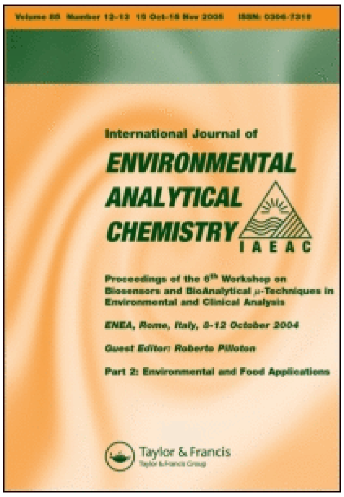

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The Use of Pollutant and Biogenic Markers as Source Discriminants of Organic Inputs to Estuarine Sediments

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The Use of Pollutant and Biogenic Markers as Source Discriminants of Organic Inputs to Estuarine Sediments

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The estuarine distributions of a variety of molecular organic markers are described and discussed in relation to sources, transport mechanisms and fates of anthropogenic and biogenic inputs to the Tamar Estuary, U.K.

Components selected for study include hydrocarbons (saturated and polycyclic aromatics), sterols, lignins and photosynthetic pigments.

Techniques used in the analyses of markers include preparative TLC and HPLC; quantification by HPLC-UV/fluorescence and capillary GC-FID with identification by HPLC—rapid scanning diode array spectrophotometry and computerised capillary GC-MS.

Non-parametric cluster analysis and multi-dimensional scaling are employed to resolve co-variability between members of different compound classes to investigate sources and processes affecting fates of the compounds in estuarine environments.

KEY WORDS: Molecular organic markers, lignins, photosynthetic pigments, sterols, hydrocarbons, estuarine sediments.

INTRODUCTION

The use of molecular organic markers forms the basis of organic geochemistry and has been applied to environmental chemistry to establish terrestrial inputs to marine waters[1–8] and to trace pollution in aquatic systems.[9–17]

Lignin compounds are phenolic polymers that occur as major constituents of the cell walls of vascular plants.[18] Their source, natural abundance, wide distribution and resistance to microbial degradation render them good terrestrial markers and *via* their phenolic aldehyde oxidation products afford characterisation of their source materials.[1,7,8,18]

Certain lipids are also regarded as markers of terrestrial organic material, including fatty acids,[2] diterpenoid acids and hydrocarbons,[3,4] *n*-alkanes[5] and sterols.[6] It has been shown that after deposition, lipids of terrestrial origin are refractory.[1,2,19,20]

Organic markers have been proposed to assess sewage pollution including polyorganosiloxanes,[11] carbohydrates (and organic carbon)[12] and α-tocopheryl acetate.[13] Coprostanol, a principal sterol of human and animal faeces,[21] has proven particularly successful and has been the most widely used indicator of sedimentary sewage contamination in recent years.[9,10,14–17]

Pollution of aquatic systems by oil has resulted in extensive research. Saturated hydrocarbons are generally analysed by gas chromatography to quantitatively establish the extent of pollution and to resolve interfering biogenic hydrocarbons (which afford additional information on natural processes).[e.g. 1,9,22,23]

Carcinogenic polycyclic aromatic hydrocarbons (PAH) represent another important class of environmental pollutants.[24] These compounds originate from petrogenic and pyrogenic sources and their transport and fate in the environment is of particular interest.[24]

Most of these compound classes and molecular markers have, in the past, been evaluated in comparative isolation and it is only in the last few years that studies have begun to inter-relate them.[1,9,23] This study represents an initial investigation in which a substantial suite of molecular markers have been quantified in sediments sampled throughout an axial transect of the Tamar Estuary, U.K. Compound classes selected for study include lignins, sterols, hydrocarbons (saturated and polycyclic aromatics) and photosynthetic pigments. To investigate the complex inter-relationships between the components, selected statistical techniques including cluster analysis[25] and multidimensional scaling[26] are introduced.

MATERIALS AND METHODS

Study area and sample collection

The geographical location of the sampling area and position of the sample sites are shown in Figure 1. Sediment core samples (2.5 cm diameter, 10–15 cm long) were collected using the gravity Butler Corer[27] on 23 April 1985 during a marine to riverine axial traverse of the Tamar Estuary. The top 3 cm sections of the cores were sampled and stored in labelled acid-cleaned jars sealed with solvent-rinsed aluminium foil-lined lids. These vessels were immediately stored in darkness over solid carbon dioxide and maintained frozen and in darkness in the laboratory until analysis.

Sample treatment

The sediments were freeze dried in darkness and the water content was determined by the loss in weight. Following thorough grinding in an agate ball mill, weighed sub-samples were removed for organic carbon and nitrogen determination and for analyses of lignins, photosynthetic pigments, sterols and hydrocarbons. Samples awaiting analyses were stored in darkness at -15°C. Sediments (0.5 g) were treated with hydrochloric acid (2M; 2 cm^3; overnight) to remove carbonates prior to carbon and nitrogen determination (Carlo Erba Elemental Analyser 1106).

All solvents used were of Fisons or Rathburns HPLC grade. Diethyl-ether for lignin analyses was treated with a saturated aqueous solution of $Fe(NH_4)_2(SO_4)_2 . 6H_2O$ to remove peroxides.[18]

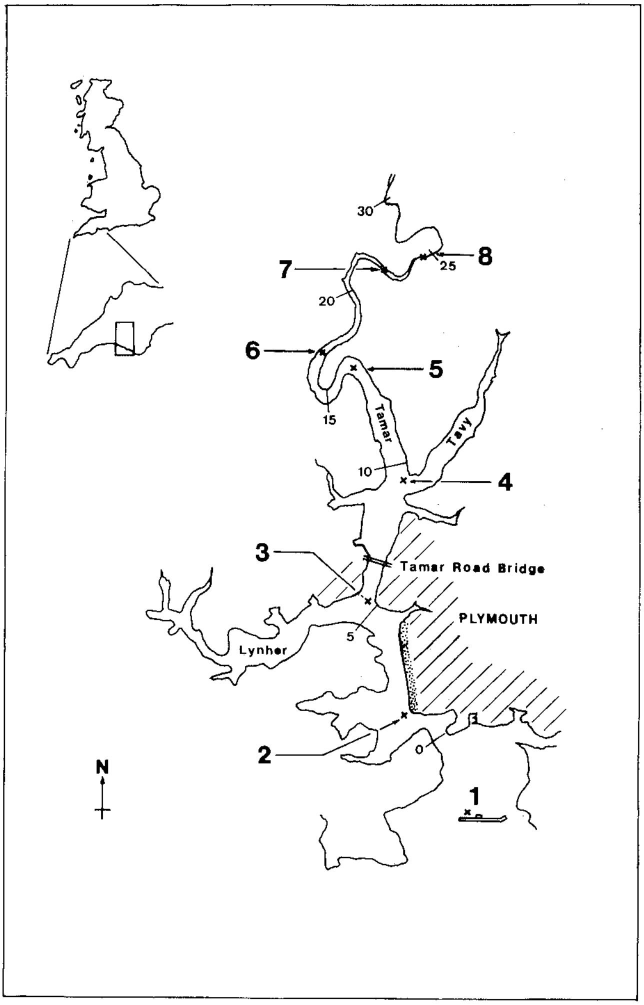

FIGURE 1 Description of the Tamar Estuary and sampling stations. Sediment sample numbers are identified by the large figures with their respective locations indicated by crosses. Distances upstream from the narrows (point 0) are shown in 5 Km intervals. Plymouth is indicated by the hatched area with the dockyards located along the estuarine foreshore demarked by stipples.

