ANALYSIS OF PETROLEUM HYDROCARBONS IN SOIL FROM VIEW OF BIOREMEDIATION PROCESS

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ABSTRACT

The pollution of the environment by petroleum hydrocarbons is the most often pollution of them all. Nevertheless, hydrocarbons present in environment can be not only of petroleum or anthropogenic origin, but of biogenic as well. Typically the hydrocarbons are presented in the environment as very complex mixtures of individual compounds with very different chemical structure, wide range of the boiling points ($\approx$800 ^{o}C) as well as with the wide range of the number of carbon atoms. Immediately they are spread in any environmental matrix the complex physical, chemical and biochemical reactions start. A lot of methods have been developed and new are permanently in progress for the monitoring and control of petroleum hydrocarbons contamination and/or soils bioremediation. Generally, all methods by whose the hydrocarbons contaminants are determined in GC-FID system do not satisfied recommendations for enough accurate and precise results. Hyphenation of capillary gas chromatography and mass selective detector operated in the selective ion monitoring mode essentially allows detailed specification of non-polar extractable hydrocarbons. Isoprenoid alkanes, alkylhomologues of aromatic hydrocarbons and polycyclic alkanes hopanes-like were investigated as markers for recognition of petroleum and biogenic contamination. $C_{30}17\alpha(H)21\beta(H)$-hopane ($C_{30}$-hopane) seems to be a suitable marker to identify hydrocarbons origin, to determine composting rates for nonpolar extractable compounds and to calculate real content of non-polar extractable compounds in final composting status on the assumption that the contamination is of mineral oil type. This is the survey into the results obtained in this field published in the literature as well as reached in our laboratory.

Key words: environment, soil, petroleum hydrocarbons, remediation

INTRODUCTION

Environmental contamination by petroleum-products from variety of sources including leaking fuel storage and crude oil spills during production, transport and distribution of petroleum products represents one of the most frequent contamination. Crude oil and oil-spill-related samples represent extreme complex mixtures in which the components range from simple alkanes to complex asphaltenic compounds the boiling points of which vary in a wide range from a few, to of several hundred degrees and the range number of carbon atoms is more than 100. As soon as oil is spilled into the environment immediately started the process of volatilization, dissolution microbial and photochemical degradation of original oil composition resulting to the change of this composition. Because soil pollution represents a hazard to water supplies and health there were developed criteria for assessing of such contamination and the technology to deal with the contaminants. Typically among such criteria the standard analytical methods and environmental limits for the assessment of pollutants are defined[1]. There are the characteristic definitions of analyzed pollutats. The dried extract of the soil samples is defined as "total solvent extractable materials" (TSEM) or "oil and grease" (O&G). After the extract treatment with a silica gel for removing polar compounds the concentration of the "total petroleum hydrocarbons" (TPH) is measured.
Hydrocarbons contamination vary in amount and content horizontally as well as vertically in the soil profile. It is therefore difficult to establish a minimum concentration below which the soil may be considered uncontaminated for any particular contaminant. The another problem is the origin of any hydrocarbons in the soil.
In the Slovak Republic the hydrocarbon contamination of soil, water, and wastes is assessed by content of so called “nonpolar extractable compounds (NEC)” according the directive of the Ministry of Environment of Slovak Republic [2]. However, the threshold values and standards accepted for hydrocarbon contaminants in some EC countries [3] differ from those accepted in the Slovak Republic. The comparison of such standards accepted for hydrocarbons contaminants in the Netherlands and in the Slovak Republic is given in Table I. There is the discrepancy between the definition of assessed contaminants as well as between the quantitative limits. EC standard considers contamination by fuels and mineral oil individually, i.e. qualitatively well defined petroleum products. Slovak standard considers qualitatively uncertain group of compounds called “nonpolar extractable compounds”. The EC threshold value above which a soil clean-up is necessary (limit C) for mineral oil, that is the most common pollution, is almost five times higher than Slovak criterion defined as a sum of NEC. It looks like the Slovak Republic being almost five times more strict for assessing such pollution than the Netherlands. In practice this discrepancy is source of legislative, economical as well as technological problems during assessing the environmental damages, environmental risks and the results of clean-up processes.

Table I. Standards adopted in the Netherlands (NL) and the Slovak Republic (SR) for soil hydrocarbons contamination (in mg.kg^{-1} dry weight)

Petroleum Products	Limits					
	A		B		C	
	NL	SR	NL	SR	NL	SR
Fuel	20	/	100	/	800	/
Mineral oil	100	/	1000	/	5000	/
Nonpolar extractable compounds (total)	/	50	/	500	/	5000

Further, these limits often are related only to contaminants being descendant from petroleum source. However, hydrocarbons present in environment can be not only of petroleum or anthropogenic origin, but of biogenic as well. Relatively high content of "non-polar extractable compounds" is determined in materials, such as mould, wood, various sludges and wastes. Green plants, including tree and bush leaves, synthethize alkanes, mainly straight-chain alkanes. As published [4], plant leaves and flowers contain of 140 to 1700 mg.kg $^{-1}$ of alkanes. Natural-derived hydrocarbons are produced as from terrestrial plants as phytoplankton in water. The latter forms hydrocarbons in amount of 300 to 15 300 mg.kg^{-1} and there are of 4 800-57 000 mg.kg^{-1} in cultivated algae. Generally, even if only 10-20% is considered leaking into atmosphere, it corresponds to about the same amount arising from municipal traffic and petroleum processing [4].

Boiling point range of hydrocarbons from biogenic sources is within the middle petroleum distillates region from C_{20} to C_{33}. Generally, odd-chain alkanes are more abundant than even-chain alkanes and branched alkanes and straight alkanes ratio is smaller than in the distribution of petroleum-derived hydrocarbons. The biogenic hydrocarbons usually show more simple chemical composition in comparison to hydrocarbons originating from the petroleum-derived sources [5,6].

Wide hydrocarbon distribution is typical of petroleum inputs and the presence of an unresolved complex mixture (UCM) of hydrocarbons, which shows up as a baseline rise in a chromatogram, is usually considered the convincing indicator of petroleum contamination.

Triterpane squalane $C_{30}H_{62}$ formed from isoprenoid alkene squalene is accounted the characteristic biogenic substance. Coming under this group other alkanes, for example phytol (chlorophyll) derived phytane and one carbon atom number smaller pristane[7] are quite abundant constituents of many natural materials[8]. However, they are also present in most petroleum sources, usually as major constituents of a much wider distribution of isoprenoid alkanes, and thus are often considered as indicators of petroleum contamination. The ratio of pristane to phytane varies between oils and in certain cases can be used to identify the source of an oil spillage[9]. Since isoprenoid alkanes exist in oils as diastereoisomers produced from thermally induced isomerisation of the initial single biological isomer, the presence of all isomers indicates petroleum contamination. Nevertheless, the separation of diastereoisomers requires employing an extremely efficient capillary column (of 3-6 x 10^5 effective plates)[10], but this approach is not appropriate for the common analyses.

