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ANALYTICAL TECHNIQUES IN THE INVESTIGATION OF PETROLEUM POLLUTANT FATE IN THE ENVIRONMENT

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Summary: Studies on the environmental fate of petroleum-type pollutants remain to be an actual scientific interdisciplinary problem. The fate of an oil type pollutant in the environment can be monitored by determining its quantity and studying its composition in the polluted samples from the same or close locality, and during different periods of time. On the other side, simulation of the natural conditions in the laboratory or by *ex situ* bioremediation with simultaneous intensification of only specified factors affecting the intensity and rate of transformation, made it possible to save time and come to relevant conclusions. This paper presents a brief overview of the analytical, primarily instrumental, techniques that is applied in featuring oil hydrocarbons, identification of oil spills in assessments of environmental impacts, and in following up the efficacy of bioremediation. This review is focused on new trends in the analysis methods of oil biomarkers, applied in our recent work.

Keywords: petroleum-type pollutants, biomarkers, environment, bioremediation, analytical techniques

1. INTRODUCTION

In spite of remarkable advancement of petroleum exploration, transport and refining technologies, petroleum and its refining products continue to be one of the most abundant environmental pollutants. Consequently, studies on the environmental fate of petroleum-type pollutants remain to be an actual scientific interdisciplinary problem. Transformation processes of petroleum-type pollutants in soils, recent sediments, alluvial sediments, ground and surface waters were studied by numerous authors [as an example: 1-3]. The fate of an oil type pollutant in the environment can be monitored the most accurately by determining its quantity and studying its composition in the polluted samples from the same or close locality, and during different periods of time. On that way a number of experiments were carried out in order to define the intensity and optimal conditions for the most efficient biodegradation (temperature, humidity, nutrients). On the other side, simulation of the natural conditions in the laboratory or by *ex situ* bioremediation with simultaneous intensification of only specified factors affecting the intensity and rate of transformation, made it possible to save time and come to relevant conclusions.

This paper presents a brief overview of the analytical, primarily instrumental, techniques that is currently applied in featuring oil hydrocarbons, identification of oil spills in assessments of environmental impacts, and in following up the efficacy of bioremediation procedures. This review is focused on new trends in the analysis methods of oil hydrocarbon biomarkers, applied in our recent work [4-9].

2. ANALYTICAL TECHNIQUES IN THE INVESTIGATION OF TRANSFORMATION PROCESSES OF PETROLEUM POLLUTANT AND PROCESSES OF BIOREMEDIATION

In this paper our recent results will be reviewed observed by investigating transformation processes of petroleum-type pollutant by determining its quantity and studying its composition in the polluted samples from

different distance of oil spill; by determining its quantity and studying its composition in the polluted samples during different periods of time from oil spill; by simulation of the natural conditions in the laboratory and by *ex situ* bioremediation with simultaneous intensification of only specified factors affecting the intensity and rate of transformation.

2.1. Transformation processes of petroleum pollutant

In order to gain insight into petroleum transformations which occur during migration, and to contribute to the elucidation of migration mechanisms in water-wet environments, the content and composition of petroleum from an accidental oil spill (near a railway station) was determined in crushed rock samples from various depths and distances from the oil spill [4]. The group composition of investigated samples was determined by column chromatography, well known methods in organic geochemistry [10]. Gas chromatographic (GC) analyses of saturated hydrocarbons were carried out with a Varian 3300 gas chromatograph using a 25 m x 0.25 mm i.d. fused silica DB-1 capillary column directly connected to an FID detector. The observed differences in the group composition (increase in NSO-compounds content with depth and distance), and in the distribution of *n*-alkanes and isoprenoids C₁₉, pristane (Pr) and C₂₀, phytane (Phyt) lead to the conclusion that petroleum migration through the crushed rock environment occurred in the presence of water by the colloidal micelle mechanisms.

The process of biodegradation of petroleum-type pollutants in underground waters from Danube alluvial sediments (the locality of Pančevo Oil Refinery) was followed through a period from November 1997 to February 2000 [5,6].