For pigment analyses, peroxide-free diethyl-ether was prepared by distillation over iron powder, followed by storage in an amber bottle with anhydrous sodium sulphate.[28]

Lignin analyses

Lignins were characterised by their cupric oxide oxidation products. The method used in the analysis was that of Hedges and Ertel.[18] Sediment (0.5 g) was accurately weighed into a PTFE lined Parr$^{(R)}$ or Valtech$^{(R)}$ bomb (25 cm^3) and a stainless steel ball agitator added. The bomb, together with a vial containing dichloromethane-extracted CuO (1.00 g; powdered) plus $Fe(NH_4)_2(SO_4)_2 . 6H_2O$ (50 mg) and a beaker containing nitrogen purged NaOH dissolved in HPLC grade water (8% wt/wt; 7 cm^3) were loaded into an Atmos Bag$^{(R)}$ (Aldrich Chemical Company). The bag was then purged with nitrogen for 1 hour. All components were then added to the sediment, the bomb assembled and after thorough shaking it was heated at 170°C (in a disused GC oven). At hourly intervals the bomb was shaken (30 seconds). After precisely 3 h it was removed and cooled under running water. The contents were transferred with washings ($NaOH/H_2O$; 8% wt/wt) to a centrifuge tube and the residual sediment separated (2000 rpm, 10 min). The residue was rinsed twice with additional $NaOH/H_2O$ (8% wt/wt). Supernatants were combined, diluted with distilled water (40 cm^3), acidified (pH 1) with HCl, and extracted three times with diethyl ether. After drying (anhydrous sodium sulphate), ethyl vanillin (0.1 mg in diethyl ether) was added as an internal standard. The solvent was then evaporated under a gentle stream of nitrogen and the residue treated to produce TMS derivatives (10:1 BSTFA/TMCS v/v, Regisil T, 100 mm^3; 2 h at 40°C). The extract was taken to dryness under a stream of nitrogen and re-dissolved in toluene for analysis by capillary gas chromatography—flame ionization detection (cGC-FID). Reagent blanks were simultaneously treated and sediment samples were analysed as duplicates to ensure good reproducibility.

cGC Analyses: Aliquots (1 mm^3) of the derivatised extracts were analysed using a Hewlett Packard 5890 gas chromatograph fitted with a splitless injector, a flame ionization detector and coupled to a Hewlett Packard 3392A Integrator. A BP1 WCOT vitreous silica column (25 m × 0.22 mm i.d.; 0.25 μm film thickness; SGE) was used under the following operating conditions: injection port 280°C; temperature programme: 100°C for 1 min (splitless), 100°C to 250°C at 4°C.min^{-1}, 250°C isothermal for 10 min; detector, FID, 290°C; carrier gas, He (1.1 Kg.cm^{-2} head pressure).

Peak identification was by co-injection of derivatised authentic standards and by computerised GC—mass spectrometry (CGCMS) using a Finnigan MAT 700 Ion Trap Detector coupled to a Carlo Erba HRGC 5300 Mega Series capillary gas chromatograph (Grob type split/splitless injector). Quantification was by peak area comparison with the internal standard, which was then corrected for FID-response.

Examples of GC-traces of a sediment extract, derivatised standard phenolic aldehydes and a reagent blank are shown in Figure 2.

Sterols and hydrocarbons

The analytical protocol used to quantify the sterols and hydrocarbons has been fully described and evaluated previously.[23] A summary of the technique is shown in Figure 3.

Sterols isolated from the total organic extract by thin-layer chromatography were derivatised to produce TMS ethers (10:1 BSTFA/TMCS v/v, Regisil T, 50 mm^3; 2 h at 40°C). The derivatised extracts were then taken to dryness using a gentle stream of nitrogen and redissolved in toluene. Aliquots (1 mm^3) were analysed using the same cGC-FID system as described for lignins but fitted with an OV1O1 WCOT vitreous silica column (24 m × 0.2 mm i.d.; 0.25 μm film thickness; SGE). Operating parameters were as follows: injection port 290°C; temperature programme: 100°C for 1 min (splitless), 100°C to 250°C at 12°C.min^{-1}, 250°C to 290°C at 3°C.min^{-1}, 290°C isothermal for 10 min; detector, FID, 300°C; carrier gas, He (1.5 Kg.cm^{-2} head pressure).

The HPLC fractions in hexane containing (i) primarily the saturated hydrocarbons[23] and (ii) the polycyclic aromatic hydrocarbons (PAH) were reduced in volume (to approximately 50 mm^3) using a gentle stream of nitrogen. Quantification of the hydrocarbons was by cGC-FID using a Carlo Erba 4160 GC fitted with a Grob type split/splitless injector, FID detector and coupled to a Spectra-Physics SP4100 Integrator. Saturated hydrocarbons were analysed using an OV1 WCOT fused silica column (25 m × 0.32 mm i.d.; 0.17 μm film

