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# Compositions and sources of extractable organic matter in Mesopotamian marshland surface sediments of Iraq: II. Polar compounds

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**Abstract** The concentrations of polar organic compounds including $n$-alkanoic acids, $n$-alkanols, steroids and triterpenoids were determined in extracts of shallow sediments from the Mesopotamian marshlands of Iraq. The sediments were collected by a stainless steel sediment corer, extracted with a dichloromethane and methanol mixture (3:1 v:v) by ultrasonic agitation and then analyzed by gas chromatography–mass spectrometric (GC–MS). The analysis results showed that the $n$-alkanoic acids ranged from $C_8$ to $C_{20}$ with concentrations of $7.8 \pm 1.2$ μg/g sample, whereas the concentrations of $n$-alkanols, which ranged from $C_{12}$ to $C_{39}$ were from $28.6 \pm 4.3$ to $121.7 \pm 18.3$ μg/g sample. The steroids and triterpenoids included stenols, stanols, stenones, stanones, tetrahymanol, tetrahymanone and extended $\beta\beta$-hopanes. The total concentrations of steroids and triterpenoids ranged from $26.8 \pm 4.1$ to $174.6 \pm 26.2$ μg/g and from $0.74 \pm 0.11$ to $11.2 \pm 1.7$ μg/g sample, respectively. The major sources of these lipids were from natural vegetation, microbial (plankton) residues and bacteria in the sediments, with some contribution from anthropogenic sources (livestock, sewage and petroleum). Further studies of these wetlands are needed to characterize the input rate, transformation and diagenesis of the organic matter and to assess its various sources.

**Keywords** Mesopotamian marshes · GC–MS · Steroids · Triterpenoids · Lipids

## Introduction

The Mesopotamian marshes of Iraq, which comprise a complex of inter-connected shallow freshwater lakes and wetlands, extend from 30 to 33°N and from 45 to 48°E and are considered as the most extensive wetland ecosystem in the Middle East (Brasington 2002; Partow 2001). The largest wetlands within this complex ecosystem are the Al-Hammar Marshes, south of the Euphrates, the Central Marshes, north of the Euphrates and west of the Tigris, and the Al-Hawizeh Marshes extending east from the Tigris into neighboring Iran (Fig. 1). These wetlands ultimately drain southeastwards into the Gulf via the Shatt Al-Arab waterway (DouAbul et al. 1988; Partow 2001). Drainage of wetlands, diversion of water supplies and dam-building are the main threats to the marshes in Iraq (Partow 2001). For instance, the once vast Central Marsh, which covered more than 300 km² in 1973, has decreased by 97%. Most of what remains are reeds growing in irrigation canals. Wetland marshes are utilized for cultivation, freshwater fisheries and livelihood of the local people
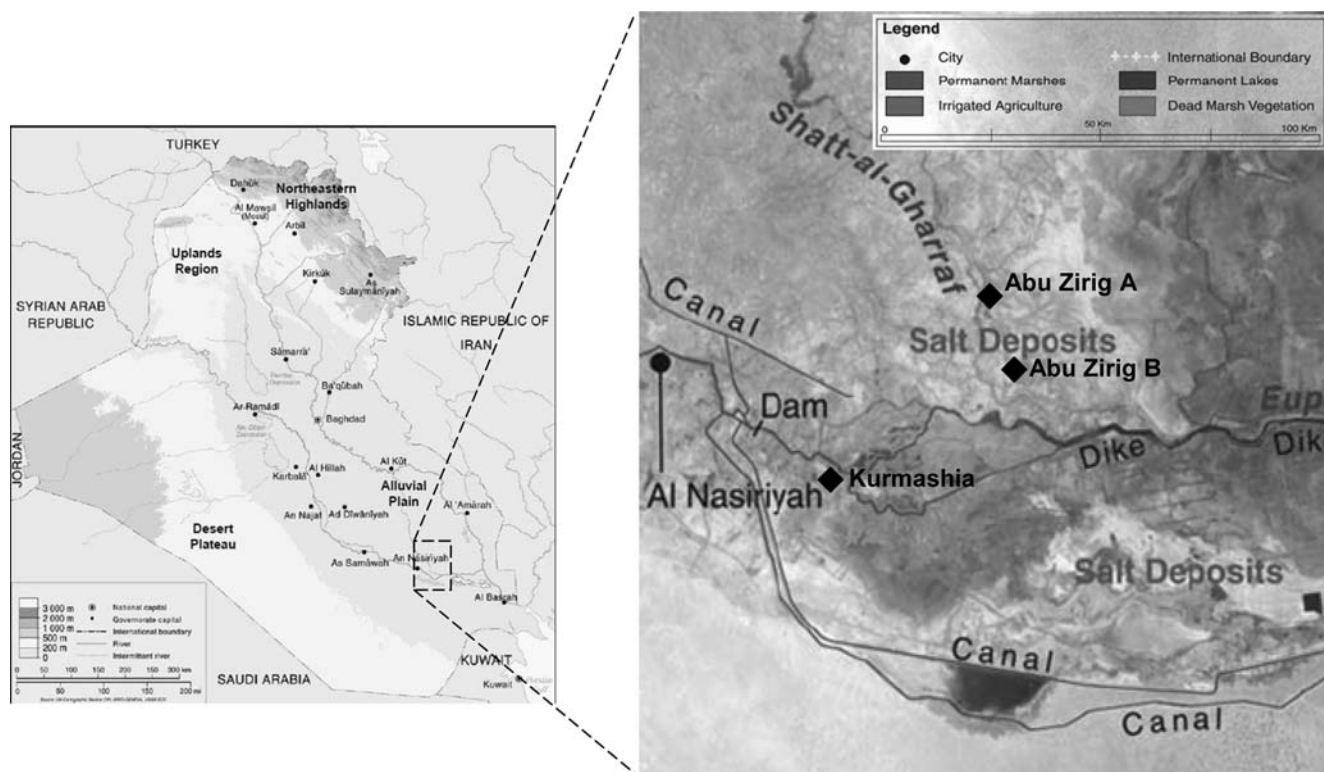
**Fig. 1** Location map of the sampling sites in the Abu Zirig and Kurmashia marshlands

(Salim 1962; Talling 1980; Thesiger 1985; Young 1983). It is postulated that only 15–20% of the drained marshes can be restored as a result of: (1) excessive salt buildup, pollution and dumping of toxic wastes and poisons during the war, (2) the severe reduction in available water, and (3) loss of the seed bank of native plant species (Partow 2001; Lawler 2005; Richardson et al. 2005).

Currently, restoration by re-flooding of drained marshes is proceeding in the Central and Al-Hammar marshlands (Lawler 2005; Partow 2001). However, uncontrolled re-flooding may have counterproductive adverse 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. One aspect of these studies is to determine the composition, concentrations and sources of the organic matter in the sediments of these marshes.

Thus, the main objectives of this study are to determine the characteristics, distribution, and concentrations of organic tracers in the extractable organic matter of shallow sediments from recently re-flooded marshlands of Iraq and identify the sources of these organic compounds. This study focuses on the polar lipids, including steroids and triterpenoids.

