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Catabolic Fingerprinting and Diversity of Bacteria in *Mollic Gleysol* Contaminated with Petroleum Substances

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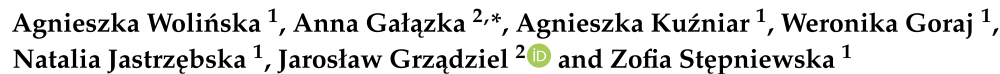

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Abstract: This study focused on the determination of both catabolic and genetic fingerprinting of bacteria inhabiting soil contaminated with car fuels. A surface layer (0–20 cm) of *Mollic Gleysol* was used for the experiment and was contaminated with car fuels—unleaded 95-octane petrol and diesel at a dose of 15 g per 10 g of soil. The experiment lasted 42 days and was performed at 20 °C. The metabolic potential of soil bacterial communities was evaluated using the Biolog EcoPlate system. The results demonstrated that petroleum substances influenced the structure of the microbial populations and their catabolic activity. The *Arthrobacter*, *Paenibacillus*, and *Pseudomonas* genera were found in diesel-contaminated soil, whilst *Bacillus* and *Microbacterium* were found in petrol-contaminated soil. *Rhodococcus* species were identified in both variants of impurities, suggesting the widest capability of car fuel degradation by this bacterial genus. The contamination with unleaded 95-octane petrol caused rapid inhibition of the metabolic activity of soil bacteria in contrast to the diesel treatment, where high metabolic activity of bacteria was observed until the end of the incubation period. Higher toxicity of petrol in comparison with diesel car fuel was evidenced.

Keywords: Biolog EcoPlate system; biodiversity; petroleum contaminants; soil; diesel; petrol

1. Introduction

Petroleum hydrocarbons (PHs) are common fuels used in extremely large quantities and represent a serious problem when released into the environment [1–3] because they contain n-alkanes, i.e., aliphatic and aromatic compounds [4]. Accidental release of PHs usually results in their persistence in soil [5–7] for decades because their biodegradation process occurs very slowly due to their recalcitrant nature [8]. The major sources of PH soil contamination are accidents during petroleum transfer, refinery wastes released into the environment, or petroleum leakage from pipelines [9]. Moubaser et al. [10] have reported that many PHs are usually highly toxic, carcinogenic, and mutagenic for both soil microorganisms and humans. The danger posed by the presence of PHs in soil is connected with their inherent properties: volatility and solubility [11]. PHs represent bioresistant organic compounds that can inhibit the metabolism of aerobic bacteria [12]. Consequently, they can exert a toxic effect on soil biodiversity and a negative impact on plants and soil health l [2,13]. Therefore, effective removal of PHs from the environment is crucial [11,14].

The risk posed by PH soil contaminants can be reduced by the application of indigenous microorganisms dedicated to bioremediation processes [3,13,15]. Being in intimate contact with the

soil, microorganisms are known to be the best indicators of soil pollution [13]. To date, various methods have been recommended to remediate polluted soil, i.e., chemical oxidation, thermal treatment, and bioremediation [11]. Soil microorganisms are economically feasible and are an effective solution for soil clean up [1,3,4]. Soil microorganisms break down complex compounds (i.e., PHs) and release inorganic nutrients that are available to plants [14,16]. In ideal conditions, PHs are completely biodegraded to carbon dioxide and water (products that are innocuous to humans and the environment); however, more frequently, biodegradation is not complete [4]. Generally, microorganisms in contaminated soil should adapt to environmental conditions as an effect of genetic mutations [3] and induction of specific enzymes, i.e., oxygenases, hydrolases, and oxidoreductases [6,17]. Nevertheless, it should be remembered that introduced microorganisms do not often survive when added to soil [1]. Therefore, introduction of new microflora dedicated to bioremediation should always be preceded by identification of autochthonic bacteria inhabiting the soil environment that use hydrocarbon pollution as a carbon source for their metabolic pathway [1,18,19].

