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Ahmed I. Rushdi
Ali A. DouAbul
Sama Samir Mohammed
Bernd R. T. Simoneit

Compositions and sources of extractable organic matter in Mesopotamian marshland surface sediments of Iraq. I: aliphatic lipids

A. I. Rushdi (✉) · B. R. T. Simoneit
Environmental and Petroleum
Geochemistry Group, College of Oceanic
and Atmospheric Sciences,
Oregon State University, Corvallis
OR 97331, USA
E-mail: arushdi@coas.oregonstate.edu
Tel.: +1-541-7375707
Fax: +1-541-7372064

A. A. DouAbul · S. S. Mohammed
Iraq Foundation, House 45, Street 3,
Area 609, Mansour, Baghdad, Iraq

B. R. T. Simoneit
Department of Chemistry,
College of Science, Oregon State
University, Corvallis
OR 97331, USA

Abstract Shallow surface sediment samples from the Mesopotamian marshlands of Iraq were collected and analyzed to determine the distribution, concentrations and sources of aliphatic lipid compounds (n-alkanes, n-alkanols, n-alkanoic acids, and methyl n-alkanoates) and molecular markers of petroleum in these wetlands. The sediments were collected using a stainless steel sediment corer, dried, extracted with a dichloromethane/methanol mixture and then analyzed by gas chromatography-mass spectrometry (GC–MS). The aliphatic lipid compounds included n-alkanes, n-alkanoic acids, n-alkanols and methyl n-alkanoates with concentrations ranged from 6.8 to 31.1 µg/g, 4.1 to 5.0 µg/g, 5.9 to 7.7 µg/g and from 0.3 to 5.9 µg/g, respectively. The major sources of aliphatic lipids were natural from waxes of higher plants (24–30%) and microbial residues (42–30%), with a significant contribution from anthropogenic sources (27–30%, petroleum), based on the organic geochemical parameters and indices. Further studies are needed to characterize the rate, accumulation and transformation of various organic matter sources before and after re-flooding of these wetlands.

**Keywords** Mesopotamian marshes · UCM · GC–MS · Lipids · Iraq

Introduction

The Tigris and Euphrates rivers have created about 15,000 km^2 of wetlands known as the Mesopotamian marshes. These wetlands comprise a complex of interconnected shallow freshwater lakes and marshlands and are considered the most extensive wetland ecosystem in the Middle East (Brasington 2002; Partow 2001). Water levels reach their maximum in early spring and then fall by as much as two meters during the hot dry summer (Al-Saaidi et al. 1981; Talling 1980). The larger wetlands within this complex ecosystem are: (a) the Al-Hammar and its associated marches south of the Euphrates, (b) the Central Marshes, a vast complex of permanent lakes and marshes north of the Euphrates and west of the Tigris, and (c) the Al-Hawizeh and its associated mar-shes extending east from the Tigris into neighboring Iran (Fig. 1). These wetlands ultimately drain southeast-wards into the Gulf via the Shatt Al-Arab waterway (DouAbul et al. 1988; Partow 2001). Iraq's very limited coastal zone consists mainly of intertidal mudflats flanked by bare silt flats, often with an intervening narrow strip of date palm gardens (Maltby 1994; Partow 2001).

The economy of many peoples living in the region has been intimately involved with the wetlands for at least 6,000 years (Thesiger 1985). Civilization was well established in Mesopotamia by the fourth millennium BC, and a sophisticated irrigation system had been developed at that time (Salim 1962). Floodplain wetlands, river banks and lake shores are utilized for the cultivation of cereals, rice or vegetables, while the rivers

Fig. 1 Location map of the sampling sites in Abu Zirig and Kurmashia marshlands
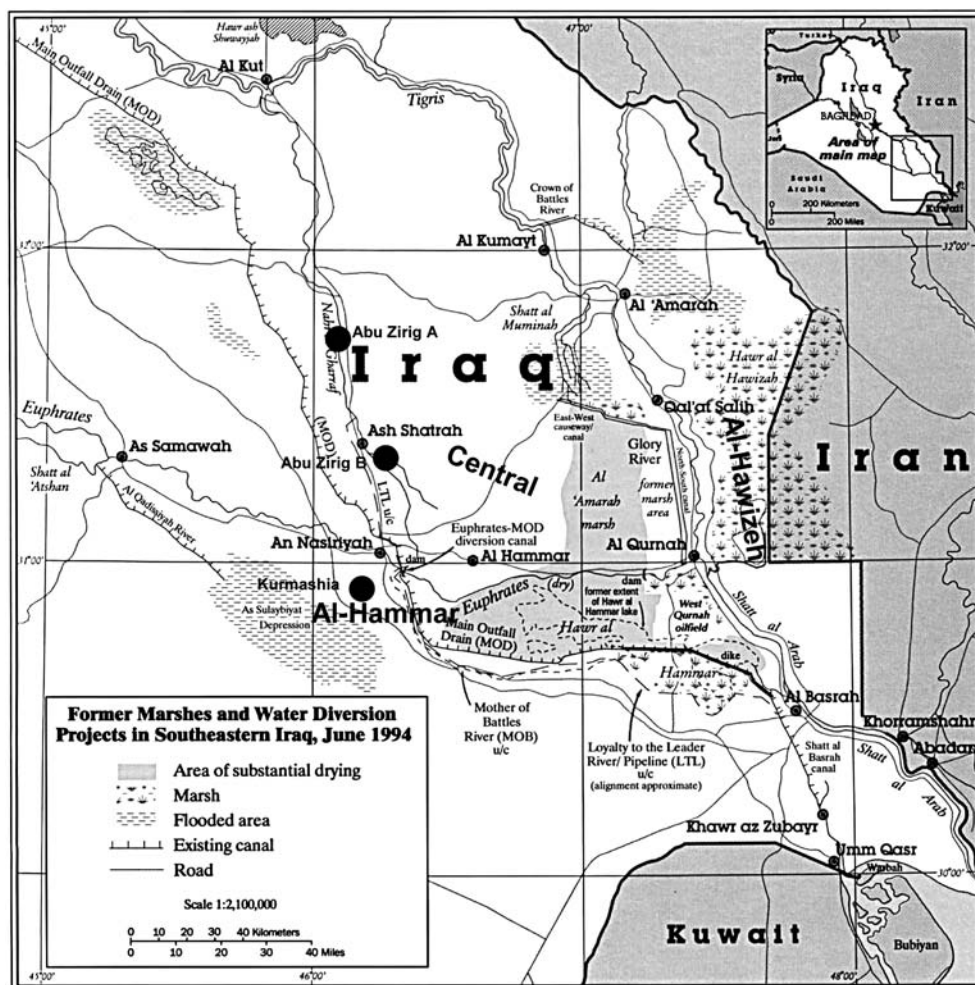
and lakes themselves support freshwater fisheries. In the vast reed-beds of Mesopotamia, marsh-dwelling communities are almost totally dependent on reeds for their construction needs. Large numbers of domestic livestock, particularly water buffalo, are allowed to graze on wetland vegetation, and aquatic plants are harvested to provide fodder during winter months. In Mesopotamia, large numbers of waterfowl are captured and sold each year, providing a livelihood for hundreds of people (Salim 1962; Thesiger 1985; Young 1983).