## Experimental

Sampling site

The sampling sites have been described in part I (Rushdi et al. 2006a). Abu Zirig marsh of the Central Marshes is located at the terminal end of the Gharraf River (Fig. 1). The main supply of water to the marsh is through the Shatt Abu Lihia river channel and other separate channels from the Gharraf River. The Abu Zirig marsh was re-flooded in April 2003 as a result of the direct action by the Ministry of Water Resources at the request of the local population.

The Kurmashia marsh is located southeast of Nasiriyah, at the head of the Al-Hammar marsh (Fig. 1). This area has become 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 mud-flats to open water around 2 m deep.

The Abu Zirig and Kurmashia marshes were chosen as examples to study the restoration processes of wetlands in Iraq because:(1) they were the first marshes to be re-flooded, (2) they are relatively small areas with limited numbers of inlets and outlets, and (3) the area has well controlled hydro-biological variables.

## Sampling and sample preparation

Sediment core samples were collected from Abu Zirig marsh and Kurmashia wetland (Fig. 1) using stainless steel gravity corers. About 3 cm$^3$ of each wet sediment sub sample was taken by a 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 each 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 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 (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 fatty acids, alcohols, steroids and triterpenoids was based on the GC–MS data (i.e., key ion fragmentograms and mass spectra). Retention times were compared with those of external standards. The *n*-alkanoic acids, *n*-alkanols, stenols, stanols, stenones, and stanones were identified primarily by their mass spectra [i.e., key ions *m/z* 117 (TMS), 103 (TMS), 129 (TMS), 215 (TMS), 124, and 231, respectively] and gas chromatographic retention times. Quantification was performed from the GC profiles using the external standard method (Rushdi et al. 2005, 2006a, b). Average

response factors were calculated for each compound. All quantifications were based on the peak areas of the compounds derived from the ion fragmentogram. The concentrations of compounds in microgram per gram sample were estimated from the peak areas in the total ion current traces.

# Results and discussion

The main features of the GC–MS data for the sediment extracts are shown in Fig. 2 and the major fatty acids, alcohols, steroid and triterpenoid compounds identified are listed in Table 1. The extracts are comprised of lipids from both autochthonous wetland and allochthonous terrigenous sources. They include aliphatic lipids (Rushdi et al. 2006a), fatty acids and alcohols, steroids, triterpenoids and traces of hopanes, which can be used to define the sources of detrital organic matter.

## Fatty acids and alcohols

Fatty acids (*n*-alkanoic acids) in fauna and flora generally range from $C_{12}$ to $C_{36}$ (mainly even carbon chain lengths) and are usually unsaturated in plants and saturated in animals. The major fatty acids in plants are the $C_{18}$ mono-, di- and tri-unsaturated forms, whereas polyunsaturated fatty acids are more common in algae than higher plants. Fatty alcohols (*n*-alkanols) have similar chain lengths, and are found mainly in plants. They have predominantly even carbon number chains because fatty alcohols are biosynthesized from fatty acids by enzymatic reduction (Lehninger 1970).

*n*-Alkanoic acids (silylated) were found to range from $C_8$ to $C_{19}$ in Abu Zirig A and B sediments and to $C_{24}$ in Kurmashia sediment, all with a $C_{max}$ at 16 (Fig. 3a), and carbon preference indices (CPI$_{e/o}$) ranging from $3.1 \pm 0.5$ to $5.3 \pm 0.8$ (Table 1). Their concentrations varied from $7.8 \pm 1.2$ to $18.6 \pm 2.8$ μg/g (Table 1). The presence of *n*-alkanoic acids ($< C_{20}$), with a strong even carbon number predominance and $C_{max}$ at 16 indicate multiple sources from mainly microbial and lesser vascular plants detritus, (Simoneit 1977, 1989). The absence of unsaturated fatty acids reflects extensive oxidation and biodegradation of the lipid input to the sediments.

The *n*-alkanols were major compounds ranging from $C_{12}$ to $C_{32}$ (Fig. 3b) with $C_{max}$ at 26, 28, or 30 for the sediment samples. Their concentrations were $28.6 \pm 4.3$ μg/g in Abu Zirig A, $47.7 \pm 7.2$ μg/g in Abu Zirig B and $121.7 \pm 18.3$ μg/g in Kurmashia sediment, with a strong even carbon numbered predominance (CPI$_{e/o}$ = $5.7 \pm 0.9$ to $7.3 \pm 1.1$, Table 1). This fatty alcohol distribution indicates an input of vascular plant wax
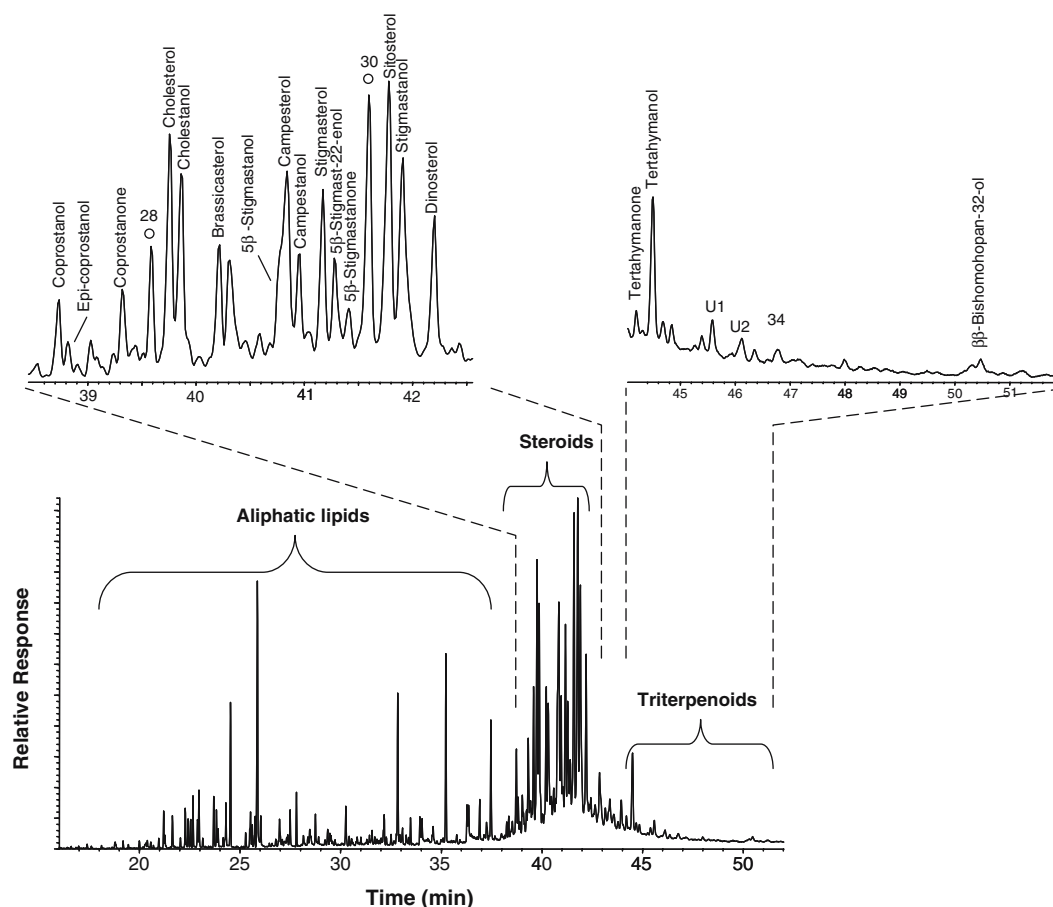
**Fig. 2** GC–MS total ion current trace of a total extract from a surface sediment sample in the Kurmashia wetland showing the major organic compounds (as TMS)

from tropical to semitropical environments (Simoneit 1977, 1989).

