Fatty Alcohols – a review of their natural synthesis and environmental distribution

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Executive Summary

The published and grey literature on the environmental occurrence, fate and behaviour of fatty alcohols has been reviewed. The principal focus has been on the natural production, which occurs in all living organisms from bacteria to man, and the profiles and concentrations of these compounds in water, soils and sediments. Their relatively non-polar nature means they are principally associated with solid phases (*e.g.* sediments) rather than dissolved in water. The major production mechanism is from the reduction of fatty acids, through aldehyde intermediates, to fatty alcohols and in many organisms to esters with fatty acids to form waxes. These waxes are used for a variety of purposed from the prevention of desiccation in the terrestrial environment to energy reserves in the marine environment. They are ubiquitous and occur in most environments around the world including the deep ocean and in sediment cores.

Due to the nature of the synthetic pathway using acetyl-CoA, most fatty alcohols are of an even chain length. Terrestrial plants utilise fatty alcohols as waxy coating and these compounds are dominated by long chain moieties with chain lengths from C_{22} to C_{32}; in contrast marine organisms synthesise smaller compounds with peak chain lengths of C_{14} to C_{16}. Bacteria also produce fatty alcohols but these can also be odd chain lengths and contain branches. This aspect of their occurrence enables them to be used as biomarkers for organic matter sources.

As well as their natural production and occurrence, fatty alcohols are also utilised in detergent formulations principally as polyethoxylates. The analytical method used to measure the concentration of the ethoxylates involves direct derivatisation with a pyridinium complex and quantification *via* LC-MS. This technique will detect free fatty alcohols as well as the ethoxylates but will not detect any of the bound alcohols such as the waxes. To detect this group, a saponification step is required. This second method in combination with the LC method will detect all of the ethoxylates and may be considered a good measure of the total fatty alcohols present in the system.

The concentration of individual fatty alcohols in the environment ranges from low values in old deep cores and the open ocean floor (undetectable to 12 $ng.g^{-1}$ DW for C_{16}) to high values near natural sources and especially in suspended particulate matter

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(2.7 mg $.g^{-1}$ DW for C_{16}); this is almost a factor of 10^6 difference in their concentrations. The short chain compounds are more readily degradable than the longer chain compounds and in many cases are removed first as a food source for bacteria. The longer chain compound may also degrade to short chain compounds with time but, in general, the $>C_{20}$ class of alcohols are better preserved in sediments than the $<C_{20}$ class.

The different compound profiles for each source has made them suitable as biomarkers and the use of multivariate statistical methods can clearly distinguish compounds from each potential source as well as sites. Principal Component Analysis (PCA) is particularly useful in this regard. Signature analysis using Partial Least Squares (PLS) analysis is successful when the marine / terrestrial sources are used to discriminate samples, however, due to the commonality of commons present in detergent formulations and the natural environmental alcohols, source partitioning on the basis of compounds alone is not as successful. When ascribing proportions to such sources, a different approach such as stable isotopes may be more appropriate.

Key issues and directions for further study which have arisen from this review include the lack of context information presented when anthropogenic alcohols are quantified; no corresponding measure of total (including wax bound) alcohols is made and this may serve as a useful indicator of the relative importance of each source. Further information is needed on the rates at which free alcohols may be derived from bound sources or fatty acid precursors both in sewage treatment plants and in the environment as a whole. These aspects will have repercussions on the toxicity and ecotoxicity of alcohols in the environment, an aspect that was not included in this review.

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Chapter 1. Definitions *(This chapter aims to introduce the family of compounds, how they are referred to, the likely structures that will be found and their chemistry from an environmental point of view).*

Names and structures

Fatty alcohol is a generic term for a range of aliphatic hydrocarbons containing a hydroxyl group, usually in the terminal position. The accepted definition of fatty alcohols states that they are naturally derived from plant or animal oils and fats and used in pharmaceutical, detergent or plastic industries (*e.g.* Dorland's Illustrated Medical Dictionary). It is possible to find the hydroxyl (–OH) group in other positions within the aliphatic chain although these secondary or tertiary alcohols are not discussed to any great extent in this treatise.

The generic structure of fatty alcohols or n-alkanols can be seen in Figure 1.1 and specific examples in Figure 1.2. The value of the *n* component is variable and is discussed below.

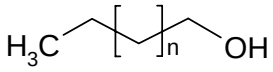

Figure 1.1 Generic structure of a fatty alcohol – the total number of carbons needs to be greater than 8 – 10 to be a "fatty" alcohol; shorter chain compounds have an appreciable water solubility.

The range of chain lengths for these n-alcohols can be from 8 to values in excess of 32 carbons. With such a wide range of chain lengths, the chemical properties and, consequently, environmental behaviour vary considerably. As well as these straight chain moieties, a range of branched chain compounds are also naturally produced by micro-organisms in the environment. The major positions for the methyl branches are on the carbons at the opposite end of the molecule to the terminal –OH. If the methyl branch is one in from the end of the molecule (ω-1) it is termed an *iso* fatty alcohol; if it is two in from the end (ω-2) it is called an *anteiso* fatty alcohol. Examples of these branches can be seen in Figure 1.2.

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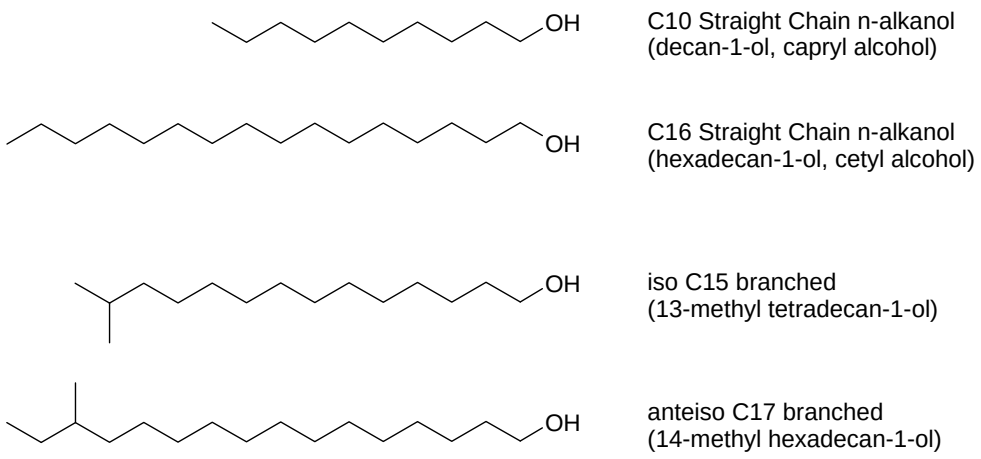

Figure 1.2 Example fatty alcohol structures. The majority found in nature are of the straight chain type with smaller amounts of the branched chain compounds also being present.

Most fatty alcohols are saturated in that they have no double bonds present in their structure. However, there are a limited number of mono-unsaturated compounds that can be found in nature. The two most common compounds are phytol (3,7,11,15 – tetramethyl-2-hexadecen-1-ol), an isoprene (Chikaraishi *et al.*, 2005) derived from the side chain of chlorophyll (Figure 1.3) and a straight chain C_{20} alcohol with a double bond in the ω9 position counted from the terminal carbon (eicos-11-en-1-ol, Figure 1.4, (Kattner *et al.*, 2003).

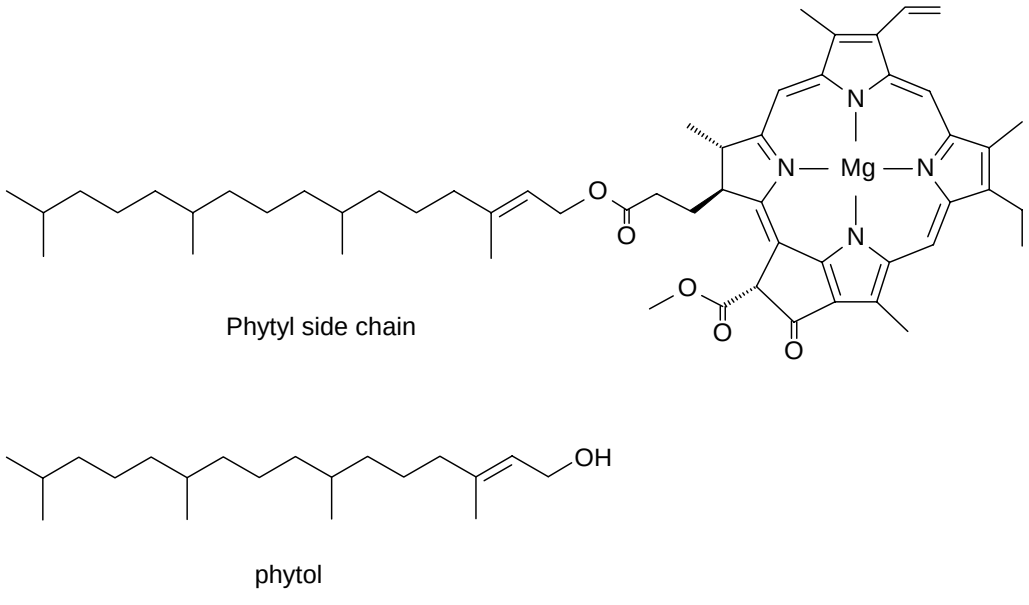

Figure 1.3 The chlorophyll a molecule with the phytyl side chain labelled. Cleavage of this chain at the COO- group produces free phytol in the environment.

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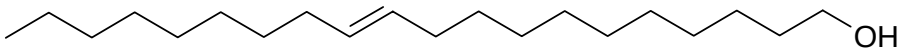

Figure 1.4 Eicos-11-en-1-ol or 20:1 fatty alcohol, one of the most frequently measured straight chain mono-unsaturated alcohol in the environment.

There have been occasional reports of polyunsaturated fatty alcohols but these are relatively rare (*e.g.* Ju and Harvey, 2004) and are confined to di-unsaturates such as 18:2. There is a group of isoprenoid lipids which may be found in bacteria which are essentially repeating isoprene subunits strung together and terminated by a hydroxyl group (Perry *et al.*, 2002). These compounds are also uncommon in environmental analyses and are not reported to any great extent.

Fatty alcohols together with many other groups of compounds have both systematic and trivial or common names. This trivial name is based on the length of the alkyl chain and the root is common between aliphatic hydrocarbons and fatty acids. These names together with the systematic name and carbon number are shown in Table 1.1.

Physico-Chemical Properties

Solubility vs. chain length

One of the key factors in determining the environmental behaviour of any compound is its water solubility; this will determine the partitioning between solid and solution phases. Compounds with low water solubility will be preferentially adsorbed to particulate matter, either settled or suspended in water. These compounds will also partition into the lipid phase of organisms and have higher bioconcentration factors. The available physico-chemical properties for the fatty alcohol series from C_4 to C_{30} are summarised in Table 1.1. These data are drawn from many sources but principally from the Beilstein Chemical Database (Elsevier MDL). The density and melting points in the summary data (Table 1.1) have a degree of uncertainty about them as some compounds, especially the longer chain and odd carbon number moieties, are less well studied. The density data are not available for all compounds.

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Table 1.1 Names and key properties of fatty alcohols from C_4 to C_{30}. The boiling point values quoted are at atmospheric pressure. $^{+}$ @ 20°C. BML = Below Measurement Limits

Systematic name	**Trivial name (*x* alc.)**	**Carbon number**	**d^{20} ($g.cm^{-3}$)**	**Melting point (°C)**	**Boiling point (°C)**	**Water Solubility (@25°C)**
Butanol	Butyl	4	0.810	-90	117	91 $ml.l^{-1}$
Pentanol	Amyl	5	0.815	-79	137.5	27 $g.l^{-1}$
Hexanol	Caproyl	6	0.815@25°C	-51.6	157	5.9 $g.l^{-1}$
Heptanol	Oenantyl	7	0.819@25°C	-34.6	175.8	1.6 $g.l^{-1}$
Octanol	Caprylic	8	0.827	-16	194	0.5 $g.l^{-1}$
Nonanol	Pelorgonyl	9	0.828	-5	215	0.1 $g.l^{-1}$
Decanol	Capryl	10	0.830	6.4	232.9	0.04 $g.l^{-1}$
Undecanol		11	0.832	15	244	0.008 $g.l^{-1+}$
Dodecanol	Lauryl	12	0.831@24°C	24	259	BML
Tridecanol		13	0.915	30	278	BML
Tetradecanol	Myristyl	14	0.824	38	289	BML
Pentadecanol		15	0.893	42		BML
Hexadecanol	Cetyl	16	0.811	49	344	BML
Heptadecanol	Margaryl	17	0.885	53		BML
Octadecanol	Stearyl	18	0.811	59	360	BML
Nonadecanol		19	0.882	62		BML
Eicosanol	Arachidyl	20	0.88	65		BML
Henicosanol		21		68.5		BML
Docosanol	Behenyl	22	0.8	70		BML
Tricosanol		23		72		BML
Tetracosanol	Lignoceryl	24		72		BML
Pentacosanol		25		75		BML
Hexacosanol		26		73		BML
Heptacosanol		27		80		BML
Octacosanol		28		81		BML
Nonacosanol		29		83.5		BML
Tricontanol		30		87		BML

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The short chain compounds (up to C_8) have appreciable water solubility and would not be classified as a "fatty" alcohol as the free compounds are more likely to be in solution than on the solid phase (abiotic or biotic). Compounds with a chain length greater than 10 carbons are essentially insoluble in water and will partition on to the solid phase in the environment.

Partitioning (K_{ow}) and sediment associations

It is usual to measure the water solubility and related factors such as Bioconcentration Factors (BCF) through the octanol – water partition coefficient (K_{ow}) or its $\log_{10}$ ($LogK_{ow}$). There is relatively little information published for *measured* K_{ow} values for fatty alcohols although there are some data from estimated from HPLC retention times (Burkhard *et al.*, 1985). Difficulties arise in the measurement of these coefficients due to the hydrophobic – hydrophilic nature of the different parts of the molecule (Figure 1.5). The hydroxyl group gives that end of the molecule a degree of water solubility while the alkyl carbon chain is hydrophobic. Therefore, these compounds sit at the interface of the octanol and water in the experimental situation.

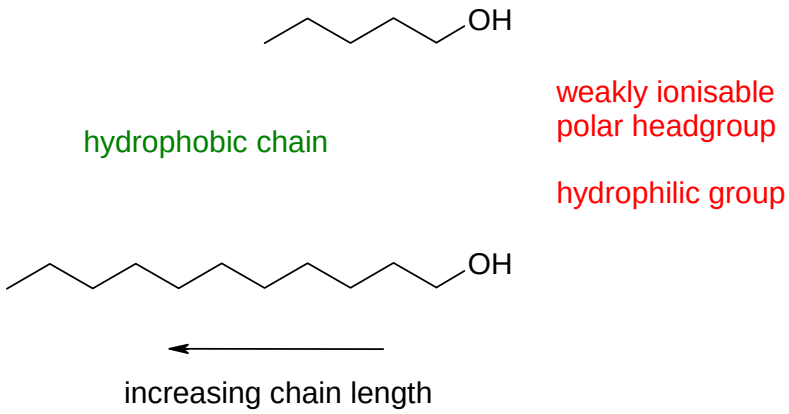

Figure 1.5 The –OH group is weakly ionisable to form $–O^-$ and H^+ and as such will "dissolve" in water. However, with increasing the alkyl chain length, the effect of this is diminished and the compound has lower water solubility. This property does allow the molecule to be used as a detergent, one of the principal anthropogenic functions of fatty alcohols.

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Table 1.2 Octanol – water partition coefficients from [a](Tewari *et al.*, 1982), [b](Burkhard *et al.*, 1985) and [c](Hansch *et al.*, 1968).

Fatty Alcohol	Carbon number	Log K_{ow}
Butanol	4	0.785[a]
		0.84[c]
Pentanol	5	1.53[a]
Hexanol	6	2.03[a]
		1.84[c]
Heptanol	7	2.57[a]
		2.34[c]
Nonanol	9	3.31[b]
		3.77[a]
Dodecanol	12	5.36[b]
Tetradecanol	14	6.03[b]
Hexadecanol	16	6.65[b]
Octadecanol	18	7.19[b]
Eicosanol	20	7.75[b]

The $LogK_{ow}$ values for compounds (Table 1.2) with a chain length greater than C_9 are above 4 (range 3 – 5 depending on view point, Sanderson, *pers comm..)* and are indicative of materials that will be preferentially absorbed to particulate matter. In most environmental situations, this means the compounds will be associated mainly with particle such as settled and suspended sediments. The nature of these particulate materials is that they will settle out to the benthos at some stage and will be transferred to the geosphere. This partitioning between the solution phase for short chain compounds and solid phase for long chain compounds may lead to the separation of mixtures such that short chain moieties will remain in solution while longer chain moieties may settle out of solution. There will also be different degradation steps possible as materials in the solid phase may enter anaerobic environments in sediments; this may lead to preservation of some materials.

The association of fatty alcohols with suspended matter will be of importance in sewage treatment plants as incoming materials may be removed from the system by

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partitioning into the solid phase which subsequently settles out. Experiments using radiolabelled alcohols with activated sewage sludge (van Compernolle *et al.*, in press) measure the time dependent partition coefficients for a range of alcohols typically used in detergent formulations (Table 1.3). The mean values can be seen in Figure 1.6; the data are presented on a log axis and a linear relationship can be seen in this figure. These values are relatively high implying that in such a system, free fatty alcohols will be actively scavenged by the particulate phase and may be removed with the sludge rather than be discharged with the liquid effluent.

Table 1.3 The partition coefficients (K_d) for fatty alcohols with activated sewage sludge suspended in river water. Data from van Compernolle *et al.* (in press).

Time (h)	C_{12}	C_{14}	C_{15}	C_{16}	C_{18}
1	4100±267	14700±645	4070±387	34100±1700	107000±6330
5	3410±119	12700±675	3820±183	33300±1600	90300±3070
16	3320±276	10500±167	3590±104	28600±1720	89900±1980
30	3100±143	10200±670	3480±77	27600±1930	82400±2970
72	3000±78	8490±916	3080±271	23800±3160	78700±5350

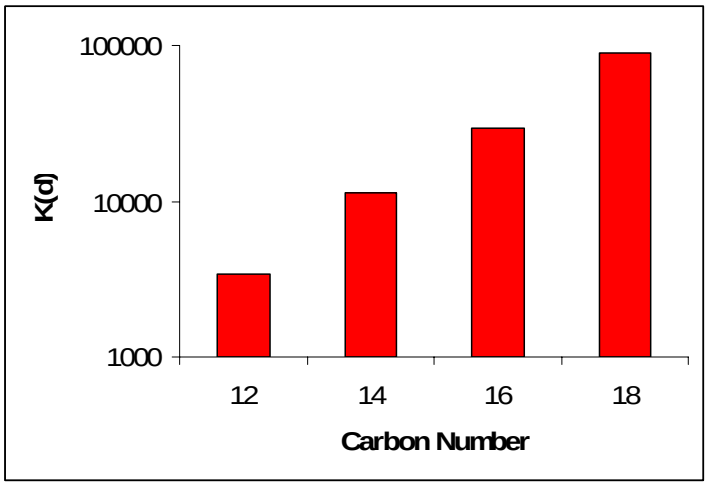

Figure 1.6. The mean K_d for the even carbon numbered fatty alcohols (data from Table 1.3). N.B. The Y-axis is a log scale.

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Chapter 2. Biological Synthesis (The biochemical mechanisms that lead to their formation highlighting the differences between bacteria, that can produce odd chain and branched compounds, with everything else that produces even chain compounds.)

The synthesis of fatty alcohols by living organisms is intimately linked to the production of fatty acids. In order to understand the types of fatty alcohols present in the environment, it is necessary to appreciate the biochemical synthetic pathways that lead to their formation in the first place.

The formation of fatty acids can progress through two major pathways; animals, fungi and some Mycobacteria use the type I synthetic pathway. In this system, the synthesis takes place within a large single protein unit and has a single product in the form of a C_{16} unsaturated fatty acid. This system has genetic coding in one location. In contrast, plants and most bacteria use a series of small discrete proteins to catalyse individual steps within the synthesis; this is termed type II fatty acid synthesis (Rock and Cronan, 1996). These proteins are genetically encoded in several different locations. Yeasts are intermediate between these two extremes where the synthesis activities take place in two separate polypeptides (Lehninger *et al.*, 1993).

Animals

Type I Fatty Acid Synthesis (FAS) occurs in animals. As well as having this initial style of fatty acid synthesis, there are a series of subsequent reactions which lead to the elongation of the primary fatty acid (hexadecanoic acid, C_{16}) to higher carbon numbers and desaturation mechanisms leading to monounsaturated products. However, animals are unable to manufacture some fatty acids and these must be obtained from plants in the diet (*e.g.* ω3 essential fatty acids).

The synthesis of fatty acids in this system occurs on a single large complex comprised of seven polypeptides. This complex acts as the focus for a series of reactions building the fatty acids up from an acetyl – CoA starter and malonyl – CoA subunits. The key components in the system can be seen in Figure 2.1. The complex performs four steps each time two carbons are added to the chain: initially CO_2 is removed from the malonyl – CoA in a condensation reaction joining the two molecules together. NADPH is used in a reduction step converting the C = O group to C – OH. This is dehydrated (removal of H_2O) making a mid-chain double bond that undergoes a final reduction step with more NADPH leading to a saturated alky chain.

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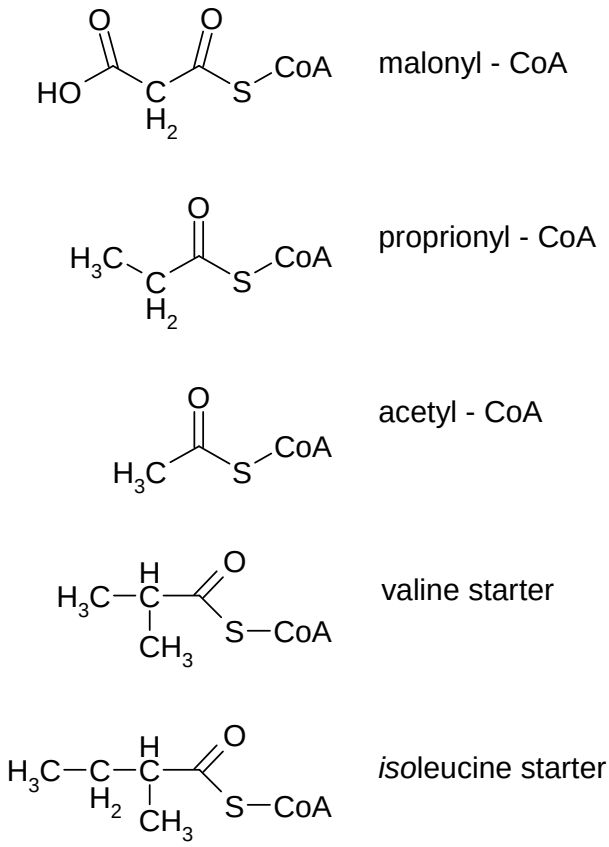

Figure 2.1 Key compounds in fatty acid synthesis. In general, plants and animals principally use acetyl – CoA as the starter while bacteria, plants and animals may sometimes use the others as well.

The net effect of this series of four sub-reactions can be seen in Figure 2.2 as the product of the first step. The process is repeated until a 16 carbon chain has been created. The completed fatty acid is then cleaved from the FAS complex and is available for further reactions. This process explains why the most common fatty acid (and frequently fatty alcohol) found in environmental systems is comprised of 16 carbons. In some cases, an extra cycle occurs and a C_{18} fatty acid is formed instead.

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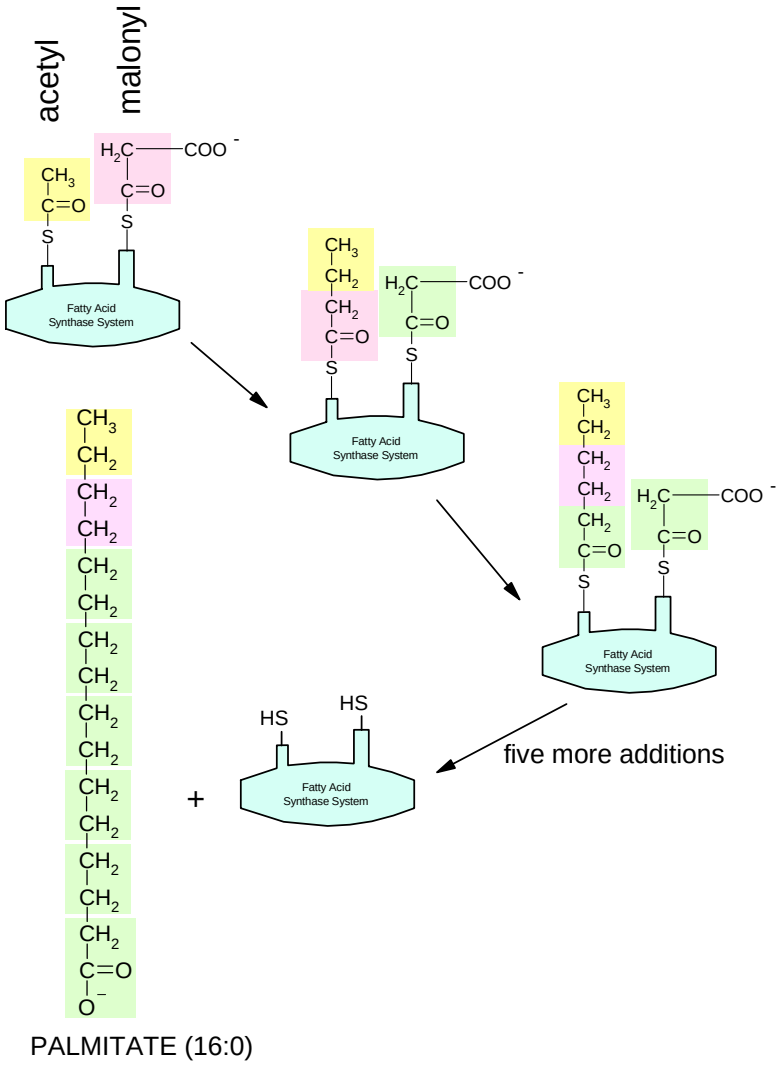

Figure 2.2 The process of palmitate (C_{16} fatty acid) synthesis through sequential addition of C_2 units from malonyl-CoA to an initial acetyl-CoA.

Unsaturated chains

In animals, fatty acyl – CoA desaturase catalyzes the removal of two hydrogen atoms from the bond between C_9 and C_{10} in either palmitic or stearic acid to provide the Δ^9 *cis* double bond in palmitoleic or oleic acid (Figure 2.3).

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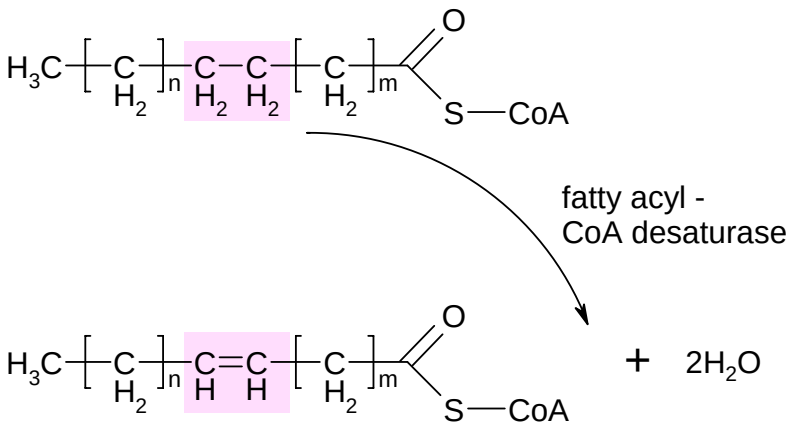

Figure 2.3 Desaturation of the acyl chain. Animals can only desaturate bonds in the Δ9 position and closer to the carboxylic acid group. Plants are able to desaturate bonds closer to the ω end of the molecule.

Plants and Bacteria

Type II FAS in bacteria and plants occurs in a similar fashion to type I but the seven different polypeptides are independent of one another. The reactions are similar to those above but the products then undergo a wider range of elongation and desaturation reactions. In the case of some plants (*e.g.* coconuts and palms), the fatty acid is cleaved before it reaches 16 carbons and up to 90% of the oil from these plants may have fatty acids between C_8 and C_{14} (Lehninger *et al.*, 1993).

Unsaturated Compounds

Unlike most animals, plants can introduce double bonds into fatty acids at locations other than the Δ^9 position; they have enzymes that act on the Δ^{12} and Δ^{15} positions of oleic acid (18:1ω9) but only when it is part of a phospholipid or phosphatidylcholine. This specificity may explain why very few polyunsaturated fatty alcohols are found.

Plants frequently contain fatty acids with three or more double bonds within the molecule. For example, the principal fatty acid within linseed oil is linolenic acid or 18:3ω3, an 18 carbon straight chain molecule with three double bonds, the first of which is in position three from the ω end of the molecule (Δ^{15}). Animals can not generally make these polyunsaturated compounds and must obtain them from their diet. Once in animals, however, they may be elongated to form a range of other biochemically active compounds such as prostaglandins (Lehninger *et al.*, 1993).

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Branched chains

Bacteria make branched chain fatty acids and alcohols (Kaneda, 1967); the orientation of the carbons in the starter complex during the initial stages of FAS determines the final structure. there are three possible orientations which yield either a straight chained odd carbon numbered compound or two branched compounds with the methyl group in the *iso* or *anteiso* position (Figure 1.2 and 2.4).

It is also possible to start the fatty acid synthesis with an amino acid (Kolattukudy *et al.*, 1976). The structure of the most appropriate molecules, valine and *iso*leucine, are shown in Figure 2.1. When valine is used, an *iso*-branched product of the FAS is formed while *iso*leucine yields *anteiso*-branched products. These compounds are the principal fatty acids in Gram-positive bacteria (Kates, 1966). The chain elongation process is the same as other higher organisms (*e.g.* Figure 2.2) but the starter compounds are different. Mid-chain branches are derived from other pathways where typically a methyl sub-unit is added across the double bond of an unsaturated compound such as oleic acid (Kates, 1966).

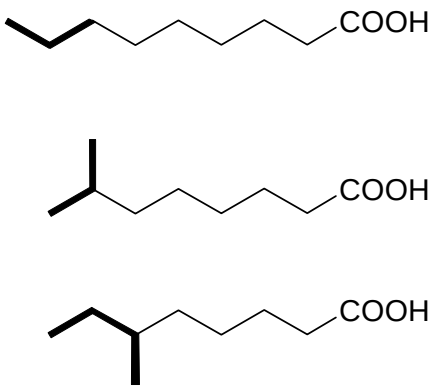

Figure 2.4 Orientation of the precursor leading to the formation of branched odd chain length fatty acids.

Fatty Acid Degradation

The C_{16} fatty acid produced by the type I and type II FAS pathways may undergo chain shortening (Figure 2.5) as well as elongation and desaturation. This is particularly important with regard to the formation of some fatty alcohols (see below) that require an appropriate fatty acid to start with. There are several enzyme systems

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involved in the process belonging to the acyl – CoA dehydrogenase family; those fatty acids with carbon chains between C_{12} and C_{18} use a long chain acyl – CoA dehydrogenase, a medium one operates on C_4 to C_{14} acids while a short chain one acts on C_4 to C_6 only (Berg *et al.*, 2002).

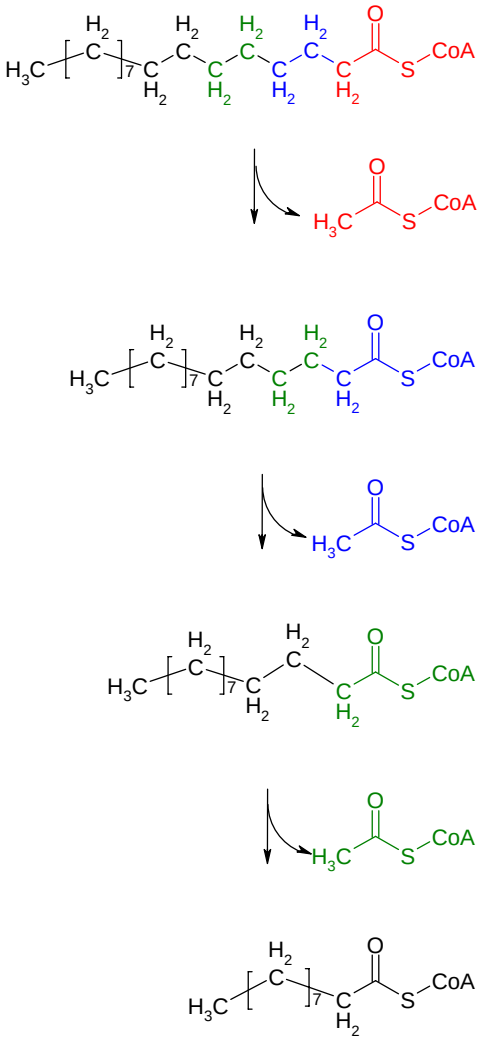

Figure 2.5 The sequence by which fatty acids (as a CoA) are shortened by fatty acyl CoA dehydrogenase. Two carbon subunits are sequentially removed.

Fatty Acyl-CoA Reductase (FAR)

Fatty alcohols have several uses within an organism; they are principally associated with waxes and storage lipids although ether lipids also contain alcohols (Metz *et al.*, 2000). Waxes are abundant neutral lipids that coat the surfaces of plants, insects and

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mammals. They are composed of long chain alcohols linked via an ester bridge to fatty acids and have the chemical property of being solid at room temperature and liquid at higher temperatures. Waxes have several essential biological roles including the preventing water loss, abrasion and infection (Cheng and Russell, 2004).

According to Cheng and Russell (2004) who studied the synthesis of wax in mammals, two catalytic steps are required to produce a wax monoester (Figure 2.6). These include a reduction step of a fatty acid to a fatty alcohol and subsequently the trans-esterification of the fatty alcohol to a fatty acid. The first step is catalyzed by the enzyme fatty acyl-CoA reductase (FAR) which uses the reducing equivalents of NAD(P)H to convert a fatty acyl-CoA into a fatty alcohol and Co-ASH. These enzymes must exist in several organisms as cDNAs specifying fatty acyl-CoA reductases have been identified in the jojoba plant, the silkworm moth, wheat and in a micro-organism (Cheng and Russell, 2004).

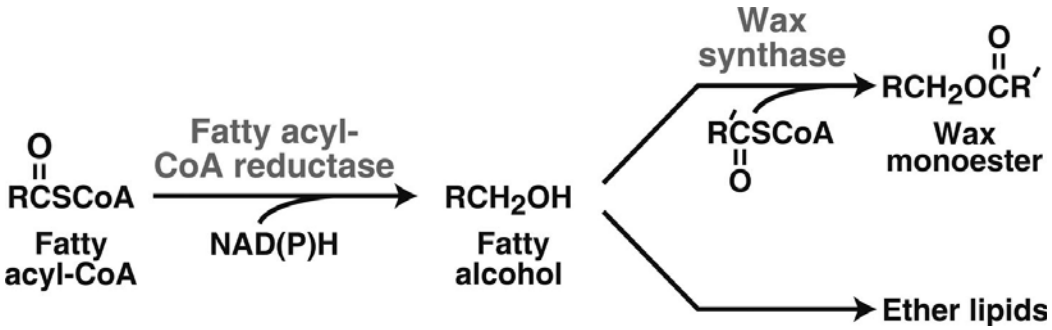

Figure 2.6 From Cheng and Russell (2004), the scheme for the production of wax esters and enzymes used in mammals and other organisms.

Fatty alcohols have two metabolic fates in mammals: incorporation into ether lipids or incorporation into waxes. Ether lipids account for ~20% of phospholipids in the human body and are synthesized in membranes by a pathway involving at least seven enzymes. The second step of this pathway is catalyzed by the enzyme alkyl-dihydroxyacetone phosphate synthase, which exchanges a fatty acid in ester linkage to dihydroxyacetone phosphate with a long chain fatty alcohol to form an alkyl ether intermediate. Once produced, ether lipids are precursors for platelet activating factor, for cannabinoid receptor ligands, and for essential membrane components in cells of the reproductive and nervous systems (Cheng and Russell, 2004).

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Metz *et al.* 2000 has summarised the biochemistry of fatty alcohol synthesis; the process has been examined in diverse organisms and it has been demonstrated that the alcohols are formed by a four-electron reduction of fatty acyl-CoA (Khan and Kolattukudy, 1973; Kolattukudy and Rogers, 1978; Bishop and Hajra, 1981; Wu *et al.*, 1981; Kolattukudy and Rogers, 1986) using a Fatty Acyl-CoA Reductase (FAR) enzyme. Although the alcohol-generating FAR reactions proceed through an aldehyde intermediate (Figure 2.7), a free aldehyde is not released (Kolattukudy, 1970). Thus, the alcohol-forming FARs are distinct from those enzymes that carry out two-electron reductions of fatty acyl-CoA and yield free fatty aldehyde as a product (Wang and Kolattukudy, 1995; Reiser and Somerville, 1997; Vioque and Kolattukudy, 1997). A further distinction is that the alcohol-forming FARs are thought to be integral membrane proteins, whereas those that carry out two-electron reductions are either soluble enzymes or have a peripheral membrane association.

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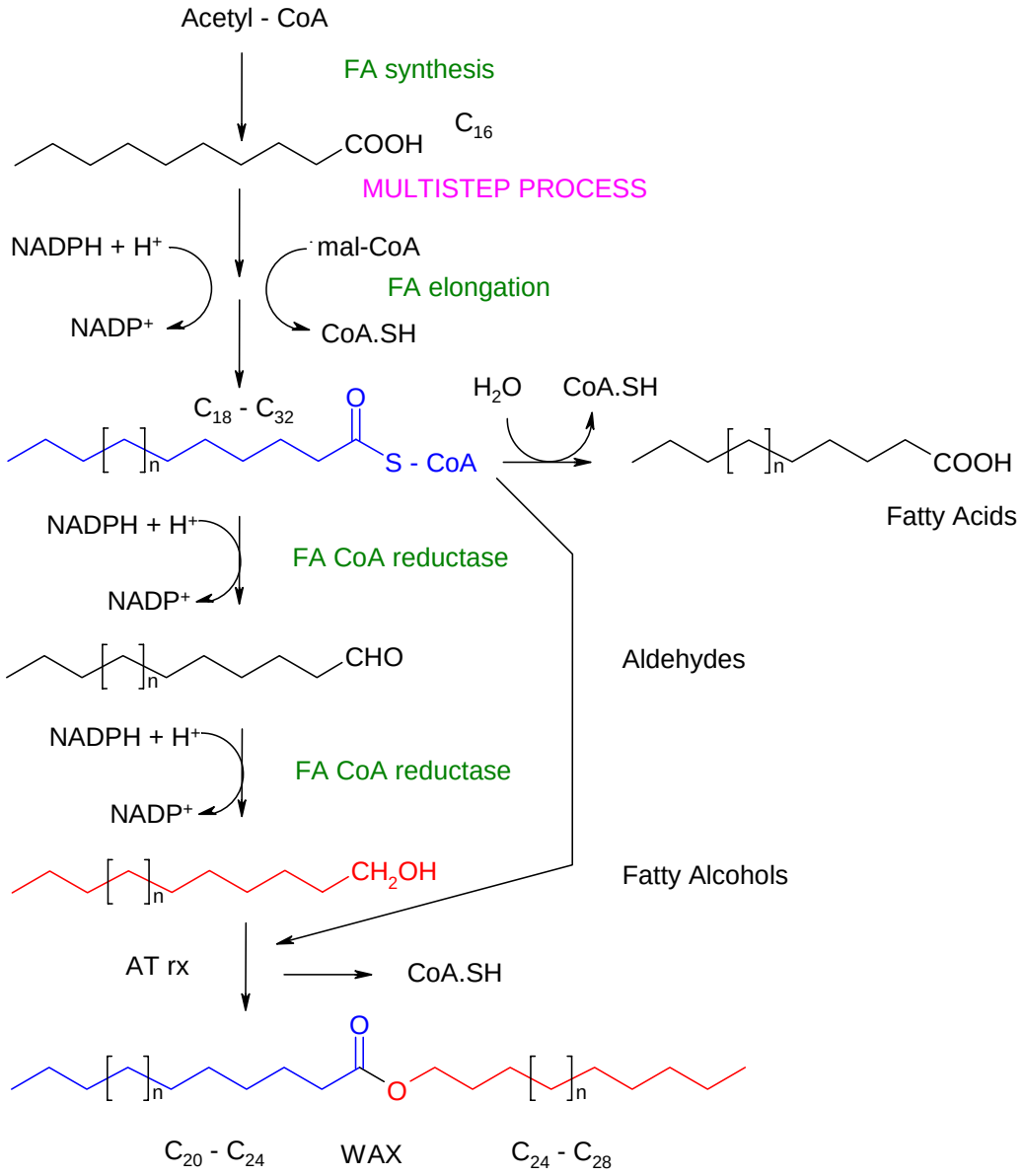

Figure 2.7 Scheme for fatty alcohol and wax production. FAS Type I produces a C_{16} fatty acid that undergoes repetitive C_2 chain elongation. These may be converted to "free" fatty acids by cleavage of the CoA.S group. Alternatively, the two step FAR process converts the carboxylic acid group to (i) an aldehyde and (ii) an alcohol. In many organisms, the acid and alcohol are combined to produce a long chain wax.

The range of fatty alcohols produced by organisms is, therefore, dependent on the fatty acids produced by the organism and the position within the synthesis pathway that the FAR reactions take place. The relative lack of polyunsaturated fatty alcohols indicates that these reactions take place before plants convert the unsaturated long chain acids to polyunsaturated acids (Figure 2.8).

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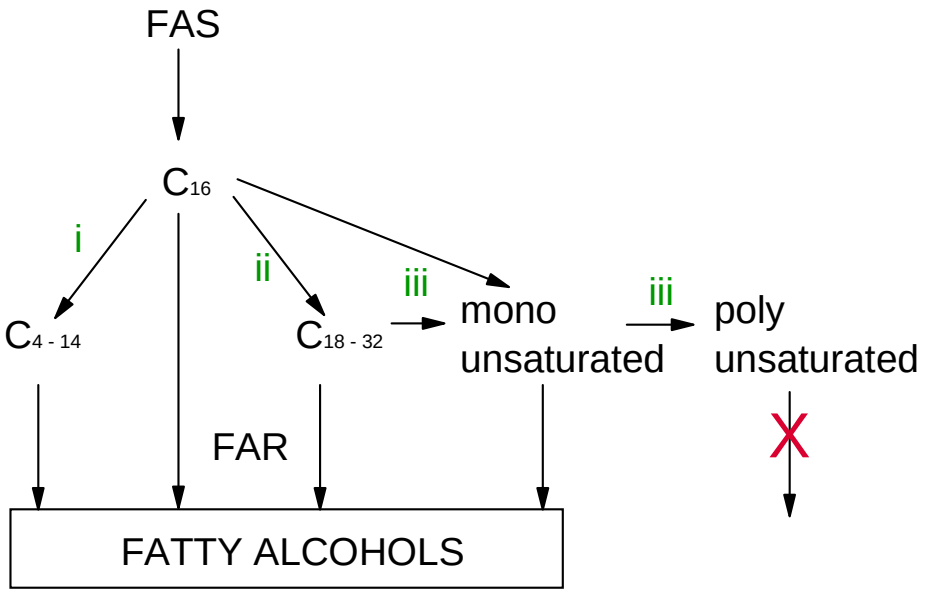

Figure 2.8 Schematic process for the formation of fatty alcohols from fatty acids. Reaction (i) is chain shortening by fatty acyl – CoA dehydrogenase; reaction (ii) is chain elongation by continued malonyl – CoA addition in plants; reaction (iii) is desaturation principally in the Δ^9, Δ^{12} and Δ^{15} positions, the latter two being in plants only.

Synthesis from carbohydrates (Copepods)

There have been several studies of lipids in copepods, a small zooplankton abundant in cool and temperate waters (Sargent *et al.*, 1976; Sargent and Falk-Petersen, 1988; Kattner and Krause, 1989; Kattner and Graeve, 1991; Kattner *et al.*, 2003). In general, copepods were heaviest and rich in lipid shortly after the spring phytoplankton bloom and it has been implied that these organisms are making the fatty acids and alcohols directly from the carbohydrate source rather than *de novo* synthesis from acetyl subunits. The fatty acid and alcohol compositions of two *Calanus* species showed high levels of C_{16} acids and 20:5 acid, which are characteristic for diatoms (Kates and Volcani, 1966; Ackman *et al.*, 1968; Kattner *et al.*, 1983). A comparison of particulate matter in the sea with the data from *Calanus finmarchicus* in spring shows that the copepod fatty acids may originate directly from the particulate material, which consists of diatoms and a substantial amount of detritus (Kattner and Krause, 1989).

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What don't we know?

1. Rate of production of free fatty alcohols from waxes in natural conditions; most alcohols are in the environment as wax esters and little information is available on the rate of conversion to free alcohols. This has a direct relevance to the measured concentration when using a non-saponifying method such as that used for polyethoxylate quantification.