One of the most serious threat to the wetlands in Iraq has been the drainage and diversion of water, as typically supply for agricultural purposes, but also in recent years, for military reasons (Partow 2001). Within the last few years, major hydrological engineering activities in and around the area of Lower Mesopotamia have resulted in the drying out of vast areas of wetlands in the Central Marshes and Al-Hammar, and could eventually lead to the disappearance of these systems (Lawler 2005; Richardson et al. 2005). Currently, less than 10% of the marshlands in Iraq remain as fully functioning wetlands because of the extensive

drainage and upstream agricultural irrigation programs on the Tigris and Euphrates rivers (DouAbul et al. 1988; Lawler 2005; Maltby 1994; Partow 2001). The Marsh Arabs, or Ma'dan, who have lived in the marshes for at least 5,000 years, have been particularly affected by these actions (Brasington 2002; Lawler 2005; Maltby 1994; Partow 2001).

Currently, restoration by re-flooding of drained marshes is proceeding in the Central and the Al-Hammar marshlands (Lawler 2005; Partow 2001). Uncontrolled re-flooding may have counterproductive impacts on the restoration processes of these wetlands. Therefore, interdisciplinary studies are necessary and essential, especially during the restoration operations that are currently taking place.

The main objectives of this study are to determine the characteristics, distribution, and concentrations of the extractable organic matter in shallow sediments of recently re-flooded marshlands of Iraq and identify the sources of these organic compounds based on key parameters and molecular marker analysis. This study focuses on the aliphatic lipids.

Experiments

Sampling site

Abu Zirig marsh of the Central Marshes is at the terminal end of the Gharraf River as shown in Fig. 1. It is north of An Nasiriyah in a natural depression. A series of embankments, built in 1920, protect the agricultural farms around the perimeter of the deep lake of Abu Zirig. The main supply of water to the marsh is through the Shatt Abu Lihia river channel, which runs through the marsh until it dissipates at the southern end into a larger marsh. In addition, the marsh is fed through three other separate channels

Fig. 2 TIC GC–MS total ion current traces of total extracts of the surface sediment samples showing the major organic components (nonpolar): **a** Abu Zirig A; **b** Abu Zirig B; and **c** Kurmashia (numbers refer to the carbon chain length, *ip* isoprenoids, and *UCM* unresolved complex mixture)
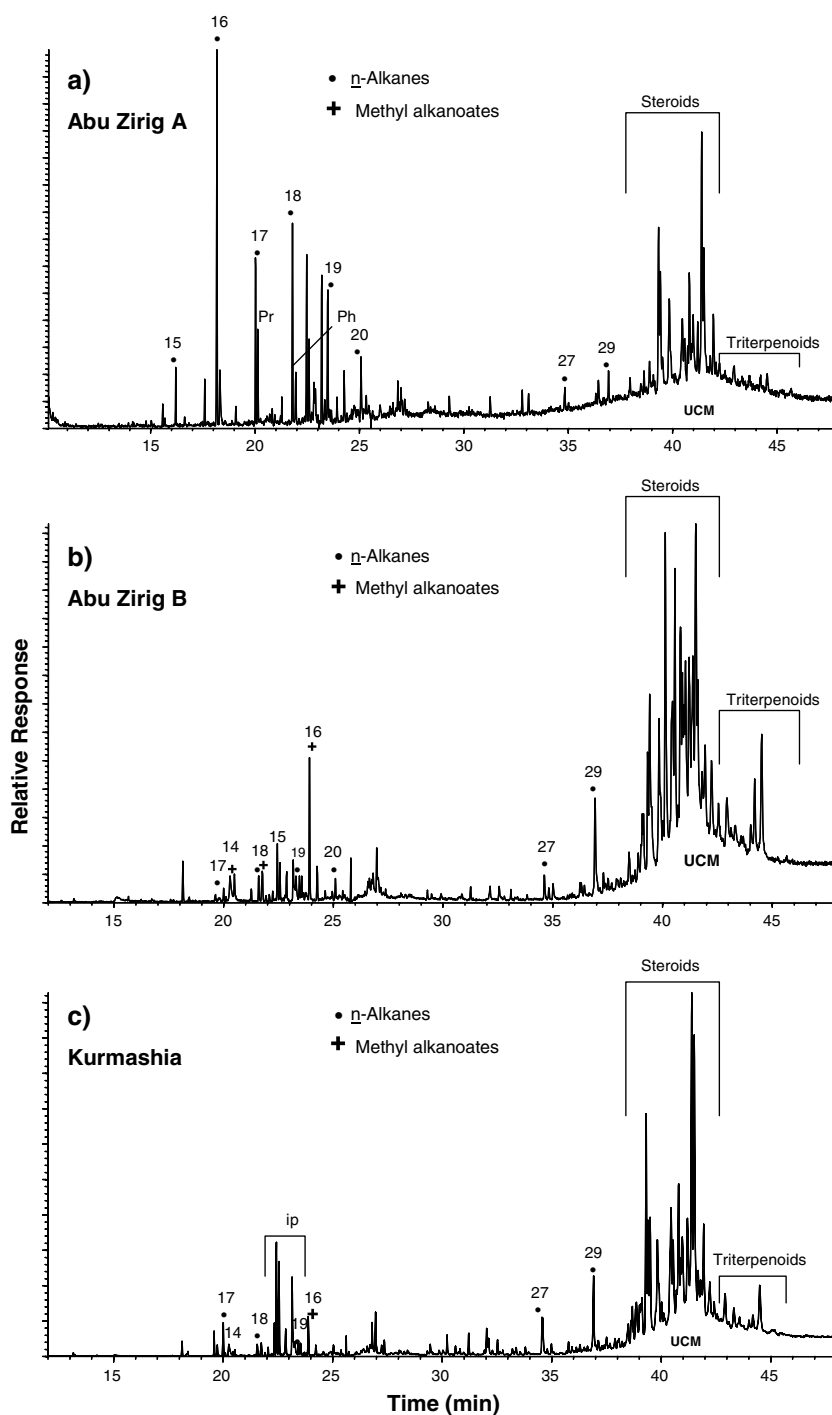
Table 1 Concentrations and geological parameters of solvent-extractable aliphatic compounds of surface sediments from the Mesopotamian marshlands, Iraq