*n*-Hentriacontan-12-ol ($C_{31}H_{63}OH$) is detectable and may indicate an origin from terrestrial vegetation. This compound has been reported in some epicuticular waxes (Gülz 1994; Oros et al. 2002; Tulloch 1976).

Steroids

Steroids occur in all ecosystems and can be utilized to identify the sources and fate of organic matter in the environment. They are derived mainly from biogenic sources and are found in appreciable quantities in animal and vegetal tissues. Steroid hydrocarbons are found in fossil fuels. The natural steroids comprise a variety of molecules as 3-hydroxysteroids and generally range from $C_{26}$ to $C_{30}$ (Moreau et al. 2002). Cholesterol (cholest-5-en-3$\beta$-ol I, R = H, all chemical structures cited are shown in Appendix), which is a major

compound in faunal lipids and also in plankton, plays an important role in regulating cell membrane permeability by reducing average fluidity (Lehninger 1970), and in the lateral organization of membranes, thus controlling the membrane protein activity (Barenholz 2002). In higher plants, the steroids are known as phytosterols with compounds ranging from $C_{28}$ to $C_{30}$ with one or two carbon–carbon double bonds, typically one in the sterol nucleus and a second in alkyl side chain. More than 200 different phytosterols have been reported in plant species (Moreau et al. 2002).

The concentrations of sterols in these samples were high in all sites (Table 1). The highest sterol concentration (42.8 ± 6.4 µg/g) was observed for Kurmashia wetland and consisted of campesterol (I, R = $\alpha CH_3$), stigmasterol (II), and sitosterol (I, R = $\beta C_2H_5$) (Fig. 4b). The general sterol distribution from the samples analyzed shows cholesterol dominant or equal to sitosterol, with brassicasterol (24-methylcholesta-5,22-dien-3$\beta$-ol, III) and dinosterol (IV) as minor components. Cholesterol (in part), dinosterol and brassicasterol are interpreted to be from algal plankton in the aquatic environment (Bode et al. 2003; de Leeuw et al. 1993; Didyk et al. 1978; Giner and Boyer 1998; Giner and Li 2001; Robinson et al. 1984).

**Table 1** Concentrations (µg/g sample) of polar compounds detected in surface sediments from Mesopotamian marshlands of Iraq