2. Rate of production of free fatty alcohols from fatty acids under natural conditions; fatty acids exist in the environment both esterified as waxes but also linked to other compounds and little is known about the exogenous production of free alcohols from these acids. There may be continued synthesis / degradation of fatty alcohols from fatty acid precursors exogenously in sediments. These rates are not quantified.

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Chapter 3. Occurrence *(How much, where and why)*

Due to the diversity of synthetic pathways outlined in Chapter 2, different organisms will contain or excrete different ranges of fatty alcohols. It is this diversity that will have a key bearing on the ability to use fatty alcohols as biomarkers for different organic matter sources.

Most fatty alcohols occur in biota as waxes, esters with fatty acids (Figure 2.6). These compounds can have relatively high carbon contents and chain lengths up to C_{64} have been observed (Tulloch, 1976). These compounds serve several purposes to the organism that produces them. In the marine environment these can be summarised in the following list (Sargent *et al.*, 1976):

1. Energy reserve
2. Metabolic water reserve
3. Buoyancy generator
4. Biosonar lens in marine mammals
5. Thermal insulator.

In the terrestrial environment, another range of functions can be ascribed to the waxes from plants (Dahl *et al.*, 2005) and insects (Buckner *et al.*, 1996; Nelson *et al.*, 1999).

1. Prevention of dessication
2. Protection from bacterial attack and
3. UV screening.

In birds, waxes are secreted by the uropygial or the preening gland and are used to maintain the condition of the feathers (Jacob, 1976). This may be for waterproofing as well deterring pests.

Bacteria

Bacteria synthesise a range of relatively short chain fatty acids and alcohols. Using FAS Type II, they synthesise compounds with chain lengths normally up to C_{18} (Zhang *et al.*, 2004). However, due to their ability to use proprionyl – CoA and other amino acids as the starter for FAS, these compounds can have odd chain lengths as

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well as even chains (Perry *et al.*, 2002). The orientation of this starter also leads to the formation of *iso* and *anteiso* branched chain compounds as well. Typical compounds are in the range of C_9 to C_{20} (Albro, 1976).

Johns *et al.* (1977) states that "branched chain fatty acids in particular have been considered to reflect a bacterial origin". Both *iso* and *anteiso* branched chain acids are common to many bacteria (O'Leary, 1962; Kates, 1964; Tornabene *et al.*, 1967) and account for up to 60% of the total fatty acids in many *Bacillus* species (Kaneda, 1967). Due to the synthetic pathway for fatty alcohols, the fatty acids should act as a good indicator of the likely fatty alcohols found in bacteria. Unlike plants, in bacteria the fatty acids are part of the cell membrane but the role of fatty alcohols is not known.

The occurrence of wax esters in bacteria from the genus Acinetobacter is principally as an energy storage reserve (Waltermann *et al.*, 2005). In bacteria, waxes are accumulated as cytoplasmic inclusions surrounded by a thin boundary layer similarly to eukaryotes. Recently, a wax ester synthase / acyl-CoA:diacylglycerol acyltransferase (WS/DGAT) was identified from *Acinetobacter calcoaceticus* which catalysed the key steps in the biosynthesis of both storage lipids (Kalscheuer and Steinbuchel, 2003). A large number of WS/DGAT-related proteins were identified in the genome sequences of triacylglycerol (TAG) and wax ester accumulating bacteria like *Mycobacterium tuberculosis*, *M. leprae*, *M. bovis*, *M. smegmatis* and *Streptomyces coelicolor*; it may be assumed that this type of enzyme is responsible for wax ester and TAG biosynthesis in all oleogenous bacteria (Daniel *et al.*, 2004).

It has been suggested that the C_{14}–C_{18} distribution of fatty acids in sediments reflects an input from bacteria (Parkes and Taylor, 1983). Further evidence for bacterial activity exists in the presence of *iso* and *anteiso* C_{15} and C_{17} acids (Leo and Parker, 1966; Boon *et al.*, 1977; Boon *et al.*, 1978; Perry *et al.*, 1979). Work on these fatty acids present in marine sediment (Parkes and Taylor, 1983) can provide a good indicator of the likely fatty alcohol series that might be seen as well; few data are available directly on the fatty alcohols in bacteria. In their experiments, Parkes and Taylor identified several short chain acids, several of which were also unsaturated. The profile of the straight chain and *iso* and *anteiso* branched compounds can be seen in Figure 3.1. The dominant compound is 16:0 in all cases but substantial amounts of

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odd chain and branched compounds are also present. Parkes and Taylor (1983) suggest that the *anteiso* C_{15} may be indicative of sulphate reducing bacteria (SRB).

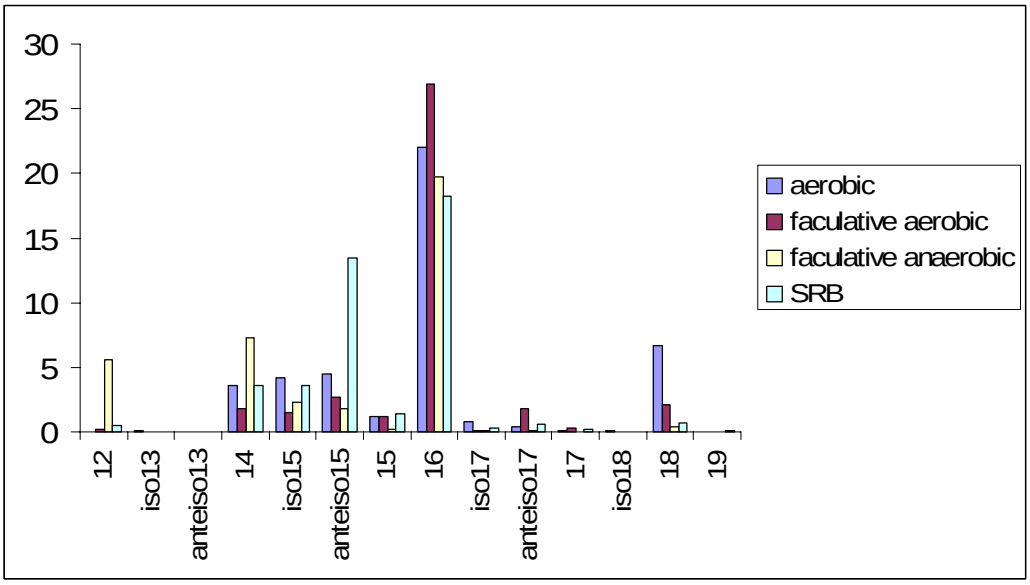

Figure 3.1 The percentage straight chain and *iso* / *anteiso* branched fatty acids in different types of marine bacteria. Data from Parkes and Taylor (1983).

Chlorophyll side chain (phytol)

One of the major fatty alcohols in the environment (*e.g*. Mudge and Norris, 1997; Jeng and Huh, 2004) is the phytol molecule derived from the side chain of chlorophyll (Figure 1.3). Chlorophyll, the major photosynthetic pigment of green plants is comprised of a tetrapyrrole ring structure co-ordinating a magnesium atom. This part of the molecule harvests the photons of incident radiation and passes it along an electron transport system. The phytyl side chain is mainly present to impart a degree of hydrophobicity to reduce the water solubility and immobilise the chlorophyll within the cells. The synthesis of the phytyl side chain is from an isoprenoid system using mevalonic-acid and does not rely on a fatty acid precursor (Chikaraishi *et al.*, 2005).

Analysis of environmental samples by saponification (see Chapter 5) will release the phytol from chlorophyll into the solvent. Therefore, the phytol may be a good indicator of the chlorophyll in the water column. This may originate in both the

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terrestrial environment from green plants or in the marine environment from phytoplankton. However, stable isotope analysis of estuarine sediment samples appears to show that the phytol in that location was entirely derived from the marine environment and not terrestrial plants (Tolosa, *unpublished data*). Therefore, if there is little transfer from the land to the sea, the phytol may be used as an indicator of primary productivity.

Free phytol can also be generated in the water column by hydrolysis of chlorophyll (Baker and Louda, 1983), during the digestive processes of copepods feeding on phytoplankton (Shuman and Lorenzen, 1975) and by senescence of diatoms (Jeffrey, 1974).

Marine Plants

Marine plants do not have substantial wax concentrations; there are few reports of long-chain alcohols in microalgae and it appears that microalgae are not a major source of these lipids in most sediments (Volkman *et al.*, 1998).

Some marine plants do have some fatty alcohols and these are reviewed by Volkman *et al.* (1998). For example, a 18:1 fatty alcohol has been found in the diatom *Skeletonema costatum* (Berge *et al.*, 1995). A series of C_{22}–C_{28} saturated n-alcohols, with even carbon numbers predominating, and a maximum at C_{26} and C_{28}, has been identified in the heterocyst glycolipids of the Cyanobacterium, *Anabaena cylindrical* (Abreu-Grobois *et al.*, 1977). The green alga, *Chlorella kessleri,* contains C_{10}–C_{20} saturated and mono-unsaturated fatty alcohols, with 16:0 most abundant, all esterified to long-chain fatty acids (Rezanka and Podojil, 1986; Rezanka *et al.*, 1986). C_{30}–C_{32} alcohols having one or two double bonds are significant constituents of the lipids of marine Eustigmatophytes of the genus Nannochloropsis (Volkman *et al.*, 1992). The freshwater Eustigmatophytes, *Vischeria punctata* contains saturated and monounsaturated n-alkanols from C_{16} to C_{28} showing a strong predominance of 22:0 and 26:1, respectively. He reports that in the alcohols data there is not a steady decline in abundances with increasing chain length, but rather a strong predominance of just a few homologues (Volkman *et al.*, 1998).

There are no reports of series of n-alkanols being found in marine macroalgae. The only report of interest is of a polyunsaturated compound found in the red alga

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Gracilaria foliifera which would not be identified in traditional environmental analyses (Hayeememon *et al.*, 1991).

Terrestrial Plant Waxes

The main function of plant waxes is to reduce water loss through evaporation. Therefore, the chain length of the waxes for this source tends to be longer than for marine animals. Typical profiles of fatty alcohols for selected plants can be seen in Figure 3.2 and 3.3.

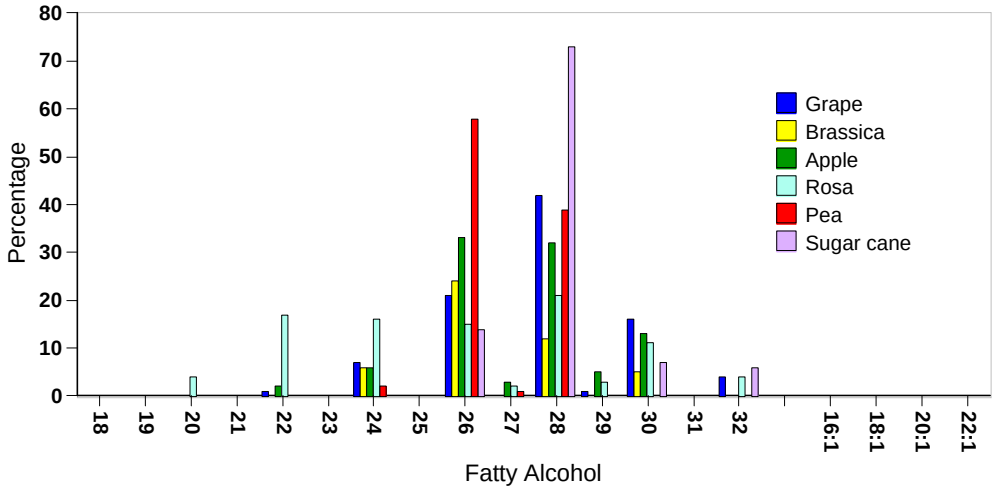

Figure 3.2 Fatty alcohols from terrestrial plants. The major fatty alcohols occur either at C_{26} or C_{28} in most cases. After (Tulloch, 1976).

As can be seen in these figures, short chain alcohols ($<C_{20}$) are not present and the most prominent chain lengths are C_{26} to C_{30}. In the example from the African grasslands (Ali *et al.*, 2005), compounds up to C_{34} are relatively common. In their study, odd chain alcohols were present but at very low concentrations and were not reported. There is also an even over odd dominance indicative of the C_2 (alkyl) chain elongation process. Few unsaturated fatty alcohols have been reported in plants unlike marine animals.

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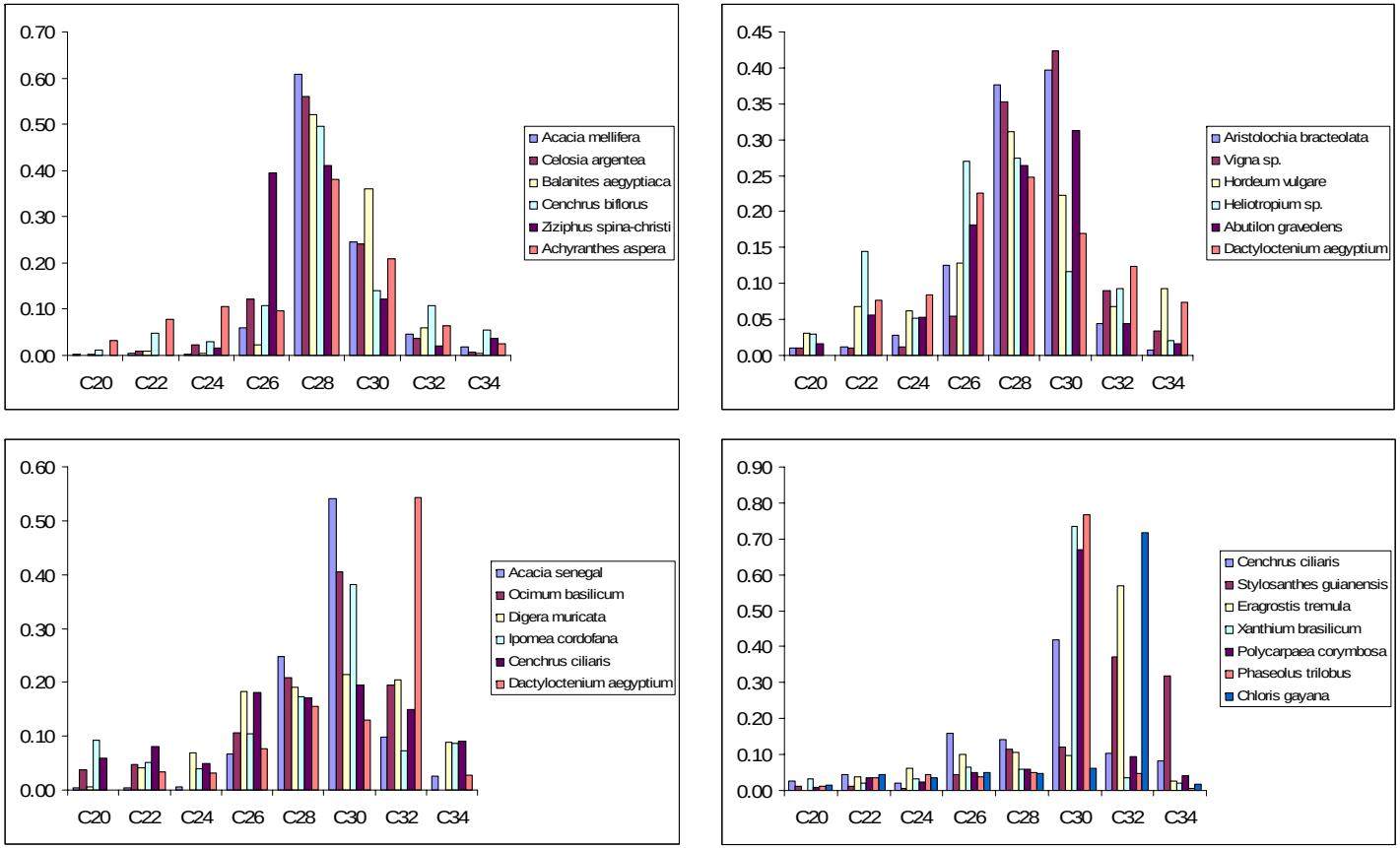

Figure 3.3 Fatty alcohol profiles (proportion data) for a range of terrestrial plants from African Grassland (Ali *et al.*, 2005). Note the differing scales on the Y-axis.

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Mosses and other peat forming plants

Some terrestrial plants live in wet areas and when they die their remains degrade *in situ* and may form peat. The typical plants of these environments are the mosses and a study by Nott (2000) investigated the fatty alcohol and other biomarker composition as part of a study of larger study of Bolton Fell Moss (Xie *et al.*, 2004 and also see Chapter 6). The profile of fatty alcohols in several moss species and other peat forming plants can be seen in Figures 3.4 to 3.6. The *Sphagnum* species (Figure 3.4) have very similar profiles although the other plants are less similar. The major alcohols are in the C_{24} – C_{32} range with very few odd chain compounds.

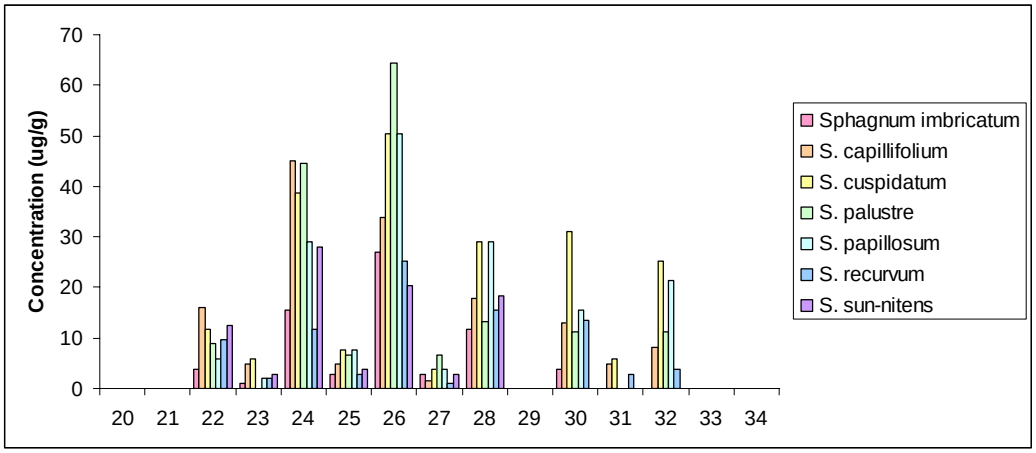

Figure 3.4 The fatty alcohol profile of mosses from the genus *Sphagnum*. Samples were collected from Bolton Fell Moss. Data after Nott (2000).

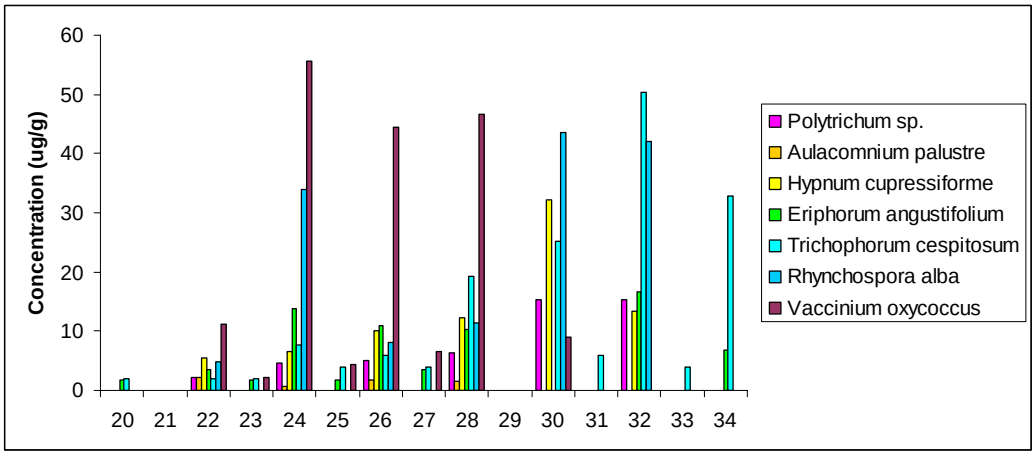

Figure 3.5 The fatty alcohol profile of peat forming plant species. Samples were collected from Bolton Fell Moss. Data after Nott (2000).

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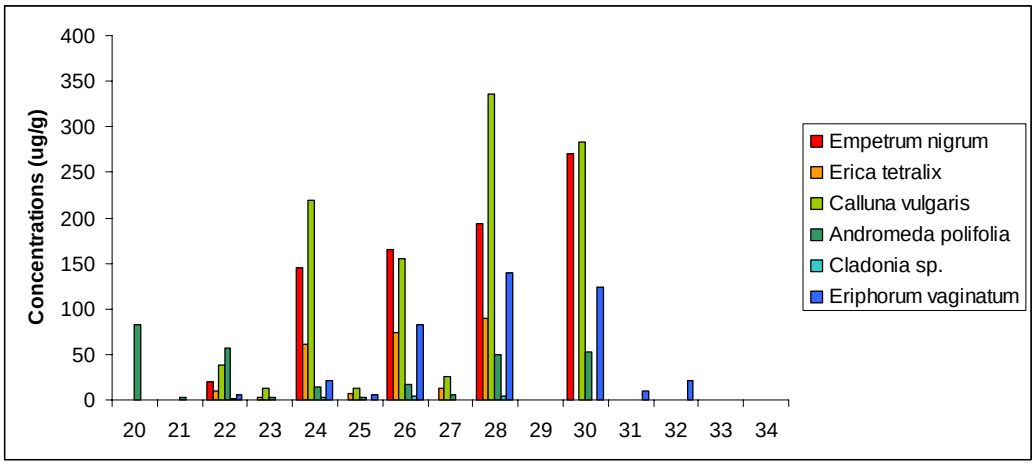

Figure 3.6 The fatty alcohol profile of peat forming plant species. Samples were collected from Bolton Fell Moss. Data after Nott (2000).

Marine animals

In contrast to terrestrial plants, marine animals principally use fatty alcohols as a storage product (Sargent *et al.*, 1976). Examples from several different organisms are shown in Figure 3.7. No saturated fatty acids with chain lengths greater than C_{19} were observed although a substantial amount of unsaturated compounds were present. As with terrestrial plants, few odd chain length compounds were present in the samples.

Work by Hamm and Rousseau (2003) on the foam found after a *Phaeocystis* bloom, showed it consisted of particulate and dissolved matter that did not contain the typical pattern of *Phaeocystis*-derived fatty acids. They found a high abundance of fatty alcohols, which are indicators of wax esters and thus zooplankton (Volkman *et al.*, 1980), in the foam; they thought this was surprising since apart from a few copepod eggs no zooplankton was found in the foam. Hamm and Rousseau (2003) suggested that the fatty alcohols may have originated from dissolved zooplanktonic wax esters, a phenomenon which has been observed earlier by Volkman *et al.* (1980) within a "milky water" event in the North Sea. Wax esters are thought to occur in high concentrations in over-wintering copepods (as a food reserve, Sargent *et al.*, (1976)), but less so in vernal zooplankton (Sargent and Falk-Petersen, 1988). However, Hamm and Rousseau (2003) demonstrated that high percentages (20-92%) of wax esters are found in the lipids of *Calanus finmarchicus* in all stages of its development.

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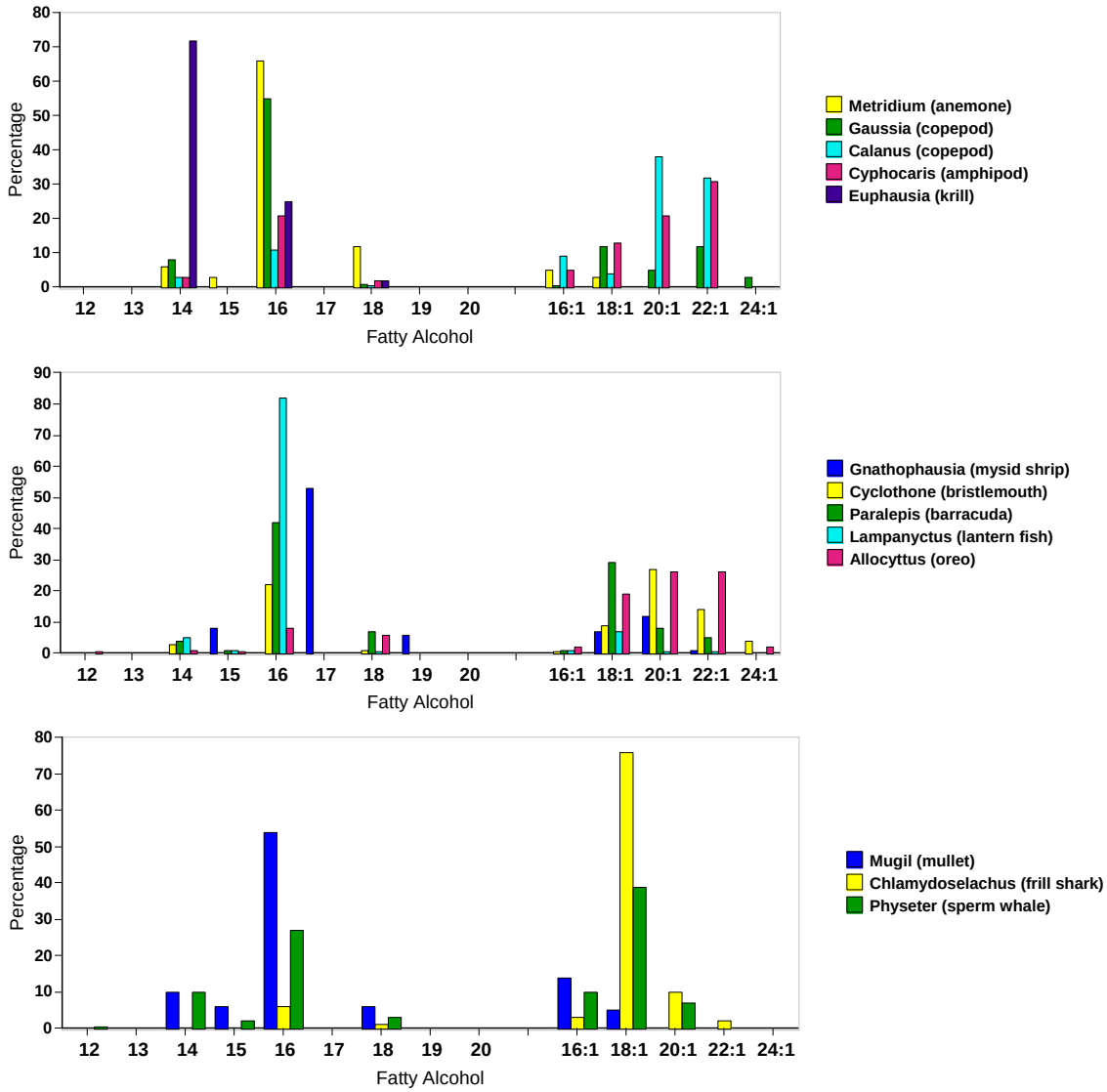

Figure 3.7 Fatty alcohol profiles from several types of marine animal. The *n*-alcohols are principally short chained with a maximum carbon chain length of 16.

Kattner and Krause (1989) also found a seasonal variation between samples of *Pseudocalanus elongatus*; those collected in spring had a relatively high percentage of short chain saturated alcohols (C_{14}+C_{16} = 87%) but this was reduced in summer (60%) and winter (30%). There was a corresponding increase in the percentage of C_{20} and C_{22} mono-unsaturated compounds (6% - 26% - 69%) through the seasons as the copepods stored the carbon as waxes. This led Hamm and Rousseau (2003) to speculate that the occurrence of the dissolved fatty alcohols in the post-*Phaeocystis* bloom indicated the mortality of a copepod population.

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Insects

The cuticular surfaces of insects are also covered by a lipid layer whose primary function is to restrict water movement across the cuticle preventing desiccation of the insect (Buckner *et al.*, 1996). The major components in the cuticular extracts of insects include hydrocarbons, wax esters, aldehydes, ketones, alcohols and acids. The quantities and composition of free cuticular lipid can vary widely among insect groups and sometimes within the developmental stages of the same species. In a study of two lepidopteran species, Buckner *et al.* (1996) found similar fatty alcohol compounds present in each although in one C_{26} had the maximum occurrence while it was C_{30} in the other (Figure 3.8). In both cases, the composition is similar to that of terrestrial plants.

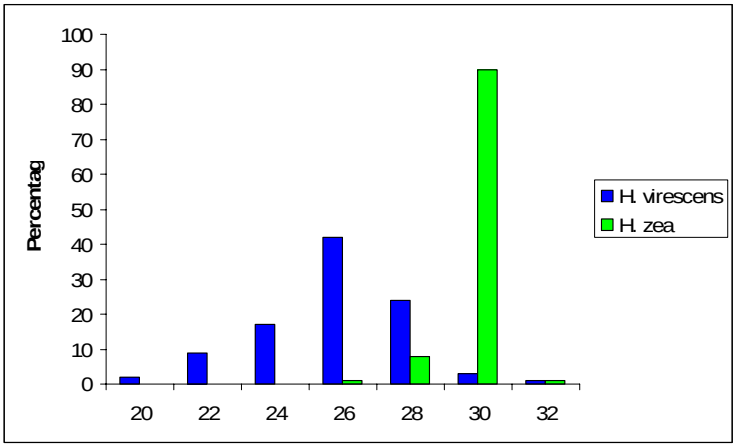

Figure 3.8 Fatty alcohol composition in the wax esters from two lepidopteran species, the tobacco budworm, *Heliothis virescens* and the corn earworm, *Helicoverpa zea*. Data after Buckner *et al.* (1996).

In general, homopteran insects produce large amounts of wax (reviewed by Nelson *et al.* (1999) . This wax is in the form of filaments or particles in many whitefly species. These particles have been identified as not being composed of wax esters but of a mixture of a long-chain aldehyde and a long-chain alcohol; for example, the greenhouse whitefly, *Trialeurodes vaporariorum* has dotriacontanal (C_{32} aldehyde) and dotriacontanol (C_{32} alcohol). In the sweetpotato whitefly, *Bemisia tabaci*, the waxes are composed of the C_{34} equivalents tetratriacontanal and tetratriacontanol. The external wax of whitefly nymphs may play a role in the parasitization or predation of nymphs which are often preferred prey over adult whiteflies. In a study of the external

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lipids of the giant whitefly, *Aleurodicus dugesii*, Nelson *et al.* (1999) found a range of alcohols present within the adult wax esters (Figure 3.9). In this figure, the peak chain lengths are similar to those found in terrestrial plants ($C_{20} - C_{30}$).

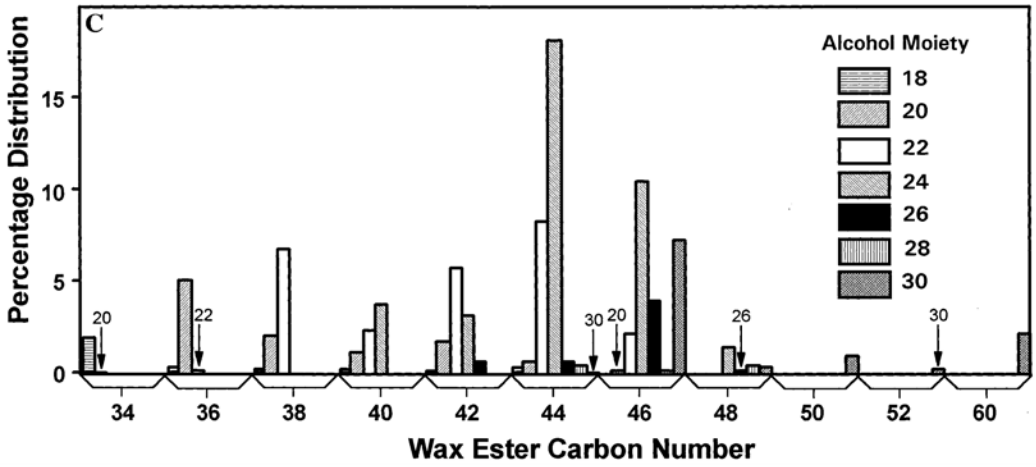

Figure 3.9 The fatty alcohol composition of wax esters in the giant whitefly, *Aleurodicus dugesii*, after Nelson *et al.* (1999).

Birds

Birds use waxes to maintain waterproofing of their feathers and resist microbial / fungal infestation. The preen gland (uropygial gland) is located under the tail feathers and secretes a simple wax derived from fatty acids and fatty alcohols. The typical profiles for the alcohols in these waxes can be seen in Figure 3.10. Unusually, many of the fatty alcohols from this source tend to have methyl branches in the 2, 4, 6 or 8 position (Jacob, 1976).

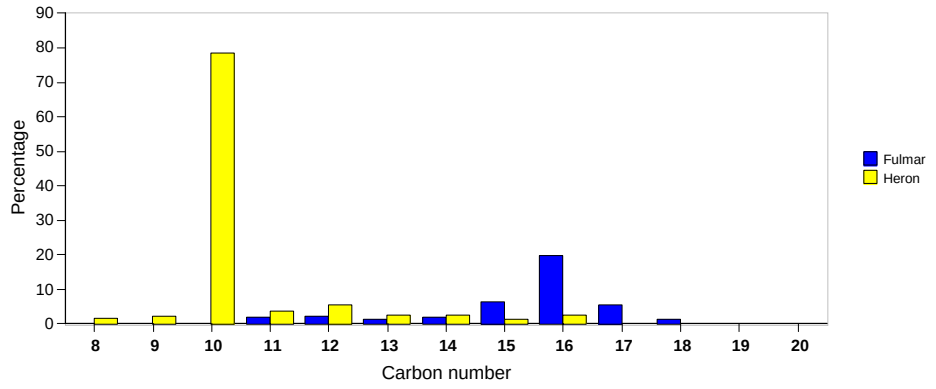

Figure 3.10 Fatty alcohols from the preen gland of selected birds. After Jacob (1976).

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Detergents

Fatty alcohols are widely used in the manufacture of detergents; there are several types with (poly)ethoxylate or sulphate adjuncts imbuing the alcohol with increased water solubility. The most frequently used class of detergents with alcohol as the non-polar component are the alcohol ethoxylates (AE); examples of typical structures are shown in Figure 3.11 and mixtures used in formulations are shown in Table 3.1.

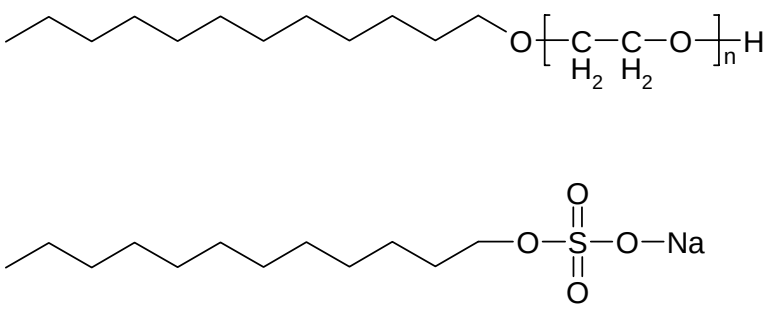

Figure 3.11 Typical structures of some alcohol based detergents; alcohol polyethoxylates where n = 0 – 20 and alcohol sulphate *e.g.* Sodium Dodecyl Sulphate (SDS).

Table 3.1 The CAS (Chemical Abstracts Service) registry number for several blends of fatty alcohols used in detergent formulations with the principal chemical species present in each.

CAS	Chemical Name	Composition
111-27-3	1-Hexanol	100% Linear; >95% C_6 [range C_6-C_{10}]; Even
111-87-5	Octyl alcohol	100% Linear; >90% C_8 [range C_6-C_{10}]; Even
85566-12-7	C_{8-10} Alcohols	100% Linear; > 80% $C_{8/10}$, C_6<=5%, C_{12}<10% [range C_6-C_{12}]; Even
112-30-1	1-Decanol	100% Linear; >90% C_{10} [range C_8-C_{12}]; Even
68603-15-6	C_{6-12} Alcohols	**Generic** 5-100% Linear; C_{6-12} alcohols [range C_{6-13}]; Even or Even & odd
		Type A. 5-95% Linear; >= 95% C_{11} [range C_9-C_{13}]; Even & odd
		Type B. >80% Linear; > 95% $C_{9/10/11}$ [range C_8-C_{12}]; Even & odd
		Type C. >80% Linear; > 95% $C_{7/8/9}$ [range C_6-C_{10}]; Even & odd
		Type D. 100% Linear; >=90% $C_{8/10}$; <10% C_6 [range C_6-C_{12}]; Even
66455-17-2	C_{9-11} Alcohols	>80% Linear; > 95% $C_{9/10/11}$ [range C_{8-12}]; Even & odd
85665-26-5	Alcohols, C_{10-12}	100% Linear; >90% $C_{10/12}$, <=5% C_{14} [range C_8-C_{16}]; Even

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112-42-5	Undecyl alcohol	>80% Linear; >95% C_{11} [C_9-C_{14}]; Even & odd
67762-25-8	C_{12-18} Alcohols	**Generic** 100% Linear; >95% $C_{12/14/16/18}$ [rangeC_8-C_{20}]; even
		Type A. 100% Linear; >50% $C_{12/14}$; >10% $C_{16/18}$ [range C_8-C_{20}]; even
		Type B. 100% Linear; >10% $C_{12/14}$; >60% $C_{16/18}$ [range C_{12}-C_{20}]; even
67762-41-8	C_{10-16} Alcohols	**Generic** 5-100% Linear; C_{10-16} alcohols [range C_{8-18}]; Even or Even & odd
		Type A. 100% Linear; >80% $C_{10/12/14}$, <10% C_{16} [range C_8-C_{18}]; Even
		Type B. 5-50% Linear; >=95% C12/13 [range C11-C14]; Even & odd
		Type C. 80-95% Linear; >95% $C_{12/13}$ [range C_{11-15}]; Even & odd
		Type D. 40-50% Linear; >95% $C_{12/13/14/15}$ [range C_{11}-C_{16}]; Even & odd
68855-56-1	C_{12-16} Alcohols	**Generic** 40-100% Linear; C_{12-16} alcohols, >95% $C_{12/13/14/15}$ [range C_8-C_{18}]; Even or Even & odd
		Type A. >40% Linear; >95% $C_{12/13/14/15}$ [range C_{10}-C_{17}]; Even & odd
		Type B. 100% Linear; >80% $C_{12/14}$, <20% C_{16} [range C_8-C_{18}]; Even
		Type C. 100% Linear; <10% C_{12}, >90% $C_{14/16}$ [range C_{10}-C_{18}]; Even
75782-86-4	C_{12-13} Alcohols	>80% Linear; >95% $C_{12/13}$ [range C_{11}-C_{15}]; Even & odd
75782-87-5	C_{14-15} Alcohols	>80% Linear; >95% $C_{14/15}$ [range C_{12-17}]; Even & odd
80206-82-2	C_{12-14} Alcohols	**Generic** 100% Linear; >95% $C_{12/14/16}$ [rangeC_6-C_{18}]; Even
		Type A. 100% Linear; >90% $C_{12/14}$ (C_{12}>C_{14}), <10% C_{16} [range C_6-C_{18}]; Even
		Type B. 100% Linear; >95% $C_{12/14}$ (C_{12}<C_{14}) [range C_8-C_{18}]; Even
63393-82-8	C_{12-15} Alcohols	**Generic** >40% Linear; >95% $C_{12/13/14/15}$ range [range C_{10}-C_{17}]; Even & odd
		Type A. >80% Linear; >95% $C_{12/13/14/15}$ range C_{10}-C_{17}]; Even & odd
		Type B. 40-50% Linear; >95% $C_{12/13/14/15}$ [range C_{11}-C_{16}]; Even & odd
112-72-1	1-Tetradecanol	100% Linear; >95% C_{14} [range C_{12}-C_{16}]; Even
68333-80-2	C_{14-16} Alcohols	**Generic** 5-95% Linear; >95% $C_{12/13/14/15}$ [range C_{11-16}]; Even & odd
		Type A. 5-95% Linear; >95% $C_{14/15}$ [range C_{12-17}]; Even & odd
		Type B. <=5% Linear; >95% $C_{12/13/14/15}$ [range C_{11}-C_{16}]; Even & odd
36653-82-4	1-Hexadecanol	100% Linear; >=95% C_{16} [range C_{14}-C_{18}]; Even
67762-27-0	C_{16-18} Alcohols	100% linear (or unstated); <10% C_{14}, >=90% $C_{16/18}$

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		[range C_{12}-C_{20}]; Even
67762-30-5	C_{14-18} Alcohols	**Generic** 100% Linear (or unstated); >95% $C_{14/16/18}$ [rangeC_{10}-C_{20}]; Even
		Type A. 100% Linear (or unstated); >=95% $C_{16/18}$ [rangeC_{12}-C_{20}]; Even
		Type B. 100% Linear (or unstated); >95% $C_{14/16/18}$ [rangeC_{10}-C_{20}]; Even
629-96-9	1-Eicosanol	>80% Linear; >=90% C_{20} [range C_{18-22}]; Even
97552-91-5	C_{18-22} Alcohol	100% Linear; >95% $C_{18/20/24}$ [range C_{16}-C_{24}]; Even
661-19-8	1-Docosanol	100% Linear; >95% C_{22}; Even
68002-94-8	C_{16-18} and C_{18} Unsaturated	100% linear; >70% $C_{16/18}$, <10% C_{14}, including 40-90% C_{18} unsaturated [range C_{12}-C_{22}]; Even
68155-00-0	Alcohols, C_{14-18} and C_{16-18}-unsatd.	Linearity unspecified; 5-50% $C_{16/18}$ saturated, 40-90% $C_{16/18}$ unsaturated [range C_{14}-C_{18}]; Even
112-70-9	1-Tridecanol	>80% Linear; >90% C_{13}, <10% C_{12} [range C_{12-14}]; Even & odd
143-28-2	9-Octadecen-1-ol, (9Z)-	100% linear; >70% $C_{16/18}$, <10% C_{14}, including >70% C18 unsaturated [range C_{12}-C_{20}]; Even
629-76-5	1-Pentadecanol	>80% Linear; >90% C_{15}, <10% C_{14} [range C_{14-15}]; Even & odd
68551-07-5	C_{8-18} Alcohols	100% Linear; 5-30% $C_{8/10}$, >60% $C_{12/14/16/18}$ [range C_8-C_{20}]; Even
90583-91-8	Tridecanol, branched and linear	5% Linear; >95% C_{13}; Odd

There are two factors determining behaviour and products in the environment from the AE; chain length of the parent alcohol and number of ethoxylates attached to the alcohol. The principal chain lengths used in detergent manufacture are C_{12} – C_{16} and C_{18} straight chain, *n*-alkanols (Modler, 2004). The length of the alcohol reflects the synthetic pathway and solubility needs of the product. No data for commercial samples are readily available but some data from the analysis of Sewage Treatment Plant (STP) effluents can be used to give an indication of the likely profile. Data from western Europe, Canada and USA are shown in Figure 3.12 (Belanger and Dorn, 2004).

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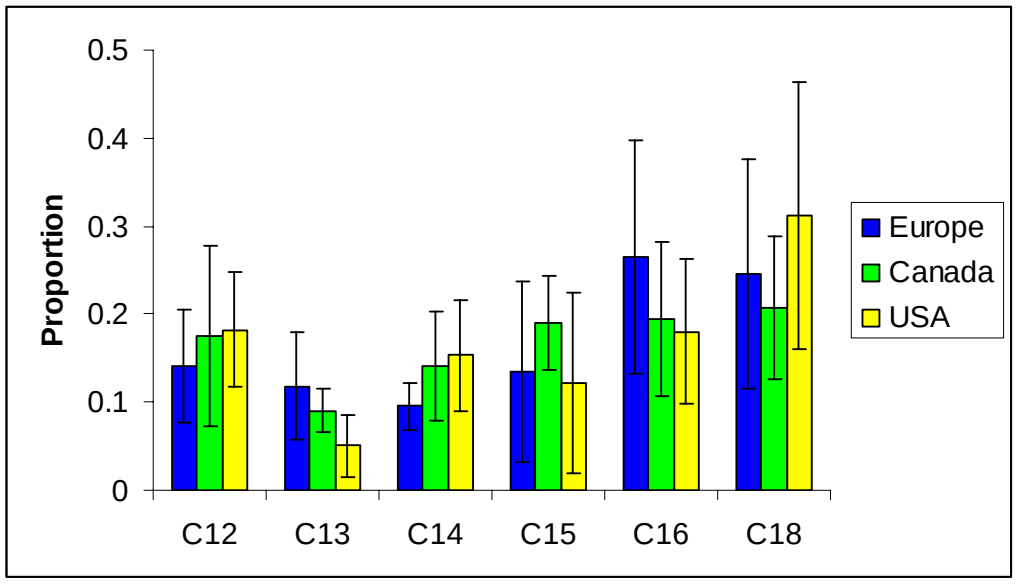

Figure 3.12 The mean free fatty alcohol chain length in STP materials (activated sludge, trickling bed filters or oxidation ditches) from Europe, Canada and USA. The error bars are 1 standard deviation. The concentration range was 0.32 – 11.2 $\mu g.L^{-1}$ in European samples; 0.29 – 14.2 $\mu g.L^{-1}$ for Canadian samples and 0.13 – 2669 $\mu g.L^{-1}$ for USA samples.