Aromatic hydrocarbons provide the further opportunity to identify contamination from the petroleum and biogenic sources. Only individual alkylbenzenes were observed in the biogenic-originated samples, for example from limonene 1-methyl-4-isopropylbenzene is formed in wastewater environment, and only individual polycyclic aromatic hydrocarbons, for example under reduction conditions perylene originates from plant material. Hellmann[4] published total amount of polycyclic aromatic hydrocarbons in plant leaves and flowers of 140-850 μg.kg^{-1} dried material. Unlike petroleum PAH distribution in biogenic as well as anthropogenic sources only unsubstituted polycyclic aromatic hydrocarbons are present and alkylhomologues of naphthalene, phenantrene, antracene, and higher polycyclic aromatics absent.

Polycyclic alkanes like hopanes, steranes, diasteranes and aromatic steroid hydrocarbons, along with compounds mentioned above, belong to the petroleum hydrocarbon markers. Polycyclic triterpanes are appropriate for the differentiation of petroleum and biogenic contamination because of their relatively high content in petroleum and high resistency

against microbial and photochemical degradation. Triterpanes are distributed in a wide range with C_{30}-hopane being usually prominent[6,11].

During the last years a large progress has been done in oil-spill clean-up techniques including physical, chemical and biological methods. Among these techniques bacterial degradation of petroleum hydrocarbons has been widely recognised. In situ bioremediation of soil contaminated with petroleum residues is based on the stimulation of the microbial transformation of contaminants by adding oxidants and nutrients. Ideally, this technique leads to the complete mineralization of contaminants[12]. However, particularly in anaerobic zones of soil the efficiency of this technique is poorly documented and the fate of the hydrocarbons remains often unknown.

The hydrocarbon biodegradation in contaminated soils is dependent upon the demands as follows: the presence of hydrocarbons degrading bacteria, the creation of optimal environmental conditions for biodegradative activity, the predominant petroleum hydrocarbon types in the contaminated matrix, the bioavailability of contaminants to degradative bacteria. In addition, the measured degree of hydrocarbons biodegradation is also dependent on the analytical methods employed to quantify the contaminant concentrations[13]. Regularly, the assessment of the efficiency of in situ bioremediation together with the decision whether or not the quality criteria are met are solely based on the analysis of residual petroleum hydrocarbons determined in total content of NEC or TPH.

As it is apparent from the facts mentioned above the recognition of biogenic-derived hydrocarbons and/or "nonpolar extractable compounds" and xenobiotics is important task from aspect of both decontamination and composting processes.

According to these facts as well as considerable economic problem with respect, that the final soil clean-up and composting statuses have to meet the exacting C limit adopted in Slovak Republic, analytical method allowing differentiation or more detailed specification of chemical composition of nonpolar extractable compounds is required.

A lot of methods have been developed and new are permanently in progress for such purpose[1]. The accuracy of soil contamination evaluation depends on representative sampling, proper preservation of samples, the effectiveness of pollutants extraction, and the performance of the laboratory analysis.

The extraction procedures include Soxhlet, ultrasonic, and the most recently developed supercritical fluid techniques. The physical state of contaminants in soil is one of the main factors determining the extraction recovery. They may be present in the soil in a very complex status[14]. The extraction of contaminants adsorbed on the soil particles and diffused into the soil particle is controlled by diffusion and sorption-desorption processes. It is time dependent and problematic. To evaluate the effectiveness of an extraction method, the addition of surrogate standard is often used. However, spiked analytes are typically extracted more easily than native analytes that were formed in the sample matrix or have been long-term exist under the environmental conditions. Since the concentration of native contaminant cannot be known in the real sample, there is no direct way to determine extraction efficiency[15]. Therefore, recovery data for real samples are generally based on the comparisons with the standard extraction methods, usually Soxhlet extraction or sonication. Both of these methods are time consuming and require the use of solvent, which creates disposal problems. Supercritical fluid extraction can be used as an effective freon and/or other extraction solvents alternative for determination of hydrocarbons in soil.

Environmental samples contain at least some water, which may either help or interfere with extraction process[16]. Drying samples with a drying agent can leads to substantial loss of volatile and semi-volatile analytes, since mixing a wet sample with drying agent can cause

substantial heating of the sample and significant loss of contaminants, i.e. tetradecane and more volatile species. Similarly, even drying the extraction solvent at room temperature can also lead to substantial losses of analytes[15].
As it was already mentioned above the major advances have been achieved in the analytical methods and techniques used for analyses of oil contaminated soils. There are many analytical techniques applicable to analysis of petroleum residues in soil[1]. The most frequently used analytical methods include gravimetry, infrared spectroscopy, gas chromatography and mass spectrometry. Based on these methods there are three classes of laboratory analyses of hydrocarbon contaminants. The first include standard methods for the sum concentration measurement of compounds representing the class of compounds e.g. total petroleum hydrocarbons - TPH. The second class include group analysis (e.g. saturates) and/or hydrocarbons typing (e.g. pentacyclic saturates). The last one is the target individual compound analysis measuring characteristics and concentration of specific compounds (e.g. C_{30} - 17α(H)21β(H) - hopane). The recognition of biogenic, anthropogenic, and petroleum hydrocarbons is essentially possible using high performance capillary gas chromatography coupled with a mass selective detector operated in SIM mode[4, 6, 11,17-19].

EXPERIMENTAL

There are the materials, reference materials, sample treatment methods and analytical methods characteristic for this type of environmental analyses and used in our laboratory.

Materials

Diesel oil and mineral oil contaminated soils (real samples) were used. Uncontaminated soils samples from the same regions were used as the reference samples. Sawdust, paper mill waste, straw, heater, tradescantia and agate leaves samples were used as the source of biogenic hydrocarbons.
Biogenic materials used in composting of oil and biological sludges (straw, bark, lop), initial biological and oil sludges, and samples prepared mixing these materials at starting and final composting status were used as model mixtures. Six composting variants have been studied:

Variant I (biological sludge: oil sludge: straw = 15.18:10.71:74.11),
Variant II (biological sludge: oil sludge: straw: lop = 15.59:11.01:55.05:18.35),
Variant III (biological sludge: oil sludge: straw: bark = 12.89:10.61:42.42:34.1),
Variant IV (average mixture from Variants I-III),
Variant V (biological sludge: straw = 21.14:78.86) and Variant VI (biological sludge: wooden shavings = 22.35:77.65).

Diesel oil, mineral oils (No R953 and No R932) (Slovnaft Ltd.) were used as the reference materials of petroleum fractions.

C_{30}-17β(H)21α(H)-hopane (Chiron AS, Trondheim, Norway) was used as the standard reference material for the determination of C_{30}-17α(H)21β(H)-hopane.

Liquid extraction and column chromatographic fractionation

Dried samples (1-10 g) were extracted using 50 ml distilled acetone thoroughly shaking for 15 minutes and subsequently a 100 ml distilled isohexane was added and shaked for next 15 minutes. Resulting slurry was left for 3 days while the equilibrium was achieved and then passed through a filter paper. Obtained extractants were divided into two equal portions. The first part was concentrated to the final volume of 1 ml using vacuum rotary evaporator and extractable compounds (TSEM) and naphthalene homologues were measured. The rest portion was brought to almost dryness using vacuum rotary evaporator, a 1 ml isohexane was added and quantitatively transferred onto the silica gel column using an additional 4 ml isohexane to complete the transfer. A chromatographic column was plugged with glass wool at the bottom and rinsed with isohexane. The column was packed with 4 g of activated silica gel (180 °C, 6 hours) with tapping to settle the silica gel. 50 ml hexane was used to elute the nonpolar extractable compounds out of the column. The hexane fraction was concentrated to the volume of 1 ml using vacuum rotary evaporator and used for analysis of nonpolar extractable compounds (TPH) and hopanes, respectively.

