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Original scientific paper

Characterisation of weathered petroleum hydrocarbons during a landfarming bioremediation study

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Abstract: Landfarming bioremediation was performed over 2 years on soil heavily polluted with weathered oil and oil derivatives: 23200 mg kg^{-1} of mineral oil, 35300 mg kg^{-1} total hydrocarbons and 8.65 mg kg^{-1} of total polycyclic aromatic hydrocarbons, PAHs. During the experiment, mineral oil, total hydrocarbon and PAH concentrations decreased by approximately 53, 27 and 72 %, respectively. A GC/MS scan was used to identify the crude oil components that persisted after the bioremediation treatment of the contaminated soil and the metabolites generated during this process. The data shows that in soil contaminated with weathered-hydrocarbons, the number of initially detected compounds after the bioremediation process further decreased over a 2-year period and, concurrently, several new compounds were observed at the end of experiment. Higher persistence was shown by heavier *n*-alkanes and branched alkanes, which could be detected over a longer period. The analysis highlighted the importance of *n*-alkanes, their substituted derivatives and PAHs as the most significant pollutants.

Keywords: weathering; bioremediation; crude oil; GC-MS fingerprint; PAH.

INTRODUCTION

Petroleum hydrocarbons contain a complex mixture of compounds that can be categorized into four fractions: saturates, aromatics, resins and asphaltenes. The saturates fraction includes straight chain alkanes, branched alkanes and cycloalkanes. The aromatic fraction contains volatile monoaromatic hydrocarbons, such as benzene, toluene, xylenes *etc*., polyaromatic hydrocarbons, naphthenoaromatics and aromatic sulphur compounds such as thiophenes and dibenzothiophenes. The resin (N, S, O) and asphaltene fractions consist of polar molecules containing nitrogen, sulphur and oxygen. Resins are amorphous solids that are

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truly dissolved in oil, whereas asphaltenes are large molecules colloidally dispersed in oil. The relative proportions of these fractions are dependent on many factors, such as the source, geological history, age, migration and alteration of crude oil.[1–4]

Of the various petroleum fractions, *n*-alkanes of intermediate length (C_{10}–C_{20}) are the preferred substrates for microorganisms and tend to be the most readily degradable, whereas shorter chain compounds are more toxic. Longer chain alkanes (C_{20}–C_{40}) are hydrophobic solids and consequently are difficult to degrade due to their poor water solubility and bioavailability, branched chain alkanes and cycloalkanes are also degraded more slowly than the corresponding normal alkanes. Highly condensed aromatic and cycloparaffinic structures, tars, bitumen and asphaltic materials have the highest boiling points and exhibit the greatest resistance to biodegradation. Asphaltenes are the product of petroleum hydrocarbons in soil that appear to be resistant to microbial degradation. It has been proposed that such residual material from oil degradation is analogous to, and could even be regarded as, humic material. Due to its inert characteristics, insolubility and similarity to humic materials, it is unlikely to be environmentally hazardous.[1,5–7]

The effectiveness of bioremediation is usually evaluated by measurement of the degradation of total oil and a limited number of individual oil compounds.[8,9] This is not always easy to interpret because as well as the concentration of individual contaminants, the composition of the oil changes as the oil degrades.[2,10] GC-MS fingerprinting analysis could be conveniently used to monitor specific classes of organic pollutants in the environment. Fingerprint analysis provides a picture of the overall pollutant composition. This data could serve as a basis for tracing the source and time of pollution, and to detect pollutants not covered by regulations and metabolites derived from pollutants.[11] The purpose of this work was to identify weathered petroleum-hydrocarbons that persisted after the bioremediation process, and metabolites generated during this process, by applying GC/MS analysis at various stages of the biotreatment process.

EXPERIMENTAL

Contaminated soil

The soil for this investigation was contaminated with oil and oil derivatives (gasoline, crude oil, kerosene, diesel and oil combustion products) as a consequence of infrastructure destruction at the Novi Sad Oil Refinery,[12,13] Serbia, and had been exposed to uncontrolled natural processes of weathering and decomposition for the last 8 years in the controlled depot of the Novi Sad Oil Refinery. The soil particle size distribution of the mineral fraction was 94.0 % sand, 4.1 % silt and 0.5 % clay, which is as expected as the area of the Novi Sad Oil Refinery was covered with a sand layer before its construction. The investigated soil had the following characteristics: 22.7 % water holding capacity, 2.6×10^{-4} cm s^{-1} permeability coefficient (k_f), pH 7.30, and 3.6 % humus content.

Landfarming

After 8 years of weathering, part of the contaminated soil (2.7 m^3) was placed in a 3×3 m wide and 0.4 m deep prismatic hole, and covered with resistant polypropylene foil to prevent contamination spreading from the landfarm (Fig. 1). With the aim of facilitating oxygen and water transport through the soil, the contaminated soil was composted with straw. The landfarm was turned twice a month and watered twice a week; moisture was maintained at approximately 50–80 % of the water holding capacity during the experiment. In addition to the stimulation of native microflora by soil aeration and irrigation, bioaugmentation was also performed with microorganisms separated from the contaminated soil and cultivated in a laboratory bioreactor. Approximately 25 L of the inoculated water from the bioreactor was used together with leaching water for weathering the landfarm.