These data shown in Figure 3.12 include natural fatty alcohols derived from a range of sources within the sewage treatment system as well as anthropogenically derived detergent alcohols (Eadsforth *et al.*, in press; Morrall *et al.*, in press).

The detergent formulation uses a series of ethoxylates up to ~20 and some representative data using two typical formulations can be seen in Figure 3.13a. Influent material to a STP will contain AEs of this general formulation. This can be compared to measurements made in STP effluent from samples in different geographic regions (Figure 3.13b). The n = 0 sample is the free fatty alcohol and is substantially higher than the ethoxylates as it contains alcohols derived from non-detergent sources as well. The distinctive EO chain pattern of the commercial material has also been lost.

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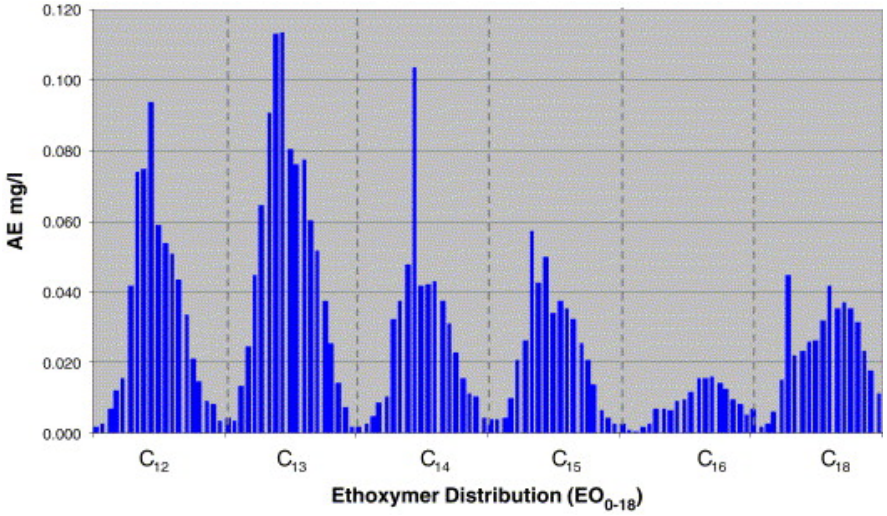

Figure 3.13a The ethoxylate chain length in a mixture of commercial detergent formulations; this is considered typical of influent material to a STP. Figure from Wind *et al.* (in press).

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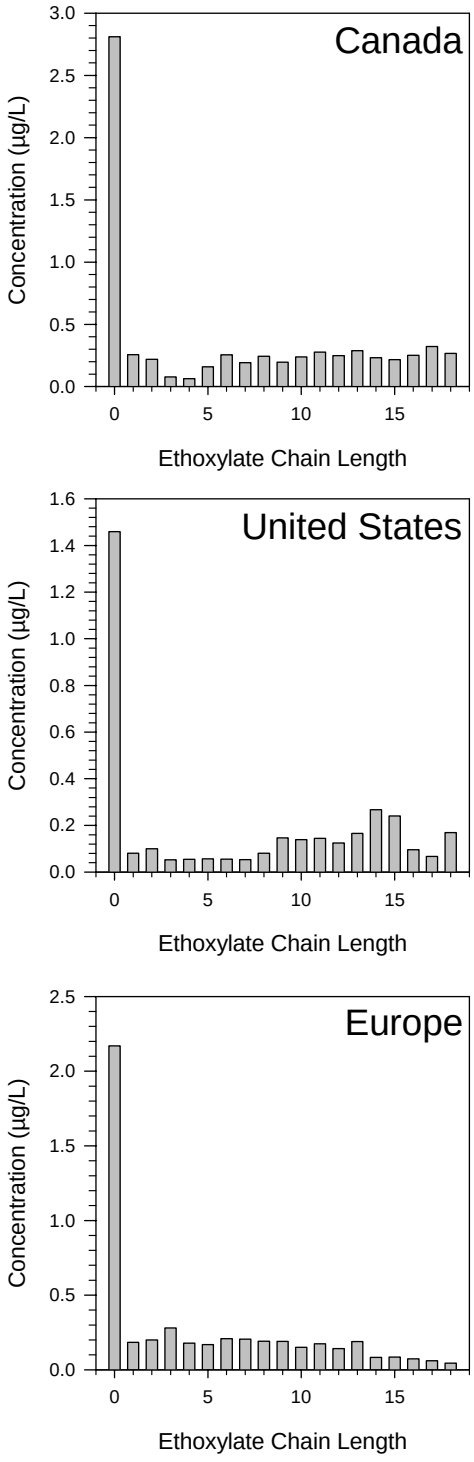

Figure 3.13b. Distribution of ethoxylate chains by region in STP effluent. Alkyl chain lengths from $C_{12\text{-}18}$ were summed per ethoxylate. Data from Eadsforth *et al.* (in press); Morrall *et al.* (in press).

The production of alcohol ethoxylates is significant with close to 1 million tonnes produced annually worldwide (Modler, 2004). The usage and production is centred in

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three regions; Japan, western Europe and North America. The production in each, where available, is summarised below.

Japan

The usage of fatty alcohols in detergents in Japan increased from 1979 to reach a peak in 1992 (Figure 3.14); since then, the consumption has stabilised at ~120 000 tonnes per year with approximately half of that quantity being imported. Their own production has declined since a peak in 1992 but has levelled off in recent years. In Japan, there has been a shift away from fatty alcohol production derived from natural (vegetable) oils (Figure 3.15) to those developed from petrochemical industries. A rapid decline in production began in 1992 but this has stabilised into a slower decline from the mid-1990's. The natural sources now include Malaysia and the Philippines with oils derived from palms and coconuts. From a tracking viewpoint, the change in feedstock will result in different isotopic signatures although with imports accounting for half of their consumption, these may be blurred.

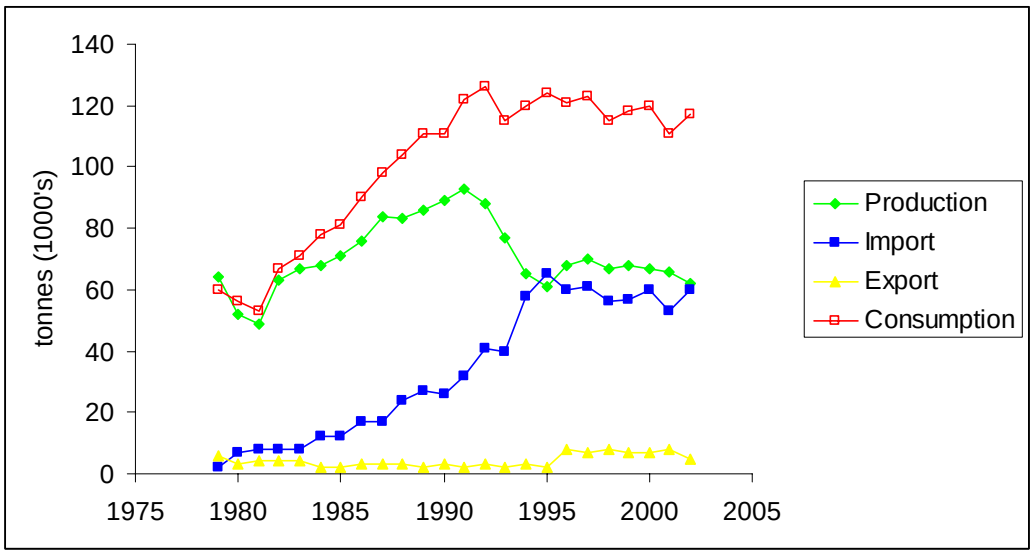

Figure 3.14 Production and usage of fatty alcohols as detergents in Japan. Data from Modler (2004).

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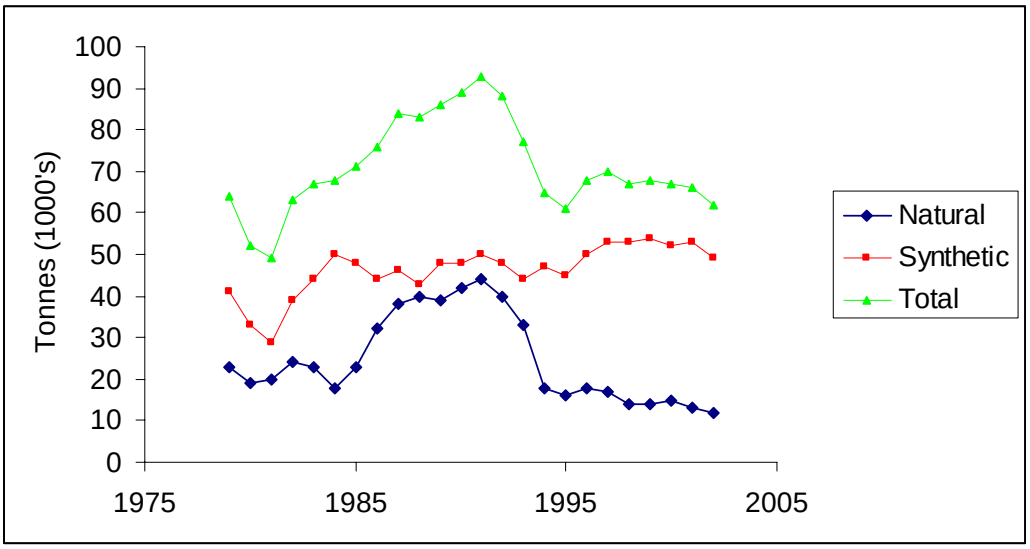

Figure 3.15 Production of fatty alcohols with Japan from natural and synthetic sources. Data from Modler (2004).

The fate of these alcohols is principally to alcohol ethoxylate detergents (Table 3.2) although sulphates have been more important in the past. This usage reflects the current trend of reducing the phosphate content in detergents and also the promotion of "green detergents" based on their perceived synthesis from natural materials rather than man-made precursors; it has also been suggested that these green compounds will degrade faster in the environment than anthropogenic compounds (Modler, 2004).

Table 3.2 The usage of fatty alcohols in thousand of tonnes in detergents in Japan with a forecast of the likely 2007 numbers. Data from Modler (2004).

JAPAN	**1992**	**1995**	**1998**	**2002**	**2007**
Alcohol Ethoxylates + AES	58	62	65	77	80
Alcohol Sulphates	42	34	21	9	6
Other Derivatives	13	16	18	21	23
Alcohols Used as such	13	12	11	10	10

Western Europe

A similar story can be seen in Western Europe (Table 3.3); most alcohols are (and have been) used in the production of polyethoxylates. The growth in production has principally been led by the displacement of Linear Alkyl Sulphonate (LAS)

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surfactants with alcohol-based surfactants; these have better compatibility with enzymes, higher efficacy in low or non-phosphate powders; in Sweden and Denmark, environmental considerations have led to their usage and there is a more favourable price vs. performance relationship compared to Linear Alkyl Benzenes (LAB).

Table 3.3 The usage of fatty alcohols in thousand of tonnes in detergents in Western Europe. There is an overall forecast growth to 2007 of 1.8%. Data from Modler (2004).

Western EUROPE	**1995**	**1998**	**1999**	**2002**	**2003**
Alcohol Ethoxylates	245	302	333	419	426
Alcohol Sulphates	73	85	91	68	69
Polymethacrylate Esters	27	29	30	31	32
Fatty Nitrogen Derivatives	11	16	20	23	24
Thiodipropionate Esters	5	5	5	5	5
Other derivatives, Alcohols used as such & C20+ alcohols	64	72	75	81	85

North America

The production of fatty alcohols for use in detergents is focussed in the USA and of those used in Canada, most originate in the USA. The production by year and type of detergent manufactured can be seen in Table 3.4. There has been a large increase in the use of alcohol ethoxylates although in recent years, this may have peaked and alcohol sulphates are increasing. The end use of these alcohol based detergents is principally within the domestic arena (~80%) with industrial applications amounting for ~20% of the total (Figure 3.16). This latter section may increase in future as detergents based on nonyl phenol polyethoxylates, which are known to have a poorer behaviour in the environment being replaced by alcohol based compounds (Modler, 2004).

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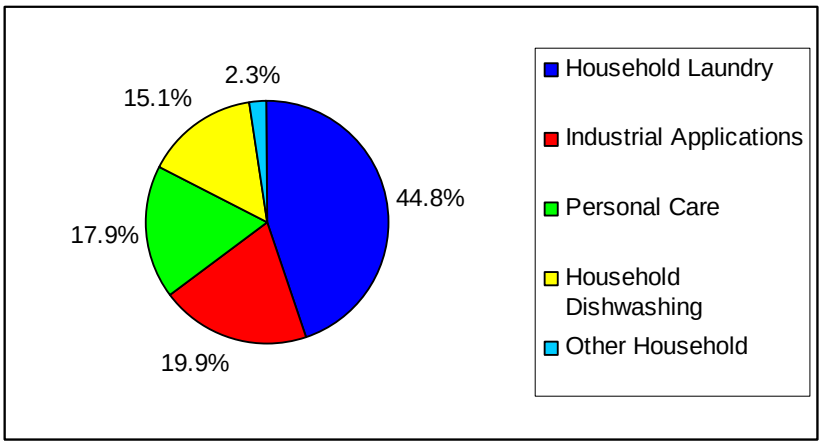

Figure 3.16 Usage of detergent fatty alcohols in North America. Data from Modler (2004).

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Table 3.4 The usage of fatty alcohols in thousand of tonnes in detergents in North America. There is an overall forecast growth to 2007 of 2.2% per annum. Data from Modler (2004).

North AMERICA	**1980**	**1985**	**1988**	**1989**	**1990**	**1991**	**1992**	**1993**	**1994**	**1995**	**1996**	**1997**	**1998**	**2000**	**2002**
Alcohol Ethoxylates	122	169	181	184	193	184	182	241	284	311	304	353	365	391	346
Alcohol Sulphates	74	67	91	83	54	71	78	34	29	29	17	27	27	25	78
Polymethacrylate Esters	27	17	15	14	14	13	13	13	16	11	12	11	12	13	15
Fatty Nitrogen Derivatives	9	7	7	7	6	6	9	11	11	11	9	9	9	10	10
Alkyl Glyceryl Ether Sulphonates	12	8	5	5	5	5	5	5	6	7	8	7	7	7	7
Thiodipropionate Esters	2	3	3	3	3	3	3	3	3	3	3	7	7	7	7
Other Derivatives	5	6	7	7	7	7	7	15	18	16	16	14	14	14	17
Free Alcohols, and All C20+ Alcohols	22	23	24	24	24	24	24	25	29	28	27	27	28	27	31
Total	272	301	332	326	306	314	320	347	396	416	396	454	470	493	513

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Chapter 4. Environmental Transformations *(Once in the environment, what happens to them? Contrasts between the short and long chain moieties which have very different degradation rates).*

All organic matter is potentially degradable in the environment and may be utilised in the short term by microbial action or over longer periods through geochemical processes leading to the formation of kerogen or oil. The debris from cell lysis, leaf fall, feeding, faecal matter and organism death generally are accumulated in the soils or surface sediments. Some material may be degraded during its settling through the water column (Lee *et al.*, 2004) and the material that reaches the sediments is likely to be altered considerably compared to the original source material. There will also be a transfer of terrestrial materials to the sea by either wind blow (Dahl *et al.*, 2005) or more usually through river flow and soil wash off.

Lipids are substantially more resistant to degradation that carbohydrates or proteins and often survive better to reach the sediments more intact that other organic matter. In general, small molecules will be more rapidly degraded than large ones and aliphatic compounds are degraded more quickly than aromatic structures. This means that waxes are likely to reach the sediments reflecting the original source material although free fatty alcohols (and acids) are less likely to do so.

Metabolism of Fatty Alcohols

Fatty alcohols are metabolised as part of system that operates on alkanes and fatty acids as well. The general scheme is shown in Figure 4.1. In the oxidation process, alkanes may be converted to alcohols and subsequently fatty acids. These then enter the β-oxidation pathway to yield a series of acetyl – CoA products (and one proprionyl – CoA in the case of odd chain compounds) and several molecules of ATP (Berg *et al.*, 2002; Soltani *et al.*, 2004). This process happens within the cell and the products of this oxidation pathway are used to provide energy, water *etc*.

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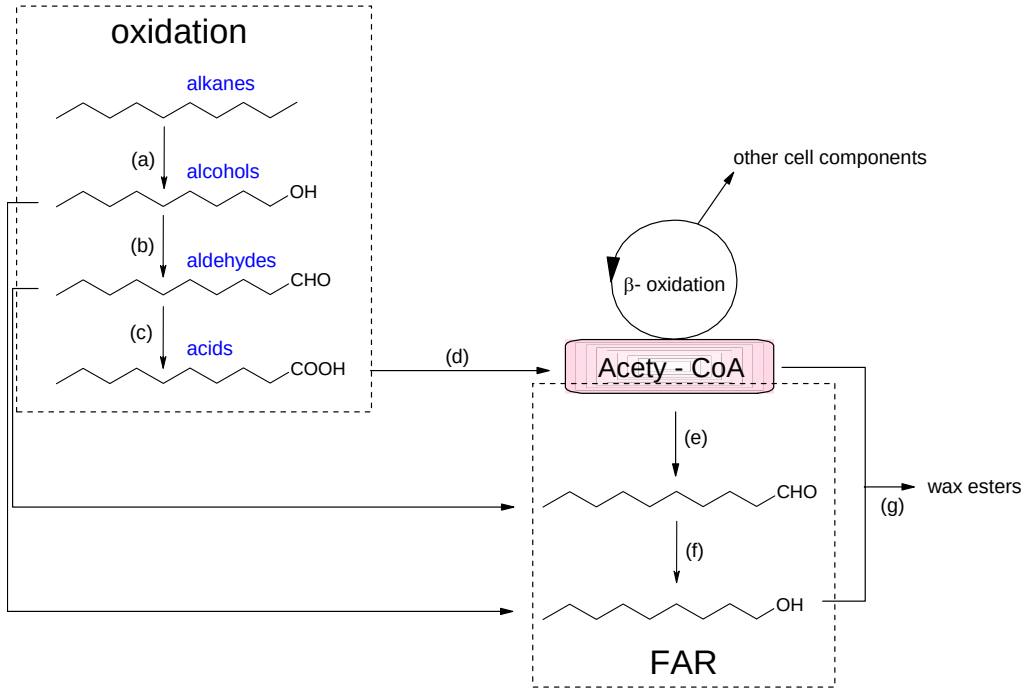

Figure 4.1 Schematic process for the metabolic degradation of fatty alcohols in *Acinetobacter* spp. The oxidation component is degrative while the FAR step builds new compounds. (a) alkane monooxygenase, (b) alcohol dehydrogenase, (c) aldehyde dehydrogenase, (d) acyl–CoA synthetase, (e) acyl–CoA reductase, (f) aldehyde reductase (alcohol dehydrogenase) and (g) acyl–CoA:alcohol transferase. Redrawn from Ishige *et al.* (2003).

Environmental transformations of alkanes and waxes may be mediated by bacteria (*e.g*. hydrocarbon degradation) and these reactions yield alcohol intermediates. It is worth noting at this point that naturally occurring bacteria are able to degrade waxes (Roper, 2004). The bacterial (*Pseudomonas oleovorans*) alkane hydroxylase system (Ishige *et al.*, 2003) that is responsible for the total oxidation of an *n*-alkane to *n*-alcohol ($RCH_3 + NADH + H^+ + O_2 \rightarrow RCH_2OH + NAD^+ + H_2O$) consists of three components: alkane hydroxylase (AlkB), rubredoxin (AlkG) and rubredoxin reductase (AlkT). AlkB is a non-heme iron integral membrane protein that catalyzes the hydroxylation reaction. AlkG transfers electrons from the NADH-dependent flavoprotein rubredoxin reductase to AlkB. The resultant alcohol is oxidized to 1-alkanoate by a membrane-bound alcohol dehydrogenase (AlkJ) and cytosolic aldehyde dehydrogenase (AlkH). 1-alkanoate is incorporated through β-oxidation via the acyl–CoA synthetase (AlkK) reaction.

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This is the case for relatively small compounds with chain lengths between C_5 and C_{12} (Ishige *et al.*, 2003). Other Gram-negative *n*-alkane degraders belonging to *Acinetobacter* grow on longer-chain *n*-alkanes. Although the reactions for the longer chain alkanes C_{12}–C_{18} are principally the same as those of *Pseudomonas oleovorans*, the organization of the genes is different (Ishige *et al.*, 2003). *Acinetobacter* sp. strain M-1 is characterized by its ability to use much longer-chain *n*-alkanes (C_{20}–C_{44}) and can degrade *n*-alkanes up to C_{60} when grown on a paraffin wax mixture (Ishige *et al.*, 2003).

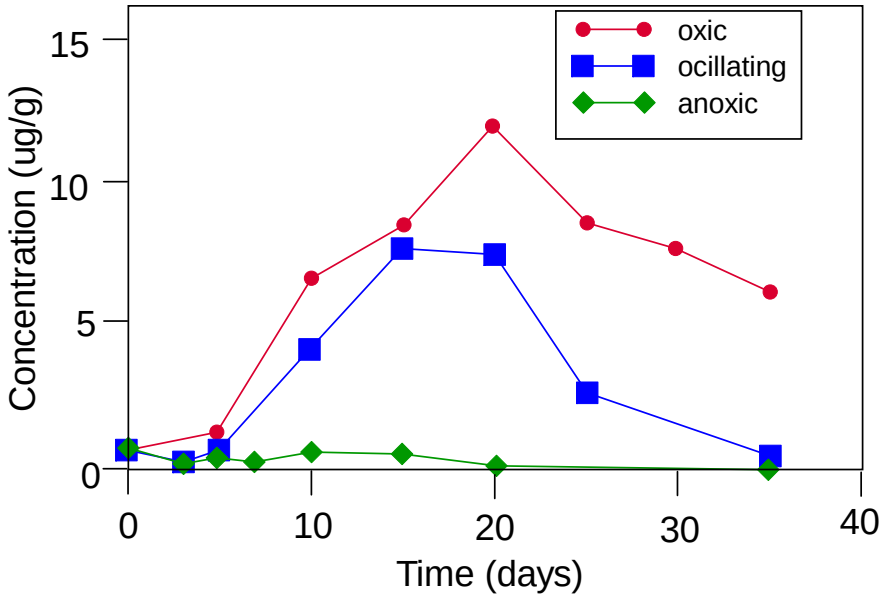

Figure 4.2 The concentration of C16 and C16:1 fatty alcohols during oxic, oscillating and anoxic incubations of sediment with the micro-alga *Nannochloropsis salina* (Caradec *et al.*, 2004).

Work by Caradec *et al.* (2004) on the degradation of fatty acids identified the production of free saturated and monounsaturated C_{16} and C_{18} fatty alcohols during anoxic and alternating oxic/anoxic incubations of algal material and natural sediments. The production of fatty alcohols coincided with a high degree of triacylglycerol hydrolysis; this supports a precursor-product relationship between fatty acids esterified to triacylglycerol and the alcohols produced. The greater accumulation of C_{16} alcohols observed under anoxic conditions (Figure 4.2) might reflect a lower efficiency of anaerobic bacteria for mineralising these compounds. For the oscillating conditions, it is possible that alcohols were produced under anoxia and consumed

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during oxic periods. This suggests that aerobic and anaerobic bacteria in the sediments used different assimilation pathways.

Relatively little information exists on the extra-cellular production of free fatty alcohols but some work on the fungus *Botrytis cinerea* by Doss (1999) and Cooper *et al.* (2000) showed that some were indeed produced with a profile compatible with direct loss after FAS and FAR (57% C_{16} and 43% C_{18} and no longer or unsaturated compounds). There was a wider range of compounds present in waxes with the majority being C_{20} or C_{28}.

Some experimental work (Larsen *et al.*, 1995; Doss, 1999)) has shown that exogenous fatty alcohols may be taken up by bacteria and in this case incorporated into bacteriochlorophyll c. Usually, mid chain length compounds were utilised ($C_{16} - C_{18}$) although they demonstrated that short chain ($C_{10} - C_{12}$) and long chain (C_{20}) compounds were also utilised.

Natural degradation

The degradation of short chain compounds occurs at a greater rate than long chain compounds (*e.g.* Haddad *et al.*, 1992). In their study of fatty acids in the marine environment, the concentration of 16:0 decreased from 185.1 $\mu g.g^{-1}$ dry weight (DW) at the surface to 4.9 $\mu g.g^{-1}$ DW at 250 cm depth in a core from Cape Lookout Bight, NC, USA. In contrast, the 28:0 fatty acid only decreased from 16.3 to 11.2 $\mu g.g^{-1}$ DW over the same depth. This indicates the general pattern of degradation for chains of alkanes, alcohols and acids. In Figure 4.3, the relatively high concentration of short chain fatty acids at the surface (~350 $\mu g.g^{-1}$) reduced to approximately 25% of this value within the top 25 cm. The corresponding long chain fatty acid concentration increased in absolute terms over the same depth interval; the change in these concentrations down the whole 250 cm was negligible.

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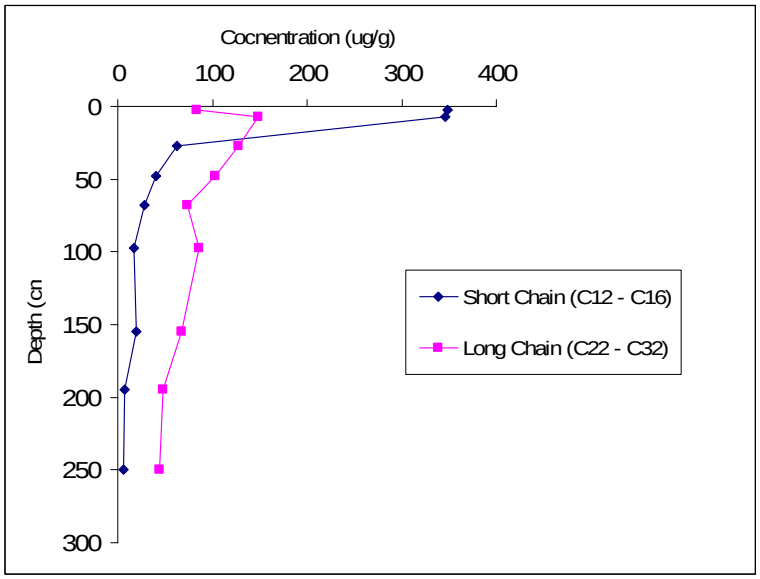

Figure 4.3 The fatty acid concentrations in a core. Data redrawn from Haddad *et al.* (1992).

Short chain moieties

Models of the relative fatty acid degradation rate have been constructed by Haddad *et al.* (1992). In general terms, there was an order of magnitude less degradation of the long chain (>C_{20}) compounds compared to the short chain (C_{18} and below) compounds. There was also a 25% increase in the degradation rate for the even chain (*e.g.* C_{14} and C_{16}) compounds compared to the odd chain equivalents (C_{15} and C_{17}, Figure 4.4).

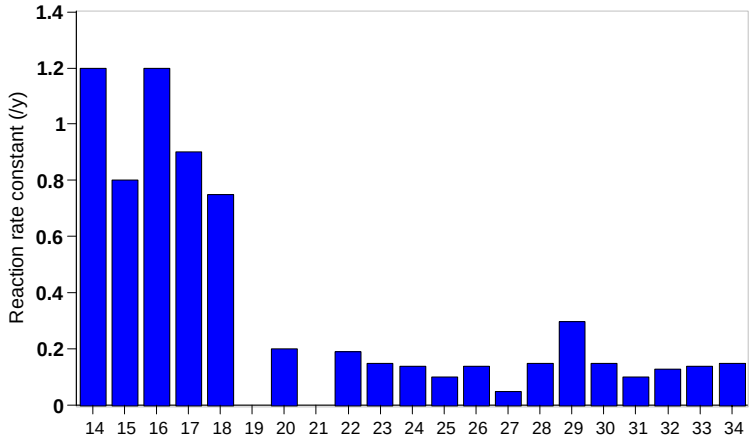

Figure 4.4 Degradation rates for fatty acids modelled by Haddad *et al.* (1992). Data have been measured from original figure for reproduction here.

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Figure 4.5 Location map for a 1.5 m core analysed for fatty alcohols and other lipid biomarkers. The sills in sea lochs tend to trap both terrestrial matter that runs off from the land as well as fine grained sediments that enter from the sea and then settle out.

In sediment samples from a core collected in a Scottish sea loch (essentially, a fjord; see Figure 4.5 for the location), the concentration of the short chain fatty alcohols generally decreased with depth (Figure 4.6a), especially in the top 20 cm, while the long chain compounds increased with depth (Figure 4.6b). The net effect of this can be seen in the short / long chain fatty alcohol ratio (Figure 4.6c). In the surface sediments there are greater concentrations of short chain, marine derived compounds often with a sub-surface maximum. This is a common feature of several sediment cores (Hotham, 2001; Mohd. Ali, 2003) and may be due to *in situ* biosynthesis by bacteria utilising the depositing organic matter.

At the deeper depths, the concentration of the longer chain compounds is greater than the short chain ones and a ratio of less than one can be measured. This may reflect two distinct processes; *in situ* degradation of the short chain compounds or a change in organic matter source from marine at the surface (recent past) to terrestrial at depth (past 500 years). The difficulty in separating these two different processes will be considered in a later chapter (statistical approaches to biomarker data).

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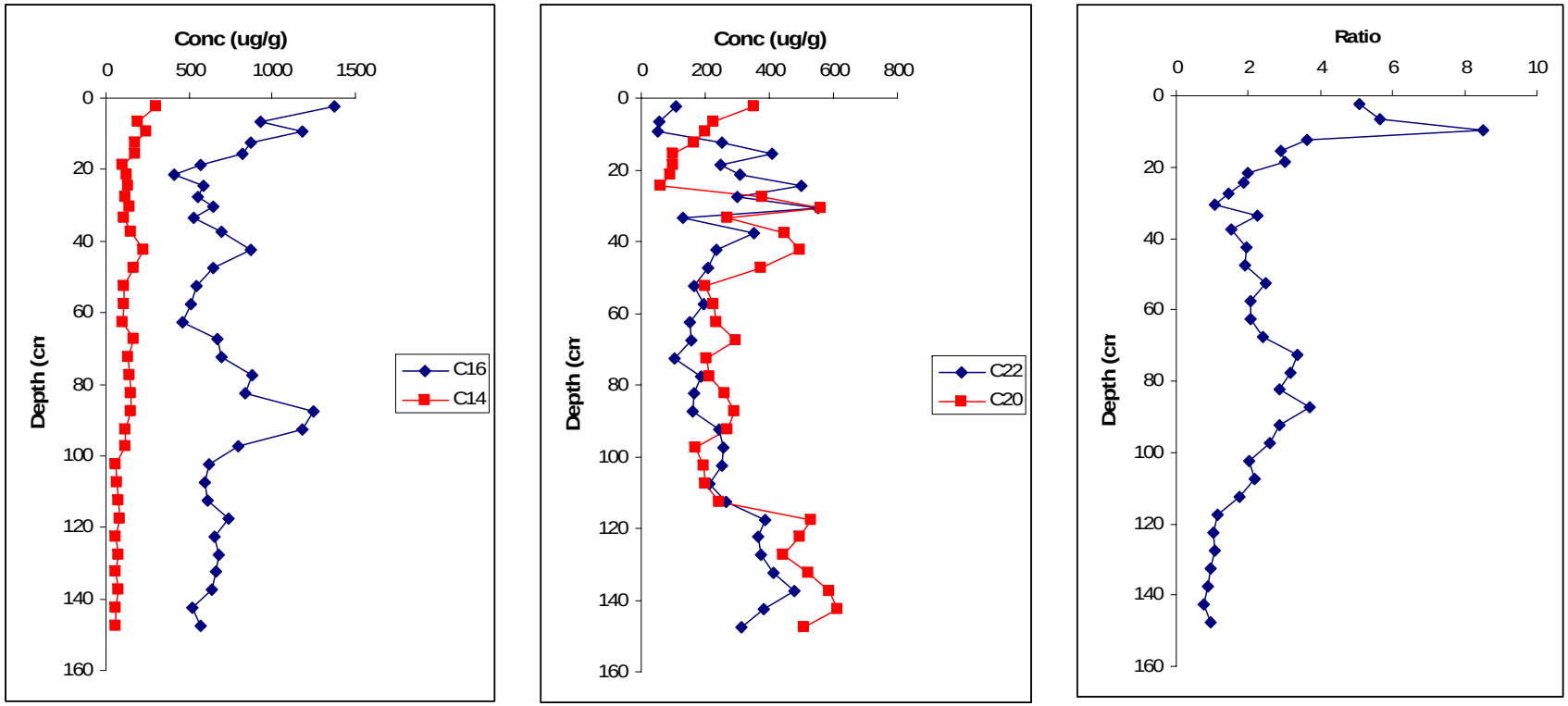

Figure 4.6 (a) Short chain and (b) long chain fatty alcohol concentrations in a core from a sea loch and (c) the ratio between the short chain fatty alcohol ($C_{12} - C_{18}$) / long chain ($C_{19} - C_{24}$) (Loch Riddon, Mohd. Ali, 2003).

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In some locations, terrestrial inputs are small and the short chain fatty alcohols dominate at all depths within the sediment core. An example of this can be seen in the Ria Formosa Lagoon, Portugal (Figure 4.7 in work by Unsworth (2001)). This is the largest lagoon in Europe and receives little terrestrial runoff for most of the year; when it does rain in November – February, the water tends to flush out any suspended materials from the lagoon (Mudge *et al.*, 1998; Mudge *et al.*, 1999). Therefore, the settled sediment is dominated by marine markers which can be seen in the short chain fatty alcohol ($C_{12} – C_{18}$) / long chain ($C_{19} – C_{24}$) ratio (Figure 4.8). The ratio is considerably greater than the data for the sea loch environment which will be receiving and trapping terrestrial organic matter. Therefore, the absence of long chain fatty alcohols indicates a marine source for the organic matter and tells the investigator significant information about sources and their deposition in the area. This makes the fatty alcohols a useful group of biomarkers in the marine environment although the sterols are also useful but from a different context (Mudge and Norris, 1997).

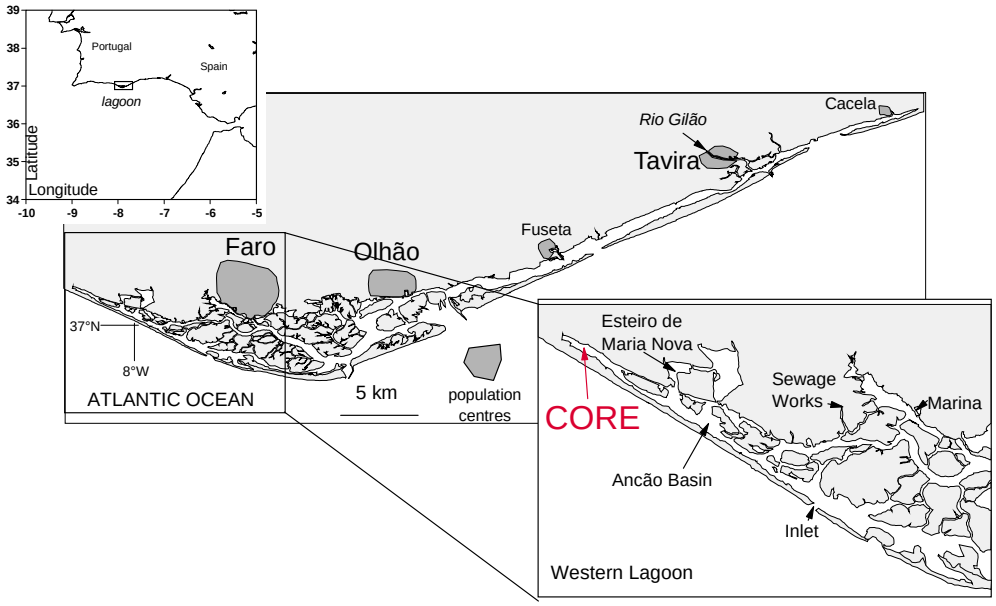

Figure 4.7 The Ria Formosa Lagoon in Portugal. Although there are rivers and other site of terrestrial run off, the region is dominated by marine derived fatty alcohols.

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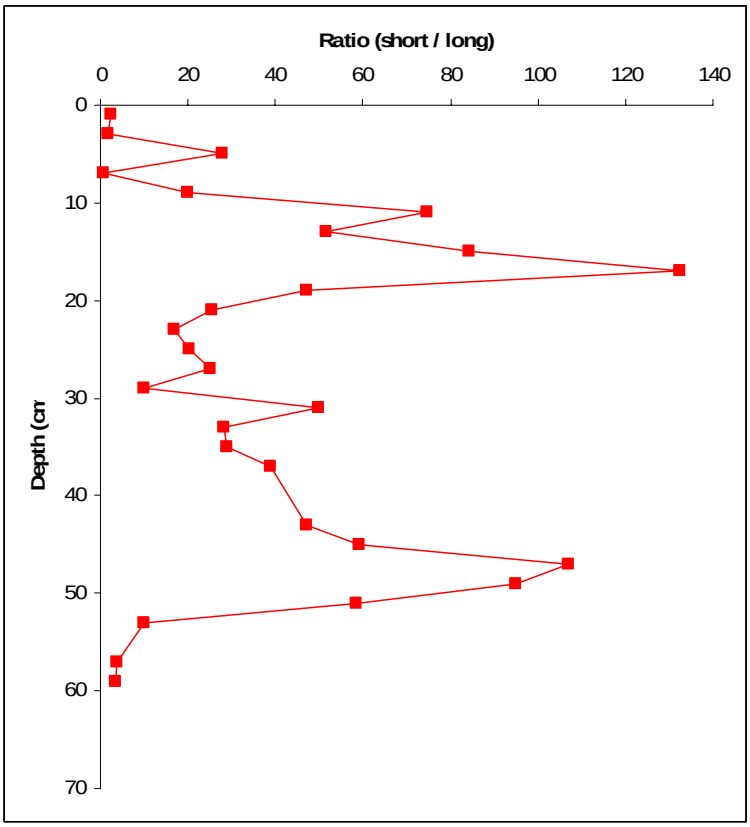

Figure 4.8 The short chain fatty alcohol (C_{12} – C_{18}) / long chain (C_{19} – C_{24}) ratio down a core from the Ria Formosa lagoon. After Unsworth (2001).

Long chain moieties

Work on the settling and settled organic matter from the equatorial Pacific Ocean (Wakeham *et al.*, 1997) has indicated that most biogenic compounds are degraded in the water column (Figure 4.9). Compounds that occurred at maximal abundance at 10-12 cm in the sediments they classed as Group IV; these were resistant to microbial degradation. Chemicals with this behaviour include three separate homologous suites of high-molecular-weight, straight-chain fatty acids, fatty alcohols and alkanes. Compounds comprising these series exhibit carbon-number predominance patterns characteristic of cuticles from vascular land plants and likely were transported to the central Pacific by wind (Gagosian and Peltzer, 1986; Prahl *et al.*, 1989).

Therefore, the longer chain fatty alcohols and acids reach the sediments and represent refractory constituents of water column particles that become magnified at depth by selective preservation as >99% of the surface produced organic carbon was respired.

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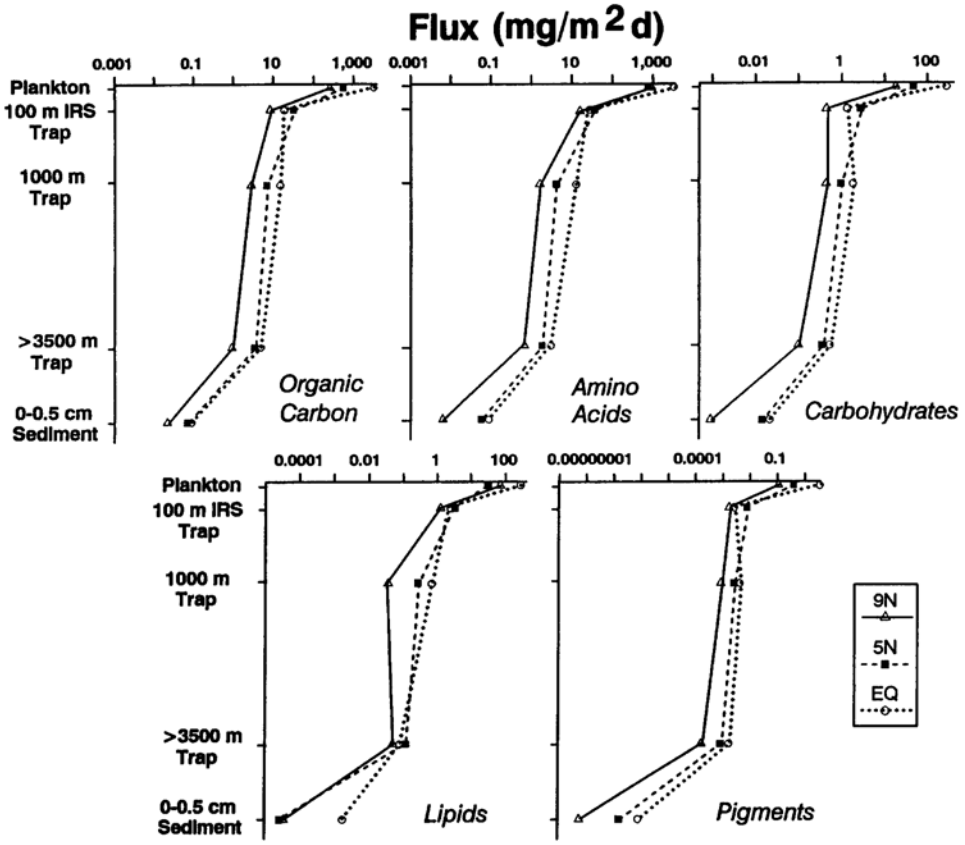

Fig. 1. Fluxes (mg/m^{2}d) of organic carbon and biochemical classes at 9°N, 5°N, and the equator. Compound class fluxes are in mg of compound, not mg C. Fluxes for net-plankton are derived from primary production rates (Barber et al., 1996) and our measurements of biochemical content of net-plankton. Floating sediment trap fluxes at 5°N and the equator are means of two surveys (there was only 1 measurement at 9°N). Fluxes into surface sediments were calculated using the sediment C_{org} content and accumulation rates (DeMaster et al., 1997) and our biochemical measurements. There are no accumulation rate data for 10–12 cm sediments.

Figure 4.9 Image from Wakeham *et al.* (1997) showing the rate at which different organic matter classes degrade with depth through the water column and then into surface sediments.

The degradation pathway for fatty alcohols follows the general scheme advanced in Figure 2.8; alkanes and alcohols are converted to fatty acids which enter the β-oxidation pathway (Soltani *et al.*, 2004). The fate of the acetyl – CoA sub-units cleaved off during this process is usually to end up as carbon dioxide.

The short term diagenesis of fatty acids in marine sediments, the ultimate fate of most organic carbon, indicated the following reactivity relationships (Haddad *et al.*, 1992): unsaturated fatty acids > branched fatty acids > saturated fatty acids. They also detected differences within the saturated fatty acid fraction such that medium chain length compounds (C_{14}-C_{19}) were degraded at rates 6-7 times faster than long chain length compounds (C_{20}-C_{34}). Results of kinetic modelling indicated that no simple relationship exists between remineralization rates and molecular weight (or carbon

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chain length) and they suggest that the preferential preservation of terrestrially derived long chain length fatty acids results from their inclusion into microbially inaccessible matrices (Haddad *et al.*, 1992).

Degradation Rate Constants

Haddad *et al.* (1992) calculated apparent degradation rate constants for n-alkanols and phytol by assuming that they are degraded by first order kinetics and at steady state and used the following equation:

$$\ln C = \ln C_0 - k\,(z/s)$$

where C = the alcohol concentration at depth

C_0 = the alcohol concentration at z = 0

k = the apparent rate constant (y^{-1})

z = core depth (cm)

s = sedimentation rate ($cm.y^{-1}$)

The apparent rate constant can be estimated from the linear regression of $\log_e$ alcohol concentrations versus z/s. Their results from the continental slope off Taiwan (354 m water depth and 0.33 $cm.y^{-1}$ sedimentation rate) are summarised in Table 4.1.

Table 4.1 Degradation rate constants (y^{-1}) for fatty alcohols in marine sediments (after Jeng *et al.* (1997). Extractable alcohols are those that can be removed from the sediment without a saponification step while the bound ones need a saponification step.

	Extractable	Bound
Phytol	0.015	0.011
n-alkanols	0.010	0.007

These values are similar to other published rates from Sun and Wakeham (1994) who measured values between 0.024 and 0.070 y^{-1} in three locations. However, re-analysis of data from Loch Riddon (Mohd. Ali, 2003) produces a slower degradation rate. Plotting of the $\log_e$ concentrations of the C_{14} and C_{16} fatty alcohols against the k/s (from the equation above) can be seen in Figure 4.10. The sedimentation rate, s, was

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calculated from the position in the sediment core of the increased PAH concentrations derived from the increased burning of coal and coke from 1750 onwards. In this core, that was 52.5 cm depth and so a sedimentation rate of 0.21 cm.y^{-1} was used.