Capillary gas chromatography

GC measurements were performed on a Hewlett Packard (HP) 5890 gas chromatograph equipped with an HP 7673 autosampler, and a flame ionization detector for extractable and nonpolar extractable compound analysis, and mass spectrometric detector HP 5971A operated in selective ion monitoring mode for analysis of naphthalene alkylhomologues (m/z 128, 142, 156, 170, 184), respectively. An 8.5 m x 0.32 mm x 0.25 μm DB-5 fused silica capillary column (J&W Scientific, Folsom, CA) was used. The carrier gas was helium with inlet pressure of 40 kPa and linear velocity of 70 $cm.s^{-1}$ at 40 °C . The injector and detector temperatures were set at 350 °C. The following temperature program was used: initial temperature of 40 °C, ramp to 340 °C at 15 °C. min^{-1} then 6 min hold for GC-FID measurements of extractable and nonpolar extractable compounds and initial temperature of 60 °C to 300 °C at 15 $°C.min^{-1}$ for GC-MSD measurements of naphthalene alkylhomologues, respectively. A 2-μl aliquot was injected in the splitless mode with a 1 min purge off. A Trace Gas Chromatograph 2000 Series (Thermoquest, CE Instruments) fitted with a mass selective detector Voyager (Finnigan) in the selective ion m/z 191 monitoring was used for hopane measurements. An 8 m x 0.32 mm x 0.4 μm OV-1 fused silica capillary column was used. The carrier gas was helium with inlet pressure of 70 kPa and linear velocity of 120 $cm.s^{-1}$ at 80 °C. The injector and detector temperatures were set at 330 °C. The following temperature program was used: initial temperature of 80 °C, ramp 1 to 200 °C at 20 $°C.min^{-1}$ and ramp 2 to 300 °C at 10 $°Cmin^{-1}$, then 10 min hold. The chromatographic column was connected with mass detector through a 1 m x 0.1 mm restrictor heated to 330 °C.

SURVEY RESULTS

DETERMINATION OF SUM CONCENTRATION

The extraction methods for soil sample preparation as well as analytical methods for assessing soil contaminated with petroleum products were standardized[1]. These methods

are based on gravimetry [EPA 9071], infrared spectroscopy [EPA 418.1] or gas chromatography of soil extract [EPA 8015 Modified]. Determination of sum hydrocarbon contamination is standard and most common procedure. According to the scheme in Figure 1[13], the extract is dried to obtain gravimetric "total solvent extractable materials" (TSEM) or "oil and grease" (O&G) concentrations. After the extract treatment with a silica gel for removing polar compounds the concentration of the "total petroleum hydrocarbons" (TPH) is measured by IR or after evaporation of extraction solvent gravimetricaly. The difference of concentrations "O&G" and "TPH" characterizes the concentration of polar compounds in the analyzed sample. In comparison with the definition of the Slovak standard NEC ("nonpolar extractable compounds") TPH parameter is the most close related even if there is the qualitative difference (petroleum hydrocarbons vs. extractable compounds).

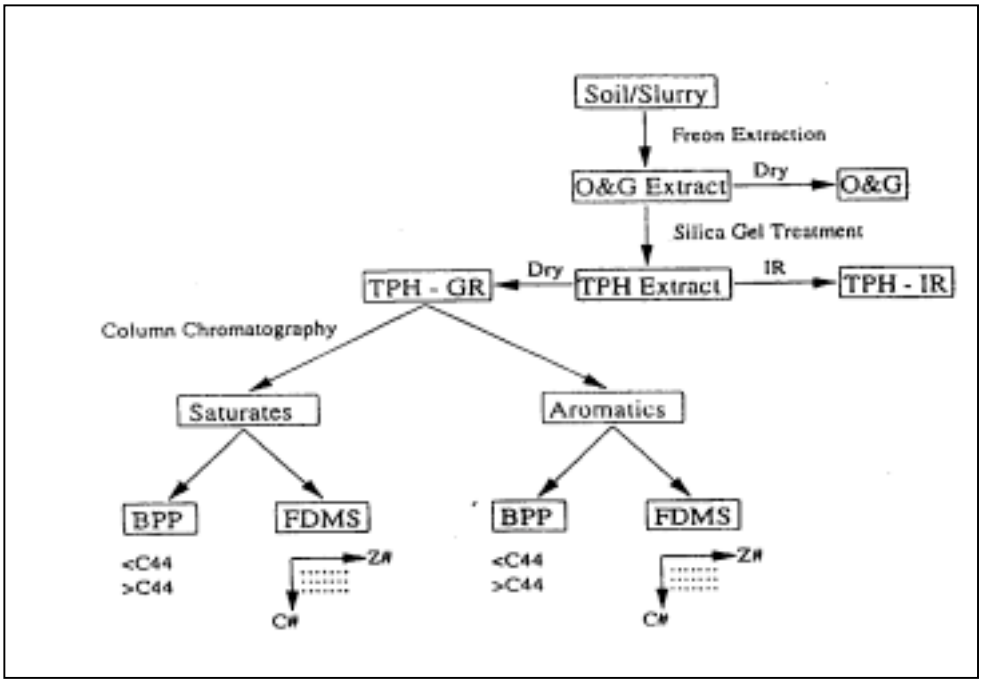

Figure 1. Comprehensive analytical characterization procedure for petroleum hydrocarbons in contaminated soils or slurries[13]

Significant concentrations of polar compounds were determined also for refined petroleum products such as pure diesel oil. These results probably reflect non-selectivity of silica gel treatment of extracts, that silica gel removes also non-polar hydrocarbons such as certain aromatics together with the non-selectivity of used analytical methods (gravimetry and infrared spectroscopy)[13].

The published results for closed land farming bioremediation of soils and slurries contaminated with various types of petroleum products and at different treatment conditions[13] enable to form some conclusions. "O&G" data show significant differences in concentrations determined in two laboratories. The NEC/TPH values obtained gravimetrically and by infrared spectroscopy are also significantly different. These differences can

be related to heterogeneous character of the soil and physical state of contaminants in soil. The extraction of contaminant adsorbed on the soil particles and diffused into the soil particles is problematic. Another cause of these differences can be the loss of volatile and semi-volatile hydrocarbons during evaporation of solvent extracts as it follows from the published data[15]. The gravimetric NEC/TPH procedure accounts only non-volatile hydrocarbons however using infrared NEC/TPH method both volatile and non-volatile hydrocarbons are measured. Moreover, TPH-IR data can be strongly affected by the composition of the calibration standard and the relative fractions of aromatics versus saturates in the NEC/TPH extract.

Recently, for quantification of the NEC/TPH extract mostly gas chromatography has been used. GC is preferred for lower quantitation limit, obtaining simulate distillation profile, and capability to identify petroleum fractions. This capability is documented on the chromatogram of separation of diesel fuel and paraffin oil in Figure 2[20].

Problematic is elution of higher molecular weight hydrocarbons from the column. Nevertheless contemporary high temperature GC allows separate hydrocarbons with number of carbon atoms to approximately 110 which are present in heavy petroleum products[21]. The example of such HTGC analysis is in Figure 3.

