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Oil pollutants in alluvial sediments – influence of the intensity of contact with ground waters on the effect of microorganisms

T. ŠOLEVIĆ[1,2], B. JOVANČIĆEVIĆ[1,2*#], M. VRVIĆ[1,2#] and H. WEHNER[3]

[1]Faculty of Chemistry, University of Belgrade, P. O. Box 158, 11001 Belgrade, [2]ICTM, Chemistry Centre, P. O. Box 815, 11001 Belgrade, Serbia and Montenegro and [3]Federal Institute for Geosciences and Natural Resources, P. O. Box 510153, Hannover, Germany

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Abstract: The influence of the intensity of interaction between oil pollutants and ground waters in alluvial sediments on the effect of microbial activity was investigated in this work. The study was based on a comparison of detailed analyses of two fractions of an oil pollutant originating from a Danube alluvial formation near the Pančevo Oil Refinery: fraction 1, separated from the aqueous layer by decantation, presumed to have been in less intensive interaction with water, and fraction 2, isolated from the aqueous emulsion by extraction with chloroform, presumed to have been in stronger interaction with water. Both fractions were shown to originate from the same type of oil pollutant. Nevertheless, significant compositional differences between the two fractions were observed. A significantly pronounced domination of even carbon number homologues of C_{18}–C_{24} *n*-alkanes in fraction 2, atypical for crude oil pollutants, compared to the corresponding distribution observed in fraction 1, suggested a more intense activity, *i.e.*, a much better effect of microorganisms in direct contact with the oil pollutant within the aqueous environment. The identification of even carbon number C_{14}–C_{18} *n*-alcohols and C_{14}–C_{18} fatty acids, as well as cholesterol, in fraction 2, suggested that microorganisms of the algal type in non-photosynthetic conditions were most probably responsible for the mentioned microbial processes.

Keywords: oil pollutants, alluvial ground waters, microbial activity, *n*-alkanes, *n*-alcohols, cholesterol, fatty acids.

INTRODUCTION

According to organic geochemical investigations, the composition of a crude oil depends on the composition and type of the organic source material, the depositional environment, thermal maturity, migration pathway and microbial degradation in reservoir rocks.[1–3] The latter was shown to occur only in the presence of water. On the other hand, investigations of the fate of oil pollutants in the environment (soil or alluvial sediment plus

* Corresponding author.
# Serbian Chemical Society active member.

ground water) have also shown that microorganisms have a significant influence on the composition of the pollutant. In both cases *n*-alkanes, the dominant components of petroleum, are preferentially removed by bacterial degradation.[4–8] However, decomposition of oil in a reservoir rock occurs within geological time (millions of years), in contrast to the decomposition of oil polutants in the environment which occurs within a relatively short time (several months to several years).

It was shown that non-photosynthetic-nutritional, unicellular, sporulating algae – *Pyrrophyta*, sometimes called "fire algae",[9] and many other types of dinoflagellates use crude oil hydrocarbons in oil polluted ground waters as a source of carbon, and degrade them by the process of β-oxidation. On the other hand, they possess the ability to biosynthesise even carbon number *n*-alkane, especially in the range C_{14}–C_{28}.[10,11] These algae accumulate *n*-alkanes in the form of lipid inclusions, often called "oil drops".[12–15]

The influence of the intensity of interaction between the oil pollutant and ground waters on the effect of microbial activity under non-photosynthetic conditions was investigated in this work. The study was based on a comparison, by detailed analysis, of two fractions of an oil pollutant: fraction 1, separated from the aqueous layer by decantation, and fraction 2, isolated from the residual aqueous emulsion by extraction. The decanted fraction was presumed to have been in weaker interaction with ground waters compared to the extracted fraction. Hence, a comparison of their chemical compositions was expected to contribute to the evaluation of the influence of the intensity of interaction between the oil pollutant and the ground waters on the effect of microbial activity under non-photosynthetic conditions.

