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Međunarodna konferencija

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ENVIRONMENTAL PROTECTION

Razmena iskustava u oblastima korozije, zaštite materijala i životne sredine

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Removal of petroleum-type pollutants from the environment by bioremediation

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Studies on the environmental fate of petroleum-type pollutants remain to be an actual scientific interdisciplinary problem. Namely, petroleum and petroleum products are complex mixtures consisting of thousands of compounds that are usually grouped into four fractions: aliphatics, aromatics, nitrogen–oxygen–sulphur (NSO) compounds and asphaltenes. When crude oil or petroleum products are accidentally released into the environment, they are immediately subject to a wide variety of weathering process (evaporation, dissolution, microbial degradation, dispersion and water–oil emulsification, photooxidation, adsorption onto suspended particulate materials, and oil–mineral aggregation). The susceptibility of hydrocarbons to microbial attack is ranked in the following order *n*-alkanes > branched alkanes > branched alkenes > *n*-alkylaromates of small molecular mass > monoaromates > cyclic alkanes > polycyclic aromates > asphaltenes. Bioremediation is a technology of cleaning and remedying the soil through biological methods by means of non-pathogenic microorganisms that feed on the contaminating substances. The microorganisms are used to reduce the complexity of organic molecules (biotransformation), or for degradation to complete mineralization (biodegradation). In this paper our recent results will be reviewed observed by simulation of the natural conditions in the laboratory and by *ex situ* bioremediation.

INTRODUCTION

Transformation processes of petroleum-type pollutants in soils, recent sediments, alluvial sediments, ground and surface waters were studied by numerous authors: in coastal marine environment, in estuarine sediments, under arctic marine conditions, on the East Mediterranean coast *etc.*

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 [1-8].

Bioremediation is a technology of cleaning and remedying the soil through biological methods by means of non-pathogenic microorganisms that feed on the contaminating substances. The microorganisms are used to reduce the complexity of organic molecules (biotransformation), or for degradation to complete mineralization (biodegradation). Some defined bacterial species are able to degrade, to a limited extent, all hydrocarbons present in heavy fuel oil or oil sludge. Some of the polluting components may be dissolved only by the coupled metabolic activity of multiple genera of microorganisms. A consortium (mixed culture) of microorganisms can conduct these complex processes of degradation, while at the same time, being more resistant, on average, to changes in the ecosystem than just a single microbial species.

In this paper our recent results will be reviewed observed by simulation of the natural conditions in the laboratory and by *ex situ* bioremediation.

Simulation of the natural conditions

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 1 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*) [9].