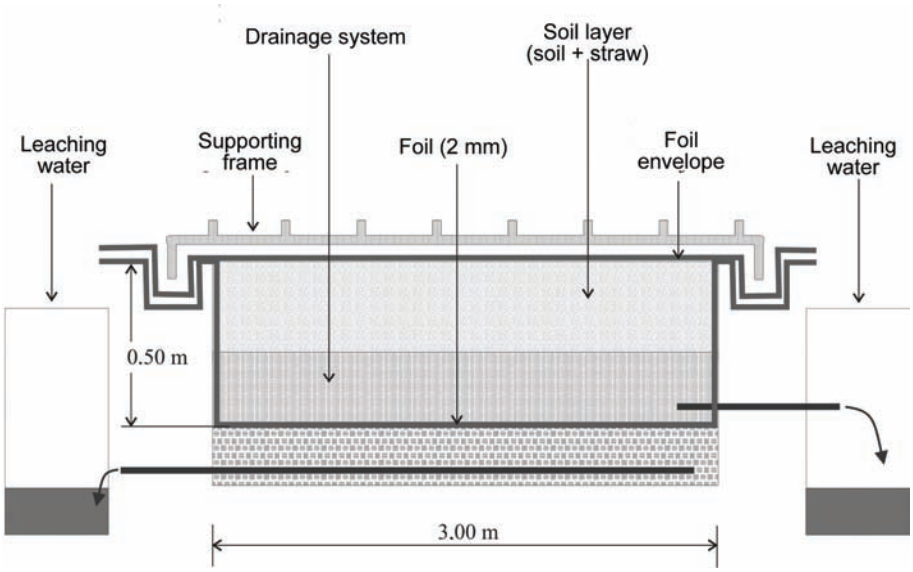


Fig. 1. Landfarm vertical cross-section.

Sampling, and chemical and microbiological analyses

For the chemical and microbiological analyses, approximately 10 soil samples were taken from the experimental field before the start of the experiment (day 0 was taken as a control sample) and after 56, 92, 128, 196, 280, 328, 443, 504, 613 and 710 days. Soil samples were homogenised and mixed, and one composite sample was made. Samples were analysed for moisture content, total hydrocarbons, mineral oil, PAHs and GC-MSD scan organic profile, and a microbiological characterisation was also performed.

Determinations of total hydrocarbons and mineral oils were performed on a Thermo Nicolet Nexus 670 FTIR instrument by standard IR spectrophotometric methods.[14] The analyses were performed by preparing approximately 7g samples according to standard methods.[15] Extraction was realised in a Soxhlet apparatus with carbon tetrachloride (100 mL) for 6 h. All compounds with hydrocarbon functionalities (both adsorbable on Al_2O_3 and mineral oils) were determined by IR spectroscopy before and after filtration of the carbon tetrachloride extract through aluminium oxide. Thus, an indication was obtained of the total hydro-

carbon moieties in the samples, regardless of other functional groups and mineral oil content. The carbon tetrachloride was of Merck grade for IR spectroscopy (≤ 99.9 %). It was checked for possible impurities before analysis by the IR method. Cross contamination of samples during preparation and analysis did not occur as demonstrated by solvent checks. The practical quantification limit (*PQL*) was 15 mg kg^{-1} dry weight for hydrocarbons. Measurements were performed as single probes. For four replicate soil analyses, the relative standard deviation (*RSD*) was 10 %.

To realise the GC/MS analysis, 7 g of soil sample was mixed with a water–methanol mixture (1:3) and extracted with dichloromethane–hexane mixture (1:1). Elemental sulphur was removed with copper powder.[16] Samples were fractionated over silica gel.[17] The GC/MS scan analysis was performed on an HP 5890GC Series II gas chromatograph with a 5971 MSD mass spectrometer in the splitless mode. The chromatographic conditions were: column 25 m×0:2 mm×0:33 μm ULTRA 2; helium flow rate 1 mL min^{-1}, injector temperature 250 °C and detector temperature 280 °C. The temperature programme was set at: 40 °C for 5 min, 4 °C min^{-1} to 130 °C hold for 2:2 min, increase by 12 °C min^{-1} to 180 °C, hold for 2:2 min, increase at 7 °C min^{-1} to 300 °C, hold 11:79 min. Qualitative analysis of the samples was performed by scanning the mass range between 35 and 550 amu, one run per sample. The interpretation of each spectrum was performed by comparison with the commercial Wiley database of spectra, using Hewlett Packard G1035A probability base matching (PBM) software for the mass spectrometric search. The performance of the MSD was evaluated using 2,6,10,15,19,23-hexamethyltetracosane, 0.93 mg mL^{-1}, and *N*-phenylbenzeneamine, 0.97 mg mL^{-1}. The PBM search results were 83 % for the former and 87 % for the latter. Quantitative PAH analyses were performed under the same chromatographic conditions used for the GC/MS scan analysis but in the selected ion monitoring (SIM) mode. Details of the method are given elsewhere.[13] The concentrations of the PAHs were calculated by the internal standard method (target ion peak areas were used for the calculation). The results were not corrected for recoveries. Replicate analyses gave results of relative *SD*s up to ±27 %.