	Abu Zirig (A) (n = 3)	Abu Zirig (B) (n = 4)	Kurmashia (n = 4)
n-Alkanes			
Range	14–31	23–31	16–31
C_{max}	16	29	29
Concentration (µg/g)	9.1 ± 2.7	6.8 ± 1.0	31.1 ± 3.3
CPI[a]	1.2 ± 0.3	7.2 ± 0.1	2.9 ± 0.4
Total wax *n*-alkanes (µg/g)	1.0 ± 0.3	5.9 ± 0.8	11.7 ± 1.3
Total wax *n*-alkanes (%)	13.0 ± 3.3	88.0 ± 0.7	38.7 ± 4.6
Pr/Ph	1.4 ± 0.1	0.44 ± 0.03	0.6 ± 0.2
n-Alkanoic acids			
Range	8–19	8–18	8–28
C_{max}	16	16	16
Concentration (µg/g)	3.2 ± 2.2	7.5 ± 3.7	25.5 ± 1.9
CPI[b]	4.5 ± 0.7	4.1 ± 0.8	5.0 ± 0.3
n-Alkanols			
Range	12–32	14–32	12–32
C_{max}	16, 30	16, 26	30
Concentration (µg/g)	18.4 ± 1.8	39.7 ± 6.8	111.4 ± 9.7
CPI[c]	7.7 ± 0.5	6.9 ± 0.5	5.9 ± 0.1
Methyl *n*-alkanoates			
Range	13–31	13–31	13–31
C_{max}	17	17	17
Concentration (µg/g)	0.34 ± 0.33	5.9 ± 2.0	4.7 ± 0.7
UCM			
Concentration (µg/g)	22.3 ± 7.0	4.0 ± 0.7	38.5 ± 5.1
Percent	31.2 ± 1.8	52.3 ± 0.9	47.6 ± 1.7
U:*n*-C_B[d]	1.7 ± 0.9	0.2 ± 0.1	1.4 ± 0.7

[a]CPI = $(\sum nC_{15} + nC_{17} + \cdots + nC_{31})/(\sum nC_{14} + nC_{16} + \cdots + nC_{30})$
[b]CPI = $(\sum nC_8 + nC_{10} + \cdots + nC_{18})/(\sum nC_9 + nC_{11} + \cdots + nC_{19})$
[c]CPI = $(\sum nC_{14} + nC_{16} + \cdots + nC_{32})/(\sum nC_{13} + nC_{15} + \cdots + nC_{31})$
[d]Ratio of UCM/total plant waxes

(al-Rumaidh, Abu Ismaisim and Abu Al-Jury), all emanating from the Gharraf River. The rivers and channels feed the marsh along its northerly perimeter. The marsh terminates at the left bank of the Euphrates River. The Abu Zirig marsh was re-flooded in April 2003 as a result of the direct action of the Ministry of Water Resources at the request of the local population.

Kurmashia marsh is located southeast of An Nasiriyah, at the head of the Al-Hammar marsh (Fig. 1). This area has been inundated since May 2003 as a result of the opening of the termini of several distributary canals from the Euphrates. Its area may reach more than 100 km², and it contains a considerable variety of plant-cover and various water depths ranging from open mud-flats to open water around 2 m deep.

Sampling and sample preparation

The Abu Zirig and Kurmashia marshes were chosen as examples to study the restoration processes in Iraq wetlands because: (a) they were the first marshes to be re-flooded; (b) they are relatively small areas with limited numbers of inlets and outlets, and (c) the area has well controlled hydro-biological variables. Sediment core samples were collected from the northern and southern parts (Fig. 1) of Abu Zirig marsh and Kurmashia wetland using a stainless steel gravity corer. About 3 cm³ of each wet sediment sub sample was taken by micro core at 5 cm intervals, dried at room temperature, then ground and sieved to obtain < 125 µm fine particles.