Detailed analysis of steranes and triterpanes was performed to serve as a basis for checking the common source of both pollutant fractions. On the other hand, the isolation and analysis of alcohols, fatty acids and cholesterol was aimed at indicating the type of microorganisms supposed to be responsible for microbial attack on the oil pollutant.

EXPERIMENTAL

Samples

The sample of ground water (alluvial formation of the River Danube) severely contaminated by an oil pollutant was taken from a piezometer within the Pančevo Oil Refinery (Serbia). The sampling depth was about 3 m, corresponding to the depth of ground water – sediment contact surface. The sample was taken into a 1 L bottle in February 2000.

Analytical methods

The upper layer of lower density, marked fraction 1, was separated from the oil polluted sample of ground water by decantation. Fraction 2 of the pollutant was extracted from the remaining aqueous phase in a separatory funnel, using chloroform as the solvent.

The bulk composition (contents of alkanes, aromatics and NSO-ompounds) and the distribution of polycyclic alkanes of the sterane and triterpane types in both fractions were determined according to the scheme given in Fig. 1a. *n*-Alkanes, alcohols and fatty acids were analyzed according to the scheme given in Fig. 1b.

The bulk composition was determined by column chromatography (adsorbents: silica gel and alumina). The saturated hydrocarbons were eluted with petroleum ether and the aromatic hydrocarbons with a petroleum ether/benzene mixture (2:1). The proportion of NSO-compounds was determined by difference.

Polycyclic alkanes of the sterane and triterpane types were analyzed by gas chromatography with a mass selective detector (GC-MSD) using the single ion monitoring (SIM) method. The steranes were identi-

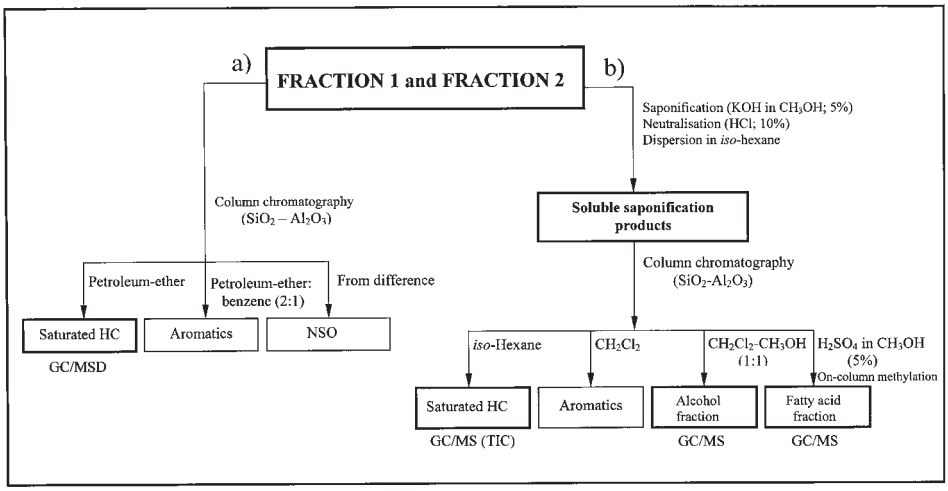

Fig. 1. Analytical procedure.

fied from *m/z* 217 and the triterpanes from *m/z* 191 ion chromatograms. A Hewlett Packard 5990, Series II, gas chromatograph fitted with a HP-5MS fused silica capillary column (temperature range: 40 – 300 ºC; heating rate: 15 ºC/min in the 40–160 ºC, and 4 ºC/min in the 160–300 ºC range) with helium as the carrier gas (flow rate 1 cm^3/min) was used. The GC was coupled to a Hewlett-Packard 5972 MSD operated at 70 eV in the 45–500 scan range.