The best-known bacteria that are able to degrade PHs include the following genera: *Arthrobacter*, *Acinetobacter*, *Bacillus*, *Brachybacterium*, *Corynebacterium*, *Geobacillus*, *Nocardia*, *Klebsiella*, *Mycobacterium*, *Microbacterium*, *Pseudomonas*, *Micrococcus*, and *Rhodococcus* [19–24]. Similarly, *Ralstonia* and *Burkholderia* have been identified as PH degraders [25]. Borowik et al. [7] have suggested that the precise estimation of the effect of petroleum substances on the natural environment is very difficult. The main reason is that the proportions between fast- and slow-growing bacteria in PH-contaminated soils are usually variable [26], and knowledge of microbial succession is necessary for soil purification from petroleum contaminants [7].

The Biolog EcoPlate technique is recommended to indicate viable microorganisms and to perform metabolic fingerprinting [16,27,28]. Since microorganisms usually react quickly to any environmental disturbances and stresses, changes in their metabolic activity could be an early signal of alterations in the ecosystem [16]. The Biolog EcoPlate is a redox method for which the variability in the rate of colour development depending on the microbial community structure indicates the rate of utilization of carbon substrates preferred by soil microbes [16]. The method is appropriate and thus recommended for the evaluation of the microbial structure in contaminated and uncontaminated sites on the basis of differences in metabolic response [16]. Yet, it should be pointed out that the Biolog technique investigates the functional diversity of the part of microbial community which is metabolically active and able to grow in plate conditions [16].

Therefore, our principal goal was to evaluate a 6-week impact of car fuel contaminants on both metabolic activity and microbial community structure in order to gain knowledge of autochthonic microflora that is able to survive in the presence of these impurities. Additionally, recognition of differences in the microbial structure depending on the car fuel type (petrol and diesel) was also studied. In our previous study, we examined the effect of petroleum-derived substances (petrol, diesel, and new and used engine oils) on selected enzymatic activity and microbial structure as a result of 1-week soil contamination [19]. In the current work, the period of petroleum impact on microorganisms was extended to 6 weeks to determine the long-term effects of pollutants on soil bacteria.

2. Materials and Methods

2.1. Soil Characteristics and Experimental Design

The surface layer (0–20 cm) of *Mollic Gleysol* taken from an agricultural meadow exploited for hay-making, located in Kosiorów village (51°13′ N; 21°51′ E), southeast Poland, was used for the experiment. The soil was a peaty soil type with acidity (pH) of 6.19, redox potential (Eh) of 480.73 mV, electric conductivity (EC) of 197.8 μS/cm^3, total organic carbon (TOC) of 27.4%, and bulk density (BD) of 0.77 Mg/m^3 [19,29]. In laboratory conditions, the soil material was contaminated with the following petroleum substances: unleaded 95-octane petrol and diesel (their basic properties, according to Orlen specification, are summarized in Table 1) at a dose of 15 g per 10 g of soil. Control samples were prepared without any petroleum substances and with the addition of 15.0 g of distilled water per 10 g

of soil. The contaminated and control soils were incubated for 6 weeks (42 days) at room temperature (20 °C). After this time, the following analyses were performed.

Table 1. The basic properties of petroleum substances (according to www.orlen.pl).

Parameter	Unit	Unleaded 95-Octane Petrol	Diesel
Carbon atoms number	–	C_5–C_{12}	C_9–C_{25}
Density	g cm^{-3}	0.72–0.78	0.82–0.85
PAH * content	%	20	11
Sulphur content	mg kg^{-1}	10	10

* PAH—Polycyclic Aromatic Hydrocarbons.

2.2. Soil DNA Extraction and Molecular Identification of Bacteria

DNA was extracted from soil samples, contaminated and control (400 mg each), after a 42-day incubation period with the application of the Gene All® Exgene™ Soil SV mini kit (GeneAll Biotechnology Co. Ltd., Seoul, Korea) according to the manufacturer's instruction. Subsequently, PCR reactions were performed. The following primers—27F (5′-AGAGTTTGATCATGGCTCAG-3′) and 518R (5′-CCAGCAGCCGCGGTAATACG-3′)—were synthesized to amplify the 16S rRNA gene. 1X Phusion Flash High-Fidelity PCR Master Mix (Thermo Scientific, Waltham, MA, USA) was applied for the PCR reaction (30 µL). The reaction conditions were as follows: initial denaturation (98 °C for 10 s), 30 cycles (98 °C for 5 s), primer annealing (53 °C for 5 s), and elongation (72 °C for 40 s). Then, the amplification products were separated by electrophoresis in a 1% agarose gel, purified, and sequenced (Genomed S.A. Warsaw, Poland). The obtained results were analyzed by BLAST online comparison (http://www.ncbi.nlm.nih.gov) for identification of the isolates.