| Compound | Composition | M.W. | Abu Zirig A | Abu Zirig B | Kurmashia |
|---|---|---|---|---|---|
| *n*-Alkanoic acids | | | | | |
| Octanoic acid | $C_8H_{16}O_2$ | 144 | 0.20 ± 0.03 | 0.82 ± 0.12 | |
| Nonanoic acid | $C_9H_{18}O_2$ | 158 | 0.59 ± 0.9 | 1.05 ± 0.16 | |
| Decanoic acid | $C_{10}H_{20}O_2$ | 172 | 0.49 ± 0.07 | 0.82 ± 0.12 | |
| Undecanoic acid | $C_{11}H_{22}O_2$ | 186 | 0.20 ± 0.03 | 0.35 ± 0.05 | 0.06 ± 0.01 |
| Dodecanoic acid | $C_{12}H_{24}O_2$ | 200 | 0.59 ± 0.09 | 1.35 ± 0.20 | 0.62 ± 0.09 |
| Tridecanoic acid | $C_{13}H_{26}O_2$ | 214 | 0.20 ± 0.03 | 0.47 ± 0.07 | 0.25 ± 0.04 |
| Tetradecanoic acid | $C_{14}H_{28}O_2$ | 228 | 0.88 ± 0.13 | 4.68 ± 0.70 | 2.24 ± 0.34 |
| Pentadecanoic acid | $C_{15}H_{30}O_2$ | 242 | 0.64 ± 0.10 | 2.22 ± 0.33 | 1.74 ± 0.26 |
| Hexadecanoic acid | $C_{16}H_{32}O_2$ | 256 | 3.32 ± 0.50 | 6.20 ± 0.93 | 9.34 ± 1.40 |
| Heptadecanoic acid | $C_{17}H_{34}O_2$ | 270 | 0.15 ± 0.02 | 0.12 ± 0.02 | 0.50 ± 0.08 |
| Octadecanoic acid | $C_{18}H_{36}O_2$ | 284 | 0.40 ± 0.06 | 0.47 ± 0.07 | 1.25 ± 0.19 |
| Nonadecanoic acid | $C_{19}H_{38}O_2$ | 298 | 0.15 ± 0.02 | | |
| Eicosanoic acid | $C_{20}H_{40}O_2$ | 312 | | | 0.50 ± 0.08 |
| Heneicosanoic acid | $C_{21}H_{42}O_2$ | 326 | | | 0.06 ± 0.01 |
| Docosanoic acid | $C_{22}H_{44}O_2$ | 340 | | | 0.87 ± 0.13 |
| Tricosanoic acid | $C_{23}H_{46}O_2$ | 354 | | | 0.03 ± 0.004 |
| Tetracosanoic acid | $C_{24}H_{48}O_2$ | 368 | | | 1.12 ± 0.17 |
| Total | | | 7.77 ± 1.18 | 18.53 ± 2.78 | 18.58 ± 2.77 |
| CPI (e/o) | | | 3.08 ± 047 | 3.40 ± 0.51 | 5.27 ± 0.79 |
| *n*-Alkanols | | | | | |
| Dodecanol | $C_{12}H_{26}O$ | 186 | 0.47 ± 0.07 | | 0.40 ± 0.06 |
| Tridecanol | $C_{13}H_{28}O$ | 200 | 0.24 ± 0.04 | | 0.54 ± 0.08 |
| Tetradecanol | $C_{14}H_{30}O$ | 214 | 1.18 ± 10.18 | 0.95 ± 0.14 | 3.48 ± 0.52 |
| Pentadecanol | $C_{15}H_{32}O$ | 228 | 0.48 ± 0.07 | 1.08 ± 0.16 | 2.68 ± 0.40 |
| Hexadecanol | $C_{16}H_{34}O$ | 242 | 3.15 ± 0.48 | 4.27 ± 0.64 | 11.65 ± 1.75 |
| Heptadecanol | $C_{17}H_{36}O$ | 256 | 0.52 ± 0.08 | 1.02 ± 0.15 | 2.28 ± 0.34 |
| Octadecanol | $C_{18}H_{38}O$ | 270 | 1.03 ± 0.16 | 1.22 ± 0.18 | 2.68 ± 0.40 |
| Nonadecanol | $C_{19}H_{40}O$ | 284 | 0.14 ± 0.02 | 0.13 ± 0.02 | 0.54 ± 0.08 |
| Eicosanol | $C_{20}H_{42}O$ | 308 | 0.52 ± 0.08 | 0.54 ± 0.08 | 2.95 ± 0.44 |
| Heneicosanol | $C_{21}H_{44}O$ | 322 | 0.19 ± 0.03 | 0.20 ± 0.03 | 1.34 ± 0.20 |
| Docosanol | $C_{22}H_{46}O$ | 326 | 2.12 ± 0.31 | 1.56 ± 0.23 | 11.51 ± 1.73 |
| Tricosanol | $C_{23}H_{48}O$ | 340 | 0.19 ± 0.03 | 0.27 ± 0.04 | 1.74 ± 0.26 |
| Tetracosanol | $C_{24}H_{50}O$ | 354 | 2.07 ± 0.31 | 2.03 ± 0.31 | 13.39 ± 2.01 |
| Pentacosanol | $C_{25}H_{52}O$ | 368 | 0.33 ± 0.05 | 0.54 ± 0.81 | 1.87 ± 0.28 |
| Hexacosanol | $C_{26}H_{54}O$ | 382 | 2.59 ± 0.39 | 4.88 ± 0.73 | 7.50 ± 1.12 |
| Heptacosanol | $C_{27}H_{56}O$ | 396 | 0.19 ± 0.03 | 0.27 ± 0.41 | 0.94 ± 0.14 |
| Octacosanol | $C_{28}H_{58}O$ | 410 | 2.77 ± 0.42 | 4.20 ± 0.63 | 9.64 ± 1.45 |
| Nonacosanol | $C_{29}H_{60}O$ | 424 | 0.24 ± 0.04 | 0.34 ± 0.05 | 1.34 ± 0.20 |
| Triacontanol | $C_{30}H_{62}O$ | 438 | 3.01 ± 0.46 | 2.81 ± 0.42 | 19.68 ± 2.95 |
| Hentriacontanol | $C_{31}H_{64}O$ | 452 | 0.12 ± 0.02 | 0.14 ± 0.02 | 0.67 ± 0.10 |
| Dotriacontanol | $C_{32}H_{66}O$ | 466 | 0.56 ± 0.09 | 0.47 ± 0.07 | 2.41 ± 0.36 |
| Tritriacontanol | $C_{33}H_{68}O$ | 480 | 0.05 ± 0.01 | | |
| Tetratriacontanol | $C_{34}H_{70}O$ | 494 | 0.19 ± 0.03 | | |
| Hentriacontan-12-ol | $C_{31}H_{64}O$ | 508 | 4.41 ± 0.37 | 18.56 ± 2.78 | 20.68 ± 3.10 |
| Phytol | $C_{20}H_{40}O$ | 306 | 1.84 ± 0.28 | 2.18 ± 0.33 | 1.82 ± 0.27 |
| Total | | | 28.55 ± 4.33 | 47.69 ± 7.15 | 121.69 ± 18.25 |
| CPI (e/o) | | | 7.33 ± 1.11 | 5.74 ± 0.86 | 6.13 ± 0.92 |
| Steroids | | | | | |
| 5α-24-Norcholest-22-enol | $C_{26}H_{44}O$ | 372 | 0.21 ± 0.03 | 1.14 ± 0.17 | 1.63 ± 0.24 |
| 5α-24-Norcholestanol | $C_{26}H_{46}O$ | 374 | 0.25 ± 0.04 | | |
| Cholesterol | $C_{27}H_{46}O$ | 386 | 2.28 ± 0.35 | 7.00 ± 1.05 | 14.51 ± 2.18 |
| Coprostanol | $C_{27}H_{48}O$ | 388 | 0.55 ± 0.08 | 2.36 ± 0.35 | 5.27 ± 0.79 |
| *Epi*-coprostanol | $C_{27}H_{48}O$ | 388 | 0.32 ± 0.05 | 2.10 ± 0.32 | 2.64 ± 0.40 |
| Cholestanol | $C_{27}H_{48}O$ | 388 | 2.42 ± 0.37 | 11.21 ± 1.62 | 13.98 ± 2.10 |
| Brassicasterol | $C_{28}H_{46}O$ | 398 | 1.42 ± 0.22 | 5.76 ± 0.86 | 9.79 ± 1.47 |
| Campesterol | $C_{28}H_{48}O$ | 400 | 1.47 ± 0.22 | 6.14 ± 0.92 | 10.12 ± 1.52 |
| 5α-Campestanol | $C_{28}H_{50}O$ | 402 | 0.98 ± 0.15 | 6.25 ± 0.94 | 7.75 ± 1.16 |
| Stigmasterol | $C_{29}H_{48}O$ | 412 | 2.02 ± 0.31 | 8.46 ± 1.27 | 11.32 ± 1.70 |
| 29-Nordinostanone | $C_{29}H_{48}O$ | 412 | 0.746 ± 0.11 | 3.60 ± 0.54 | 4.26 ± 0.64 |
| 5β-Stigmast-22-en-3α-ol | $C_{29}H_{50}O$ | 414 | T | 1.91 ± 0.29 | T |
| 5β-Stigmast-22-en-3β-ol | $C_{29}H_{50}O$ | 414 | 1.33 ± 0.20 | 5.60 ± 0.84 | 8.79 ± 1.32 |
| Sitosterol | $C_{29}H_{50}O$ | 414 | 3.60 ± 0.55 | 9.64 ± 1.45 | 21.06 ± 3.15 |

**Table 1** (Contd.)

| Compound | Composition | M.W. | Abu Zirig A | Abu Zirig B | Kurmashia |
|---|---|---|---|---|---|
| Sitostanone | $C_{29}H_{50}O$ | 414 | $0.78 \pm 0.12$ | $1.13 \pm 0.17$ | $5.15 \pm 7730.77$ |
| 5α-Stigmastanol | $C_{29}H_{52}O$ | 416 | $2.94 \pm 0.45$ | $10.43 \pm 1.56$ | $19.58 \pm 2.94$ |
| 5β-Stigmastan-3β-ol $\pm$ 5β-Stigmastan-3α-ol | $C_{29}H_{52}O$ | 416 | $4.41 \pm 0.67$ | $18.56 \pm 2.78$ | $20.68 \pm 3.10$ |
| Dinostanone | $C_{30}H_{50}O$ | 426 | $0.48 \pm 73$ | $3.14 \pm 0.47$ | $3.82 \pm 0.57$ |
| Dinosterol | $C_{30}H_{52}O$ | 428 | $0.61 \pm 0.09$ | $6.27 \pm 0.94$ | $14.32 \pm 2.15$ |
| Peridinosterol | $C_{30}H_{52}O$ | 428 | | $3.21 \pm 0.48$ | |
| 7-Hydroxystigmastan-3-one | $C_{29}H_{50}O_2$ | 430 | | $3.26 \pm 0.49$ | |
| Total | | | $26.81 \pm 4.07$ | $117.15 \pm 17.57$ | $174.62 \pm 26.19$ |
| Triterpenoids | | | | | |
| Gammacerene | $C_{30}H_{50}$ | 410 | | | |
| Tetrahymanone | $C_{30}H_{50}O$ | 426 | T | $1.76 \pm 0.26$ | $2.61 \pm 0.39$ |
| Tetrahymanol | $C_{30}H_{52}O$ | 428 | $0.74 \pm 0.11$ | $4.25 \pm 0.34$ | $7.53 \pm 1.13$ |
| 17β(H),21β(H)-Bishomohopan-22-ol | $C_{32}H_{56}O$ | 456 | T | $0.79 \pm 0.12$ | $1.07 \pm 0.16$ |
| Total | | | $0.74 \pm 0.11$ | $6.79 \pm 1.02$ | $11.20 \pm 1.68$ |
| Unknowns | | | | | |
| U1 | | | | $3.41 \pm 0.51$ | $4.20 \pm 0.63$ |
| U1 | | | | $2.10 \pm 0.32$ | $3.00 \pm 0.54$ |
| Total | | | | $5.51 \pm 0.83$ | $7.20 \pm 1.08$ |
| UCM | | | $22.31 \pm 3.11$ | $52.34 \pm 7.4$ | $72.90 \pm 10.68$ |