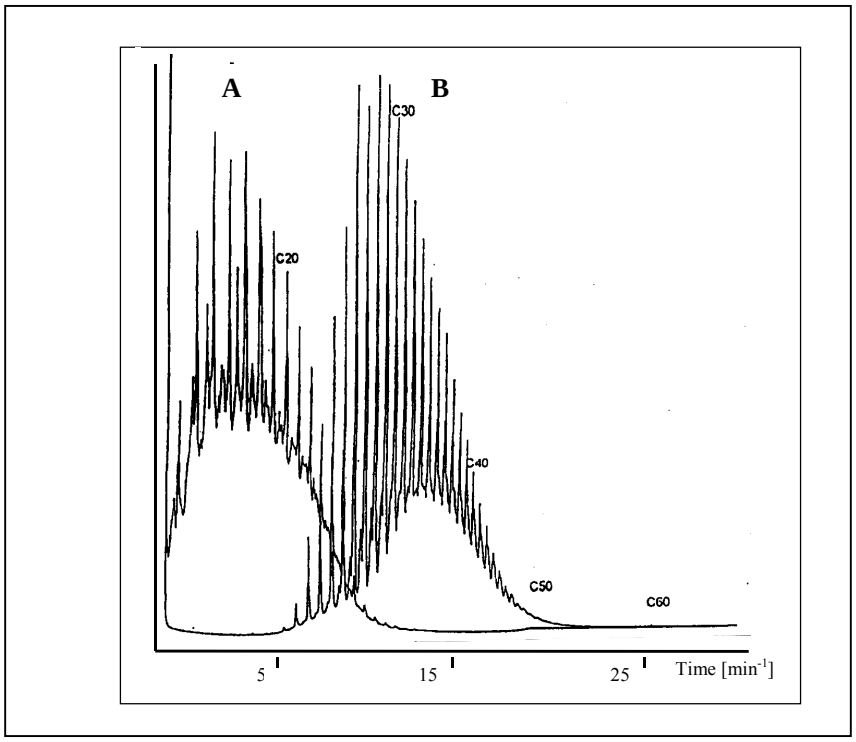

Figure 2. GC chromatogram of diesel fuel (A) and paraffine wax (B)

Published data[13] show, that hydrocarbons were degraded in range approximately of 50 up to 90 %, when high degradation degree is valid for lighter oil fractions such as diesel oil. The remaining concentration of total petroleum hydrocarbons residues after bioremediation is substantially higher than threshold C value in Table I. The other results for in situ

bioremediation of soil contaminated with lower concentrations of petroleum products are presented in Table II[22]. In both cases, maximum biodegradation efficiencies are similar (approx. 90 %), however only in the case of lower soil contamination the clean-up procedure leads to residues hydrocarbon concentration in soil smaller than threshold C value of Slovak standard.

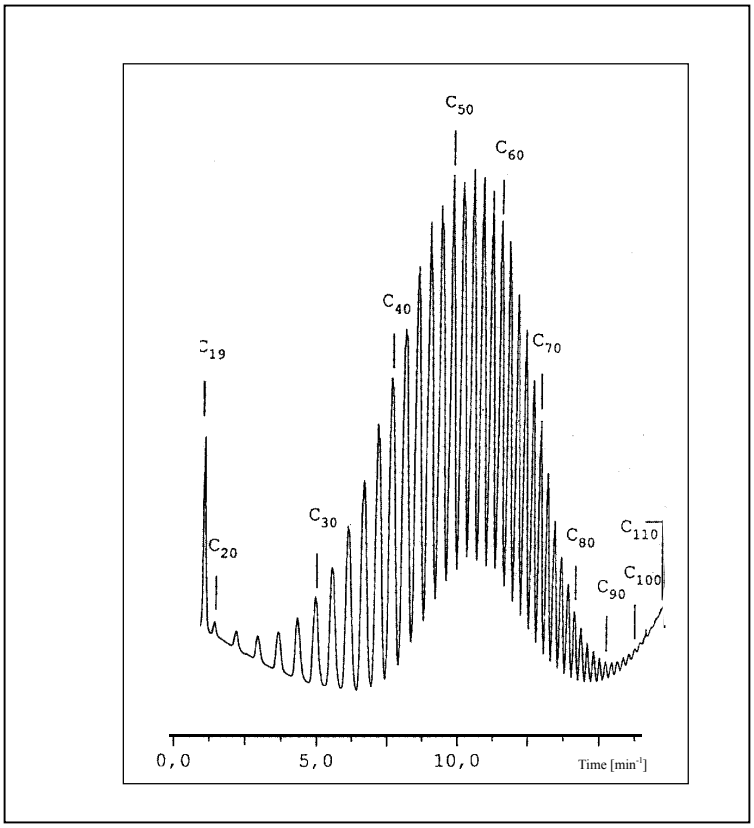

Figure 3. HTGC-FID chromatogram of Polywax 655. Chromatographic column 1.5m x 0.53mm x 0.075 μm OV-1, initial temperature 80 ^{o}C, temperature ramp 20 ^{o}C.min^{-1} to 425 ^{o}C, 2 min. hold

The another problem is to recognise biogenic and petroleum hydrocarbons in contaminated soil. The example and illustration of such problem is in Figure 4. As mentioned above, the biogenic hydrocarbons usually show more simple chemical composition in comparison with hydrocarbons originating from the petroleum-derived sources. Comparing GC-FID chromatograms of petroleum (Figure 4A) and biogenic-derived (straw - Figure 4B) samples confirms this fact. On the other hand, GC-FID chromatogram of wooden sawdust in Figure 4C does not show simple constituents pattern. In some cases when the material of natural origin is used there is the chance for the another confusion. In Figure 4D there is the chromatogram of NEC/TPH of the paper mill waste. Consisting of only water, cellulose and inorganic filling (kaoline) this particular material is the source of NEC/TPH with the concentrations typically far over C limit of Slovak standard. Therefore it is officially

considered as the special waste of high environmental risk! Its deposition is specially regulated and controlled while sometimes before it was used for the composting. Hence, information about the contamination origin only due to the unresolved complex mixture is present or not in the chromatogram may not be sufficient, and in some instances even confusing.