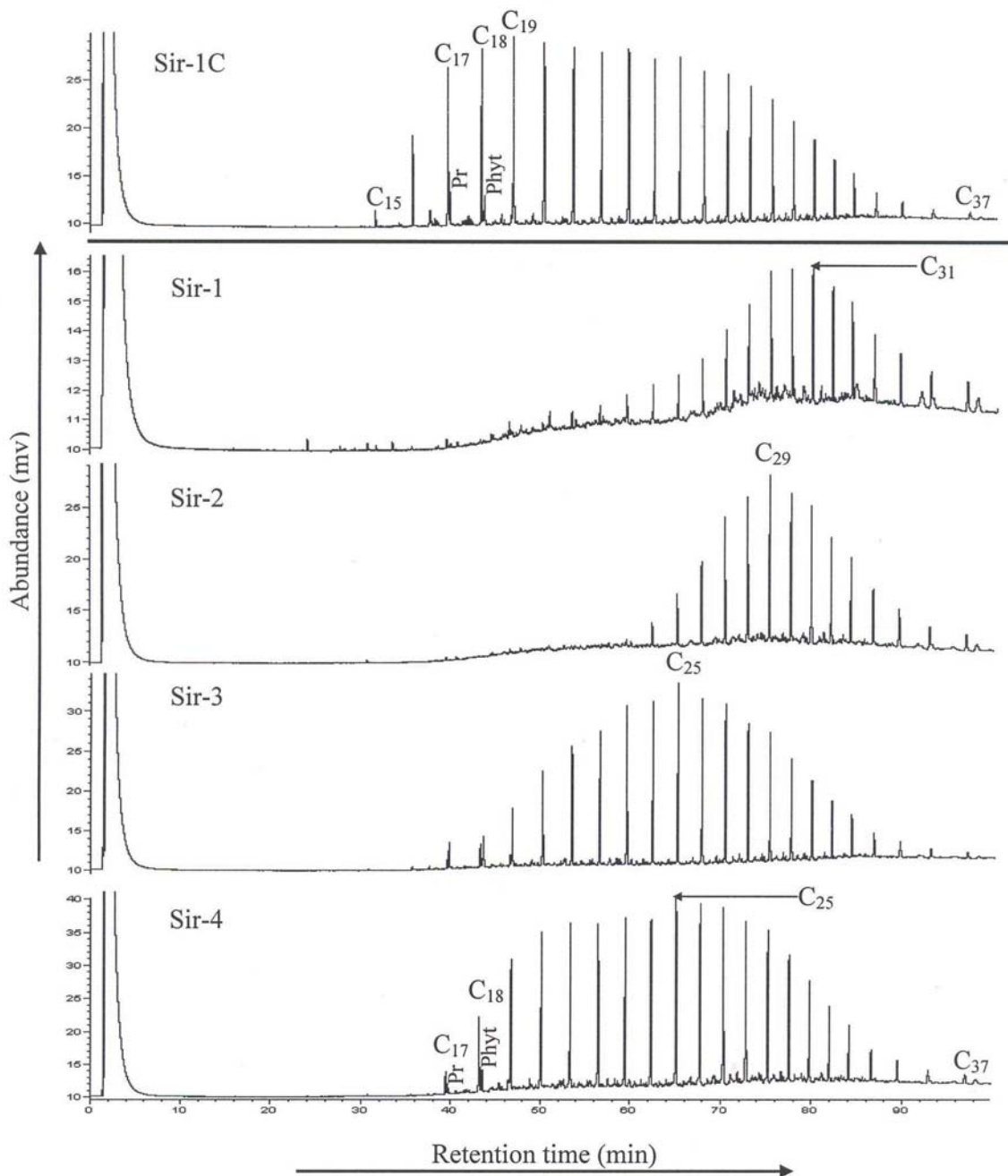


Figure 1. Gas chromatograms of the alkane fractions derived from paraffinic type petroleum of Sirakovo after 90 days of simulated biodegradation with *Phormidium foveolarum*, *Achanthes minutissima*, *Nitzschia communis* and *Chlorella communis* [9].

n-Alkanes and isoprenoid aliphatic alkanes, pristane and phytane, in the aliphatic fractions, were analysed using gas chromatography (GC). Separation of samples into chemical compound classes is shown in Figure 2 [2]. 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.

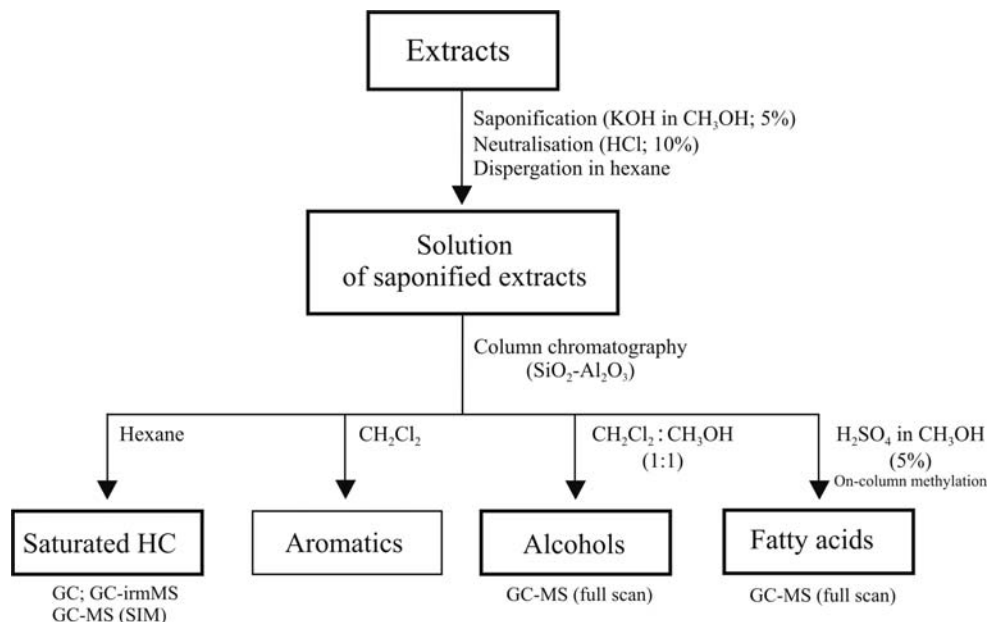


Figure 2. Separation of samples (extracts) into chemical compound classes [2].
GC-irmMS: GC - isotope ratio monitoring - MS; SIM: Single Ion Monitoring; HC: hydrocarbons.

