What contribution do detergent alcohols make to sewage discharges and the marine environment?

Stephen M Mudge

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Abstract

To investigate the potential sources for fatty alcohols arriving at a WWTP and entering the receiving waters, a study was conducted in North Wales in the catchment of the Treborth treatment plant. Two dimensional stable isotope analyses (^{13}C and ^{2}H) had been shown to be a suitable analytical tool in an earlier study and so was used here to separate the different sources of fatty alcohols. Since the fatty alcohols may arise from natural biological synthesis as well as from synthetic production, this approach was deemed the most appropriate as the stable isotopes were characteristic of the sources.

Samples were collected from four soils, four marine sediments, four detergents used in the catchment and at different parts of the WWTP. Samples were collected to establish the temporal variability of both the influent and effluent. The solid samples were refluxed in KOH in methanol, partitioned into hexane and derivatised with BSTFA to form the trimethylsilyl esters of the fatty alcohols. Liquid samples had KOH added to induce saponification and were then extracted by liquid – liquid separation into hexane. All environmental samples had an internal standard added to allow quantification of the compounds. Detergent samples were extracted by conversion of the alkyl chain to an iodide.

All samples were analysed by traditional gas chromatography – mass spectrometry to identify and quantify the compounds present. The samples were then analysed by stable isotope ratio mass spectrometry for both the ^{13}C and ^{2}H in each compound.

The fatty alcohol profiles varied according to the source but there was much commonality in the detergent range of C_{12} to C_{18}. Soil samples had fatty alcohols up to C_{28} in length but the majority were shorter in chain length. Marine samples principally had compounds in the C_{12} to C_{18} chain length range. There was variability in both the concentration of fatty alcohols in the influent as well as the profile. However, the C_{12} dominated overall. The total concentrations decreased in the liquid phases through the treatment works with the majority of the compounds accumulating in the sludge (biosolids).

The ^{13}C signal alone was good enough to separate terrestrial from marine sources in the environment but it does not separate faecal sources from either natural or oil-based detergents. Natural plant based detergents have $\delta^{13}C$ values between -26 and -32‰ while oil-based detergents occupy a range between -25 and -30‰. The corresponding $\delta^{2}H$ values are -250‰ for natural sourced materials and -50‰ for oil-based detergents which does enable these two sources to be separated.

Of the supplied detergents which are typical of the local catchment, samples 3 and 4 appear to exclusively derived from oil-based raw materials while detergents 1 and 2 have C_{12} and C_{14} components from natural sources combined with some oil-based longer chain fatty alcohols.

The influent to the WWTP contained fatty alcohols which originated mainly from faecal sources and natural surfactants (~75%) with a smaller amount potentially derived from oil-based surfactants (~25%). This mixture is compound specific and only realistically applies to the C_{12} and maybe the C_{14} fatty alcohols. Longer chain compounds do not appear commonly in the influent. The effluents from the WWTP contained mainly short chain compounds with a chain length less than C_{16}. Their $\delta^{2}H$ stable

isotope signature was different to the other potential sources examined and suggests bacterial synthesis during the treatment processes.

The sludge produced from the WWTP had relatively high concentrations of fatty alcohols as would be expected from their low water solubility. The stable isotopic signatures were consistent with a mixture of faecal and detergent sources although this again was variable depending on the particular compound examined. For instance, the C_{18} does not appear to have any detergent influence although the C_{13} might have but this compound is also synthesised by bacteria within the WWTP. The sludge in this area is routinely spread on agricultural land as a fertiliser and may find its way back into the sea via land runoff.

The marine sediment samples had fatty alcohols that are typical of marine production (short chain) and with stable isotope values that indicate exclusive marine production for the C_{14} with potentially mixed terrestrial for the C_{16} and C_{18} compounds. Therefore, the fatty alcohols in the marine sediments are not the same as those that were discharged in the liquid effluent and these fatty alcohols were not the ones that entered the works through the influent but were synthesised or recycled within the works.

Only the $\delta^{13}C$ value is available for the C_{12} and this might indicate some detergent contribution to this system for that compound alone at one site. It is also possible that Combined Sewer Overflows (CSOs) might contribute direct surface water runoff to that location.

On the basis of the mean WWTP discharge rates and the mean C_{12} concentration in the effluent, this works would contribute ~300 g of C_{12} per day to the receiving waters with a total of around 640 g of fatty alcohols per day (in ~10000 m^3 of water) but it must be stressed that these are not the same compounds that entered through the influent.

Introduction

Fatty alcohols are widely produced by bacteria, plants and animals for a variety of purposes (Sargent et al., 1976) including an energy reserve, a source of metabolic water, a buoyancy generator, in the composition of biosonar lenses in marine mammals and as a thermal insulator. Land plants (Dahl et al., 2005) and insects (Nelson et al., 1999) may also use fatty alcohols in the form of waxes for the prevention of desiccation, protection from bacterial attack and UV screening (a full review can be seen in Mudge et al., 2008. In general, terrestrial plants produce long chain compounds with carbon chain lengths greater than 20. In turn, organisms that consume these plants also tend to have similar chain length profiles. These long chain compounds have higher melting points and are better able to protect the organisms where volatilisation is a possibility. In comparison, marine organisms do not have the same problem with atmospheric exposure and tend to have shorter chain compounds typically from C_{10} to C_{18}.

Due to the synthetic pathway by which these compounds are formed, most higher organisms tend to have even carbon numbered straight chains such as C_{10}, C_{12} and C_{14}. Bacteria, however, may use a slightly different initial starting compound in the synthesis and can form odd chain lengths as well as branched chain compounds (Perry et al., 2002), typically in the *iso* and *anteiso* positions. It is rare to find naturally produced secondary alcohols and primary (terminal hydroxyl) forms are most common.

Detergent formulations have included fatty alcohols for a number of years either as alcohol ethoxylates (AE) or alcohol ethoxysulphates (AES). The chain length of the compounds used in these formulations has typically been in the C_{10} to C_{18} region with some mid-chain methyl branches possible as well as straight chain moieties (Mudge et al., 2008). These alcohols may be sourced from both natural materials such as palm oils or from *de novo* synthesis from oil components (Matheson, 1996). The majority of these compounds are functionally identical to the natural fatty alcohols produced by bacteria, plants and animals.

Fatty alcohols may enter the marine environment from a range of sources including both natural production by animals and plants as well as the use of man-made products such as liquid detergents and cosmetics. Terrestrial runoff may deliver long chain plant and insect waxes both associated with the parent biological material or after partial degradation in soils. Marine organisms may synthesise fatty alcohols directly or they may be formed *in situ* through the degradation of other organic matter. Waste water treatment plants (WWTP) collect surface water drainage containing soils and plant materials as well as faecal matter, food waste and anthropogenic fatty alcohols used in cleaning or cosmetic formulations. These compounds may be altered during passage to the influent works of the WWTP, within the WWTP itself and also be removed with the solid phase sludges (biosolids) so the final effluent may have a different suite of compounds. The discharges would combine with the natural materials in the marine environment from runoff and *in situ* production.

It has been shown during Phase I of this work that stable isotopes of carbon and hydrogen can be used to develop a fingerprint for the different source materials (Figure 1). The carbon-13 content of molecules can vary depending on the synthetic pathway which may preferentially favour the lighter (^{12}C) or heavier (^{13}C) isotope. These isotopically light or heavy compounds retain their fingerprint unless

involved in reactions which may exchange elements. The same is true of the hydrogen isotope ^{2}H (deuterium) and the two elements together can provide a fingerprint that can be used in source apportionment.

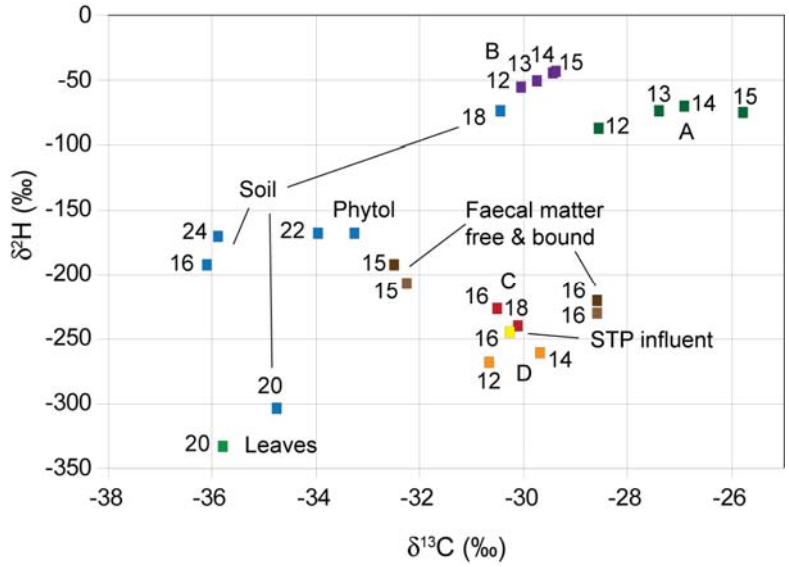

Figure 1. The two dimensional stable isotope ($\delta^{2}H$ and $\delta^{13}C$) signatures for samples from the Phase I study. The oil derived surfactant fatty alcohols are labelled A and B while those derived from plant materials are labelled C and D.

