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Ancient polycyclic aromatic hydrocarbons in modern soils : ¹³C, ¹⁴C and biomarker evidence

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Abstract- Polycyclic aromatic hydrocarbons (PAHs) of soils from two maize fields were analyzed for molecular structure by gas chromatography-mass spectrometry, for ¹³C/¹²C ratios by isotope ratio mass spectrometry and for ¹⁴C age by accelerator mass spectrometry. The molecular investigation indicates that the aromatic fractions are dominated by pyrolytic inputs. Calculation of molecular maturity parameters based on hopanes and PAHs gives values typical of high thermal stress such as exhibited by high rank coals and petroleum with equivalent vitrinite reflectances of ~ 0.6-1.5%. The interpretations based on molecular compositions are corroborated by isotopic data. Specifically, both the absence of ¹³C/¹²C variation of aromatic fractions during 23 years of maize cropping, and a ¹⁴C age greater than 9820 years demonstrate that these substances are not derived from aromatization of modern plant material or soil biomass. Together, these results show that soil PAHs are mainly of pyrolytic origin, e.g. exogenous (airborne) contributions of fossil fuel combustion products and (old) vegetation fires, with a small contribution of uncombusted fossil fuels. This approach using molecular and isotope information represents a promising way to study the fate of ancient, reworked, fossil organic matter in modern media such as soils, sediments, plants, air and food.

Key words - carbon-13, carbon-14, soil aromatic hydrocarbons, hopane, maturity parameters, pollution.

INTRODUCTION

The occurrence of PAHs in modern media such as soils, sediments, aerosols, waters, animals and plants is of increasing environmental concern because these compounds may exhibit mutagenic and carcinogenic effects towards living organisms (Hites and Biemann, 1972, Youngblood and Blumer, 1975, Lunde and Bjorseth, 1977, Smith, 1977, Laflamme and Hites, 1978, 1979, Windsor and Hites, 1979, White, 1986, Pahlman and Pelkonen, 1987, Mac Elroy *et al.*, 1989, Trendel *et al.*, 1989, Lipiatou and Salot, 1991, Simonich and Hites, 1994, Garrigues *et al.*, 1993, 1995). The advent of high resolution analytical techniques such as capillary gas chromatography-mass spectrometry in the 70's has allowed the molecular structure of a wide array of PAHs to be elucidated. Nonetheless, with the exception of few minor PAH classes such as steroid and triterpenoid derivatives that bear enough structural information to be related to biological substances (e.g. Douglas and Mair, 1965, Laflamme and Hites, 1979, Mackenzie *et al.*, 1982, Trendel *et al.*, 1989, Lichtfouse and Albrecht, 1994), the sources and the transport processes leading to the presence of most PAHs in modern environments are rarely clear.

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In soils, for example, PAHs may be indigenous as a result of rapid aromatization of biological compounds during humification processes (Trendel *et al.*, 1989). They can also reflect contributions from ancient hydrocarbon sources, such as petroleum and coal residues (Tissot and Welte, 1984), provided that the soil has been initially formed by weathering of a petroleum source-rock or coal seam. Alternatively, several exogenous sources can be considered including migration of petroleum from deeper formations, fall of airborne particles, and various spills (Lunde and Bjorseth, 1977, Smith, 1977, Windsor and Hites, 1979, Tissot and Welte, 1984). Geochemists have also tried to distinguish on structural grounds petrogenic sources, that account for the direct contribution of fossil fuel, from pyrolytic or pyrogenic sources formed at relatively high temperature by incomplete combustion of fossil fuels, e.g. car exhausts, industrial emissions, and vegetation fires (Youngblood and Blumer, 1975, Wise *et al.*, 1988, Garrigues *et al.*, 1995). Moreover, although a substantial amount of PAHs could be formed recently (e.g. from modern vegetation fires), the high chemical stability of their aromatic systems means that these molecules could have ancient sources such as ancient vegetation fires and fossil fuels that are somehow preserved in the modern environment.