Microbiological characterisation. Bacteria counts in the contaminated soil and the leaching water used for watering the landfarm were determined by the indirect (cultivation) method on solid agar medium.[18] A series of dilutions was made first in 0.1 % sodium pyrophosphate solution with intensive shaking on a Vortex shaker, and the solutions were inoculated on the nutritive medium. After incubation (5 to 7 days at 26–28°C), the colonies were counted. The study encompassed several important groups of bacteria: organotrophs, facultative oligotrophs, lipolytic bacteria, and hydrocarbon-oxidizing bacteria.[19]

RESULTS AND DISCUSSION

Total hydrocarbon and mineral oil degradation

During the 2-year landfarming bioremediation treatment of the soil heavily polluted with weathered oil and oil derivates, the mineral oil and total hydrocarbons content decreased by approximately 53 % (from 23.2 to 10.8 g kg^{-1}) and 27 % (from 35.3 to 25.8 g kg^{-1}), respectively (Fig. 2). The rate limiting step in the biodegradation pathways of alkanes and alkenes and most other hydrocarbons is the initial oxidation. In this case, the higher degradation rate of the mineral oil compared to the total hydrocarbons degradation rate is a consequence of the significant contribution of poorly degradable material (humus material, lignin, as-

phaltenes, *etc*.) to the total hydrocarbon degradation rate. Additionally, the most intensive degradation of mineral oil was observed in the first and in the last six month periods of the landfarming bioremediation process. In between, a long period of stagnation was observed, as a consequence of the degradation of easily degradable short-chain alkanes and alkenes in the initial period of the bioremediation process, and then a long lag period for microbiological adaptation for the oxidation of poorly degradable long chain alkanes, branched alkanes and cyclic alkanes. In the case of the total hydrocarbons, the most rapid degradation was observed in the first six months of the bioremediation process, with the total hydrocarbon content remaining relatively constant after this period. This is also a consequence of the presence of poorly degradable material, such as humus material, lignin, asphaltenes *etc*.

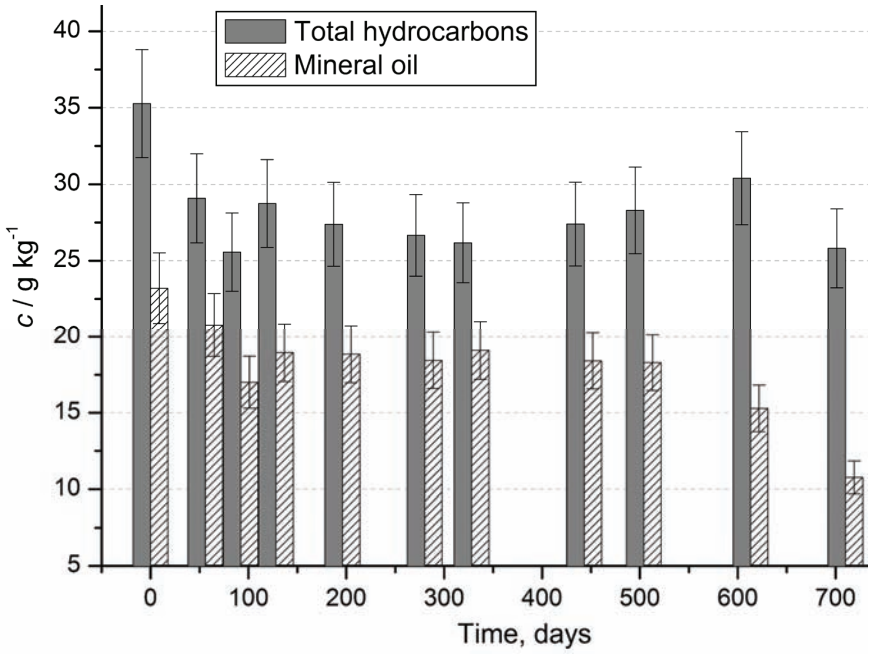


Fig. 2. Concentration change of total hydrocarbons and mineral oil.
Error bars represent a relative *SD* of 10 %.

PAH Degradation

The results for the changing concentrations over time of the total PAHs and the sums of PAHs with three, four, and five–six rings are presented in Fig. 3. During the observation period, the amount of PAHs with 3 rings decreased by 97 % (from 485 to 13 μg kg^{-1}), with 4 rings by 72 % (from 4854 to 1344 μg kg^{-1}) and for the 5–6 rings by 70 % (from 3300 to 998 μg kg^{-1}), with the approximate

total amount of PAHs during this period decreasing by 72 %. The highest degradation of PAHs was obtained for PAHs with a smaller number of rings, as a consequence of their simple structure relative to PAHs with a larger number of rings. This is in agreement with literature findings.[6,20] Additionally, it is important to stress at this point that the concentration of PAHs with 3 rings was approximately ten times lower than the concentration of PAHs with a larger number of rings at the start of the landfarming bioremediation process. This is due to the higher degradation rate of PAHs with a smaller number of rings during the uncontrolled weathering processes which occurred in the 8 previous years.