Extraction

About 5 g of sediment sample was extracted three times using ultrasonic agitation for a 15 min period each with 30 mL of dichloromethane and 10 mL of methanol. The extraction was carried out in a 150 mL precleaned beaker. The extract was then filtered using a filtration unit containing an annealed glass fiber filter for the removal of sediment particles. The filtrate was first concentrated on a rotary evaporator and then reduced using a stream of dry nitrogen gas to a volume of approximately 200 µL. The volume was then adjusted to 500 µL exactly by addition of dichloromethane: methanol (3:1, v:v).

Instrumental analysis

Gas chromatography-mass spectrometry (GC–MS) was carried out with a Hewlett-Packard 6890 gas chromatograph coupled to a 5973 Mass Selective Detector, using a DB-5MS (*Agilent*) fused silica capillary column (30 m×0.25 mm i.d., 0.25 µm film thickness) and helium as carrier gas. The GC was temperature programmed from 65°C (2 min initial time) to 310°C at 6°C min⁻¹ (isothermal for 20 min final time) and the MS was operated in the electron impact mode at 70 eV ion source energy. Mass spectrometric data were acquired and processed using the GC–MS ChemStation data system.

Identification and quantification

The identification of *n*-alkanes was based on the GC–MS data (i.e., m/z 85 fragmentograms and mass spectra). Retention times were compared with those of external standards. The identities of *n*-alkanoic acids, *n*-alkanols and methyl *n*-alkanoates are based primarily

on their mass spectra (i.e., key ions m/z 117, 103 and 74, respectively) and gas chromatographic retention times. Quantification was performed from the GC profiles using the external standard method with authentic compounds of each homologous series (Rushdi et al. 2006). Average response factors were calculated for each compound. All quantifications were based on the compound areas derived from the ion fragmentogram.

Results and discussion

The main features of the GC–MS data for the sediment extracts are shown in Fig. 2. Major aliphatic compounds identified are listed in Table 1. The extracts comprise lipids and biomarkers from both autochthonous wetland and allochthonous terrigenous sources, and contain considerable amounts of anthropogenic organic compounds. These include n-alkanes, n-fatty acids, methyl n-alkanoates, n-alkanols, steroids and titerpenoids, which can be utilized to identify originating sources (Simoneit 1984, 1985). Accordingly, comparisons are possible between known sources and observed organic carbon mixtures in environment.

n-Alkanes

n-Alkanes, which occur in all ecosystems, are especially suitable for assessing the origin, source and fate of organic matter in the environment. They are derived from both biogenic and anthropogenic sources and can be differentiated based on their distribution pattern in samples. Key parameters associated with n-alkane sources and characteristics are the well-established carbon preference index (CPI) and carbon number maximum (C_{max}) (Simoneit 1977, 1978). For sediment samples from the marshes, the dominant n-alkanes are in the range of C_{14} to C_{31}. With concentrations of 9.1 ± 2.7 µg/g dry weight (dw) for Abu Zirig site A, 6.8 ± 1.0 µg/g dw for Abu Zirig B and up to 31.1 ± 3.3 µg/g dw for the Kurmashia site (Fig. 3a). They increase in concentration from north to south.

The C_{max} of the most abundant n-alkane can also be used as an indicator of relative source input (Simoneit 2002). The sediment samples have an even C_{max} at 16 for Abu Zirig A and an odd C_{max} at 29 for Abu Zirig B and Kurmashia (Fig. 4). An even C_{max} at 16 or 18 is not common for sediments and only a few microbial organisms produce direct inputs of predominantly even-carbon chain n-alkanes (Bird and Lynch 1974; Davis

