Quantification of petrogenic PAH in marine sediment using molecular stable carbon isotopic ratio measurement

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Compound-specific carbon isotope analyses were performed on polycyclic aromatic hydrocarbons (phenanthrene and methylphenanthrenes) isolated from marine sediments contaminated with petroleum. Isotopic composition measurements of individual methylphenanthrenes were subject to important uncertainties because they were not completely separated by gas chromatographic separation. Nevertheless the isotopic composition of the sum of methylphenanthrenes was measured with good reproducibility. As petrogenic PAHs and PAHs present in the sediment before the petroleum contamination have different isotopic compositions it was possible to quantitatively source apportion methylphenanthrenes in contaminated sediment with a good precision and reliability, using the isotopic composition of the sum of methylphenanthrenes and a simple mass balance calculation.

Introduction

Polycyclic Aromatic Hydrocarbons (PAHs) are ubiquitous organic pollutants because of their chemical stability and the multiplicity of their sources. PAHs can be introduced into the environment via incomplete organic matter combustion (pyrolytic origin), oil spill and natural oil leakage (petrogenic origin) and via natural precursor transformations during early diagenesis processes (diagenetic origin). PAH source and behaviour elucidation presents an ecotoxicological interest as metabolisation products of PAHs show mutagenic and carcinogenic properties.

Approaches based on molecular PAH fingerprint interpretation, on the study of relative isomers distribution and on the study of the abundance of alkylated compounds allow some qualitative source differentiations in recent polluted sediments [1-3]. Nevertheless some biotic and abiotic processes such as biodegradation, evaporation, chemical reactions and photooxydation can selectively alter molecular patterns [3,4]. In this respect, qualitative and even more quantitative source assessment of PAHs using solely molecular considerations can then be hazardous in some cases, especially for weathered samples.

In this context, the use of molecular carbon isotopic composition ($^{12}C/^{13}C$), which depends on the carbon source used for the synthesis of the compound, on the biosynthesis pathway and on the environmental conditions [5] (geographical origin, temperature...), should allow to improve PAH source apportionment [6].

Due to the analytical improvements of the last decade, it is now possible to get access easily to the isotopic ratios of individual compounds in a complex mixture by the use of gas chromatography/isotope ratio mass spectrometry (GC/IRMS) [7]. In this technique, individual compounds eluting from a gas chromatograph are converted to CO_2 and H_2O in a combustion furnace heated at 940 °C containing CuO and Platinum wires. H_2O is then trapped via a naphion membrane. Interfering nitrogen oxides resulting from the combustion are reduced to N_2 in a reduction furnace heated at 600 °C containing Cu. Purified CO_2 is then directly introduced into a magnetic mass spectrometer continuously monitoring ions having 44 ($^{12}C^{16}O_2$), 45 ($^{13}C^{16}O_2$ and $^{12}C^{17}O^{16}O$), 46 ($^{12}C^{18}O^{16}O/^{13}C^{17}O^{16}O$ and $^{12}C^{17}O_2$) as ratio *m/z*. The isotopic composition is then calculated using the ratio 44/45 *m/z* and 44/46 *m/z* for the correction of ^{17}O contribution to the 45 *m/z* signal. The isotopic ratio is then reported in terms of δ-notation ($\delta^{13}C$), giving the permil deviation of the isotopic ratio of a sample relative to the international standard, a fossil belemnite from the Pee Dee formation (PDB).

In order to evaluate the potentialities of this isotopic PAH source apportionment strategy, the isotopic composition of phenanthrene and methylphenanthrenes was monitored during an *in-situ* oil spill simulation experiment. In this study, a known quantity of petroleum was intentionally introduced at the top of the sedimentary column. In those samples both natural and petrogenic PAHs were present. The aim of this study was to quantify petrogenic PAHs present in the samples all along a natural biorestoration period.

Experimental

In-situ bioremediation experiment

In the cove of Carteau, close to the mouth of the Rhone River (Mediterranean coast of France) 10 cm diameter and

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25 cm long PVC tubes were placed in order to delimit a biotope and biocenose fraction. A 1 cm thick freezed slice of sieved sediment coming from the experimental site was placed on the top of each corer. Some of those slices were contaminated with petroleum (blended Arabian light distilled at 250 °C, 40 g of BAL 250 per kg of dry sediment) and some others were free of petroleum, giving respectively contaminated cores and control ones. Three contaminated and control cores were sampled every six months. Each core was cut in 2 cm thick slices.

In this study, isotopic compositions of phenanthrene and methylphenanthrenes in the petroleum used for the contamination (BAL 250), in the 2 to 4 cm layer of a reference core and three contaminated cores sampled after six, twelve and eighteen months of experiment were measured.