Except for point-source pollution events such as petroleum spills, the influence of human activities on PAH assemblages is rather difficult to assess, especially when PAHs occur at very low concentrations. In that respect, it should be borne in mind that the *major* part of petroleum formed in deep rocks over geological periods of time has not been trapped in geological pools but has migrated, and is still slowly migrating toward modern environments through soils and sediments without any anthropogenic influence. Therefore, PAH in modern media are likely to occur as mixtures of several sources, either indigenous or exogenous, either petrogenic or pyrolytic, either ancient or modern, either anthropogenic or from natural sources. In recent investigations, we found that molecular and isotope analysis could be a means to decipher the origins of soil organic molecules (Lichtfouse and Eglinton, 1995; Lichtfouse and Budzinski, 1995; Lichtfouse *et al.*, 1995; Lichtfouse, 1995). Here, a similar approach provides several novel insights into the provenance of PAHs in soils.

EXPERIMENTAL

Detailed crop soil characteristics are described elsewhere (Lichtfouse *et al.*, 1995b, Lichtfouse and Eglinton, 1995). Soil samples were cored through the 0-30 cm ploughed horizon of two experimental cultivated fields, one at La Minière (48°45'N, 2°05'E, ~ 15 Kms from Paris), near Versailles, and the other at Boigneville (48°20'N, 2°20'E, ~ 50 Kms from Paris), Essone, France. Both sites are rural, but the La Minière field should be under higher influence of urban pollutants due to its position near Paris suburbs with a higher density of highways. Both fields have been cropped without any organic amendments. At La Minière (field A), time 0 (Table 2) refers to uncultivated soils, from the same field, previously cultivated solely with C₃ plants, mainly *Triticum aestivum* (wheat). At Boigneville (field B), time 0 refers to soils, from the same field, cultivated with *Triticum aestivum*. These fields were cultivated at one crop per year with the C₄ plant *Zea mays* (maize) from 1987 to 1992 at La Minière, and from 1970 to 1993 at Boigneville, thus labelling soil organic matter at natural abundance with ¹³C-enriched carbon (Lichtfouse, 1995).

Table 1. $\delta^{13}\text{C}$ of total organic carbon (TOC) and aromatic fractions (PAH) from crop field at *La Minière*, France (A, 1987-92) and at *Boigneville*, France (B, 1970-93).

Time (yrs)	TOC		PAH	
	A	B	A	B
0	-26.28	-25.92	-25.99	-27.04
4.33	-24.96		-25.93	
20		-20.74		-27.13
23		-20.33		-27.15

Dried soils were sieved to 2 mm, finely ground and extracted ultrasonically (30 min, 30°C, 3 X) with CHCl_3 -MeOH (3/1 v/v). Extracts were fractionated into neutral and acid fractions by passage through silica gel impregnated with KOH followed by HCO_2H acidification (McCarthy and Duthie, 1962). Neutral fractions were separated into hydrocarbon-ester ($R_F > \sim 0.85$), ketone ($R_F \sim 0.28$ -0.85), alcohol ($R_F \sim 0.05$ -0.28) and polar ($R_F < \sim 0.05$) fractions by silica-gel thin layer chromatography (0.25 mm silica thickness), using CH_2Cl_2 as developer and dibenz(a,c)anthracene, friedelin and cholesterol as reference compounds. Hydrocarbon-ester fractions were fractionated into aliphatic ($R_F > \sim 0.89$), aromatic ($R_F \sim 0.09$ -0.89) and ester fractions ($R_F < \sim 0.09$) by silica-gel thin layer chromatography, with *n*-hexane as developer and using *n*-octacosane, 1-phenyldodecane, 2-methylphenanthrene and dibenz(a,c)anthracene as reference compounds.

$\delta^{13}\text{C}$ of total organic C and of aromatic fractions were measured by continuous flow-isotope ratio monitoring mass spectrometry (Lichtfouse *et al.*, 1995b) and are expressed in per mil. relative to the Pee Dee Belemnite standard: $\delta^{13}\text{C} = [({}^{13}\text{C}/{}^{12}\text{C}_{\text{sample}}/{}^{13}\text{C}/{}^{12}\text{C}_{\text{std}}) - 1] \times 10^3$. Aromatic and aliphatic fractions were analysed by gas chromatography-mass spectrometry (GC-MS). For aromatic fractions, conditions were : HP 5890 series II gas chromatograph, splitless injection, 30 m x 0.25 mm i.d. fused silica column, 5%phenyl-95%methylpolysiloxane phase (0.25 μm thickness), He flow 1 ml/min, temperature : 50-290°C at 5°/min then 20 min isothermal, electronic impact (70 eV), MSD 5970 mass spectrometer, 1.2 scans/sec. GC-MS analyses were performed in full scan mode (50-650 uma) or single ion monitoring using the molecular ions of the studied PAHs : 178, 190, 192, 202, 228, 252, 276, 278. For aliphatic fractions, conditions were similar except : 50 m x 0.32 mm i.d. fused silica column, 100%methylpolysiloxane phase (0.53 μm thickness), He flow 1.9 ml/min, temperature : 50-100°C at 15°/min, 100-310°C at 3°/min then 60 min isothermal, 50-650 uma scan mode. Identification of hydrocarbons was performed by comparison of their chromatographic and spectroscopic characteristics with literature data, pure standards and data from mature sediments and petroleums analysed in the same conditions. A 700 μg sample of the aromatic fraction from the soil cultivated for 4.3 yrs were analysed for radiocarbon by accelerator mass spectrometry, as described by Lichtfouse and Eglinton (1995). This analysis gave a value of 9820 ± 70 yrs BP corresponding to a fraction Modern of 29.45%.