Table II. Initial and final concentrations of NEC/TPH for in situ biodegraded petroleum contaminated soil samples

Initial [ppm]	Final [ppm]	Treatment period [months]	Efficiency [%]
4 800	360	6	93
4 250	590	4	86
8 000	1 300	5	84
16 030	2 500	8	84

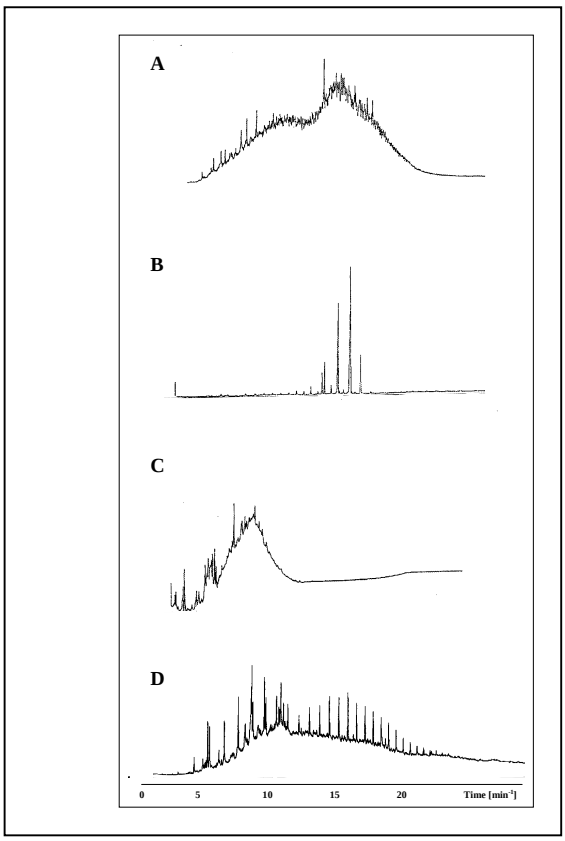

Figure 4. GC-FID chromatograms of petroleum- (A) and biogenicderived (B - straw, C - wooden sawdust, D – paper mill waste) samples performed on an 8.5 m x 0.32 mm x 0.25 μm DB-5 fused silica capillary column; initial temperature 40 °C, rate 15 °C.min^{-1} to 340 °C, then 6 min hold

From these results and from other published data it follows that use of sum petroleum residues concentration determinations is insufficient for meaningful assessing of bioremediation process because:

A) analytical methods used for the determination of the sum of NEC/TPH are not enough proper for the qualitative characterization of analysed compounds, i.e. they do not satisfy the recognition of petroleum products contamination and biogenic one;
B) biodegradation is significantly affected by the chemical structure of hydrocarbons;

Providing a group and hydrocarbons typing composition analysis of the entire soil ex-tracts offers a significant improvement of the analysis of petroleum residues.

GROUP AND TYPE ANALYSIS OF HYDROCARBONS

Various adsorbents (silica gel, alumina, florisil, combination of silica and aluminal and various eluting solvents have been used to fractionate oil extract into saturate, aromatic and polar fractions using column chromatography. According to the scheme in Figure 1[13] dried NEC/TPH extract was redissolved in cyclopentane and placed on the top of a glass column packed with silica gel. The saturate hydrocarbons were eluted with n-pentane, the aromatic compounds using n-pentane-benzene (60-40 %) solvent and polar fraction was eluted with benzene-isopropyl alcohol (80%-20%) solvent mixture. The total mass of each fraction was determined gravimetrically. Both saturate and aromatic fractions were further characterized by a field desorption mass spectrometry (FDMS) for providing compositional data based on both carbon number (or molecular weight) and hydrogen deficiency characterized by z-number[13]. This measurement allows to obtain the approximate molecular ring structure of hydrocarbons. From the dependence of the concentration of biodegraded straight-chain and branched alkanes (z = 2) on their number of carbon atoms for initial and final concentrations follows that these alkanes are completely biodegraded when number of carbon atoms is lower than 22, or partially degraded when this one is higher than 22.
It was obtained that at least 70 % et al. of $>C_{44}$ saturates and 25% of $>C_{44}$ aromatics were removed during the bioremediation treatment[13]. From these data it is evident that even aqueous insoluble hydrocarbons can be biodegraded to a significant extent. It is possible that bacteria are able to solubilize and emulsify heavy hydrocarbons with the help of microbial surfactants produced in response to these substrates or that larger hydrocarbon droplets are taken up via cell contact[23].
For heavily weathered soil samples from former refinery area an other analytical protocol was used by Pollard et al.[24]. In that scheme, after asphaltenes precipitation from extract using n-pentane, the extract is separated on saturates, monoaromatics, diaromatics, polyaromatics and polar compounds using column chromatography on silica + alumina. The quantitative results of this group analysis for heavily weathered petroleum products show general agreement with group separation results for the bitumen standard. The increase of the asphaltene content in weathered oils suggest that some petroleum fractions are transformed into polymeric asphaltenes during biotransformation. GC simulated distillation profiles of the extract fractions indicates the boiling point range over which they are distillated (up to 720 °C atmospheric equivalent boiling point, or up to C_{100}). It provide

also the information relating to the relative resistance of saturate, aromatic and polar fractions to the degradation in these wastes. Such procedure allows high temperature GC on an aluminium clad Quadrex column 8m x 0.53mm I.D. x 0.15 mm film thickness of 5% phenyl silicone stationary phase. The measurements were done at temperature program 55-420 °C with the speed of 10 °C.min^{-1}.
The observed persistence of polar constituents in soil microcosms suggests that some heterocylic components are also sufficiently resistant to biodegradation[25].
From the group and hydrocarbons typing analysis follows that the heavily degraded hydrocarbon residues in soil have a character of heavy oils suggesting limited potential biotreatibility of residual contamination. The resistance to the microbial degradation is also the function of individual compound structure. The capability of used analytical methods to recognise petroleum products and biogenic hydrocarbons is limited.

SPECIFIC TARGET COMPOUND DETERMINATION

The effects of spilled oil on the environment are strongly related not only to the gross amount of oil, but also to the levels of key toxic compounds. Hyphenation of high-performance capillary gas chromatography with mass spectrometry in the scan and mainly in the selected ion monitoring modes is useful for identification, characterization and quantitation of the oil components. Petroleum fingerprinting hydrocarbons are were published[26]. More than 100 saturated hydrocarbons, 50 alkylbenzenes, 60 polycyclic aromatic hydrocarbons (PAHs) and 50 biomarker compounds were analyzed in oils samples and various oil-spill-related environmental samples.
A lightly degraded oil is usually indicated by partial depletion of normal alkanes. A moderately degraded oil is often indicated by heavy loss of alkanes and partial loss of lighter polycyclic aromatic hydrocarbons. Isoprenoid alkanes are very prone to extensive weathering and/or biodegradation[13] as shown in chromatograms of extract of soil contaminated with diesel oil in Figure 5. The chromatogram 5A illustrates only recently contaminated soil, whereas the chromatogram 5D contamination subjected to environmental conditions for several months. Figure 5 clearly demonstrates that after straight-line alkanes and branched alkanes are completely lost, fully degradation of isoprenoid alkanes occurs. For this reason their use as markers for differentiation of petroleum and biogenic derived hydrocarbons from composting process is limited.
From extensively degraded oil samples normal and branched alkanes might be completely lost[26] and polycyclic aromatic hydrocarbons (PAHs) could be highly degraded. Nevertheless aromatic hydrocarbons represent further potential markers for identification of biogenic and petroleum contamination. Sometimes PAHs are considered as the most important analytes in an oil/spill natural resource damage assessment[26]. Alkylated PAHs are the most abundant PAH compounds in oil and they are more persist than their parent compounds. The ratios for isomeric PAHs during weathering are relatively consistent, however during biodegradation the ratios of the isomers change within the same isomeric PAH group and can be used as markers of biodegradation in some occasions[26].
There were, unlike published data[4], phenantrene, fluorantene and pyrene found out within this work in all investigated samples of heather, tradescancy and agate leaves with the typical predominance of phenantrene. Antracene, benzo(a)antracene, chrysene and benzo(k)fluorantene were present at lower levels. The total amount of polycyclic aromatic hydrocarbons ranges from 105 to 945 μg.kg^{-1} what is in a good agreement with literature[4].