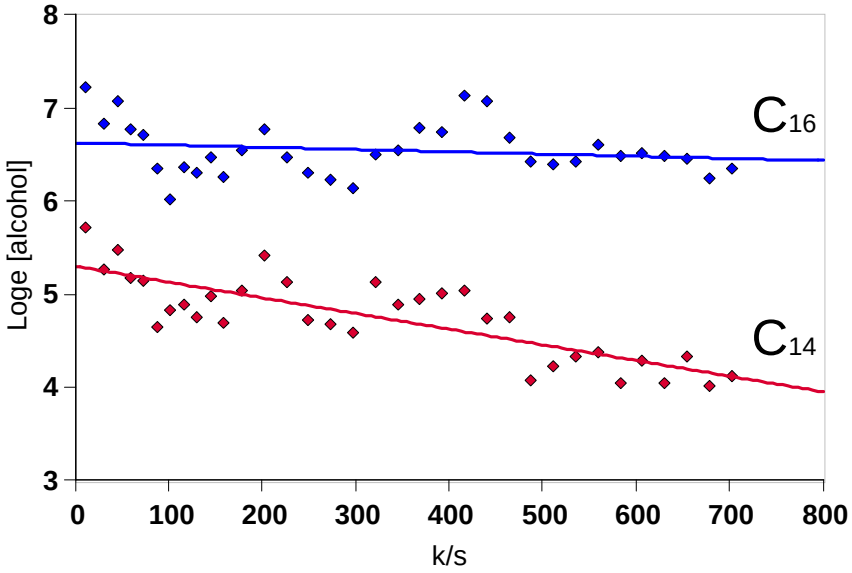

Figure 4.10 Log_e of the C_{14} and C_{16} fatty alcohol concentrations from a Scottish sea loch. A sedimentation rate of 0.21 cm.y^{-1} was calculated from the PAH profile based on the beginning of the industrial revolution circa 1750.

The calculated slope for the C_{14} was 0.002 y^{-1}, less than previously reported values for bound fatty alcohols shown in Table 4.1 (Jeng *et al.*, 1997). This may be due to several reasons; incorrect sedimentation rates although other analyses have confirmed the rate, better preservation in this particular site due to low bacterial activity or altered input fluxes through time. The latter appears to be most likely as other biomarker signatures change with time due to increased anthropogenic organic matter deposition after the initial industrialisation period. The degradation rate of the C_{16} was almost an order of magnitude less than the C_{14}. This may be due to its increased chain length (Jeng *et al.*, 1997) or *in situ* production by biota. Shorter chain alcohols were only present in the top few centimetres of the sediment core (*e.g.* C_{12} to 12.5 cm) and have, therefore, degraded much quicker than the rate reported here.

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Phytol degradation

The degradation of phytol in the Loch Riddon marine core can be seen in Figure 4.11. The calculated degradation rate in this case was 0.005 which was half of values of Jeng *et al.* (1997). Again, the values could be compromised by increased primary productivity in the more recent past, fuelled by the anthropogenically derived nutrients introduced as Glasgow expanded in the post-industrial revolution period.

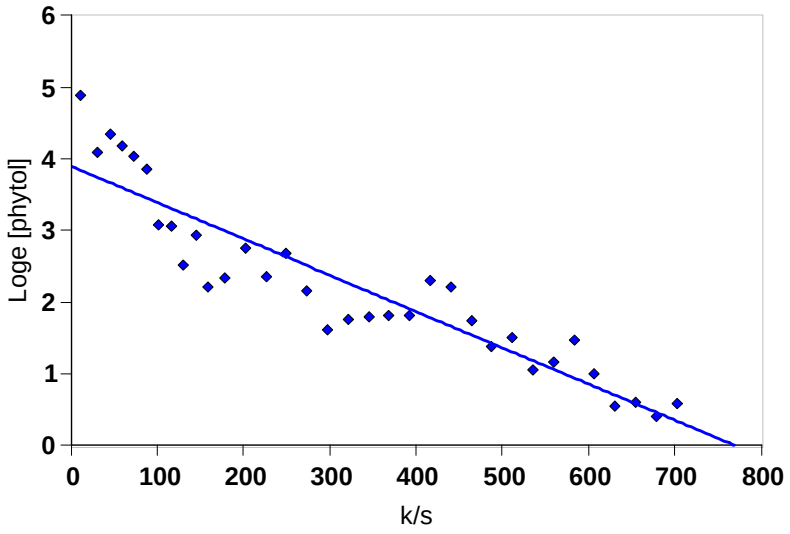

Figure 4.11 Log_e of the phytol concentrations from a Scottish sea loch. A sedimentation rate of 0.21 $cm.y^{-1}$ was used.

The rate of phytol degradation compared to straight chain, saturated alcohols is greater; Jeng *et al.* (1997) put this down to the unsaturated nature of the molecule and compared their results with those of Sun *et al.* (1997) who studied fatty acids in oxic and anoxic marine sediments.

Effect of chemical associations on transformation rates

"Natural" fatty alcohols in STPs

In a study on the potential origin of organic matter on Blackpool Beach (see location map in Chapter 7), analyses of fatty alcohols were made of the influent and effluent of the STP serving the area (Mudge, 2001). The profile of these chemicals can be seen in

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Figure 4.12; the concentration in the influent material (liquid / liquid extraction of total after addition of KOH) is significantly greater than for the effluent indicating degradation / transformation of the materials during treatment.

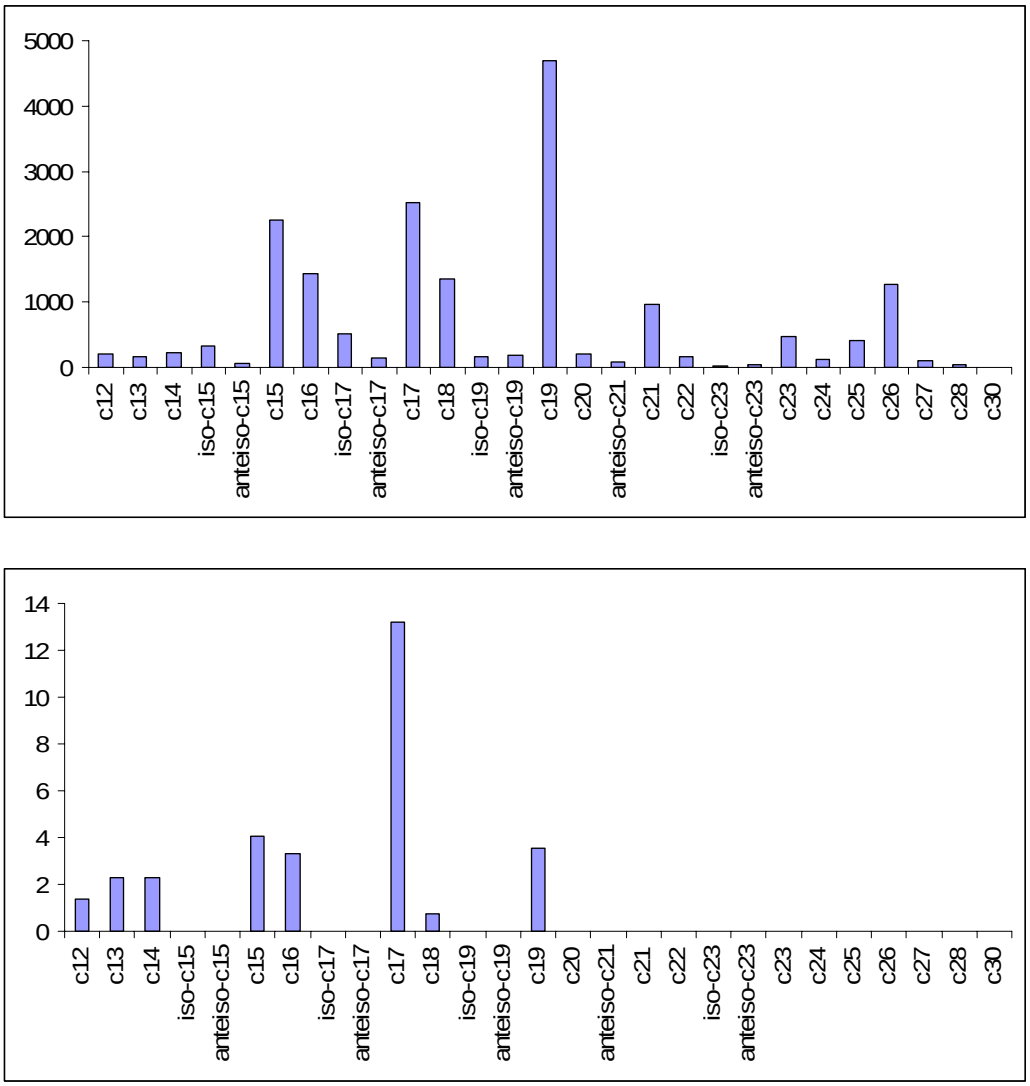

Figure 4.12 (a) Fatty alcohols in the influent and (b) effluent of the STP serving Blackpool, UK. ($\mu g.L^{-1}$).

The final effluent concentration is 0.16% of the initial influent fatty alcohol concentration. In both cases, the profile is dominated by odd carbon chain alcohols; C_{19} in the influent and C_{17} in the effluent. This indicates the presence of high bacterial biomass as would be expected in these materials. The influent also has a range of long

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chain alcohols up to C_{28} indicative of terrestrial plant matter. These would be associated with waste food material and terrestrial runoff incorporated in to the sewer. These materials are absent from the effluent with the longest chain alcohol detected being C_{19}. The proportion of the short chain $C_{12} - C_{14}$ compounds goes up due to the metabolism of the longer moieties or removal of these compounds with settling particulate matter. The lack of branched chain fatty alcohols is of interest as these are normally associated with bacteria (Parkes and Taylor, 1983). Their absence may indicate a low biomass of the particular organisms that produce these *iso* and *anteiso* branches, they are more readily metabolised or simply that their presence is below the limit of detection.

This pattern of degradation does not follow that seen in the environment; in natural sediments the longer chain waxy materials are preserved and can be seen to considerable depths in cores. In the STP, the long chain ones are removed and the proportion of short chain ones increases. The origin of the $C_{12} - C_{14}$ compounds in this system can not be determined from these analyses alone as they may have originated from another, non-biological source such as detergents although these also degrade in STPs. Further work using compound specific $\delta^{13}C$ may elucidate the origin of the short chain compounds in the effluent.

Anthropogenic fatty alcohols in STPs

The different chemical form of fatty alcohols entering the waste water system from detergents may lead to different transformations during treatment and ultimately change the fate of the compounds. The detergent fatty alcohols are principally in the form of polyethoxylates (see Chapter 3) and as such are considerably more "available" than bound fatty alcohols, usually in the form of waxes. Itrich and Federle (2004) studied the effect of both the alcohol chain length and the number of ethoxylate groups used in the hydrophilic section of the molecule. As with previous studies (Steber and Wierich, 1983; Kravetz *et al.*, 1984; Battersby *et al.*, 2001), they utilised radiolabelled homologues and determined the fate of the radioactive ^{14}C. The study used a poisoned (control) activated sludge systems and in this system >98% of the introduced radiolabelled ethoxylates were present as the parent molecule at the end of the experiment. In contrast, in the biologically active system no ^{14}C was recovered as the parent ethoxylate and 68.7% was measured in the trapped CO_2. Of

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the remainder, 7-14% of the initial ^{14}C from the α carbon of the alcohol was associated with the solids, potentially as free fatty alcohols (Itrich and Federle, 2004).

What don't we know?

1. What is the origin of $C_{12} - C_{14}$ in effluent; do they come from (a) detergents, (b) naturally present from raw source, (c) degradation of longer chain compounds and (d) some mix of all these processes? This could be addressed by measuring the compound specific $\delta^{13}C$ values for the alcohols in the influent and effluent.

2. What is the degradation rate in the short term (top 10 cm of sediment) at high resolution? We often see higher than surface values in the top few centimetres of a core… are these being produced *in situ* by degradation of long chain compounds, sediment compaction increasing apparent concentration or bacterial biomass?

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Chapter 5. Analytical methods *(How do most people measure these compounds? Are there major differences between methods that may bias one set of results against another?)*

Overview of Methods

There are two major approaches to the analysis of fatty alcohols in the environment. In one case, free fatty alcohols and polyethoxylates are measured (principally) by a liquid chromatography (LC) technique and in the other ester linked compounds are saponified and analysed by gas chromatography. The former method seems to have become the standard for the detergent industry while environmental analytical laboratories are using the latter. This may lead to an issue of "context"; results generated by the former method will not include the ester linked compounds which form the bulk of the natural fatty alcohols. Therefore, no measure is made of the relative proportion of fatty alcohols in the environment derived from each source.

Methods for analysis of free fatty alcohols (and ethoxylates)

The basis for this method is the derivatisation of the terminal –OH group with 2-fluoro-N-methylpyridinium p-toluenesulfonate (Pyr+), giving the molecule a net cationic charge (Dunphy *et al.*, 2001). The schematic of the reaction can be seen in Figure 5.1. Since all terminal –OH groups are derivatised, all species including the free fatty alcohols and those with only one ethoxylate can be effectively detected by electrospray Mass Spectrometry (MS).

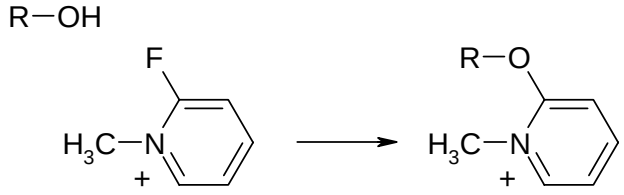

Figure 5.1 The derivatisation of the terminal –OH group on a fatty alcohol or an ethoxylate derivative with Pyr+.

The typical ethoxylates used in detergents have aliphatic alcohols with chain lengths between C_{12} and C_{18} (except C_{17}) with ethoxylate (EO) hydrophilic components typically up to EO_{20}. The most sensitive detector configuration for such a suite of

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compounds is single ion monitoring (SIM) and a list of the principal ions of each species can be seen in Table 5.1 (after Dunphy *et al.* (2001).

Table 5.1 key ions (*m/z*) to be used in the identification of Pyr+ derivatised polyethoxylates of fatty alcohols.

EOx	C_{12}	C_{13}	C_{14}	C_{15}	C_{16}	C_{18}
0	278	292	306	320	334	362
1	322	336	350	364	378	406
2	366	380	394	408	422	450
3	410	424	438	452	466	494
4	454	468	482	496	510	538
5	498	512	526	540	554	582
6	542	556	570	584	598	626
7	586	600	614	628	642	670
8	630	644	658	672	686	714
9	674	688	702	716	730	758
10	718	732	746	760	774	802
11	762	776	790	804	818	846
12	806	820	834	848	862	890
13	850	864	878	892	906	934
14	894	908	922	936	950	978
15	938	952	966	980	994	1022
16	982	996	1010	1024	1038	1066
17	1026	1040	1054	1068	1082	1110
18	1070	1084	1098	1112	1126	1154

The limits of detection for individual homologues in their study ranged from 0.1 $ng.l^{-1}$ to an estimated 22 $ng.l^{-1}$ but most values were between 3 and 5 $ng.l^{-1}$ (Dunphy *et al.*, 2001). When these values are summed, it is possible to calculate the limit of quantitation for all alcohol ethoxylates in effluent samples; a value of 1.7 $\mu g.l^{-1}$ was suggested for the commercial mixtures of these ethoxylates used in their study.

Environmental Samples

Due to the non-polar nature of fatty alcohols either as free alcohols or as wax esters, the compounds will be present in the sedimentary phase either in suspension or settled (Mudge and Duce, 2005). Therefore, most analyses concentrate on this phase when determining environmental concentrations. The association of the compounds within the sediment is not homogenous and several distinct phases may exist. The method

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chosen for extracting these compounds will, therefore, determine what components are quantified.

The analysis of fatty alcohols in environmental samples falls into two camps; those who extract directly into a non-polar organic solvent and those who saponify the sediment directly. The scheme for these two methods can be seen in Figure 5.2. The principal division is in the use of KOH for saponification directly on the sediment or only after lipid extraction with DCM / MeOH. The different routes will yield different values and profiles as the fatty alcohols may be associated with different matrices in the sediment and these will change with source and age.

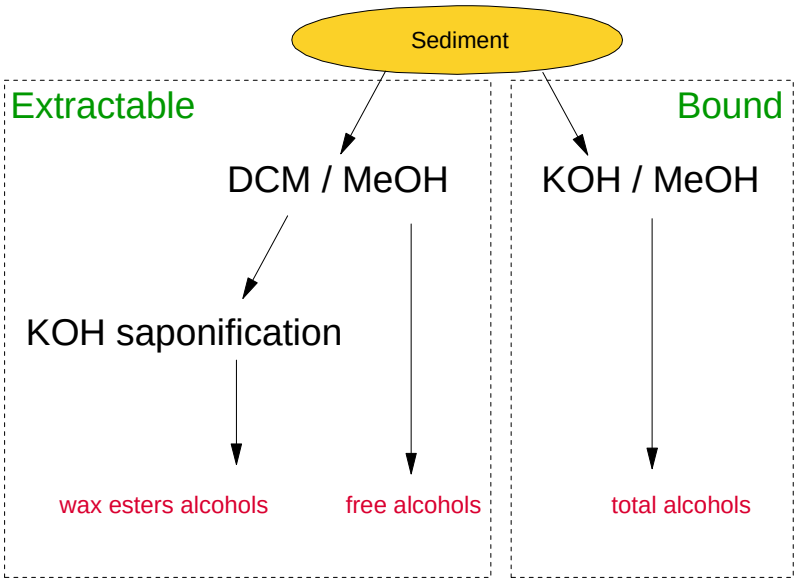

Figure 5.2 Typical extraction protocols for fatty alcohols. The major division is whether the sediment sample is treated directly with KOH to remove all bound compounds or is it only used after solvent extraction.

The "extractable" component is removed by dissolving it into either a DCM or chloroform mix with 1:1 methanol (MeOH) after the methods of Folch *et al.* (1957). These extractable lipids may then be saponified to break any ester linkages leaving the free lipid in solution, sometimes as its sodium or potassium salt. In the case of waxes, a fatty acid and a fatty alcohol are produced (Figure 5.3). These may then be extracted separately from each other. Typically, the neutral lipids will include the sterols and fatty alcohols and these may be extracted directly into a non-polar solvent such as hexane (Chikaraishi and Naraoka, 2005). The fatty acids may also be

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extracted, if required, by titrating the KOH / MeOH fraction back to an acid pH with HCl. These polar lipids may then be extracted into 9:1 hexane / diethyl ether (Chikaraishi and Naraoka, 2005) or 1:4 DCM / hexane (Mudge *et al.*, 1998). The alcohols and sterols are then generally derivatised to form the trimethylsilyl (TMS) ethers. Several reagents are available for this purpose and the most commonly used is BSFTA (*bis*-(trimethylsilyl) trifluoroacetamide).

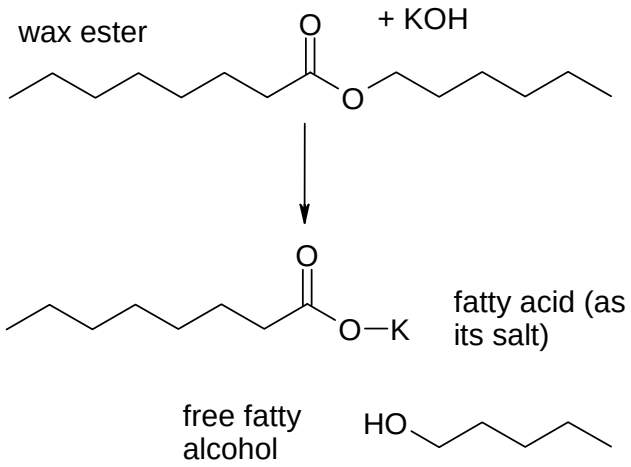

Figure 5.3 The saponification process leaving free fatty alcohols in solution.

The free alcohols may be directly derivatised as above and analysed in the same manner as other lipids.

The method for bound (wax ester, geolipids, *etc.*) fatty alcohols involves direct saponification of the sediment with alkaline methanol. Moist sediment or biological material is boiled in 6% KOH in methanol (*w/v*) for approximately 4 hours. Unpublished work has shown that this is sufficient for >95% extraction of the bound sterols. After allowing the extract to cool, the solids should be separated from the liquor by either centrifugation or filtration. If using centrifugation, glass centrifuge tubes of nominally 40 ml capacity should be balanced within 1 g and spun at 2500 rpm for ~5 min or until the solids have separated from the liquor. For improved recovery, the solids can be re-suspended in methanol, re-centrifuged and the liquid combined with the initial extract.

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The clear liquor from sediment extractions ranges in colour from pale yellow to dark brown can be poured into a glass separating funnel using a glass funnel to aid in the transfer. Addition of 20 – 30 ml of hexane to the liquor and shaking will extract the non-polar lipids that now includes any free fatty alcohols originally present plus and bound (wax derived) compounds. The polar compounds including the fatty acids will be left behind in the alkaline methanol. As well as the fatty alcohols, the sterols and PAHs will preferentially partition into the hexane (Mudge and Norris, 1997). Derivatisation is by use of BSTFA as above.

The most useful instrumental method for fatty alcohols from environmental samples is gas chromatography – mass spectrometry (GC-MS). The analytical column should be of the DB-5, HP-5, BPX-5 variety although better baselines have been seen using a high temperature column such as SGE's (Scientific Glass Engineering, Australia) HT-5. The temperature programme needs to go to about 360°C to ensure removal of all compounds from the column and at these elevated temperatures increased column bleed can become troublesome with some columns. Typical column lengths are 30 to 60 m and the best separations can be seen with narrow bores and thin films (0.25 mm and 0.1μm).

A typical temperature programme should start at 60°C, increasing at 15°C min^{-1} to 300°C, then at 5°C min^{-1} to a maximum of 360°C. Other gradients are possible and may be recommended if the sterols and polyaromatic hydrocarbons are to be quantified as well. The mass spectrometer (MS) is best configured for electron impact ionisation at 70eV and a mass scan range of 45-545 *m/z* per second. If better detection limits are required, the mass spectrometer may be operated in the single ion monitoring (SIM) mode using the fragments of $M^+ - CH_3$ as the identifier. A list of key ions is shown Table 5.2.

An example of the GC trace with selected fragments for the homologous series can be seen in Figure 5.4. The major ions from Table 5.2 are shown for the $C_{14} – C_{18}$ compounds above the total ion count (TIC). Examples of the mass spectra for alcohols from real samples are shown in Figure 5.5.

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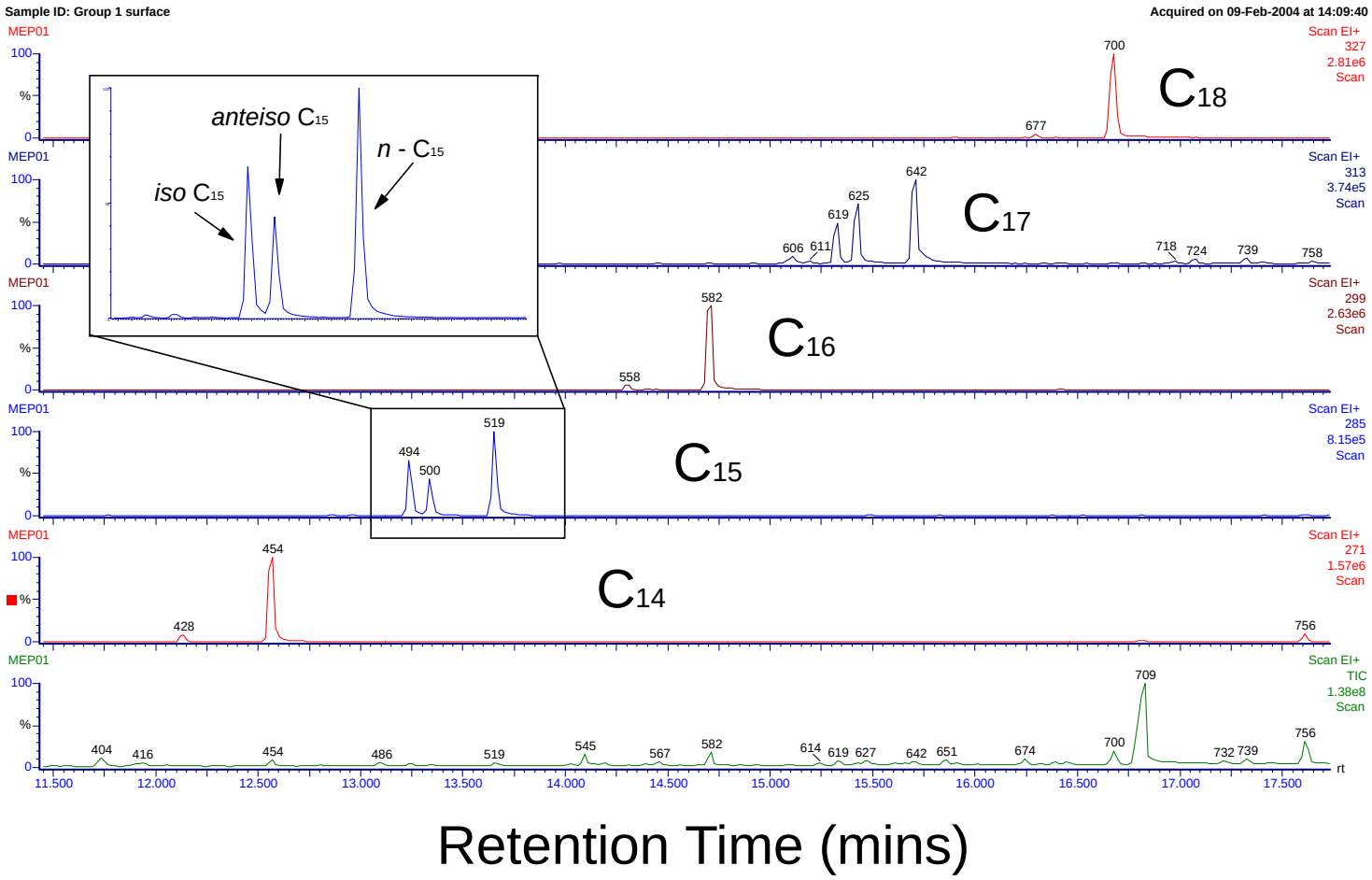

Figure 5.4 GC trace for an alkaline saponification of surface sediment (Menai Strait, North Wales) with key ions for a range of fatty alcohols. The inset shows the branched chain compounds for the odd carbon numbered species.

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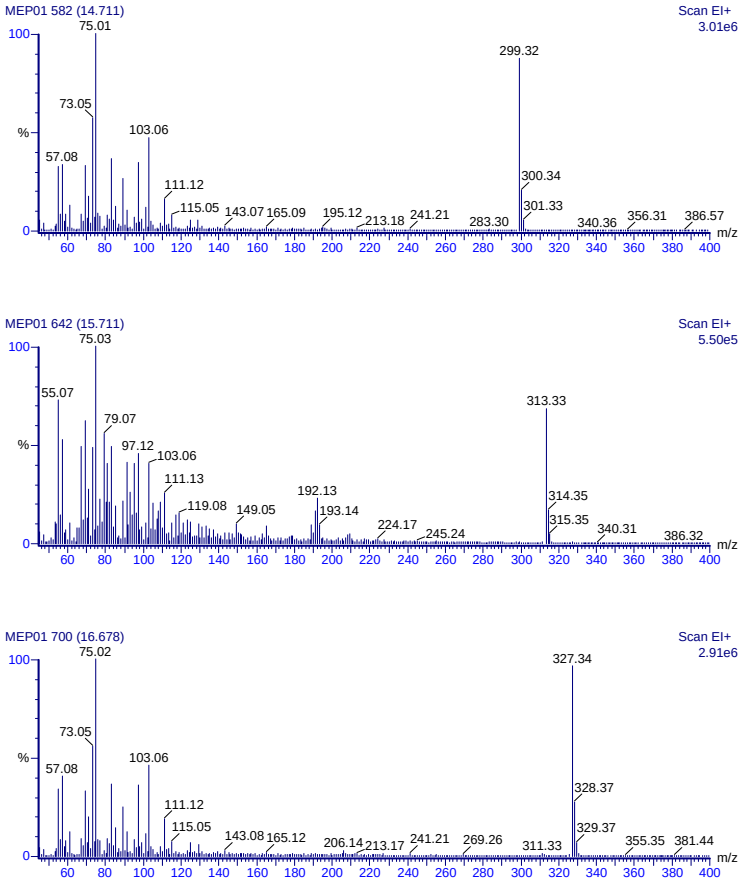

Figure 5.5 Mass spectra of the C_{16}, C_{17} and C_{18} *n*-alcohols. The characteristic ions (M^+ - 15) can clearly be seen in each case.

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Table 5.2 A list of the key fragments ($M^+ - CH_3$) for fatty alcohol identification by GC-MS analysis. The *iso* and *anteiso* branched components are also included with the parent C_n *m/z*.

Carbon Number	*m/z*	Carbon Number	*m/z*
10	215	20	369
11	229	21	383
12	243	22	397
13	257	23	411
14	271	24	425
15	285	25	439
16	299	26	453
17	313	27	467
18	327	28	481
19	341	29	495
20	355	30	509

Comment on inter-laboratory comparisons

No reports of inter-laboratory comparisons have been found. It is possible that since these compounds are not routinely reported, that one has not yet been carried out. Considering the different extraction routes possible (non-polar solvent then saponification vs direct saponification into a polar solvent), effort may need to be directed in this direction. A further aspect is the magnitude of the free alcohols derived from anthropogenic sources compared to naturally derived wax alcohols.

What don't we know?

1. Context – relative proportion of alcohols coming from natural & detergent sources in (a) STP effluents and (b) the environment generally.

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Chapter 6. Environmental Concentrations *(What's out there? Has it changed much (core data) and are there "patterns" in the distributions that tell us about sources or degradation pathways).*

This chapter sets out to describe the environmental concentrations of fatty alcohols around the world. It is approached by initially reviewing a range of studies that have measured such compounds and then synthesising these data to determine if patterns exist that may be explainable by geographic location or proximity to anthropogenic sources. One medium that falls between the concentrations in organisms (Chapter 3) and the concentrations in inert environmental samples (this Chapter) is faecal matter either from land drainage or through sewage treatment plants (STPs). These sources are considered in Chapter 4 on Environmental Transformations.

Compared to other groups of compounds, fatty alcohols have received relatively little attention in environmental studies. This may be due to the occurrence of better lipid biomarkers for determining source (*e.g.* the sterols, see Mudge and Norris (1997)) or more sensitive indicators of degradation (*e.g.* the fatty acids, see Haddad *et al.* (1992). On the positive side, however, fatty alcohols are extracted along with sterols and other biomarkers and so many studies do generate these data. Of these, few use the alcohols in a diagnostic manner and the data are only touched upon in many papers or remain in unpublished Masters or Doctoral theses. In compiling this Chapter, as many original datasets as possible have been collected and the raw data are presented in the Appendix (Microsoft Excel file included). For each study, a brief overview of the purpose is given followed by some concentration data and distributions.

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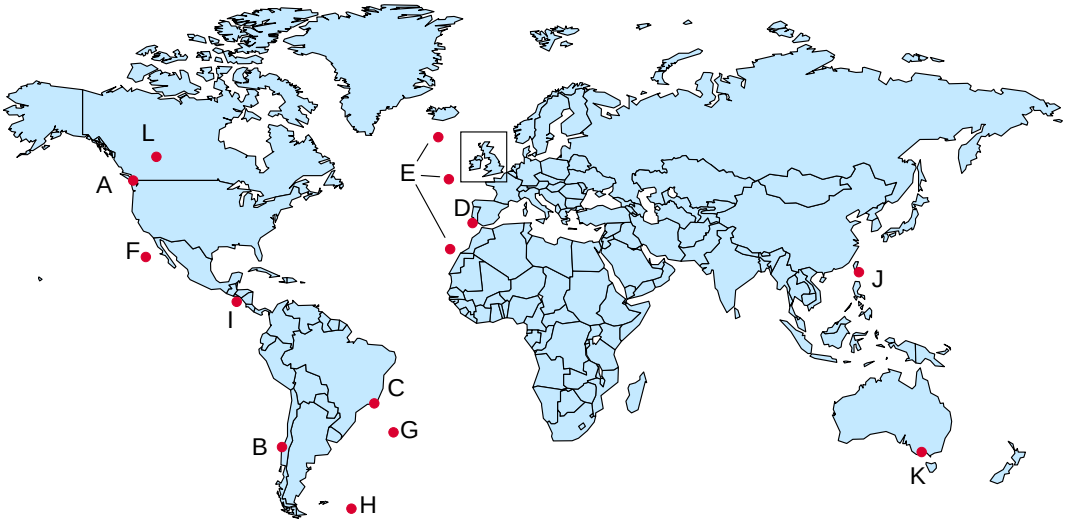

Figure 6.1 Map of study locations with reported fatty alcohol data. Data locations for the UK are shown in a separate figure (Figure 6.2)

Table 6.1 Study locations, sample dates and authors of fatty alcohol data relating to the map in Figure 6.1.

Code	Location	Country	Sample type	Date	Reference
A	Victoria Harbour, BC	Canada	Estuarine surface sediments	July, 1998	(Mudge and Lintern, 1999)
B1	Concepcíon Bay	Chile	Marine surface sediments	January, 2000	(Seguel *et al.*, 2001)
B2	San Vicente Bay	Chile	Marine surface sediments	January, 1998	(Mudge and Seguel, 1999)
C	Rio de Janeiro	Brasil	Embayment surface sediments	December, 2000	Mudge (unpub)
D1	Arade Estuary	Portugal	Estuarine surface sediments	June, 1997	(Mudge *et al.*, 1998)
D2	Ria Formosa lagoon	Portugal	Lagoonal surface sediments	June, 1995	Mudge (unpub)
D3	Ria Formosa lagoon	Portugal	Lagoonal suspended	June, 2002	(Mudge and Duce, 2005)

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			sediments		
D4	Ria Formosa lagoon	Portugal	Shallow core	June, 2001	(Unsworth, 2001)
E	E. North Atlantic		Oceanic cores	Summers of 1990 - 1991	(Madureira, 1994)
F	San Miguel Gap, California	USA	Oceanic cores	Early 1980's(?)	(McEvoy, 1983)
G	Rio Grande Rise (516F of leg 72 ODP)	Brazil	Oceanic cores	Early 1980's(?)	(Howell, 1984)
H	Falkland Plateau (511 of leg 71 ODP)	S. Atlantic	Oceanic cores	Early 1980's(?)	(Howell, 1984)
I	Guatemalan Basin (Legs 66 & 67 ODP)	Central America	Oceanic cores	Early 1980's(?)	(Howell, 1984)
J1	Continental slope, SW of Taiwan	Taiwan	Oceanic surface sediments	1996(?)	(Jeng *et al.*, 1997)
J2	East China Sea, N of Taiwan	Taiwan	Oceanic surface sediments	May – June, 1999	(Jeng and Huh, 2004)
K	Pasture land, Southern Australia	Australia	Freshwater runoff	November, 2000	(Nash *et al.*, 2005)
L	Prairie Zone soils, Alberta	Canada	Terrestrial soils	2003(?)	(Otto *et al.*, 2005)

The majority of studies of environmental fatty alcohols investigate sediments or soils. This is directly related to the non-polar nature of these compounds either as free fatty alcohols or as bound wax esters. In both cases, very small proportions of the totals will be in the dissolved phase; if water samples are taken, the fatty alcohols are present predominantly on the suspended particulate matter.

This is in direct contrast to the occurrence of fatty alcohols polyethoxylates; the long repeating chain of $–CH_2CH_2O–$ dramatically increases the water solubility and will alter the transport of the compounds accordingly. However, there will be ether cleavage, shortening of the ethoxylate chain and production from alcohol sulphates through time in sewage treatment plants (Itrich and Federle, 2004); with these

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processes the water solubility decreases and the compounds will eventually become free fatty alcohols and partition on to the solid phase accordingly. Therefore, studies of sedimentary material will contain fatty alcohols derived from both natural and anthropogenic sources but the transport paths may be different.

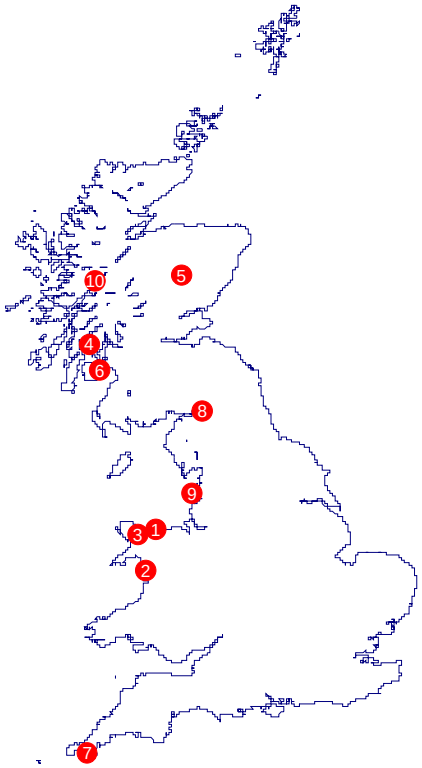

Figure 6.2 Locations of fatty alcohol data collected in the UK

Table 6.2 UK data locations

Code	Location	Country	Media	Date	Reference
1	Conwy Estuary	Wales	Estuarine core	April, 1997	(Mohd. Ali, 2003)
2	Mawddach Estuary	Wales	Estuarine surface sediments	June, 1996	(Mohd. Ali, 2003)
3	Menai Strait	Wales	Marine surface sediments	1996 – 2005	Mudge (unpub)
4	Loch Riddon	Scotland	Marine core	May, 1998	(Mohd. Ali, 2003)
5	Lochnagar	Scotland	Lake core		(Scott,

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					2004)
6	Clyde Sea	Scotland	Marine surface sediments	May, 1998	(Mohd. Ali, 2003)
7	Loe Pool	England	Brackish lagoon		(Pickering, 1987)
8	Bolton Fell	England	Peat core		(Nott, 2000)
9	Blackpool	England	Beach sediments	Summer, 2000	(Mudge, 2001)
10a	Loch Lochy	Scotland	Freshwater core	November, 2000	(Hotham, 2001)
10b	Loch Eil	Scotland	Marine core	November, 2000	(Hotham, 2001)

Global Locations

A. Victoria Harbour, BC – Surface Sediments

A study (Mudge and Lintern, 1999) was undertaken in Victoria Harbour, BC, Canada (Figure 6.3) to assess the extent of sewage contamination; bacterial studies had suggested the upper reaches were unaffected by materials discharged near the harbour. The study investigated sterols principally but also reported fatty acids and fatty alcohols. GC-MS analysis of the samples identified 37 fatty alcohols in surface sediment samples after alkaline saponification. In these samples, the C_{22} fatty alcohol had the highest mean concentration (193 $\mu g.g^{-1}$) and may have been directly related to the presence of wood chips in the samples. The distribution between long and short chain fatty alcohols changes between sites in the system with more short chain fatty alcohols near the marine end (C_{14}), longer chain fatty alcohols in upper reaches (C_{22} & C_{24}) with a mixture of mid-length compounds in the intervening sections (C_{16}, C_{18} & C_{20} dominant) (Figure 6.4). The results are not as clear-cut as implied since terrestrial organic matter in the form of by-products from wood processing could be found at all sites.

This unusual heterogeneity can be seen in the traditional marker ratios such as C_{24} / C_{16} where the longer chain alcohol is derived from terrestrial plants and the latter one from marine organisms. Figure 6.5 shows this ratio for samples organised according

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to their distance from the sea; samples to the left are closest to the marine end while those at the right of the figure are from the upper reaches. There is no gradient in this marker which shows a wide range of values with no obvious trend probably due to the wood chip distribution. Concentrations of these fatty alcohols were significantly greater than in many of the other similar sediment studies where wood chips were absent.

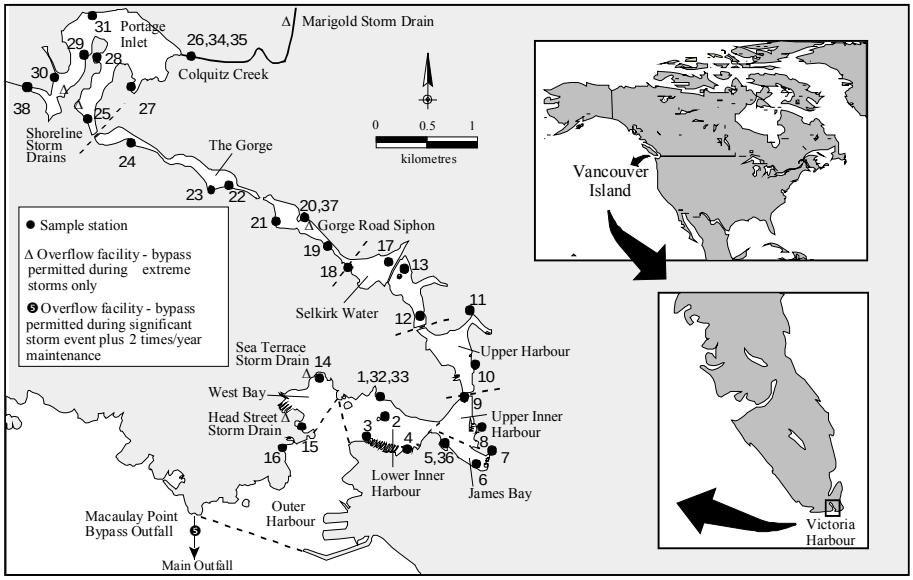

Figure 6.3 The sampling locations (1-38) in Victoria Harbour cited in (Mudge and Lintern, 1999).

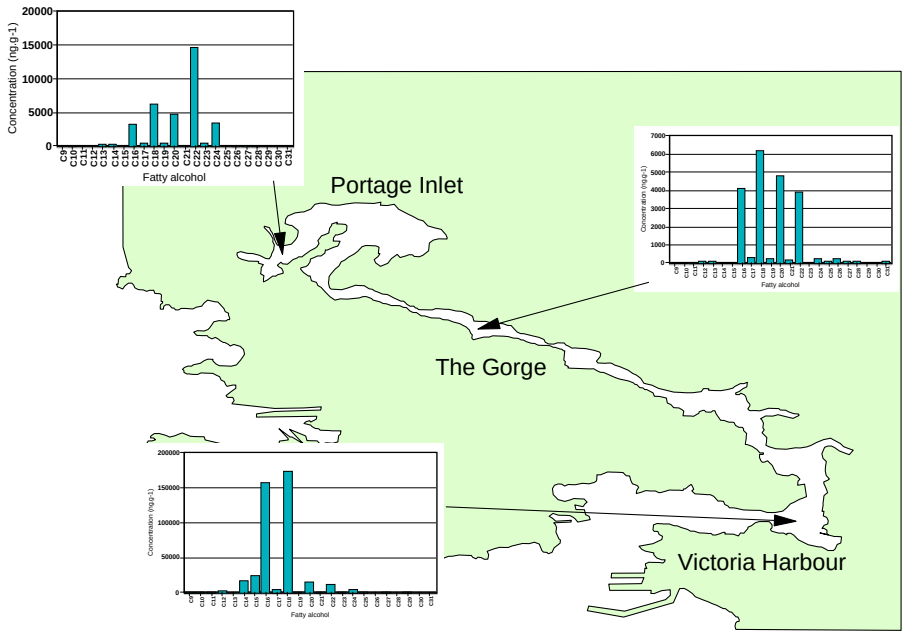

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Figure 6.4 Distribution of fatty alcohols in surface sediment samples from Victoria Harbour, BC, Canada. Representative samples show the change from short chain moieties near the seaward end to longer chains in the upper reaches (Mudge and Lintern, 1999).

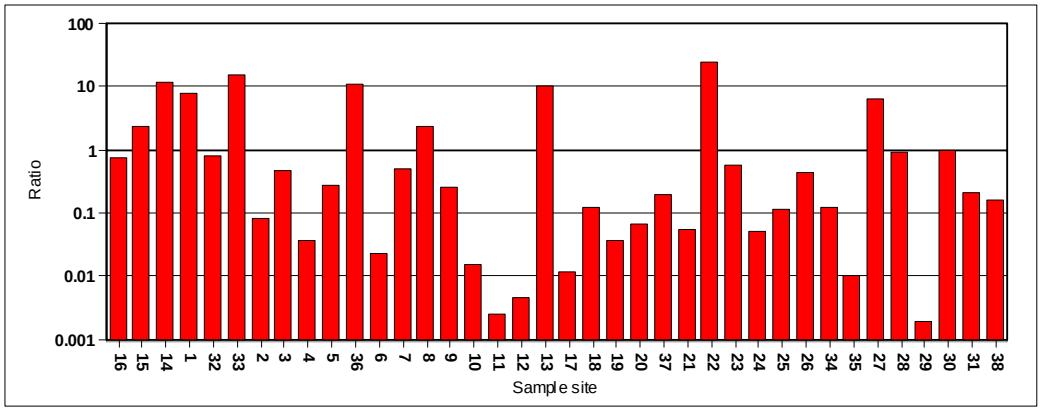

Figure 6.5 The ratio of C_{24} to C_{16} in surface sediment samples from Victoria Harbour. The data are organised by increasing distance from the sea (from left to right). Sample locations may be seen in Figure 6.3.