The amount of enrichment or depletion of ^{13}C and ^{2}H in a compound relative to a standard (Vienna Pee Dee Belemnite (PDB) in the case of ^{13}C and Standard Marine Ocean Water (SMOW) for ^{2}H) is usually expressed in the form of $\delta^{13}C$ and $\delta^{2}H$ (Philp & Kuder, 2008). This is calculated from the equation:

$$\delta^{13}C = \left(\frac{\left(\frac{^{13}C}{^{12}C}\right)_{sample}}{\left(\frac{^{13}C}{^{12}C}\right)_{standard}} - 1 \right) \times 1000‰$$

where the ^{13}C and ^{12}C are the isotopic content of the compounds in the sample and standard. These values are usually reported as their ratios hence the name stable isotope RATIO mass spectrometry.

The Phase I project was able to discriminate between sources showing that this approach is valid and useable in the context of source apportionment. In Phase I, the sampling and analysis was conducted to determine if the stable isotopes could distinguish between natural and synthetic fatty alcohols. The fatty alcohol profiles analysed by GC-MS for the synthetic raw materials (Lial and Neodol) are very different from the natural materials as these mixtures are rich in mid-chain branched compounds which are essentially absent from the natural environment and all sewage samples. In all the environmental fatty

alcohol analyses reported (Mudge et al., 2008), no mid-chain branched compounds were identified and only the *iso* and *anteiso* moieties were seen. The δ^2H and $\delta^{13}C$ stable isotope values for these two synthetic detergent materials also had different values to each other and samples taken from the WWTP. However, this cannot be said for the two detergent material samples that were derived from palm oil (Lorol and Stenol). Since they have biological precursors, they had similar fatty acid / alcohol profiles as natural materials and also had similar but not identical stable isotope values. Therefore, consumer products made with these formulations will have similar stable isotopic signatures and may be more difficult to distinguish from fatty alcohols in other sample extracts.

Of great interest, however, was the value of the C_{16} fatty alcohol in the marine sediment – essentially the short term sink for the WWTP discharge. Although the hydrogen values could not be obtained, the carbon stable isotope value could; this value (-24‰) was significantly greater than all the other samples measured and is indicative of marine faunal production and suggests very little anthropogenic or sewage derived fatty alcohol input at this site.

This second phase of work aims to provide a source apportionment for all the potential sources that may contribute to the marine environment adjacent to the outfall of a WWTP using the stable isotope method and a linear mixing model.

Materials and Methods

Potential Source Materials

Samples of raw fatty alcohols used in the formulation of detergents and cosmetics were provided from the manufacturers. These had been analysed in the first phase and analyses were repeated to ensure consistency between results. These compounds required no special treatment and were simply derivatised at 60°C for 10 min with BSTFA from Sigma Aldrich (the same batch as used in Phase 1) to form the fatty alcohol – TMS ethers.

Final products were selected after a qualitative survey of the different brands of liquid detergents available in the major supermarket serving the catchment of the Treborth WWTP. On the basis of this survey, four liquid formulations containing fatty alcohols, two hand dishwashing liquids and two liquid laundry detergents, were selected and provided simply labelled 1 – 4 for analysis. These fatty alcohols were extracted as their alkyl iodides using the following methodology:

1. 100 µl of the detergent sample was added to a Reactivial together with 2 ml of 55% hydrogen iodide. The mixture was shaken and heated to 130°C for 100 min with intermittent agitation.

2. After cooling, the mixture was transferred to a 100 ml separation funnel with 2 x 2 ml water and 3 x 3 ml pentane washes to ensure all materials were transferred. The mixture was vigorously shaken and sufficient 0.1 N sodium thiosulphate was added to mop up any free iodine thus making the solution colourless. The mixture was shaken for a further 30 s.

3. The lower aqueous phase was run off and the upper pentane phase retained and rewashed with 15 ml of sodium thiosulphate.

4. In the case of detergents 2 and 3, this resulted in a clear pentane phase within a few minutes. This was then separated from the lower aqueous phase, re-washed with water (2 x 15 ml), taken to dryness under a stream of nitrogen and re-dissolved in 1 ml of hexane.

5. Detergents 1 and 4 produced an emulsion on shaking which did not separate cleanly even after 24 h. Therefore, the lower aqueous phase was removed and the emulsion layer was transferred to a glass centrifuge tube and spun briefly at 2500 rpm. The now clear pentane phase was removed by pipette and taken to dryness under a stream of nitrogen. The sample was then re-dissolved in 1 ml of hexane.

Sediments and Soils

Soil samples were collected from land that would potentially contribute to the Menai Strait, North Wales. Soils were collected as surface scrapes from an arable field, a pasture field, within a deciduous wood and within a coniferous wood. The location of the samples can be seen in Figure 2 and photographs can are in the Appendix. In each case, ~200 ml of soils was collected. The marine sediment samples were collected in a similar fashion along a transect from the discharge point of the WWTP. The straight line distances to the discharge point can be seen in Table 1.

Table 1. Key sample data

Sample Description	OS GB Sheet SH Eastings	Northings	Distance from outfall (m)
SOILS			
Deciduous	54391	71546	
Conifer	54293	71513	
Pasture	54286	71658	
Arable	54257	74753	
SEDIMENTS			
St. Mary's Church	53698	71073	250
Swellies	54487	71566	1000
Church Island	55212	71791	1700
Ynys Faelog	55929	72110	2700

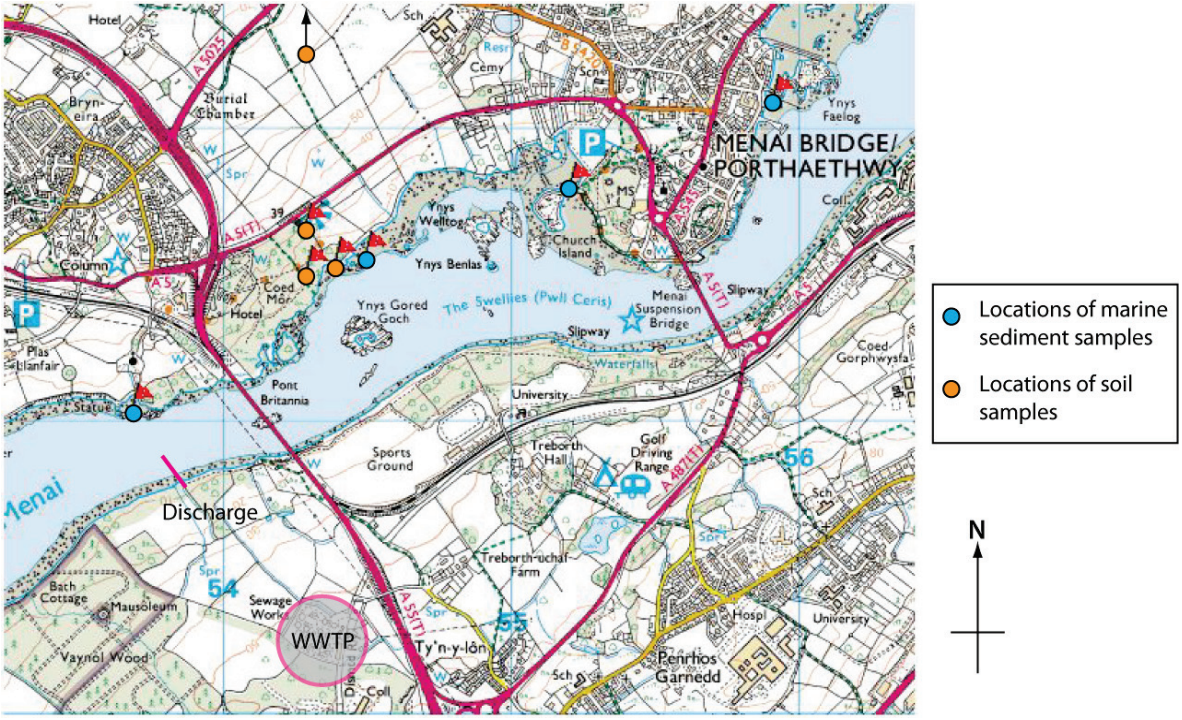

Figure 2. Sampling locations for the soil and marine sediment samples.

All samples were returned to the laboratory in a cool box and ~100 g wet weight was extracted using the following protocol (Mudge & Norris, 1997).

1. Approximately 100 g wet weight was weighed accurately to two decimal places and placed in a round bottom flask. An internal standard was added (1 ml of a 1.02 $mg.ml^{-1}$ solution of 2-dodecanol (Figure 3) from Sigma Aldrich in methanol) together with 80 – 100 ml of 6% (w/v) potassium hydroxide in methanol.

2. The sample was refluxed for four hours. After cooling, the liquid was drained into glass centrifuge tubes and spun at 2500 rpm for 5 min to settle the solids and produce a clear liquor.

3. The supernatant was poured into a separating funnel and the non-polar (lipid) compounds extracted into hexane twice. The combined hexane phases were rotary evaporated to <5 ml and finally taken to dryness under a stream of nitrogen.

4. The lipids were derivatised at 60°C with ~5 drops of BSTFA for 2 h to ensure complete derivatisation of the secondary alcohol. Excess BSTFA was evaporated under nitrogen and the final samples re-dissolved in 1 ml of hexane.