As mentioned in the introduction, alkylhomologues of naphthalene, phenantrene, antracene and higher aromatics are characteristic compounds in the petroleum-derived hydrocarbon distribution. Figure 6 represents GC-MSD chromatograms for naphthalene alkylhomologues determined in the petroleum (A) and biogenic-derived (B) samples. Unfortunately, as well parent PAHs as their alkyl homologues could be highly degraded. In such cases, identification through recognition of alkanes and/or PAH distribution pattern would be rather difficult.

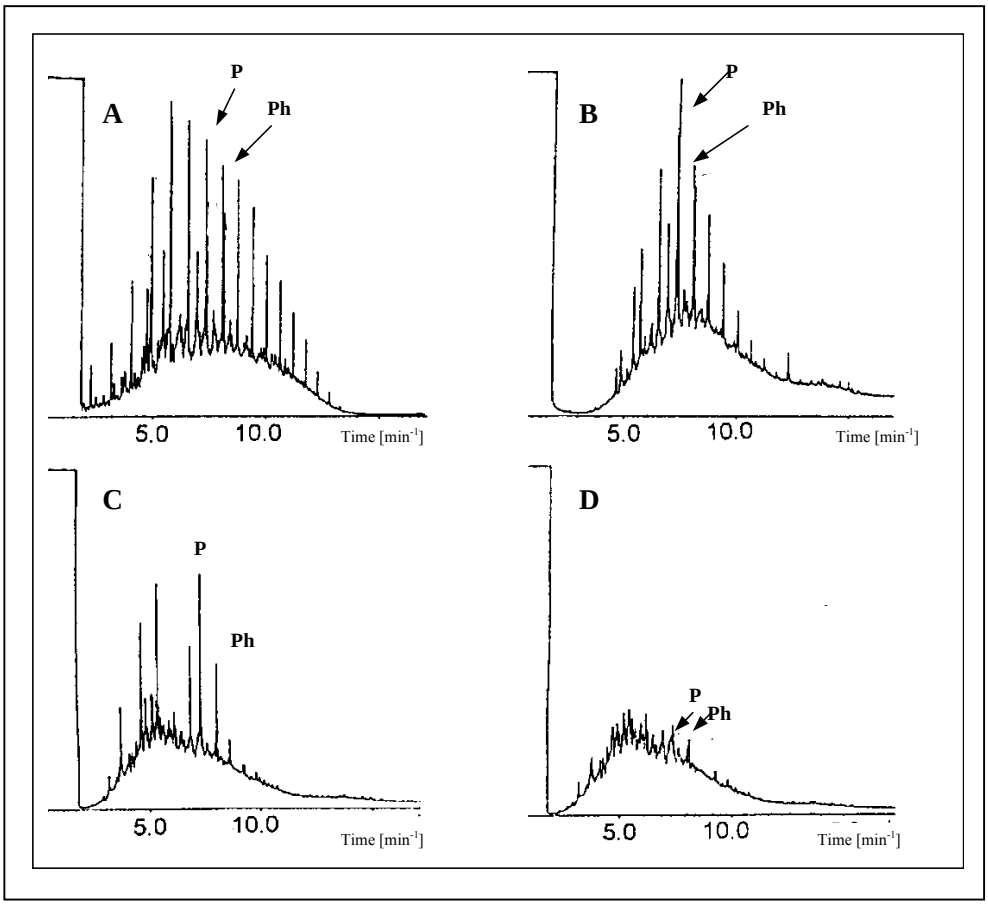

Figure 5. GC-FID chromatograms of soil extracts contaminated with diesel oil as a function of contamination ageing performed on an 8.5m x 0.32mm x 0.25μm DB-5 fused silica capillary column; initial temperature 40 ^{o}C, rate 15 ^{o}C.min^{-1} to 320 ^{o}C; P – pristane, Ph - phytane

It seems that analysis of hopanes is necessary for assessment of composting process, and generally most abundant pentacyclic hopane C_{30}-17α(H)21β (H)-hopane (C_{30}-hopane) was investigated. C_{30}-hopane is highly degradation resistant compound that remains in the samples after long-term weathering and/or biodegradation. Therefore C_{30}-hopane has been utilized for assessment of the weathering and degradation rate of highly degraded

samples[27]. As only oxygen derivatives were found in the biogenic materials[28] we suggested using this compound for identification of petroleum contamination in the samples from composting of oil and biological sludges.

As an biodegradable matter becomes more degraded, the content of C_{30}-hopane increases relatively to more easily degraded constituents. On the basis of C_{30}-hopane analysis in the starting-point sample and sample after composting process finished the degree of oil degradation can be calculated. Drawback of this approach consists in only oil contaminated samples applicability. Figure 7 shows GC-MSD chromatograms for selective ion (m/z 191) monitoring of heavy oil standard (A), petroleum (oil sludge, B) and biogenic-originated sample (wooden sawdust, C) as well as biological sludge sample (D). The absence of hopanes in the biogenic sample (C) is evident. On the basis of these analyses, we assumed that in the initial biological sludge sample (as the sample title also could indicate) hopanes would not be present. The GC-MSD/SIM chromatogram (m/z 191) of biological sludge in Figure 7D, however, exhibits the pattern typical of triterpanic compounds. During biological treatment of wastewater they likely adsorb onto the sludge particles, and as these compounds are not degraded their content relatively increased.