B1. Concepcíon Bay, Chile

B2. San Vicente Bay, Chile

In a study of two anthropogenically contaminated bays in Chile (Figure 6.6), sub-tidal surface sediments were collected and extracted by alkaline saponification (Mudge and Seguel, 1997). Fatty alcohol data were collected together with alkanes, fatty acids and sterols. Within the alcohol data, sediment concentrations were similar to those found elsewhere although the profile was not quite as expected (Figure 6.7).

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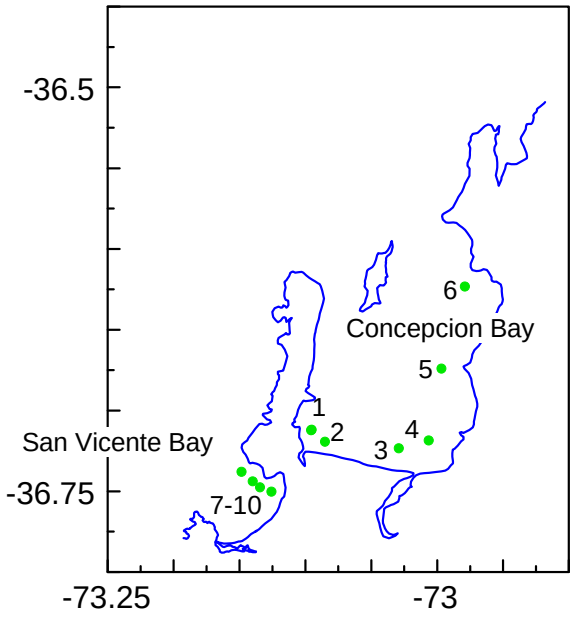

Figure 6.6 Sampling locations for sub-tidal surface sediments in Chile (Mudge and Seguel, 1997).

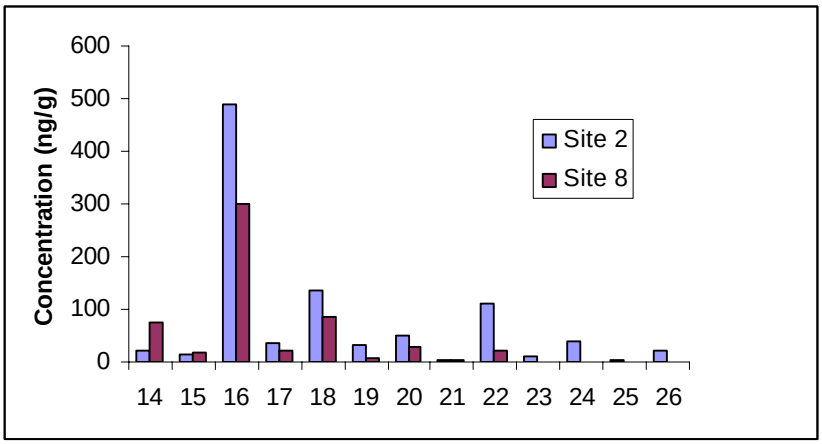

Figure 6.7 Fatty alcohol profile for two samples, one from Concepcíon Bay (Site 2) and one from San Vicente Bay (site 8).

All of the samples were relatively depleted in long chain (>C_{20}) compounds. These waters are fully saline but Concepcíon Bay does receive terrestrial runoff. The surrounding areas are densely wooded and long chain compounds might be expected. However, as Figure 6.7 indicates, the short chain C_{16} dominates in both bays indicating a strongly marine signature. The lack of a terrestrial signature both in the fatty alcohol and sterol data prompted a further investigation of this bay (Seguel *et al.*,

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2001). Sub-tidal surface sediments were again collected but only from Concepcíon Bay (Figure 6.8).

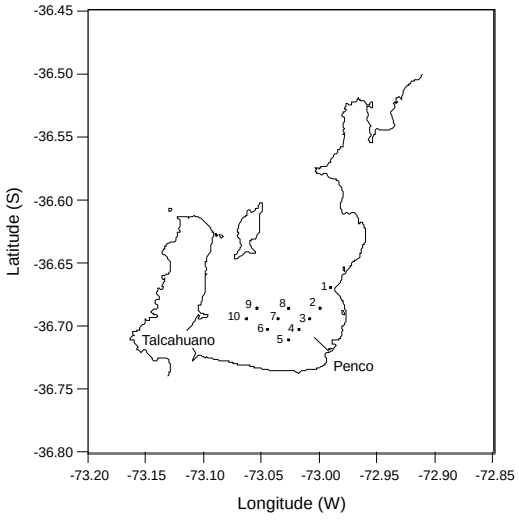

Figure 6.8 Sample locations for sediments in Concepcíon Bay.

These Concepcíon Bay sediments were soft, black, reducing muds, rich in organic matter with a thin overlying flocculent layer with anoxic conditions prevailing at the sediment-water interface during most of the year (Farías *et al.*, 1996). Domestic wastewater from several small outfalls, the Andalien River and from two pipes, has long been discharged in different places of Concepcíon Bay, although a primary sewage treatment plant has been built to serve the nearby coastal city of Penco – Lirquen (Lepez, 1996). The effluent discharge pipe extends 1300m into the bay; the depth of the water column at the disposal site is 25m, with a discharge rate of 95 litres per second, 14m above the seabed.

All samples had high concentrations of phytol; this compound was the most abundant fatty alcohol with a range of 0.1 to 98.6 $\mu g.g^{-1}$ dry wt. This suggested that the study area had high productivity of micro-algae. Straight chain fatty alcohols between C_{12} and C_{30} were identified in the samples together with several short chain, odd carbon number branched compounds. In general, sites close to the sewage disposal had the highest total concentrations especially in the top 0 to 15 cm of the sediment column and ranged from 0.1 to 10 $\mu g.g^{-1}$ dry wt (excluding phytol). Small amounts of the branched fatty alcohols *iso*C15:0, *iso*C17:0 and the corresponding *anteiso* forms were also found; these fatty alcohols had a clear prevalence at sites 9 to 10 (see Figure 6.8 for location) in the 0 to 15 cm range (9.1 to 38.4% of total alcohols excluding phytol).

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In all samples there was an even-over-odd dominance in the *n*-alcohols with fatty alcohols C_{16}, C_{26} and C_{28} dominating in all sites. The ratio between odd chain length and even chain length of saturated fatty alcohols $(\sum C_{13} - C_{29}) / (\sum C_{14} - C_{30})$ has been used as an indicator of bacterial activity; in Concepcíon Bay this ratio was essentially small at most sites, although the range was from 0.01 to 0.52. Although most ratios had low values, sites 9 and 10 had the highest values of this bacterial indicator between 5 to 10 cm and sites 3 and 7 had the highest values at 10 to 20 cm.

The ratio between long and short chain saturated fatty alcohols can be used to indicate the terrestrial input into the system; values greater than 1 are indicative of long chain fatty alcohols potentially from terrestrial sources. However, short chain compounds are more readily degraded than long chain ones and this may alter the ratio. The distribution of this ratio $(\sum C_{19} - C_{30}) / (\sum C_{12} - C_{18})$ in Concepcíon Bay showed that sites 2 to 6 close to the sewer had the highest values indicating mainly terrestrial sources (Figure 6.9); this was also true of the sterol biomarker ratio although this value did not exceed 0.4. This may indicate a possible terrestrial runoff source associated with the Andalien River or associated with the sewage input itself.

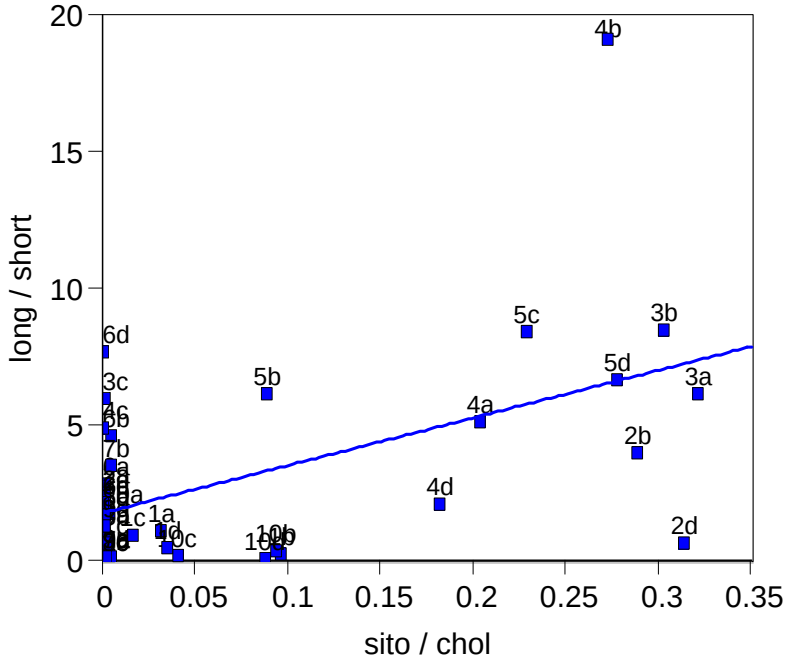

Figure 6.9 The long (>C_{18}) / short (<C_{19}) ratio plotted against the sterol marker for terrestrial organic matter (β-sitosterol / cholesterol).

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C. Rio de Janeiro – surface sediments in a contaminated bay

Rio de Janeiro is located on the South Atlantic coast of Brazil and around the Guanabara Bay. The bay has a surface area of 384 km^2 and a perimeter of 131 km. The mean water depth is 5.7m and has a max depth of 30m in the entrance channel such that 50% flushing of water in the bay takes 11.4 days. To the north, it is bordered by 90 km^2 of fringing mangroves, of which 43 km^2 is an Environmentally Protected Area. The bay receives wastes and runoff from the catchment; sewage from nearly 8 million people discharges directly into bay with little treatment and there is considerable waste from industry and shipping. The port area is continuously dredged.

In an unpublished study, Mudge and Neto collected surface sediments with a grab from a small boat. Samples were sealed and returned to the UK for analysis after alkaline saponification. The full fatty alcohol results are presented in the appendix. The ratio C_{22} / C_{16} is presented spatially in Figure 6.10 as a classed posting together with the C_{16} concentration.

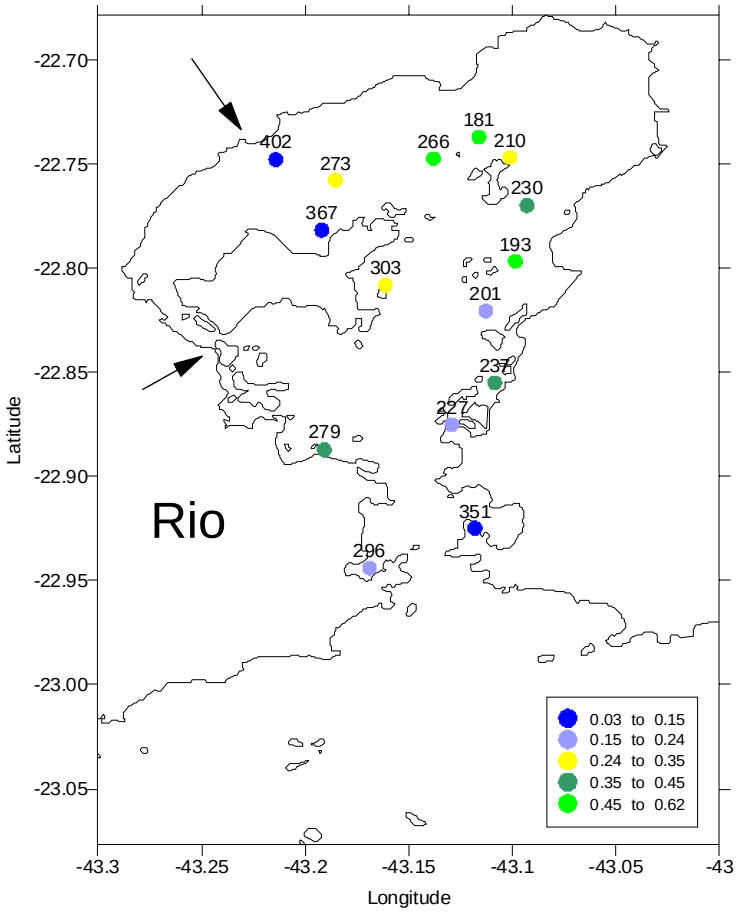

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Figure 6.10 Spatial distribution of the C_{22} / C_{16} ratio in Guanabara Bay, Brazil shown as a classed posting. Each sampling site is also labelled with the C_{16} concentration in $ng.g^{-1}$.

The highest values of the ratio indicating terrestrial organic matter are located in the north east of the bay adjacent to the mangrove swamps. The lowest values are toward the outer reaches most influenced by the sea and in the north west corner near the outfalls from the city and the large oil refinery belonging to PetroBras. The reason for these sites being high in C_{16} is not immediately obvious except that it also has the highest sterol sewage indicators as well. A comparison of the fatty alcohol profiles at two of the sites (Figure 6.11) shows how similar they are despite site 10 having the greatest 5β-coprostanol / cholesterol ratio, an indicator of human sewage and site 15 having a value almost seven times less. It is possible that a component of these alcohols may have been derived from an anthropogenic source rather than a natural one.

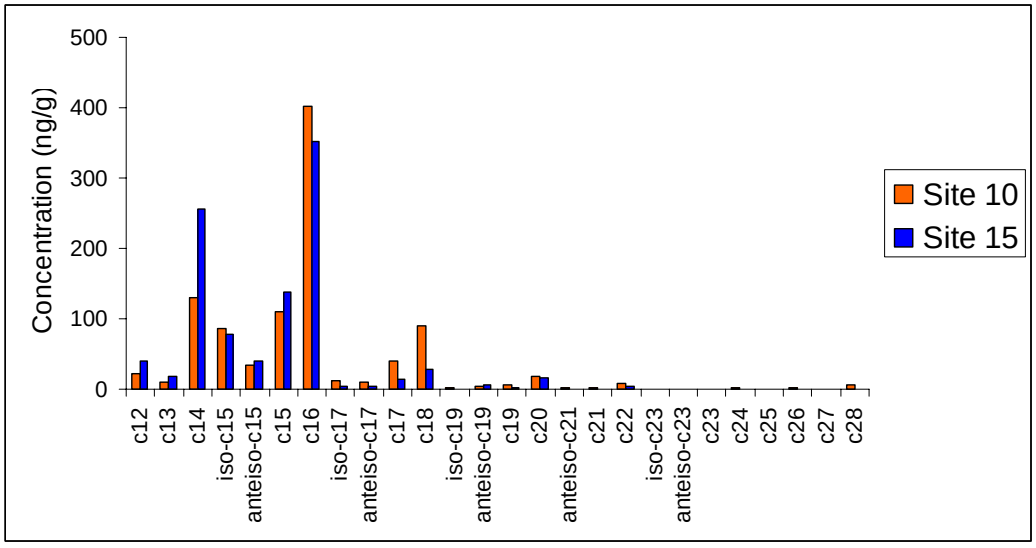

Figure 6.11 The concentration profile for fatty alcohols at site 10 near the oil refinery and sewage discharge compared to site 15 near the mouth of the bay.

D1. Arade Estuary

The Arade Estuary in Southern Portugal comprises the River Arade and the tributaries Odelouca and Boina (Figure 6.12). In the early 1990's, there was a marked decline in the environmental quality of the waters due to effluent from agriculture, aquaculture,

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fish canning, boat building and sewage from the towns and villages within the watershed of the estuary. In 1993, the authorities responsible for management of this region, in conjunction with the European Union, funded a viability study for improving the environment of the estuary. Part of the study included a proposal for a dredging program to allowing navigation of boats of up to 2 metres draught on the Arade at any stage of the tide between Portimão and Silves.

A study was undertaken (Mudge *et al.*, 1998) to investigate the state of the estuarine system in the summer of 1997 using lipid biomarkers. The study focussed on the sterol compounds as indicators of sewage, the distribution of which can be seen in the contour map of Figure 6.12. Fatty alcohols were also measured and the data were patchy and only ratios reported; the C_{22} / C_{16} ratio was used to indicate terrestrial matter but in this case the values indicted greatest influence in the middle reaches (sites 4 – 7) near the river junctions. At this location, the estuary was wide and shallow and many vascular plants grew that may have been the source of the long chain compounds. At these mid-estuary sites, the ratio was approximately 1.0 compared to an average of ~0.13 at the remaining sites.

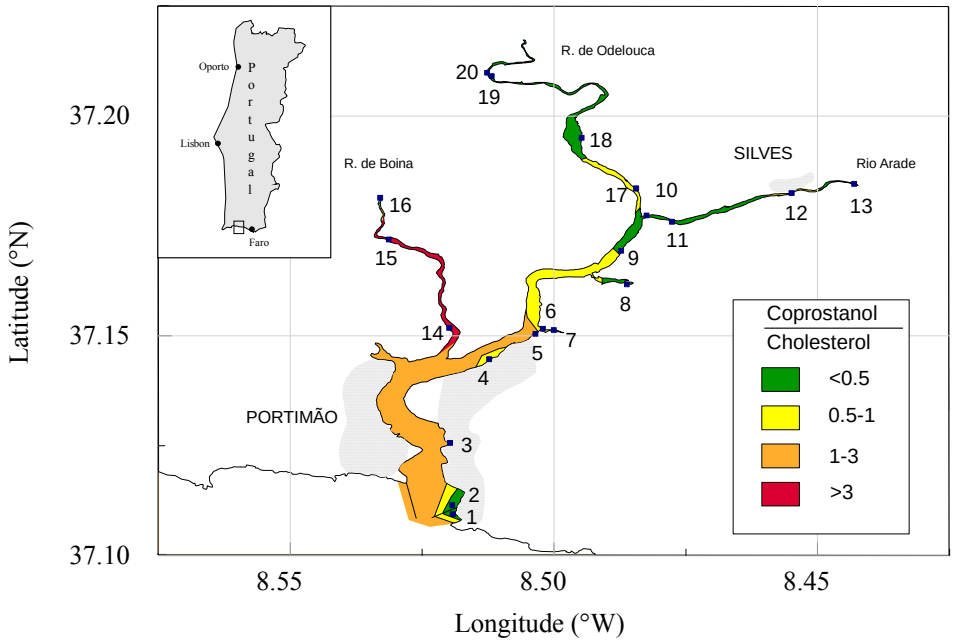

Figure 6.12 Location map of the Arade Estuary, Portugal and the 5β-coprostanol / cholesterol ratio, an indicator of human sewage.

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D2. Ria Formosa lagoon – surface sediments

The Ria Formosa lagoon in southern Portugal is the largest lagoon in Europe with a length of ~55km. The system receives inputs from several sources including domestic sewage from the towns and cities of the coastal zone, especially Faro; there is terrestrial runoff of nutrients as the hinterland is intensively farmed but riverine input is small and confined to a few rivers of which only one runs throughout the entire year. There have been several studies of the system for a range of contaminants including PCBs (Barreira *et al.*, 2005), sterols (Mudge *et al.*, 1999), fatty acids (Mudge *et al.*, 1998), nutrients (Newton and Mudge, 2005) and metals (Bebianno, 1995). As part of an earlier study, Mudge & co-workers collected surface sediments and analysed these after alkaline saponification for sterols and fatty acids, the results of which have been reported in the literature. However, they also collected aliphatic and aromatic hydrocarbons and fatty alcohols. These data have yet to be reported. The fatty alcohol data are presented here and as raw concentrations in the Appendix.

Fatty alcohols ranging from C_9 to C_{32} were measured together with a number of branched chain compounds: C_{16} was present in the highest concentration with a maximum value of 1348 $ng.g^{-1}$ DW. An example of the profile for two different sites can be seen in Figure 6.13. Site 35 is close to the major sewage outfall for the city of Faro and has high faecal sterol markers (Mudge *et al.*, 1999) while site 43 is close to the wide sandy entrance to the North Atlantic at Armona. The data from Figure 6.13 show how there are more long chain (C_{20}+) compounds in the inner site influenced by the sewage discharge than the outer site which is marginally enriched in the short chain compounds compared to site 35.

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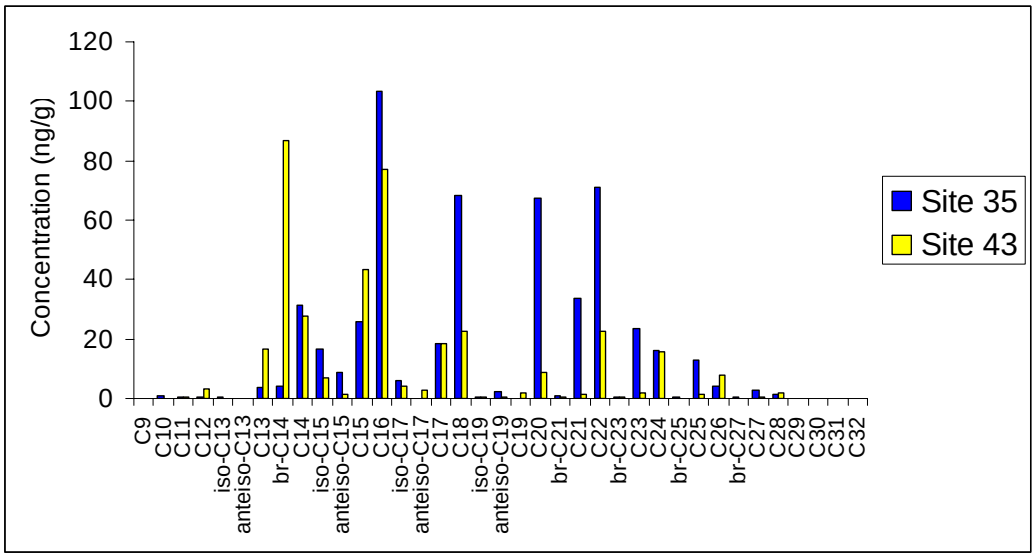

Figure 6.13 The fatty alcohol profile for two contrasting sites in the Ria Formosa lagoon.

It is possible to view these data spatially to determine the regions where compounds are associating. Figure 6.14a presents the sum of the $C_9 – C_{14}$ fatty alcohol concentrations, generally assumed to be the marine component while Figure 6.14b shows the ratio of $C_{24} – C_{32}$ / $C_9 – C_{14}$, high values of which should indicate the most likely terrestrially derived organic matter accumulation sites. Highest concentrations (blue circles) of the short chain compounds can be seen in the Armona inlet which supports a community of sand dwelling organisms including micro-algae (Mudge *et al.*, 1998); low values (red circles) can be found in a diversity of habitats – in the river (site 14), in the main navigation channel (which is dredged, sites 24 – 26) and near seagrass beds (sites 3 – 4 to the west and 39 – 40 in the centre of the lagoon).

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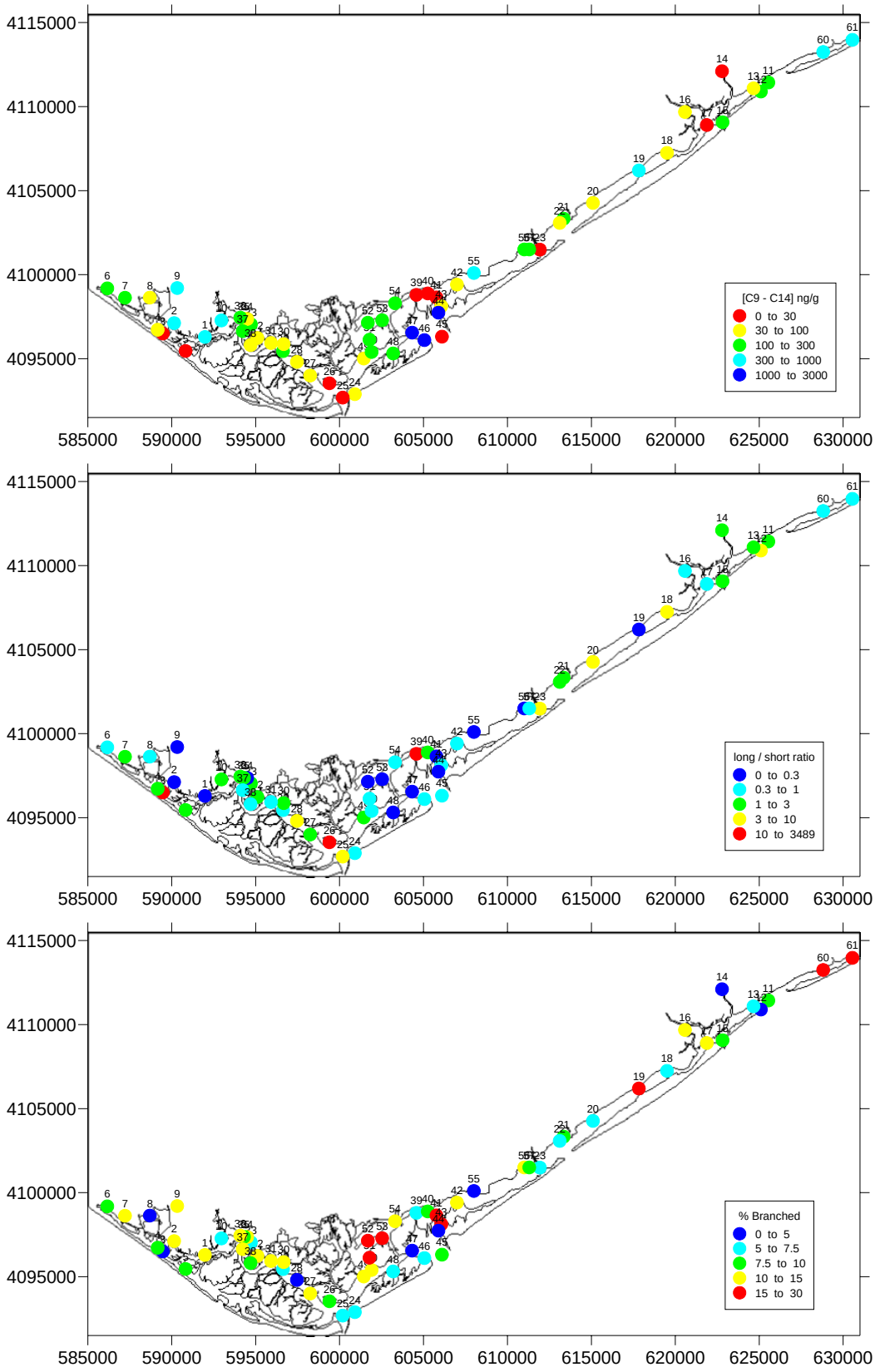

Figure 6.14 (a) the concentration of the short chain fatty alcohols, (b) the long / short ratio and (c) the percentage branched chains across the Ria Formosa, Portugal.

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One of the measures of bacterial activity in any system is the percentage of all fatty alcohols in a branched configuration. In these samples, this measure can be seen in Figure 6.14c. The highest values were associated with sites that are known to be areas of fine grain sediment accumulation and the sediments may be anaerobic at depth. As with other studies, the fatty alcohols in this form do not appear to be very good indicators of sewage inputs unlike the sterols (Mudge *et al.*, 1999) however, when examined used multivariate techniques (Chapter 7), greater discrimination between sites can be seen.

D3. Ria Formosa lagoon – suspended and settled sediments

A further study was undertaken to determine the sources and transport paths of sewage derived materials in this lagoon. The data are report in Mudge and Duce (2005). Key factors in this study were the collection and analysis of suspended particulate matter and its relationship with settled sediments and potential origins of the organic matter. The concentrations of fatty alcohols were significantly greater in the potential source materials (*e.g.* sewage disposal sites) than either the suspended particulate matter or the settled sediments (Figure 6.15). This decrease is not surprising given the labile nature of these compounds.

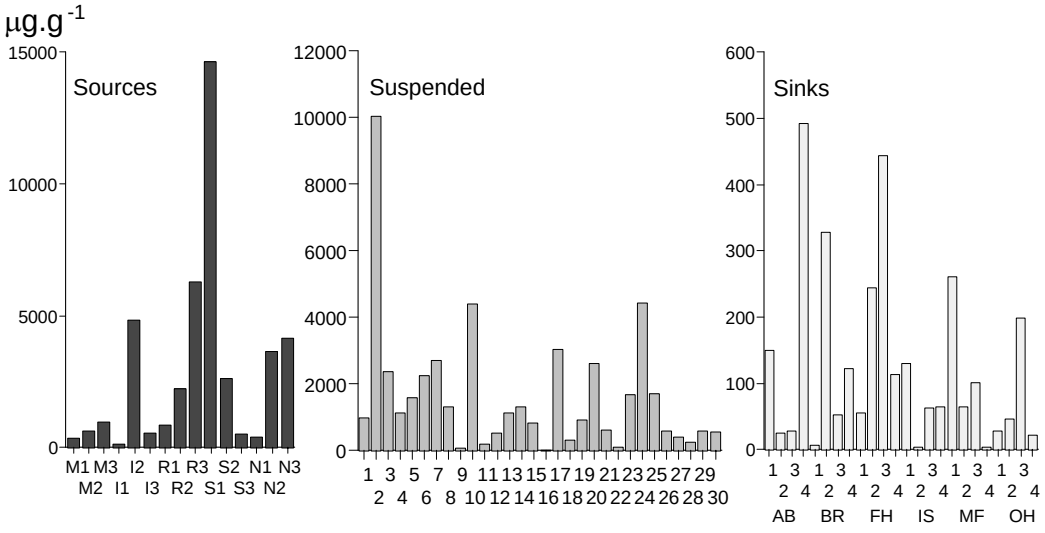

Figure 6.15 Total fatty alcohol concentrations in potential source materials, suspended sediments and settled sediments (sinks) in the Ria Formosa Lagoon, Portugal. (Data from Mudge and Duce (2005)).

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The fatty alcohols may also give source information based on the ratios of key components such as long chain (terrestrial) compounds and short chain (marine) compounds. Plots of these measures suggested a subtle change in profile between the source and suspended matter and the settled sediments where the relative proportion of the long chain compounds increased (Figure 6.16). This is due in part to the relative resistance of these compounds to degradation.

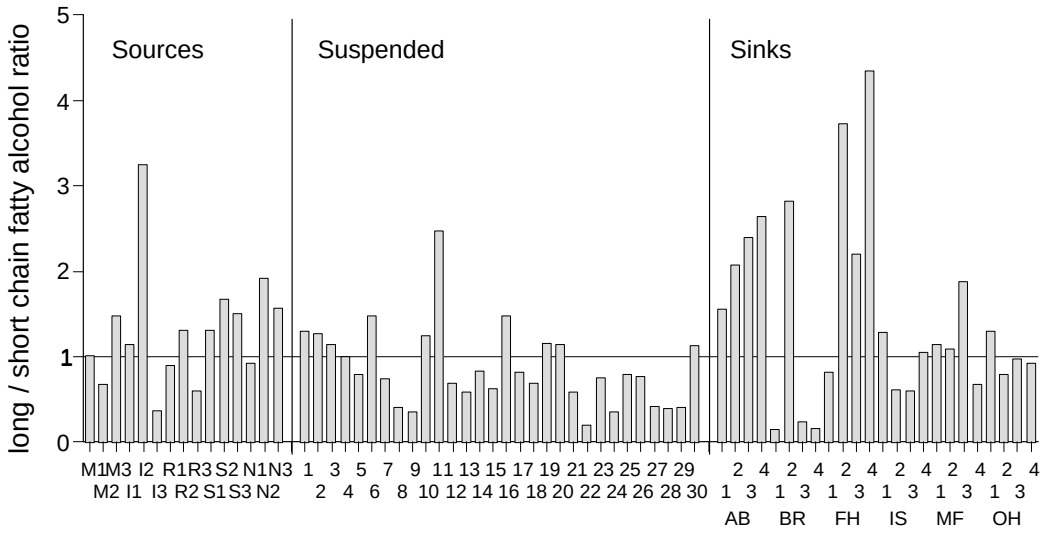

Figure 6.16 The ratio of long chain and short chain fatty alcohols in the sedimentary matter from the Ria Formosa.

The odd chain compounds may also be used to indicate bacterial sources. This ratio is not particularly diagnostic in this situation as Figure 6.17 shows; all sources types and all other sediments have mixed ratios with some showing high values of the ratio. In these cases, the bacterial biomass may be high although there is no clear trend across the locations.

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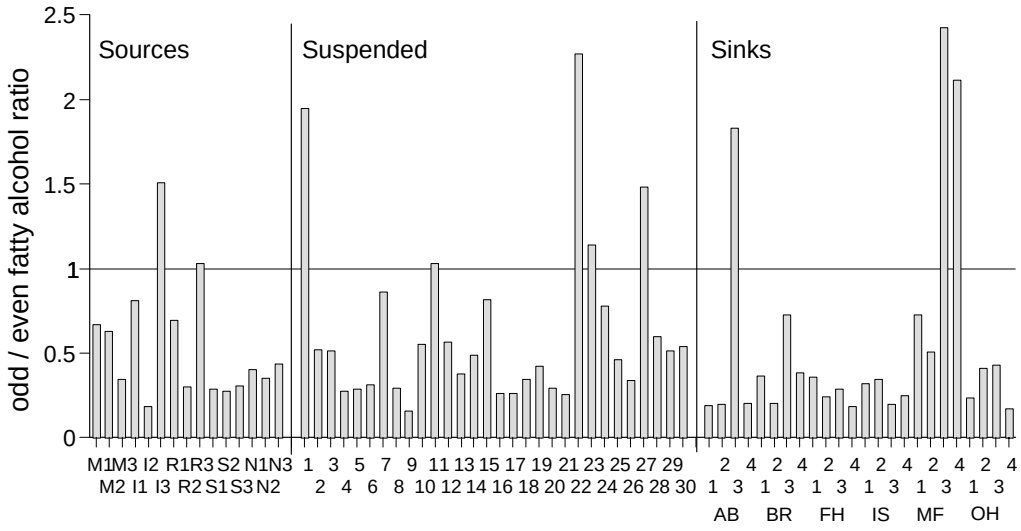

Figure 6.17 The odd chain to even chain ratio for fatty alcohols in the sedimentary matter from the Ria Formosa.

D4. Ria Formosa lagoon – shallow core from intertidal sediments

In a study of the potential environmental changes that are recorded in the sediments, (Unsworth, 2001) collected a core from the productive Ria Formosa Lagoon and extracted sterols, fatty alcohols and PAHs. The concentration data for key compounds are shown in Figure 6.18. The region is regularly bioturbated in the search for shellfish and few undisturbed core sites were available. The short chain compounds are present in relatively low concentrations compared to other sites in the lagoon (Mudge and Duce, 2005) and decrease with depth as might be expected from their degradability. The longer chain compounds are present in much lower concentrations as there is very little terrestrial runoff in to this coastal lagoon. Even so, the concentrations do decrease with depth indicating a degree of degradation with time.

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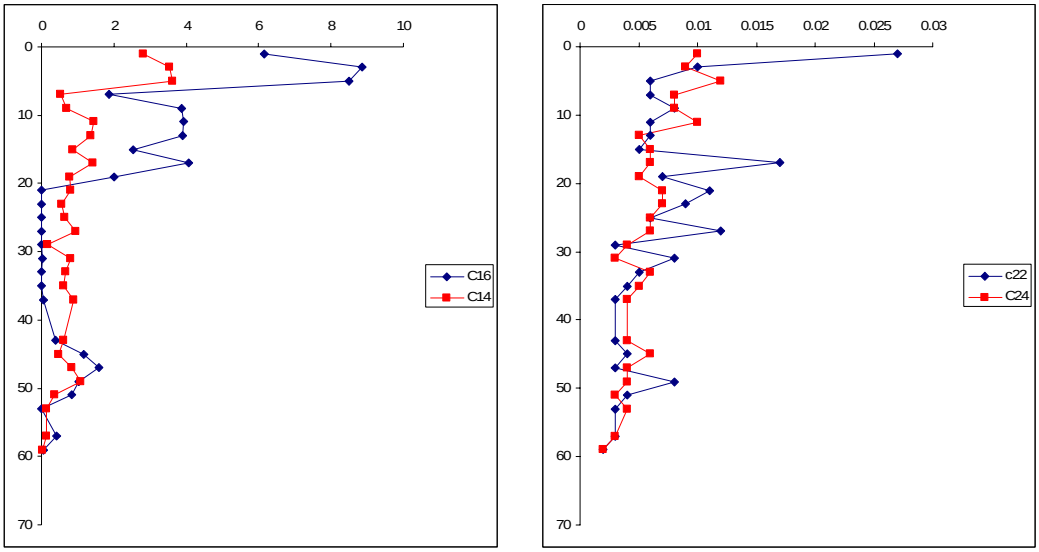

Figure 6.18 The concentration ($\mu g.g^{-1}$) of (a) short chain and (b) long chain fatty alcohols in a short core (60 cm) in the productive Ria Formosa lagoon, Portugal. (Data after Unsworth (2001).

E. Eastern North Atlantic

In a Ph.D. study at Bristol University, Madureira studied the lipids present within five cores from the eastern North Atlantic (see Figure 6.1 for approximate locations or Madureira's thesis, (Madureira, 1994)). The results include a suite of fatty alcohols from C_{16} to C_{28} obtained from the sediment cores after alkaline saponification. The raw concentration data is presented in the Appendix and in Figures 6.19 and 6.20. The distribution of the alcohols at each of the sites is biased toward the long chain moieties with maximal concentrations in the C_{22} to C_{26} length range although the long / short chain ratio does indicate enrichment in the short chain marine compounds near the surface.

If the $\log_e$ of the C_{16} and C_{26} compounds for an example core are taken, a plot against the accumulation rate of the sediment can give an indication of the degradation rate (Figure 6.21). In this case, the rate of loss of the C_{16} is greater than the C_{26} as might be expected from the chain length information. Here, the sedimentation rate has been assumed ($3mm.y^{-1}$) but since it is only used to compare the relative rates, the exact value is of less importance.

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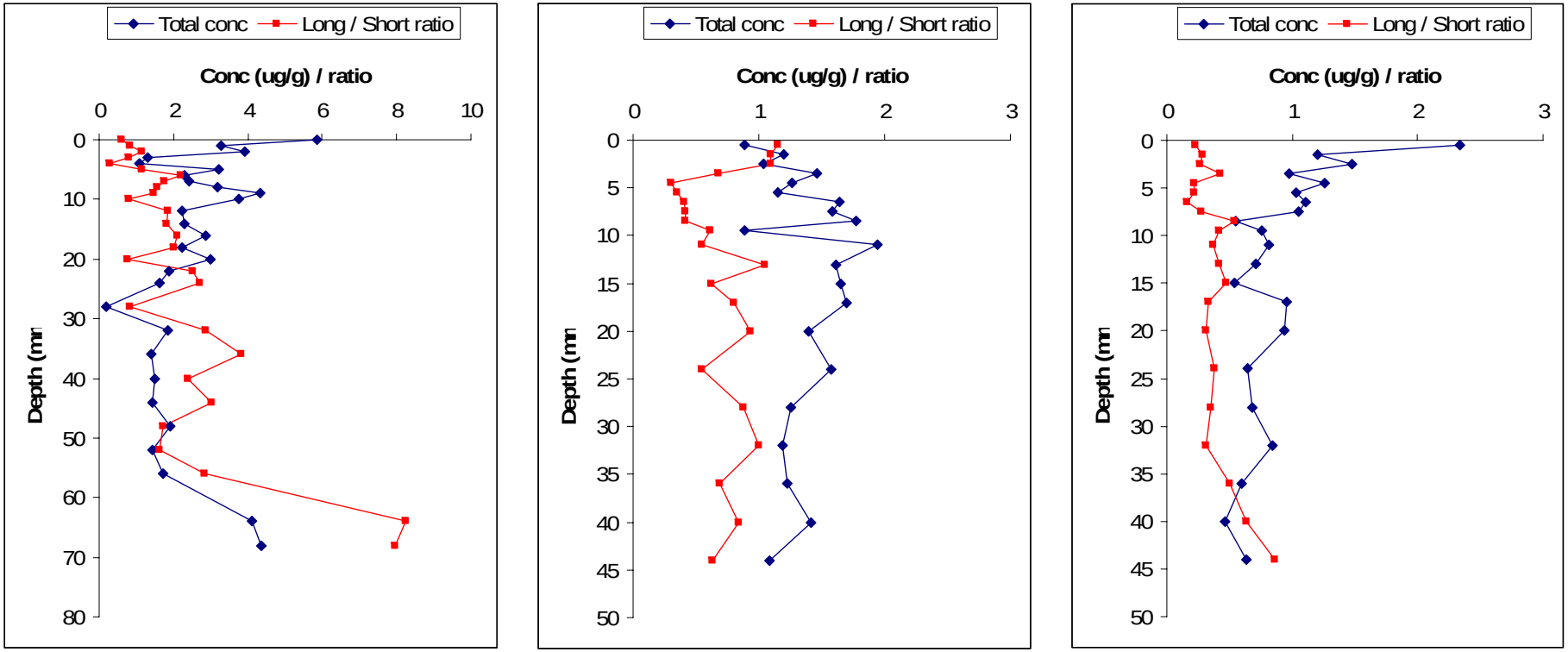

Figure 6.19 The total fatty alcohol concentration ($\mu g.g^{-1}$) and long ($C_{22} - C_{28}$) / short ($C_{16} - C_{20}$) ratio in three eastern North Atlantic cores. (a) 61N, (b) 59N and (c) 48N. Data after Madureira (1994).

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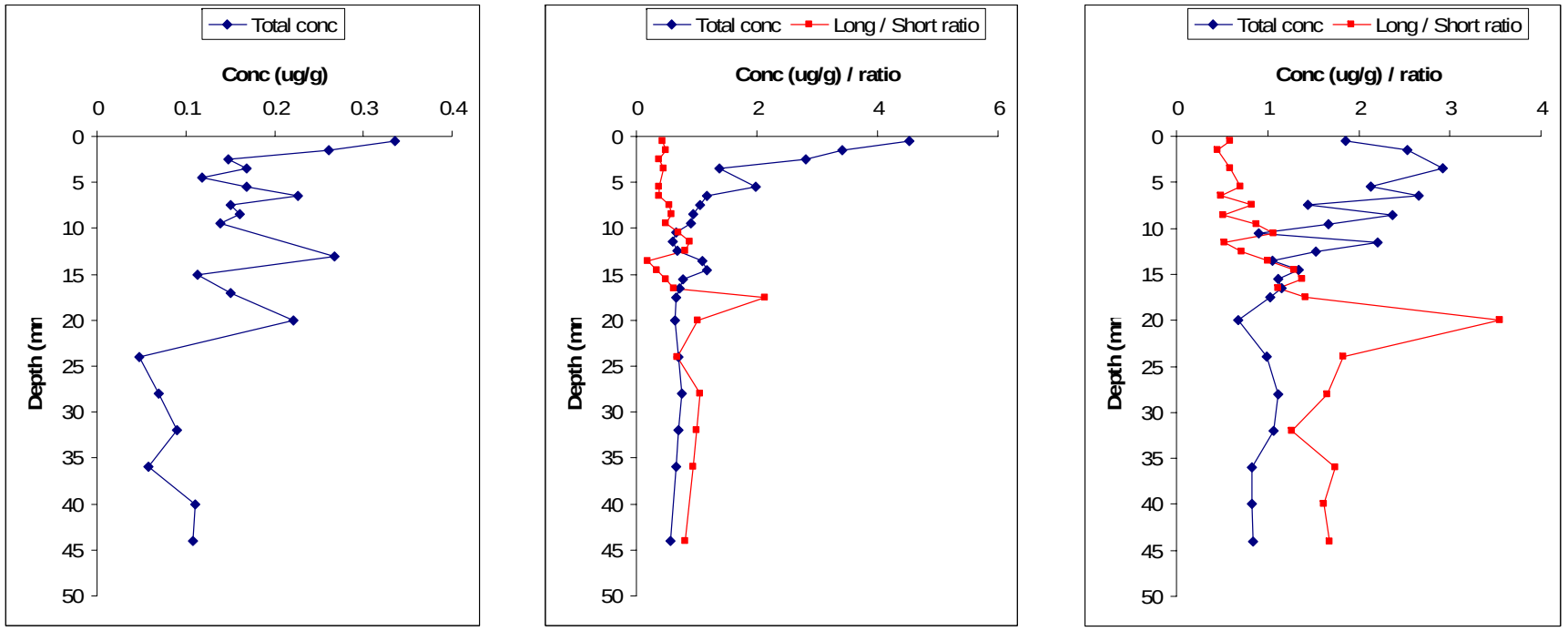

Figure 6.20 The total fatty alcohol concentration ($\mu g.g^{-1}$) and long ($C_{22} - C_{28}$) / short ($C_{16} - C_{20}$) ratio (where possible) in three eastern North Atlantic cores. (a) Bound lipids in 48N, (b) 32N and (c) 18N. Data after Madureira (1994).