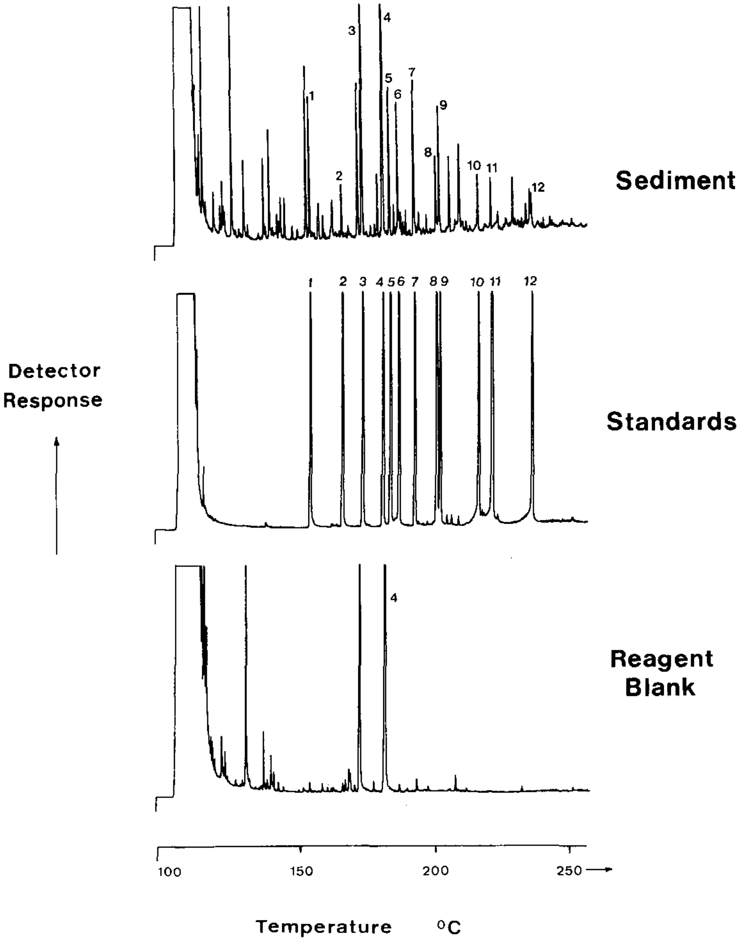

FIGURE 2 Examples of capillary GC-FID chromatograms of derivatised aliquots of: (i) cupric oxide oxidation products isolated from a Tamar Estuary sediment (ii) standard phenolic aldehydes ,(iii) an analytical reagent "blank". Peak numbers correspond to: 1, ρ-hydroxybenzaldehyde; 2, ρ-hydroxyacetophenone; 3. vanillin; 4, ethylvanillin (internal standard); 5, acetovanillone (+co-eluting compounds in the oxidised sediment extract); 6, ρ-hydroxybenzoic acid; 7, syringaldehyde; 8, acetosyringone; 9, vanillic acid; 10, syringic acid; 11, ρ-coumaric acid; 12, ferulic acid. Full analytical conditions are described in the text.

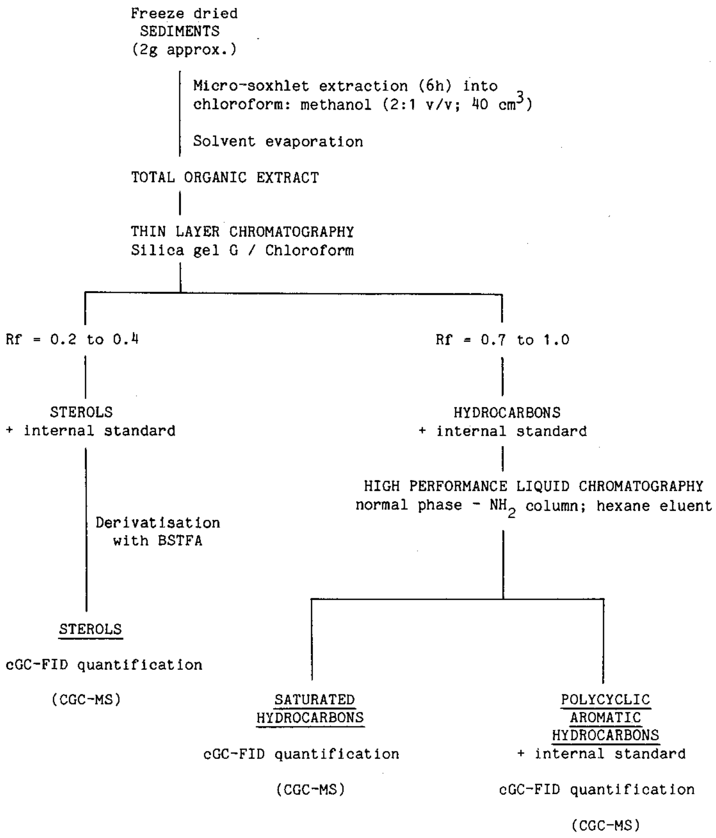

FIGURE 3 A summary of the analytical protocol selected to quantify sterols, saturated hydrocarbons and polycyclic aromatic hydrocarbons in the estuarine sediment samples (as described by Readman *et al.*).[23]

thickness; Hewlett Packard Ultra Series) and PAH using an SE54 WCOT fused silica column (25 m × 0.32 mm i.d.; 0.17 μm film thickness; Hewlett Packard Ultra Series). Operating parameters for both saturates and PAH were as follows: injection port 290°C; temperature programme: 50°C for 1 min (40 sec splitless), 50°C to 290°C at 6°C.min^{-1}, 290°C isothermal for 10 min; detector, FID, 300°C; carrier gas, He (0.7 Kg.cm^{-2} head pressure).

Quantification of sterols and hydrocarbons was by peak area comparison with the internal standards (5α cholestane for sterols and

2,21 dimethyldocosane for saturated hydrocarbons and PAH). Corrections were made for relative FID responses, contributions from reagents and recovery efficiencies.

Peak identification was achieved by co-injection of authentic standards and by CGCMS (Finnigan MAT 700 Ion Trap Detector coupled to a Carlo Erba HRGC 5300 Mega Series capillary gas chromatograph—Grob type split/splitless injector).

Examples of cGC-FID chromatograms of sterol and hydrocarbon extracts of Tamar Estuary sediments are shown in Figure 4.

Photosynthetic pigments

Photosynthetic pigments were analysed by HPLC using the method of Mantoura and Llewellyn.[28,29] Weighed (0.300 g) sub-samples of the freeze dried, ground sediments (stored at —15°C in darkness) were extracted by pulverisation into acetone:water (90:10; 2.00 cm^3) immediately prior to analysis by HPLC. Extracts were centrifuged (3000 rpm, 4 min) and the supernatant ion-paired with solution P (3:1 v/v) (solution P = 1.5% tetrabutyl ammonium acetate + 7.7% ammonium acetate in water). Following equilibration (1 min), aliquots (100 mm^3) were introduced *via* a fixed loop valve (Rheodyne 7125) onto a Hypersil C_{18} reverse-phase column (Shandon, 25 cm × 5 mm i.d.; 5 μm). Solvent was delivered (2 $cm^3 . min^{-1}$) using a programmable gradient pump (ACS model 300 + 750/37) with a 10 min linear gradient (0–100%) elution scheme (primary 80:10:10 v/v methanol/water/solution P; secondary 60:40 v/v methanol/acetone), and maintaining isocratic elution with the secondary solvent until completion of the chromatogram. Pigments were detected by absorbance at 440 nm using a diode array spectrophotometer (Hewlett Packard 1040A) and by fluorescence ($\lambda_{ex} = 430$–440 nm, $\lambda_{em} > 600$ nm; Dupont 838). An example of a pigment separation is shown in Figure 5. Preparation and sources of "standard" pigments together with information on peak identification using rapid-scanning diode array spectra have been detailed by Mantoura and Llewellyn.[28,29]