Saturated hydrocarbon fractions from 5 investigated oil samples were isolated again by column chromatography [10]. Distributions of *n*-alkanes and isoprenoids C₁₉, pristane (Pr) and C₂₀, phytane (Phyt) in these fractions were defined by GC (Varian 3300, Figure 1).

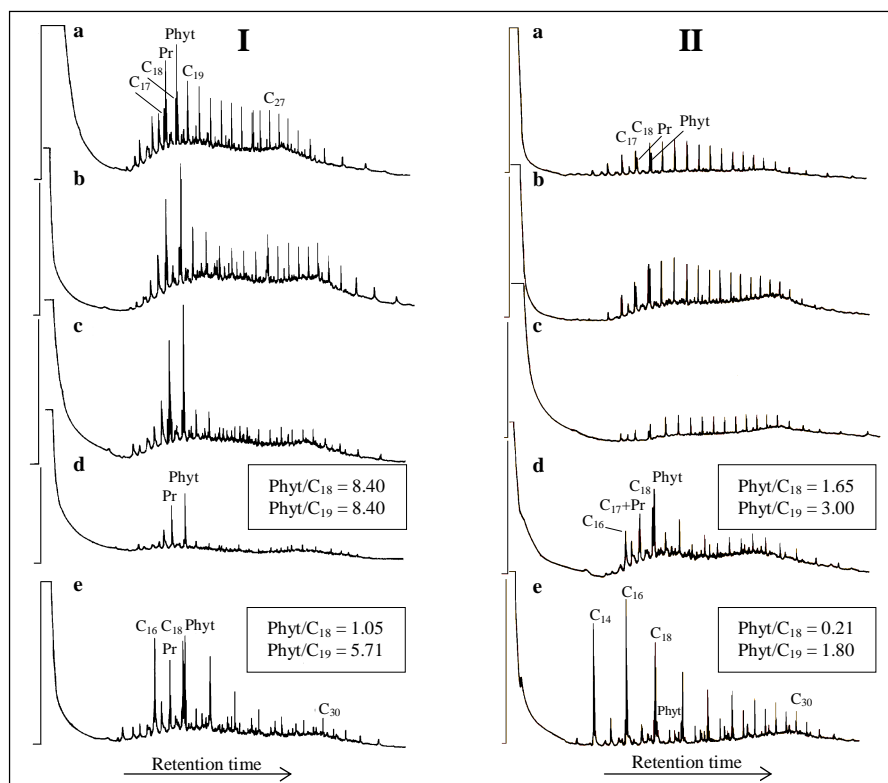


Figure 1: GC analyses of alkanes from oil polluted alluvial ground waters (Pančevo locality, I and II wells): November 1997 (a), May 1998 (b), September 1998 (c), September 1999 (d) and in February 2000 (samples e) [5,6].

In the period from November 1997, when the first sample was taken, to February 2000, when the fifth sample was taken, important changes of the chemical composition were obvious (especially in well I). Relative contribution of *n*-alkanes as compared to pristane and phytane in sample Ia indicated changes defined as "initial petroleum biodegradation". Gas chromatogram of sample Ib showed that in the period from November 1997 to May next year the amount of *n*-alkanes relative to isoprenoids was reduced, a phenomenon typical for biodegradation intensity in geochemical literature defined as "very slight" or "minimal biodegradation" [11,12].

Later, in September 1998 (sample Ic), the amount of *n*-alkanes was still smaller. Finally, during next one year, *n*-alkanes were almost completely degraded (sample Id). In a relative short period of time, from September 1999 to February 2000, the alkane fraction of the petroleum-type pollutant suffered an unexpected change (sample Ie, Figure 1). Namely, in this fraction of the pollutant new even carbon-number C₁₆ to C₃₀ *n*-alkanes were observed. It was supposed that these even carbon-number *n*-alkanes were biosynthesized by some microorganisms (*Desulfovibrio desulfuricans*, *Corynebacterium sp.*, *Escherichia coli*, *Rhizopus stolonifer* or *Penicillium sp.* [13]). Consequently, the biosynthesis of even carbon-number C₁₆-C₃₀ *n*-alkanes was tried to confirm by detailed analysis of extracts obtained from samples d and e (Figure 2 [7]). Saturated hydrocarbons, alcohols and fatty acids were analyzed by gas chromatography-mass spectrometry (GC-MS, Figure 3). A Hewlett Packard 5890 gas chromatograph was used, fitted with a SGE BPX-5 capillary column (30 m x 0.25 mm ID x 0.25 μm df; temperature program 80 °C, isothermal time 3 min, heating up to 320 °C with a heating rate of 4 °C/min). It was coupled to a Finnigan Mat 8222 mass spectrometer (mass resolution, R=1000), operating at 70 eV. Helium was used as the carrier gas (flow rate 1.5 ml/min). The most relevant peaks in the chromatograms of the fractions were identified on the basis of total mass spectra, using mass spectra data bases.