The dominance of sitosterol in the samples is interpreted to originate from terrigenous sources (Barbier et al. 1981; Simoneit et al. 1983; Moreau et al. 2002). The ratio of $C_{27}/(C_{27 + 28 + 29})$ [cholesterol/(cholesterol + campesterol + stigmasterol + sitosterol)] in a sample can be used as an indicator of the contribution from faunal sources. These ratios ranged from 0.21 to 0.35 with mean values of $0.21 \pm 0.07$ for Abu Zirig A and Abu Zirig B and $0.35 \pm 0.07$ for Kurmashia (Table 2). The relatively high ratio for Kurmashia indicates that organic matter from faunal sources is more significant there than in the Abu Zirig wetlands. Ergosterol, an important sterol from yeast and fungi (Baraja-Aceves et al. 2002; Charcosset and Chauvet 2001) was not detectable in any sample.

Stanols, the fully-saturated sterols, occur at trace levels in many plant species and at significant levels in tissues of a few cereal species (Moreau et al. 2002). They are generally produced by hydrogenation of sterols (Lehninger 1970). Stanols occur in dinoflagellates but are not common in other marine microalgae (Robinson et al. 1984). Dinoflagellates are often the major direct source of 5α-stanols (e.g., dinosterol, IV) in marine sediments (Robinson et al. 1984). The 5β-stanols (e.g., coprostanol V, R = H, and epimer, *epi*-coprostanol VI, R = H) are major components of the total sterols in carnivore feces (Chris et al. 2001) and are derived from anaerobic bacterial alteration of sterols. The 5β-phytostanols (e.g., 5β-stigmastan-3β-ol, V, R = βC₂H₅ and epimer VI, R = βC₂H₅), also formed by anaerobic bacterial alteration of the precursors, are eliminated in feces of herbivores (Rogge et al. 2006).

Stanols were significant in these samples (Fig. 4b, Table 1) with the highest concentration in the Kurmashia

wetlands. Their occurrence indicates microbial alteration of sterols, where coprostanol generally is due to sewage sources and the 5β-campestanols and 5β-stigmastanols
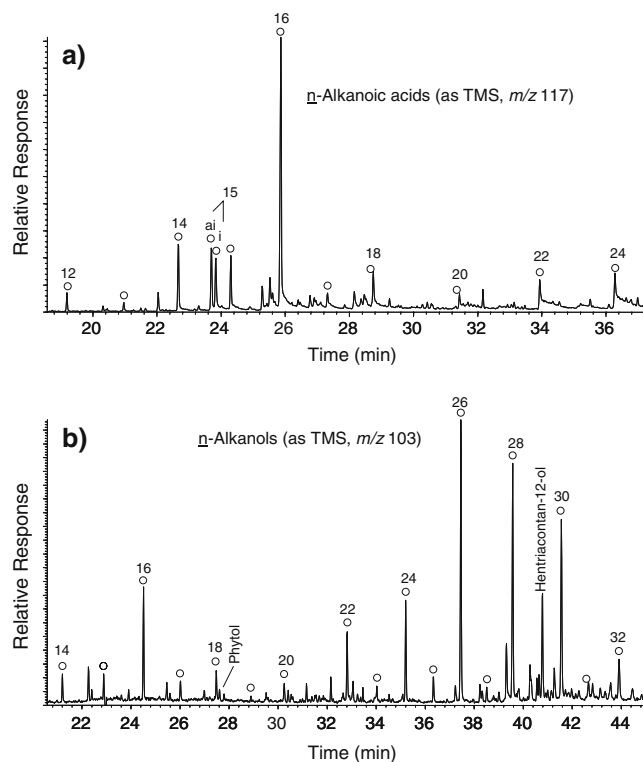


**Fig. 3** Examples of GC–MS key ion pots for: **a** *n*-alkanoic acids (as TMS, *m/z* 117) and **b** *n*-alkanols (as TMS, *m/z* 103) in an extract from Kurmashia and Abu Zirig B sediments, respectively

are from animal wastes (Fernandes et al. 1999; Chris et al. 2001; Rogge et al. 2006). The ratio of cpr/(cpr + chl) [coprostanol/(coprostanol + cholestanol)] can be used to evaluate the influence of sewage on the wetlands. These computed ratios for the sediment samples ranged from 0.17 to 0.27 with mean values of $0.19 \pm 0.03$ for Abu Zirig A, $0.17 \pm 0.02$ for Abu Zirig B and $0.27 \pm 0.04$ for Kurmashia (Table 2), and confirm an input of organic matter from domestic sewage to these wetland areas with the highest input in Kurmashia. The assessment of the influence of livestock operations on the wetlands can be done by the ratio of the $5\beta$-phytostanols to the sum of the $5\beta$-phytostanols plus coprostanol and epimer [$5\beta C_{28 + 29}/(5\beta C_{28 + 29} + 5\beta C_{27})$]. These ratios for the sediment samples ranged from $0.86 \pm 0.12$ to $0.91 \pm 0.13$ (Table 2) and indicate a more dominant input from herbivore waste than sewage, especially for Abu Zirig A.