Quantification of chlorophylls, phaeophorbides and phaeophytins was by fluorescence relative to chlorophyll a, for which the system had been previously calibrated. Suitable corrections were introduced for differences in the fluorescence response of the individual compounds. Peridinin, fucoxanthin and lutein were quantified by their

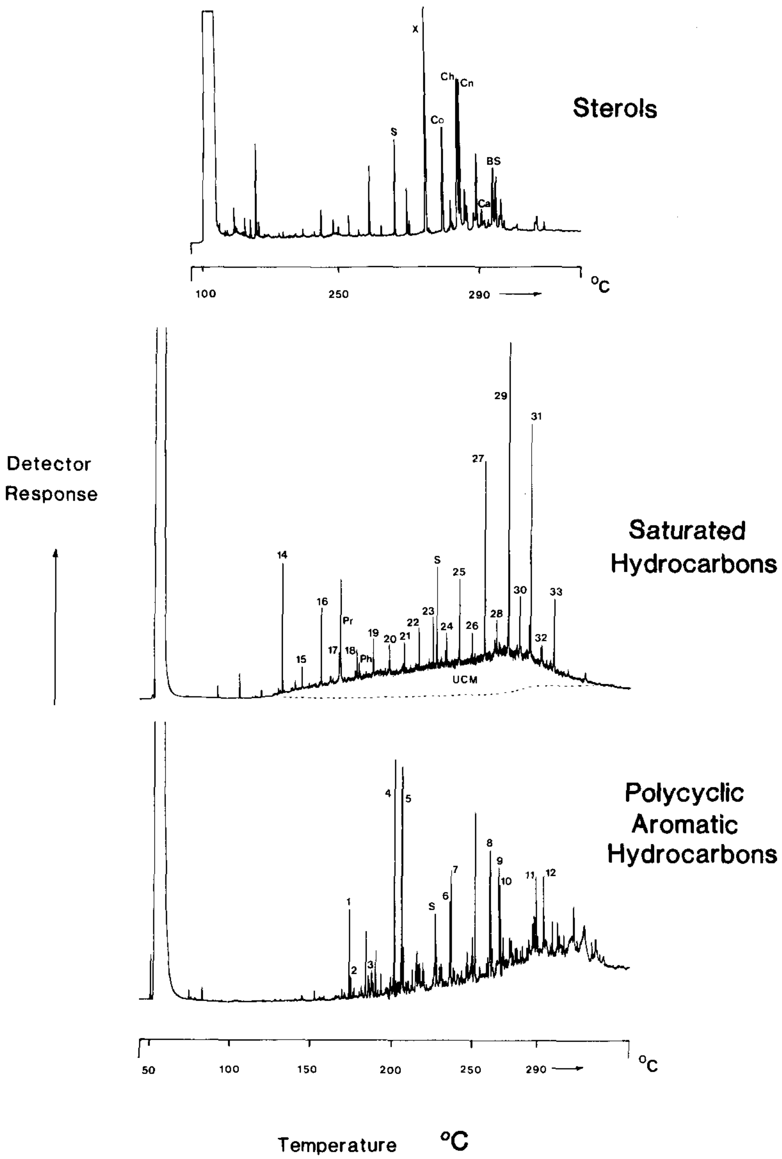

FIGURE 4 Examples of GC-FID chromatograms of (i) sterols (chromatographed as TMS—derivatives) (ii) saturated hydrocarbons and (iii) polycyclic aromatic hydrocarbons isolated from Tamar Estuary sediments. Peak abbreviations and figures correspond to: STEROLS... Co, coprostanol; Ch, cholesterol; Cn, cholestanol (CGCMS indicate this peak to be impute); Ca, campesterol; BS, β-sitosterol; S, 5α cholestane internal standard; x, indicated by CGCMS to be an aliphatic alcohol.[23] SATURATED HYDROCARBONS... *n*-alkanes are indicated simply by their carbon number (see Table II); S, 2,21-dimethyldocosane internal standard; Pr, pristane; Ph, phytane; UCM = unresolved complex mixture. POLYCYCLIC AROMATIC HYDROCARBONS... 1, phenanthrene; 2, anthracene; 3, methyl-phenanthrenes and anthracenes; 4, fluoranthene; 5, pyrene; 6, benz(a)anthracene; 7, chrysene/triphenylene; 8, benzo-fluoranthenes; 9, benzo(e)pyrene; 10, benzo(a)pyrene; 11, indeno(1,2,3-cd)pyrene; 12, benzo(ghi)perylene; S indicates the internal standard (2,21-dimethyldocosane). Full analytical conditions are described in the text.

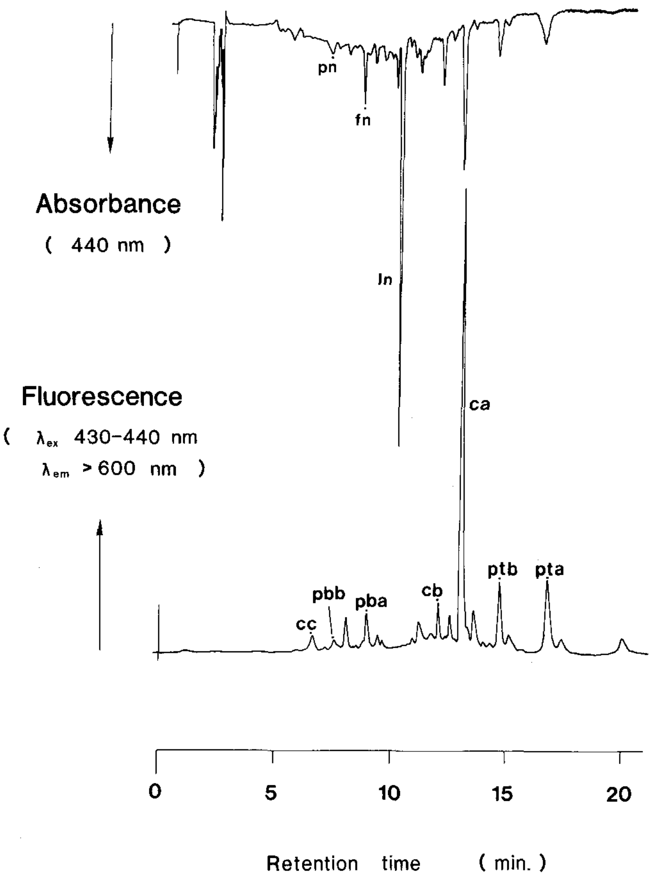

FIGURE 5 An example of ion-pair reverse-phase HPLC absorbance (440 nm) and fluorescence (λ_{ex} = 430–440 nm, λ_{em} > 600 nm) chromatograms of photosynthetic pigments isolated from a Tamar Estuary sediment. Peak abbreviations correspond to: cc, chlorophyll $c_1 + c_2$; pbb, phaeophorbide b; pba, phaeophorbide a; cb, chlorophyll b; ca, chlorophyll a; ptb, phaeophytin b; pta, phaeophytin a; pn, peridinin; fn, fucoxanthin; ln, lutein. Full analytical conditions are described in the text.

absorbance at 440 nm following calibration of the system using authentic standards.

