline or engine oil. Group IV consists of elements that were analyzed to discover any gasoline or engine oil impurities, i.e., lead or cadmium.

Table 10. The mean concentration of the studied elements from the groups A, B, and D_5. The results include the measurement of a fiber from each group. The mean concentration and standard deviation of the elements in the solid particles are based on the results of numerous analysis points.

		A 44 Points			**B 48 Points**			**D_5 15 Points**	
	Fiber	Mean Mass %	SD	Fiber	Mean Mass %	SD	Fiber	Mean Mass %	SD
C	53.74	62.53	9.80	49.55	67.07	9.12	23.69	36.14	10.22
O	25.08	17.50	5.33	25.90	16.30	5.84	41.12	29.00	5.80
F	7.92	7.76	1.38	11.89	7.38	1.64	17.98	15.18	3.97
Na	1.07	1.02	0.29	1.63	1.06	0.37	1.83	2.11	0.75
Al	3.74	2.26	1.74	1.03	0.66	0.43	11.74	2.26	1.24
Mg	nd	0.20	0.27	nd	0.15	0.09	1.66	0.20	0.10
Si	7.04	4.72	1.65	8.15	4.43	1.73	nd	7.16	3.25
P	nd	0.37	0.26	nd	0.54	0.49	nd	nd	nd
S	nd	1.17	1.83	0.52	0.16	0.10	nd	0.43	0.34
K	0.34	0.34	0.18	nd	0.30	0.12	0.36	0.57	0.32
Ca	0.23	0.53	1.03	0.36	0.45	0.59	0.39	1.75	2.56
Cr	nd	0.36	0.22	nd	5.13	0.00	nd	3.62	0.00
Fe	nd	1.64	2.70	nd	2.86	2.54	nd	9.47	3.64
Ni	nd	1.09	0.85	nd	1.09	0.56	nd	1.75	0.00
Cu	nd	0.61	0.39	nd	1.18	2.20	nd	0.78	0.49
Zn	0.56	0.30	0.15	0.61	0.38	0.37	0.78	0.55	0.34
Ba	0.29	1.23	2.05	0.36	0.20	0.12	0.45	0.47	0.37
Cl	nd	0.09	0.04	nd	0.07	0.00	nd	0.23	0.13
Ti	nd	1.78	0.99	nd	0.26	0.05	nd	nd	nd
Zr	nd	nd	nd	nd	0.08	0.02	nd	nd	nd
Sb	nd	0.09	0.03	nd	nd	nd	nd	nd	nd
Mn	nd	0.08	0.00	nd	nd	nd	nd	0.34	0.00
Co	nd	0.13	0.00	nd	nd	nd	nd	nd	nd
Nb	nd	nd	nd	nd	nd	nd	nd	0.49	0.00
Bi	nd	nd	nd	nd	nd	nd	nd	1.06	0.41

SD—standard deviation; nd—not detected.

Table 11. Grouped elements from the analysis.

Element	**Wavelength (nm)**	**Intensity**	**Detected in**	**Possible Interferences**
Group I				
Na	568.820	516.8	All studied groups	REEs
	589.592	81,616.0		-
Mg	518.360	19,330.3		REEs
Al	394.401	117,785.1		REEs
	396.152	259,034.3		-
K	769.897	56,824.4		-
Ca	616.217	12,565.2		REEs
Group II				
Cr	425.433	273,540.4	All studied groups	-
Fe	371.993	16,875.6		Ti, REEs
Zn	213.857	37,285.1		-
Ba	614.171	651,323.8		-

Table 11. *Cont.*

Element	Wavelength (nm)	Intensity	Detected in	Possible Interferences
Group III				
Ti	334.941	141,419.6	A; B	REEs
Mn	403.307	183,876.2	A; D_1	Mn, REEs
Cu	324.754	477,458.5	All studied groups	-
Bi	306.772	32,659.3	D_1	Ti, V, REEs
Co	340.512	35,116.9	A	Pd, Zr, REEs
Ni	305.082	28,111.5	All studied groups	V
Group IV				
Be	265.062	22,793.5	Not detected	REEs
Ag	328.068	461,835.6		-
Cd	508.582	1582.8		Sc, Y
Pb	363.957	5504.8		REEs

REEs—rare earth elements.