RESULTS AND DISCUSSION

The molecular structures of some PAHs and hopanes cited in this study are shown in Scheme 1

Scheme 1. Molecular structures of polycyclic aromatic hydrocarbons (PAHs) and hopanes

Aromatic hydrocarbon distributions

Aromatic fractions are present both at La Minière near Versailles and Boigneville in Essone crop fields in concentrations of ~ 13 mg/kg of dried soil at La Minière and ~ 7 mg/kg at Boigneville (total organic C $\sim 1\%$), which indicates a non-point source contamination rather than a massive spill. These concentrations were found to be constant with increasing time of cropping, thus suggesting a regular supply of PAHs to the soil.

The distribution of individual PAH are similar in all soil samples, and show the predominance of only a few parent PAHs of high molecular weight (Figure 1). Such a fingerprint is typical of combustion products and aerosols (Lunde and Bjorseth, 1977, Laflamme and Hites, 1978), rather than of a direct contribution of petroleum-based fuels since the latter are usually much more complex containing low molecular-weight PAH and alkylated homologues (Radke *et al.*, 1982, 1984, Radke, 1987).

Figure 1. Aromatic hydrocarbons in crop soils. Single ion monitoring at m/z 178, 190, 192, 202, 228, 252, 276 and 278 of a typical aromatic fraction analysed by gas chromatography-mass spectrometry. **1** alkylnaphthalenes, **2** phenanthrene, **3** methylphenanthrenes, **4** fluoranthene, **5** pyrene, **6** chrysene, **7** benzo[fluoranthenes], **8** benzo[e]pyrene, **9** indeno[1,2,3-c,d]pyrene, **10** benzo[g,h,i]perylene.

Further evidence for a major contribution of pyrolytic products is given by 1) the high abundances of PAH with an alicyclic five-membered ring (Simoneit *et al.*, 1990); 2) a high fluoranthene/pyrene concentration ratio of ~ 2.3 (Sicre *et al.*, 1987 and refs. therein), 3) the occurrence of 4,5-methylenepheneanthrene, a typical pyrolytic product commonly absent in uncombusted fossil fuels, at concentrations in the same order of magnitude as methylphenanthrene isomers (Figure 2), and 3) the occurrence of benzo[g,h,i]perylene, a compound correlated with automobile exhaust (Currie *et al.*, 1994).

Nonetheless, the absence of anthracene derivatives, that are also usually encountered in pyrolytic products (Laflamme and Hites, 1978, Simoneit *et al.*, 1990, Lichtfouse *et al.*, 1994) points to a small contribution of uncombusted fossil fuels to soil PAH. Finally, we did not detect any biogenic PAH, such as plant triterpene derivatives that are formed by rapid aromatization in recent muds and sediments (Laflamme and Hites, 1979, Trendel *et al.*, 1989). Therefore, these structural data show that crop soil PAH have mainly a pyrolytic origin with a small contribution of petrogenic compounds. The latter is clearly evidenced by the identification of trace amounts of C_{27} , C_{29} , C_{30} - C_{32} $\alpha\beta$ -hopane in all soil samples (Figure 3). The $17\alpha,21\beta$ configuration of these "geomarkers" is typical of thermally mature organic matter from sedimentary rocks and petroleum (Van Dorsselaer *et al.*, 1974, Dastillung and Albrecht, 1976, Ourisson *et al.*, 1979) but would not be expected to survive combustion processes.