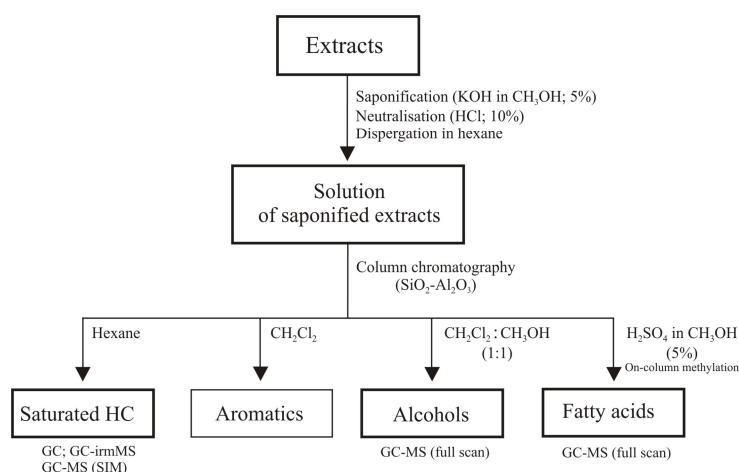


Figure 2: Separation of samples (extracts) d and e into chemical compound classes [7].
GC-irmMS: GC - isotope ratio monitoring - MS; SIM: Single Ion Monitoring; HC: hydrocarbons.

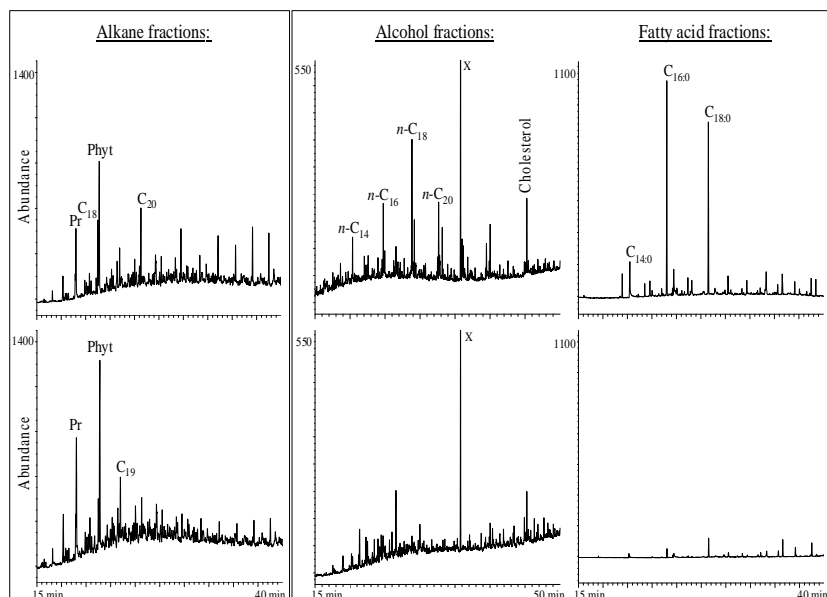


Figure 3: Chromatograms of GC-MS analyses of fractions of alkanes, alcohols and fatty acids (their methyl-esters), isolated from extracts I d (below) and Ie (above) [7].