In group I, sodium and aluminum have two different wavelengths to cover both lower and high concentrations in the sample. The less intense wavelength responses of 568.820 nm and 394.401 nm are dedicated for high concentrations of the elements that, in the case of the more intense signal, could cause detection over the limit. The more intense wavelength is better in trace amounts. Otherwise, the signal might be too low to be distinguishable from the background noise.

4. Conclusions

Solid particles from vehicles with different masses, engines, and gearboxes have diverse chemical compositions. The main factors leading to a difference in the result, i.e., the concentration of PAHs or anions, are the model year of the vehicle and the fuel used.

1. In theory, the difference between the gearbox types should be visible among the vehicles studied, favoring the automatic gearbox, which should emit fewer particles and fewer organic compounds. The results show no such indication, which could be due to the considerable experience of the test driver and the use of the driver's aid. For a decisive answer, this phenomenon should be further studied, preferably during standardized Real Driving Emissions tests.
2. The impact of engine displacement on studied PAH emissions was inconsistent. Considering emissions from group C vehicles, the cars with 1.4 L engines overall emit more micro-contaminants, while group D vehicles have the reverse tendency. In the case of anion analysis, the engine displacement also did not have a significant impact on emissions.
3. The studied groups of vehicles show a significant difference in anion concentration. Small family cars have up to 7% anions, while SUVs have from 30 to 57%. The analysis shows mostly nitrites (from 10 to 142 μg/km), nitrates (24 to 124 μg/km), and sulfate (43 to 313 μg/km). The phosphates were detected only in groups A and B, strongly suggesting that they originate from engine oil.
4. SEM/EDS analysis provided information on the physical and chemical properties of the solid particles, such as their diameter and their elemental composition. The most abundant elements are carbon and oxygen. For vehicles from 2017, carbon and oxygen reach around 65% and 17%, respectively; for newer cars, they reach 36% and 29%. C and O originate from the combustion of fuel and engine oil. The presence of other elements was related to the gasoline and engine oil additives (and impurities) and the abrasion of the engine.
5. EDS can be performed together with an electron microscopic observation (point analysis or mapping procedure), but it is only a semi-quantitative analysis, delivering a result at the percentage level, which would be insufficient in this particular case.

6. An MP-AES methodology was developed to provide preliminary information on the elemental composition of the particles. The analyzed metals were divided into three groups, depending on their signal intensity and possible interferences. Besides rare earth metals, which might be only random pollutants in some samples, other metals such as titanium or vanadium might interfere with the iron or nickel signal.

In some cases, the instruments used for this study have similar areas of analysis, but it is important to note that the techniques used are not interchangeable.

- The IC provides information only about water-soluble phosphates, while the EDS includes phosphorus in the organic matter as well.
- The GC-MS provides information only on the selected, most toxic compounds, inter alia PAHs and their derivatives, while EDS shows total carbon concentration in the specimen.
- The preliminary MP-AES study shows that this method can very quickly identify the mean concentrations of the elements, but not in the detailed way that SEM/EDS does. The method should be selected giving consideration to the aim of the study, to avoid unnecessary costs.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/en15155732/s1, E5 fuel specification and E10 gasoline fuel certificate.

Author Contributions: Conceptualization, W.P., J.G., K.S.-S. and M.M.; methodology, M.M., W.P. and P.B.; software, M.M.; validation, W.P., K.S.-S., M.M. and P.B.; formal analysis, W.P. and M.M.; investigation, W.P.; resources, W.P., K.S.-S., M.M., J.G. and P.B.; data curation, W.P. and M.M.; writing—original draft preparation, W.P.; writing—review and editing, M.M., J.G. and P.B.; visualization, W.P.; supervision, J.G., K.S.-S. and M.M.; project administration, W.P., J.G. and K.S.-S.; funding acquisition, J.G., W.P., M.M. and K.S.-S. All authors have read and agreed to the published version of the manuscript.

Funding: The authors gratefully acknowledge the funding support given by AGH UST in Krakow within the subvention project No. 16.16.210.476. The research project was partially supported by the program “Excellence initiative—research university” for the AGH University of Science and Technology.

Data Availability Statement: The information about methods and results is listed in the text. Any other information is duly available from the first author on reasonable request.