2.3. Community Level Physiological Profiling (CLPP) Analysis Using Biolog EcoPlates

The metabolic potential of the soil bacterial communities was evaluated using the Biolog EcoPlate system (Biolog Inc., Hayward, CA, USA), composed of 31 carbon sources and water blanks [28–30]. The soil suspension for the inoculation of the wells was prepared as follows: 1 g of soil (contaminated and control, after a 42-day incubation period) was weighed, transferred to conical flasks holding 99 cm^3 of sterile 0.9% NaCl each, vortexed (150 rpm for 30 min at 25 °C), and cooled (30 min at 4 °C) as described by Pohland and Owen [31]. Afterwards, the inoculates (120 µL) were transferred in each Biolog EcoPlate well and incubated in the dark (28 °C). The experiment included three replications. The results were read on the MicroStation ID system by Biolog®. The tetrazolium violet reduction rate was used to measure the utilization rate [32]. The activities of soil microorganisms are based on all carbon sources and on grouped sources defined as amines and amides, amino acids, carbohydrate, carboxylic acid, and polymers [31]. The data collected for the whole period of EcoPlate™ incubation (0–214 h) were used to calculate the total carbon source utilization patterns [29].

3. Results

3.1. Catabolic Diversity of Autochthonic Bacteria Inhabiting Soil Contaminated with Car Fuels

The Biolog EcoPlate technique was applied to characterize changes in the soil community as an effect of car fuel contamination. The ability of soil bacteria to use particular groups of organic compounds is illustrated in Figure 1.

It was found that the control and diesel-contaminated soils displayed a similar trend in the utilization of carbon sources, whereas different preferences for carbon utilization were demonstrated by microflora inhabiting the petrol-contaminated soil. In the case of bacteria present in the control and diesel treatments, carboxylic acids (CA) were the most favored source of carbon and their utilization amounted to 21%–57% and 31%–44%, respectively. Amino acids (AA) were utilized at the levels of 21%–25% and 16%–24% in the control and diesel combinations. Carbohydrates (CH) were also an

eagerly metabolized source of carbon and their utilization oscillated in the range of 12%–21% (in the control) and 21%–28% (in the diesel-contaminated soil). The utilization of polymers (P) achieved the level of 10%–12% in the control and 11%–20% in the diesel-contaminated soil. Amines and amides (AD) were the least preferred source of carbon for the bacteria from the control and diesel-contaminated soils, with the utilization percent of 1%–5% and 1%–9%, respectively.

Completely different bacterial preferences in the use of carbon sources were found in the presence of petrol contamination (Figure 1). In this case, CH were the most favourable carbon substrates (18%–59%). However, during 1 h of the experiment, bacteria in the petrol-contaminated soil started to use P with metabolic effectiveness of 51%. During the other hours (from 24 to 214 h), P were used in the range of 12%–29%.

Bacteria form the petrol-contaminated environment readily metabolized CA (11%–30%) and AA (1%–18%) as well. AD seemed to be the least preferred carbon source and their utilization amounted to only 1%–3%. At 96 h, this carbon source was not used at all.

The analysis of the carbon utilization patterns with the Biolog Ecoplate technique for control and contaminated soils are shown in Figure 2. The heat maps clearly reveal differences in the microbial activity between the treatments. Intensive utilization of the carbon substrates began to increase at 168–214, 120–144, and 168 h of incubation for the control, diesel, and petrol treatments, respectively.