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).

***ex situ* Bioremediation**

The example of *ex situ* soil bioremediation, performed at the locality of the Oil Refinery in Pančevo (alluvial formation of the Danube river (Serbia), polluted with the oil type pollutant is presented by Beškoski *et al.* [10]. 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 3). 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.

The experiments of biostimulation, bioventilation and reinoculation of autochthonous microbial consortium also were carried out at the locality of the Oil Refinery in Pančevo during the six-month period (May - November 2006) [11]. The changes in the quantity and composition of the pollutant, or the bioremediation effect, were monitored by analysis of the samples of the polluted soil taken in time spans of two weeks. In that way, from the beginning until the end of the experiment, 12 samples were collected and marked with P₁-P₁₂ (Pančevo 1 - Pančevo 12).

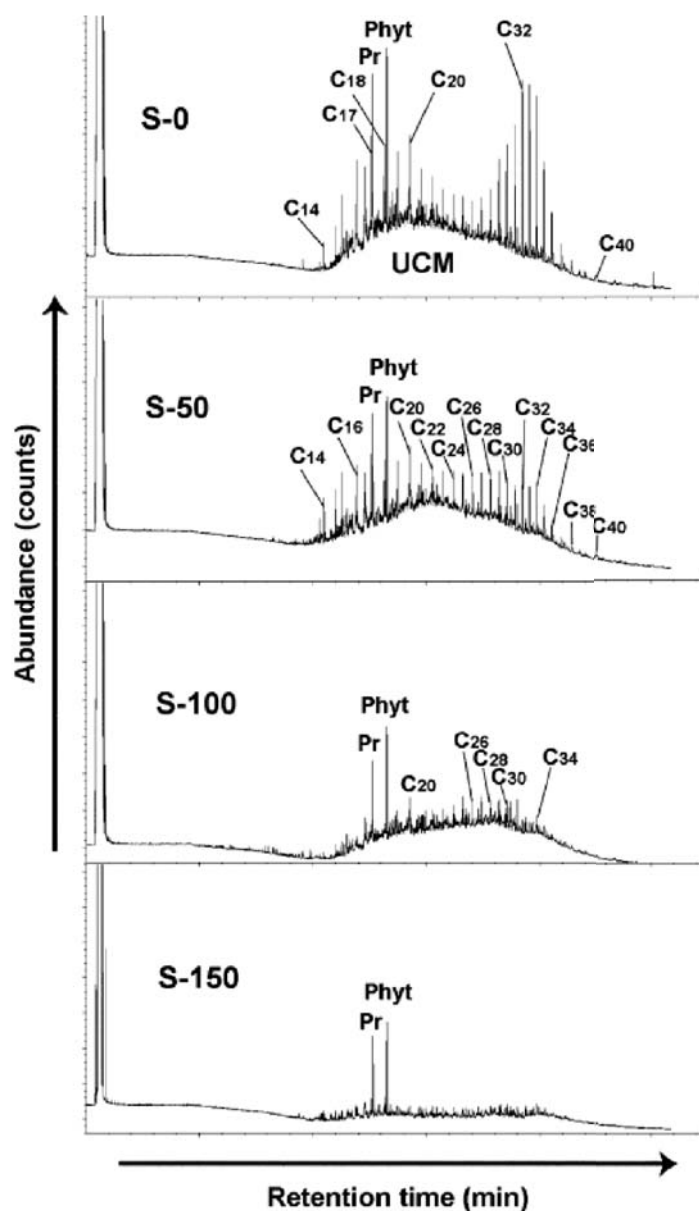


Figure 3. Chromatograms of the TPH extracts of the treated samples during the bioremediation experiment after 0, 50, 100 and 150 days; Pr: Pristane; Phyt: Phytane; [10].

The results obtained showed that more significant changes in the composition of the oil pollutant occurred only during the last phases of the experiment (P₈-P₁₂; Figure 4). The activity of microorganisms was reflected in the increase of quantity of the polar oil fractions, first of all fatty acid fractions. In that way the quantity of total eluate increased, and the quantity of the insoluble residue was reduced to minimum, by which the oil pollutant was transferred to a form which can be removed more efficiently and more completely from the soil, as a segment of the environment.