**Table 2** Biomarker parameters and indices for surface sediments from the Mesopotamian marshlands of Iraq

| | Abu Zirig A ($n = 3$) | Abu Zirig B ($n = 4$) | Kurmashia ($n = 4$) |
|---|---|---|---|
| *n*-Alkanoic acids | | | |
| Range | 8–19 | 8–18 | 11–24 |
| $C_{max}$ | 16 | 16 | 16 |
| $CPI^a_{(e/o)}$ | $3.1 \pm 0.5$ | $3.4 \pm 0.5$ | $5.3 \pm 0.8$ |
| *n*-Alkanols | | | |
| Range | 12–34 | 14–32 | 12–32 |
| $C_{max}$ | 30 | 26 | 30 |
| $CPI^a_{(e/o)}$ | $7.3 \pm 1.1$ | $5.7 \pm 0.9$ | $6.1 \pm 0.9$ |
| Sterols | | | |
| Range | 27–29 | 27–30 | 27–30 |
| $C_{max}$ | 29 | 29 | 29 |
| $C_{27}/C^b_{27 + 28 + 29}$ | $0.21 \pm 0.07$ | $0.21 \pm 0.05$ | $0.35 \pm 0.07$ |
| Stanols | | | |
| Range | 27–29 | 27–29 | 27–29 |
| $C_{max}$ | 29 | 29 | 29 |
| cpr/(cpr + chl)$^c$ | $0.19 \pm 0.03$ | $0.17 \pm 0.02$ | $0.27 \pm 0.04$ |
| $5\beta C_{28 + 29}/(5\beta C_{28 + 29} + 5\beta C_{27})^d$ | $0.91 \pm 0.13$ | $0.89 \pm 0.12$ | $0.86 \pm 0.12$ |
| Stenones | | | |
| Range | 27–29 | | |
| $C_{max}$ | 27 | | |
| Stanones | | | |
| Range | 27–29 | | |
| $C_{max}$ | 27 | | |

[a]Even carbon numbers/odd carbon numbers = $(\Sigma C_{12} + ... + C_{18})/(\Sigma C_{11} + ... + C_{17})$ (for *n*-alkanoic acids) = $(\Sigma C_{14} + ... + C_{32})/(\Sigma C_{15} + ... + C_{33})$ (for *n*-alkanols)
[b]Cholesterol/(cholesterol + campesterol + stigmasterol + sitosterol) ratio
[c]Coprostanol/(coprostanol + cholestanol) ratio
[d]All $C_{28}$ and $C_{29}$ $\beta$-stanols/(all $C_{28}$ and $C_{29}$ $\beta$-stanols + coprostanol + *epi*-coprostanol)
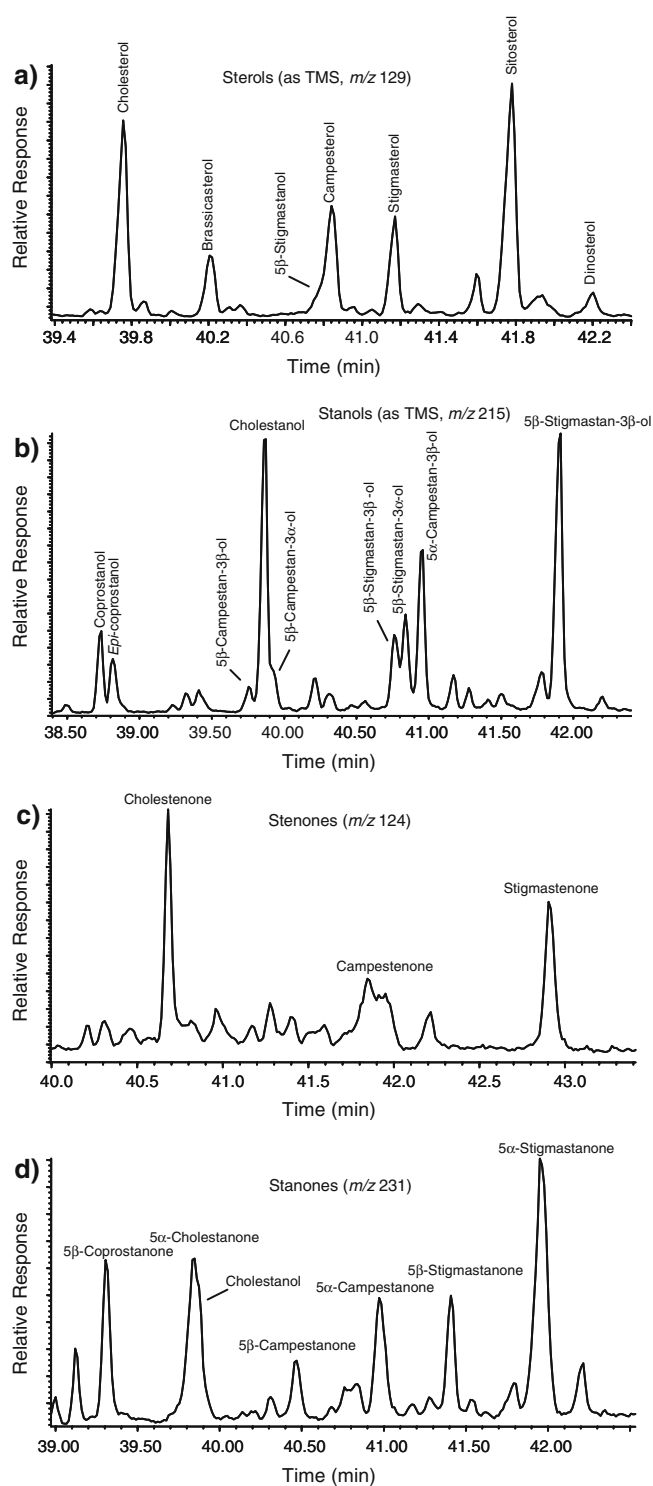


**Fig. 4** Examples of GC–MS key ion plots for: **a** sterols (as TMS, *m/z* 129), **b** stanols (as TMS, *m/z* 215), **c** stenones (*m/z* 124), and **d** stanones (*m/z* 231) in the extract of a sediment sample from Kurmashia wetland

Alteration of sterols by accelerated diagenesis yields stenones (VII) and stanones (VIII) with the same carbon number range from $C_{27}$ to $C_{29}$. These were found only in the wetland of Abu Zirig A (Fig. 4, Table 1). The $C_{28}$ stenones, 24-methylcholesta-4,22-dien-3-one and 24-methylcholest-22-en-3-one were not detectable in most samples, although their precursor brassicasterol (III) was found. The presence of stenones and stanones in Abu Zirig A can be interpreted to indicate an admixture of deeper sediments with the surface sediments due to turbulent resuspension during flooding.