It was found that sample Ie, containing remarkable amounts of even *n*-alkane homologues, contained, as well, in the alcoholic fraction, a homologous series of even carbon-number alcohols in a C₁₄ to C₂₀ range and a relatively significant amount of cholesterol. On the other hand, sample Id, which did not contain any significant amounts

of neither odd nor even *n*-alkane homologues, did not contain alcohols nor higher fatty acids. Even carbon-number alcohols and fatty acids observed in sample Ie were taken as a proof of the presence of particular microorganisms, i.e., of unicellular, nonphotosynthetic algae of *Pyrrophyta* type [14].

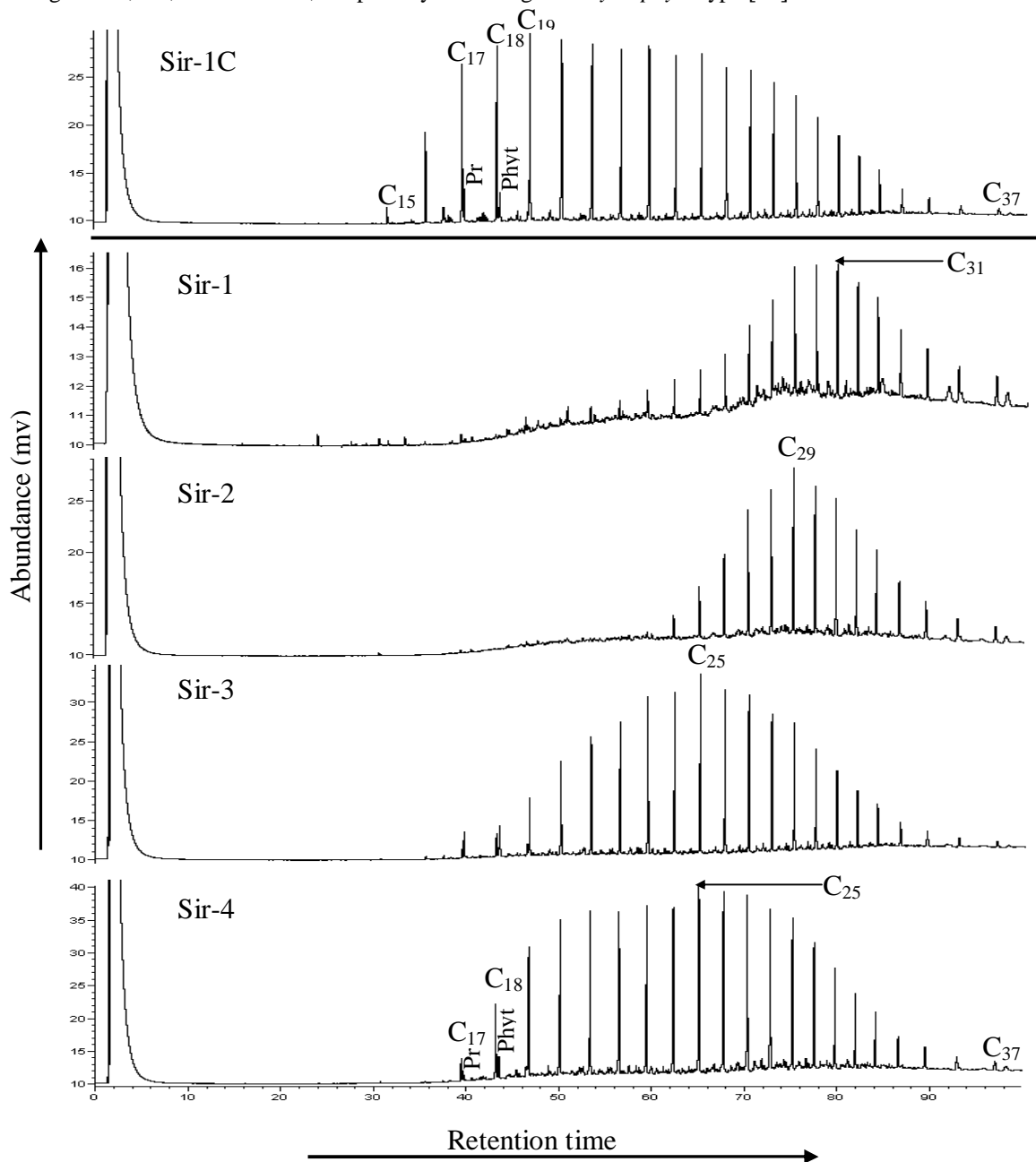


Figure 4: Gas chromatograms of the alkane fractions derived from paraffinic type petroleum of Sirakovo after 90 days of simulated biodegradation with *Phormidium foveolarum*, *Achanthes minutissima*, *Nitzschia communis* and *Chlorella communis* [8].