Acknowledgments: The authors thank DEng Katarzyna Berent and DEng Andrzej Sławek from the Academic Centre for Materials and Nanotechnology AGH for help with the SEM/EDS, the staff of the BOSMAL Emissions Testing Laboratory for conducting a series of vehicle emissions tests on the chassis dynamometer, and DEng Joe Woodburn for the thorough proofreading of this paper.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

BAT	Best Available Techniques
CRM	Certified reference material
DI	Direct injection
DISI	Direct injection spark ignition
EC	Elemental carbon
EDS	Energy-dispersive spectroscopy
GC-MS	Gas chromatography with mass spectroscopy
GDI	Gasoline direct injection
GPF	Gasoline particulate filter
HCs	Hydrocarbons
IC	Ion chromatography
MCs	Micro-contaminants
MP-AES	Microwave plasma atomic emission spectroscopy

NMHC	Non-methane hydrocarbons
OC	Organic carbon
PAHs	Polycyclic aromatic hydrocarbons
PFI	Port fuel injection
PM	Particulate matter
PMP	Particle Measurement Programme
PN	Particle number
REEs	Rare earth elements
SD	Standard deviation
SEM	Scanning electron microscopy
SI	Spark ignition
SIM	Single ion monitoring
SUV	Sport utility vehicle
TEF	Toxic equivalency factor
TEQ	Toxic equivalent
TWC	Three-way catalyst
UNECE	United Nations Economic Commission for Europe
UV	Ultraviolet
WHO	World Health Organisation
WLTC	Worldwide Harmonized Light-Duty Vehicle Test Cycle
WLTP	Worldwide Harmonized Light-Duty Vehicle Test Procedure

References

1. Wards Intelligence. *World Vehicles in Operation by Country 2013–2017*; Informa Tech Automotive Group: Southfield, MI, USA, 2018.
2. ACEA. *The Automobile Industry Pocket Guide*; European Automobile Manufacturers' Association: Brussels, Belgium, 2021.
3. Odysse-Mure. *Sectoral Profile—Transport*; Enerdata: Grenoble, France, 2018; Volume 1, pp. 1–8.
4. World Health Organisation. *Health Effects of Particulate Matter*; WHO Regional Office for Europe: Copenhagen, Denmark, 2013.
5. European Commission. Directive 2010/75/eu of the European Parliament and of the Council of 24 November 2010 on Industrial Emissions (Integrated Pollution Prevention and Control). *Off. J. Eur. Communities* **2010**, *2010*, 1–103. Available online: https://eur-lex.europa.eu/legal-content/EN/TXT/HTML/?uri=CELEX:32010L0075&from=EN (accessed on 10 December 2021).
6. European Commission. *Communication COM/2020/562: Stepping Up Europe's 2030 Climate Ambition*; European Comission Communication: Brussels, Belgium, 2020.
7. *Ministry of Environment of the Republic of Poland Regulation of the Minister of the Environment of 24 August 2012 on the Levels of Certain Substances in the Air*; Dziennik Ustaw: Warsaw, Poland, 2012. Available online: https://isap.sejm.gov.pl/isap.nsf/DocDetails.xsp?id=WDU20120001031 (accessed on 1 November 2021).
8. Woodburn, J.; Bielaczyc, P.; Giechaskiel, B. Particulate Exhaust Emissions in the WLTP/RDE Era. *SAE Tech. Pap.* **2022**, *1*, 1021.
9. European Parliament, and Council of the European Union. Regulation (EC) No 715/2007 of the European Parliament and of the Council. *Off. J. Eur. Communities* **2006**, *2006*, 1–54. Available online: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2004:139:0001:0054:en:PDF (accessed on 14 November 2021).
10. Bielaczyc, P.; Woodburn, J.; Szczotka, A. Investigations into Particulate Emissions from Euro 5 Passenger Cars with DISI Engines Tested at Multiple Ambient Temperatures. *SAE Tech. Pap.* **2015**, *24*, 2517. [CrossRef]
11. Bielaczyc, P.; Szczotka, A.; Woodburn, J. Particulate matter emissions from vehicles featuring direct injection spark ignition engines. In Proceedings of the 35th FISITA World Automotive Congress, Maastricht, The Netherlands, 2–6 June 2014.
12. Bielaczyc, P.; Woodburn, J.; Szczotka, A. Particulate Emissions from European Vehicles Featuring Direct Injection Spark Ignition Engines Tested Under Laboratory Conditions. *SAE Int. J. Fuels Lubr.* **2014**, *7*, 580–590. [CrossRef]
13. Bielaczyc, P.; Woodburn, J.; Szczotka, A. Low Ambient Temperature Cold Start Emissions of Gaseous and Solid Pollutants from Euro 5 Vehicles featuring Direct and Indirect Injection Spark-Ignition Engines. *SAE Int. J. Fuels Lubr.* **2013**, *6*, 968–976. [CrossRef]
14. Bielaczyc, P.; Merkisz, J.; Pielecha, J.; Woodburn, J. RDE-Compliant PEMS Testing of a Gasoline Euro 6d-TEMP Passenger Car at Two Ambient Temperatures with a Focus on the Cold Start Effect. *SAE Tech. Pap.* **2020**, *3*, 1–9. [CrossRef]
15. Pacura, W.; Szramowiat-Sala, K.; Berent, K.; Sławek, A.; Gołaś, J. The possibilities of GPF Surface modification in the aspect of micro-contaminants removal. *Energy Rep.* **2022**, *8*, 9261–9269. [CrossRef]
16. Bielaczyc, P.; Woodburn, J. Trends in Automotive Emission Legislation: Impact on LD Engine Development, Fuels, Lubricants and Test Methods: A Global View, with a Focus on WLTP and RDE Regulations. *Emiss. Control Sci. Technol.* **2019**, *5*, 86–98. [CrossRef]
17. Bielaczyc, P.; Szczotka, A.; Woodburn, J. The Impact of Fuel Ethanol Content on Particulate Emissions from Light-Duty Vehicles Featuring Spark Ignition Engines. *SAE Int. J. Fuels Lubr.* **2014**, *7*, 224–235. [CrossRef]
18. Bielaczyc, P.; Woodburn, J.; Klimkiewicz, D.; Pajdowski, P.; Szczotka, A. An examination of the effect of ethanol–gasoline blends' physicochemical properties on emissions from a light-duty spark ignition engine. *Fuel Process. Technol.* **2013**, *107*, 50–63. [CrossRef]

