



**HAL**  
open science

## Organic geochemistry of sewage sludge. I. Lipid fractionation by thin layer chromatography

Cécile Payet, Carine Bryselbout, Jean-Louis Morel, Eric Lichtfouse

### ► To cite this version:

Cécile Payet, Carine Bryselbout, Jean-Louis Morel, Eric Lichtfouse. Organic geochemistry of sewage sludge. I. Lipid fractionation by thin layer chromatography. *Analisis*, 1999, 27 (5), pp.396-398. hal-00262460

**HAL Id: hal-00262460**

**<https://hal.science/hal-00262460>**

Submitted on 11 Mar 2008

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Revised version

Analisis/European Journal of Analytical Chemistry 27, 396-398, 1999.

<http://www.edpsciences.org/articles/analisis/abs/1999/05/contents/contents.html>

Correspondence: Dr. Eric Lichtfouse, INRA-CMSE-PME, 17, rue Sully, 21000 Dijon, France

Eric.Lichtfouse@dijon.inra.fr

## **Organic Geochemistry of Sewage Sludge. I. Lipid Fractionation by Thin Layer Chromatography**

Cécile PAYET, Carine BRYSELBOUT, Jean-Louis MOREL and Éric LICHTFOUSE\*  
Laboratoire Sols et Environnement, INRA/ENSAIA-INPL, BP 172, 54505 Vandoeuvre-Lès-Nancy, France.

### **Abstract**

This article reports detailed procedures : 1) to decrease organic contamination of samples in the laboratory; 2) to extract sewage sludge with methylene chloride; and 3) to fractionate extracts by silica-gel thin layer chromatography (TLC) into fractions enriched in alkanes, aromatic hydrocarbons, ketones, alcohols and fatty acids, eluting either hexane or methylene chloride.

*Cet article décrit de façon détaillée : 1) des procédures visant à minimiser la contamination organique des échantillons au laboratoire; 2) l'extraction au chlorure de méthylène de boues de station d'épuration; et 3) le fractionnement des extraits par chromatographie sur couche mince (CCM) de silice en fractions enrichies en alcanes, en hydrocarbures aromatiques, en cétones, en alcools et en acides gras, en éluant soit de l'hexane, soit du chlorure de méthylène.*

### **INTRODUCTION**

Sewage sludge contain various organic substances inherited from the decay of urban waste waters. Molecular studies of sewage sludge have been mainly focused on xenobiotics because sludge can be recycled as fertilisers for agricultural production [1]. Nonetheless, knowledge on the source, transformation and fate of sludge molecules is scarce [2]. Here, we report the first step of a molecular study aiming at the systematic identification of sewage sludge lipids.

### **DECONTAMINATION**

Solvents were distilled in glass. Glass funnels, 250 ml Pyrex round-bottom flasks, 600 ml Pyrex beakers, steel mortar and rotary evaporator were vigorously washed with dish detergent, hot water (7 x), distilled water (3 x), allowed to dry at room temperature, then washed with distilled methylene chloride. Cellulose acetate filters, 10 ml glass vials, TLC glass jam-pots, aluminium foil, 2 ml screw-top vials, steel spatulas, cotton wool, pipette Pasteur and teflon-plugged glass syringes were washed with distilled methylene chloride prior use. 10 x 5 cm silica-gel 60 pre-coated F-254 glass TLC Merck plates were used. After labelling the top of the plates with a lead pencil, the plates were washed by elution of distilled ethyl acetate, allowed to dry on aluminium foil under a ventilated hood, reactivated 2 h at 50°C, 2 h at 80°C, 2 h at 110°C in a ventilated oven, then allowed to cool at room temperature. The aim of this step is to concentrate the

---

\* Author for correspondence <lichtfouse@ensaia.u-nancy.fr>

contaminants at the top 0.5 cm of the plates. The plates were handled with fingers touching only the top 0.5 cm.

### EXTRACTION

Sludge (~ 300 g) were freeze-dried then finely ground 1 min using a steel-ball shaking mortar. ~ 60 g of dried sludge were extracted 3 times with methylene chloride (120 ml, 60 ml, 60 ml) in a 600 ml Pyrex beaker dipped 15 min into a sonication water-bath. The liquid phase was transferred onto a cellulose acetate filter fitted on a glass funnel, using a spatula to guide the liquid flow. The filtrates were collected in a 250 ml round-bottom flask, concentrated at nearly 1 ml at 30°C under vacuum, transferred into a 10 ml glass vial with a 1 ml teflon-plugged syringe, allowed to dry under a ventilated hood, then kept at - 20°C.

### FRACTIONATION

A sub-sample of the extract (~ 0.5 mg) was prepared by dilution with methylene chloride, transferred into a 2 ml glass vial, then concentrated under a ventilated hood. Vertical lines were drawn with a lead pencil at 1.5 cm (migration start) and 9 cm (end) on the TLC plates. A simple rising device allowed the absence of contact between the ruler and the silica-gel. The extract in CH<sub>2</sub>Cl<sub>2</sub> was spot-transferred onto the 1.5 cm line of the plate with a glass capillary, allowed to dry 3 min, then eluted either with hexane or CH<sub>2</sub>Cl<sub>2</sub>. Pure standards of increasing polarity (*n*-octacosane, 2-methylphenanthrene, 1,2:3,4-dibenzanthracene, friedelin, cholesterol) were eluted on a separate TLC plate. Standards were revealed by UV (254 nm), then by short dipping into a 5% v/v EtOH-H<sub>2</sub>SO<sub>4</sub> solution followed by hot-plate heating (~ 250°C) under a ventilated hood. R<sub>f</sub> values refers as ratios of migration length substance versus solvent.