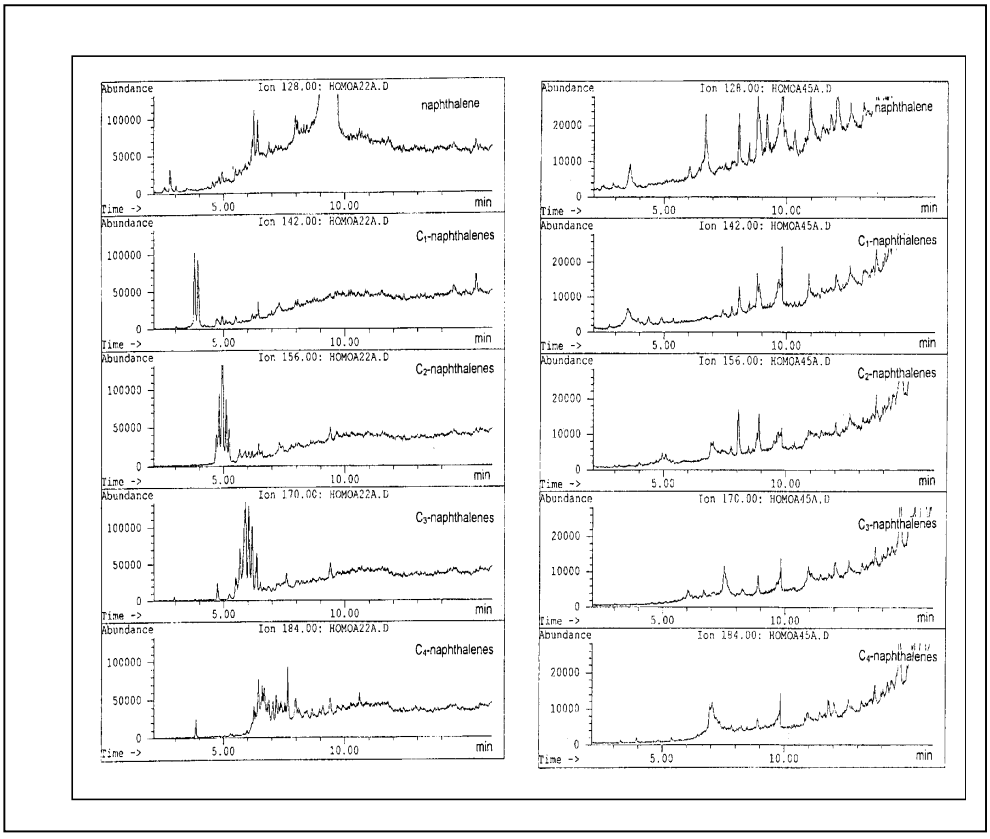

Figure 6. GC-MSD/SIM chromatogram of petroleum-derived sample (oil sludge) (A) and biogenic-derived sample (wooden sawdust)(B) monitoring naphthalene alkylhomologues performed on a 8.5m x 0.32mm x 0.25μm DB-5 fused silica capillary column; initial temperature 60 ^{o}C, rate 15 ^{o}C.min^{-1} to 300 ^{o}C.

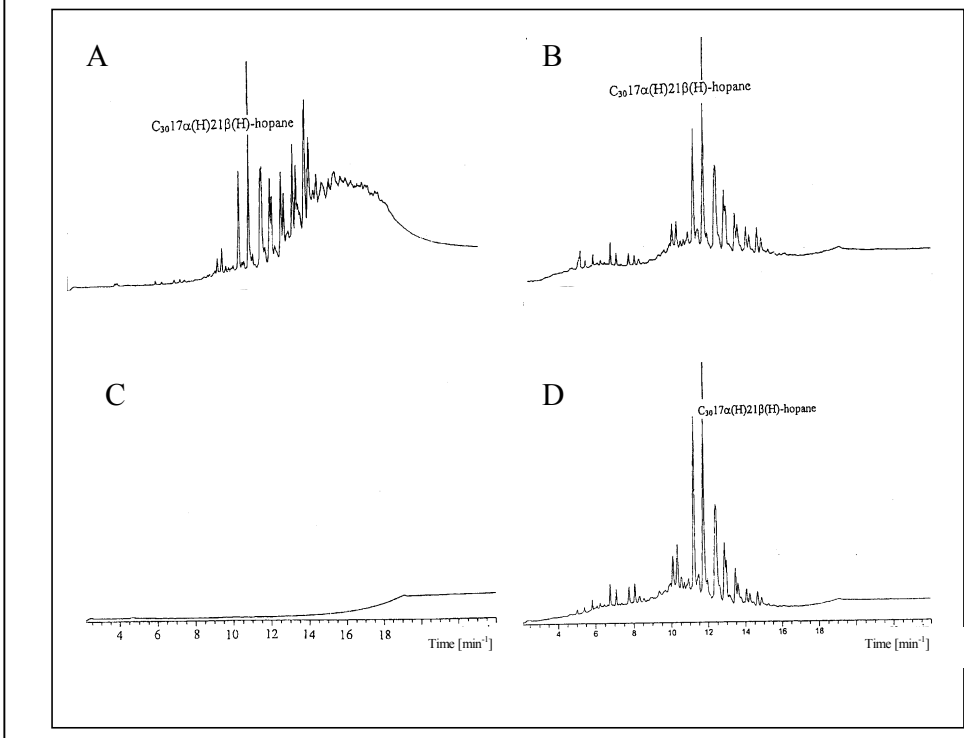

Figure 7. GC-MSD/SIM (m/z = 191) chromatogram of heavy oil standard (A), petroleum (B-oil sludge), biogenic-derived sample (wooden sawdust)(C) and biological sludge (D) samples performed on an 8m x 0.32mm x 0.4μm OV-1 fused silica capillary column; initial temperature 80 ^{o}C, rate 20 ^{o}C.min^{-1} to 200 ^{o}C and then to 330 ^{o}C at 10 ^{o}C.min^{-1} and 10 min. hold.

Table III shows the content of C_{30}-hopane determined in the sludge samples and initial composting mixtures from pilot-scale composting experiment, quantified using the external standard C_{30}-17β(H)21α(H)-hopane (Chiron AS, Trondheim, Norway), content of nonpolar extractable compounds (NEC/TPH) calculated on the basis of known content of C_{30}-hopane in the sample and in heavy mineral oil R953 (Slovnaft, Ltd.) (TPH^{1}), and NEC/TPH content determined in GC-FID system (TPH^{2}). It is apparent the higher content of C_{30}-hopane in biological sludge in comparison with oil sludge by one order of magnitude, similarly the higher NEC/TPH content was determined in the biological sludge. TPH^{1} contents are several times higher than TPH^{2} in all analyzed samples, so it can be expressed the assumption the sludge samples entering the composting process are themselves degraded to the certain extent. Further, C_{30}-hopane content differs in the individual petroleum fractions, hence, this way determined value must be influenced by the character of oil fraction standard used. For instance, in heavy oil utilized for the quantification is of 450 μg C_{30}-17α(H)21β(H)-hopane in 1 g oil, in light oil sample R932(Slovnaft, Ltd.) is C_{30}-hopane

present at level of 125 μg/g oil. Moreover, elution maximum of compounds present in composting mixtures is in C_{30}- hopane region, between elution maximums for light and heavy oil, what means that the content of C_{30}-hopane in the source samples could be several times higher. Taking into consideration these facts, it is obvious that the availability of fresh petroleum fraction, which both contains C_{30}-hopane and has a similar distillation profile with contaminating fraction is a key presumption for the determination of petroleum originated hydrocarbons in the samples from composting process.

Table III. Content of C_{30}-hopane and NEC/TPHs in analysed samples from composting process.

Sample	C_{30}-hopane [μg/g sample]	TPH[1] [μg/g sample]	TPH[2] [μg/g sample]
Biological sludge	45	100 000	15 000
Oil sludge	4.5	10 000	3 300
Variant I	9.2	20 400	3 800
Variant II	6.5	14 400	3 600
Variant III	4.7	10 400	4 100
Variant IV	4.2	9 300	3 000

In Table IV are given contents of extractable compounds (TSEM) and nonpolar extractable compounds (NEC/TPH) determined by GC-FID system, and content of C_{30}-hopane determined by GC-MSD in the sample and corresponding TSEM and NEC/TPH fractions for the six studied composted sets, decrease percentage and calculated actual TSEM and NEC/TPH contents in samples after completing the composting process.

As petroleum contamination in the samples from composting process is assessed and controlled thorough the content of nonpolar extractable compounds, parameter that characterizes the success of compostation should logically be decrease in the content of nonpolar extractable compounds in final samples. In spite of this, the increase in NEC/TPH content can be observed in final-point samples for variants I and III, although the composted mixture appearance and odour properties clearly indicated the compostation had occurred.