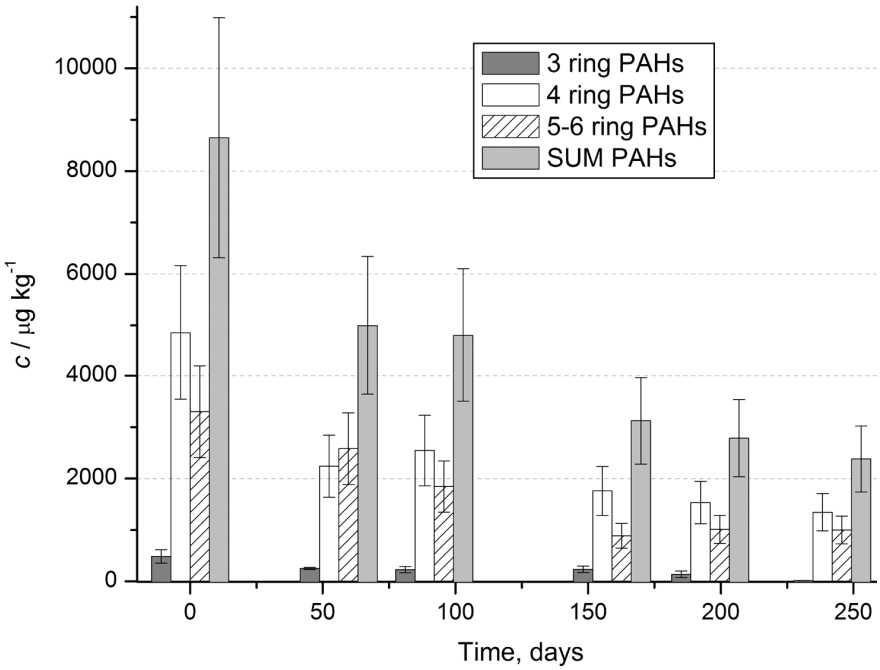


Fig. 3. PAHs in the contaminated soil during the experiment. Error bars represent a relative *SD* of 27 %.

GC/MS Scan qualitative analysis

The compounds detected by GC/MS analysis of the extracts of the various soil samples taken during the landfarming bioremediation process are given in Table I, with only the main compounds from the hit-lists of the PBM search presented. Additionally, the *TIC* and *m/z* 85 SIM ion chromatograms for some representative samples are presented in Fig. 4. The data reflect the fact that the soil used in this investigation was sampled from the dumping area of a refinery where the initial pollutants were of very diverse composition, *i.e.*, a mixture of crude oil, masut, diesel, middle distillates, heavy distillates, kerosene, *etc*.[12] The

TABLE I. Compounds detected in soil samples during the bioremediation process; Quality of the PBM search results quoted as percentages (first compounds from the hit lists)

Detected compound	Time, days						Detected compound	Time, days					
	0	92	280	443	613	710		0	92	280	443	613	710
Tridecane					64		Pentacosane, 13-undecyl-					80	
Tetradecane	93		90		81		5-Undecene, 7-methyl-(*Z*)						35
Pentadecane	96		45		76		2-Undecene, 4,5-dimethyl-						50
Hexadecane	97	60	47	53	72		1,4-Hexadiene, 2,3,4,5-tetra-methyl-				27		
Heptadecane	95						1,6-Decadiene, 2,6,9-trimethyl-(*E*)-				30		27
Octadecane	97	76			62		1,4-Undeca-diene, (*Z*)-				35		47
Nonadecane	95	60			49		Benzene, methyl-				91		
Eicosane	95	59	72				Benzamine, 4-butyl-			27			
Heneicosane	91		78				Benzaldehyde, 4-ethyl-					53	
Docosane	97	64	60		91		1,2-Benzenedi-carboxylic acid			83			
Tricosane	91	93			87		Benzene, 1,1'--methylene-bis(3-methyl)-					35	
Tetracosane	93	84				87	1,3-Benzenedi-amine, 4-methoxy-				38		
Pentacosane	95	96		60		95	Dibenz(*B*,*F*)-azepine	38			64	72	
Hexacosane	89						Phenol, 2,4-bis-(1,1-dimethyl-ethyl)-	38					
Heptacosane	91					49	Anthracene, 1,4-dimethyl-	22					
Octacosane	93						Anthracene, 9-dodecyltetra-decahydro-	27		37			18
Nanocosane	96					87	Phenanthrene, 2,3,5-trimethyl-			40			
Hentriacontane	94				38		2-Methyl-chrysene	59				38	
Tetratriacontane	98				87		Cyclopenta[*cd*]-pyrene	43					