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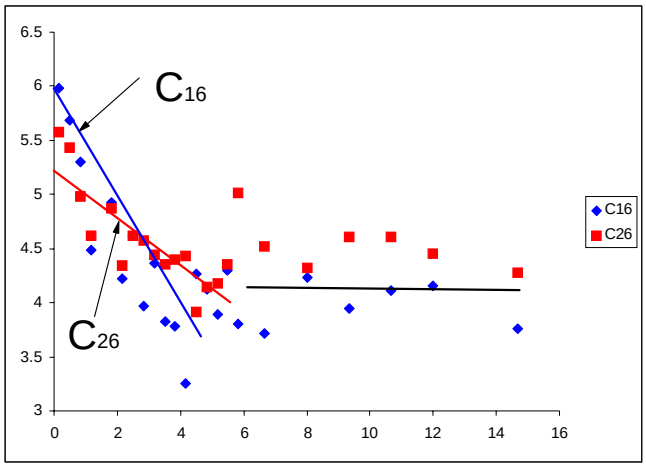

Figure 6.21 The relative degradation rates shown by a plot of the loge of the C_{16} and C_{26} fatty alcohols vs. a sediment accumulation rate (assumed to be 3mm.y^{-1}). Data for site 32N from Madureira (1994).

F. San Miguel Gap, California – long core

McEvoy (1983) conducted a study on lipids from a long core collected off the Californian coast. In his study, McEvoy first extracted lipids into a DCM : methanol solvent (2:1) and then saponified the lipids collected. Therefore, any wax bound fatty alcohols that were extractable in the DCM : methanol will be quantified in this method. Only bound, unextractable with DCM : methanol compounds will not be included.

The results are shown in Figure 6.22. Concentrations near the surface were almost 1000 ng.g^{-1} but decreased with depth. By 500m, no fatty alcohols were detectable although fatty acids could be recovered all the way down to 1000m below the surface.

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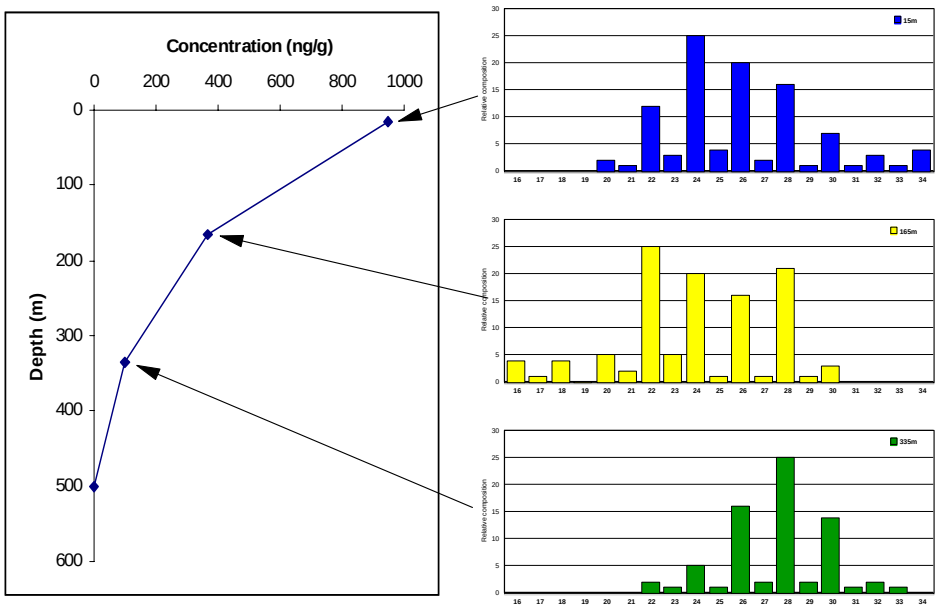

Figure 6.22 The total fatty alcohol in core sediments from the San Miguel Gap, California Data after McEvoy (1983).

As well as the concentrations decreasing down core, the profile of fatty alcohols also changed reflecting the preferential degradation of short chain compounds. The profile near the surface (Figure 6.22) shows C_{24} to be present in the highest proportion of the total alcohols which shifts towards C_{28} in the 335m sample. The intermediate sample has a different profile with a bimodal distribution and more short chain compounds present as well.

G. Rio Grande Rise (516F of leg 72 ODP), Brazil

As part of a series of analyses, Howell (1984) analysed the lipids in a core taken from the Rio Grande Rise off the coast of Brazil. This was part of Leg 72 of the Ocean Drilling Programme (ODP) cruise to this region. Sediments were extracted by DCM : methanol : hexane (2:1:1) initially and then saponified with KOH in methanol. The results shown in Figure 6.23 indicate a very substantial marine signature with a peak occurrence at C_{16} tailing to near zero by C_{22}; this indicates the almost complete absence of terrestrial organic matter with long chain fatty alcohols at this site. The concentration of the C_{16} alcohol is also substantially less (67 $ng.g^{-1}$) than those observed in more coastal environments (*e.g.* Ria Formosa up to 1348 $ng.g^{-1}$)

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indicating a limited *in situ* production or regeneration in the water column before reaching the sediments.

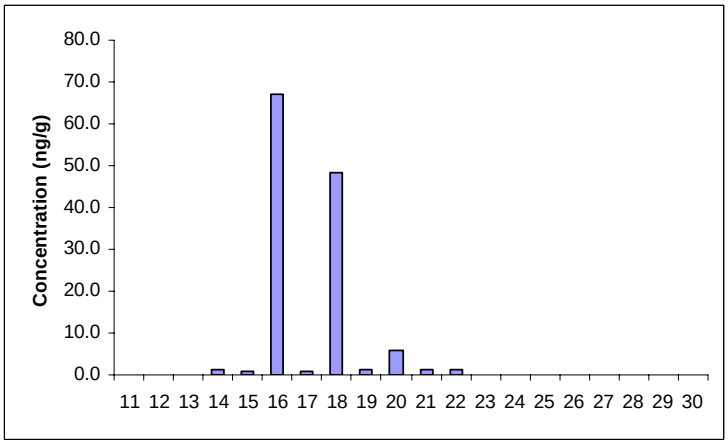

Figure 6.23 Fatty alcohol profile from sediments off Brazil on the Rio Grande Rise (after Howell (1984).

H. Falkland Plateau (511 of leg 71 ODP), S. Atlantic

Howell also studied a site to the east of the Falkland Islands on the side of Maurice Ewing Bank (Howell, 1984). After similar extractions and analyses, the results generated a profile even more biased toward short chain compounds than the sample off Brazil (Figure 6.24). Four profiles were averaged to give this picture but all show a dominance of the C_{12} with little or no long chain alcohols present. This again must represent an entirely marine signature with no transport of terrestrial organic matter obvious in the samples.

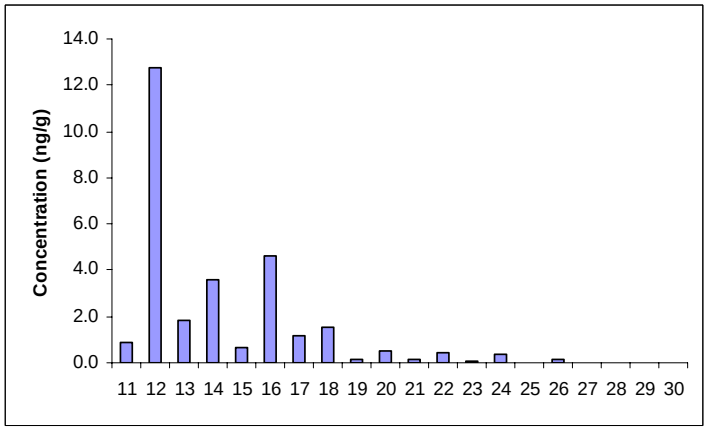

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Figure 6.24 The fatty alcohol profile from sediments on the Falklands Plateau (after Howell (1984).

I. Guatemalan Basin (Legs 66 & 67 ODP), Central America

A third group of samples from a tropical, near shore location in the Mid-America Trench off Mexico and Guatemala were analysed as before (Howell, 1984). These sites were substantially closer to shore even though they were collected from deep waters around the 2000m isobath. As a consequence of this proximity to a coastal tropical environment, the terrestrial markers are dominant and the total concentrations are substantially greater than those measured in the offshore sites. The profile mean profile expressed as proportions of the total concentration due to wide changes in concentration (from 337 to 3380 $ng.g^{-1}$) can be seen in Figure 6.25.

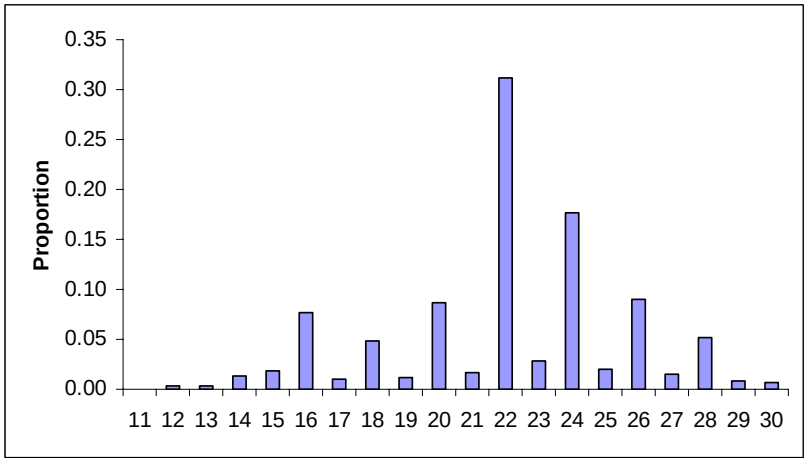

Figure 6.25 The fatty alcohol profile from sediments in the Guatemalan Basin (after Howell (1984).

Although a minor peak in the C_{16} can be seen in these data, the C_{22} is considerably more important a contributor to the total alcohols present. It may be concluded from these three studies that the proximity to terrestrial runoff is crucial in determining the fatty alcohol profile and not water depth. Marine production at remote oceanic sites is small compared to the terrestrial influence from continental runoff.

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J1. Continental slope, SW of Taiwan

In a study of degradation, Jeng *et al.* (1997) collected a box core from the continental slope of Taiwan in 354m of water. The sediments were sectioned every 4 cm and the fatty alcohols were extracted in a hexane : chloroform (2:3) solvent, partitioned in to chloroform : methanol (4:1) and hydrolysed overnight (presumably with KOH). Fatty alcohols from C_{14} to C_{28} were reported although the authors used C_{19} as an internal standard which is likely to be present in the samples naturally as well; no correction was made for this. The total concentrations in this core can be seen in Figure 6.26; high concentrations near the surface (1839 $ng.g^{-1}$ in the 0 – 4 cm fraction) decrease relatively rapidly to ~800 $ng.g^{-1}$ by 10cm and degrade with a slower rate at deeper depths. From these data, the authors calculate the degradation rate of fatty alcohols as between 0.010 and 0.007 y^{-1}; these aspects are reported in more detail in Chapter 4.

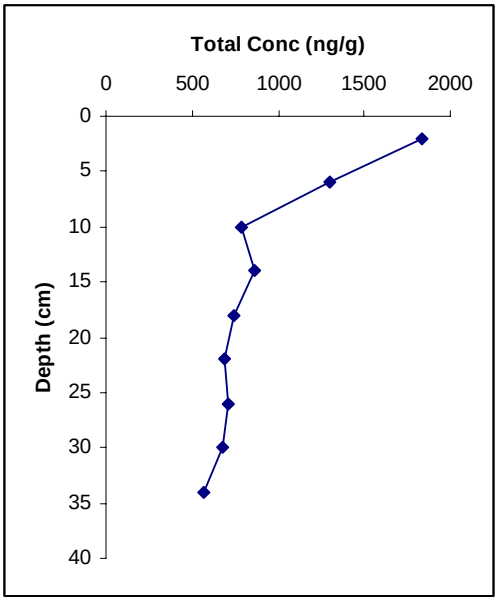

Figure 6.26 Total fatty alcohol concentration in a short core on the continental shelf off Taiwan (after Jeng *et al.* (1997)).

J2. East China Sea, N of Taiwan

In a follow study investigating the source of organic matter in the East China Sea, Jeng and Huh (2004) collected suspended and settled sediments from a range of sites. After Soxhlet extraction with DCM : methanol (1:1) and saponification with KOH in methanol, the samples were fractionated on a silica gel column. The data from the

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suspended particles is partly presented in Figure 6.27; here, the ratio of C_{24} to C_{16} is shown and it indicates the relative enrichment of the long chain compounds in the near shore samples compared to the offshore samples. The concentrations of the fatty alcohols are significantly greater in the suspended particles compared to settled sediments and reach 166 $\mu g.g^{-1}$ for the C_{22} at site 18.

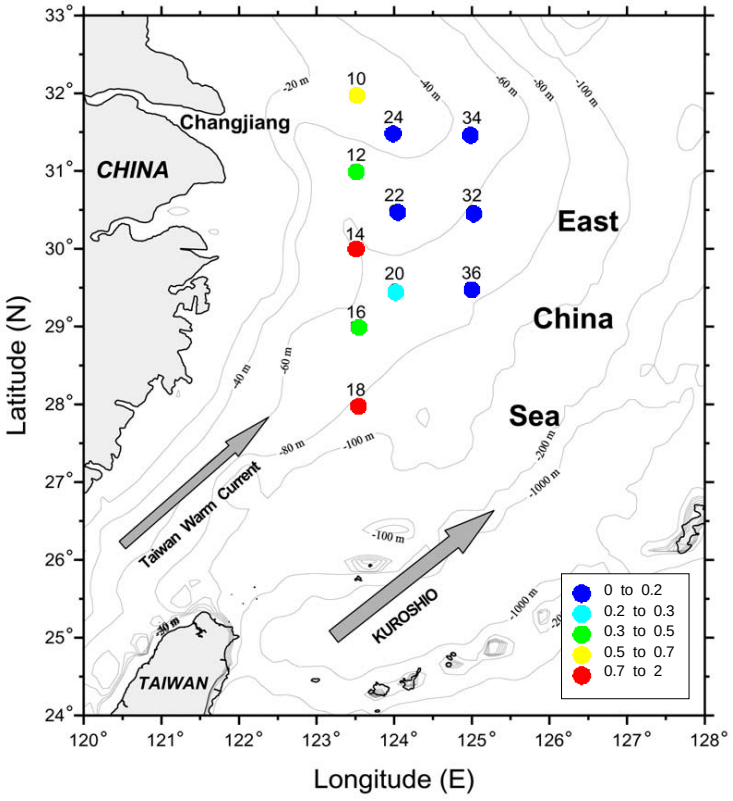

Figure 6.27 The ratio between C_{24} and C_{16} in the suspended particulate matter collected in the East China Sea (after Jeng and Huh (2004).

With regard to the settled (bottom) sediment, the maximum concentration reached 476 $ng.g^{-1}$ for C_{22} at site 36… three orders of magnitude less than the suspended particulate concentration. The ratio between the terrestrial biomarker is also different with only one value in this latter case <1.0. This is most likely due to the more rapid degradation of the short chain compounds in the sediment compared to the longer chain alcohols. The pattern of distribution between the two marker ratios is also different suggesting that the surface waters suspended particulate matter does not reflect the final depositing sediments.

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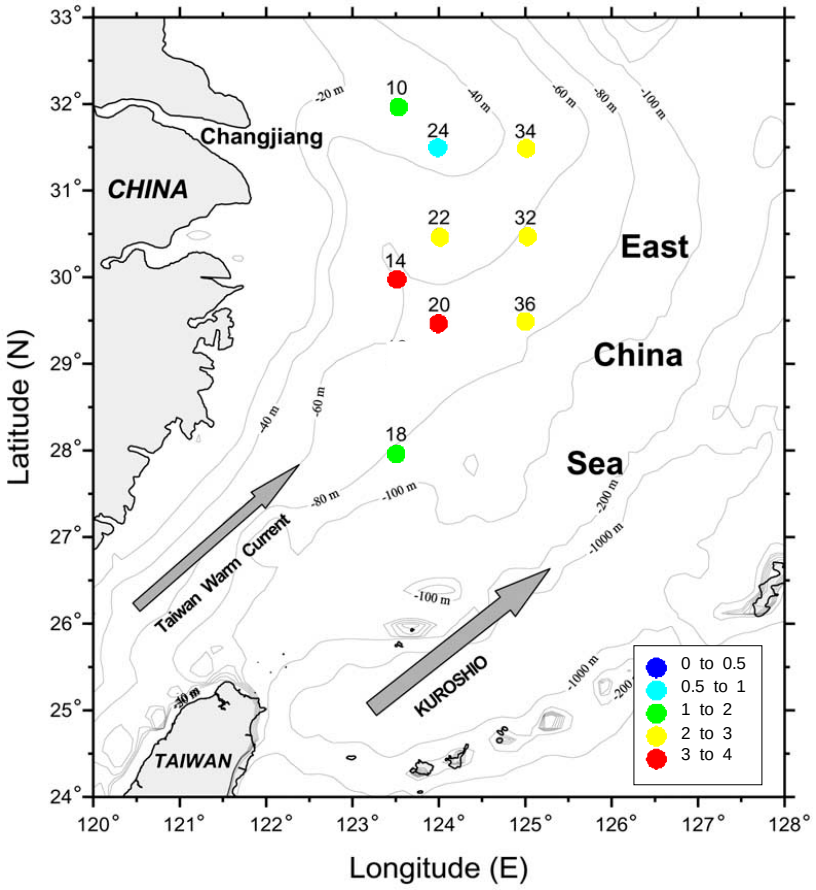

Figure 6.28 The C_{24} / C_{16} ratio in settled sediments from the East China Sea. Notice the change of scale compared to Figure 6.27 (after Jeng and Huh (2004).

The authors of the work (Jeng and Huh, 2004) suggest that the region is dominated by marine production although the settled sediment fatty alcohol profiles do not reflect this. In the two extreme cases of the ratio shown in Figure 6.28, the concentration profile indicates a substantial terrestrial component (Figure 6.29). If these data are compared to the oceanic core samples of Howell (1984), considerable differences can be seen. The Chang Jiang (Yangtze River) is the largest river in Asia and the third longest in the world. It might be expected that considerable amounts of terrestrially derived organic matter would be entering the East China Sea by this route, depositing on the continental shelf off the mouth. This may provide the long chain fatty alcohols seen in the samples.

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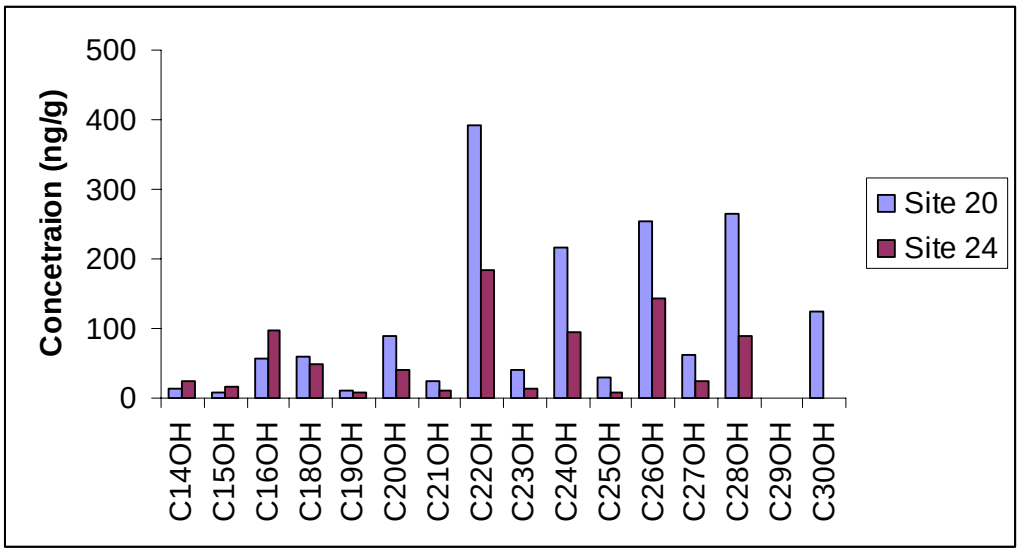

Figure 6.29 The fatty alcohol profile for surface sediments from the East China Sea. Data after Jeng and Huh (2004). Site numbers refer to locations shown in Figure 6.27.

K. Pasture land, Southern Australia

In a study of the faecal material of grazing animals in Australia, Nash *et al.* (2005) quantified a few fatty alcohols in surface water runoff from pastureland. Somewhat surprisingly, the authors only report alcohols in the $C_{26} - C_{32}$ range although this may be due to concentrating their efforts on the sterols which elute on a typical GC run in the same region. Therefore, the absence of $C_{16} - C_{25}$ in these data does not mean they were not present, just not quantified.

The data are presented in log (x + 1) concentration form and have been converted back to true concentrations (Figure 6.30) for a typical sample pair. These data indicate a predominance of the C_{26} fatty alcohol with smaller amounts of the other even chain length compounds; no odd chain length compounds were reported.

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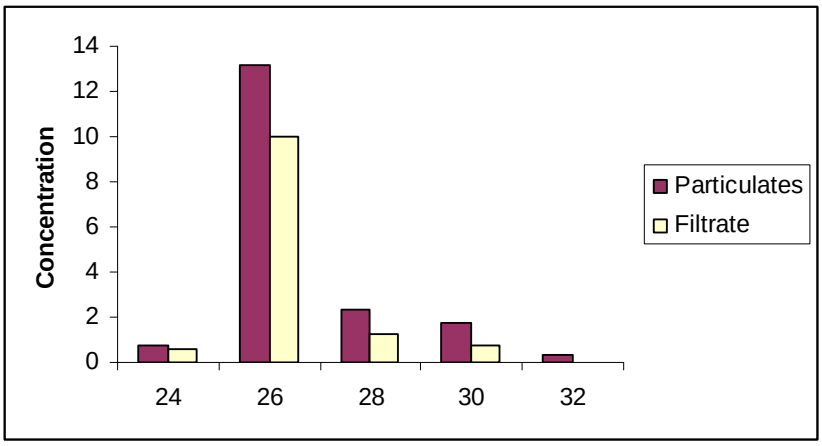

Figure 6.30 Concentration of long chain fatty alcohols in runoff from pastureland in Australia (after Nash *et al.*, 2005). Units are $\mu g.g^{-1}$ for the particulate fraction and $\mu g.L^{-1}$ for the filtrate.

L. Prairie Zone soils, Alberta, Canada

Soils can act as the short term repository for many biologically derived compounds. Due to the relative stability of waxes, these may be preserved for several years. In most cases, these are derived from terrestrial plants and so the profile is similar to that of local plant species (Figure 6.31 after Otto *et al.*, 2005).

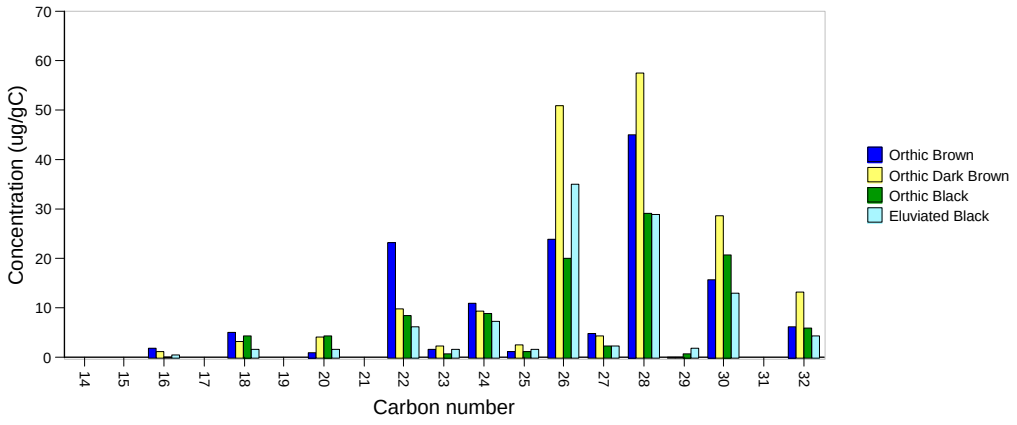

Figure 6.31 Fatty alcohols in Canadian soils. After Otto *et al.*, 2005).

In other locations, it may be that the waxes are derived from animals such as sheep (Roper, 2004); in this case, the presence of these waxes may reduce the fertility of the soil and methods are required to reduce their content prior to cultivation.

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UK Studies

As well as the studies reviewed above, several have been undertaken in the UK. Most of these are concentrated on marine samples and also on the western side of the UK (Figure 6.2). Some of these data are taken from unpublished Ph.D. theses, M.Sc. dissertations and even an Undergraduate Honours project.

1. Conwy Estuary – Core (50 cm)

As part of a Ph.D. project investigating the use of multivariate statistics with biomarkers, Mohd. Ali (2003) collected a core from the Conwy Estuary, North Wales. This was a muddy location 3 km from the mouth of the estuary. The system receives terrestrial organic matter from the extensive mixed forests in the catchment as well as domestic and a small amount of industrial sewage from the towns of Conwy and Llandudno. Fatty alcohols from C_{14} – C_{29} were present in the core samples after an alkaline saponification extraction procedure (Mudge and Norris, 1997). Concentration data for this and other studies is presented in the Appendix

Near the surface, short chain compounds (*e.g.* C_{16}) dominated but at depth, longer chain compounds increased in importance (Figure 6.32). The concentrations were in the order of ng.g^{-1} dry weight with a maximum value of 939 ng.g^{-1}DW. The mean chain length increased from 19.5 at the surface to almost 22 by 50 cm depth..

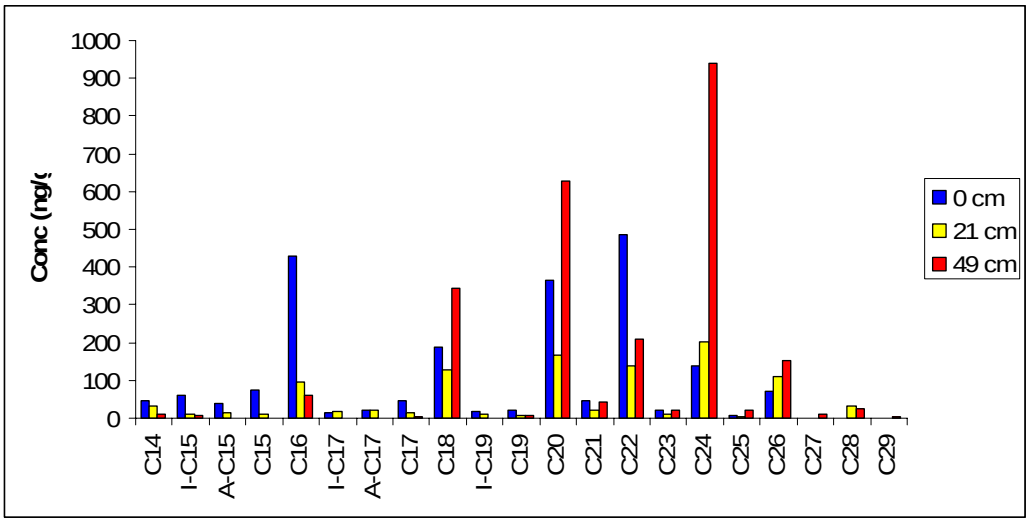

Figure 6.32 Fatty alcohol profiles from a core in the Conwy Estuary, North Wales.

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There were several other changes in alcohol profile down the core with the percentage of branched (*iso* and *anteiso*) chain compounds reaching a sub-surface maximum (11.2% at 9 cm) and decreasing to almost zero at depths >40 cm (Figure 6.33). This sub-surface maximum is a relatively common occurrence in cores and may be due to increased bacterial biomass feeding on the organic matter as it rains out of the water column. The oxygen content of the sediments also decreases with depth in cores and there may be a change from aerobic to anaerobic bacterial groups which have a different fatty alcohol suite.

At the deeper depths, the samples were dominated by the longer chain compounds from terrestrial plants. These waxy compounds are generally more resistant to degradation and are preserved in the core. This is unlike the observation made for alcohols is the STP effluent (Figure 4.10) where long chain compounds were metabolised during treatment. The concentration also increases as more deposited material is compressed into a smaller section and since these compounds are not degrading over that time scale, the concentration increases on a per gram dry weight basis.

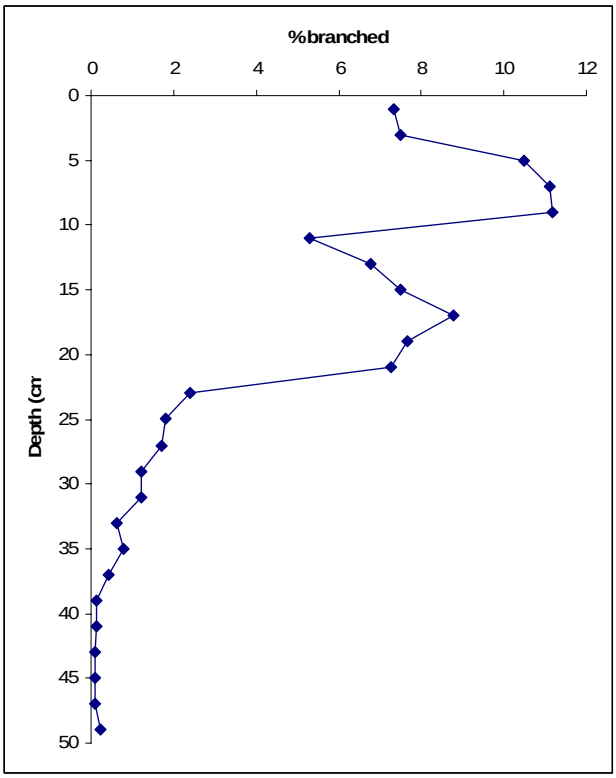

Figure 6.33 Chain in percentage branched fatty alcohols down the core from Conwy Estuary, North Wales.

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The degradation rate may be studied in a similar manner to other sites (see Chapter 4). However, no estimate was made of the sedimentation rate in this system and so a value of 0.3 cm y^{-1} has been adopted. A plot of the phytol degradation can be seen in Figure 6.34. The trendline provides a measure of the degradation rate (= 0.019 y^{-1}) and is consistent with that measured by (Jeng *et al.*, 1997) for the extractable phytol. Considerable variation may be present in this value depending on the true sedimentation rate.

Further measures of the rate of change either due to change in source or preferential degradation of the short chain compounds can be seen by using the C_{24} / C_{14} ratio (Figure 6.35). In this case, low values near the surface indicate a relatively high C_{14} composition which declines with increasing depth or age. In the deepest sample, the amount of C_{24} completely dominates the C_{14}.

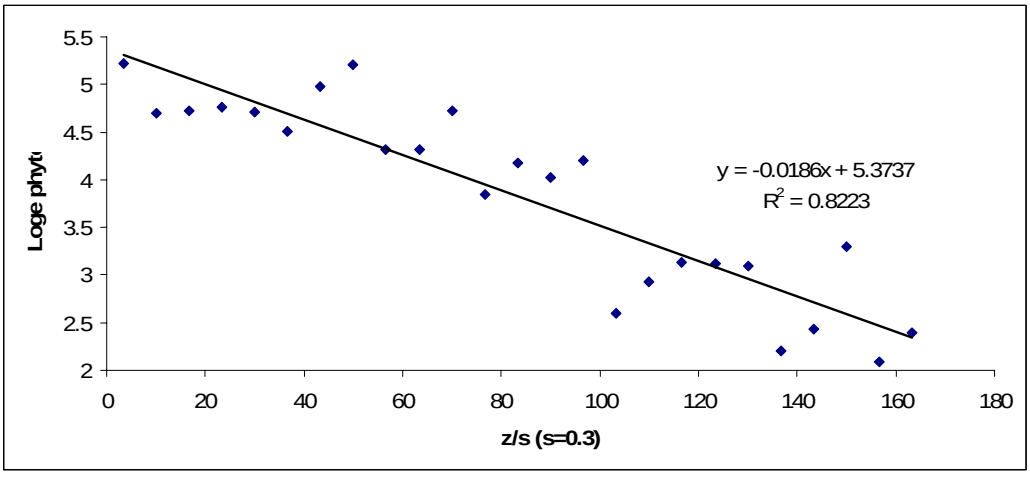

Figure 6.34 Log_e of the phytol concentrations from a Conwy core. A sedimentation rate of 0.3 cm.y^{-1} was used.

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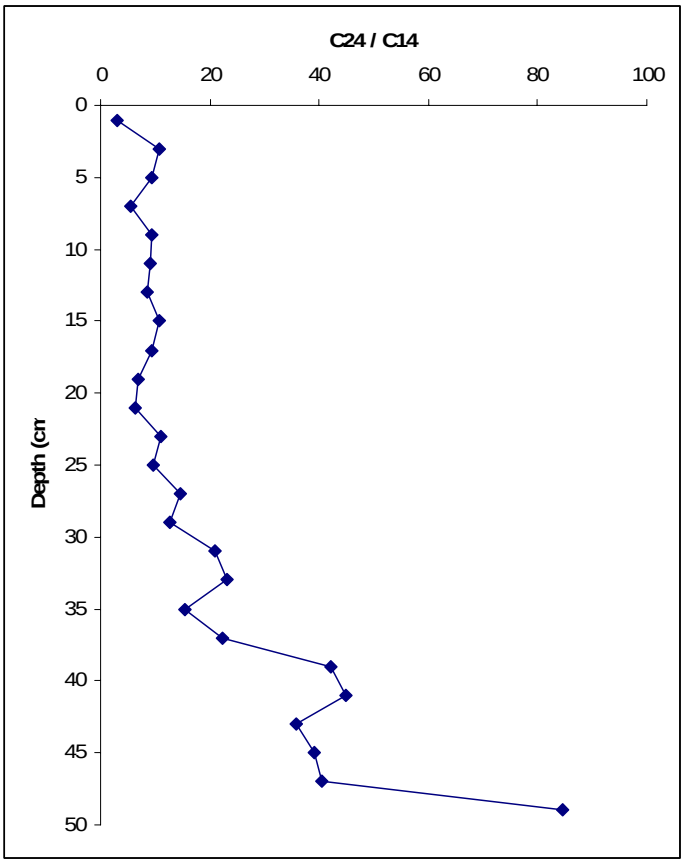

Figure 6.35 The C_{24} / C_{14} ratio as a measure of either (a) change in source from marine to terrestrial with increasing depth or (b) selective degradation of the short chain alcohol.

2. Mawddach Estuary – surface sediments.

A further part of Masni Mohd. Ali's Ph.D. project (Mohd. Ali, 2003) investigated the surface sediment distribution of a range of lipid biomarkers including fatty alcohols, fatty acids and sterols in the Mawddach Estuary. This is a relatively clean, sandy location with no industrialisation although there are several domestic sewer outflows into the system. Surface scrapes of sediments were taken at the locations indicated in the map in Figure 6.36. Samples were collected above the tidal limit to ensure a terrestrial signature was obtained.

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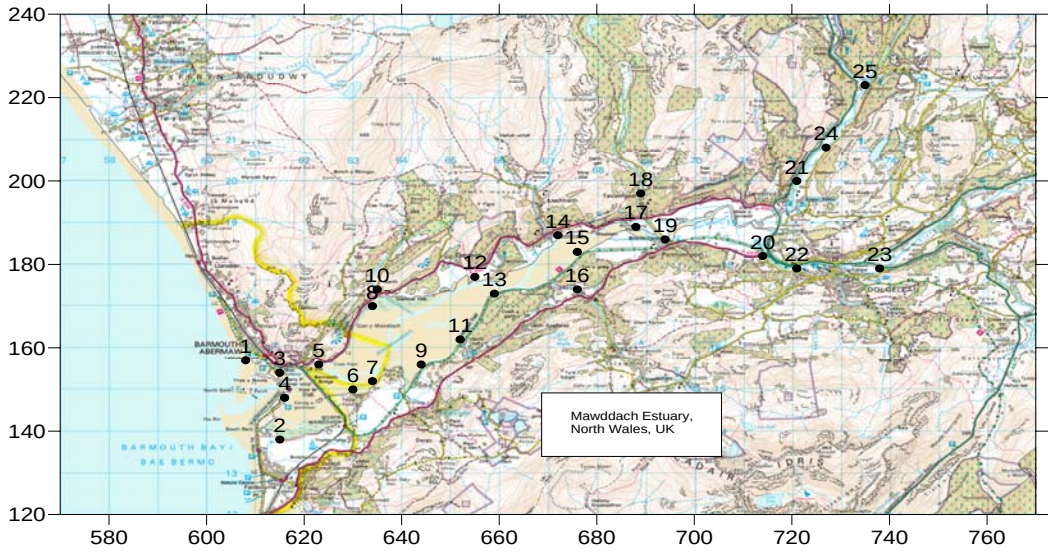

Figure 6.36 Sample location map for sites in the Mawddach Estuary, UK. The sites to the west of the area are above the tidal limit.

The profile of fatty alcohols in the various samples changes in response to several environmental parameters. The principal factor is the salinity as marine samples (high salinity) should be dominated by short chain compounds and terrestrial samples (low salinity) will have long chain compounds. Samples from the middle reaches of the estuary will have a mixture of both. Examples of this can be seen in Figure 6.37. This change in signature from short chain at the mouth to long chain above the tidal limit leads to a change in the mean chain length for all the n-alkanols in the samples (Figure 6.38).

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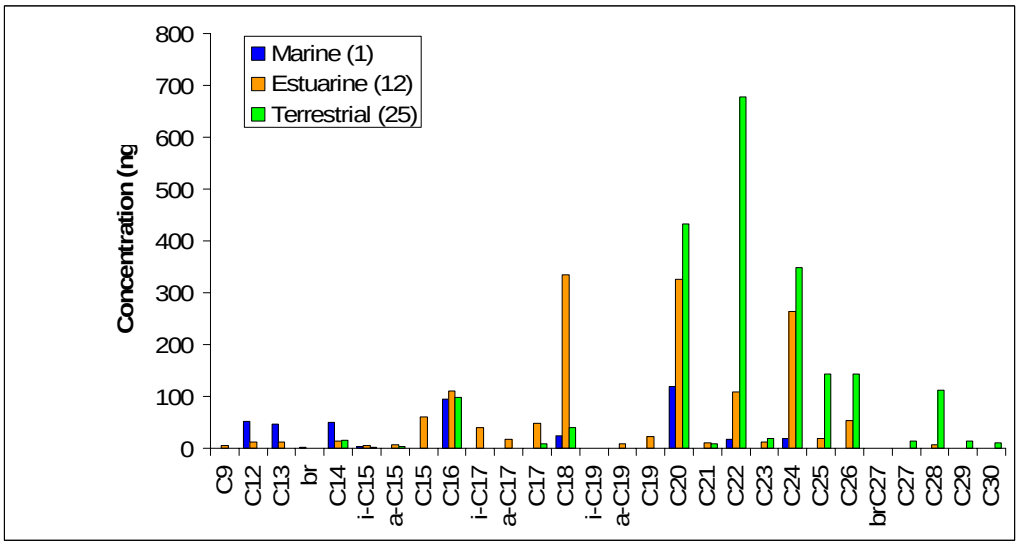

Figure 6.37 Profiles of fatty alcohols in samples from the Mawddach Estuary. Samples shown are from the marine environment (site 1), mid-estuary (site 12) and above the tidal limit (site 25).

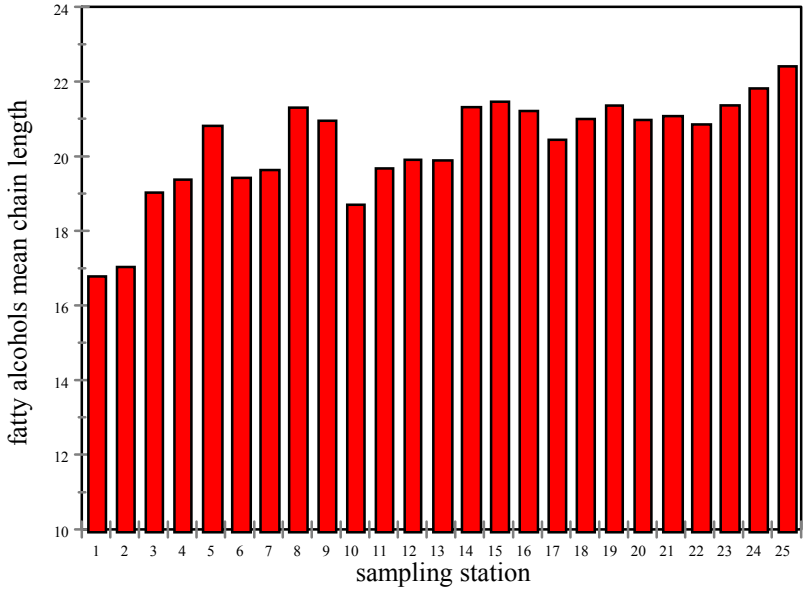

Figure 6.38 Mean chain length on the n-alkanols through the Mawddach Estuary from the marine environment (site 1) to above the tidal limit (*e.g.* site 25).

The differences in the profile enable signatures to be developed for the different potential sources of organic matter in the system. This will be considered in more detail in Chapter 7.

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3. Menai Strait

As part of an ongoing study of lipid biomarkers in the sediments of Menai Bridge, fatty alcohols and sterols are measured in short cores from intertidal mud annually. The results of the surface sediment alcohol profile extracted by alkaline saponification are shown in Figure 6.39. In all years, there is a weak bimodal distribution with peak concentrations in the $C_{22} – C_{26}$ region representing terrestrial organic matter inputs and again in the $C_{16} – C_{18}$ region from marine animal synthesis. The concentrations varied between years but reached a peak of ~500 $ng.g^{-1}$ wet weight for the C_{22} in 2004. Branched chain compounds are also present together with odd chain length alcohols indicating the presence of bacterial biomass.

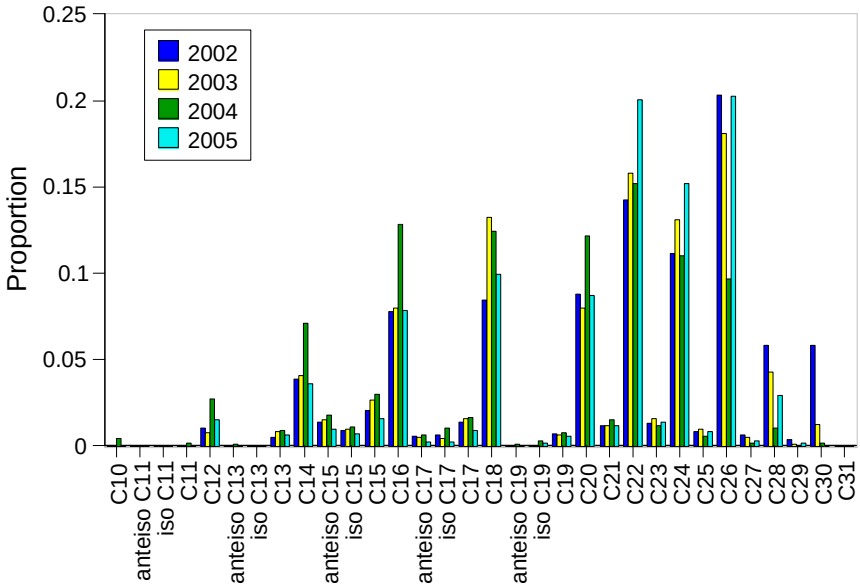

Figure 6.39 Fatty alcohol profile expressed as proportions for surface sediment in the Menai Strait collect in February 2002 – 2005.

Plots of the core data for each year show a characteristic high concentration at the surface which decreases with depth; this clearly represents the *in situ* degradation of this organic matter by the naturally present bacterial population.

4. Loch Riddon, Scotland – mid-length core

To investigate the long term changes of lipid biomarkers (Mohd. Ali, 2003), a series of cores from the sea lochs surrounding the Clyde sea area were collected in 1998.

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Detailed analysis was conducted on a 1.5 m core from Loch Riddon (Figure 4.5) where sterols, fatty acids and fatty alcohols were measured after alkaline saponification.

The change in alcohol profile can be seen in Figure 6.40; short chain compounds again dominate in the surface samples and decrease with depth while the longer chain compounds come to dominate at the bottom of the core. The increase in concentration of the C_{22} compounds is not as marked as for the Conwy Estuary as this catchment has relatively little wooded areas and so the terrestrial component entering the system is smaller. Even so, the compounds are still preserved in the 145 – 150 cm section which is in the order of 1000 years BP based on the PAH profile.

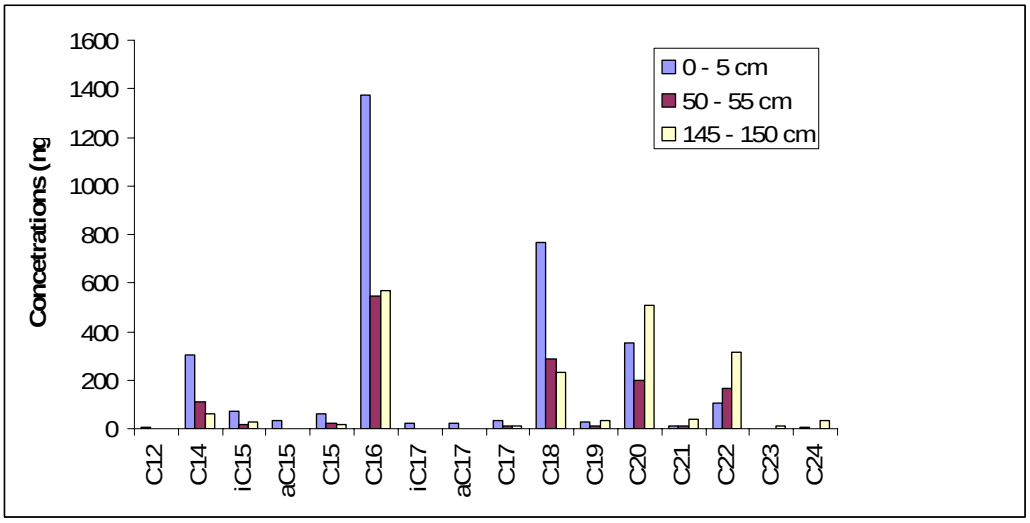

Figure 6.40 Fatty alcohol profiles for the surface, middle and bottom of a 150 cm core from Loch Riddon, Scotland.