Decrease [%] calculated using the content of $C_{30}17\alpha(H)21\beta$ (H)-hopane in the starting and final samples ranges from 10.7 to 50% for analyzed variants. In spite of almost the same ratio of slugdes (biological + oil sludge) to nutrients (straw + bark + lop) for all samples, degradation rates rather vary. It is interesting that the higher decreases (40.5% for Variant III and 50% for Variant IV) are of use in samples where the bark was added, what indicates quite easy bark biodegradability. Given values however represent the total diminution of composted mixture, what is confirmed comparing thus value for Variant IV (50%) with those determined by weighting (43.6%), and do not reflect the effect of composting on the parameter we observe (TSEM and particularly NEC/TPH), because only the portion of whole matter is included in certain parameter. For instance, decrease percentage calculated using determined amount of C_{30}-hopane in initial and final sample for Variant I is 10.7%. According to the TSEM value only negligible decrease was observed and as already mentioned, NEC/TPH content even enhanced. For these reasons it is necessary to relate the content of C_{30}-hopane to the amount of extractable or nonpolar extractable compounds in the sample. This approach allows to calculate the loss of TSEM of 10.2%, substances expressed as nonpolar extractable compounds remained almost without changes in amount.

Table IV. TSEM, NEC/TPHs and C_{30}-hopane contents in analysed composted samples

Variant I	**Starting-point sample**	**Final sample**	**Decrease [%]**	**Actual content**
TSEM [µg/g sample]	7560	7600	0	6790
NEC/TPH [µg/g sample]	3800	4200	Increase	3750
C_{30}-hopane [µg/g sample]	9.2	10.3	10.7	
C_{30}-hopane [µg/g TSEM]	1217	1355	10.2	
C_{30}-hopane [µg/g TPH]	2421	2452	1.3	
Variant II	**Starting-point sample**	**Final sample**	**Decrease [%]**	**Actual content**
TSEM [µg/g sample]	8810	5600	38.7	4730
NEC/TPH [µg/g sample]	3600	3500	2.8	3000
C_{30}-hopane [µg/g sample]	6.5	7.7	15.6	
C_{30}-hopane [µg/g TSEM]	738	1375	46.3	
C_{30}-hopane [µg/g TPH]	1806	2200	17.9	
Variant III	**Starting-point sample**	**Final sample**	**Decrease [%]**	**Actual content**
TSEM [µg/g sample]	9440	5300	43.8	3150
NEC/TPH [µg/g sample]	4100	2900	29.3	1730
C_{30}-hopane [µg/g sample]	4.7	7.9	40.5	
C_{30}-hopane [µg/g TSEM]	498	1491	66.6	
C_{30}-hopane [µg/g TPH]	1146	2724	57.9	
Variant IV	**Starting-point sample**	**Final sample**	**Decrease [%]**	**Actual content**
TSEM [µg/g sample]	6760	5350	20.9	2680
NEC/TPH [µg/g sample]	3000	3800	Increase	1960
C_{30}-hopane [µg/g sample]	4.2	8.4	50.0	
C_{30}-hopane [µg/g TSEM]	621	1570	60.4	
C_{30}-hopane [µg/g TPH]	1444	2211	34.7	
Variant V	**Starting-point sample**	**Final sample**	**Decrease [%]**	**Actual content**
TSEM [µg/g sample]	9480	7200	24.0	5200
NEC/TPH [µg/g sample]	3290	3000	8.8	2170
C_{30}-hopane [µg/g sample]	5.6	7.75	27.7	
C_{30}-hopane [µg/g TSEM]	591	1077	45.1	
C_{30}-hopane [µg/g TPH]	1702	2582	34.1	
Variant VI	**Starting-point sample**	**Final sample**	**Decrease [%]**	**Actual content**
TSEM [µg/g sample]	9850	6000	39.1	4900
NEC/TPH [µg/g sample]	3370	3000	11.1	2460
C_{30}-hopane [µg/g sample]	5.9	7.2	18.1	
C_{30}-hopane [µg/g TSEM]	598	1200	50.2	
C_{30}-hopane [µg/g TPH]	1749	2400	27.1	

CONCLUSIONS

TSEM decrease of 10.2% represents only 0.08% from the total matter amount, and only 0.005% responds to NEC/TPH loss of this value. 10.6% left represents the loss of organic matter not included in TSEM value. The result interpretation for other composting variants is similar.

It is apparent that the parameters TSEM and NEC/TPH are not appropriate for the monitoring of composting process, as far as these values are not related to the matter decrease during composting process. In other words, the content of extractable and nonpolar extractable compounds is necessary to know in order to be possible to calculate their actual content in final composting mixture on the basis of known amount of C_{30}-hopane in the initial and final sample. Finally, it is needed to remark that the actual contents of extractable and nonpolar extractable compounds in final status mixtures shown in Table IV can still include the compounds having biogenic origin because it is almost impossible to determine the degradation rates for petroleum and biogenic hydrocarbons individually

In the case of heavily degraded oil samples the analysis of biomarkers as highly degradation resistant compounds can give some information about biodegradation characteristics.

Hydrocarbon contamination determined as content of nonpolar extractable compounds in environmental samples can originate from petroleum as well as biogenic sources. Use of standardized methods by sum measurement of compounds concentration that represent a class of compounds, reported as total solvent extractable materials (TSEM), "oil and grease" (O&G) or nonpolar extractable compounds/total petroleum hydrocarbons (NEC/TPH) parameters, is insufficient for meaningful assessing of bioremediation process. That is because degree of petroleum hydrocarbons biodegradation is lagerly affected by their structure type and molecular weight distribution and the qualitative characterization of analysed compounds is not satisfied.

Resolution of petroleum and biogenic-derived hydrocarbons within nonpolar extractable compounds is practically impossible using infrared spectroscopy and limited using capillary gas chromatography with flame ionization detector. In recent years, new analytical methods has been developed to determine selected hydrocarbon groups and types as well as individual hydrocarbons. These procedures involve group separation, boiling point distribution or GC simulated distillation, hydrocarbons typing by field desorption mass spectrometry and determination of target individual petroleum compounds by GC/MS-SIM. Hyphenation of capillary gas chromatography and mass selective detector operated in the selective ion monitoring mode essentially allows detailed specification of nonpolar extractable hydrocarbons origin. Isoprenoid alkanes, alkylhomologues of aromatic hydrocarbons and polycyclic alkanes hopanes-like can be figured as markers for recognition of petroleum and biogenic contamination. Due to the quite rapid degradation rate for isoprenoids as well as aromatics, their use for this purpose is limited in weathered and/or degraded soils. C_{30}-hopane seems to be a suitable marker to identify the contaminant origin, to determine degradation rates of nonpolar extractable compounds and to calculate actual content of nonpolar extractable compounds in final composting mixture, on the assumption the contamination is of mineral oil type and fresh petroleum fraction with similar distillation profile with contaminating fraction is available.

Obtained more detailed chemical composition of hydrocarbons contamination has particular significance for the assessing potential biotreatibility of waste soil and providing an effective clean up strategy. These data are important for assessing changes in the chemical

composition of the spilled oil during biodegradation process, especially when there is a prolonged period of degradation, as well as for identification of source of spilled petroleum product and for assessing the damage of the natural resources with a spilled oil, too.
There is of high importance that properly defined assessment and strategy based on enough high accuracy and precision of used analytical methods can help not only to clean-up environmental damages but to improve the environment and help to save reasonable economic resources. Taking into consideration increasing deficit of organic matter in soil, composting of particular waste (petroleum hydrocarbons contaminated soils and/or another materials, paper mills waste, biological sludges, etc.) and adding this processed material into soil shows more economical than costly scrap-heaps.

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