TABLE I. Continued

Detected compound	Time, days						Detected compound	Time, days					
	0	92	280	443	613	710		0	92	280	443	613	710
Hexatriacontane	97			83	58	50	Naphthalene, 2-(1,1-dimethyl-ethyl)-	25					
3-Dodecene, (*Z*)-					64	43	2,6-Naphthalenedione, octahydro-1,1,8-		38				18
7-Tetradecene, (*Z*)-					91		1*H*-Inden-5-ol, 2,2-dihydro-		25		78	59	
1-Octadecene, (*E*)-					35		Cyclopropane, 1-(1,2-dimethyl-propyl)-	25					64
3-Octadecene, (*E*)-					38		Cyclopentane, 1,3-dimethyl-2--8(1-methyl)-	53			38		
9-Eicosene, (*E*)-					74		Cyclopentene, 3,3-Dimethyl-2-isopropyl-			25			
Nonane, 5-butyl-	76					27	Cyclopentane, 1-butyl-2-propyl-			35			
Decane, 2-methyl-					64		Cyclohexene, 3,3,5-trimethyl-				45	64	43
Decane, 3,6-dimethyl-	72						Cyclohexane, pentyl-	50					
Decane, 2,6,8--trimethyl-					90	72	Cyclohexane, octyl-					86	
Undecane, 4,6-dimethyl-	80		78	64	87	47	Cyclohexane, undecyl-	96		43			
Dodecane, 2,6,10-trimethyl-		72				60	Cyclohexane, eicosyl-		43				
Tridecane, 5-propyl-	83				74	22	Cyclohexane, (1,3-dimethyl-butyl)-	42		38			
Pentadecane, 3-methyl-			76				Cyclohexane, (3,3-dimethyl-pentyl)-				53		
Pentadecane, 2,6,10-trimethyl-	94	95			90	72	Cyclohexane, 1,2-diethyl-3--methyl-				58	59	
Pentadecane, 2,6,10,14-tetramethyl-	93	95	93	96	99	95	Cyclohexane, 1-(1,5-dimethyl-hexyl)-4-			47			

TABLE I. Continued

Detected compound	Time, days						Detected compound	Time, days					
	0	92	280	443	613	710		0	92	280	443	613	710
Hexadecane, 3-methyl-	64		46		62	64	Cyclohexane, 1-(cyclohexyl-methyl)-2-e					49	
Hexadecane, 2,6,10,14-tetra-methyl-	99	91	94	94	91	97	Cyclohexane, 1, 1′-(1-methyl-1, 3-propane)-			30		55	49
Heptadecane, 2,6-dimethyl-	94		53			87	Cyclododecane					89	89
Heptadecane, 2,6,10,15-tetra-methyl-	78	90	53	46	90	86	Cycloundecane, (1-methylethyl)-				45		
Heptadecane, 9-octyl-	83	66		80	83		Cyclohexa-decane				43		
Octadecane, 2,6-dimethyl-	38		49				Cycloeicosane					87	87
Nonadecane, 2,6,10,14-tet-ramethyl-	64	81	86	55	83		Androstane, (5*α*, 14*β*)-		50	11			
Nonadecane, 2-methyl-5-pro-pyl-			59				Pregnane	53	43	59	83	64	64
Heneicosane, 3-methyl-		91					Cholestane	59	95	91			
Heneicosane, 11-decyl-	91						2-Pntanone, 4-cyclohexyl-iden-3,3-diene-				32		
Docosane, 11-decyl-	91						Isoquinoline, 1,2,2,4-tetra-hydro-7-methyl-			52			
Tricosane, 2-methyl-			70				Camphor					45	
Tetracosane, 2,6,10,15,19,23--hexamethyl-	94	86	72				1*H*-Pyrazole, 1,3,5-trimethyl-			43	43		
Total (both columns)								49	22	33	25	42	29
In total with qual ≥70 %								34	13	6	7	21	11

untreated weathered soil sample contained a large variety of straight-chain hydrocarbons and their methyl derivatives (both those with even and odd numbers of C atoms), many of which persisted during the treatment. However, many of the aromatic hydrocarbons found in the untreated soil, mainly substituted polycyclic aromatic hydrocarbons, were not detected in the samples of treated soil, thus showing lower persistence than the alkanes. The absence of volatile components may indicate that the contamination was caused by heavier oil fractions, but is

most likely caused by the loss of lighter components by evaporation and biodegradation during the uncontrolled aging process in the dumping area of the refinery. According to a previous investigation, the *n*-alkanes ≤ C20 disappear very quickly, leaving behind isoprenoid structures;[21] this was confirmed by the presented data. At the start of the process, the soil sample contained a large variety of straight-chain hydrocarbons and their methyl derivates. Many of these compounds, in particular branched alkanes, were also detected after the treatment. Several new compounds were found at the end of the experiment, including mainly unsaturated *n*-alkenes and different derivates of cycloalkanes. It was very difficult to establish which compounds originated exclusively from the spilled oil and which were of natural origin, but data clearly showed that the number of organic compounds extracted from the soil decreased during the treatment process.