Statistical analyses of the results

The large number of possible paired correlations between the variables quantified requires non-parametric procedures for their statistical appraisal.[30] Initially, Pearson product-moment correlation coefficients (r) were calculated for all pairs of variables to assess the covariability of individual constituents. Following compilation of the coefficients into a data matrix, cluster analysis[25] was performed to graphically summarise the correlations between individual variables in a similarity dendrogram (Figure 6). Multi-Dimensional Scaling (MDS)[26] was also computed to summarise correlations in the data as points in a two dimensional MDS space; the closer the points, the stronger the correlations between those variables (Figure 7). (The origin, orientation and scale of the MDS plot are arbitrary).[30] Mantoura *et al.*[30] have critically evaluated the use and merits of MDS in environmental organic chemistry.

To investigate the statistical validity of the correlations, fifty data matrices of identical size to the environmental data set were configured by randomly re-distributing figures within individual columns of the environmental data matrix. Only 6.42% (4.21% + ve; 2.21% − ve) of correlations within these data sets exhibited coefficients outside the range −0.7 to +0.7. This compares with 24.37% (23.03% + ve; 1.34% − ve) in the environmental data set.

RESULTS AND DISCUSSION

Concentrations of the variables quantified are listed in Tables I and II and are statistically appraised by cluster analysis and MDS in Figures 6 and 7 respectively.

Biogenic components

Most biogenic compounds are isolated from anthropogenic inputs at a similarity level of approximately 40% (Figure 6). A cluster with similarity >70% includes total and all individual lignin oxidation

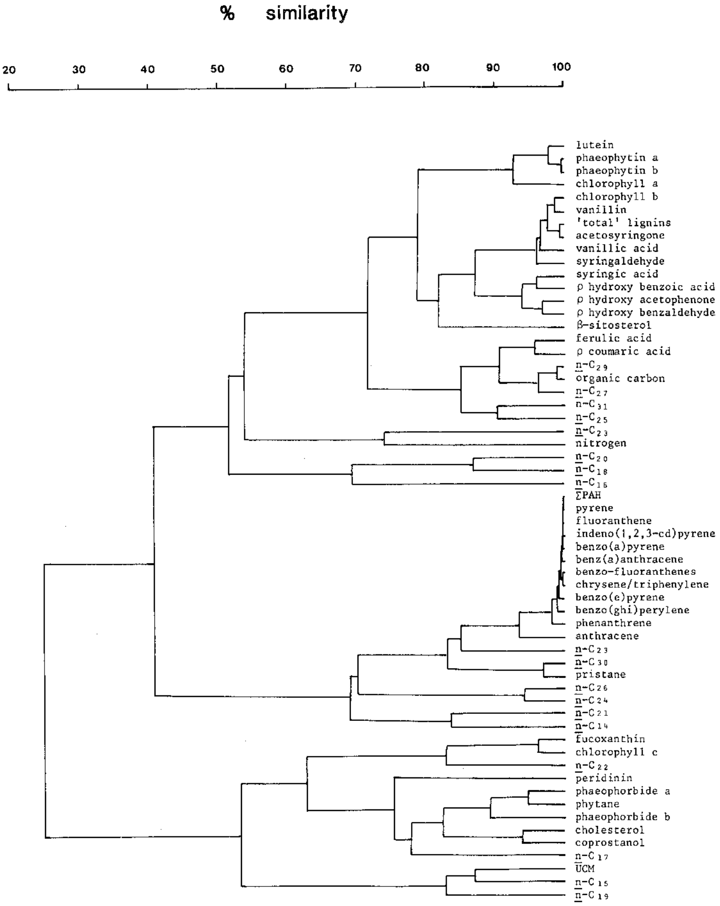

FIGURE 6 Cluster analysis dendrogram of the variables quantified in the Tamar Estuary sediment samples. A scale indicating percentage similarity between the variables is shown at the top of the diagram.

products, the higher plant waxes (n-C_{25}, n-C_{27}, n-C_{29} and n-C_{31}),[1,22,33] β-sitosterol (the major sterol found in higher plants),[6] chlorophylls a and b, phaeophytins a and b and lutein. Organic carbon is also included in this cluster.

Concentrations of lignin oxidation products (Table I) are comparable with previously reported levels for freshwater[8,18] and coastal[1,8] sediments. Summed values of the quantified vanillyl,

TABLE I

Concentrations[a] of organic carbon, nitrogen, sterols, lignin oxidation products and photosynthetic pigments in surface sediments sampled from the Tamar Estuary

Compound	Abbreviation[b]	Sample site							
		1	2	3	4	5	6	7	8
Organic carbon (%)	OC	1.380	2.010	1.072	3.650	3.999	3.002	4.376	4.027
Nitrogen (%)	N	0.230	0.250	0.097	0.340	0.248	0.286	0.307	0.294
Sterols									
Coprostanol	Co	2.4	16.8	1.0	5.1	3.9	1.0	4.4	0.6
Cholesterol	Ch	3.7	8.6	1.2	3.0	2.5	0.7	1.4	1.0
β-sitosterol	Bs	2.3	5.6	2.2	14.6	15.9	4.4	6.5	9.7
Lignin oxidation products[c]									
ρ-hydroxybenzaldehyde	P-ALD	21	24	10	58	57	23	56	74
ρ-hydroxyacetophenone	P-KET	4	3	3	32	25	10	26	35
ρ-hydroxybenzoic acid	P-ACD	12	19	7	76	47	28	66	70
vanillin	V-ALD	45	50	45	301	182	76	168	170
vanillic acid	V-ACD	12	15	15	134	58	25	52	70

syringaldehyde	S-ALD	15	20	18	120	59	30	60	43
acetosyringone	S-KET	6	10	3	55	30	14	36	32
syringic acid	S-ACD	3	7	4	36	28	12	29	26
ρ-coumaric acid	C-PCA	5	8	9	21	32	14	42	33
ferulic acid	C-FA	3	5	7	13	21	6	21	15
TOTAL LIGNINS[d]	L	126	161	121	846	539	238	556	568
Photosynthetic pigments									
chlorophyll a	ca	2.34	3.98	1.67	15.89	15.45	2.78	10.50	3.52
chlorophyll b	cb	0.85	1.21	0.68	6.45	4.24	1.47	3.16	2.95
chlorophyll c_1+c_2	cc	0.33	0.31	0.08	0.69	0.61	0.05	0.33	0.12
phaeophytin a	pta	1.14	0.98	0.92	11.80	12.97	4.11	12.12	5.42
phaeophytin b	ptb	0.44	0.28	0.19	4.95	5.88	1.68	5.56	2.10
phaeophorbide a	pba	2.24	5.32	0.51	3.72	3.06	0.15	1.33	0.29
phaeophorbide b	pbb	0.46	0.71	0.09	0.69	0.62	0.05	0.05	0.05
peridinin	pn	0.11	0.40	0.01	0.26	0.39	0.03	0.21	0.01
fucoxanthin	fn	0.63	0.89	0.17	1.37	1.09	0.08	0.55	0.08
lutein	ln	0.48	0.97	0.41	5.65	6.77	1.03	6.77	1.59

[a] Concentrations in $\mu g.(g\ dry\ sediment)^{-1}$ except for organic carbon and nitrogen which are expressed as %.