Fig. 3 The mean concentrations of **a** n-alkanes, **b** wax n-alkanes, **c** n-alkanoic acids, **d** methyl n-alkanoates, **e** n-alkanols, and **f** UCM
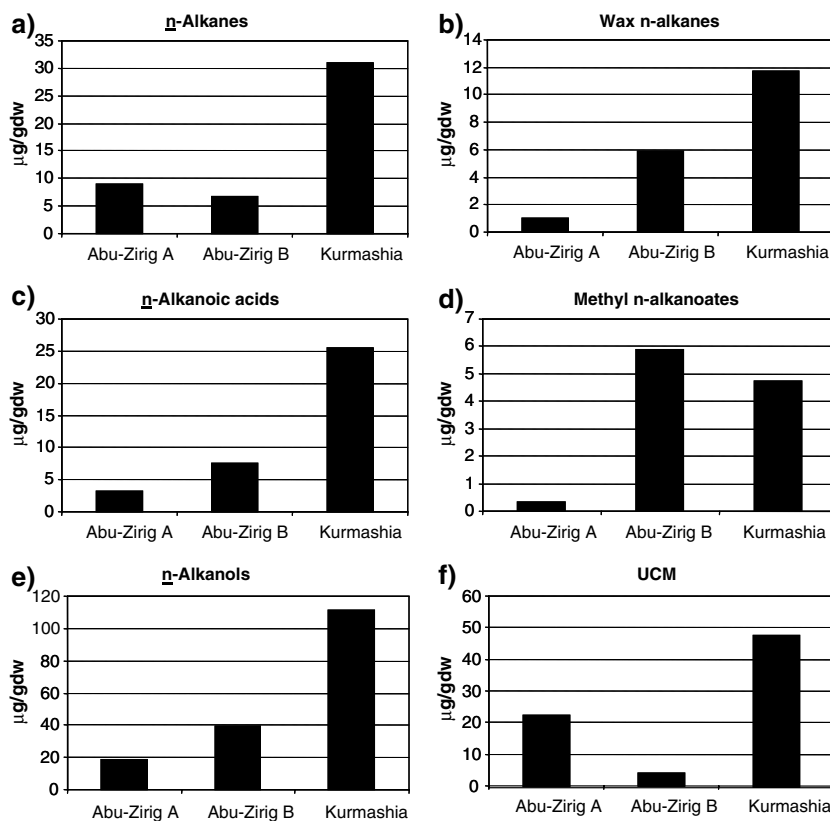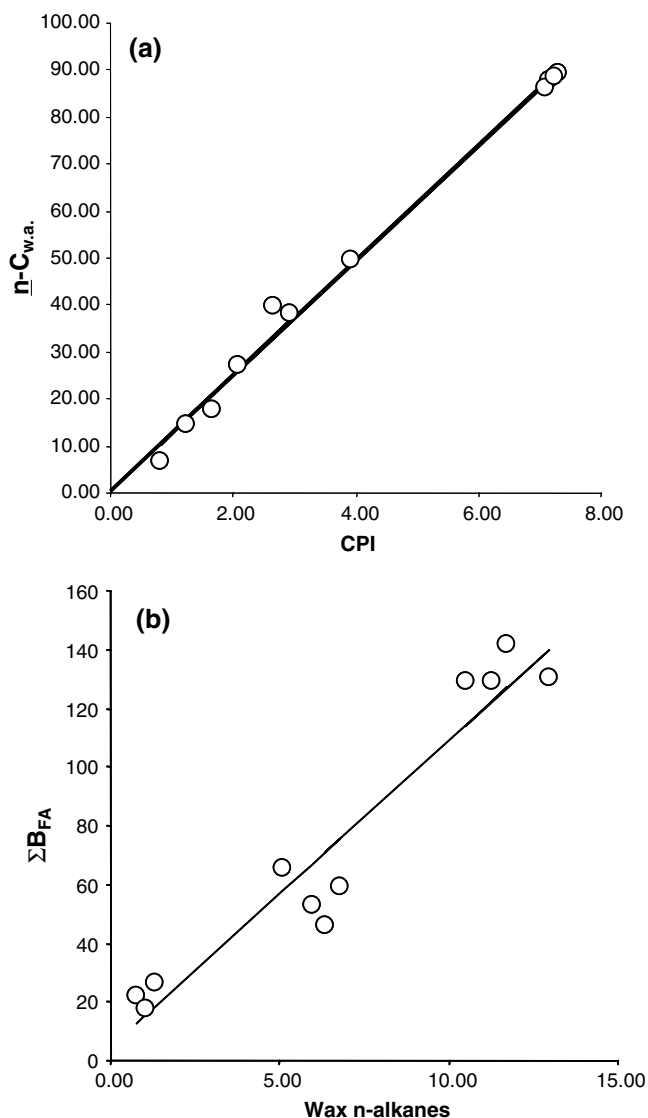
Fig. 4 Examples of typical GC–MS key ion plots for *n*-alkanes in the sediment samples, (*m/z* 85): **a** Abu Zirig A, **b** Abu Zirig B, and **c** Kurmashia (numbers refer to carbon chain length)
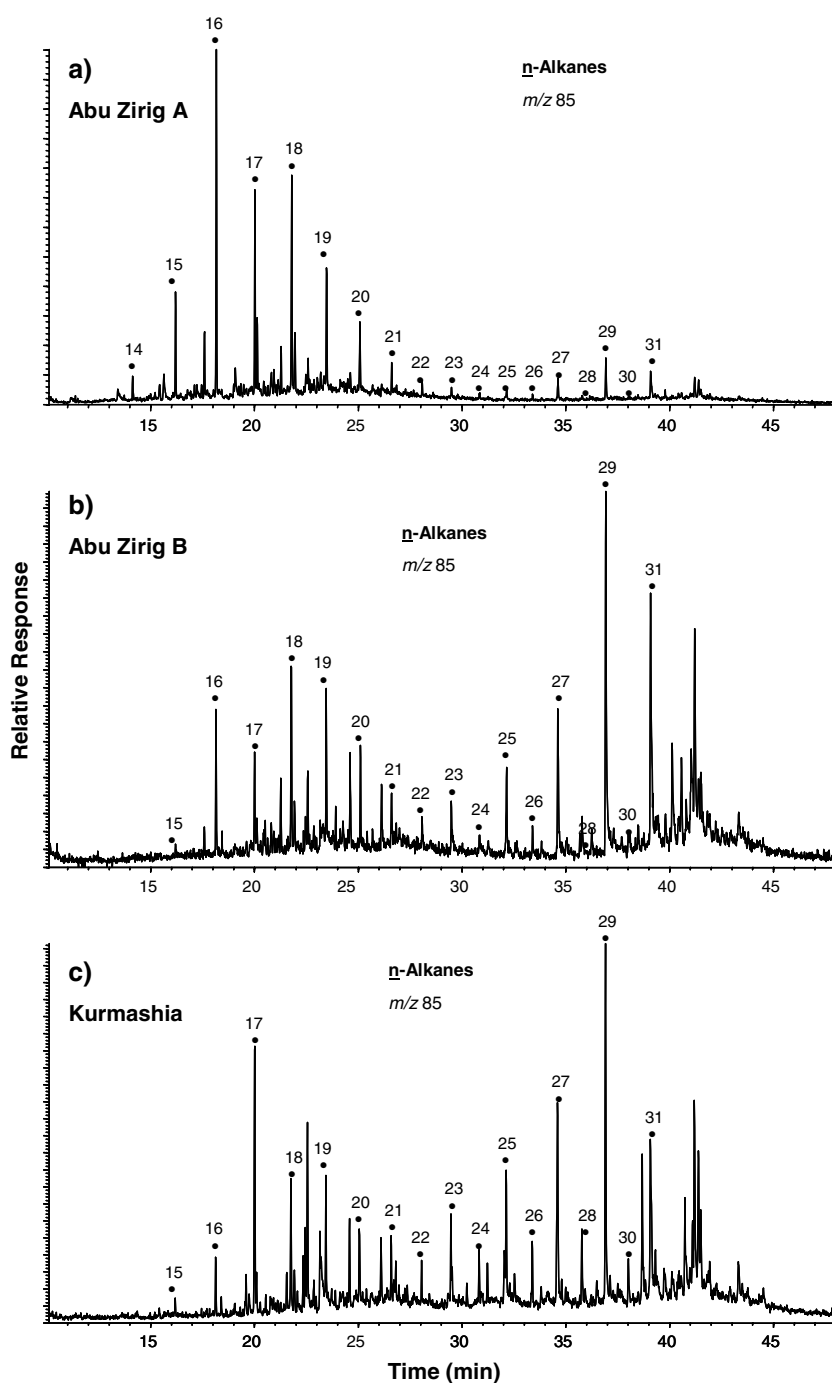
1968). Even-carbon predominances have been reported in sediments from the Black Sea (Debyser et al. 1977) and the Arabian Gulf (Grimalt et al. 1985). This was attributed to the anoxic depositional conditions, which promote the bacterial reduction of fatty acids (C_{14}–C_{18}), yielding *n*-alkanes that maximize at C_{16}–C_{18}. The even-carbon number predominances are less pronounced in samples from Kurmashia. The samples from Kurmashia and Abu Zirig have a C_{max} at 29, which is characteristic of biogenic sources from higher plant waxes (Fig. 4). This higher molecular weight *n*-alkane distribution supports the interpretation that plant waxes of tropical vegetation have a high C_{max} (Simoneit 1978). The CPI values for the *n*-alkanes of these samples are 1.24 ± 0.25