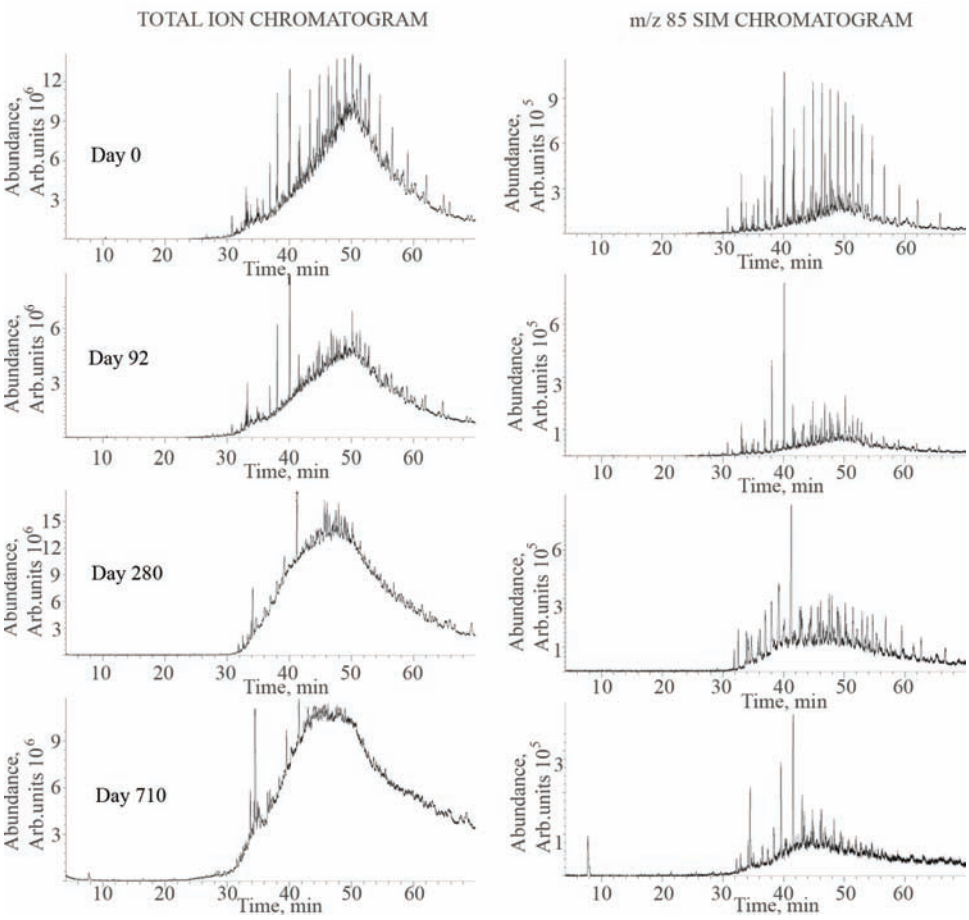


Fig. 4. TIC and *m/z* 85 SIM ion chromatograms for some representative samples.

In the original soil samples, 34 compounds were identified exhibiting a PBM ≥70 %. After the first six months of bioremediation, the number of compounds dropped significantly to 13 (≈ 62 %), after this period the number of detected compounds remained approximately constant. At the end of the treatment, the number of compounds in the treated soil was 11, of which 6 compounds were newly formed. However, a better insight into the bioremediation processes can be obtained by examining changes in the number of compounds in the different groups of compounds (PBM ≥70 %).

The number of the straight chain alkanes (PBM ≥ 70%), 19 at the beginning of the experiment, dropped to 4 (≈ 79 % reduction in the number of compound) in the first six months, and while the number of compounds remained mainly constant to the end of the process. It is important to note that after 710 days in the soil, only heavier straight chain alkanes remained in the soil. With respect to the branched alkanes, the number of detected compounds (PBM ≥ 70 %) in the untreated soil was 12, 8 after the first six months of treatment and remained mainly constant until near the end of the experiment, when the number dropped to 6 compounds, 3 of which were newly formed. Significantly, highly branched alkanes such as 2,6,10-trimethyl-pentadecane, 2,6,10,14-tetramethyl-entadecane, 2,6,10,15-tetramethyl-heptadecane, 2,6,10,15-tetramethyl-heptadecane and 2,6,10,14-tetramethyl-nonadecane were present in the soil sample from the start to the end of the experiment, as a consequence of their poor biodegradation characteristics. This is in accordance with the literature.[1,11,22,23] Additionally, a few unsaturated straight chain and branched alkenes were detected in the last six months of the treatment, in which a significantly higher number of compounds was also detected, indicating the formation of metabolites during the biodegradation processes occurring in the investigated soil.