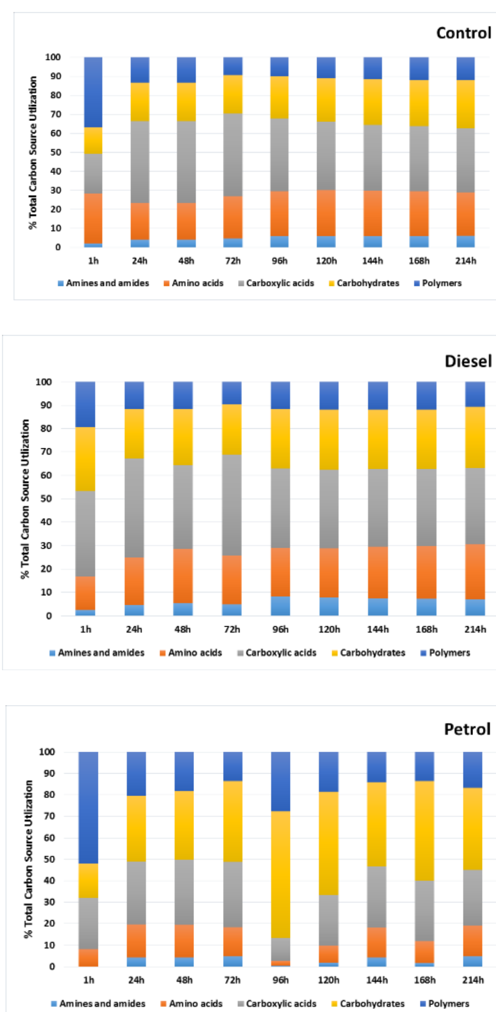

Figure 1. Total carbon source utilization response (%) tracked for control and soils contaminated with diesel and petrol for the different carbon substrate groups.