19. Zervas, E.; Montagne, A.X.; Lahaye, J. C1−C5 Organic Acid Emissions from an SI Engine: Influence of Fuel and Air/Fuel Equivalence Ratio. *Environ. Sci. Technol.* **2001**, *35*, 2746–2751. [CrossRef]
20. Bock, N.R.; Baum, M.M.; Moss, J.A.; Castonguay, A.; Jocic, S.; Northrop, W.F. Dicarboxylic acid emissions from a GDI engine equipped with a catalytic gasoline particulate filter. *Fuel* **2020**, *275*, 117940. [CrossRef]
21. Rodriguez, F.; Dornoff, J. Beyond NOx: Emission of unregulated pollutants from a modern gasoline car. *Int. Counc. Clean Transp.* **2019**, *5*, 1–37.
22. Zhang, Z.; Wen, M.; Cui, Y.; Ming, Z.; Wang, T.; Zhang, C.; Ampah, J.D.; Jin, C.; Huang, H.; Liu, H. Effects of Methanol Application on Carbon Emissions and Pollutant Emissions Using a Passenger Vehicle. *Processes* **2022**, *10*, 525. [CrossRef]
23. Liu, H.; Wang, X.; Zhang, D.; Dong, F.; Liu, X.; Yang, Y.; Huang, H.; Wang, Y.; Wang, Q.; Zheng, Z. Investigation on Blending Effects of Gasoline Fuel with N-Butanol, DMF, and Ethanol on the Fuel Consumption and Harmful Emissions in a GDI Vehicle. *Energies* **2019**, *12*, 1845. [CrossRef]
24. Sharma, N.; Agarwal, A.K. Gasoline Direct Injection Engines and Particulate Emissions. In *Air Pollution and Control*; Sharma, N., Agarwal, A.K., Eastwood, P., Gupta, T., Singh, A.P., Eds.; Springer: Singapore, 2018; pp. 87–105. [CrossRef]
25. Merkisz, J.; Pielecha, J. *Nanoparticle Emissions from Combustion Engines*; Springer: Poznań, Poland, 2015. [CrossRef]
26. Bielaczyc, P. Global development of emissions reduction strategies from light duty vehicles. *IOP Conf. Ser. Earth Environ. Sci.* **2019**, *214*, 012139. [CrossRef]
27. European Commission. *Down To Ten—Measuring Automotive Exhaust Particles Down to 10 Nanometres*; Project No. 724085; Cordis: Luxembourg City, Luxembourg, 2020; Available online: https://cordis.europa.eu/project/id/724085/ (accessed on 8 December 2021).
28. Di Iorio, S.; Catapano, F.; Sementa, P.; Vaglieco, B.M.; Nicol, G.; Sgroi, M.F. Sub-23 nm Particle Emissions from Gasoline Direct Injection Vehicles and Engines: Sampling and Measure. *SAE Tech. Pap.* **2020**, *03*, 1–9. [CrossRef]
29. Giechaskiel, B.; Mamakos, A.; Woodburn, J.; Szczotka, A.; Bielaczyc, P. Evaluation of a 10 nm Particle Number Portable Emissions Measurement System (PEMS). *Sensors* **2019**, *19*, 5531. [CrossRef]
30. Giechaskiel, B.; Riccobono, F.; Vlachos, T.; Mendoza-Villafuerte, P.; Suarez-Bertoa, R.; Fontaras, G.; Bonnel, P.; Weiss, M. Vehicle Emission Factors of Solid Nanoparticles in the Laboratory and on the Road Using Portable Emission Measurement Systems (PEMS). *Front. Environ. Sci.* **2015**, *3*, 1–17. [CrossRef]
31. Tomašek, I.; Horwell, C.J.; Bisig, C.; Damby, D.E.; Comte, P.; Czerwinski, J.; Petri-Fink, A.; Clift, M.J.; Drasler, B.; Rothen-Rutishauser, B. Respiratory hazard assessment of combined exposure to complete gasoline exhaust and respirable volcanic ash in a multicellular human lung model at the air-liquid interface. *Environ. Pollut.* **2018**, *238*, 977–987. [CrossRef] [PubMed]