[b] Abbreviations used in the multidimensional scaling figure (Figure 8).

[c] Mean values of duplicate analyses. Variation about the mean averaged ±6.2%.

[d] Total lignins refers to the sum of the individual phenolic-aldehydes listed.

TABLE II

Concentrations[a] of hydrocarbons in surface sediments sampled from the Tamar Estuary

Compound	Abbreviation[b]	Sample site							
		1	2	3	4	5	6	7	8
Saturates									
n-C_{14}	14	0.72	0.60	0.31	0.32	0.38	0.41	1.14	0.95
n-C_{15}	15	0.14	0.22	0.11	bdl	0.08	0.67	0.18	0.13
n-C_{16}	16	0.38	0.42	0.61	0.21	0.17	0.38	0.65	0.44
n-C_{17}	17	0.12	0.20	0.09	0.17	0.13	0.13	0.12	0.14
pristane	Pr	0.41	0.77	0.12	0.67	0.81	0.26	0.78	1.57
n-C_{18}	18	0.10	0.22	0.37	0.06	0.07	0.20	0.25	0.08
phytane	Ph	bdl	0.23	0.07	0.18	0.10	bdl	bdl	0.05
n-C_{19}	19	bdl	0.20	0.05	bdl	0.05	0.07	0.26	0.07
n-C_{20}	20	0.17	0.24	0.34	0.08	0.06	0.18	0.13	0.07
n-C_{21}	21	bdl	0.30	0.06	0.09	0.10	0.13	0.27	0.30
n-C_{22}	22	0.09	0.16	0.35	0.07	0.18	0.33	0.29	0.34
n-C_{23}	23	0.09	0.12	0.11	0.23	0.22	0.29	0.32	0.77
n-C_{24}	24	0.17	bdl	0.04	0.08	0.24	0.19	0.18	0.33
n-C_{25}	25	0.17	0.51	0.13	0.66	0.56	0.43	0.79	0.97
n-C_{26}	26	0.15	bdl	0.04	0.05	0.13	0.11	0.13	0.22

n-C_{27}	27	0.39	0.70	0.21	1.03	1.49	0.94	1.82	1.78
n-C_{28}	28	0.12	0.14	bdl	0.44	0.13	0.17	0.11	0.21
n-C_{29}	29	0.56	0.94	0.34	1.94	2.10	1.59	2.79	2.26
n-C_{30}	30	0.25	0.31	0.06	0.44	0.43	0.17	0.41	0.74
n-C_{31}	31	0.40	1.37	0.34	1.73	1.39	1.39	2.65	1.93
U.C.M.[c]	UCM	77.10	118.08	32.21	89.18	70.20	23.89	103.72	63.43
Polycyclic aromatics	PAH								
phenanthrene	phe	0.28	0.15	0.01	0.17	0.13	0.04	0.46	0.71
anthracene	ant	0.07	0.03	0.03	0.08	0.03	0.04	0.18	bdl
fluoranthene	flu	0.60	0.37	0.04	0.55	0.36	0.09	1.20	2.26
pyrene	pyr	0.52	0.33	0.06	0.54	0.37	0.10	1.16	2.05
benz(a)anthracene	baa	0.23	0.13	0.03	0.25	0.15	0.05	0.47	0.79
chrysene/triphenylene	c/t	0.29	0.17	0.04	0.28	0.23	0.05	0.73	1.50
benzo-fluoranthenes	b-f	0.56	0.32	0.06	0.47	0.43	0.10	1.22	2.33
benzo(e)pyrene	bep	0.29	0.19	0.04	0.21	0.15	0.04	0.78	1.40
benzo(a)pyrene	bap	0.28	0.21	0.06	0.36	0.22	0.06	0.69	1.23
indeno(1,2,3-cd)pyrene	ip	0.22	0.17	0.03	0.24	0.20	0.04	0.53	0.98
benzo(ghi)perylene	bgp	0.19	0.14	0.03	0.23	0.15	0.06	0.55	0.82
TOTAL PAH	$\sum$PAH	3.53	2.21	0.43	3.38	2.42	0.67	7.97	14.07

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[a] Concentrations in μg.(g dry sediment)$^{-1}$.

[b] Abbreviations used in the multi-dimensional scaling figure (Figure 8).

[c] UCM = unresolved compex mixture in cGC-FID chromatograms (the UCM is estimated from its area relative to that of the internal standard).

bdl = below detection limit.

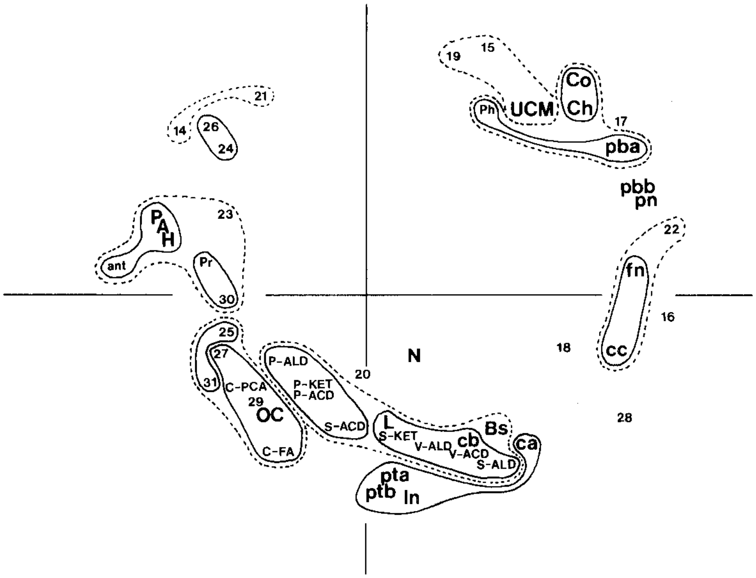

FIGURE 7 Multi-dimensional scaling analysis (MDS) of the variables quantified in the Tamar Estuary sediment samples. Clusters with similarities >90% and >80% (as shown by cluster analysis) are circumscribed by "——" and "– – –" respectively. The abbreviations used to indicate positions of the variables are identified in Tables I and II.

syringyl and cinnamyl lignins normalised to sedimentary organic carbon[1,8,31] are listed in Table III. Concentrations range from 0.57 to 1.86 mg.(100 mg organic carbon)$^{-1}$ ($\bar{x}=0.94, \delta_{n-1}=0.41$). High concentrations recorded for the mid-estuarine sediments (Stations 3 to 5; Figure 1; Table III), probably indicate that the estuary acts as a sink for lignins.