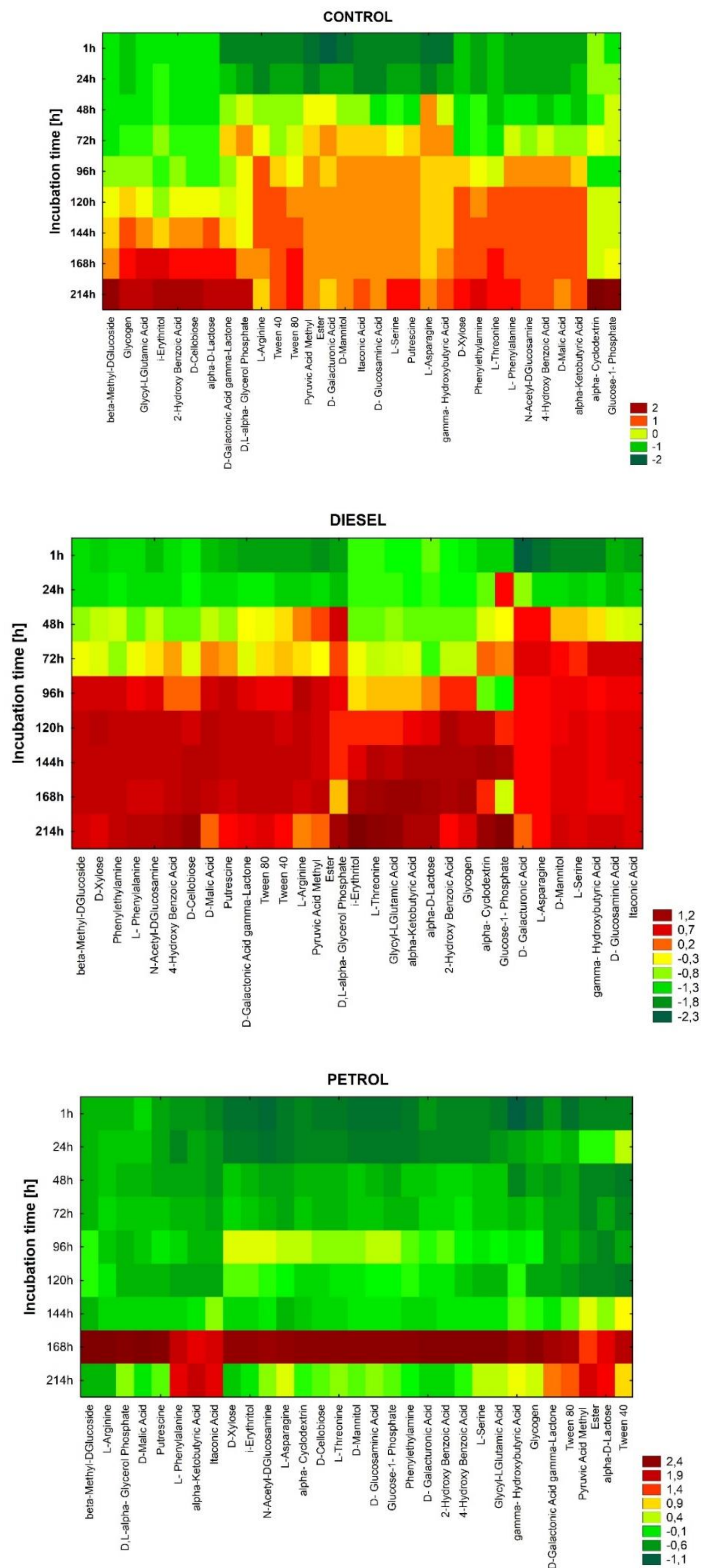

Figure 2. Biolog EcoPlate^{TM} diagram of the intensity of carbon substrate utilization for control and soil contaminated with petrol and diesel.

In the control sample, the bacteria first started to metabolize L-arginine after 96 h, whilst utilization of tween 40, D-xylose, L-threonine, L-phenylalanine, *N*-acetyl-D-glucosamine, 4-hydoxy benzoic acid, D-malic acid, and alpha-ketobutyric acid was noted after 120 h (Figure 2). Maximal intensification of carbon substrate utilization was registered between 168 and 214 h, when bacteria from the uncontaminated soil metabolized beta-methyl-D-glucoside, glycogen, glycyl-L-glutamic acid, i-erythritol, 2-hydroxy benzoic acid, D-cellobiose, alpha-D-lactose, D-galactonic acid gamma-lactone, D,L-alpha glycerol phosphate, tween 80, L-serine, putrescine, D-xylose, phenylethylamine, L-threonine, L-phenylalanine, alpha-cyclodextrin, and glucose-1-phosphate.

Carbon substrate utilization in the diesel-contaminated soil started from 24 h when the bacteria metabolized glucose-1-phosphate (Figure 2). The maximum metabolic activity was recorded from 96 h of incubation to the end of the experiment (214 h). At this time, bacteria that lived in the presence of diesel contamination most frequently metabolized the following carbon substrates: D-galacturonic acid, L-asparagine, D-mannitol, L-serine, gamma-hydroxybutyric acid, D-glucosoaminic acid, itaconic acid, L-threonine, i-erythritol, glycyl-L-glutamic acid, alpha-D-lactose, alpha-ketobutyric acid, D-cellobiose, 4-hydroxybenozic acid, *N*-acetyl-D-glucosoamine, L-phyenyloalanine, phenylethylamine, D-xylose, and beta-methyl-D-glucoside.

As presented in Figure 2, the heat map obtained for the petrol-contaminated soil differed significantly from the other two and indicated strong inhibition of bacterial activity resulting from petrol contamination. In this case, the metabolic activity was recorded only at 168–214 h of incubation, when the bacteria metabolized, in particular, L-phenylalanine, alpha-ketobutyric acid, itaconic acid, pyruvic acid methyl ester, and alpha-D-lactose. After 168 h incubation, the bacteria intensively metabolized all sources of carbon available on the Biolog plate (Figure 2). However, their metabolic activity was afterwards inhibited by petrol contamination, which evidenced a more toxic effect exerted by the unleaded 95-octane petrol in comparison to the diesel contamination.

3.2. Genetic Diversity of Autochthonic Bacteria Inhabiting Soil Contaminated with Car Fuels

The variability in the microbial preferences for utilization of the various carbon sources was confirmed by the differences in the microbial structure in the studied soil. Changes in the microbial community following contamination with petrol and diesel as well as in the uncontaminated (control) soils were monitored in the PCR reaction using the 16S rRNA gene (Figure 3). In each case, 545-bp PCR products were obtained.

The differences in the microbial community response depending on the contaminant type (petrol or diesel) are presented in Table 2.