Figure 2. Alkylphenanthrene in crop soils. Reconstructed ion current at m/z 192 (upper trace) and 190 (lower trace) showing respectively methylphenanthrene isomers (**11**, **12**, **14**, **15**) and 4,5-methylenepheneanthrene (**13**). **11**: 3-methylphenanthrene, **12**: 2-methylphenanthrene, **14**: 9-methylphenanthrene, **15**: 1-methylphenanthrene. The strong predominance of the 2-methyl and 3-methyl isomers is typical of mature fossil fuels. Note the high relative abundance of 4,5-methylenepheneanthrene, a typical pyrolytic marker.

Figure 3. Trace fossil hopanes in crop soils. Total ion current (TIC) and reconstructed ion current (m/z 191) of a typical alkane-alkene fraction of soil extracts. The upper trace shows hopanes homologues. The lower trace shows wax n -alkanes. Note the very low abundance of hopanes relative to n -alkanes. Numbers refer to n -alkane carbon number. **A**: 18α -22,29,30-trisnorhopane (C_{27} , Ts), **B**: 17α -22,29,30-trisnorhopane (C_{27} , Tm), **C**: 17α ,21 β -30-norhopane (C_{29}), **D**: 17α ,21 β -hopane (C_{30}), **E**: 17α ,21 β -homohopane (C_{31}), 22S and 22R epimers, **F**: 17α ,21 β -bishomohopane (C_{32}), 22S and 22R epimers. All these compounds are typical of mature fossil fuels.

PAH and hopane maturity parameters

During the last 20 years, numerous parameters based on relative abundances of biomarker isomers have been designed to evaluate the transformation of organic matter in sediments and petroleum (Peters and Moldowan, 1993). In an overlooked but elegant and pioneering work, Dastillung and Albrecht (1976) demonstrated the value of hopanoid isomers to infer the presence of fossil material in surface sediments. In our study, several molecular parameters based on hopane and PAH isomers were calculated to evaluate the maturity of the non-point source contamination of soils (Table 2). All molecular parameters show values consistent with sedimentary organic matter from high maturity samples such as deeply buried sedimentary rocks, petroleum and high-rank coals. Moreover, the equivalent vitrinite reflectance values shows clearly that this fossil contribution has reached the zone of petroleum generation. This contribution of ancient carbon is further confirmed by ^{14}C analysis (see below). Alternatively, high values of PAH maturity parameters may result from the ‘artificial’ maturation of organic matter during pyrolytic processes such as vegetation fires and fuel burning.

Table 2. Molecular maturity parameters in modern crop soils. The maturity scale is roughly divided into: low (early diagenesis), medium (late diagenesis) and high (catagenesis). The equivalent vitrinite reflectance R_0 (%) is evaluated either by comparison with literature data or by calculation from maturity parameters. References : a) Seifert and Moldowan, 1978, b) Peters and Moldowan, 1993, c) Mackenzie *et al.*, 1980, 1982, d) Radke *et al.*, 1982, e) Radke *et al.*, 1986, 1990, f) Radke *et al.*, 1994, g) Garrigues *et al.*, 1988, Lichtfouse *et al.*, 1994. Compound identifications refer to assignments in Figures 2-3. MN : methylnaphthalene, EN : ethylnaphthalene, DMN : dimethylnaphthalene, MP : methylphenanthrene.

Formula	Compound	Value	Maturity	R_0	Refs.
Tm/Ts	A,B	0.73	High		a,b
C ₃₁ S/(S+R)	E	59%	High	> 0.6	c
C ₃₂ S/(S+R)	F	65%	High	> 0.6	c
2-MN/1-MN		2.2	High	1-1.5	d,e
2-EN/1-EN		1.5	High	0.6-1.2	d,e
(2,6- + 2,7-DMN)/ 1,5-DMN)		5.0	High	1.4	d,f
2-MP/1-MP	12,15	1.8	High	> 1.2	d
(2-MP +3-MP)/ (1- +9- +4-MP)	11,12,14,15	1.5	High	> 1.2	g