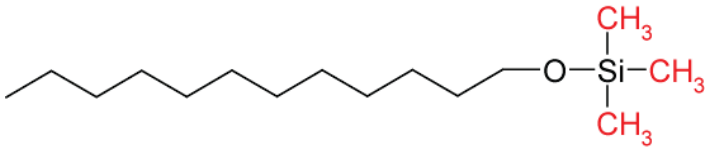

Figure 3. The atoms highlighted in red have been added as part of the TMS group and will contribute to the overall $\delta^{13}C$ and $\delta^{2}H$ values. Therefore, a correction needs to be applied to calculate the original molecule values.

Waste Water Treatment Plant Samples

The majority of the samples collected within the WWTP were liquids with suspended solids. The only exception was the sludge samples collected and processed in a similar manner to the sediments and soils detailed above. Samples were collected at several points within the WWTP and at different times to assess the temporal variability. A schematic showing the position of samples within the treatment cycle at the WWTP can be seen in Figure 4.

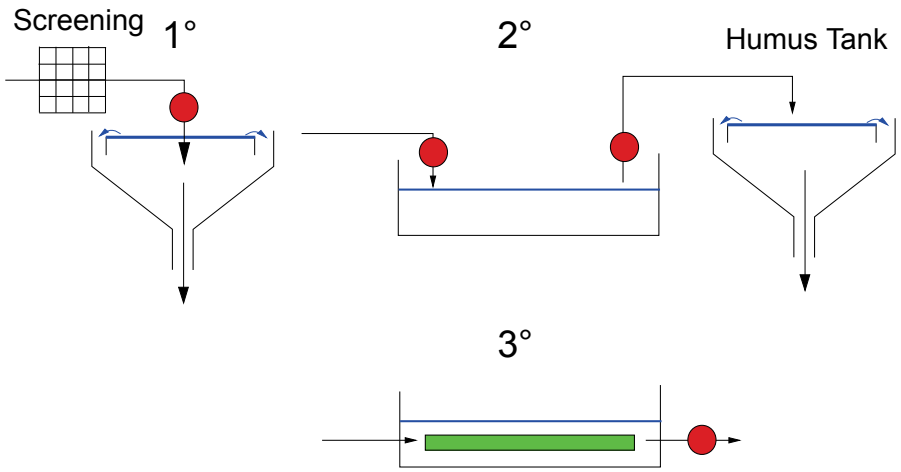

Figure 4. Schematic layout of the Treborth WWTP serving the majority of the population adjacent to the Menai Strait. The red spots indicate the sampling locations.

An aerial view (from Google Earth) showing the treatment steps and the sample locations is shown in Figure 5. During the two week sampling period, the storm tanks were not used and only one of the primary settlement tanks was in use. Rainfall in the catchment was measured with a rain gauge located 4.5 km to the north east. Samples of the influent were collected from the central feed to the primary settlement tank on Monday at 09.00, 12.30 and 16.00 and at 09.00 on Tuesday, Wednesday, Thursday and Friday. Exact dates can be seen in Table 2.

Post primary treatment and post secondary treatment samples were taken on two consecutive days from both of the parallel oxidation ditches. Final effluent was collected on a Monday, Wednesday and Friday from the Environment Agency designated sampling point after the UV disinfection stage. Sludge samples were collected on two different days from different holding bays. At this works, the sludge

samples are treated with lime (>pH 12 for 2 h) and are usually applied directly to agricultural land as a fertiliser.

Table 2. Key sample data for WWTP. The sludge samples are expressed in dry weight.

Sample	Date	Volume (ml) or *Dry Weight (g)
Influent	3rd March 2009	1000
Influent	4th March 2009	1000
Influent	5th March 2009	1000
Influent	6th March 2009	1000
Influent	9th March 2009 – 09.00	1000
Influent	9th March 2009 – 12.30	1000
Influent	9th March 2009 – 16.00	1000
Sludge*	5th March 2009	9.98
Sludge*	10th March 2009	11.55
Post Primary	10th March 2009	1000
Post Primary	11th March 2009	1000
Post Secondary	10th March 2009	1000
Post Secondary	11th March 2009	1000
Final Effluent	11th March 2009	2000
Final Effluent	13th March 2009	2000
Final Effluent	16th March 2009	2000

The extraction method for the liquid samples required a different approach to that expected as it proved not feasible to filter the samples either in the field or in the laboratory within a sensible time. The protocol used was as follows:

1. On return to the laboratory, 15 g of KOH was added to one litre of the liquid samples together with 2 ml of the internal standard. The mixture was shaken and left for 24 h at room temperature to allow an *in situ* saponification to occur. The sample was shaken periodically throughout the 24 h period.

2. The whole sample was poured into a 1 l separating funnel and ~100 ml of hexane was added. The sample was shaken, allowed to settle and the lower aqueous phase drawn off. The hexane phase was collected and the aqueous phase returned to the separating funnel.

3. A further 100 ml of hexane was added to the sample and re-extracted. The hexane phases were combined.

4. The samples were reduced to <5 ml through rotary evaporation and finally taken to dryness under a stream of nitrogen.

5. The lipids were derivatised with BSTFA for 2 h at 60°C, taken to dryness again before being re-dissolved in 1 ml of hexane.

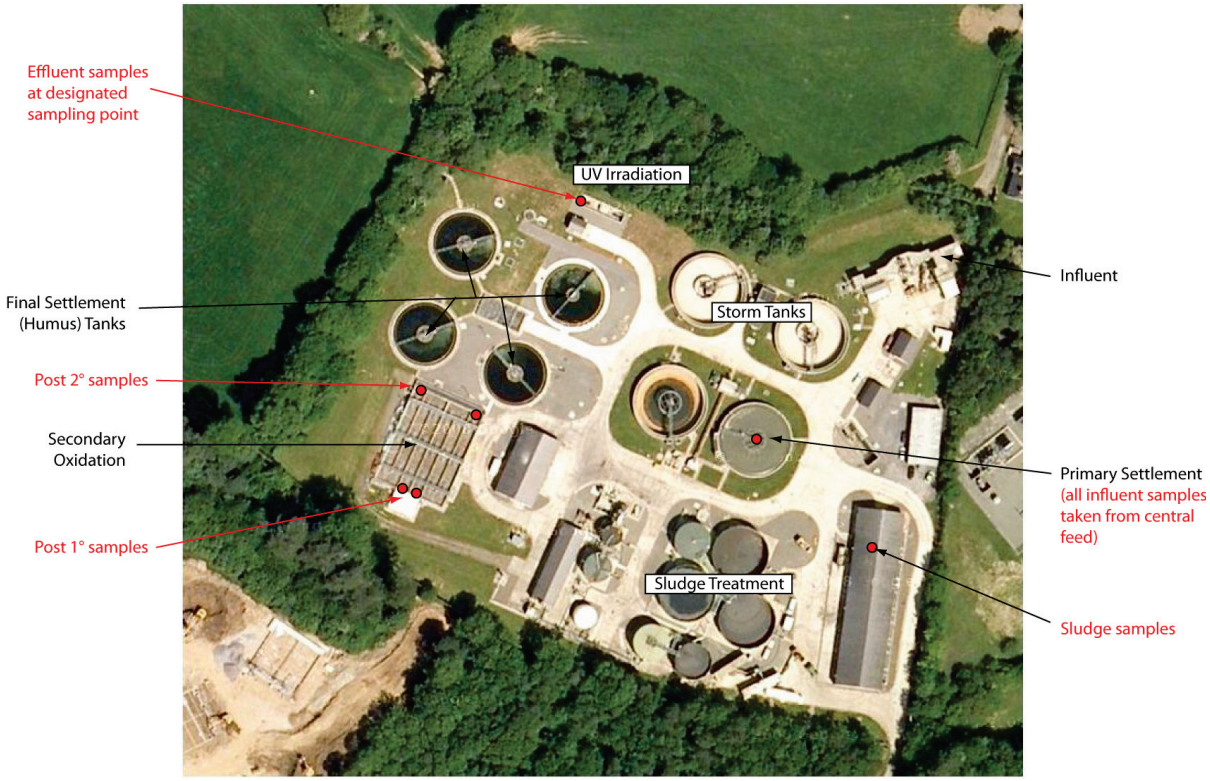

Figure 5. An aerial view of the Treborth WWTP with the location of the different processes and sampling points indicated.

Analysis

Gas Chromatography – Mass Spectrometry

All samples were analysed by GC-MS to identify and quantify the fatty alcohols; the internal standard was used to provide an internal calibration. For each sample, 1 µl was injected into a Fisons MD800 GC-MS. The on column injector was used with the following conditions:

1. ZB5HT-Inferno (Phenomenex) column, 30 m x 0.32 mm ID x 0.1 µm film thickness.
2. Temperature programme of injection at 60°C, held for 2 min, 10°C per min to 360°C with a final hold of 6 min.
3. The mass spectrometer scanned from 45 to 590 *m/z* per second with an ion energy of 70 eV.
4. All spectra were processed with the Masslab 1.4 software.