It is interesting to note that the C_{14} compounds are still present in the bottom sample despite being ~1000 years old. The rate of degradation in this system may be less than for the Conwy Estuary due to the deeper cold nature of the sample location and lack of bioturbation.

The ratio between short chain ($<C_{19}$) and long chain ($>C_{18}$) compounds can be seen in Figure 6.41; the surface samples have values between 5 and 8 while the deeper sections are tending to a ratio of 1.0. The rate of change of this ratio tends to imply that the degradation rate is slower than that in the Conwy Estuary.

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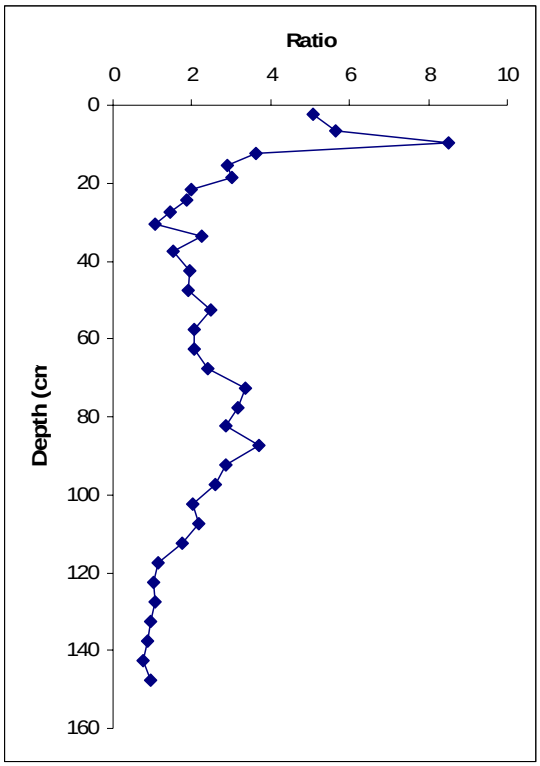

Figure 6.41 The ratio of short chain to long chain fatty alcohols down the Loch Riddon core.

5. Lochnagar, Scotland.

Lochnagar is a mountain lake 788m above sea level in the Scottish Highlands close to Balmoral in Aberdeenshire, UK. This system was investigated by Scott (2004) as part of a study into sedimentary biomarker records of climate variability in the Holocene period. The main results are reported in Dalton *et al.* (2005). A small number of results are reported in a core with maximal concentrations in the C_{22} to C_{30} chain length: the mean concentration for these long chain terrestrial plant derived compounds was 0.3 – 0.4 mg.g TOC^{-1}. There was a strong even over odd dominance at all chain lengths (Figure 6.42) with the shorter C_{15} and C_{17} compounds being below detection limits. Scott concludes that these compounds are principally derived from higher plant sources including peat, the degraded product of many plants in such upland areas. In Scott's analysis of local peat, the C_{22} fatty alcohol was the most abundant compound (Scott, 2004). The relative absence of short chain compounds in

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the core Scott attributes to a small *in situ* production by algae and dominance of allochthonous (terrestrial higher plants) organic matter.

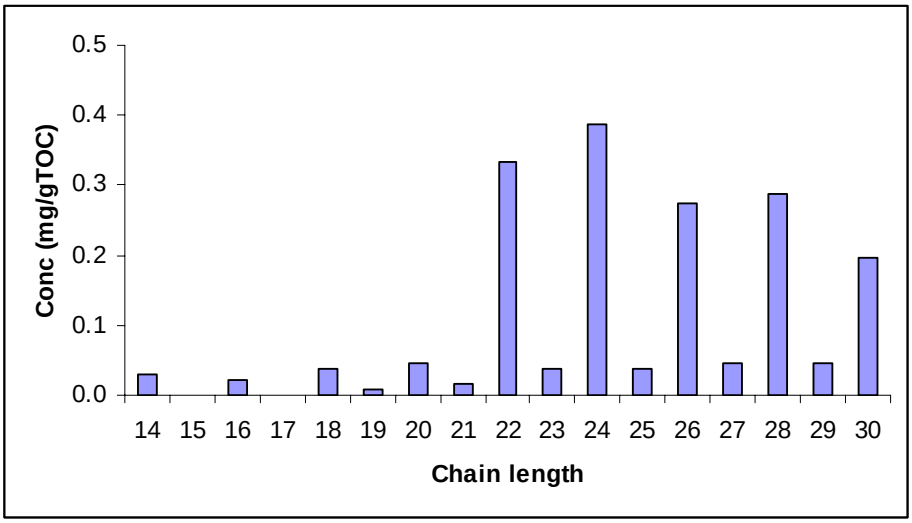

Figure 6.42 The mean fatty alcohol distribution in a core from Lochnagar, Scotland. (after Scott, 2004).

6. Clyde Sea, Scotland

The waters of the Clyde Sea area (Figure 6.43) receive inputs from rivers of southern Scotland that must first flow through the sea lochs; the sills marking the mouth of the lochs trapped much of the sedimentary matter leaving only small amounts to be transported to the sea. The Clyde River on which the city of Glasgow is built receives much anthropogenic waste from industrial and domestic sources. As well as *in situ* (autochthonous) production by the marine biota, sewage wastes have been disposed of through pipes to the sea and sludge disposal sites. Samples were collected from range of sites throughout the system from surface grabs during 1998 (Mohd. Ali, 2003). All samples were extracted by alkaline saponification and fatty alcohols quantified along with fatty acids and sterols.

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Figure 6.43 The Clyde Sea area of Scotland. Major inputs come from the sea lochs to the north and the Clyde River to the north east.

The concentrations of fatty alcohols ranged from below detection limit for some of the short chain compounds to 2216 ng.g^{-1} DW for C_{22}. An example of the distribution can be seen in Figure 6.44 which shows a classed posting of the C_{16} and C_{22} compounds in the surface sediments.

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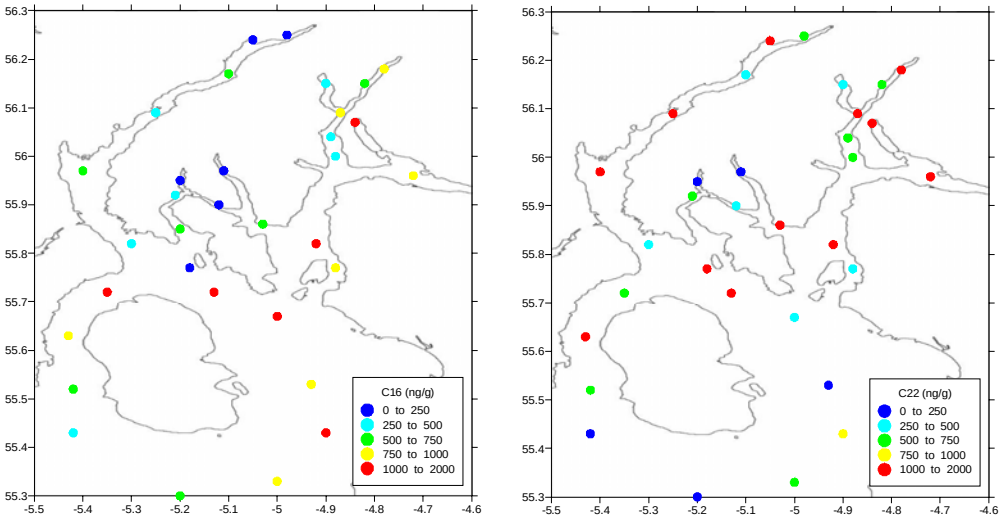

Figure 6.44 Spatial distribution of the C_{16} and C_{22} in the surface sediments of the Clyde Sea.

The distributions are somewhat complex with no clear trend from a marine through to terrestrial gradient. This may be due to the mixing of waste waters and domestic effluent with river water before discharge into the sea area. The sea lochs also trap many compounds from both marine and terrestrial sources and as the data from the Loch Riddon core shows, degradation may be slower here than in shallower waters. However, there are generally higher concentrations of the short chain compounds from C_{11} to C_{16} in the southern more marine areas and higher long chain (e.g. C_{22}) compounds in the sea lochs (Figure 6.44). The branched chain compounds also show complex patterns and are best investigated with multivariate statistical methods reviewed in Chapter 7.

From a biomarker viewpoint, in this location fatty alcohols were not as useful in the tracking of organic matter as the sterols and fatty acids (Mohd. Ali, 2003). This is discussed further when considering PCA and PLS analysis in Chapter 7.

7. Loe Pool, Cornwall

Loe Pool is a eutrophic coastal lake in the south west of England; it has received nutrient inputs from agriculture in the recent past and has start having blooms of *Hydrodictyon reticulatum*, a fast-spreading nuisance green alga (John *et al.*, 1998). A study was conducted on the sediments of the pool by Pickering (1987) and fatty alcohols were measured. The mean profile of the alcohols in the sediments can be

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seen in Figure 6.45. These samples were taken before the first reports of the nuisance blooms of the green alga which became dominant in 1993, (Flory and Hawley, 1994) although cyanobacterial blooms had been present earlier.

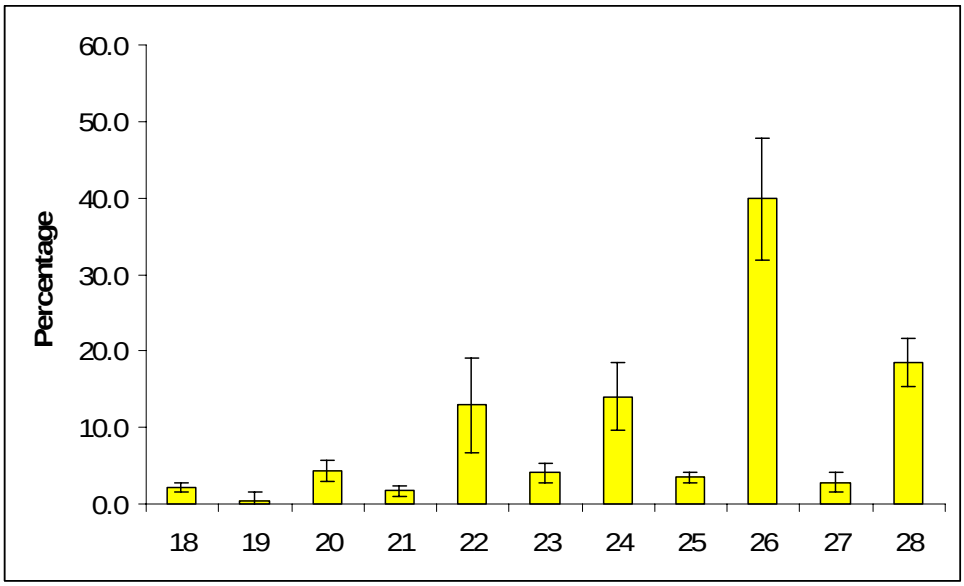

Figure 6.45 The fatty alcohol profile for sediment laminations in Loe Pool, Cornwall. N = 6. (Data after Pickering, 1987).

The distribution of alcohols is typical of a freshwater system with significant amounts of the $C_{22} - C_{28}$ fatty alcohols and almost no short chain compounds present. The total concentrations reached 330 $\mu g.g^{-1}$ DW of sediment suggesting a high loading of organic matter (Pickering, 1987).

8. Bolton Fell Moss, Cumbria

As part of a study in to an ombrotrophic ("rain nourished") mire, Avsejs (2001) investigated the lipids in short cores (30 cm ≡ 160 years BP) of peat. The full results are reported in (Nott *et al.*, 2000; Avsejs *et al.*, 2002; Xie *et al.*, 2004). The location of this peat bog is in Northern England close to the Scottish border (Figure 6.46). In his work, Avsejs (2001) extracted samples without alkaline saponification and so these data are only free, extractable fatty alcohols. The fatty alcohol profile of the samples were very similar and the mean values (n=7) are presented in Figure 6.47; both C_{22} and C_{24} were present in high concentrations relative to the other compounds. The results are expressed in $\mu g.g^{-1}$ of dry peat since the mineral content of these samples

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will be low. The highest value was 226 μg.g^{-1} of dry peat for C_{22} in a sample dominated by *Eriophorum vaginatum*.

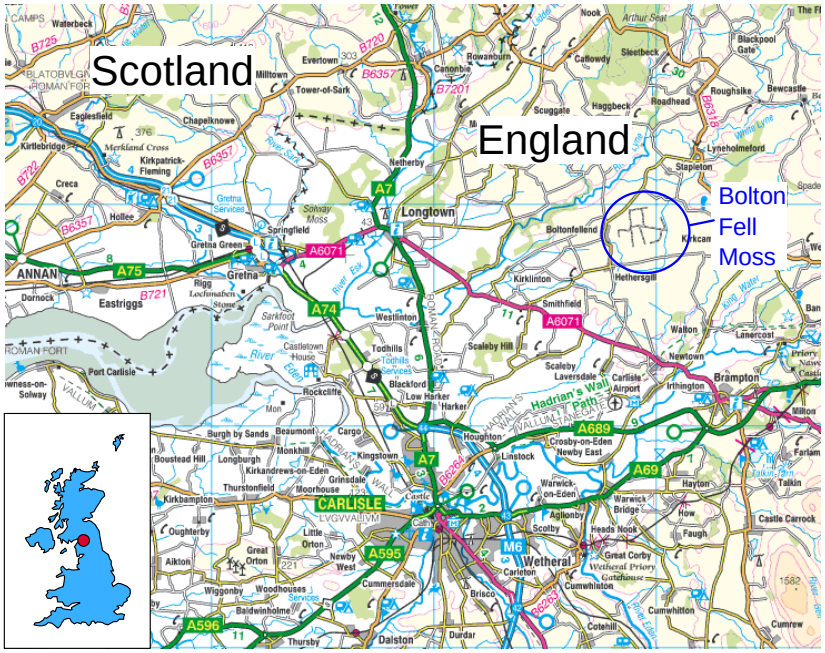

Figure 6.46 Location map for Bolton Fell Moss, a peat bog in Northern England.

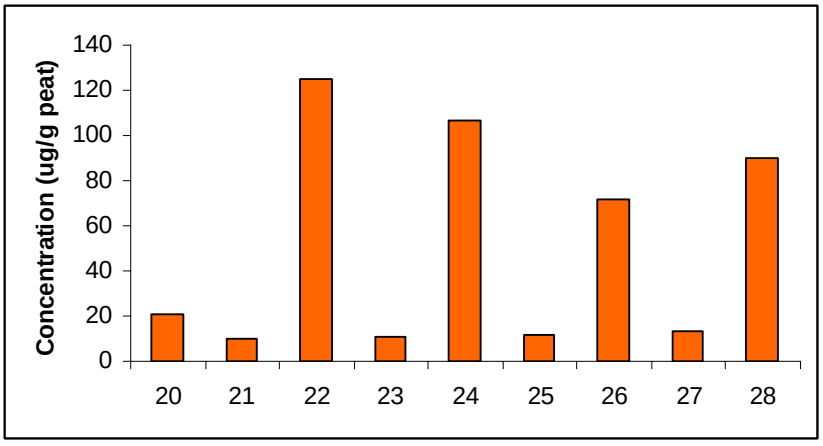

Figure 6.47 The mean concentration of fatty alcohols measured in seven peat cores (after Avsejs, 2001).

9. Blackpool Beach – see Chapter 7.

A full treatment of the data from this location is given in the following chapter as it was used to develop chemical signatures to identify the source of faecal contamination on a beach (Mudge, 2001).

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10a. Loch Lochy, Scotland – a freshwater deep loch core

10b. Loch Eil, Scotland – a mid-depth (~70m) seawater loch core

Cores were collected from the R.V. *Prince Madog* in a freshwater and saltwater loch that were only 16 km apart. The freshwater core was retrieved from 116m water depth and the saltwater one from 70m. Both cores were analysed for a range of chemical and physico-chemical parameters including lipids which were reported by (Hotham, 2001). The concentration data can be found in the Appendix.

The concentrations were significantly lower than those found in the shallow water cores probably due to increased degradation during settlement of the organic matter through the water column and lower production / input.

One of the interesting features of the data is the age of some of the compounds found; the freshwater core base was tentatively dated to be around 8000 year BP due to encountering the post-glacial clay layer typical of this area. Most compounds were quantifiable even at this age / depth although the concentrations were very small (*e.g.* 0.0627 $ng.g^{-1}$ for C_{12}). The seawater core was younger at an equivalent depth due to higher deposition rates. Near-surface concentrations in the seawater core were less than in the freshwater core although at greater depths they were similar (Figure 6.48).

The branched chain compounds in both cores had an interesting profile (Figure 6.49); due to lower deposition rate in the freshwater (FW) core, no sub-surface peak in values could be seen. It could, however, be seen in the seawater core (SW). The trend also showed increasing percentages with increasing depth which may appear counter-intuitive. This is likely to be due to the slower degradation rate of the branched compounds compared to their straight chain equivalents. Therefore, the net effect is for the percentage branched to increase. Inspection of the concentration data does show that in the freshwater core the concentrations decrease with increasing depth. This is less clear in the seawater core where increased concentrations are seen in the last two samples. The reason for this is not clear.

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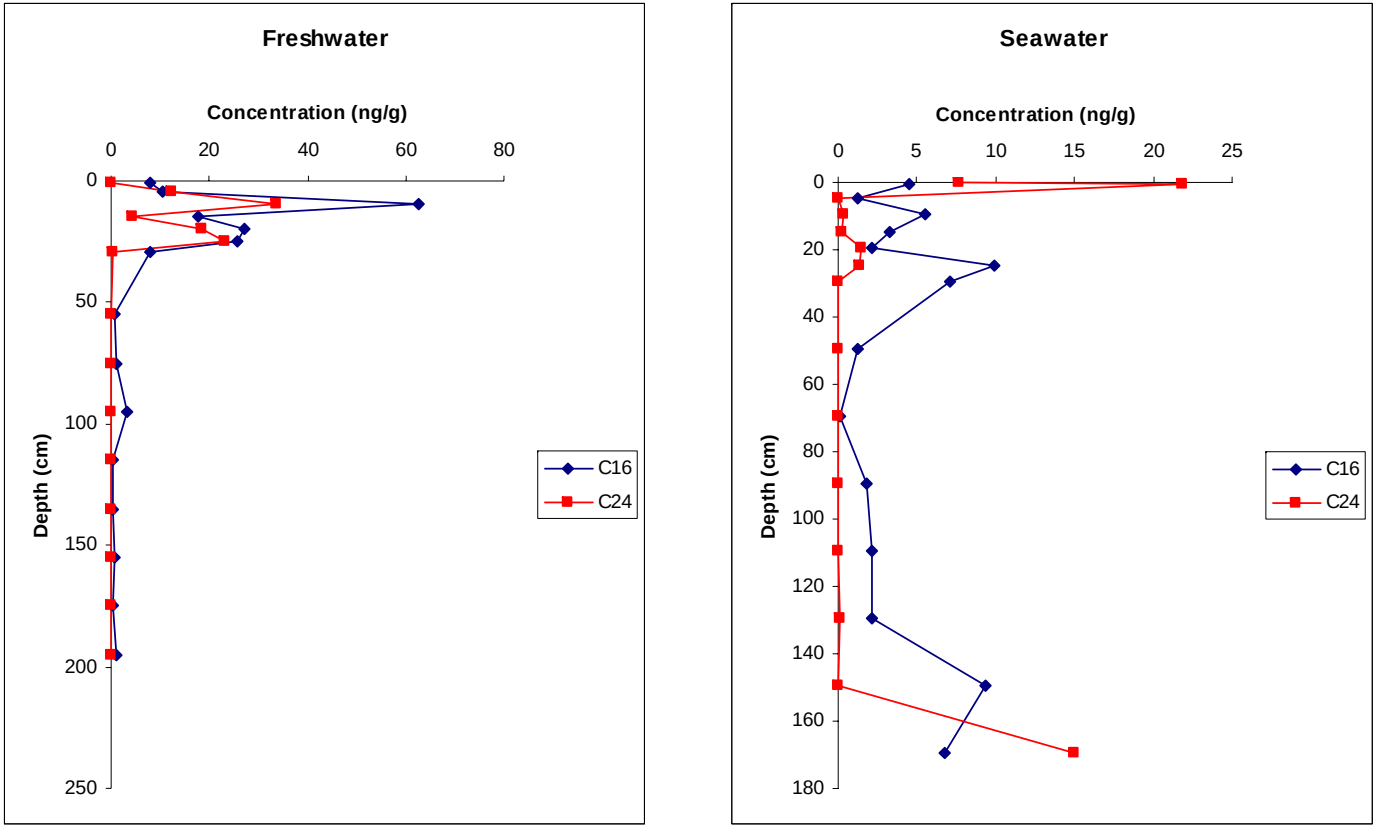

Figure 6.48 Concentrations of a short chain (C_{16}) and long chain (C_{24}) fatty alcohol in a freshwater and seawater core (after Hotham, 2001).

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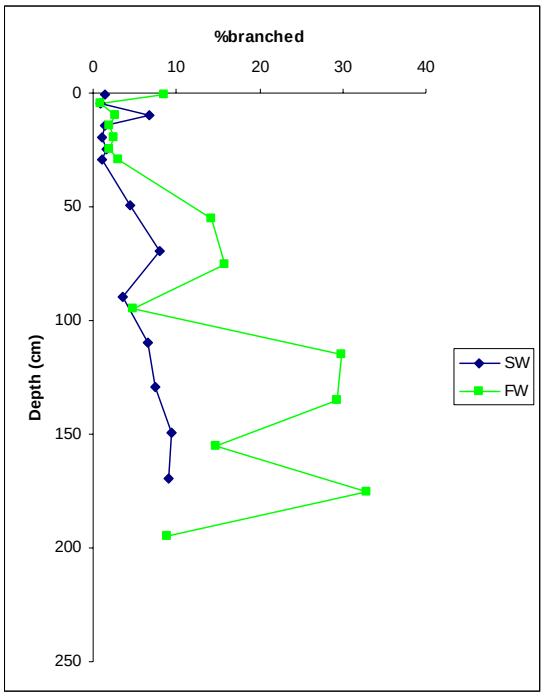

Figure 6.49 Profile of the percentage branched compounds present in the two cores.

Summary

The concentrations of fatty alcohols measured across many parts of the world are surprising similar. In most marine cases the C_{16} straight chain compounds dominates with secondary maxima in the C_{22} to C_{24} region depending on the proximity to terrestrial sources of organic matter. The C_{16} may be expected to be high as it is the end of the initial fatty acid synthesis pathway from which many fatty alcohols are made. The concentrations in suspended matter are significantly greater than those in the settled sediment as the organic matter content is usually higher. These materials may also have undergone less bacterial degradation and be more representative of local sources. The maxima are also greater in the near shore and estuarine / lagoonal regions due to the high *in situ* productivity and the presence of terrestrial plant waxes. This is reflected in the high C_{22} or C_{24} values. However, due to the preservation of the long chain moieties, these compounds may be found at locations remote to their origin, both in time (down core) and space.

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Table 6.3 The range of maximum concentrations for C_{16} and $C_{22/24}$ fatty alcohols measured at a range of locations around the world.

Environment	Maximum C_{16} concentrations	Maximum $C_{22/24}$ concentrations	Number of Studies
Coastal Marine Sediments	402 – 1961 $ng.g^{-1}$ DW	112 – 5500 $ng.g^{-1}$ DW	7
Estuaries & Lagoons	1384 – 8890 $ng.g^{-1}$ DW	27 – 818 $ng.g^{-1}$ DW	8
Ocean Sediments	12 – 404 $ng.g^{-1}$ DW	635 – 1000 $ng.g^{-1}$ DW	6
Suspended Sediments	ND – 2737 $\mu g.g^{-1}$ DW	166 – 1847 $\mu g.g^{-1}$ DW	2
Freshwater	ND	740 $ng.g^{-1}$ DW	2
Lake Sediments	63 $ng.g^{-1}$ DW	98 $ng.g^{-1}$ DW	1
Upland Soils	ND	125 $\mu g.g^{-1}$ DW	1

ND = not determined

In terrestrial environments where degrading materials may accumulate (*e.g.* peat bogs), the concentration of the long chain compounds may reach 125 $\mu g.g^{-1}$ DW. In these environments, the concentration of the shorter chain C_{16} alcohol was less than the limit of detection.

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Chapter 7. Multivariate Statistics *(What extra information can be gained from these MVS methods? PLS can give quantitative estimates of each source input).*

Chemometric methods of use with fatty alcohols

There are several multivariate statistical methods that may be of use when interpreting large datasets containing fatty alcohol data. These include;

1. Principal Component Analysis (PCA)
2. Partial Least Squares (PLS)
3. Cluster Analysis
4. Factor Analysis
5. Multi Dimensional Scaling (MDS)

The advantage of each of these methods is the ability to take all the compounds and sample observations at once and determine structure within the data. These methods are now relatively widely used in environmental biomarker analysis and may be of use with fatty alcohols alone or as part of a wider dataset including other compounds.

PCA

PCA of environmental fatty alcohol concentrations or proportions may be expected to identify those compounds that co-vary. In a two dimensional plot of the loadings using the first two components (PC1 and PC2), those compounds which have the same source or behaviour will aggregate together. An example of this can be seen in Figure 7.1; these data are proportion data from samples collected on Blackpool Beach, NW England during a 13 week period including part of the designated bathing season (European Bathing Waters Directive, 76/160/EEC). Eleven locations were measured on each occasion (Mudge, 2001). Potential source materials of faecal matter to the beach were also measured including faeces from cows, donkeys and sheep; influent and effluent to the major sewage treatment plant and surface water drains.

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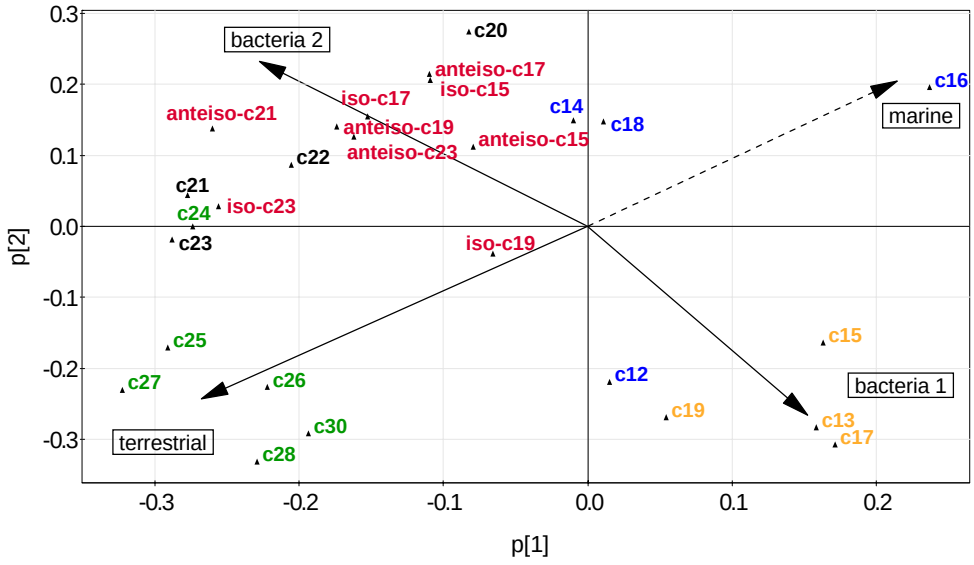

Figure 7.1 Two dimensional loadings plot for fatty alcohols from Blackpool Beach. Four potential source aggregations can be seen; terrestrial dominated by the long chain odd and even carbon compounds, a weak marine vector generally positive of PC1 opposite terrestrial, and two bacterial vectors, 1 and 2, characterised by either odd carbon compounds or branched compounds.

The major axis (PC1 explaining 19.2% of the variance in the data) appears to be a marine – terrestrial axis. However, the aggregation of the potential marine animal fatty alcohols is rather weak and is shown with a dashed arrow. PC2 (12.3%) divides two groups of compounds that are usually classified as bacterial in origin. The short chain odd carbon compounds load negatively on PC2 and positively on PC1 (showing a marine bacterial source?) while all the branched compounds are to be found in the opposite quadrant (terrestrial source?).

These putative source vectors can now be laid on the sample location aggregations, the scores plot (Figure 7.2). The potential sources to this region (colour coded in the figure), show that the influent material to the STP (Cmarin1 – 3), Cows and Sheep3 fall along the terrestrial vector determined from the loadings plot. In the lower right quadrant, the samples are principally effluent from the STP (Cmaref1 – 3), river samples (RIB1 – 3) and surface water drains. The loadings plot shows these samples to be enriched in the short chain odd carbon fatty alcohols. In comparison, the data in the upper left quadrant designated bacteria 2 due to the presence of *iso* and *anteiso*

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branched fatty alcohols, are many of the environmental samples and none of the potential sources. The general location of Blackpool can be seen in Figure 7.3.

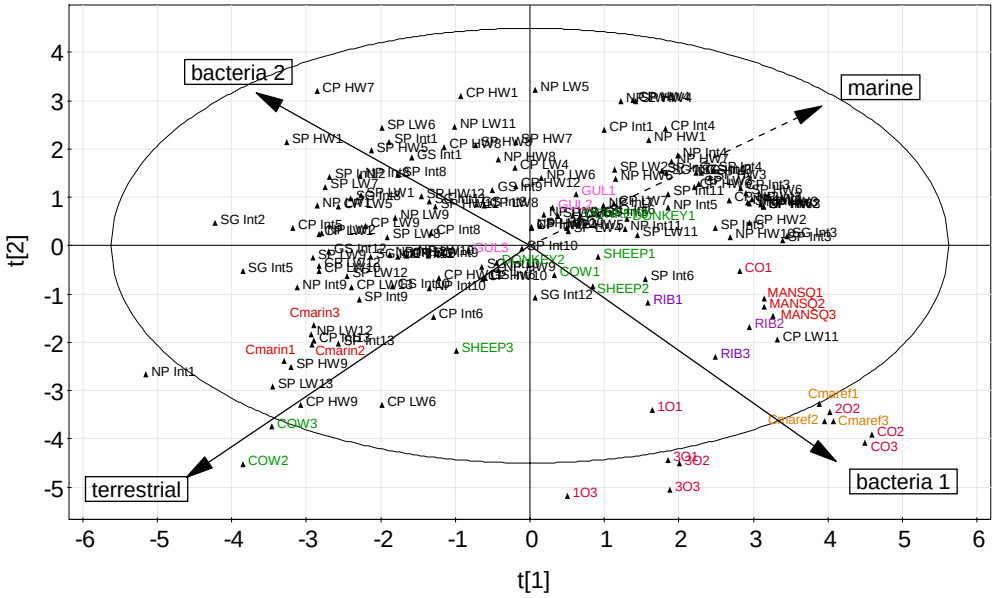

Figure 7.2 The scores plot for the data from Blackpool Beach with the putative sources from the loadings plot overlaid. The coloured sample names indicate the triplicate analyses of potential source material to the area.

Figure 7.3 Location map for the samples collected on Blackpool Beach

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Another example of compound aggregation can be seen in a set of core data from Loch Riddon in Scotland (see Figure 6.40 for a location map). The data were collected as part of a Ph.D. programme by Masni Mohd. Ali (2003) and the papers are in preparation.

As part of the pre-treatment of the data, all values were converted to proportions to remove the concentration effect and used without transformation. The loadings plot (Figure 7.4) shows the main axis (PC1, 45.6%) is a marine – terrestrial axis with the short chain compounds to the right and longer chain moieties to the left of the figure. The second PC (18.1%) separates bacterial related (branched and odd chain length) compounds giving rise to two populations as before. In this case, as the data are from a core, it is possible to see a sequence through time. The scores plot (Figure 7.5) shows a sequence indicated by the line. The top samples are relatively rich in short chain alcohols, C_{12} and C_{14} together with phytol, a chlorophyll marker, and 20:1 the storage lipid prominent in copepods, the main organism in the zooplankton. This is consistent with relatively fresh marine inputs. Samples immediately below the surface have a trend towards increasing *anteiso* C_{15}, a bacterial marker. It is likely that this reflects the microbiological community utilising the organic matter as it enters the sediments.

As the depth increases, the sequence moves towards the bottom of the scores plot towards a second set of bacterial markers, *iso* C_{15} and straight chain C_{15}. Interestingly, the C_{16} is also part of this group. This may be a bacterial group more tolerant of lower oxygen tensions, potentially even anaerobic communities, although the redox potential was not measured at the time of sampling. At depths greater than 100 cm, the samples tend towards the left of the scores plot and become enriched in the longer chain alcohols, both odd and even chain lengths. These are characteristic of terrestrial environments.

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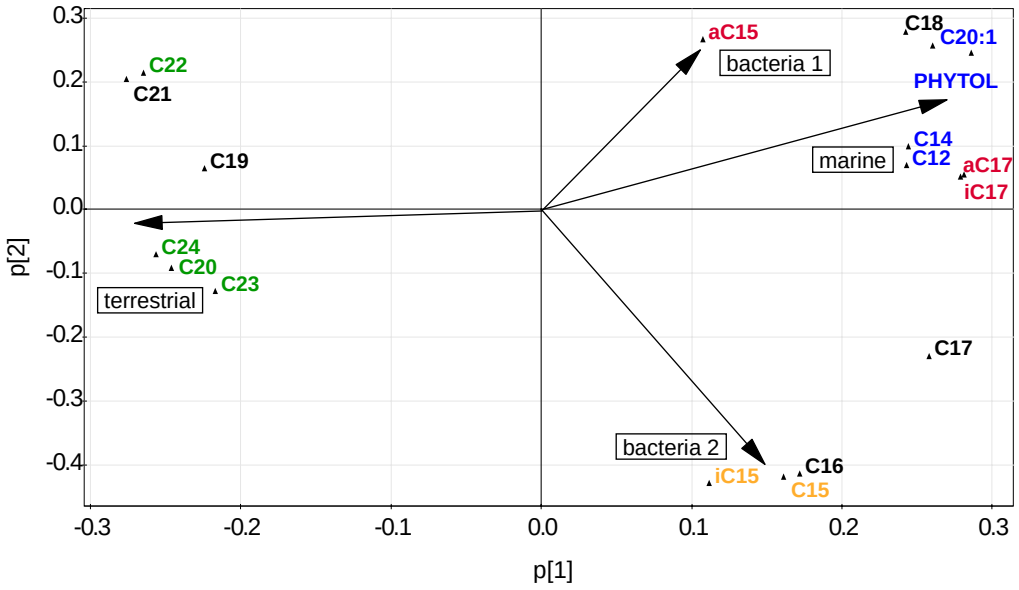

Figure 7.4 Loadings plot for fatty alcohols from a 1.5 m core collected from Loch Riddon, Scotland.

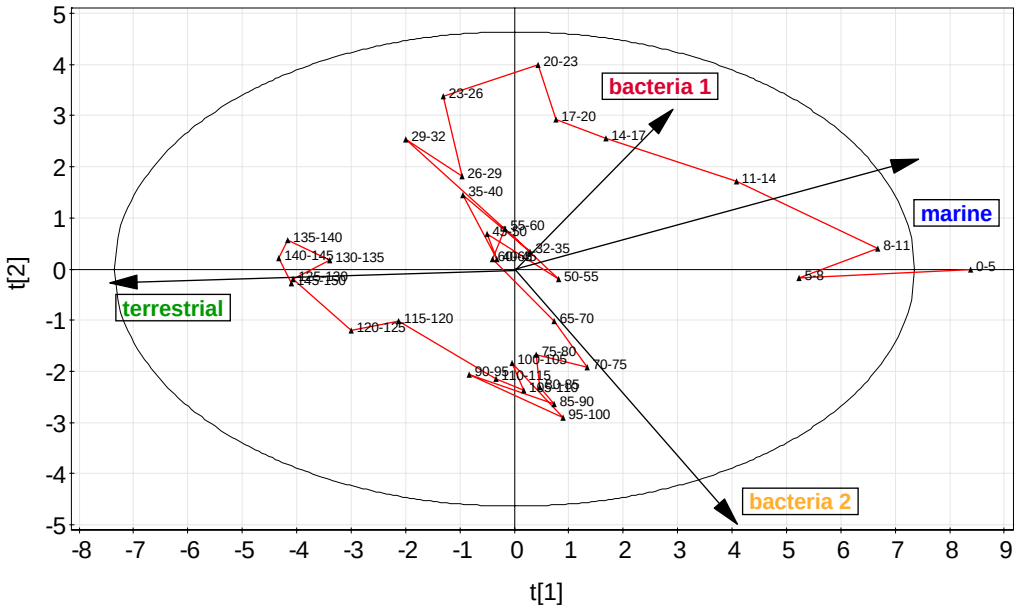

Figure 7.5 The scores plot for samples down a core. The labels refer to the sampling depth (in cm). The top of the core is to the right and the bottom to the left.

It is possible to explain the trends in the data as changes in source – samples near the surface are dominated by marine inputs which have replaced older deposits of principally terrestrial origin. However, it is also possible to interpret the data in terms

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of differential degradation rates; the waxy long chain compounds are more stable and less readily degraded in the marine environment compared to short chain (marine derived) compounds. These data alone do not enable a definite answer to be obtained. However, other biomarkers are more resistant to degradation and may be used to provide secondary evidence. A key terrestrial compound that is relatively stable is β-sitosterol (24-ethyl cholesterol) formed in the secondary thickening of higher plants (Mudge and Norris, 1997). A good marker is the ratio of this compound to cholesterol which is commonly though of as being a marine marker (Mudge and Norris, 1997). A cross plot using the sterol ratio against the fatty alcohol ratio can be seen in Figure 7.6; different regions can be seen corresponding to different depths in the core. Three regions can be identified on the plot: A – surface samples where the rates of change of the two markers are essentially the same; B – a region where the sterol marker changes while the fatty alcohol one does not (this coincides with the change from bacterial group 1 to 2 on the PCA scores plot); C – a region where the markers agree but the rate of change is less in the alcohol marker than might be expected from the sterol data.

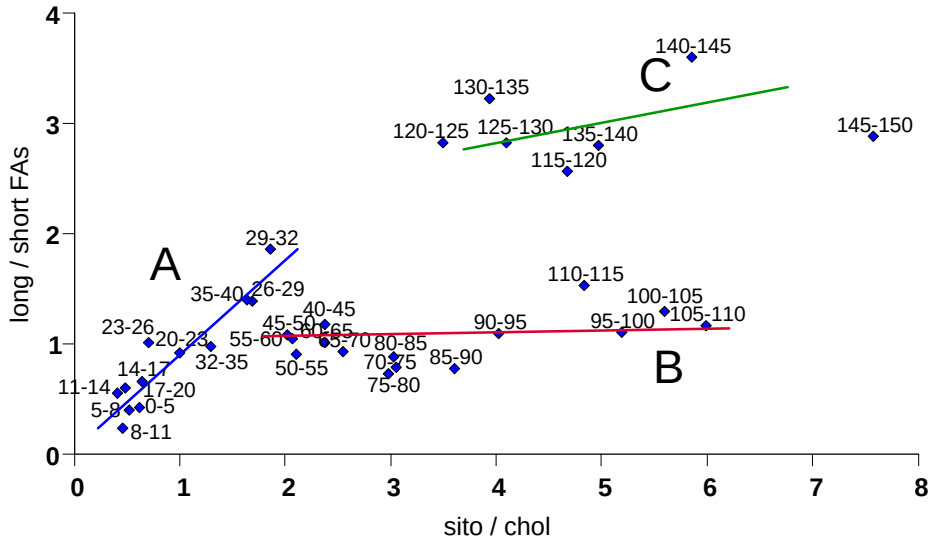

Figure 7.6 The relatively resistant sterol marker for terrestrial plant matter (sito / chol) plotted against the straight chain fatty alcohol marker ($\Sigma(C_{19} - C_{24}) / \Sigma(C_{12} - C_{18})$). See text for explanation of the region codes.

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The Principal Components Analysis in these two examples demonstrates the usefulness of these statistical methods in identifying the relationships between both compounds and sample sites. These data do not appear to show any enhanced concentrations of fatty alcohols that may be derived from anthropogenic usage; if there were high concentrations of detergent based alcohols or alcohol ethoxylates entering the system through the wastewater system, there would be more short chain compounds ($C_{12} - C_{16} + C_{18}$) in the samples. Figure 7.1 (the loadings plot from Blackpool Beach) does show increased prevalence of the odd chain fatty alcohols C_{13} to C_{19} but this includes the C_{17} which is specifically absent in the anthropogenic formulations (see Table 3.1) and also does not have the even chain components which are located elsewhere in the figure. It may be concluded from this information that these compounds are derived from natural sources rather than anthropogenic ones.

Similarly, in the core data, the surface samples are enriched in what superficially may be considered to be anthropogenic derived compounds but this group also includes phytol derived from chlorophyll and 20:1, the storage lipid from many zooplankton. Again, it must be concluded from these data that the fatty alcohols are essentially natural in origin in these two systems.

Another feature of PCA is the ability to look at the contribution each compound makes towards the overall "signature"; this can be seen in contributions plots. An example of this for the effluent from the STP at Blackpool Beach can be seen in Figure 7.7. If this effluent was enriched in fatty alcohols derived from anthropogenic sources, it might be expected to see high contributions from the $C_{12} - C_{16} + C_{18}$ compounds; in this case, the C_{17} is most prominent and the C_{16} and C_{18} are depleted relative to the average projection.

The internal relationship between each of the compounds in the sample allows a signature to be developed from the chemical contribution. This can be achieved in a semi-quantitative manner using the statistical technique of Partial Least Squares or PLS (see below).

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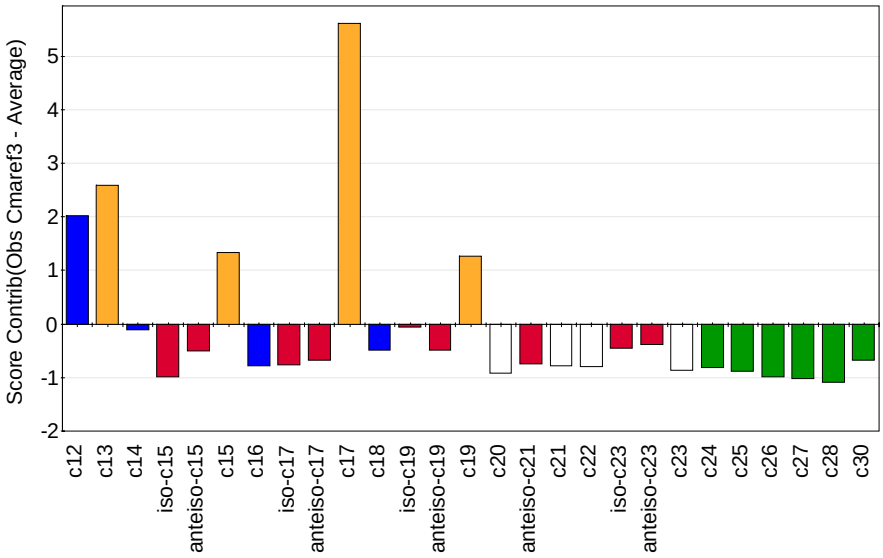

Figure 7.7 The contributions plot for the STP effluent relative to the average loadings. This sample was enriched in odd carbon number, straight chain fatty alcohols, especially the C_{17}.

A number of methods are available to improved discrimination in PCA including the use of proportion data (removes the concentration effect), log transformation (to improve normality) and addition of small values (to remove zeros by adding 50% of the limit of detection – useful if doing log transforms). An example of such improvements can be seen in Figure 7.8. The loadings for fatty alcohols from the Ria Formosa can be seen in 7.8a after a log transformation and the corresponding scores plot is in Figure 7.8b. These figures show a clear separation of compounds according to source with short chain alcohols to the right indicating marine materials and long chain alcohols to the left indicating terrestrial plants. The branched chain compounds show a range of associations indicating potential origins or different environments.

The locations in the scores plot (Figure 7.8b) also clearly separate according to their chemical composition and indicate the marine influenced locations compared to the terrestrial ones. Those sites adjacent to the sewage outfalls (*e.g.* 32-34) are located in the area associated with branched compounds in Figure 7.8a.