¹³C analysis

Using a separate approach, we examined soil organic matter in which *Zea mays* had been cultivated in two fields during 5 and 23 years (Table 2, Figure 4). After 23 years of maize cultivation, the isotope value of bulk soil organic carbon has increased by +6‰, giving evidence for the input of maize-derived C into soil organic components. The contribution of maize-derived C within each soil component can be calculated using the following equation (Lichtfouse, 1995, 1997) :

$$M = 100 \cdot (\delta - \delta_0) / (\delta_m - \delta_0)$$

where M is the percentage of maize-derived C of the soil organic component, δ is the isotope value of this component at any time of maize cultivation, δ_0 is the isotope value of this component before maize cultivation and δ_m is the isotope value of this component in maize. Thus, for bulk soil carbon, M reaches 42 % after 23 years of cultivation ($\delta_m = -12.51\text{‰}$). This significant input of maize C into soil organic substances contrasts sharply with the absence of significant isotope variation of PAH fractions observed in the two fields. Such a finding clearly shows that most of the soil PAHs were not labelled by maize C, and are therefore not biological products from plant, soil biomass and their humification products. An autotrophic contribution, e.g. from algae growing sporadically on the soil surface after rains, can be excluded because these organisms grow not only on atmospheric CO₂ but also on soil CO₂ which should be progressively labelled with ¹³C-enriched C from maize decomposition products. Moreover, the $\delta^{13}\text{C}$ value of PAH fractions, $\sim -27\text{‰}$, is not in agreement with a derivation from aromatized plant triterpenes. Indeed, given the $\delta^{13}\text{C}$ value of $\sim -36.5\text{‰}$ of C₂₇₋₃₃ *n*-alkanes from wheat growing these fields (Lichtfouse *et al.*, 1995b), one can roughly estimate a value of -35.0‰ for associated triterpenoids on biosynthetic grounds, as discussed by Hayes (1993). Further aromatization of those substances during humification processes is not expected to give notable isotopic changes (Freeman *et al.*, 1994).

Figure 4. Isotope variations of total organic carbon (TOC) and aromatic fractions (PAH) from soils planted with maize at fields of La Minière (circles) and Boigneville (squares). TOC is strongly labeled by ¹³C-enriched C from *Zea mays*, whereas aromatic hydrocarbons do not show any isotope variation. δ_0 refers to soils planted with C₃ plants. δ refers to soils planted with *Zea mays* at one crop per year.

¹⁴C analysis

We dated one aromatic fraction by accelerator mass spectrometry, and found a value of 9820 ± 70 years BP with a fraction Modern (fM) of less than 29‰ (see discussion by Lichtfouse and Eglinton, 1995). The average age of crop soil PAH is actually older than 9820 years because the convention for reporting fM is based on 1950 as 100% modern and does not take into account the large input of ¹⁴C from bomb testing after 1950. This age really is in full agreement with the ancient origin of soil PAH. Two main sources can therefore be suggested based on our different approaches : first, a major input of pyrolytic products from combustion of fossil fuels. Nonetheless, a minor contribution from products of burning vegetation cannot be ruled out. Second, a minor contribution of (uncombusted) fossil fuels.

Derivation of PAHs from the initial soil rock is possible but very unlikely because 1) initial PAH would have been rapidly removed by extensive water washing, oxidation and biodegradation during cropping practices (Campbell, 1978, Anderson and Coleman, 1985, Arrouays and Pelissier, 1994), and 2) the constant concentration of soil PAHs during cultivation calls for a regular input, e.g. from aerosols and tractor exhausts. In this respect, it has been shown that terrestrial plants could be a notable sink of atmospheric particles and associated PAHs by adsorption on cuticular waxes (Smith, 1977, Simonich and Hites, 1994). Such a process should therefore rapidly introduce airborne particles into our soils, given the high turnover of plants by cropping practices. Alternatively, a regular, upward migration of trace amounts of fossil fuels from deeper formations is possible because the soils studied are situated on the Paris Basin, which is well-known for its petroleum production (Tissot *et al.*, 1971, Lichtfouse *et al.*, 1994).

Whatever the precise origin of soil PAHs, our approach using molecular and isotope information has clearly disclosed that soil PAHs are ancient and must represent a major, allochthonous contribution of pyrolytic products derived most likely from airborne particulates. It should also be noted that the absence of isotope variation of a component in a system subjected to isotope changes (in this case the shift from C₃ to C₄ vegetation) can give useful insights on its origin. Moreover, molecular maturity parameters, that have been developed by organic geochemists in the last 30 years to study recent and ancient sediments, are promising tools to track the fate of fossil fuels in the modern environment.

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