Compound Specific Stable Isotope Ratio Mass Spectrometry

Samples from the Phase I study were analysed at the University of Oklahoma. For these Phase II analyses, all samples were taken to the Scottish Crop Research Institute in Dundee, Scotland for analysis on a Thermo Delta V Plus Stable Isotope Ratio Mass Spectrometer. As a Quality Assurance check, the

surfactants from Phase I were analysed in Phase II to demonstrate the similarity of results between laboratories. This can be seen in the final results figure where the results from the two Phases are combined. For each sample, 1 µl was injected for carbon-13 and 2 – 3 µl for hydrogen-2 analysis into a split – splitless port under the following conditions:

1. DB-5MS (J&W) column, 30 m x 0.32 mm ID x 0.25 µm film thickness.
2. Temperature programme of injection at 60°C, held for 2 min, 6°C per min to 320°C with a final hold of 5 min.
3. The GC column output was split and directed into an ion trap mass spectrometer (ITQ-900) as well as the Thermo Delta V Plus Stable Isotope Ratio Mass Spectrometer. The GC conditions were: Injector 250°C, splitless for 0.5 min; carrier flow 1.2 $mL.min^{-1}$ (constant flow); oven: 60°C for 2 min, $6°C.min^{-1}$ to 320°C, 320°C with a final isothermal hold of 5 min.
4. MS conditions were EI mode, ion source at 200°C, transfer line at 300°C, scan range 50 - 650 amu.
5. IRMS conditions were emission 1.5 mA at an electron energy of 124 eV.
6. All spectra were processed with the Xcalibur 2.0.7 and Isodat 3.0 software.

Results

General

The location and general characteristics of the samples can be seen in Table 1. Photographs of the soil and sediment sampling sites together with the WWTP can be seen in the Appendix.

The rainfall during the two week sampling period can be seen graphically in Figure 6.

At no time during the two week sampling period was the influent flow rate sufficient to demand the use of more than one of the primary settlement tanks. On one day (4th March 2009), the precipitation was principally as snow.

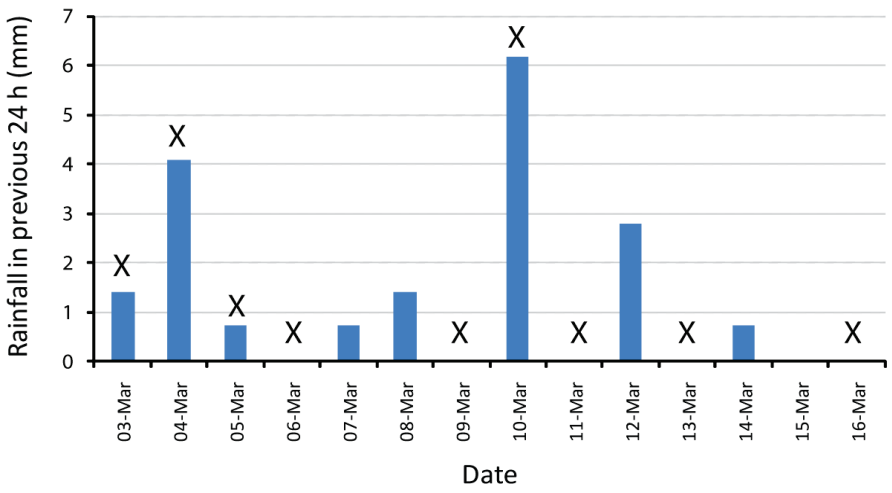

Figure 6. Rainfall in the catchment for the 24 h prior to each sampling day. Sampling days are indicated with a X.

Fatty Alcohol Profiles and Concentrations

The GC-MS trace for the standard and one of the samples can be seen in Figure 7. In the standard, the underivatised 2-OH dodecanol can be seen together with the TMS derivatised form. The mass spectrum of the TMS form is also included showing the principal fragment at 117 *m/z* due to the loss of the TMS – O – $CHCH_3$ fragment. The M^+ - 15 (loss of a methyl group) is also a diagnostic ion and usually used in quantification.

As well as the expected C_{12} compounds in the standard, there was also a C_{11} fatty alcohol (1-undecanol as its TMS ether) and another unidentified compound which may be 3-dodecanol. There was no 1-dodecanol as its TMS ether in the standard and so no contribution to any that may be in the environmental samples. The environmental sample shown in the lower pane indicates no underivatised 2-dodecanol indicating complete derivatisation of the standard, a hindered secondary alcohol. There is also a good separation in time between the primary and secondary alcohols and, therefore, no interference making calculation of the areas relatively easy. The part derivatisation of the 2-OH dodecanol was also used to calculate the contribution that the carbon and hydrogen atoms of the TMS group made to the final molecule.

The main fatty alcohols of interest elute between 10 and 22 min with the sterols eluting after this time. Sterols are structural components of cells and commonly include cholesterol in animals and its 24 ethyl derivative in terrestrial plants. As well as synthesising some of these compounds, humans ingest them as part of their diet. Therefore, these materials together with their altered products occur in faecal matter. In the sample shown (Figures 7 and 8), peaks of 5β-coprostanol, the main faecal stanol of humans, cholesterol and 24 ethyl coprostanol (derived from plant matter in the human gut) can be clearly seen. A small peak of the terrestrial plant sterol sitosterol (24 ethyl cholesterol) can be identified at scan number 1235 (24.5 min).

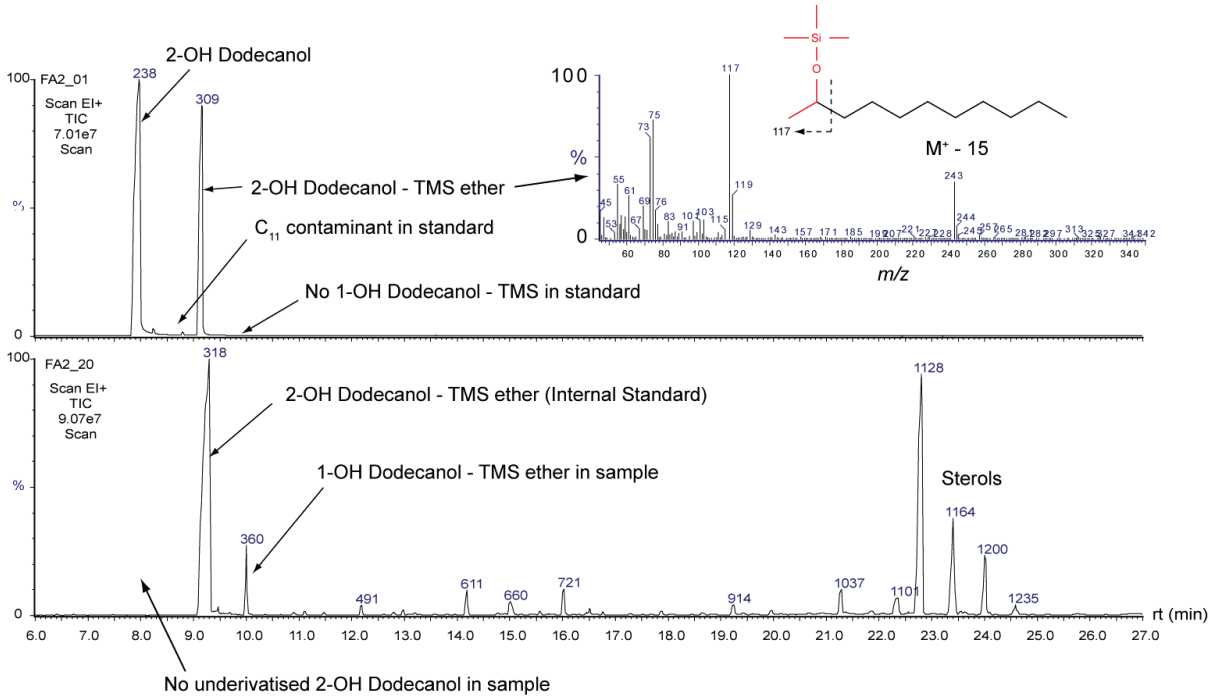

Figure 7. The GC trace of the part derivatised standard in the upper panel together with the mass spectrum of the TMS ether. The lower panel shows one of the samples including the derivatised standard.

The compounds present in the waste water samples are labelled in Figure 8. A full suite of straight chain odd and even carbon numbered alcohols are present. Small quantities of branched odd carbon chain compounds are also present (not shown).

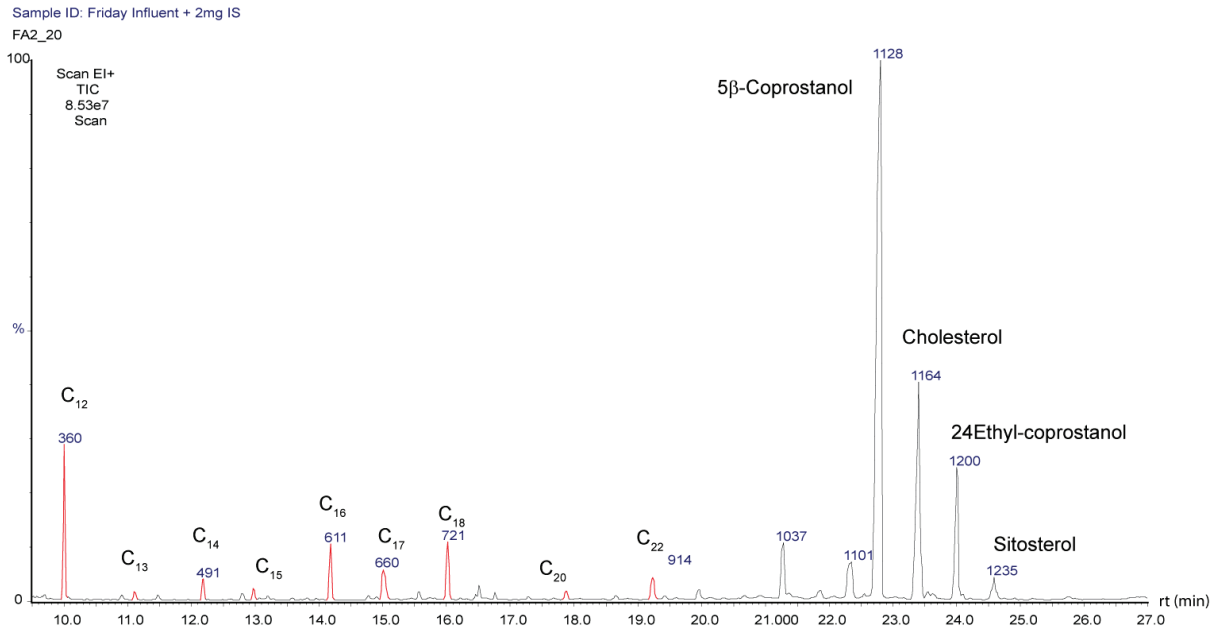

Figure 8. Fatty alcohols and sterols present in the waste water samples.