Mollic Gleysol uncontaminated with the car fuels was inhabited by bacterial representatives of the genera *Micrococcus*, *Lysinobacterium*, and *Bacillus*. In the presence of unleaded 95-octane petrol, the following genera of indigenous bacteria were identified: *Rhodococcus*, *Bacillus*, and *Microbacterium*. At the same time, the soil contaminated with diesel were characterized by higher biodiversity than those treated with petrol, and the presence of *Rhodococcus*, *Arthrobacter*, *Paenibacillus*, and *Pseudomonas* representatives was confirmed.

The variability of autochthonic microflora in the control (uncontaminated) and after treatments with car fuels (petrol and diesel) is summarized in Table 3.

Table 2. Identification of isolates based on 16S rDNA sequencing data.

Sample ID	Bacterial Genera	Isolate Name in GenBank Database	Similarity (%)	Accession Number
CONTROL	*Micrococcus*	*Micrococcus* sp. *CNJ719 PL04*	97	DQ448712
		Micrococcus sp. *76A3b*	97	KJ744024
	Lysinobacterium	*Lysinobacterium fusiformis*	99	KY515463
		Lysinobacterium sp. *enrichment culture*	99	JX992646
		Lysinobacterium sphaericus	99	KY673682
	Bacillus	*Bacillus cereus*	99	JN411317
		Bacillus thuringiensis	98	KY368122
		Bacillus tovonensis	98	KY352881
PETROL	*Rhodococcus*	*Rhodococcus erythropolis strain HL-1*	98	KM670434
		Rhodococcus sp. *A35*	98	FM986394
		Rhodococcus globerulus	97	AB828263
	Bacillus	*Bacillus anthracis*	97	KJ721202
		Bacillus mycoides	97	KU877669
		Bacillus safensis	99	KY400282
		Bacillus pumilus	99	DQ837545
	Microbacterium	*Microbacterium* sp. *IP5*	98	GU726546
		Microbacterium oxydans	97	LN890176
DIESEL	*Rhodococcus*	*Rhodococcus erythropolis strain HS9*	98	AY168587
		Rhodococcus sp. *DSD51Y*	98	AB847903
	Arthrobacter	*Arthrobacter* sp. *ES3-54*	99	AB496410
		Arthrobacter humicola	99	NR041546
		Arthrobacter oxydans	99	KP235208
	Paenibacillus	*Paenibacillus* sp. *4-21*	98	HM537156
		Bacillus sp. *DB87*	98	HM566967
		Paenibacillus xylanexedens	98	KX156222
		Paenibacillus pabuli	98	JX293322
	Pseudomonas	*Pseudomonas* sp.	99	KR673340
		Pseudomonas thiveralensis	99	KY457754
		Pseudomonas fluorescens	99	KT223372

Table 3. Variability of autochthonic microflora in the control (uncontaminated) combination and depending on the addition of car fuels (petrol, diesel).

Bacterial Genera	Control	Unleaded 95-Octane Petrol	Diesel
Micrococcus	+	−	−
Lysinobacterium	+	−	−
Bacillus	+	+	−
Rhodococcus	−	+	+
Microbacterium	−	+	−
Arthrobacter	−	−	+
Paenibacillus	−	−	+
Pseudomonas	−	−	+

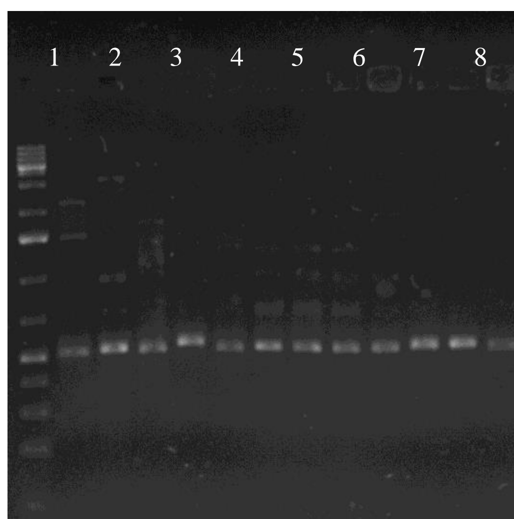

Figure 3. Agarose gel electrophoresis of PCR products (gene fragment 16S rRNA): Line 1—molecular mass marker DNA 100–10,000 bp, 2–4 control samples, 5–9 samples contaminated with unleaded 95-octane petrol, 10–13 samples contaminated with diesel.