32. Styszko, K.; Samek, L.; Szramowiat, K.; Korzeniewska, A.; Kubisty, K.; Rakoczy-Lelek, R.; Kistler, M.; Giebl, A.K. Oxidative potential of PM10 and PM2.5 collected at high air pollution site related to chemical composition: Krakow case study. *Air Qual. Atmos. Health* **2017**, *10*, 1123–1137. [CrossRef]
33. Cochran, R.; Dongari, N.; Jeong, H.; Beránek, J.; Haddadi, S.; Shipp, J.; Kubátová, A. Determination of polycyclic aromatic hydrocarbons and their oxy-, nitro-, and hydroxy-oxidation products. *Anal. Chim. Acta* **2012**, *740*, 93–103. [CrossRef] [PubMed]
34. Bandowe, B.A.M.; Meusel, H. Nitrated polycyclic aromatic hydrocarbons (nitro-PAHs) in the environment—A review. *Sci. Total Environ.* **2017**, *581*, 237–257. [CrossRef]
35. Raza, M.; Chen, L.; Leach, F.; Ding, S. A Review of Particulate Number (PN) Emissions from Gasoline Direct Injection (GDI) Engines and Their Control Techniques. *Energies* **2018**, *11*, 1417. [CrossRef]
36. Bielaczyc, P. *Particulate Number (PN) & Gaseous Emissions in the EU Automotive Emissions Context. Expert Panel Discussion EU/China Emissions Regulations*; SAE World Congress Experience: Detroit, MI, USA, 2019.
37. Giechaskiel, B.; Woodburn, J.; Szczotka, A.; Bielaczyc, P. Particulate Matter (PM) Emissions of Euro 5 and Euro 6 Vehicles Using Systems with Evaporation Tube or Catalytic Stripper and 23 nm or 10 nm Counters. *SAE Tech. Pap.* **2020**, *3*, 1–22. [CrossRef]
38. Bielaczyc, P.; Woodburn, J.; Joshi, A. World-wide trends in powertrain system development in light of emissions legislation, fuels, lubricants, and test methods. *Combust. Engines* **2021**, *184*, 57–71.
39. Bielaczyc, P.; Szczotka, A.; Woodburn, J. Investigations into Exhaust Particulate Emissions from Multiple Vehicle Types Running on Two Chassis Dynamometer Driving Cycles. *SAE Tech. Pap.* **2017**, *7*, 1–6. [CrossRef]
40. Giechaskiel, B.; Lähde, T.; Clairotte, M.; Valverde, V.; Pavlovic, J.; Suarez-Bertoa, R.; Grigoratos, T.; Zardini, A.A.; Perujo, A.; Marotta, A. Post Euro 6/VI activities focusing on particle number. *SAE World Congr. Exp.* **2019**, *1*, 1–24. [CrossRef]
41. Lähde, T.; Giechaskiel, B.; Martini, G.; Woodburn, J.; Bielaczyc, P.; Schreiber, D.; Huber, M.; Fittavolini, C.; Pellegrini, L.; Kirchner, U.; et al. Solid, sub-23nm particle number emission measurement inter-laboratory comparison exercise. *JRC Tech. Rep.* **2021**, *12*, 1–113, ISBN 978-92- 76-40950-2, JRC126191.
42. Lähde, T.; Giechaskiel, B.; Martini, G.; Woodburn, J.; Bielaczyc, P.; Schreiber, D.; Huber, M.; Eggenschwiler, P.D.; Fittavolini, C.; Florio, S.; et al. Reproducibility of the 10-nm Solid Particle Number Methodology for Light-Duty Vehicles Exhaust Measurements. *Atmosphere* **2022**, *13*, 872. [CrossRef]
43. Agarwal, A.K.; Dhar, A.; Sharma, N.; Shukla, P.C. *Engine Exhaust Particulates*; Springer: Uttar Pradesh, India, 2019. [CrossRef]
44. Fabiańska, M.; Kozielska, B.; Bielaczyc, P.; Woodburn, J.; Konieczyński, J. Geochemical markers and polycyclic aromatic hydrocarbons in solvent extracts from diesel engine particulate matter. *Environ. Sci. Pollut. Res.* **2016**, *23*, 6999–7011. [CrossRef] [PubMed]