In addition to the above target compounds, the environmental samples also contained a range of other compounds. These includes a wider range of sterols from diverse sources and in the case of one soil sample, significant polyaromatic hydrocarbons (PAHs) as well as the chlorophyll derived fatty alcohol phytol (Chikaraishi et al., 2005), were present (Figure 9).

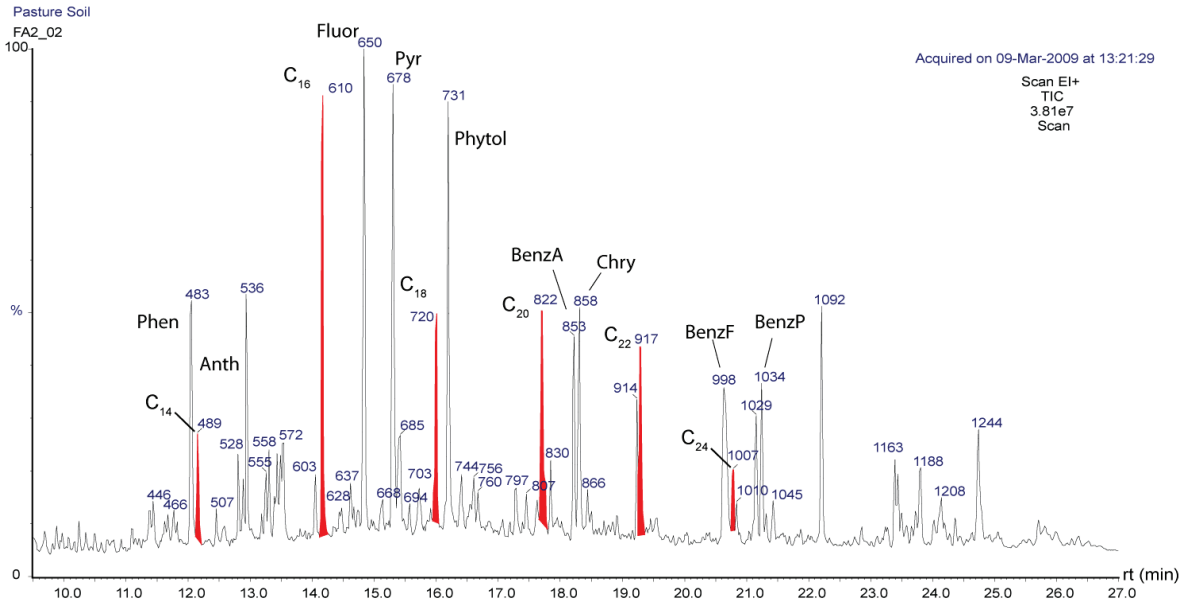

Figure 9. The pasture soil sample indicating the presence of the fatty alcohols highlighted in red and several other compounds including the PAHs (source unknown). (Phen = phenathrene; Anth = anthracene; Fluor = fluoranthene; Pyr = pyrene; BenzA = benzanthracene; Chry = chrysene; BenzF = benzofluoranthenes; BenzP = benzopyrenes).

The fatty alcohol profiles of the raw materials and the surfactant products can be seen in Figure 10.

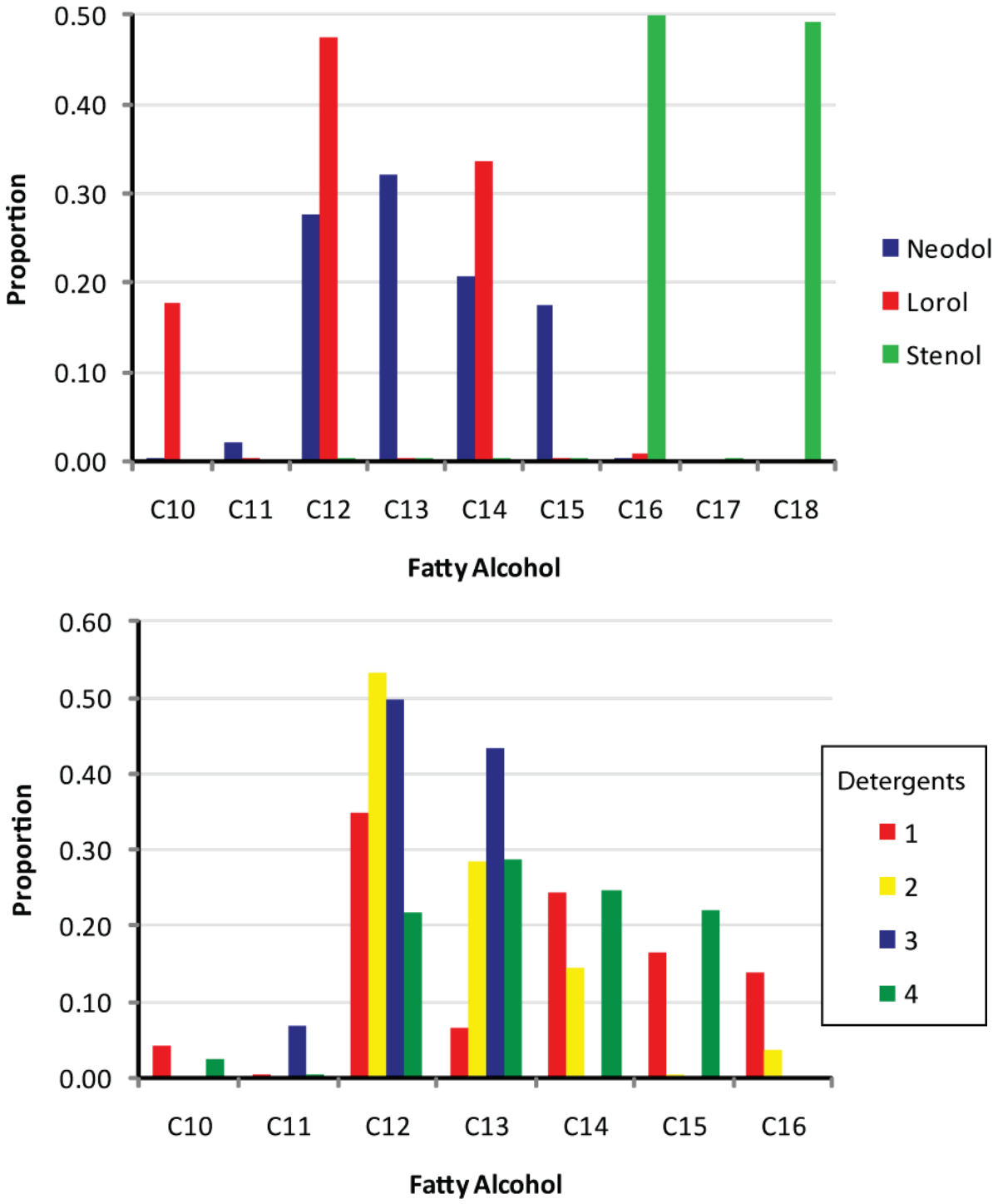

Figure 10. The fatty alcohol profile for each of (a) the raw materials and (b) detergents provided.

The fatty alcohols used for manufacturing consumer products fall into two major groups; those synthesised from oil (Neodol) and those synthesised from natural plant materials (Lorol and Stenol). This sourcing is reflected in the fatty alcohol profiles as the synthetic materials have high proportions of odd

chain length fatty alcohols not usually seen in the natural products which are essentially restricted to even carbon numbered compounds.

For the final detergent formulations analysed as their iodides, substantial amounts of odd carbon chain alkyl iodides were identified and the profile indicates that all of these consumer products were made with synthetic fatty alcohols. One of the methods for quantifying the odd chain composition in a series of alkanes, alcohols or acids is to use the Carbon Preference Index or CPI. This is calculated as the sum of the odd chain compounds in the series divided by the sum of the even chain compounds (or *vice versa* depending on what is being shown). If there was no preference between odd and even, the ratio would be 1.0; more odd would generate ratios greater than one and less odd, smaller than one.

The Carbon Preference Index (CPI) for the two naturally-derived fatty alcohol source materials (Lorol and Stenol) were both 0.0 while that of the oil-derived one (Neodol) was 1.1. The four detergents had fatty alcohol CPIs of 0.3, 0.4, 1.0 and 1.0 respectively and indicate that the first two may be a mix of two different raw materials while the second two appear to be derived from Neodol or a similar raw material.

The fatty alcohol profiles for influent samples collected from the WWTP can be seen in Figures 11 – 12. The figures indicate two major patterns; in the case of three of the 09.00 samples, the C_{12} dominates the trace while in the others, the C_{16} and C_{18} are increasingly important contributors to the total profile.