It was demonstrated that the autochthonic microbial structure was rearranged depending on the petroleum contaminant type because different bacterial genera and species were identified in the studied combinations.

Although *Bacillus* was found both in the control and the petrol-contaminated soil, the differences in the bacterial structure were demonstrated at the species level. *Bacillus cereus*, *B. thuringiensis*, and *B. toyonensis* inhabited the uncontaminated soil, whilst the presence of *B. anthracis*, *B. mycoides*, *B. safensis*, and *B. pumilus* was evidenced in the petrol-contaminated *Mollic Gleysol*. Analogous observations were reported for bacteria of the genus *Rhodococcus*, the presence of which was demonstrated both in the petrol- and diesel-contaminated soil. There were differences in the bacterial community structure at the species level, i.e., *R. erythropolis* strain HL-1, *R.* sp. A35, and *R. globerulus* were identified in the presence of the petrol contaminant, whilst *R. erythropolis* strain HS9 and *R.* sp. DSD51Y were evidenced in the presence of diesel contamination.

4. Discussion

We previously reported that emergence of *Rhodococcus* was a consequence of the 1-week incubation of *Mollic Gleysol* with petrol and diesel [19]. The current study evaluated the effect of longer (6-week) soil exposition to petroleum substances applied to test the resistance of bacteria to long-lasting PH pollution. Labud et al. [33] reported that the effect of hydrocarbon toxicity is affected by the hydrocarbon type. PHs can enter the soil environment with falling dust and they are usually strongly absorbed by the soil surface and thus hardly biodegradable [34].

The fact that diesel is a less toxic source of carbon than other automobile oils was noted by Alrumman et al. [2] and Wolińska et al. [19]. This was also proved in the current study by the Biolog EcoPlate analysis via which functional diversity was estimated. Community level physiological profiling (CLPP) yields the metabolic fingerprint of bacteria present in the soil environment and indicates the numbers of defined substrates that are oxidized by autochthonic microflora [4]. We found that the metabolic activity of bacteria inhabiting soil contaminated with diesel fuel was higher than those from the petrol-treated soil, where the functional diversity was activated only at 168 h of the incubation period.

Similarly, the biodiversity of bacteria was higher in the diesel-contaminated soil in comparison to the petrol treatment (Table 3). Also, Borowik et al. [7] confirmed that soil contaminated with diesel was characterized by higher dehydrogenase activity than the control, whilst petrol was found to act as a dehydrogenase inhibitor [12].

It should be mentioned here that dehydrogenases are intracellular enzymes present in viable microbial cells only that indicate soil biological activity [12,35]. They are considered to be pollution indicators, as they have no ability to accumulate in the extracellular environment [12]. Gałązka and Gałązka [14] noted that, besides dehydrogenases, alkaline and acid phosphatases displayed higher activity in chernozem and rendzina polluted with PHs. However, a study reported that soil contamination with diesel and petrol inhibited the nitrification process and that diesel oil was a much more powerful inhibitor of nitrification than petrol [5]. The experiment also lasted 42 days and at the last day nitrification was reduced by 99% and 88% for diesel and petrol contamination, respectively [5].