Additional analyses of fractions 1 and 2 were carried out in order to isolate alcohols and fatty acids (Fig. 1b). The samples were saponified with 5 % solution of KOH in methanol, and neutralized (after standing overnight) with 10 % hydrochloric acid. The products were dissolved in a mixture of dichloromethane (containing 1 % methanol) and *iso*-hexane (1:40), and fractionated by column chromatography using alumina and silica gel as adsorbents. The saturated hydrocarbon fraction was eluted with *iso*-hexane, the aromatic fraction with dichloromethane, the third, alcohol fraction, with a mixture of dichloromethane and methanol (1:1), and the fourth, fatty acid fraction, with a 5 % sulphuric acid solution in methanol. The fourth fraction was additionally treated with a 20 % aqueous solution of sodium chloride and *iso*-hexane, in order to isolate the organic compounds. *n*-Alkanes in the hydrocarbon fractions were analyzed by GC-MSD (TIC). Alcohols and fatty acids were analyzed by gas chromatography-mass spectrometry (GC-MS). The fatty acids were quantified as methyl-esters formed during elution. A Hewlett Packard 6890 gas chromatograph was used, equipped with a Gerstel Cold Injection System, CIS 3, and a column splitter leading to two capillary columns coated with DB-5MS. Helium was used as the carrier gas (flow rate 1.3 cm^3/min). One column was connected with a flame ionisation detector (FID), and the other was coupled to a Finnigan MAT 95 S mass spectrometer (mass resolution $R = 1500$). The relevant peaks in the chromatograms were identified on the basis of the total mass spectra, compared with a mass spectra data base (Wiley 6th Ed.).

RESULTS AND DISCUSSION

The bulk compositions of fractions 1 and 2 are shown in Table I.

While it is generally necessary to analyse a sedimentary organic matter thoroughly and, according to its composition, estimate whether it is native or pollutants, the large quantity of organic matter in the aluvial sediment sample investigated in this paper was practical proof of its anthropogenic origin. The bulk compositions of fractions 1 and 2, *i.e.*, the characteristic domination of saturated hydrocarbons over aromatics and NSO-compounds, clearly confirmed such a conclusion.

TABLE I. Bulk composition of fraction 1 and fraction 2

Sample	Content of organic matter	Alkanes/%	Aromatics/%	NSO-compounds/%
Fraction 1	Decanted oil	40.6	28.0	31.4
Fraction 2	60 mg/L (relative to aqueous layer)	48.3	23.2	28.5

A thorough analysis of both fractions, particularly of the *n*-alkanes, was planned in order to estimate the influence of the intensity of interaction of the oil pollutant with ground water on the microbial activity under non-photosynthetic conditions. However, a prerequisite for such a study was to determine whether the two fractions did originate from the same type of oil pollutant. This was done by comparing the sterane and triterpane analyses.

Steranes and triterpanes

The observed sterane and triterpane distributions in both samples were typical for oils (Fig. 2). For example, in the case of the steranes, besides biolipid, C_{27}–C_{29} αα (20R) isomers (peaks 2, 3 and 6), geolipid isomers, such as diasteranes, as well as C_{27}–C_{29} steranes with the hydrogen atoms at C_{14} and C_{17} in the β-position and S-configurations at C_{20} (*e.g.*, peaks 1, 4 and 5), were observed. As far as the triterpanes are concerned, besides oleanane and gammacerane (O and G, Fig. 2), the thermodynamically most stable hopanes (*e.g.*, peaks a, c and e, isomer 22S) and their less stable isomers (*e.g.*, peaks b, d and e, isomer