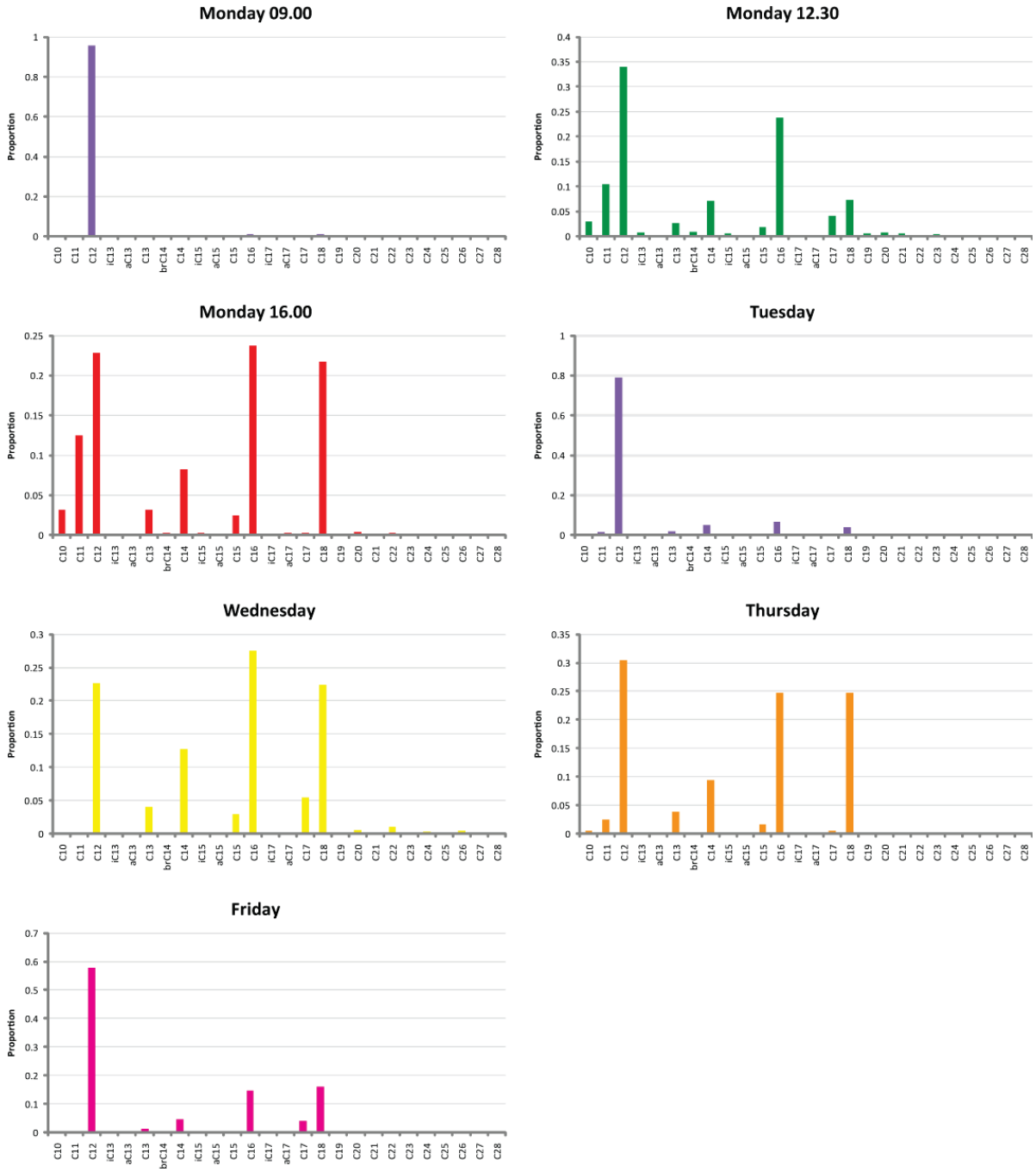

Figure 11. Variability in the fatty alcohol profile for the influent across a week. All samples from Tuesday to Friday were collected at 09.00.

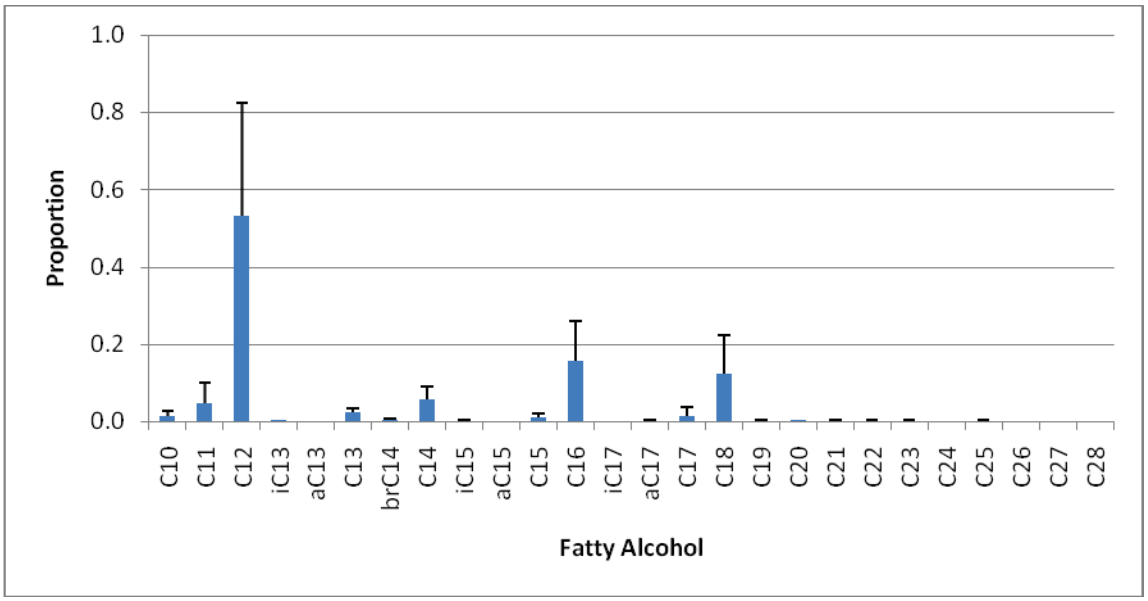

Figure 12. The mean fatty alcohol profile for the influent with one standard deviation indicated.

The fatty alcohol profiles of the WWTP influent are dominated by the C_{12} straight chain moiety followed by the C_{16} and C_{18} compounds. The profile is weighted towards the even carbon short chain compounds with few plant derived long chain compounds present. This is typical of animal derived material. Small amounts of odd chain length and branched compounds are also present indicative of bacterial presence. There is a degree of variability in the data which can be seen by the size of the one standard deviation error bars added to Figure 12, the mean condition in the influent. The total concentration also changed across the sampling period with no obvious pattern (Figure 13).

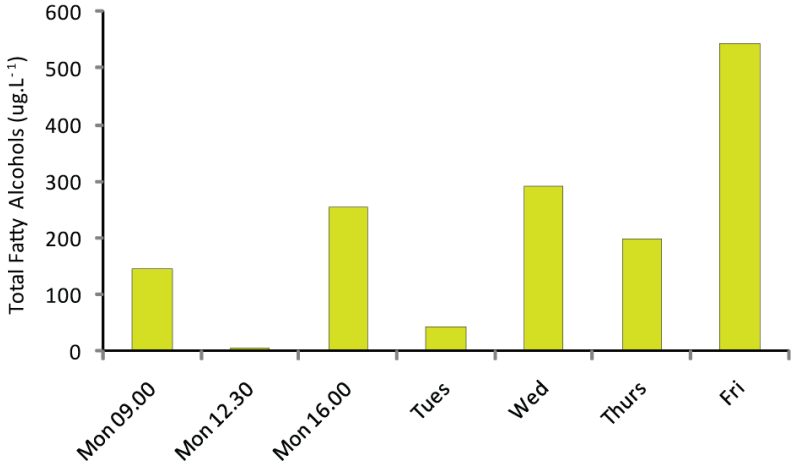

Figure 13. Total fatty alcohol concentrations in the influent. Samples from Tuesday to Friday were collected at 09.00.

The total concentration of fatty alcohols in the samples was very variable across the WWTP and may reflect the concentration of suspended solids in each sample. In some parts of the world, the "wash day" phenomenon still exists; some communities have a particular day set aside for doing laundry, typically Monday. Figure 13 shows the concentration of total fatty alcohols increasing across the working week and not peaking on Monday. The breakdown of concentrations for each fatty alcohol in each sample may be found in the Appendix. The summary data and example profiles are presented below.