The bioremediation potential of the aerobic zymogenous microorganisms in soil (Danube alluvium, Pančevo, Serbia) for crude oil biodegradation was investigated in the paper by Šolević et al. [12]. A mixture of paraffinic types of oils was used as the substrate. The laboratory experiment of the simulated oil biodegradation lasted 15, 30, 45, 60 and 75 days. From these extracts, the hydrocarbons were isolated by column chromatography and analyzed by gas chromatography–mass spectrometry (GC–MS). *n*-Alkanes, isoprenoids, phenanthrene and its derivatives with one and two

methyl groups were quantitatively analyzed. The investigated microorganisms showed the highest bioremediation potential in the biodegradation of *n*-alkanes and isoprenoids. A considerably high bioremediation potential was confirmed in the biodegradation of phenanthrene. Low bioremediation potential of these microorganisms was proven in the case of polycyclic alkanes of the sterane and triterpane types and dimethyl phenanthrenes.

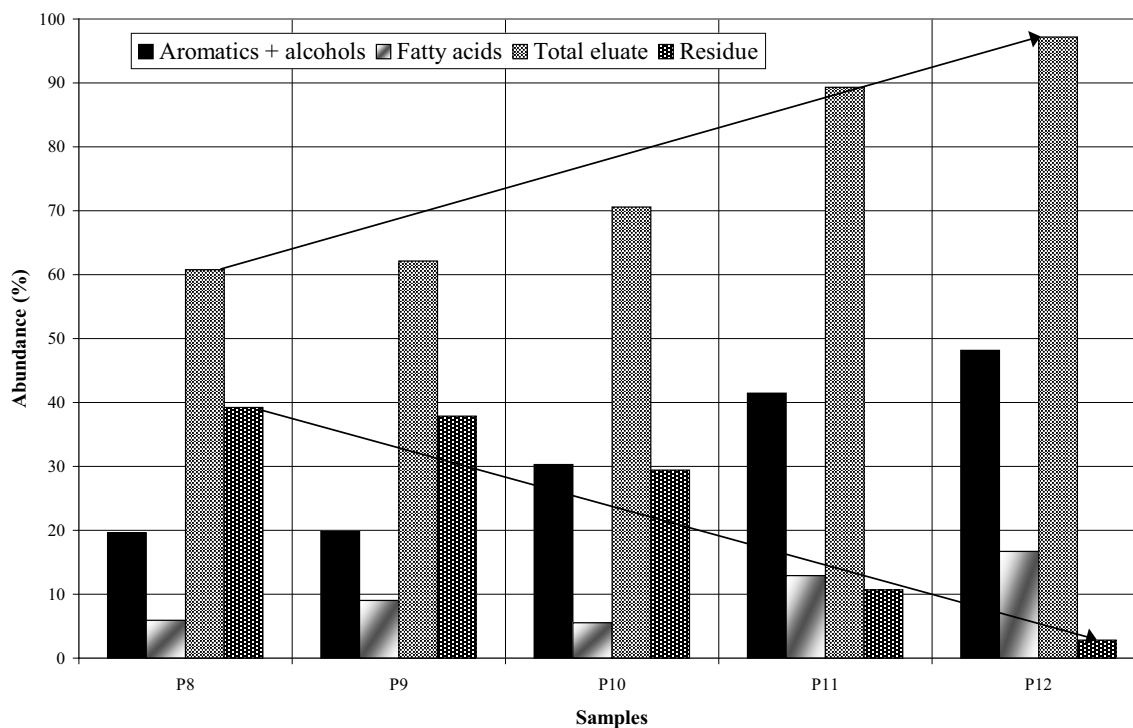


Figure 4. Contents of total aromatics and alcohols, fatty acids, total eluate and the column residue for samples P₈-P₁₂ [11].

The opposite trend of changes phenanthrene and methyl phenanthrenes was defined in paper by Novaković *et al.* [13]. During the period from September 2009. to March 2010., the biodegradation of soil contaminated with heavy residual fuel oil (mazut) was conducted. The crude oil-polluted soil was excavated contaminated soil from an energy power plant. Due to a break-down of the energy power plant, the soil had been polluted with heavy fuel oil and sediment from a heavy oil reservoir for a year.

In this study the changes in the distribution of phenanthrene and its methyl isomers (mono-, di- and tri-) during bioremediation of soils contaminated with heavy residual fuel oil (mazut) were investigated (Figure 5).

The results of bioremediation experiment of soil that was treated with biomass (re-inoculation) and nutrients (biostimulation) were compared with the results of biodegradation of soil which was not subjected to these processes of stimulation. *Pseudomonas aeruginos*, *Rhodococcus sp.*, *Pseudomonas sp.*, *Pseudomonas fluorescens*, *Sphingomonas paucimobilis*, *Pseudomonas luteola*, *Achromobacter denitrificans*, *Stenotrophomonas maltophilia* and *Aeromonas hydrophila*. cultures of zymogenous microorganisms were used.