2.2. Bioremediation of petroleum pollutant

The fate of a petroleum-type pollutant in environmental water may be foreseen on the basis of laboratory simulation experiments of microbiological degradation of petroleum using microorganism consortiums similar to those typical for the natural environment, activated on a corresponding nutrient base. As an example, Figure 4 shows a gas chromatogram of the alkane fraction of a paraffinic-type crude oil originating from Serbia oil field (Sir-1C), and gas chromatograms of alkane fractions of the same crude oil isolated after 90 days of simulated biodegradation on an inorganic "Knop" base under daylight (Sir-1) and in absence of light (Sir-2), as well as on a "Bujon" organic base under daylight (Sir-3) and in darkness (Sir-4). The experiments were carried out with microorganisms consortium similar to that one identified as dominant in the investigated surface sewage water in

the channel of the Pančevo Oil Refinery (*Phormidium foveolarum*, *Achanthes minutissima*, *Nitzschia communis*, *Chlorella communis*) [8].

Detailed description of analytical procedure was already discussed (Figure 2). *n*-Alkanes and isoprenoid aliphatic alkanes, pristane and phytane, in the aliphatic fractions, were analysed using gas chromatography (GC). A Carlo Erba GC8000 gas chromatograph fitted with a flame ionisation detector, FID, and a ZB-5 capillary column was used. Peaks were identified on the basis of the standard mixture of *n*-alkanes by comparison of retention times.

In an experiment on a Knop base, which maximally correspond to natural conditions, by biodegradation under daylight (Sir-1), *n*-alkanes were almost completely degraded. In darkness, the degradation was less effective (Sir-2). In experiments on a Bujon base, *n*-alkanes were found to be much less degraded (Sir-3 and Sir-4).

The experiment of *ex situ* soil bioremediation was performed at the locality of Pančevo polluted with the oil type pollutant [9]. The experiments of biostimulation, bioventilation and reinoculation of autochthonous microbial consortium were carried out during 150 days period. In this case GC techniques allowed the evaluation of the efficiency of bioremediation (Figure 5). The chromatograms gave qualitative and semiquantitative information on the changes in the composition hydrocarbons in the samples. Around 50% of *n*-alkanes in the size range of C₂₉–C₃₅ were biodegraded during the first 50 days. *n*-Alkanes in the range of C₁₄–C₂₀ were degraded completely by 100 days, followed by complete degradation of C₂₀–C₃₆ by 150 days.

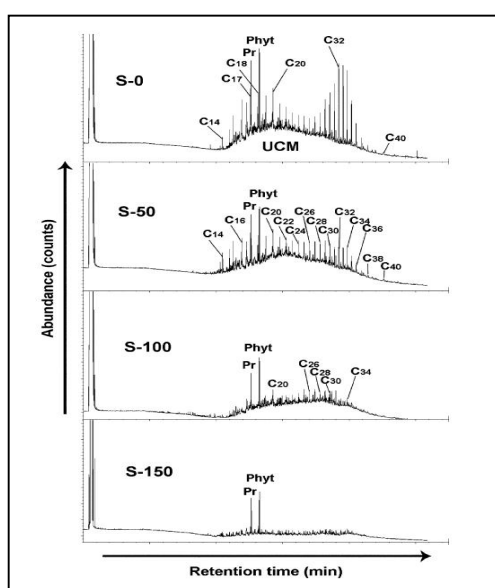


Figure 9. GC analyses of extracts during the *ex situ* bioremediation experiment after 0, 50, 100 and 150 days [9].

3. CONCLUSION

Studying the fate of petroleum type pollutants in the environment and procedures for its removal (including bioremediation is one of the most important) is impossible without the use of modern methods of instrumental analysis. In this paper an attempt was made to emphasize the importance of gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS).

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