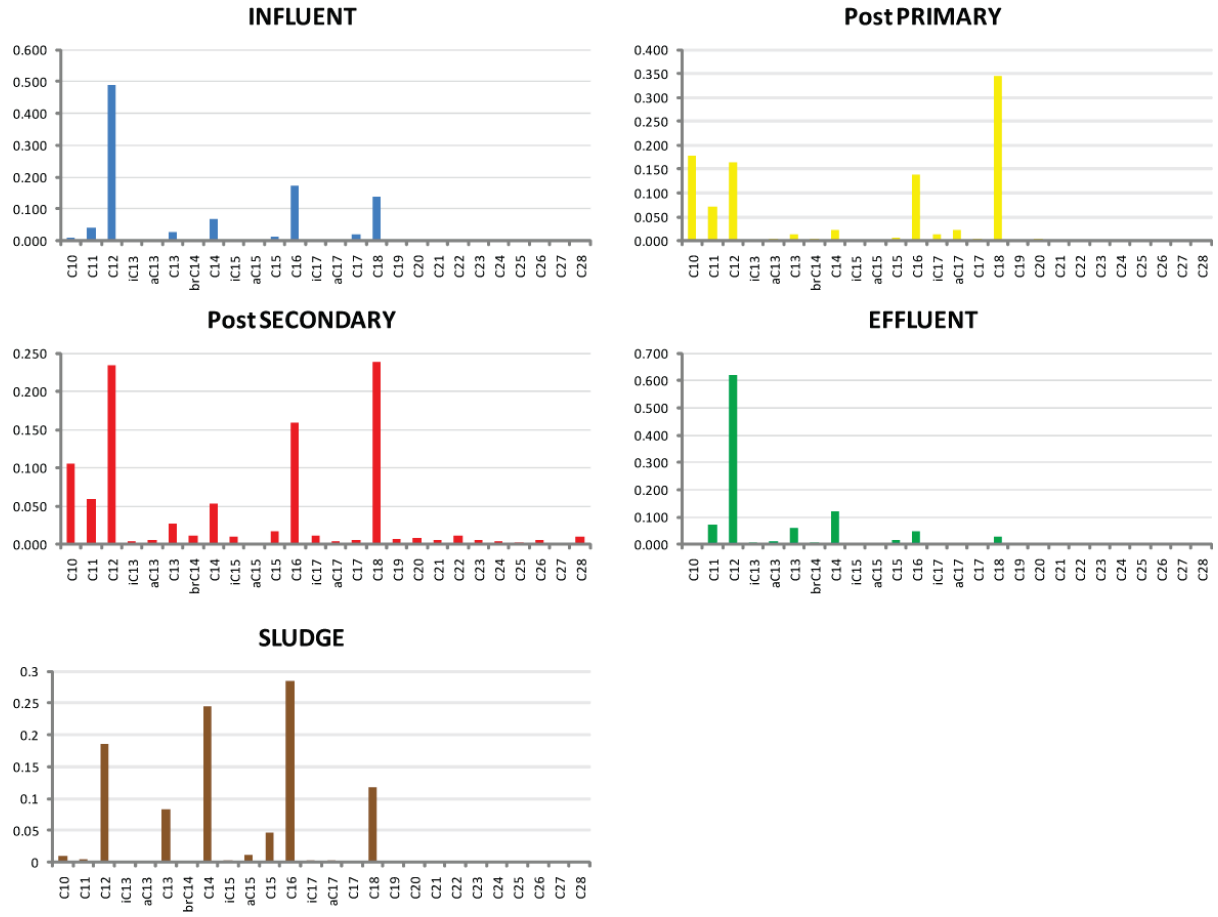

Figure 14. The mean fatty alcohol profiles for the different stages within the WWTP.

The influent and effluent samples are dominated by the C_{12} fatty alcohol. In comparison, the intermediate samples have a lower proportion of the C_{12} with a greater spread of compounds with the C_{18} making the greatest contribution. It is also worth noting an increase in the C_{10} and C_{11} compounds which may be from direct bacterial synthesis or chain shortening after β-oxidation. The later stages have a greater odd and branched chain contribution suggesting greater bacteria components. These materials may have originated within the WWTP as their magnitude in generally lower in the influent samples. The concentration of total fatty alcohols generally decreased as the waste waters passed through the WWTP (Figure 15). The concentration in the final lime-treated sludge is significantly greater than in the liquid samples especially since it is expressed in per gram units rather than per litre.

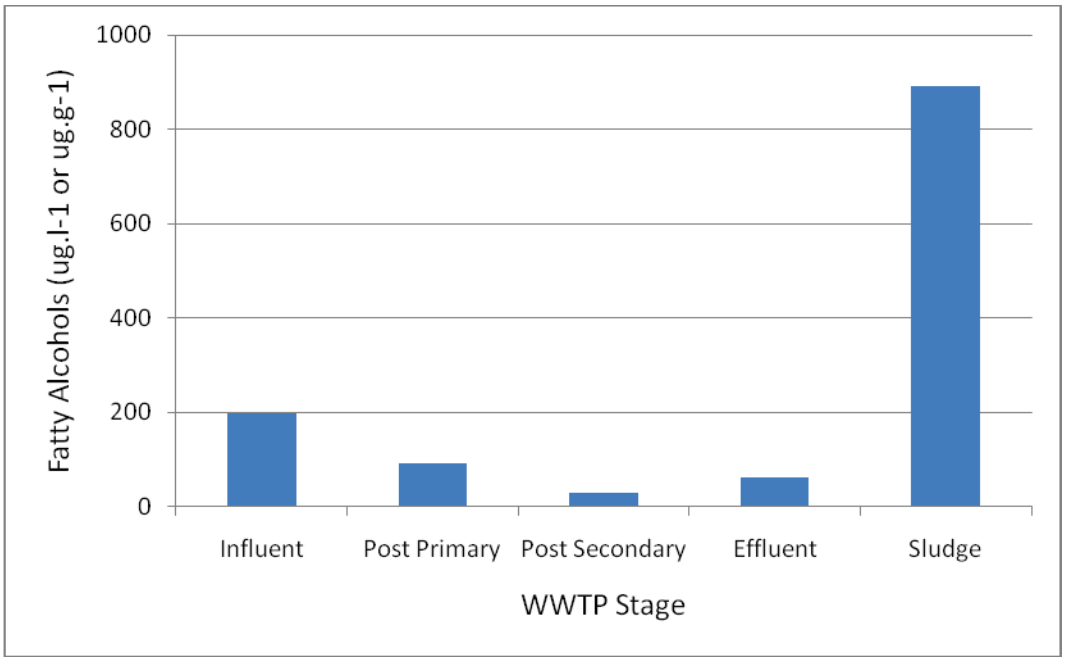

Figure 15. Concentration of total fatty alcohols at each stage in the WWTP. NB, the concentration in the sludge is expressed in $\mu g.g^{-1}$ while all the liquid samples are in $\mu g.l^{-1}$; this is a factor of 1000 difference when expressed by volume.

Using the best available flow rate data for the influent and effluent parts of the WWTP (100 and 120 $l.sec^{-1}$ respectively), it is possible to calculate the loadings. There is an increase in the effluent over the influent as the works receives partly dewatered sludges from outlying WWTPs. Based on these flows, the influent receives a total of 1.8 $kg.day^{-1}$ of fatty alcohols and the effluent discharges 640 $g.day^{-1}$ to the marine environment of which the C_{12} makes up ~50%.

The fatty alcohol profiles of the soil samples can be seen in Figure 16. No two samples had the same profile indicating a wide range of fatty alcohols. The conifer soil was rich in short chain compounds while the pasture had a wide range of compounds up to C_{28}.

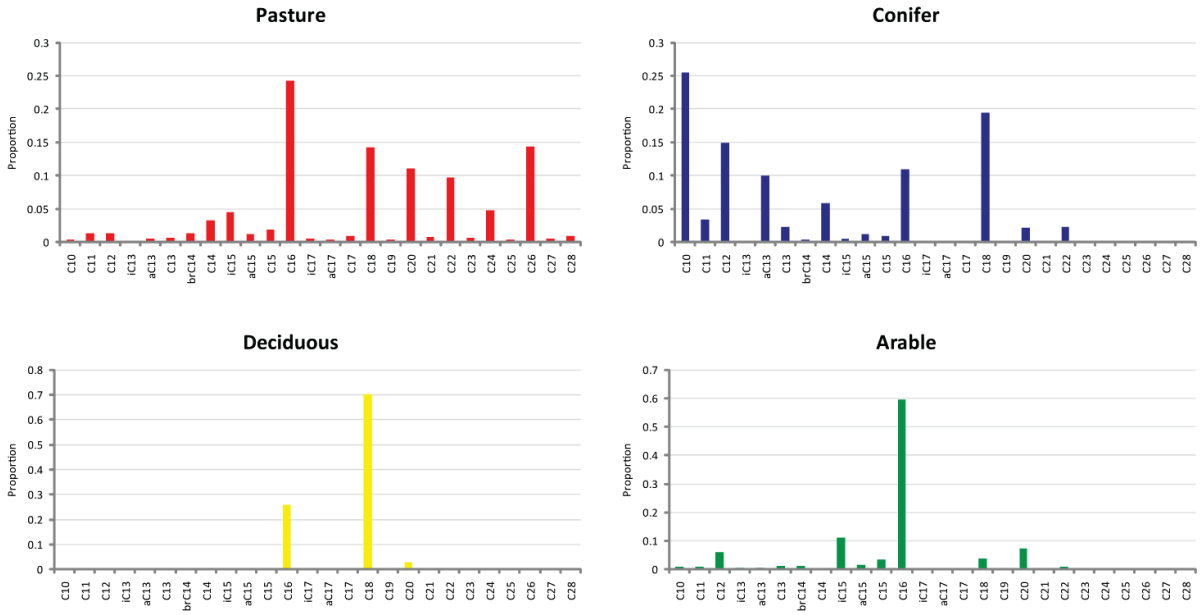

Figure 16. The fatty alcohol profile in the soil samples.