45. Fabiańska, M.J.; Kozielska, B.; Konieczyński, J.; Bielaczyc, P. Occurrence of organic phosphates in particulate matter of the vehicle exhausts and outdoor environment—A case study. *Environ. Pollut.* **2018**, *244*, 351–360. [CrossRef] [PubMed]
46. Baresel, C.; Harding, M.; Fång, J. Ultrafiltration/Granulated Active Carbon-Biofilter: Efficient Removal of a Broad Range of Micropollutants. *Appl. Sci.* **2019**, *9*, 710. [CrossRef]
47. Miralles-Cuevas, S.; De la Obra, I.; Gualda-Alonso, E.; Soriano-Molina, P.; López, J.C.; Pérez, J.S. Simultaneous Disinfection and Organic Microcontaminant Removal by UVC-LED-Driven Advanced Oxidation Processes. *Water* **2021**, *13*, 1507. [CrossRef]
48. Peralta-Maraver, I.; Posselt, M.; Perkins, D.M.; Robertson, A.L. Mapping Micro-Pollutants and Their Impacts on the Size Structure of Streambed Communities. *Water* **2019**, *11*, 2610. [CrossRef]
49. Keyte, I.J.; Harrison, R.M.; Lammel, G. Chemical reactivity and long-range transport potential of polycyclic aromatic hydrocarbons—A review. *Chem. Soc. Rev.* **2013**, *42*, 9333–9391. [CrossRef]
50. Zhao, T.; Yang, L.; Huang, Q.; Zhang, W.; Duan, S.; Gao, H.; Wang, W. PM2.5-bound polycyclic aromatic hydrocarbons (PAHs) and nitrated-PAHs (NPAHs) emitted by gasoline vehicles: Characterization and health risk assessment. *Sci. Total Environ.* **2020**, *727*, 138631. [CrossRef]
51. Lu, W.; Yang, L.; Chen, J.; Wang, X.; Li, H.; Zhu, Y.; Wen, L.; Xu, C.; Zhang, J.; Zhu, T.; et al. Identification of concentrations and sources of PM2.5-bound PAHs in North China during haze episodes in 2013. *Air Qual. Atmos. Health* **2015**, *9*, 823–833. [CrossRef]
52. Kumar, A.V.; Kothiyal, N.C.; Kumari, S.; Mehra, R.; Parkash, A.; Sinha, R.R.; Tayagi, S.K.; Gaba, R. Determination of some carcinogenic PAHs with toxic equivalency factor along roadside soil within a fast developing northern city of India. *J. Earth Syst. Sci.* **2014**, *123*, 479–489. [CrossRef]
53. Makhniashvili, I. Nitrowe pochodne wielopierścieniowych węglowodorów aromatycznych w środowisku. *Bezpieczeństwo Pr. Nauka I Prakt.* **2003**, *3*, 17–20.
54. Borillo, G.C.; Tadano, Y.S.; Godoi, A.F.L.; Pauliquevis, T.; Sarmiento, H.; Rempel, D.; Yamamoto, C.I.; Marchi, M.R.R.; Potgieter-Vermaak, S.; Godoi, R.H.M. Polycyclic Aromatic Hydrocarbons (PAHs) and nitrated analogs associated to particulate matter emission from a Euro V-SCR engine fuelled with diesel/biodiesel blends. *Sci. Total Environ.* **2018**, *644*, 675–682. [CrossRef] [PubMed]