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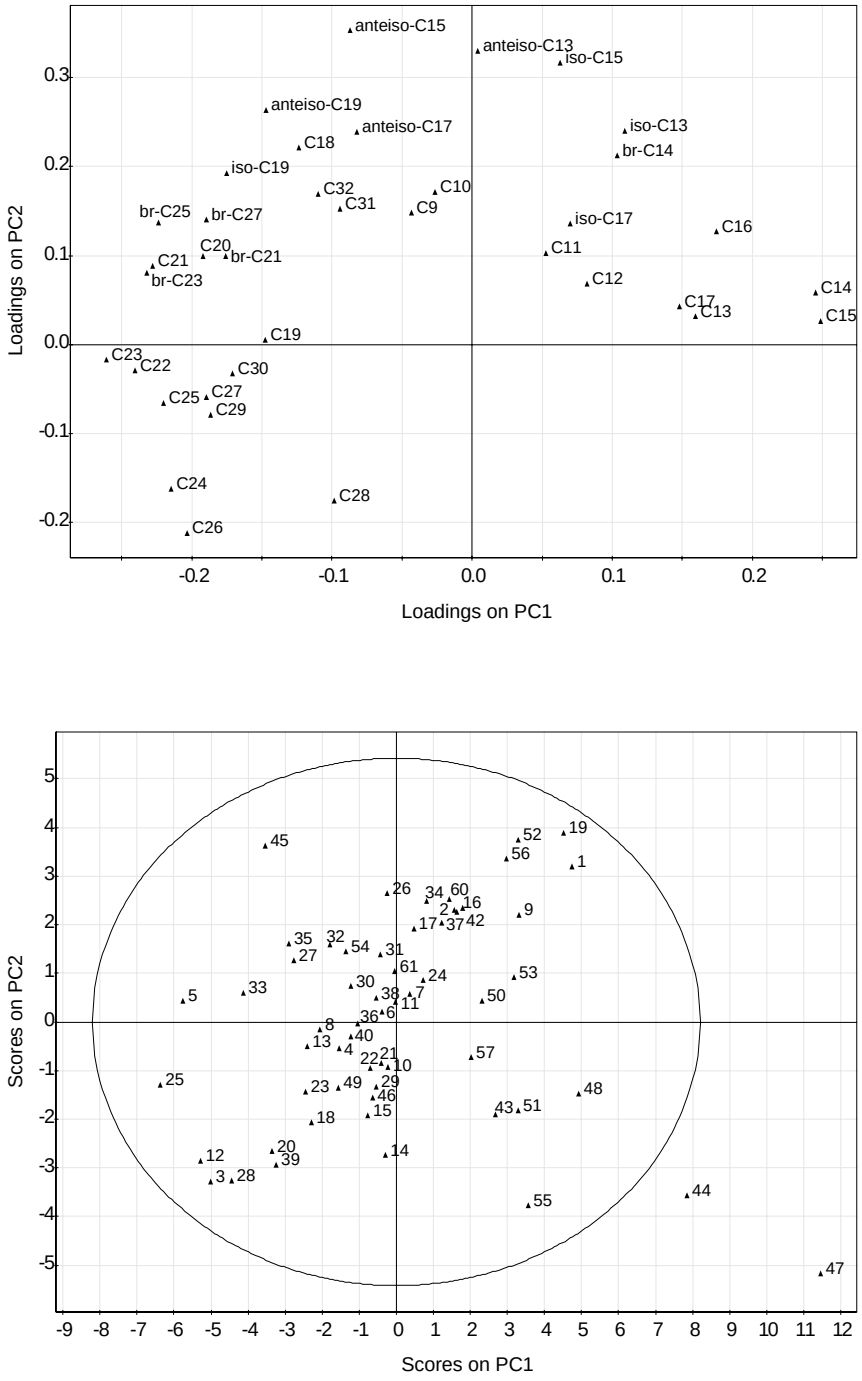

Figure 7.8. The (a) loadings and (b) scores plots for fatty alcohols from the Ria Formosa lagoon after log transformation and Principal Components Analysis. (Data from Mudge, *unpubl*.)

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PLS

The PLS technique was developed by Wold (Wold *et al.*, 1984) and has evolved into a powerful tool in environmental forensics (*e.g.* Yunker *et al.*, 1995; Mudge and Seguel, 1999). In essence, PLS performs PCA on data which are defined as the signature such as the STP effluent seen in Figure 7.2 (Geladi and Kowalski, 1986). This dataset which can be chemical concentrations, physical attributes or biological community information is called the X-Block and ideally will be a pure source sample but could be made up of environmental samples which have a high proportion of a single source such as effluents from sewage treatment plants or fatty alcohol product distributions from laboratory analysis. Since the samples come from the same source, although the concentrations may vary, PCA will generate a Principal Component 1 (PC1) that explains most of the variance in the analytical data, typically >90%. This projection or vector in *n*-dimensional space where *n* is the number of chemical compounds analysed can be described by a series of loading factors on each compound; those compounds which have a major impact on PC1 will have high loadings (either positive or negative) whereas those compounds which are relatively unimportant and therefore, do not have a major influence on the data, will have values close to zero. PC2 is fitted orthogonal to the first component so there is no component of PC1 influencing PC2. Once the first two PCs have been elucidated, their projection can be described in terms of the two sets of loadings.

These projections, which represent the signature defined in terms of the chemical compounds used, can now be applied to the environmental data (Y-Block). The amount of variance explained or predicted by each X-Block signature can be quantified. This can be shown graphically either through a scatter plot of the weightings on each sample or as the total variance explained (Mudge, 2002). If the signature is similar to that of the environmental data, a high value for the explained variance is produced. Conversely, if a poor fit is produced, the explained variance is also small. Each signature can be fitted in turn and all are fitted independently of each other. If none of them explain the variation seen in the data, the fits will be small in every case. A fuller treatment of the PLS methodology including the matrix manipulations used can be found in Geladi and Kowalski (1986).

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The advantage of PLS over other methods (*e.g*. simple ratios) is the way it uses all compounds and develops a signature based on the internal relationships between each one (Figure 7.7). In general, the more compounds that are used, the better the specificity of the signature. However, if several of the compounds are common between sources (*e.g.* all samples have lots of C_{16} which is nothing to do with the source discrimination), it may be better to reduce the number of compounds used to decrease the amount of overlap between signatures (Mudge *et al.*, 2003).

PCA can be used independently of the PLS technique to determine the number of potential sources that may be present in the Y-Block. The scores plot from such an analysis will group sites according to their chemical composition; those that co-vary are likely to have the same or a similar source. Inspection of the groupings may provide an insight into the number of sources although care must be exercised when dealing with mixtures of variable composition. This PCA technique may also be used to explore the source data and determine the groupings within the possible source materials.

Using the fatty alcohol data obtained from cow faecal matter in the Ribble catchment, which may contribute to Blackpool Beach, the amount of variance that that signature will predict in the beach samples (Y-Block) can be seen in Figure 7.9.

These results show that for some samples the predictable variance is zero while for others it can be as high as 87%. Most results are around the 30% region. This implies that using the fatty alcohols, it is possible to predict this amount of variance in the beach samples by using the domesticated farm animal's faecal matter. This is due to the similarity between the fatty alcohol profile in the samples and the cow faeces. By looking at the PCA loadings figure (Figure 7.1), this is due to the amount of long chain compounds which load negatively on PC1.

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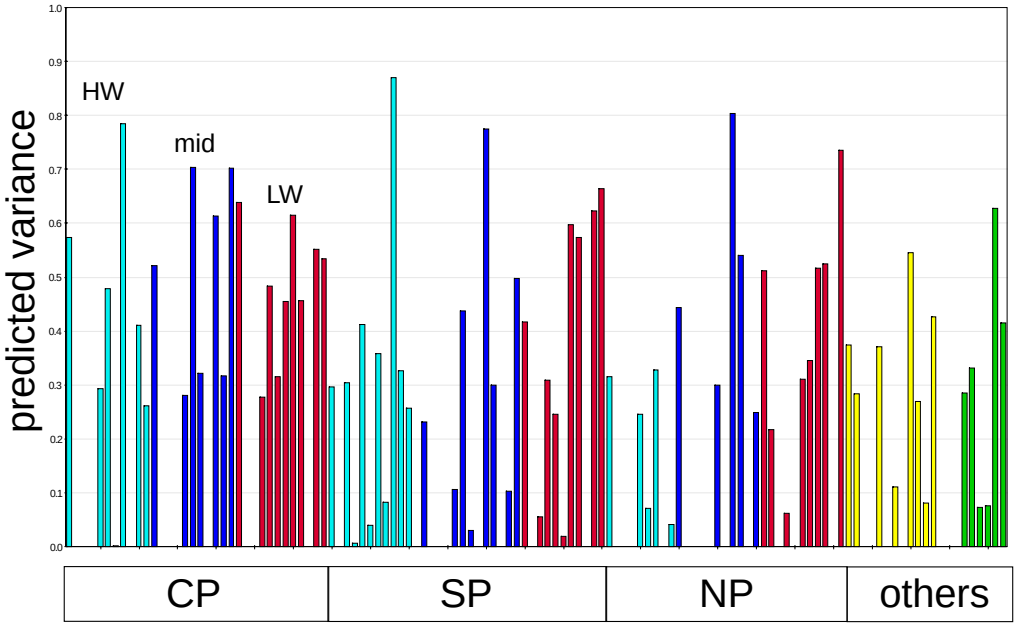

Figure 7.9 The amount of variance in beach samples (the Y-Block) using the fatty alcohols measured in faecal matter from domesticated animals (cows *etc.*). CP = central pier, SP = south pier, NP = north pier. The pale blue bars are samples collected at the high water mark (HW), dark blue from the mid tide level (mid) and red from the low water (LW). Each bar is from a different week.

Given a suitable set of samples which characterise the fatty alcohols derived from detergents, it would be possible to assess the contribution that this source made to any environmental fatty alcohol profile. As a first attempt, the data used to generate Figure 3.12 has been used to explain the profiles from the Ria Formosa lagoon in Portugal. The predictable variance can be seen in Figure 7.10 for each of the sample sites. The initial observation is that the values are considerably greater than might be expected in reality – to expect that more than 10% of the compounds come from detergents in such a system is not feasible. This highlights the inadequacy of using a simple chemical profile approach. As the chemical used in the detergents are similar to those found in nature, a degree of overlap is to be expected. A better approach may be a constrained least squares approach such as that used by Burns *et al.* (1997).

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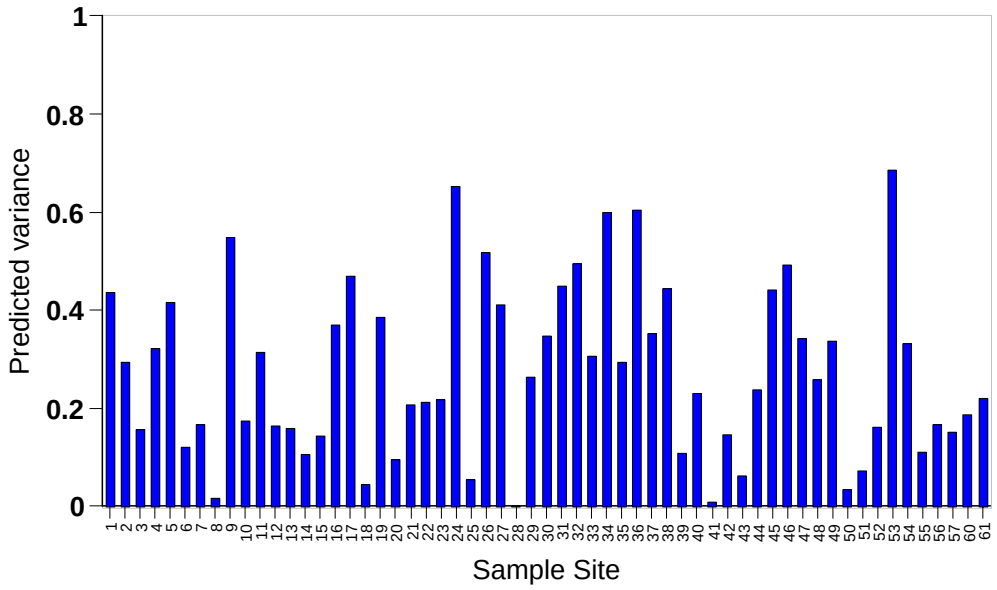

Figure 7.10 The amount of variance in a dataset from the Ria Formosa predictable from the alcohol signature from a series of European STPs. The values are significantly greater than might be reasonably be expected.

Further investigation of the use of signatures needs to be made so as to accurately predict the anthropogenic component in environmental samples. The best approaches for this may be through the use of compound specific stable isotope analysis as there is likely to be significant differences between the marine produced compounds and those developed on land either as natural terrestrial materials or detergents.

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Recommendations

This review has highlighted several aspects of the environmental chemistry of fatty alcohols that are not yet either done or reported. Some of these are summarised at the end of each chapter but key issues are:

1. How much of the fatty alcohol load present in any STP effluent is due to anthropogenic detergents? This requires consideration of the degradation of these materials with such a system together with the natural inputs and transformations. Free fatty alcohols may be formed from fatty acids or from the degradation of waxes; in both cases, analysis of the free alcohols will include a proportion of these compounds and so this analysis may be over-estimating how much of the fatty alcohols come from detergents. A more specific approach needs to be adopted such as compound specific stable isotope analysis.
2. The method of analysis for AEs will include natural alcohols as well although not wax bound compounds. However, no measure is made of these and this free component as a proportion of the total alcohols present, which have the potential to be free fatty alcohols at some stage, is not presented… this would give context to any value in an effluent. When measuring alcohols in effluents, the saponification method should be used along side to determine the total alcohols in the system.
3. What are the environmental transformations of fatty alcohols? It is likely that the free alcohols will degrade quicker than the bound alcohols as waxes. When assessing the environmental half life, it is important to consider the potential for production of compounds from bound sources or *de novo* synthesis by bacteria. This will be important in toxicity / ecotoxicity assessments where a time factor is involved.

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Appendix 1.

DETERGENT ALCOHOLS – A SUMMARY OF FEEDSTOCKS, PROCESSES AND END PRODUCTS

By Allen M. Nielsen

Detergent range alcohols are defined as alcohols containing twelve or more carbons (commonly restricted to the C_{12}-C_{18} range) and used mainly in detergent applications. These alcohols are commercially produced in a number of ways, but the resulting products are usually classified according to the source of raw materials used to produce them. There are two general categories: those derived from fats and oils (*oleochemical)* and those derived from crude oil, natural gas, natural gas liquids or coal (petrochemical*)*.

OLEOCHEMCIAL BASED ALCOHOLS

In natural fats and oils the hydrocarbon chains have already been formed in the raw material. Biological processes in living organisms synthesize long carbon chains in the form of triglycerides. From plant and animal oils the triglycerides are separated and chemically converted into key alcohol intermediates. Coconut oil and palm kernel oil are preferred for the production of C_{12}-C_{14} chain lengths. Animal fats (tallow) and palm oil are preferred for the production of C_{16}-C_{18} chain lengths (Table A1).

Table A1. Composition of Natural Triglycerides (wt %)

Triglyceride Fat or Oil	Caprylic	Capric	Lauric	Myristic	Myristoleic	Pentadecanoic	Palmitic	Palmitoleic	Margaric	Stearic	Oleic	Linoleic	Linolenic
	C8	C10	C12	C14	C14	C15	C16	C16	C17	C18	C18	C18	C18
Tallow				3.2	1.0	0.4	26.4	2.6	0.9	26.9	36.7	(1)	
Palm				0.9			46.6			4.1	39.3	9.1	
Coconut	8.0	6.7	51.3	16.2			7.6			2.7	5.9	1.6	
Palm Kernel	4.0	5.0	50.0	15.0			7.0	0.5		2.0	15.0		1.0

Fatty Acids

In general, the ester linkage in the triglyceride molecules can be severed in two ways. In one process steam is used to hydrolyze the triglycerides to yield fatty acids and glycerin. (Figure A1)

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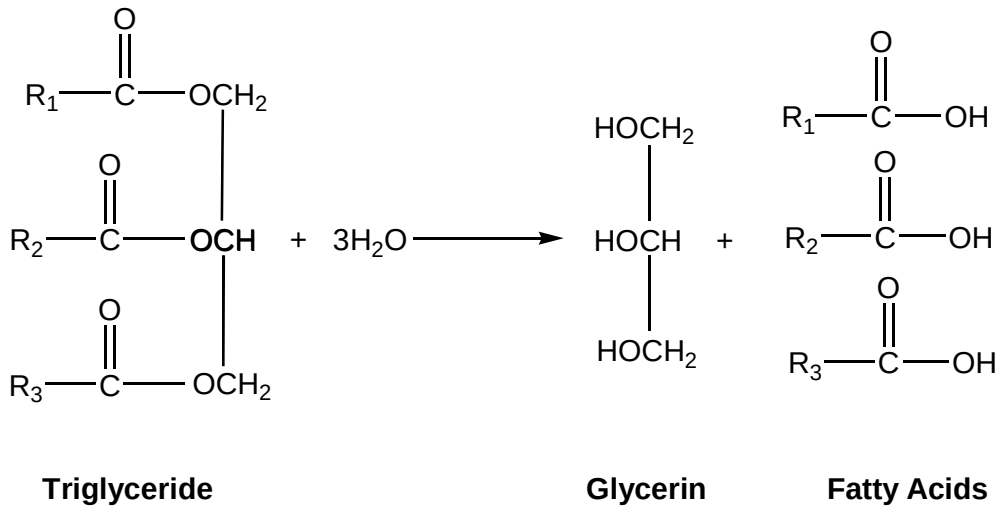

Figure A1. Fatty Acid Production (Fat Splitting)

Fatty Methyl Esters

In the other process methanol is used to transesterify the triglycerides to yield fatty methyl esters and glycerin. (Figure A2)

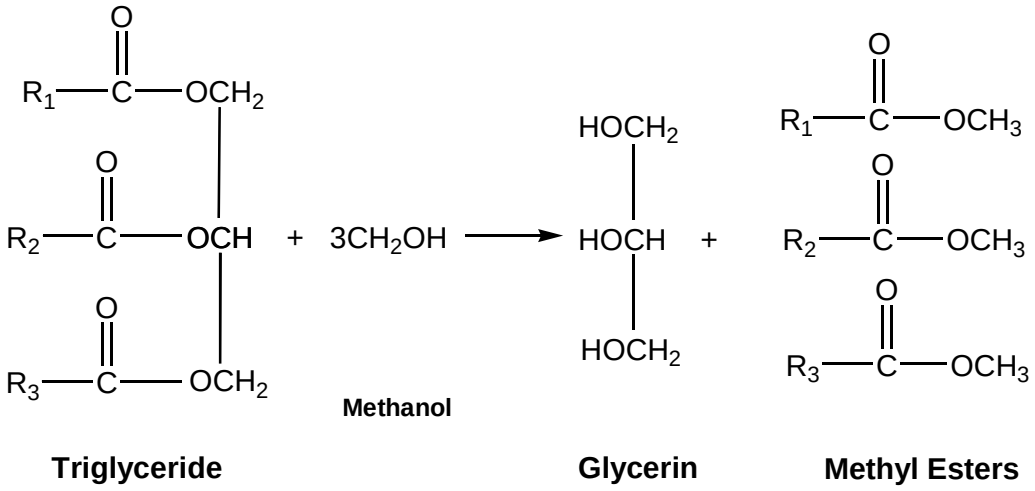

Figure A2. Methyl Ester Production

Oleo Chemical Fatty Alcohols

Oleo chemical fatty alcohols of the C_{12} to C_{18} chain lengths are produced by the hydrogenation of both fatty methyl esters and fatty acids (Figures A3 and A4).

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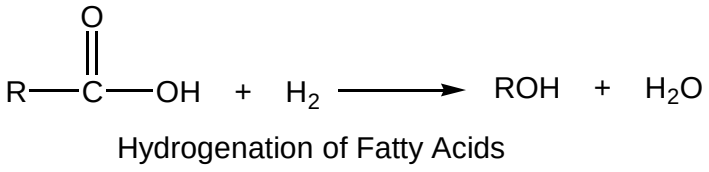
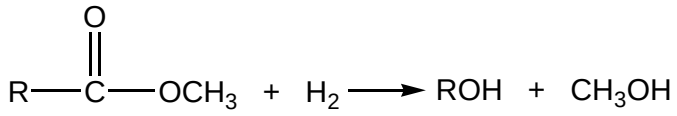


Hydrogenation of Fatty Acids



Hydrogenation of Methyl Esters

Figure A3. Oleo Chemical Alcohol Production

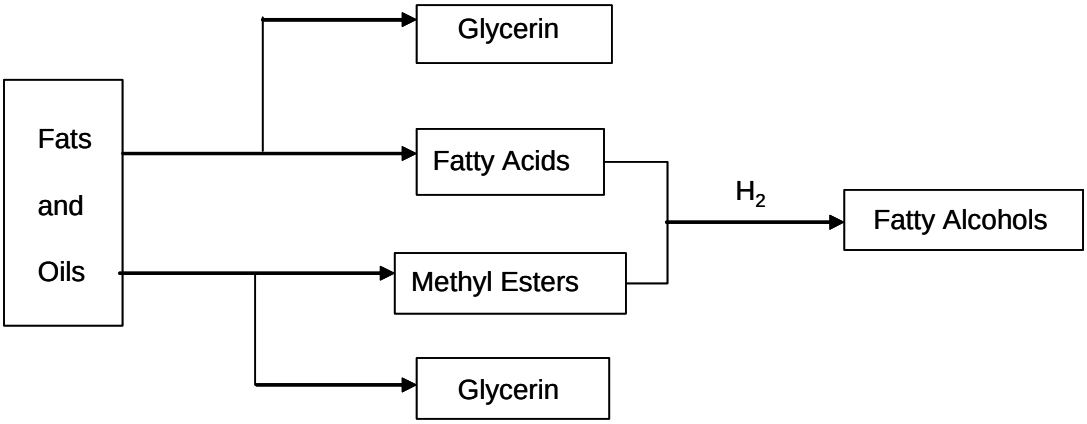


Figure A4. Summary of Oleo Chemical Alcohol Production

These alcohols are even carbon chain lengths, >99% linear, primary alcohols. Fatty alcohols are important oleochemcial-based surfactant intermediates. From them are made many surfactant products including alcohol sulphates, alcohol ethoxylates and alcohol ether sulphates

PETROCHEMCIAL BASED ALCOHOLS

Alcohols based on Petroleum

Linear hydrocarbon chains or normal paraffin can be extracted from petroleum fractions. Kerosene and gas oil are different boiling fractions of petroleum that contain hydrocarbons of the C_{10}-C_{16} and higher chain lengths.

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Normal Paraffin

Kerosene is an important hydrocarbon source. Using molecular sieve separation process such as MOLEX OR ISOSIV, the linear or normal paraffin are separated from the branched and cyclic hydrocarbons. The normal paraffin is distilled into various cuts and the branched/cyclic hydrocarbon stream or raffinate is sold as an upgraded fuel (Figure A5).

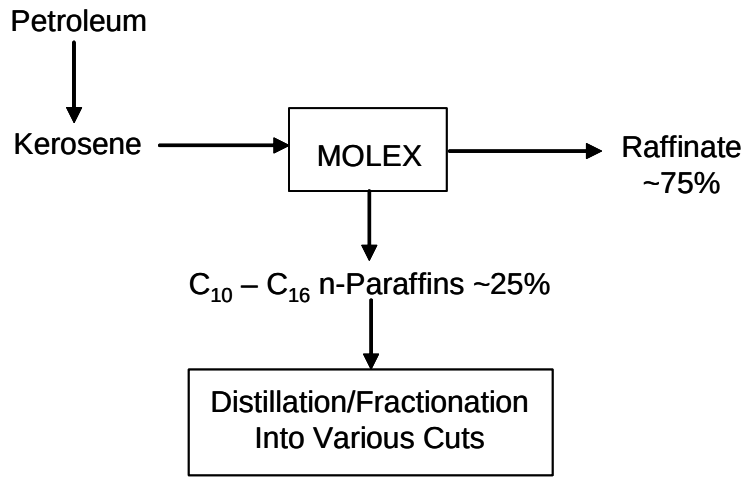

Figure A5. Normal Paraffin Production

Internal Olefins

Pure Internal olefins can be produced from normal linear paraffin. In the combined PACOL/OLEX process, dilute PACOL olefins are concentrated by the OLEX process to about 96% internal olefins (Figures A6 and A7).

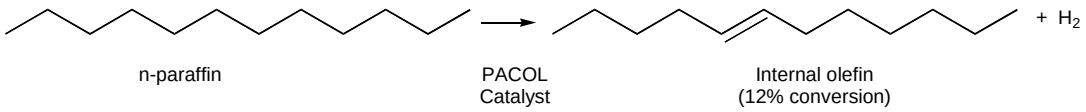

Figure A6. PACOL Dehydrogenation of n-Paraffins

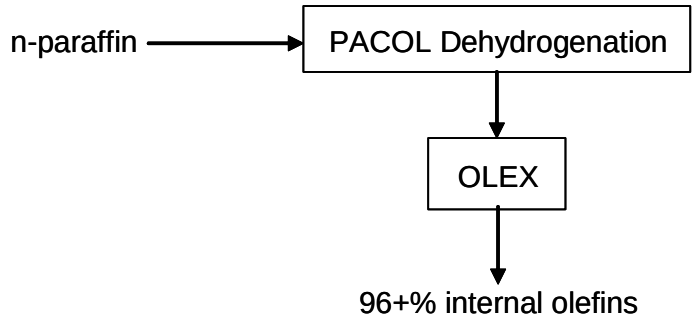

Figure A7. PACOL/OLEX Internal Olefins

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Conventional OXO Alcohols Based on Internal Olefins

Internal olefins can be converted to conventional OXO alcohols. In contrast to oleo chemical based alcohols, OXO alcohols have both odd and even carbon chain lengths and they have up to 50% branching at the second carbon position.

OXO (Hydroformylation Reaction)

The OXO reaction as applied to the synthesis of detergent-range alcohols involves the reaction of olefins with synthesis gas (CO/H_2) in the presence of an OXO catalyst to yield higher alcohols. The sequence of steps includes: hydroformylation, catalyst removal and recycle, aldehyde distillation, aldehyde hydrogenation and purification of the product alcohols as shown in Figure A8.

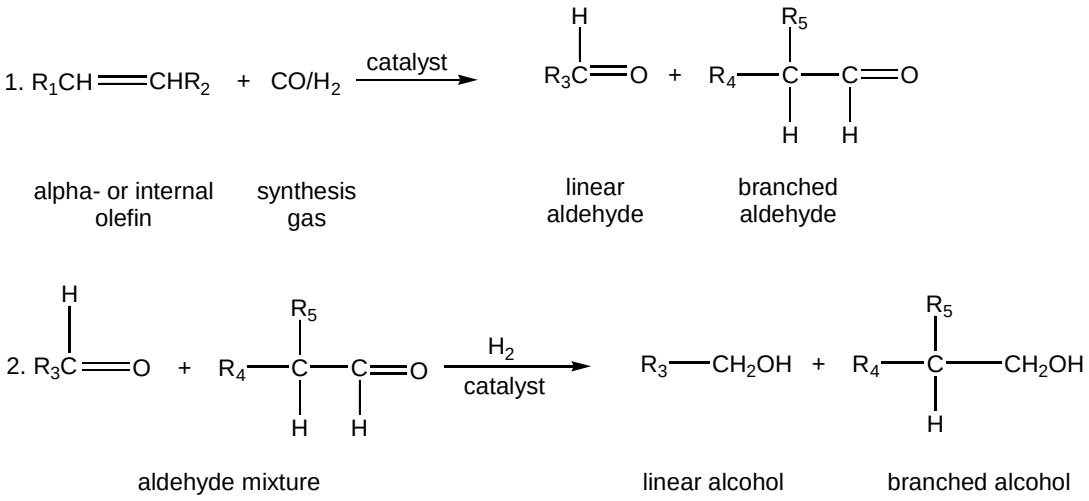

Figure A8. OXO Process

Alcohols based on Ethylene

Ziegler Ethylene Growth Process

Ethylene is used as a building block to form long hydrocarbon chains. This process employs what is called a growth reaction to make hydrocarbon chains from C_2 to C_{20} in length. Hydrocarbon chains are grown by adding ethylene units to an organometallic compound such as triethyl aluminium. The ethylene units are inserted between the growing alkyl chains and the aluminium, producing trialkyl aluminium or growth product as noted in Figure A9.

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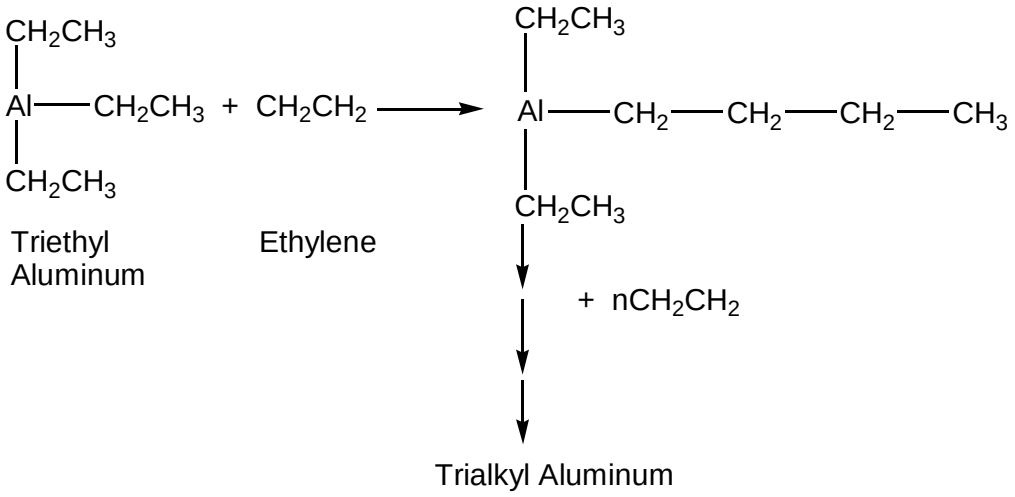

Figure A9. Ziegler Ethylene Growth Process

Ziegler Alcohols

Further processing of the growth product yields linear primary alcohols. In the Ziegler alcohol process linear, even-carbon-chain fatty alcohols are produced from the growth product by controlled oxidation followed by hydrolysis. For a given chain length, these alcohols are essentially identical to natural alcohols, having linear, even-carbon-chain length primary structures. A stoichiometric amount of aluminium is used in this process that eventually is converted into high-purity alumina after hydrolysis (Figures A10 and A11).

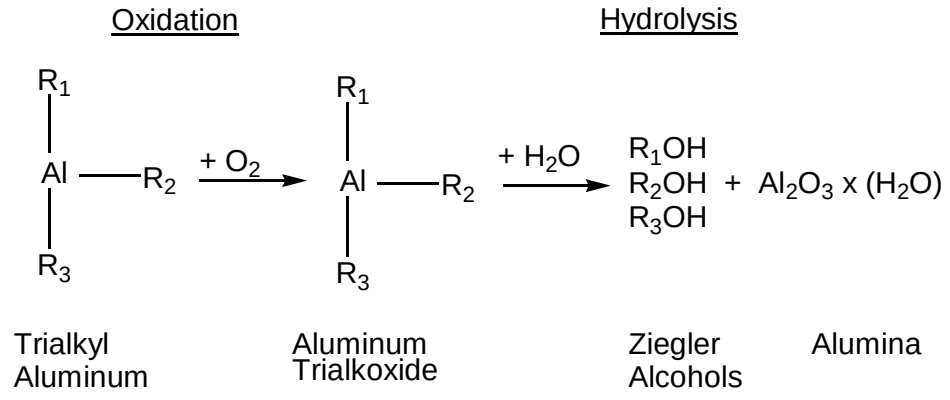

Figure A10. Ziegler Alcohol Process Chemistry

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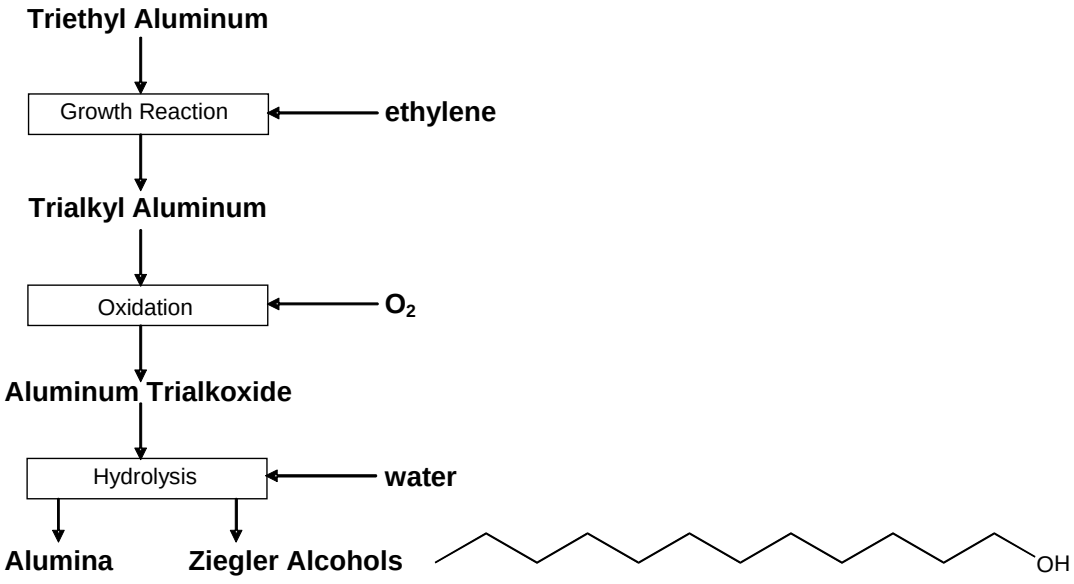

Figure A11. Ziegler Alcohol Process

Modified OXO Alcohols

SHOP Alpha Olefins

The SHOP process employs an ethylene oligomerization reaction to make alpha olefins. In the first part of the process linear, even-carbon-chain alpha olefins are produced. As with other ethylene growth reactions, the olefins are produced in a broad distribution of carbon chain lengths. Some of these chain lengths are more desirable as alpha olefin products than others and are separated by distillation and sold.

SHOP Internal Olefins and Modified OXO Alcohols

In the second part of the SHOP process, alpha olefins of the less desirable chain lengths are converted to linear internal olefins in a complicated process called isomerization/disproportionation/metathesis. The internal olefins produced in this process have both odd and even chain lengths in the range of C_{10} to C_{14} as summarized in Figure A12.

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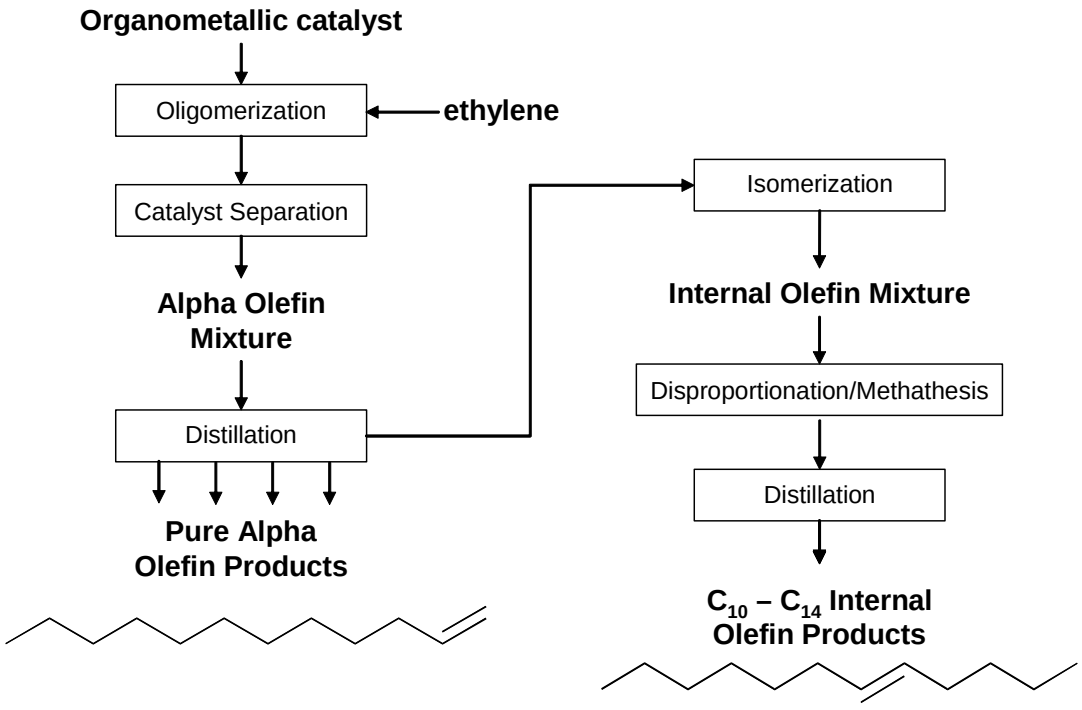

Figure A12. SHOP Olefin Process

Internal olefins from the SHOP process are converted in the Modified OXO process which produces alcohols having 20% branching. (Figure A13)

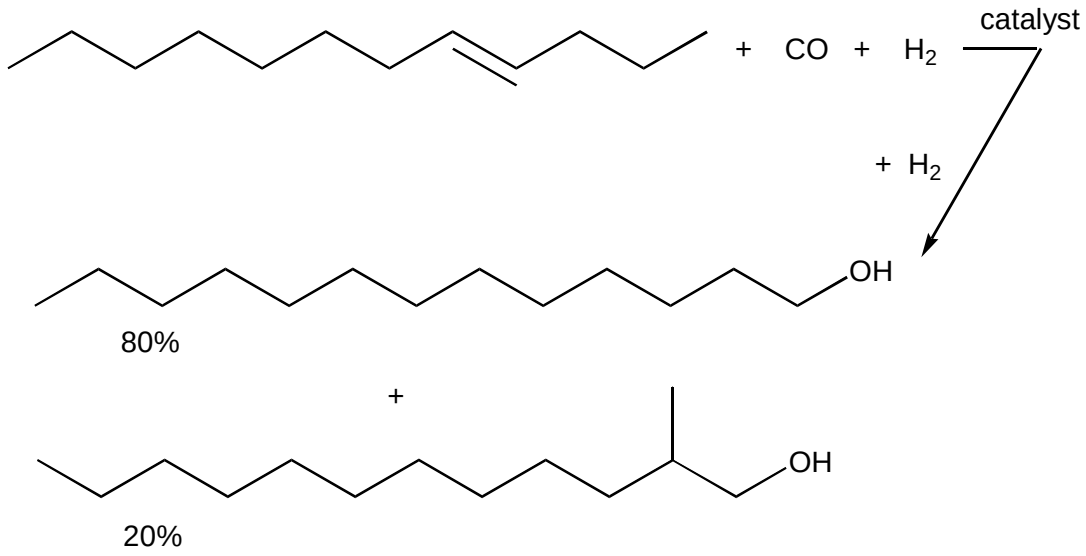

Figure A13. Modified OXO Alcohol Process

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Shell's commercial mono-methyl branched alcohol is in the $C_{16} - C_{17}$ range. The starting material in the upper left hand side of Figure A14 is a linear internal olefin (IO). The top reaction shows the route to traditional NEODOL® Alcohols from an IO. The lower reactions show how Shell converts a linear internal olefin into a branched IO. Standard modified-OXO chemistry converts the branched IO into a branched primary alcohol. Each of these structures depicts one of many possible isomers. The commercial product is >95 % branched, but fully biodegradable.

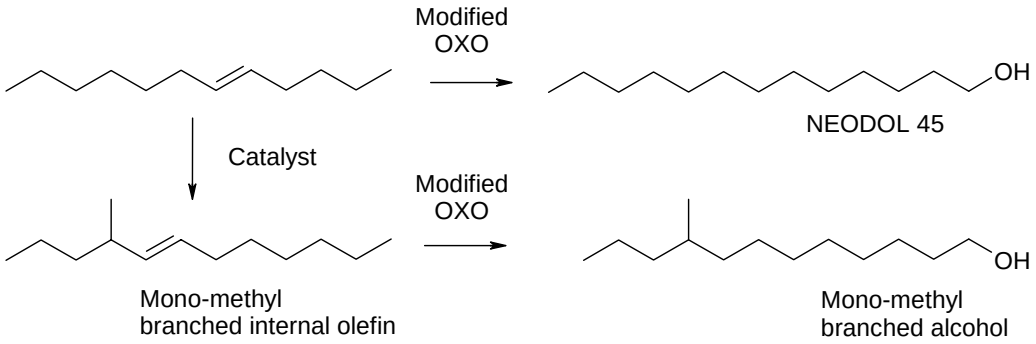

Figure A14. Modified OXO process for mid-range alcohols.

OXO Alcohols Derived from Fischer-Tropsch Alpha Olefins

The process begins with the production of synthesis (syngas) from either coal or natural gas. Syngas is then converted to a liquid hydrocarbon stream in the Fischer-Tropsch (F-T) process (Figure A15).

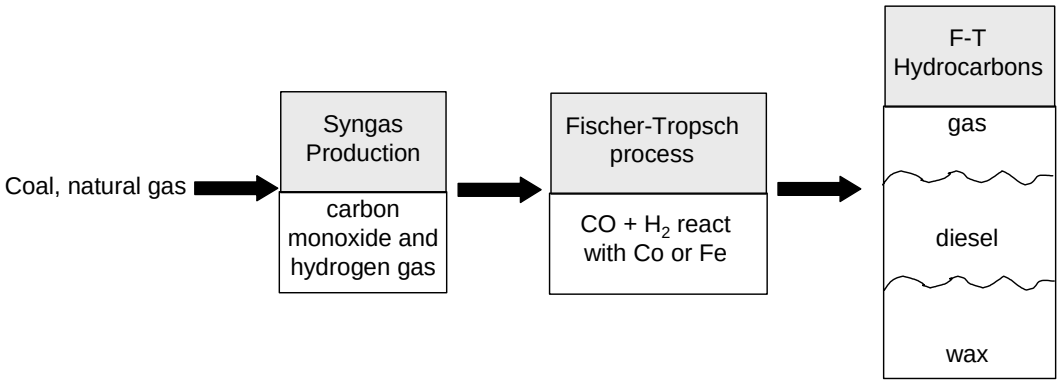

Figure A15. Fischer-Tropsch Process

This stream consists of both even and odd chain-length hydrocarbons in a Shultz-Flory distribution, the principal components are alpha olefins. The C_{11}, C_{12} hydrocarbons are separated by distillation. Hydroformylation, using CO and H_2, then serves to select the olefinic portion of that stream to make long-chain aldehydes. Further hydrogenation and purification yield the F-T oxo 1213 alcohol. F-T alcohols are 50% branched, but randomly branched because of methyl-branching in the precursor olefins. The process flow is summarized in Figure A16.

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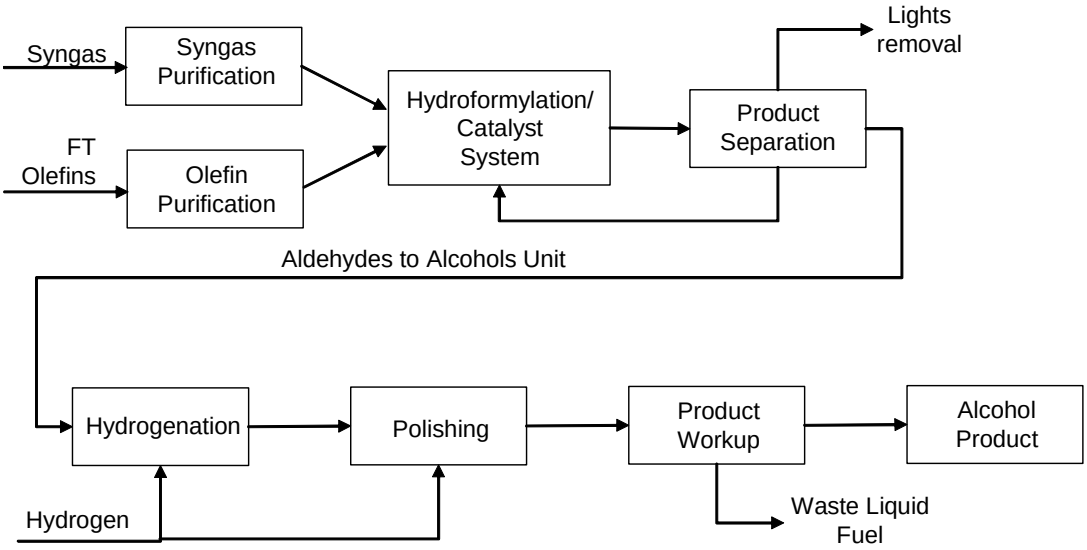

Figure A16. Fischer-Tropsch OXO Alcohol Process

SUMMARY

A comparison of the major detergent alcohols is shown below in Table A2.

Table A2. Comparison of Detergent Alcohols

Alcohol Type	Raw Material	Carbon Chain Distribution	Percent Linear
Oleo chemical	coconut oil, PKO	C_{10} - C_{18} even only	100%
Ziegler	ethylene	C_2 - C_{20} even only	98%
Modified OXO	ethylene	C_{12} - C_{15} even and odd	80%
Regular OXO	n-paraffin	C_{12} - C_{15} even and odd	50%

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