### Triterpenoids

Triterpenoids were present in all samples and included mainly tetrahymanol (IX), tetrahymanone (X), traces of gammacer-2-ene (XI) and $17\beta(H),21\beta(H)$-bishomohopan-32-ol (XII) (Fig. 5). The concentrations of total triterpenoids ranged from 0.7 to 11.2 µg/g with mean values of $0.74 \pm 0.11$ µg/g for Abu Zirig A, $6.8 \pm 1.0$ µg/g for Abu Zirig B and $11.2 \pm 1.68$ µg/g for Kurmashia (Table 1). These compounds, which are mainly derived from microbiota, are usually major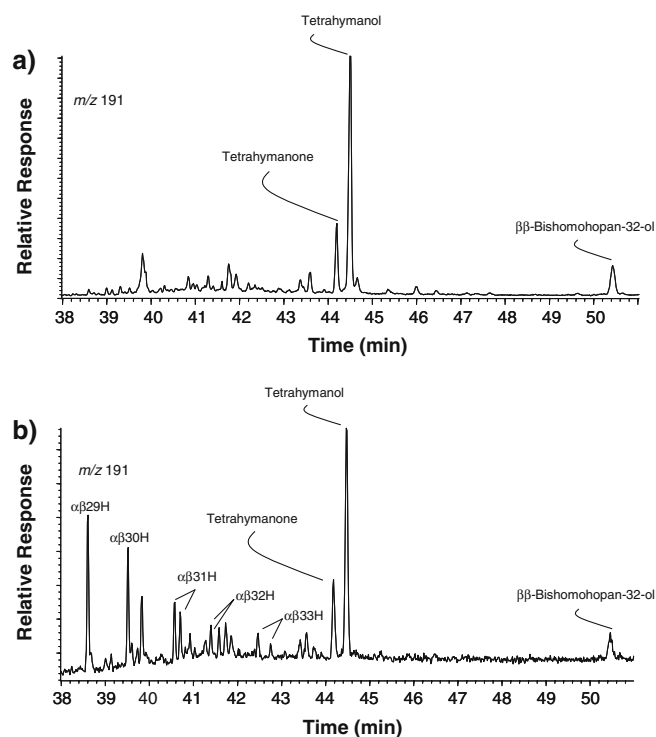 components in sediments of aquatic environments (Ourisson et al. 1979; Brassell et al. 1983; Philp 1985; Venkatesan 1989). Tetrahymanol occurs mainly in the protozoan *Tetrahymena* (Mallory et al. 1963; Holz and Conner 1973), in some ferns (Zander et al. 1969), and in cultures of the anaerobic rumen fungus *Piromonas communis* (Kemp et al. 1984). Thus, tetrahymanol with its derivatives tetrahymanone and gammacer-2-ene indicate a major input of organic detritus from aquatic plankton to the sediments. The $\beta\beta$-bishomohopan-32-ol is an early diagenetic product from bacteriohopanepolyol indicating oxidation or bacterial reworking of organic detritus. Triterpenoid markers from higher vascular plants such as $\alpha$- and $\beta$-amyrin, fernenol, or taraxerol were not detected in these samples.

Traces of petroleum biomarkers (hopanes) were found only in Abu Zirig A and B wetland samples mainly as the $\alpha\beta$-hopanes ranging from $C_{29}$ to $C_{33}$ with a $C_{max}$ at 29 (Fig. 5b). Steranes were not detectable in any of these samples.

### Major organic compound sources

The main sources of lipid compounds in these marshland sediments are from terrestrial vascular plants, lacustrine and anthropogenic inputs. The terrestrial higher plant input can be estimated as the sum of the polar lipids, i.e., *n*-alkanoic acids and *n*-alkanols with C-chain lengths > $C_{20}$, as well as $C_{28}$ and $C_{29}$ sterols. On the other hand, the autochthonous lacustrine sources from microbial activity are the total lipids with C-chain lengths < $C_{20}$, plus cholestanols, tetrahymanol, tetrahymanone and $\beta\beta$-bishomohopa-
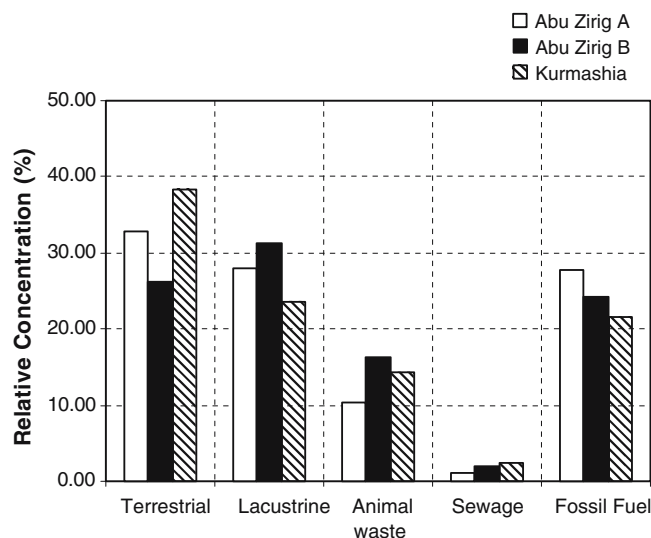


Fig. 5 Examples of GC–MS key ion plots for triterpenoids ($m/z$ 191) in the extracts of sediment samples from: **a** Kurmashia wetland, and **b** Abu Zirig A wetland



Fig. 6 The relative concentrations (as %) of the different organic compound sources in the marshlands of Iraq