55. Li, X.; Zheng, Y.; Guan, C.; Cheung, C.S.; Huang, Z. Effect of biodiesel on PAH, OPAH, and NPAH emissions from a direct injection diesel engine. *Environ. Sci. Pollut. Res.* **2018**, *25*, 34131–34138. [CrossRef] [PubMed]
56. McCaffery, C.; Durbin, T.D.; Johnson, K.C.; Karavalakis, G. The effect of ethanol and iso-butanol blends on polycyclic aromatic hydrocarbon (PAH) emissions from PFI and GDI vehicles. *Atmos. Pollut. Res.* **2020**, *11*, 2056–2067. [CrossRef]
57. Alanen, J.; Saukko, E.; Lehtoranta, K.; Murtonen, T.; Timonen, H.; Hillamo, R.; Karjalainen, P.; Kuuluvainen, H.; Harra, J.; Keskinen, J.; et al. The formation and physical properties of the particle emissions from a natural gas engine. *Fuel* **2015**, *162*, 155–161. [CrossRef]
58. Überall, A.; Otte, R.; Eilts, P.; Krahl, J. A literature research about particle emissions from engines with direct gasoline injection and the potential to reduce these emissions. *Fuel* **2015**, *147*, 203–207. [CrossRef]
59. Hao, X.; Zhang, X.; Cao, X.; Shen, X.; Shi, J.; Yao, Z. Characterization and carcinogenic risk assessment of polycyclic aromatic and nitro-polycyclic aromatic hydrocarbons in exhaust emission from gasoline passenger cars using on-road measurements in Beijing, China. *Sci. Total Environ.* **2018**, *645*, 347–355. [CrossRef]
60. Pacura, W.; Szramowiat-Sala, K.; Gołaś, J.; Bielaczyc, P. Advanced analysis of solid particles. *Powertrains Fuels Lubr. Digit. Summit Pittsbg.* **2020**, *12*, 682. Available online: https://saemobilus.sae.org/content/12682 (accessed on 25 September 2020).
61. Szramowiat, K.; Woodburn, J.; Pacura, W.; Berent, K.; Bielaczyć, P.; Gołaś, J. Engine-generated solid particles—A case study. *Combust. Engines* **2018**, *174*, 33–39. [CrossRef]
62. Bielaczyc, P.; Klimkiewicz, D.; Woodburn, J.; Szczotka, A. Exhaust emission testing methods—BOSMAL's legislative and development emission testing laboratories. *Combust. Engines* **2019**, *178*, 88–98. [CrossRef]
63. European Commission. Commission Regulation (EU) 2017/1151 of 1 June 2017. *Off. J. Eur. Communities* **2017**, *2017*, 1–643. Available online: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32017R1151 (accessed on 5 December 2021).
64. European Commission. Commission Regulation (EU) 2018/1832 of 5 November 2018. *Off. J. Eur. Communities* **2018**, *2018*, 1–314. Available online: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32018R1832 (accessed on 5 December 2021).
65. *National Center for Biotechnology Information*; PubChem. US National Institutes of Health: Bethesda, MD, USA, 2021. Available online: https://pubchem.ncbi.nlm.nih.gov (accessed on 7 September 2021).