TLC elution of extracts with hexane allowed to fractionate alkane- and aromatic hydrocarbon enriched fractions (**Figure 1**). TLC elution of extracts with CH<sub>2</sub>Cl<sub>2</sub> allowed to fractionate ketone-, alcohol- and fatty acid enriched fractions (**Figure 2**). The fractions were recovered from the plates by scratching the silica gel toward aluminium foil with a spatula, by transferring the silica gel via a glass funnel (fitted with a teflon tube) on a pipette Pasteur plugged with cotton wool [3], then by elution of a solvent (~ 2 ml) into a 2 ml vial using CH<sub>2</sub>Cl<sub>2</sub> except for the fatty acid fraction (ethyl acetate). Analyses of the fractions by gas chromatography coupled to mass spectrometry will be reported elsewhere.

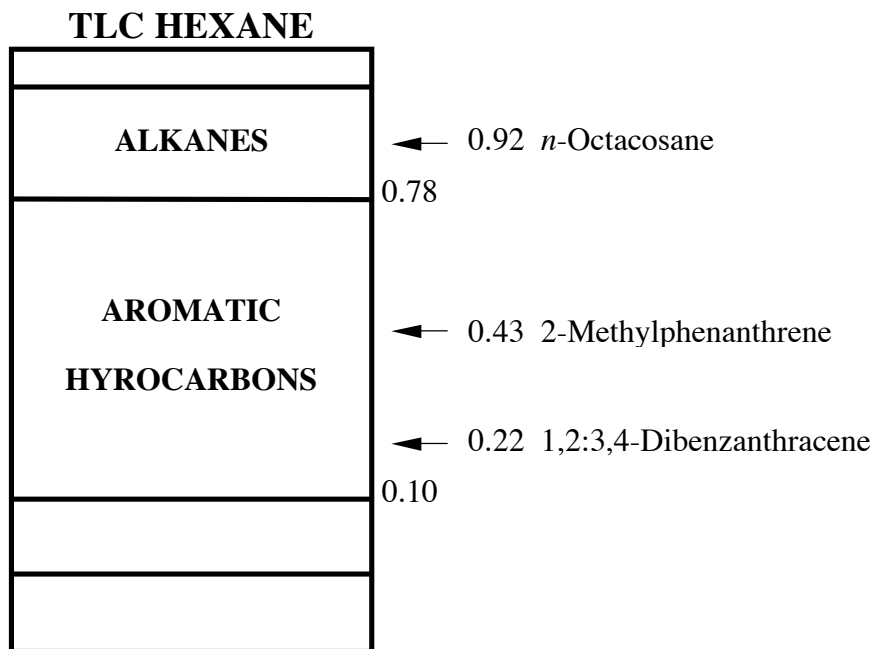


Figure 1. Fractionation of sewage sludge  $\text{CH}_2\text{Cl}_2$  extracts into alkane- and aromatic enriched fractions by thin layer chromatography (TLC) eluting hexane.

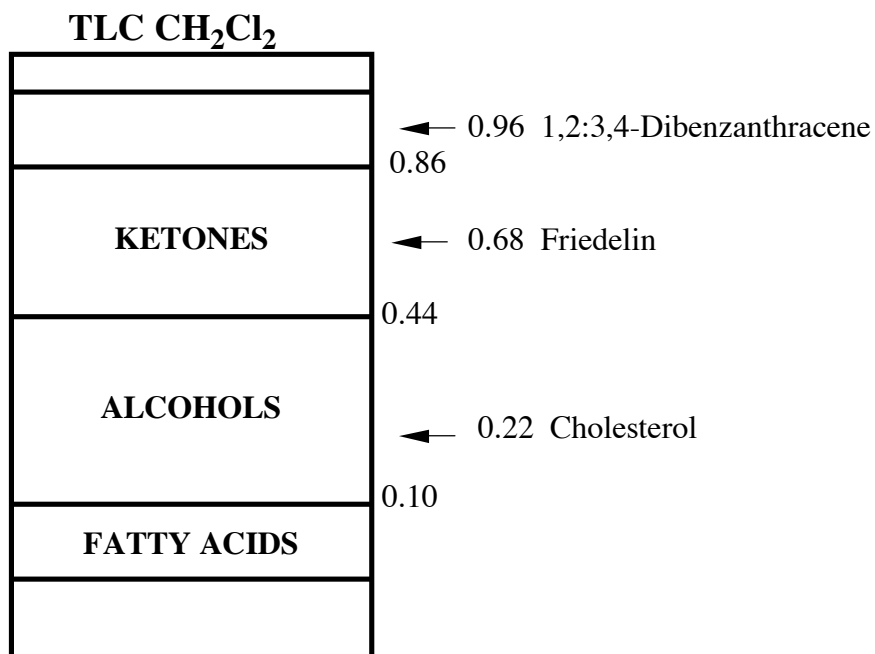


Figure 2. Fractionation of sewage sludge  $\text{CH}_2\text{Cl}_2$  extracts into ketone-, alcohol- and fatty acid enriched fractions by thin layer chromatography (TLC) eluting  $\text{CH}_2\text{Cl}_2$ .

## REFERENCES

- 1 Wilson SC, Alcock RE, Sewart AP, Jones KC, J Environ Qual, 1997, 26, 1467-1477 and refs. therein. Edwards NT J Environ Qual, 1993, 12, 427-441.
- 2 Hatcher PG, McGillivray PA, Environ Sci & Technol, 1979, 10, 1225-1229.
- 3 Henner P, Schwartz C, Lichtfouse E, Analisis, 1997, 25, M51-M52.