According to these results, it can be drawn a conclusion that this process of applied bioremediation generally resulted in an increase of the concentrations of phenanthrene, but also its lower methyl "homologue" comparing to the higher homologues.

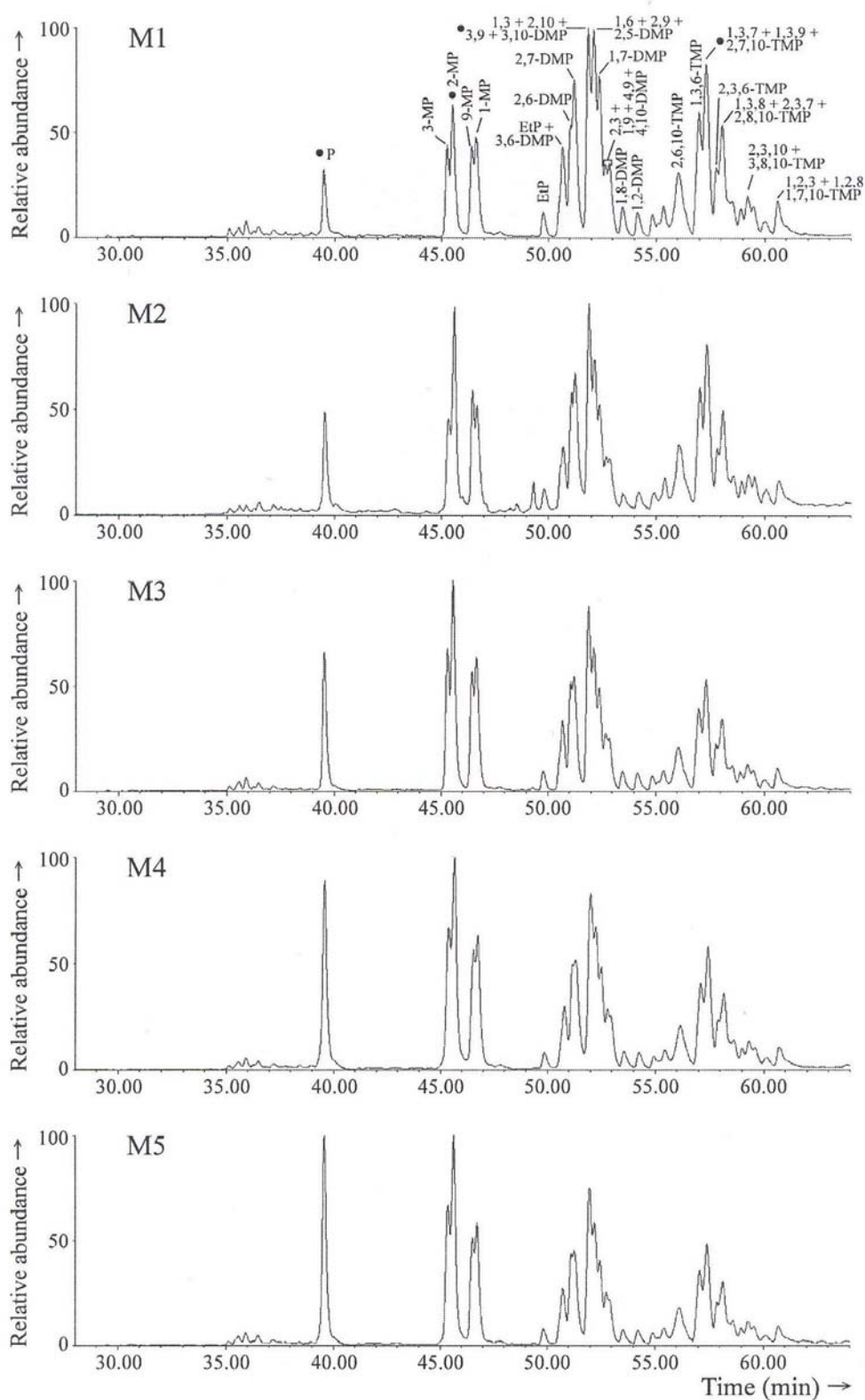


Figure 5. Mass fragmentograms of phenanthrene (P, m/z 178), methyl-phenanthrenes (MP, m/z 192), dimethyl-phenanthrenes (DMP, m/z 206) and trimethyl-phenanthrenes (TMP, m/z 220), obtained by GC-MS analysis (using the single ion monitoring, SIM method) of aromatic fractions isolated from 5 extracts of heavy residual fuel oil (the period from September 2009 to March 2010)[13].

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