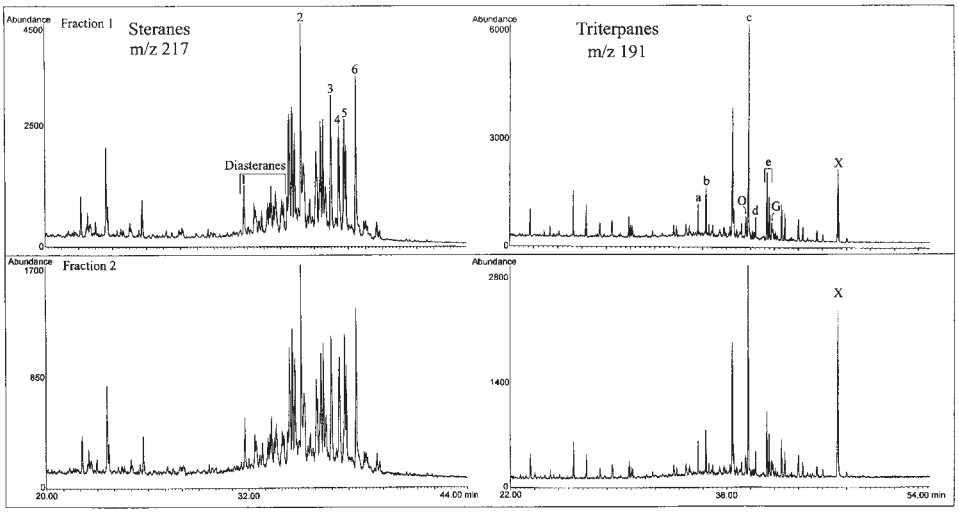

Fig. 2. Ion chromatograms of steranes (*m/z* 217) and triterpanes (*m/z* 191) of the alkane fractions isolated from fraction 1 and fraction 2. 1 – C_{27}–13β(H), 17α(H) diasterane (20S); 2 – C_{27}–C_{14}α(H), 17α(H) sterane (20R); 3 – C_{28}–14α(H), 17α(H) sterane (20R); 4 – C_{29}–14α(H), 17α(H) sterane (20S); 5 – C_{29}–14β(H), 17β(H) sterane (20R); 6 – C_{29}–14α(H), 17α(H) sterane (20R); a – C_{27}–18α(H)-22,29,30-trisnorhopane (Ts); b – C_{27}–17α(H)-22,29,30–trisnorhopane (Tm); c – C_{30}–17α(H), 21β(H) hopane; d – C_{30}–17β(H), 21α(H) moretane; e – C_{31}–17α(H), 21β(H) homohopanes (22S and 22R); O – oleanane; G – gammacerane; x – unidentified peak. (Identification of the corresponding peaks was discussed in previous papers).[16]

22R), were observed in ratios characteristic for oils.

The identical distributions of steranes and triterpanes in fractions 1 and 2 confirmed the same type of oil pollutant to be the precursor of both samples. This observation justified detailed comparative analysis of the *n*-alkanes in both samples.

n-Alkanes

The gas chromatogram of the alkane fraction isolated from sample 1 (Fig. 3a) was characterized by a pronounced domination of isoprenoid aliphatic alkanes, pristane (Pr) and phytane (Phyt). The peaks originating from *n*-alkanes were substantially smaller. Such a proportion of *n*-alkanes relative to isoprenoids is a distinctive feature of oils that had been exposed to microbial degradation of minimal to moderate intensity. However, it was debatable whether the investigated ground water was contaminated with oil that had been previously biodegraded in its reservoir rock or the process of microbial degradation had taken place in the alluvial formation.