Sample preparation

The freeze-dried 2 cm slices of sediment were sieved at 2 mm and then extracted using microwave assisted extraction (10 min, 30 W) [8]. Methylene chloride (Scharlau, HPLC grade) was the extraction solvent. The sample was filtered and the total organic extract was reduced to a small volume using a rotary evaporator. The extracts were then purified on a florisil column to eliminate polar compounds. In order to be able to measure isotopic compositions of methylphenanthrenes it was necessary to separate them from methyldibenzothiophenes as these two classes of compounds coelute during gas chromatographic separation using a classical apolar capillary column. The phenanthrenic fraction was individualized by high pressure liquid chromatography separation on aminosilane phase (Spherisorb, 5 µm, 25 cm, 4.6 mm ID) with pentane (Scharlau, HPLC grade) as eluent. The integrity of the phenanthrenic fraction was controlled checking the absence of phenanthrene and methylphenanthrenes in both the dibenzothiophenic and tetra- aromatic fraction by Gas Chromatography/Mass Spectrometry (GC/MS) analysis. The absence of compounds coeluting with phenanthrenic compounds was controlled by full scan GC/MS analysis.

The absence of isotopic fractionation during sample preparation was shown measuring the isotopic composition of some commercial 2-MP (Aldrich, 95%) before and after the application of the analytical procedure, giving respectively –25.04 ± 0.12‰ and –25.15 ± 0.15‰.

GC / IRMS analysis

Isotopic analyses of individual PAHs were carried out using an HP 5890 Series II Plus gas chromatograph interfaced via a CuO furnace (940 °C) and a hygroscopic membrane (nafion) to a Delta Plus isotopic ratio mass spectrometer from Fimigan corporation.

Injections were performed in the splitless mode. The injector temperature was maintained at 270 °C. The GC temperature program was from 50 °C to 180 °C (2 min) at 10 °C min^{-1}, to 230 °C at 2 °C min^{-1} and to 290 °C at 10 °C min^{-1}. The carrier gas was helium (flow rate: 1.35 mL min^{-1}). The capillary column used was a SGE BPX5: 60 m × 0.22 mm ID × 0.25 µm film thickness.

For calculation purposes CO_2 reference gas was automatically introduced into the isotopic ratio mass spectrometer in a series of pulses at the beginning and the end of each analysis. The reproducibility of the individual isotopic measurements, determined through repeated analyses of 2-methylphenanthrene standard, was ± 0.25‰. Precision reported in this study is based on multiple analyses (at least two analyses) of each sample.

Results and discussion

Isotopic compositions of phenanthrene and methylphenanthrenes from the petroleum, the 2 to 4 cm slice of a control core and of a contaminated core sampled after six, twelve and eighteen months of experiment are reported in figure 1.

Isotopic values of methylphenanthrenes were measured with some relatively important uncertainties, between 0.1 and 2.80‰. These non reproducible results are mainly due to the incomplete resolution of methylphenanthrenes as illustrated in figure 2. The lower part of figure 2 represents the *m/z* 44 chromatographic trace of the BAL 250 phenanthrenic fraction. This trace is analogous to that obtained by flame ionisation detection in conventional GC (peak intensities are a function of carbon concentration). The upper part of figure 2 is the *m/z* 45 to *m/z* 44 ratio trace. The inflection of the *m/z* 45 to *m/z* 44 ratio trace observed for each peak illustrates the fact that during GC separation an isotopic fractionation occurs. Molecules containing ^{13}C elute earlier due to vapor - pressure isotope effect and interactions with the GC column stationary phase [9]. It is then necessary to

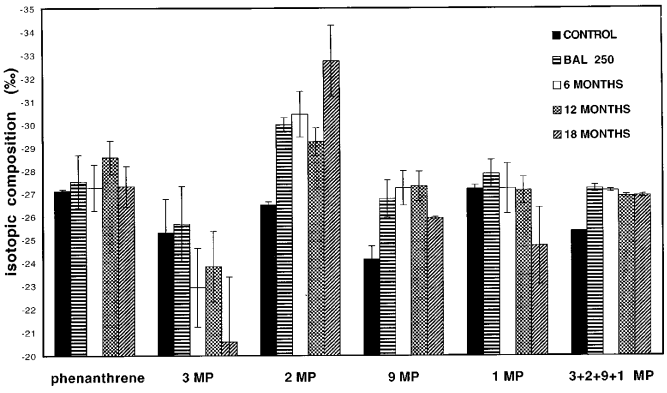

Figure 1. Isotopic composition of phenanthrene, of individual methylphenanthrenes and of the sum of methylphenanthrenes present in the petroleum and in the 2 to 4 cm slice of a reference core and three contaminated cores sampled respectively after six, twelve and eighteen months of experiment.

integrate each chromatographic peak totally in order to measure accurately isotopic compositions. When two compounds coelute as is the case for 3-MP and 2-MP, when the first peak is integrated, the ^{13}C enriched beginning of the second peak is taken into account but not the ^{12}C enriched end of the first peak, giving an artificially ^{13}C enriched isotopic composition. For the second peak an artificial ^{13}C depletion is then measured. This bias is illustrated in figure 1, indeed the more the isotopic composition of the 3-MP (first peak) is ^{13}C enriched the more the isotopic composition of the 2-MP (second peak) is ^{13}C depleted.