Fig. 5 Correlation between **a** CPI and % plant wax *n*-alkanes (*n*-$C_{w.a.}$) and **b** wax *n*-alkanes and total *n*-alkanols, *n*-alkanoic acids, and methyl *n*-alkanoates (ΣB_{FA}) for all wetland sediments

alkanes and their percentage of the total *n*-alkanes were calculated as the sum of $nC_{25} + nC_{27} + nC_{29} + nC_{31}$ (Table 1). The amounts of plant wax are $12.9 \pm 3.3\%$ in Abu Zirig A, $87.9 \pm 0.7\%$ in the Abu Zirig B and $38.6 \pm 4.5\%$ in Kurmashia samples. The percentage of plant wax *n*-alkanes is lower in sediment samples from Abu Zirig site A than from the other sites, which confirms lesser plant wax input there. The CPI shows a positive correlation with the percentage of *n*-alkanes from higher plant wax (Fig. 5). This supports the effectiveness of CPI as an indicator of biogenic sources from plant waxes versus other biogenic and anthropogenic inputs. The following equation was derived for these sediment samples (Fig. 5):

$$\% \, n\text{-}C_{\text{w.a.}} = 0.57 + 12.23 \ \ \text{CPI} \tag{1}$$

where % *n*-$C_{w.a.}$ is the relative concentration of *n*-alkanes in percentage of plant wax and CPI is the carbon preference index. This equation can be applied to calculate the percentage of plant wax *n*-alkanes by using CPI only for the marsh sediments.

Pristane (2,6,10,14-tetramethylpentadecane) and phytane (2,6,10,14-tetramethylhexadecane) are produced from the geochemical diagenesis of phytol (3,7,11,15-tetramethyl-2-hexadecen-1-ol), which is presumably produced from the hydrolysis of chlorophyll found in phototrophs (Bendoraitis et al. 1962; Maxwell et al. 1971; Ikan et al. 1975; Didyk et al. 1978), and from other microbial sources. Pristane and phytane are present in crude oil diesel fuel, lubricating oil, and in both auto and diesel engine exhaust (Simoneit 1984, 1985). The concentrations of the isoprenoid hydrocarbons in these sediments are presented in Table 1. The presence of pristane and phytane coupled with the unresolved complex mixture (UCM) generally confirm the contamination by residues from petroleum and petroleum products (Simoneit 1984, 1985).

(Abu Zirig A), 7.20 ± 0.04 (Abu Zirig B) and 2.90 ± 0.39 (Kurmashia) (Table 1). The lower CPI and C_{max} for the *n*-alkanes in the Abu Zirig A samples indicate that microbial sources are major, whereas the *n*-alkanes in the Abu Zirig B and Kurmashia samples with higher CPI and C_{max} at 29 are derived from higher plant waxes with a minor contribution of microbial inputs. Plant wax *n*-alkanes have a C_{max} in the range of 25–31, which varied depending on the plant species as well as the season and locality (e.g., Abas and Simoneit 1998; Eglinton and Hamilton 1967; Mazurek et al. 1991; Rogge et al. 1993; Simoneit and Mazurek 1982; Stephanou and Stratigakis 1993). To better evaluate the relative input from various sources, the concentrations of plant wax *n*-

n-Alkanoic acids, methyl *n*-alkanoates and *n*-alkanols

The concentrations of *n*-alkanoic acids are 3.18 ± 2.19 µg/g dw for Abu Zirig A, 7.49 ± 3.69 µg/g dw for Abu Zirig B, and 25.49 ± 1.91 for µg/g dw Kurmashia sediment samples (Table 1, Fig. 3c). They range from C_8 to C_{18} with a maximum concentration at C_{16} for both Abu Zirig A and B sites. For the Kurmashia site, they range from C_8 to C_{28} also with a maximum concentration at C_{16} (Fig. 6a). The methyl *n*-alkanoate concentrations are 0.34 ± 0.33 µg/g dw for Abu Zirig A, 5.88 ± 1.95 µg/g dw for Abu Zirig B and 4.72 ± 0.7 µg/g dw for Kurmashia (Fig. 3d). In all samples, they range from C_{11} to C_{31} with a maximum concentration at C_{17} (as acids range is C_{23}–C_{30}, $C_{max} = 16$) (Fig. 6b). Methyl *n*-alkanoates may be natural or form by transesterification during extraction.