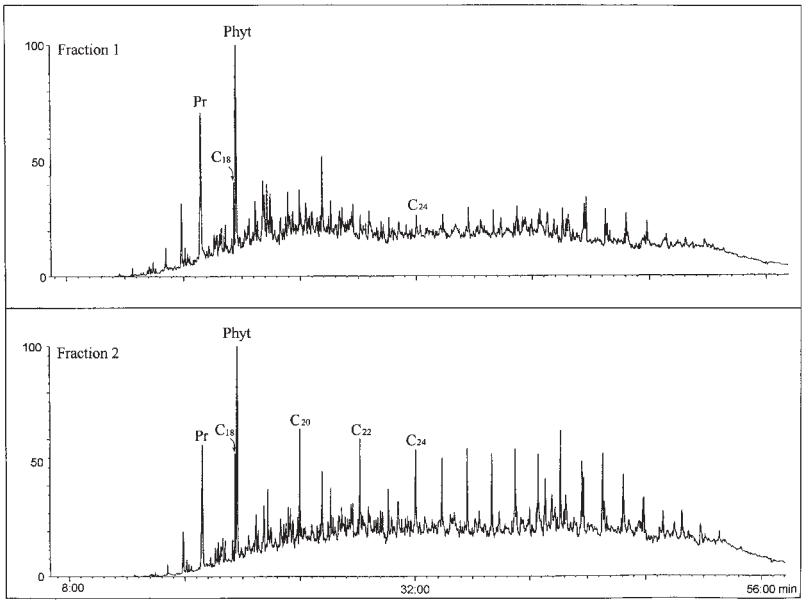

Fig. 3. Gas chromatograms of the saturated hydrocarbon fractions isolated from the oil polluted water sample (fraction 1 and fraction 2). Pr-pristane; Phyt-phytane.

The gas chromatogram of alkane fraction isolated from sample 2 (Fig. 3b) was also characterized by a domination of the isoprenoids, pristane and phytane but, in addition to this, peaks originating from C_{18}–C_{24} even-carbon number homologues of *n*-alkanes (in contrast to odd carbon number homologues) were atypically outstanding. Hence, clear differences were suggested between the distributions of *n*-alkanes in fractions 1 and 2 (Fig. 3).

It was presumed that this difference resulted from different intensities of microbial activity to which fractions 1 and 2 of the oil pollutant had been exposed. Therefore, the alco-

hols and fatty acids in both fractions were analyzed in order to explain the source of the dominance of even carbon number *n*-alkanes.

Alcohols and fatty acids

Distribution of alcohols and fatty acids isolated from fractions 1 and 2 are shown in Fig. 4.

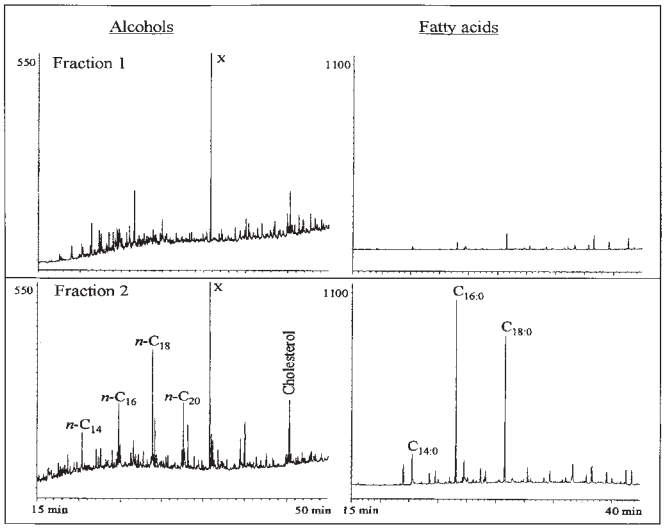

Fig. 4. Chromatograms of GC-MS analyses of alcohols and fatty acids isolated from fractions 1 and 2. X – unidentifies peak.

Distinct differences between fractions 1 and 2 were observed in the composition of alcohols and fatty acids. The chromatogram of fraction 2 alcohols is dominated by even carbon number *n*-alcohols (C_{14}–C_{20}; Fig. 4). Furthermore, the cholesterol peak was also pronounced. On the other hand, *n*-alcohols were not observed in noticeable amounts in fraction 1 and the cholesterol peak was less expressed. Likewise, the fatty acids isolated from fraction 2 were dominated by even carbon number homologues of *n*-fatty acids (C_{14}–C_{18}). In fraction 1 these fatty acids were observed only in minor concentrations (Fig. 4).