Nevertheless using the isotopic composition of the sum of methylphenanthrenes, which presents uncertainties smaller than 0.2‰, it was possible to quantitatively source apportion methylphenanthrenes in contaminated sediments.

First of all, methylphenanthrenes present initially at the experimental site and in the petroleum used in this experiment have different isotopic compositions, respectively –25.35 ± 0.01‰ and –27.18 ± 0.16‰. This difference is due to the fact that PAHs from those two samples are from different origins. PAHs from the BAL 250 are obviously from petrogenic origin whereas PAHs from the experimental site sediment are from pyrolytic origin as indicated by the GC fingerprint of the aromatic fraction of the sediment extract showing that alkylated PAHs are much less abundant than parental ones and the equal abundance of both low and high molecular weight PAHs.

So each contaminated sediment PAH can be regarded as a mixture of two sources: pyrolytic and petrogenic. Then using a simple mass balance calculation, the percentage of methylphenanthrenes from petroleum origin present in a contaminated slice can be calculated from equation (1):

$$\%MP_{pet} = 100 \times (\delta c - \delta i) / (\delta p - \delta i) \qquad (1)$$

where δc is the isotopic composition of methylphenanthrenes from the contaminated slice, δi is the isotopic composition of methylphenanthrenes from the control core and δp is the isotopic composition of methylphenanthrenes present in the petroleum.

This quantitative approach is valid only if the isotopic composition of organic compounds is not affected by the different processes degrading them. Huang et al. [10] have shown the absence of carbon isotope fractionation of individual *n*-alkanes during a 23-year field decomposition experiment. As biodegradation is the major process suspected to affect PAHs in our experiment, we have tested the isotopic effect of biodegradation on 2-methylphenanthrene using a pure bacterial strain. The isotopic composition of 2MP has remained constant all along the 15 days of the experiment although 90% of the 2MP was degraded.

Using equation (1), the calculated percentages of methylphenanthrenes from petrogenic origin present in the 2 to 4 cm slice of the 6, 12 and 18 month old cores are respectively 95.1 ± 12.3%, 82.1 ± 11.9% and 83.2 ± 11.2%. The percentage of methylphenanthrenes from petrogenic origin in the same cores determined via the quantification of methylphenanthrenes present both in the contaminated core and the reference core sampled at the same time are respectively 94.6%, 82.9% and 93.1%. Those two sets of results are in good agreement.

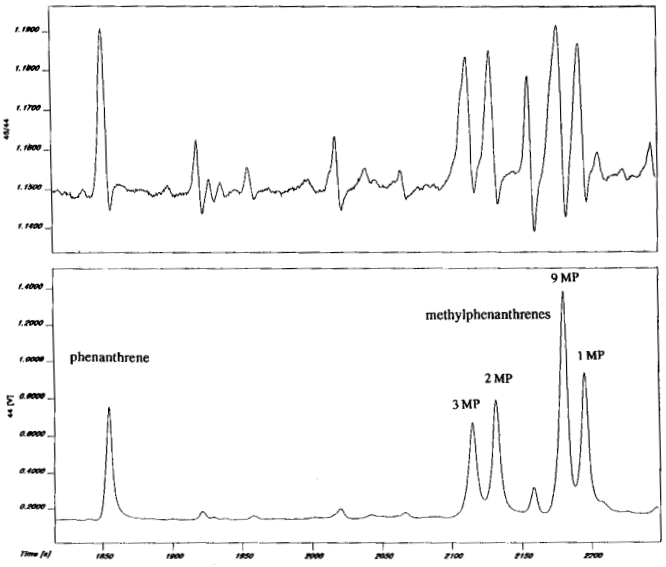

Figure 2. Part of the isotopic-ratio-monitoring gas chromatogram of the phenanthrenic fraction of BAL 250. Peaks represent CO_2 produced by the combustion of phenanthrene and methylphenanthrenes. The lower trace shows the *m/z* 44 ion current as a function of time and the upper trace depicts the *m/z* 45 to *m/z* 44 ion-current ratio.

Conclusions

This study has pointed out the fact that when complete gas chromatographic separation of compounds is not achieved, as it is the case for methylphenanthrenes, it is impossible to accurately measure isotopic composition of individual compounds by gas chromatography/isotope ratio mass spectrometry. Nevertheless using the isotopic composition of the sum of methylphenanthrenes it has been possible to quantitatively source apportion petrogenic residual methylphenanthrenes in oil contaminated sediment. This isotopic approach allows to quantify PAH sources using only one reference sample not necessarily collected at the same time and exactly at the same place, as the isotopic composition is much more spatially and temporally homogeneous than natural PAH concentrations.

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