Fig. 6 Examples of typical GC–MS key ion plots for polar compound series found in the sand and soil samples: **a** Abu Zirig A, m/z 117 n-alkanoic acids; **b** Abu Zirig B, m/z 87 methyl n-alkanoates; and **c** Kurmashia, m/z 103 n-alkanols (numbers refer to carbon chain length, polar compounds are derivatized with silylating reagent, BSTFA to TMS)
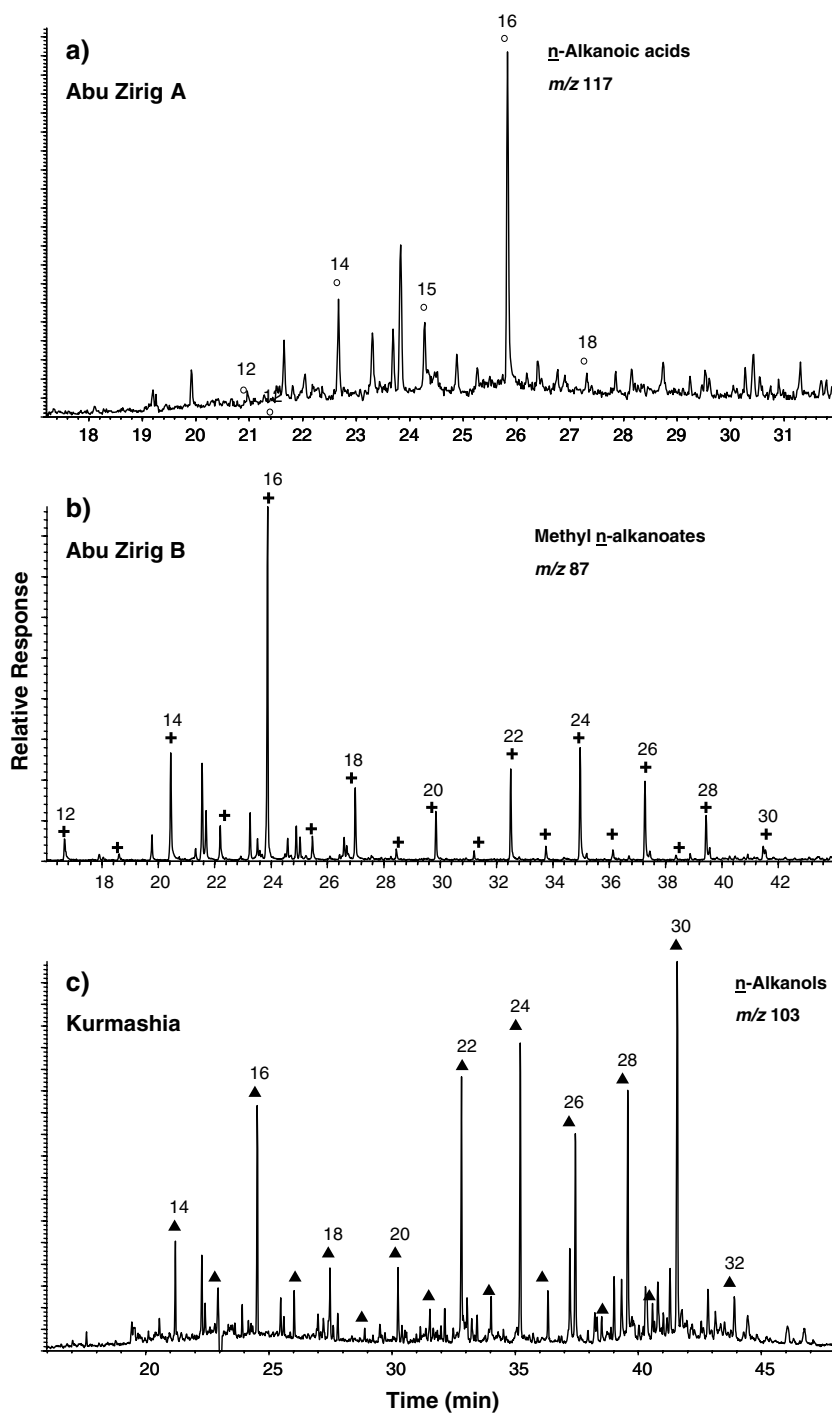
The n-alkanols range from C_{12} to C_{32} with essentially no odd numbered homologs and C_{max} mainly at 30 (e.g., Fig. 6c). They are the major compounds in the derivatized total extracts of the sediment samples (Fig. 3e). The concentrations are relatively high with mean values of 18.41 ± 1.83 μg/g dw in samples from Abu Zirig A, 39.74 ± 6.82 μg/g dw in samples from Abu Zirig B and 111.40 ± 9.73 μg/g dw in Kumashia site (Table 1, Fig. 3). The n-alkanol distributions indicate the major source from plant wax with a minor microbial component (Simoneit 1978).