Hence, in addition to the even carbon number *n*-alkane dominance, fraction 2 differed from fraction 1 by the presence of even carbon number *n*-alcohols (C_{14}–C_{20}), a higher proportion of cholesterol and a higher content of even carbon number *n*-fatty acids (C_{14}–C_{18}). The identified acids and alcohols are known to be characteristic of procaryotic microorganisms (bacteria) as well as eucaryotic microorganisms – algae.[11] However, cholesterol, which was more pronounced in fraction 2 than in fraction 1, is not typical for bacteria. This fact suggests that algae (*e.g.*, the mentioned dinoflagellates – *Pyrrophyta*) might be responsible for the transformation of the pollutant *n*-alkanes into dominant even carbon number *n*-alkanes, as well as the appearance of the other microbial degradation products observed in the investigated ground water.

CONCLUSIONS

Two fractions of a piezometer oil pollutant sample (from an alluvial sediment near the Pančevo Oil Rafinery), *i.e.*, fraction 1, obtained by decantation of the oil layer, and fraction 2, representing the extract from the residual aqueous layer, were analyzed in detail in study, with the aim of estimating the influence of the intensity of their contact with ground water on the effect of microorganisms. Both fractions were shown to originate from the same type of pollutant.

A more pronounced domination of even carbon number homologues of C_{18}–C_{24} *n*-alkanes, as well as a higher amount of other metabolites in fraction 2 suggested a more intense activity of microorganisms in direct contact with the oil pollutant in the aqueous environment. Identification of even carbon number *n*-alcohols and fatty acids, as well as cholesterol, in fraction 2, indicated that microorganisms of the algal type under non-photosynthetic conditions were most probably responsible for the microbial attack on the oil pollutant. The relatively low concentration of metabolites observed in the aqueous layer suggests the latter processes were rather restricted, probably partly due to a limited contact of oil pollutant with the ground water, resulting, among other factors, from the lack of turbulence in this type of sediment.

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ИЗВОД

НАФТНИ ЗАГАЂИВАЧИ У АЛУВИЈАЛНИМ СЕДИМЕНТИМА – УТИЦАЈ ИНТЕНЗИТЕТА КОНТАКТА СА ПОДЗЕМНИМ ВОДАМА НА ДЕЈСТВО МИКРООРГАНИЗАМА

ТАТЈАНА ШОЛЕВИЋ[1,2], БРАНИМИР ЈОВАНЧИЋЕВИЋ[1,2], МИРОСЛАВ ВРВИЋ[1,2] и HERMANN WEHNER[3]

[1]Хемијски факултет, Универзитет у Београду, п. пр. 158, Београд, [2]Центар за хемију ИХТМ, п. пр. 815, Београд, Југославија и [3]Federal Institute for Geosciences and Natural Resources, P. O. Box 510153, Hannover, Germany

Процењиван је утицај интензитета интеракције између нафтног загађивача и подземне воде алувијалне формације на интензитет микробиолошког дејства. У том циљу поређени су састави две фракције нафтног загађивача из једног пијезометра (круг Рафинерије нафте Панчево, алувијална формација реке Дунав): фракције 1, одвојене од воде декантовањем (слабија интеракција са водом) и фракције 2, издвојене из воде екстракцијом (јача интеракција са водом). Доказано је да обе фракције воде порекло од истог нафтног загађивача. Знатно већа обилност нормалних алкана са изразитом доминацијом парних хомолога (C_{18}–C_{24}) у фракцији 2 него у фракцији 1 указује на интензивнију активност микроорганизама у воденој средини. Алкохоли нормалног низа са парним бројем угљеникових атома (C_{14}–C_{20}), холестерол и масне киселине нормалног низа са парним бројем угљеникових атома (C_{14}–C_{18}) у фракцији 2 доказ су да су за микробилошку активност у нефотосинтетичким условима одговорни микроорганизми алгалног типа.

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