Many bacteria and fungi have been described as being able to utilize PHs via cometabolism [1,3,36]. Microorganisms that have the ability to degrade PHs are referred to as hydrocarbonoclastic or oleophilic microflora [8]. Environments contaminated with PHs are home for many phylogenetically and metabolically diverse groups of microorganisms [8], which was also evidenced in our study. The most common groups summarized by Fernández-Luqueño et al. [1] were represented by the following bacterial genera, i.e., *Achromobacter*, *Acidovorax*, *Aeromonas*, *Alcaligenes*, *Arthrobacter*, *Bacillus*, *Burkholderia*, *Cellulomonas*, *Flavobacterium*, *Methylomonas*, *Pseudomonas*, *Paracoccus*, *Ralstonia*, *Rhizobium*, *Rhodococcus*, *Sphingomonas*, *Shewanella*, and *Staphylococcus*. Some of these genera were also identified in our study as *Bacillus*, *Rhodococcus*, *Pseudomonas*, and *Arthrobacter*. Hamamura et al. [22] indicated that *Rhodococcus*-like populations were involved in PH degradation in a majority of soils, whilst the presence of *Bacillus* was noted by Yousaf et al. [37], White et al. [38], Peng et al. [39], and Martin-Sanchez et al. [40]. Also, the current study confirmed this fact, as we identified the presence of *Rhodococcus* in soils contaminated with unleaded 95-octane petrol and with diesel. Moreover, the presence of *Pseudomonas* in diesel-contaminated soil was found both in our study and in other works [22,37,39,41]. Various bacteria of the genus *Pseudomonas*, e.g., *P. aeruginosa*, have frequently been found in PH-contaminated soils [13,42,43]. It was indicated that members of *Pseudomonas* have a complex system of enzymes and thus demonstrated differentiation in both metabolic and physiological properties. It is also known that *Pseudomonas* has the ability to produce glycolipids and biosurfactants [43,44] and is often recommended as effective in bioremediation processes. Martin-Sanchez et al. [40] isolated 28 different bacterial strains from diesel-contaminated soil samples and identified 7 bacterial genera able to degrade PHs: *Bacillus*, *Lactobacillus*, *Lysinibacillus*, *Burkholderia*, *Citrobacter*, *Acetobacter*, *Enterococcus*, and *Stenotrophomonas*. However, the presence of *Bacillus* was noted in our study in the control and petrol-contaminated treatment, whereas *Lysinibacillus* was identified in the control sample only. The ability of *Arthrobacter* and *Microbacterium* to degrade alkanes in a petroleum-oil-contaminated environment via alkane hydroxylase encoding genes has also been reported [37]. In our study, *Arthrobacter* was found in the diesel-contaminated soil, whilst the presence of *Microbacterium* was proved in the petrol-contaminated soil. In general, many soil microbial consortia are involved in restoration of the biological balance in soil contaminated with hydrocarbons. Borowik et al. [17] summarized that the following bacterial genera are dominant in PH-contaminated soils: *Staphylococcus*, *Acinetobacter*, *Pseudomonas*, *Bacillus*, and *Paenibacillus*. The last of these genera was identified in the current study in the presence of diesel contamination.

5. Conclusions

Biological degradation of PHs in the soil environment is performed by a microbial consortium; thus, the knowledge of autochthonic microflora response to car fuel contaminants is extremely desirable. The microbial communities in the studied treatments were genetically and metabolically diverse. We found that the bacterial genera *Bacillus*, *Rhodococcus*, *Microbacterium*, *Arthrobacter*, *Paenibacillus*, and *Pseudomonas* have the potential to be considered as car fuel degraders. However, it is clear from our study that the autochthonic microbial structure is dependent on the petroleum contaminant type. A higher petrol toxicity to soil autochthonic bacteria than that of diesel was found. Additionally, the presence of *Bacillus* and *Microbacterium* was detected in the soil contaminated with unleaded 95-octane petrol, whereas the presence of *Arthrobacter*, *Paenibacillus*, and *Pseudomonas*

was confirmed in the soil contaminated with diesel. The presence of *Rhodococcus* was identified in both variants of impurities, which means that this bacterial genus has the widest capability for car fuel degradation. Uncontaminated *Mollic Gleysol* was inhabited by *Micrococcus*, *Lysinibacterium*, and *Bacillus* representatives.

The metabolic activity of bacteria directly depended on the type of petroleum products as well. The unleaded 95-octane petrol contamination caused rapid inhibition of the metabolic activity of the soil bacteria. This proves that autochthonic bacteria are reluctant to use hydrocarbons contained in petrol as an additional source of carbon. The situation was different with regard to the 6-week pollution of soil with diesel. In this case, high metabolic activity of bacteria was observed, which was maintained until the end of the incubation period. Interestingly, this activity was higher than in the control (uncontaminated) soil, which proves that PHs included in diesel are characterized by a preferred carbon content for soil bacteria. We have found that the utilization gradation of the carbon sources was as follows: carboxylic acids > carbohydrates > amino acids > polymers > amines and amides.

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

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