The n-alkanols and/or n-alkanoic acids < C_{20} in the extractable lipids or bound in the organic detritus (e.g., as part of membrane residues) could be precursors for

the even-C-chain length *n*-alkanes in sediments as mentioned previously. The *n*-alkanoic acids (both free and as methyl esters) and *n*-alkanols detected in these samples have strong even carbon number predominances > C_{12}, which indicate that these compounds are of a biotic origin (Kolattukudy et al. 1976; Simoneit 1978). The distributions of *n*-alkanoic acids and *n*-alkanols from C_{12} to C_{18} indicate a microbial origin and those from C_{20} to C_{32} reflect an origin from higher plant wax (Simoneit 1978). Palmitic acid is the dominant saturated homolog and is interpreted to be derived from both microbial and terrestrial higher plant sources.

The input of organic matter from terrestrial sources is indicated by the positive correlation between the concentrations of plant wax *n*-alkanes against the sum of the concentrations of *n*-alkanols, *n*-alkanoic acids and methyl *n*-alkanoates (Fig. 5b).

Unresolved complex mixture (UCM)

GC traces of lipid compounds obtained from sediment samples usually contain a number of unresolved branched and cyclic hydrocarbons. These hydrocarbons are derived mainly from oil spills and/or fossil fuel utilization and register in the GC traces as a signal above the baseline with the resolved compounds superimposed. This is known as the UCM of branched and cyclic compounds (Simoneit 1984, 1985). Exhaust from gasoline autos exhibits a narrow UCM, while diesel vehicle exhaust shows an UCM with two maxima, which is derived both from the fuel (lower C_{max}) and the lubricating oil (higher C_{max}) (Simoneit 1984, 1985). Biogenic hydrocarbons derived from higher plants exhibit no UCM (Simoneit and Mazurek 1982). Anaerobic alteration of microbial detritus can yield an UCM with a C_{max} at 19–22 (Simoneit et al. 1979). Thus, the UCM derived solely from fossil fuel utilization can be compared as a ratio of UCM to plant wax *n*-alkanes, *n*-alkanoic acids, *n*-alkanols and *n*-methyl alkanoates (U:*n*-C_B ratio) and used to assess the level of contamination by oil spills and input of petroleum related products. The concentrations of UCM and the U:*n*-C_B values are given in Table 1. They are higher for Abu Zirig A samples, which are contaminated by biodegraded petroleum residues (Peters and Moldowan 1993). Hopane biomarkers were detected in trace amounts only in Abu Zirig A samples, whereas as sterane biomarkers were not detectable.

From the previous discussion, it is obvious that Abu Zirig A samples are more enriched in *n*-alkanes, *n*-alkanols and *n*-alkanoic acids derived from microbial activity as a result of recent re-flooding operations. In Kurmashia sediments, the biogenic input of *n*-alkanes, *n*-alkanols, *n*-alkanoic acids and methyl *n*-alkanoates from higher plants are more dominant.

It is apparent that the main sources of lipid compounds in these wetland sediments are vascular plant, microbial and anthropogenic contributions. The fraction of terrestrial higher plant inputs can be estimated as the sum of the extractable lipid compounds with C-chain lengths > C_{20}. On the other hand, the microbial sources are the sum of all lipids with C-chain lengths < C_{20}. In addition, C_{25}, C_{27}, C_{29} and C_{31} *n*-alkanes are treated as inputs from terrestrial plant waxes, whereas C_{14}–C_{20} are treated as sources from microbial detritus. The anthropogenic (mainly petroleum) sources are the sum of pristane, phytane and UCM. The results in Fig. 7 show that both terrestrial higher plants (24–30%) and microbial detritus (42–49%) are major sources of organic lipids in these sediments. Anthropogenic inputs from petroleum products are also significant sources of hydrocarbons (UCM) in these wetland sediments and ranging from 27 to 30%.

Conclusions

The solvent-extractable aliphatic lipids present in sediments of the Abu Zirig and Kurmashia wetland marshes of Iraq have been characterized using GC–MS techniques. The microbial input is more apparent than from terrestrial higher plants and petrogenic inputs in the Abu Zirig B and Kurmashia samples compared to Abu Zirig A samples. This is supported by the decrease in CPI values of the *n*-alkanes and the shift of C_{max} to 18, indicative of microbial contributions for both Abu Zirig B and Kurmashia samples. The *n*-alkanes < C_{20} in these samples are derived mainly from microbial residues due to the recent re-flooding. In Abu Zirig A and B, the contribution from oil related operations or spills is detectable; it is lowest in Kurmashia. This is supported by the higher UCM value for the Abu Zirig A samples.
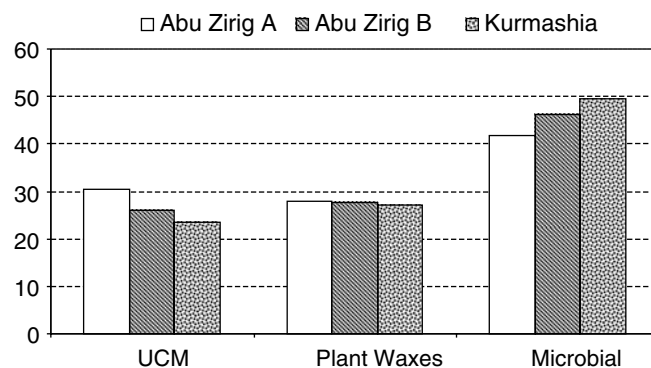
Fig. 7 *Bar plot* showing the fractions of various sources of organic lipids in sediment samples from Abu Zirig and Kurmashia marshlands

The presence of *n*-alkanes (odd carbon dominance $> C_{25}$), with the *n*-alkanoic acids, methyl *n*-alkanoates and *n*-alkanols ($> C_{20}$) indicates terrestrial higher plant wax sources. This is supported by the strong even carbon number predominance for *n*-alkanols ($C_{max} = 30$), *n*-alkanoic acids ($> C_{20}$) and methyl *n*-alkanoates ($> C_{20}$). The presence of pristane, phytane, together with the UCM reflects the contamination by petroleum residues.

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