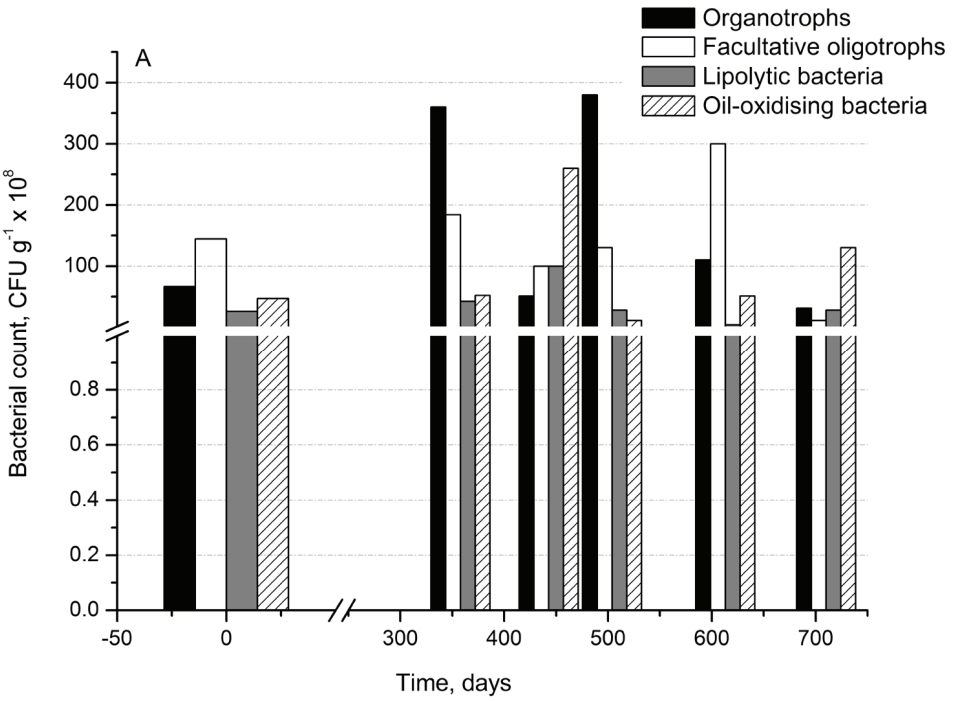
A certain number of substituted polyaromatic hydrocarbons were detected in the treated and untreated soil, but the search results for all these compounds were mainly below 70 %. Significantly, most of the originally present substituted PAHs were degraded after the first six months. A large number of different cycloalkanes were also detected, but the search results for most of these compounds were below 70%. Most of these compounds were not originally present in the untreated soil; hence, it could be concluded that they were the metabolic products of straight chain and branched hydrocarbon biodegradation.

The lowest concentration of each compound in the soil that is detectable by GC-MS could be estimated based roughly on the following. Assuming that the detection limit for the detector is approximately 500 ng for each compound and knowing that 7 g soil were extracted, that the residues were dissolved in 500 μL, and the volume injected was 2 μL, the lowest concentration of any given pollutant for effective identification should be of the order of 20 mg kg^{-1}. Thus, it is likely that there are many compounds present in oil, such as substituted deriva-

tives of alicyclic and polycyclic aromatic compounds, which are not included in standard mixtures used for routine analyses and thus not detected by GC/MS--SIM analyses, but which were detected by GC/MS scan analysis. This suggests that for bioremediation purposes, it is necessary to also apply this approach for more detailed identification of such components.

Characterization of the microbial population

The change in the counts of bacterial strains in the soil during the bioremediation experiment is presented in Fig. 5. Microbiological analyses confirmed that the bacterial populations in the landfarming were involved in the removal processes of the soil contaminants by degrading them.[19] High counts (10^8–10^9 CFU g^{-1}) of all investigated groups of bacteria were detected in the soil. It is significant that the counts of bacteria during the treatment were considerably greater than at the beginning of the experiment, which indicates that the landfarming treatment contributes to increasing the microbial degradation of hydrocarbons and increases biomass. This was confirmed by the decreasing hydrocarbon concentration during the experiment. At the end of the experiment, the counts of all the investigated groups of bacteria were reduced, and since there was still some available nitrogen and phosphorous for bacterial growth, this is probably a consequence of the changing composition of the mineral oil; after the easily degradable short-chain alkanes and alkenes had been degraded, long chain alkanes