## Abu Zirig A

Animal waste
10%

Natural, 61%

Anthropogenic
29%

## Abu Zirig B

Animal waste
16%

Natural, 58%

Anthropogenic
26%

## Kurmashia

Animal waste
16%

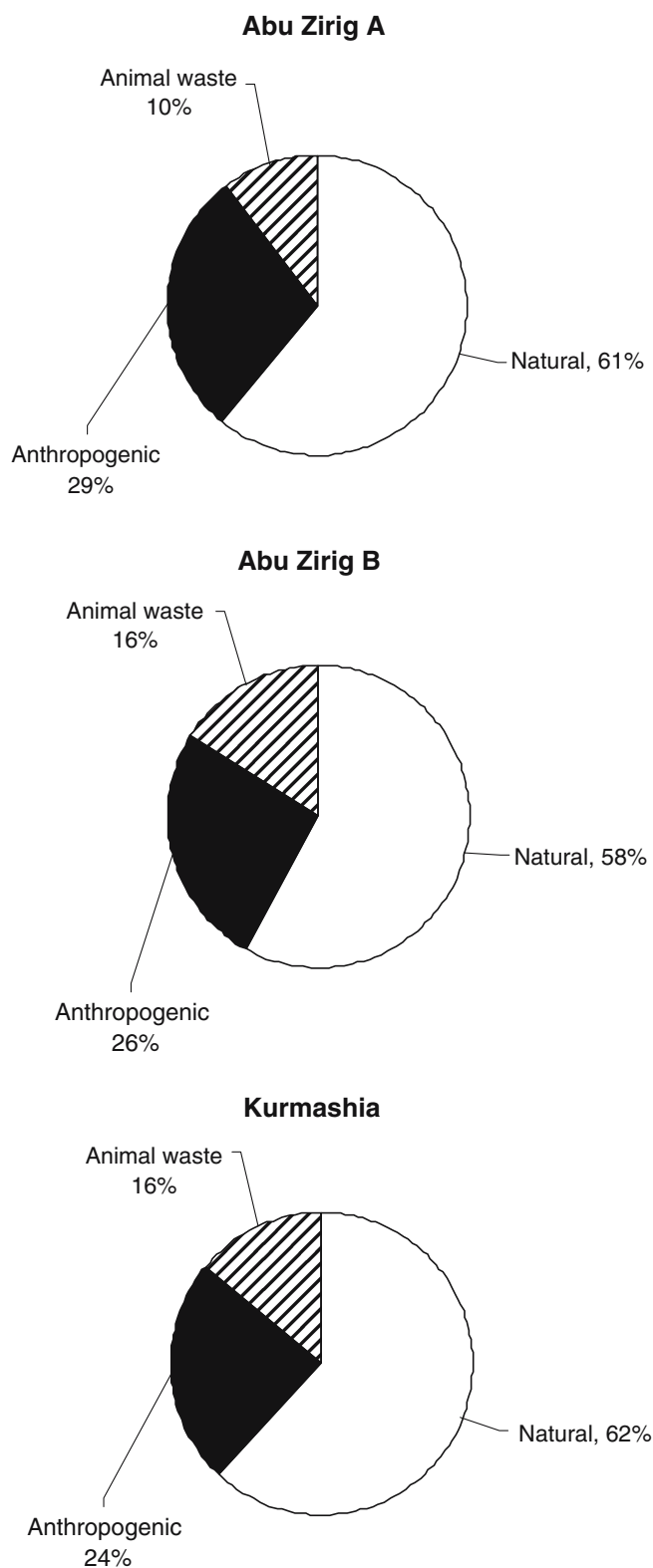Natural, 62%

Anthropogenic
24%

**Fig. 7** The percent fractions of organic matter from natural, anthropogenic and animal husbandry sources in the marshlands of Iraq
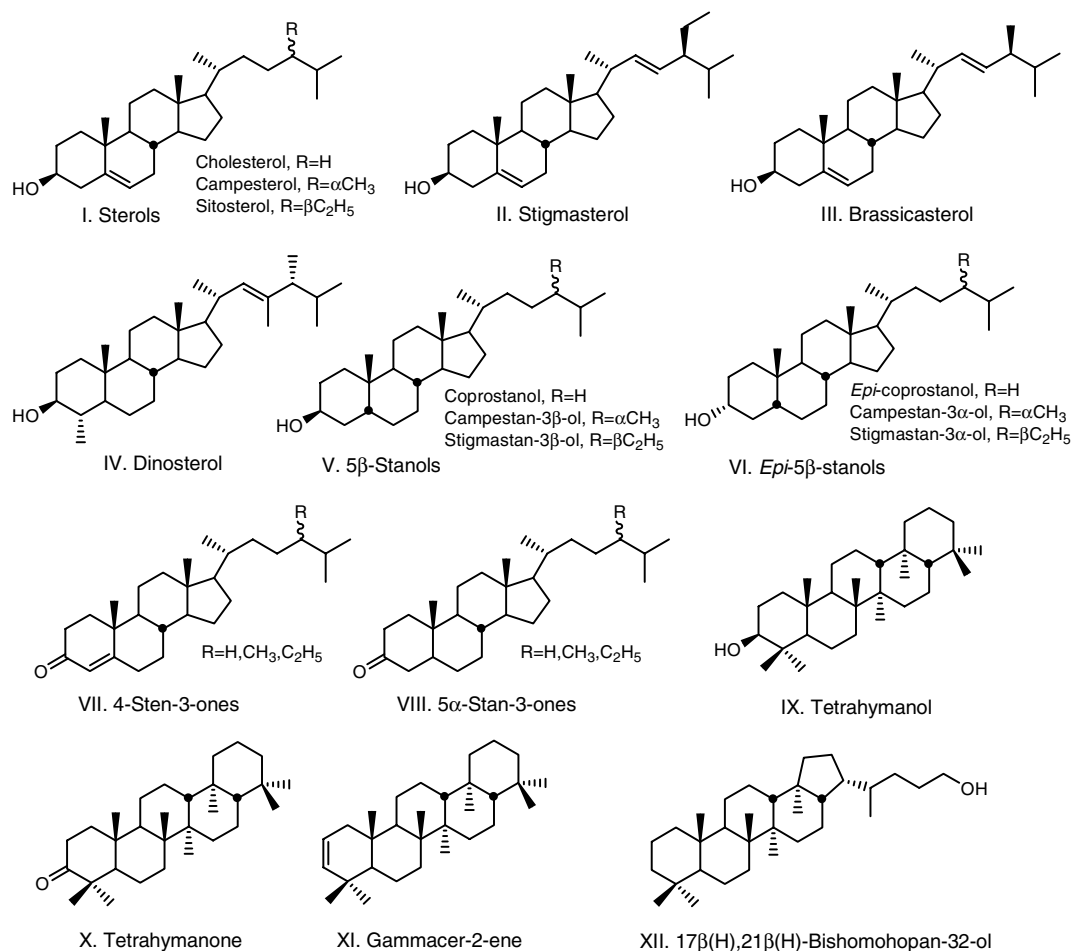
nol. The fraction attributed to animal waste is the sum of the campestanols and stigmastanols, whereas the sewage fraction is the total of coprostanol and *epi*-coprostanol. The anthropogenic (mainly petroleum) sources are the sum of the UCM, pristane, phytane and hopanes. These results are summarized in Fig. 6 and show that both terrestrial higher plants wax (26–38%) and lacustrine lipids (23–31%) are major sources. Waste from animal husbandry is a significant input (10–16%), whereas sewage is the lowest input (1–2%). Anthropogenic input from petroleum utilization is also significant as a source of hydrocarbons (UCM) in these wetland sediments and ranges from 21 to 28%. Thus, natural components (i.e., terrestrial and lacustrine) are the major source of organic lipids in these wetlands (58–62%), with anthropogenic (24–29%) and animal waste as secondary inputs (10–16%) (Fig. 7).

## Conclusion

The analyses of sediment samples from recently re-flooded areas of wetlands in Iraq show that natural biogenic organic compounds are the major contributors of the organic matter with minor inputs from anthropogenic sources. The natural sources of organic compounds are mainly from surrounding vegetation (i.e., higher plant wax) and autochthonous production (algae/plankton) and organic matter alteration by bacteria and fungi. The organic compounds from vegetation are indicated by the presence of high amounts of sterols $> C_{28}$ including campesterol, stigmasterol and sitosterol. A significant algal/plankton input is evident by the presence of tetrahymanol, tetrahymanone, cholesterol, and dinosterol. Minor bacterial sources are indicated by the presence of $\beta\beta$-bishomohopan-32-ol. The occurrence of coprostanol and *epi*-coprostanol indicate sewage pollution and the presence of 5$\beta$-phytostanols supports a significant input from animal husbandry. A trace input from petroleum is confirmed by the presence of hopane biomarkers.

# Appendix: Chemical structures cited



Cholesterol, R=H
Campesterol, R=αCH₃
Sitosterol, R=βC₂H₅
I. Sterols

II. Stigmasterol

III. Brassicasterol

IV. Dinosterol

Coprostanol, R=H
Campestan-3β-ol, R=αCH₃
Stigmastan-3β-ol, R=βC₂H₅
V. 5β-Stanols

*Epi*-coprostanol, R=H
Campestan-3α-ol, R=αCH₃
Stigmastan-3α-ol, R=βC₂H₅
VI. *Epi*-5β-stanols

R=H,CH₃,C₂H₅
VII. 4-Sten-3-ones

R=H,CH₃,C₂H₅
VIII. 5α-Stan-3-ones

IX. Tetrahymanol

X. Tetrahymanone

XI. Gammacer-2-ene

XII. 17β(H),21β(H)-Bishomohopan-32-ol

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