Upon CuO oxidation, only angiosperm tissues produce syringyl phenols in any appreciable amounts and only non-woody tissues (leaves, needles and grasses) yield cinnamyl phenols.[31] All gymnosperm and angiosperm woods and non-woody tissues yield vanillyl phenols as oxidation products.[8,32] Owing to the fact that acetovanillone was not quantified in the Tamar Estuary sediments (because of co-eluting interferences), comparison with previous results which use summed vanillyl phenols[e.g. 31] is not possible. By investigating ratios between selected individual compounds, however, characteristics of the lignins can be compared (Table III). V-ACD/V-ALD

TABLE III

Lignin parameters calculated from the Tamar Estuary Sediments

Sample station	Λ[a]	$\frac{\text{V-ACD}^b}{\text{V-ALD}}$	$\frac{\text{S-ALD}^b}{\text{V-ALD}}$	$\frac{\text{C-PCA}^b}{\text{V-ALD}}$
1	0.65	0.27	0.33	0.11
2	0.57	0.30	0.40	0.16
3	0.94	0.33	0.40	0.20
4	1.86	0.44	0.40	0.07
5	1.03	0.32	0.32	0.18
6	0.59	0.33	0.40	0.18
7	0.93	0.31	0.36	0.25
8	0.97	0.41	0.25	0.19

[a] Weight (mg) of sum of vanillyl (vanillin + vanillic acid but excluding acetovanillone—which was not quantified), syringyl (syringaldehyde + acetosyringone + syringic acid) and cinnamyl (ρ coumaric acid + ferulic acid) phenols normalised to 100 mg of sedimentary organic carbon.

[b] Abbreviations are identified in Table I.

has been proposed[1] as an indicator of the degree of aerobic degradation of lignins (V-ACD/V-ALD increases with degradation). With the execptions of samples 4 and 8, this ratio is remarkably constant (0.31 ± 0.02). Higher values recorded at stations 4 and 8 might indicate increased degradation. Also noted at station 4 (which contains the highest recorded concentration of lignins) is a low value (0.07) for the ratio C-PCA/V-ALD (= 0.18 ± 0.04 for the remaining sites) (Table III). No significant change is, however, apparent for the ratio S-ALD/V-ALD (Table III). This information indicates enrichment of V-ACD and V-ALD, with a corresponding increase in S-ALD but not in C-PCA. An increased proportion of degraded woody angiosperm tissues could produce the observed differences. The increased V-ACD/V-ALD ratio at the fresh water site 8 (Table III), coincides with a substantial decrease in the S-ALD/V-ALD ratio (Table III). This might speculatively indicate inclusion of degraded gynmosperm lignins.

Cluster analyses (Figure 6) and MDS (Figure 7) segregate the lignin oxidation products into three groups at a 90% similarity level. The first contains V-ALD, V-ACD, S-ALD, S-KET and TOTAL lignins and quantitatively represents the largest grouping. This confirms general uniformity of vanillyl/syringyl phenols. S-ACD, P-ALD, P-KET and P-ACD are separated from the first group at a similarity level of 87%. ρ-hydroxyl phenols are known to have quantitatively important non-lignin sources which can limit their use as geochemical tracers.[32] The third group comprises the cinnamyl phenols (C-PCA and C-FA) which only arise from oxidation of non-woody tissues (leaves, needles and grasses). As might be expected, the major plant waxes n-C_{27} and n-C_{29} (together with n-C_{25} and n-C_{31} at 84% similarity) are grouped with these acids. Organic carbon is also incorporated into this cluster.

Ertel and Hedges[31] have recently demonstrated the complex distribution of lignins within density fractions and humic substances of a sediment. In combination with the hydrodynamic, physico-chemical and biological gradients encountered within the estuary, further analyses are clearly required to fully appraise the distributions.

β-sitosterol (the major sterol synthesised by higher plants)[6] is grouped by cluster analysis at 82% similarity with the lignin group containing V-ALD, V-ACD, S-ALD, S-KET and L, endorsing its vascular plant origin. Cholesterol and coprostanol are segregated from β-sitosterol at 25% similarity (Figure 6).

Concentrations of chlorophylls a and b and phaeophytins a and b (Table I) co-vary ($r > 0.83$). Correlation coefficients (r) between these pigments and lutein (Table I) exceed 0.8. MDS (Figure 7) clusters this group with the lignin oxidation products and β-sitosterol [similarity = 78% (Figure 6)], implying a terrestrial or riverine primary source of these pigments. The results might also suggest some preservation of chlorophylls a and b in the anoxic sediments with primary degradation (*via* de-metallation) to form the phaeophytins.[33] It is unlikely that epibenthic diatoms are responsible for introducing chlorophylls to the sediments because of the very low correlation ($r < 0.5$) with chlorophyll c. The high correlation of lutein with this group (~ 0.9) agrees with a higher plant/chlorophyte source of this carotenoid.[34]

The herbivorous grazing of chlorophylls is thought to result in their demetallation and removal of their phytol side chains to form phaeophorbides.[35] Statistical isolation of phaeophorbides a and b from their parent chlorophylls (Figures 6 and 7) is compatible with a degradation mechanism which is unrelated to chlorophyll source (such as that described).

Chlorophylls c_1 and c_2 covary with fucoxanthin ($r = 0.95$) confirming their documented primary common sources (diatoms and brown fucoids).[34]

The statistical segregation of peridinin from the other photosynthetic pigments is in accord with its dinoflagellate source.[34]

With the exception of the higher plant waxes (n-$C_{25,27,29,31}$) (as discussed in relation to lignins), no significant trends are apparent between the *n*-alkanes (Figures 6 and 7). These saturated hydrocarbons have a multiplicity of biogenic[36] (e.g. phytoplankton, [37,38] benthic algae,[39] bacteria[38] etc.) and anthropogenic (oil)[36] sources. Distributions of the compounds are further complicated by differential transport and fates[36,40] such as volatilisation, aqueous/particulate partitioning and microbial degradation.

Anthropogenic components

Pollutants are isolated (at approximately 40% similarity) from the terrestrial biogenic inputs by cluster analysis and MDS (Figures 6 and 7). To investigate the estuarine distributions of anthropogenic components, selected organic markers have been plotted against sample stations (Figure 8). The pollutant markers chosen are coprostanol as an indicator of sewage,[9,10,14–17] the saturated hydrocarbon "UCM" (Figure 4) to indicate chronic/degraded oil contamination,[22,41–43] and total PAH.