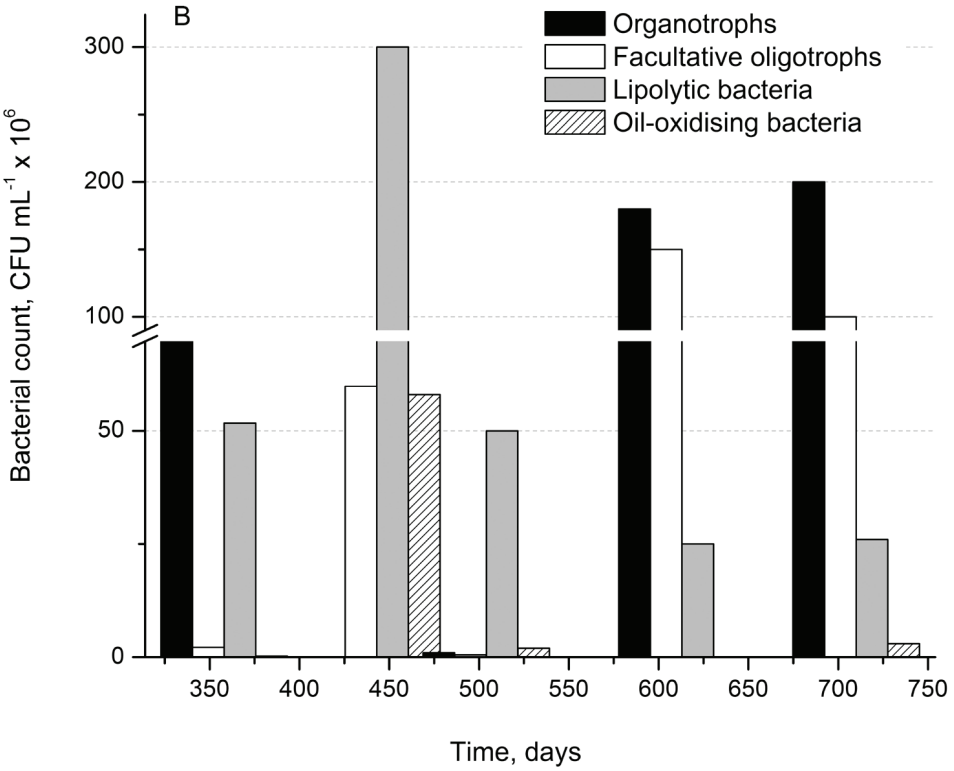


Fig. 5. Bacterial count in the soil (A) and water (B) during the experiment.

and cyclic alkanes remained. The bacteria count in the leaching water was much higher than in the initial period of the experiment, 10^7–10^8 CFU per mL of leaching water. This also confirms the microbiological degradation of hydrocarbons during the landfarming treatment.

CONCLUSIONS

During the observation period of bioremediation in the landfarm, the concentration of hydrocarbons significantly decreased. During the experiment, the contents of mineral oil and total hydrocarbons decreased by approximately 53 and 27 %, respectively, and the concentration of PAHs decreased by about 72 %. Based on GC/MS characterization of aged-hydrocarbons contaminated soil, the number of initially detected compounds after the bioremediation process further decreased during the investigation period: at the start of the experiment, the number of detected compounds was 34 and after 710 days it was 11, about 32 % of the original number, and included 6 that were newly formed. With respect to the nature of the compounds detected, three groups of organic compounds appeared to be most prominent in the contaminated soil, acyclic, substituted polycyclic aro-

matic hydrocarbons and different cycloalkanes derivates. Higher persistence was found for the heavier *n*-alkanes and branched alkanes, which were detectable over a long period.

The dominant microflora was the physiological group facultative oligotrophs, which indicates a satisfactory process of soil self-cleansing. During the experiment, lipolytic and oil-oxidising bacteria significantly increased, indicating that the hydrocarbon biodegradation processes had intensified.

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И З В О Д

КАРАКТЕРИЗАЦИЈА УГЉОВОДОНИКА СТАРОГ НАФТНОГ ЗАГАЂЕЊА ТОКОМ БИОРЕМЕДИЈАЦИОНОГ ТРЕТМАНА ПОВРШИНСКОМ ОБРАДОМ

СНЕЖАНА МАЛЕТИЋ, СРЂАН РОНЧЕВИЋ, БОЖО ДАЛМАЦИЈА, ЈАСМИНА АГБАБА, MALCOLM WATSON, АЛЕКСАНДРА ТУБИЋ и СВЕТЛАНА УГАРЧИНА ПЕРОВИЋ

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Биоремедијација површинском обрадом вршена је 2 године на земљишту загађеном изузетно високим концентрацијама старог загађења нафте и њених деривата: 23200 mg kg^{-1} минералних уља, 35300 mg kg^{-1} укупних угљоводоника и 8,65 mg kg^{-1} укупних PAH-ова. Током експеримента концентрација минералних уља, укупних угљоводоника и PAH-ова је опала за око 53, 27 и 72 %, респективно. GC/MS *scan* анализа је коришћена за идентификацију нафтних угљоводоника који заостају након биоремедијационог третмана загађеног земљишта, као и насталих метаболита током овог процеса. Резултати су показали да у земљишту загађеном старим нафтним загађењем, број иницијално детектованих једињења након 2 године биоремедијационог третмана опада, при чему истовремено долази до формирања неколико нових једињења. Показано је да виши угљоводоници имају већу презистентност и могли су бити детектовани током дугог временског периода. GC/MS *scan* анализа је омогућила праћење различите биорезградљивости *n*-алкана и њихових супституисаних деривата, као и полицикличних ароматичних угљоводоника у земљишту загађеном нафтом и њеним дериватима током процеса биоремедијације.

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