Concentrations of coprostanol throughout the estuary range typically from 0.6 to 5.1 μg.(g dry sediment)$^{-1}$ (Table I; Figure 8). These levels are comparable with those reported for the Severn Estuary[16] [0.9 to 3.1 μg.(g dry sediment)$^{-1}$], the Clyde Estuary[10] [typically 0.1 to 6 μg.(g dry sediment)$^{-1}$] and New York Bight[14] [4.8 and 5.2 μg.(g dry sediment)$^{-1}$]. The highest concentration of coprostanol in the Tamar [16.8 μg.(g dry sediment)$^{-1}$; Figure 8; Table I] indicates chronic sewage contamination[10,23] at sample site 2 (located adjacent to urban Plymouth; Figure 1). Subtle variations in concentration throughout the remainder of the estuary (Figure 8), probably relate to sewage inputs and the diffusive transport patterns from the outfalls. The distribution of cholesterol is shown to covary with coprostanol ($r = 0.94$) indicating that faecal pollution is also the primary source of this sterol in the Tamar.

The dominant individual components in all saturated hydrocarbon cGC-FID chromatograms (as exemplified in Figure 4) are the

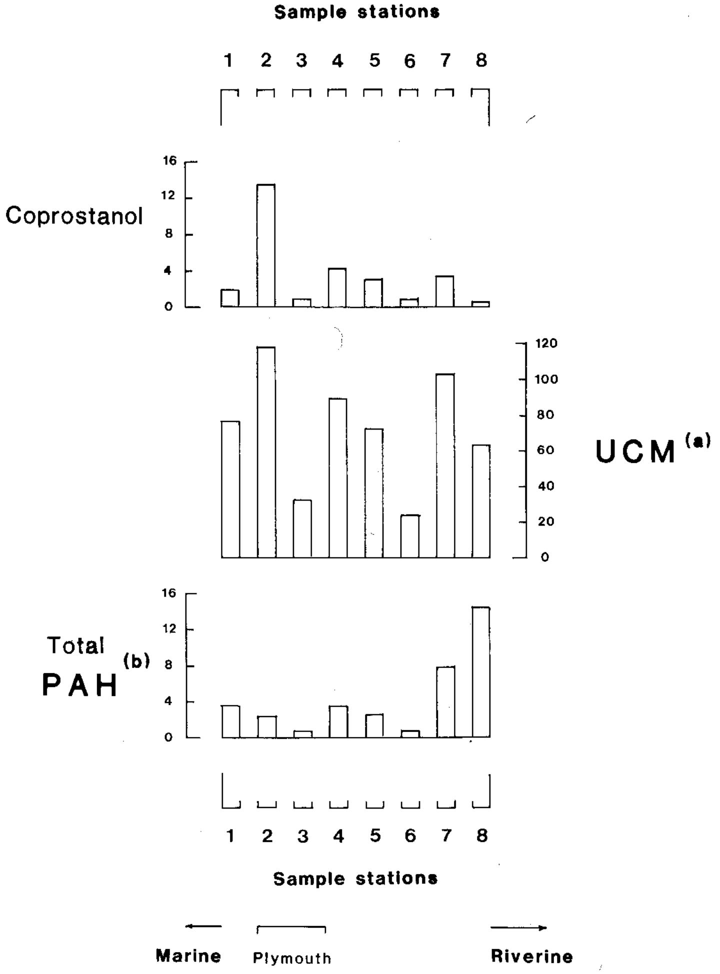

FIGURE 8 Concentrations [μg.(g dry sediment)$^{-1}$] of (i) coprostanol, (ii) the unresolved complex mixture (UCM) in the saturated hydrocarbon cGC-FID chromatograms and (iii) total polycyclic aromatic hydrocarbons (PAH) at the eight sampling stations in the Tamar Estuary (see Figure 1). Station numbers are indicated at the top and bottom of the diagram. Marine and freshwater regions are indicated. The position of Plymouth is shown. (a) the UCM was estimated from its area relative to that of the internal standard. (b) "total" PAH is the sum of the individual compounds quantified (Table II).

biogenic n-$C_{25,27,29,31}$ alkanes. Contamination of the sediments by recent inputs of undegraded petroleum[22,23,41] appears to be negligible. Sedimentary UCM concentrations (which can be used to investigate chronic/degraded oil contamination[22,41–43]) are listed in Table II and are plotted against sample station in Figure 8. Concentrations are shown to co-vary with coprostanol ($r = 0.75$). The increase in UCM at station 2 does not, however, reflect the exceptional increase in coprostanol (Figure 8). MDS confirms covariation of the two variables (Figure 7), although the high Pearson product-moment correlation coefficient (r) of 0.75 is not apparent in the cluster analysis (Figure 6). The similarities of distribution probably indicate that a primary source of the UCM is associated with waste-water effluents.

The dominance of unsubstituted parent compounds in all PAH cGC-FID chromatograms (as exemplified in Figure 4) indicates that the primary source of PAH to the Tamar is associated with combustion.[23,24,44,45] Very similar cGC-FID chromatograms have appeared in the literature.[e.g. 22,44,45] Concentrations of individual PAH (Table II) range from 0.01 to 2.26 $\mu g.(g\ \text{dry sediment})^{-1}$, which are comparable with levels previously reported for the Tamar Estuary.[46]

The distribution of total PAH with sample station (Figure 8) reveals highest concentrations at the riverine end, with a decrease in concentrations down-estuary. A similar distribution has been reported previously.[46] This earlier survey also identified elevated concentrations adjacent to Plymouth (between sample stations 2 and 3—Figure 1).

PAH were statistically isolated from all other variables at a similarity of approximately 80%, the vast majority being segregated at below 70% similarity (Figure 6). Correlations between individual PAH were typically in excess of 96% similarity (Figure 6) which indicates a highly uniform composition throughout the estuary. This is confirmed by MDS (Figure 7) which positions the compounds (with the exception of anthracene) in a tight cluster within which the individual PAH are superimposed. The uniformity of composition suggests that compound selective transport and degradation does not occur. Readman *et al.*[47] have shown that sedimentary PAH in the Tamar are not available for aqueous/particulate partitioning according to thermodynamic theory. This observation can be explained if the PAH are occluded within particulates. The same theory would explain the apparent absence of discrimination by compound-selective fates.

CONCLUSIONS

The initial investigation described in this work has demonstrated that the combined analyses of a series of molecular marker compounds can afford extensive information regarding sources, transport mechanisms and fates of both biogenic and anthropogenic organic components within estuarine environments. Complex correlations associated with such a large suite of environmental variables are well suited to statistical appraisal by the non-parametric techniques of cluster analysis and multidimensional scaling, which enable rapid assimilation of results and assist their interpretation.

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