In general, the major fatty alcohols in these soils were the C_{16} and C_{18} which is unlike many terrestrial samples where the longer chain compounds are more likely (Mudge et al., 2008). The prevalence of the shorter chain compounds may be related to the climate or proximity to the coast. In contrast to these terrestrial samples, the marine sediments from the Menai Strait (Figure 17) are dominated by the C_{12}, C_{14} and C_{16} fatty alcohols. This is the expected profile for fatty alcohols in the marine environment where desiccation is not an issue (Mudge et al., 2008).

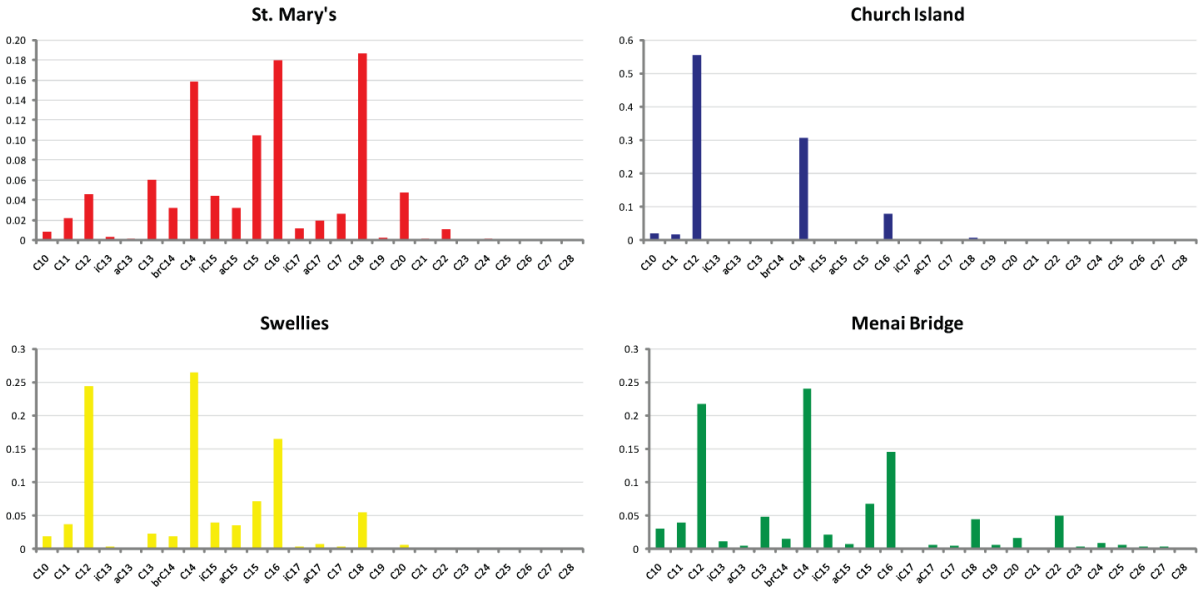

Figure 17. The fatty alcohol profile in the marine sediment samples. The samples are arranged in order of increasing distance from the WWTP discharge.

One of the best ways to view all of the complex composition data in one figure is through the use of Principal Components Analysis (PCA). PCA takes a large number of potentially correlated variables (compounds) and reduces them to a small number of uncorrelated principal components (PCs). If compounds have the same source, they will co-vary and have similar loadings in a PCA figure. Likewise, if samples have similar compositions, they will cluster together in the scores plot.

All the profile data were converted to proportions to remove any concentration effects and these values were log transformed to improve normality of the distribution before analysis. The scores for each sample are shown in Figure 18 together with the relative composition as a bar chart for selected samples (indicative of that zone of the figure). The detergent samples all indicated a similarity of profile to that of Neodol, an oil-based suite of fatty alcohols used in the manufacture of alcohol ethoxylates surfactants. This suggests that the bulk of the fatty alcohols used in the formulation may be from this source although detergent 1 may also have a component that is derived from the natural derived raw materials.

The effluent samples are relatively enriched in the short chain fatty alcohols (37 $\mu g.l^{-1}$ for the C_{12} representing 60% of the total compared with 96 $\mu g.l^{-1}$ for the C_{12} in the influent representing 45% of the total fatty alcohols) and depleted in the long chain compounds which are more characteristic of terrestrial environments. In contrast, the deciduous soil sample is depleted in the short chain compounds and enriched in selected long chain fatty alcohols. The marine sample has a mixture of both and potentially indicates a mixed source.

The samples from the WWTP separate on Figure 18 indicating a significant change in composition as sewage passes through the works. The influent samples appear to have a mixture of both short and long chain fatty alcohols while the sludge and effluent have diverging profiles but relative enrichment in both the short chain and bacterially derived odd chain compounds.

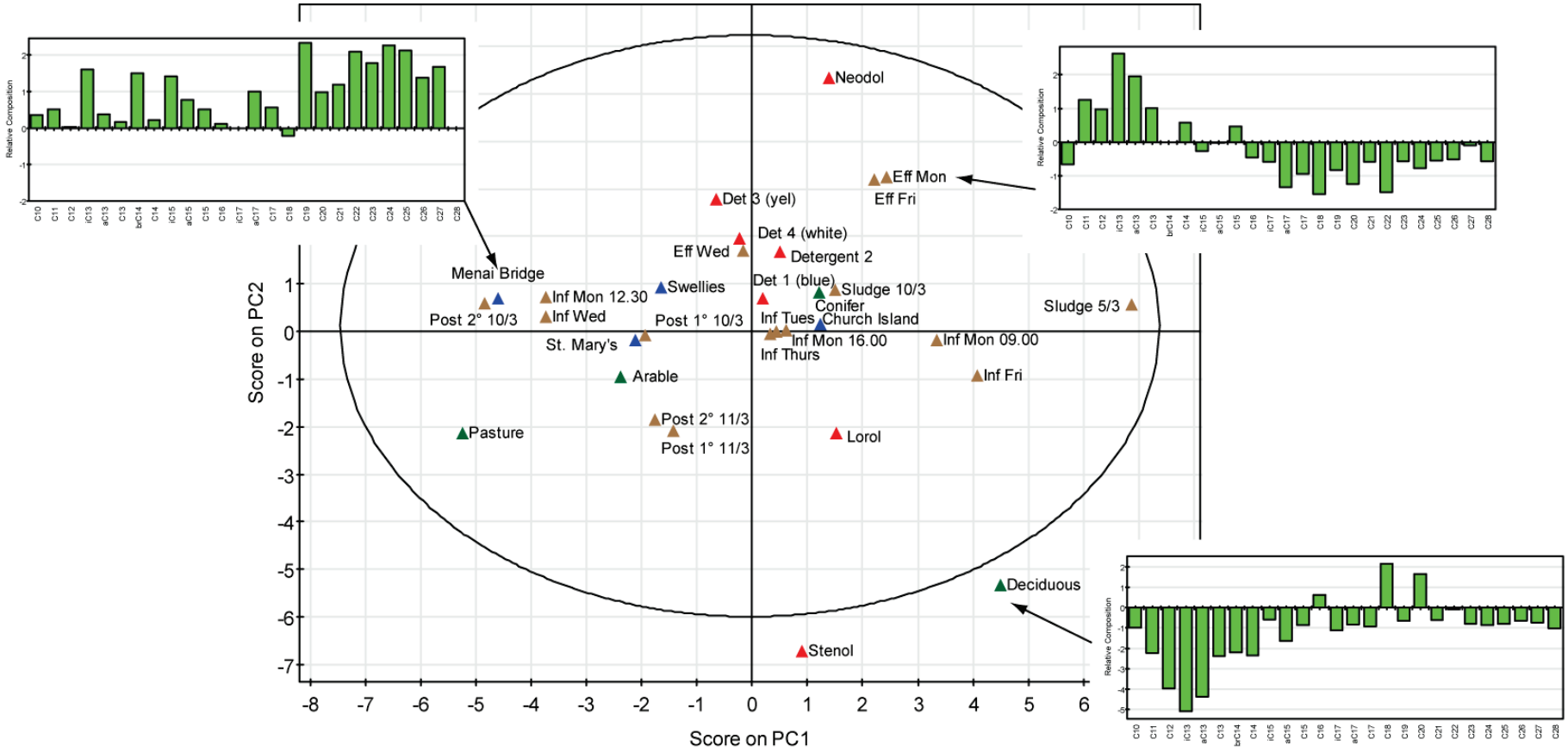

Figure 18. The Scores plot from PCA of the fatty alcohol profiles. The inset bar charts indicate the relative composition for selected samples.

Stable Isotopes

The addition of the trimethyl silyl (TMS) group added three carbon and nine hydrogen atoms to the molecules that were analysed by mass spectrometry. The $\delta^{13}C$ and $\delta^{2}H$ values for this group were calculated from the initial standard that was part derivatised to provide both compounds in a single analysis. The GC trace can be seen in Figure 7. The TMS $\delta^{13}C$ and $\delta^{2}H$ values were calculated through the fractional addition equation:

$$\frac{\text{No. of carbons}_{\text{FA-TMS}}}{\text{Total No. of carbons}} \times \delta^{13}C_{\text{FA-TMS}} = \frac{\text{No. of carbons}_{\text{TMS}}}{\text{Total No. of carbons}} \times \delta^{13}C_{\text{TMS}} + \frac{\text{No. of carbons}_{\text{FA}}}{\text{Total No. of carbons}} \times \delta^{13}C_{\text{FA}}$$

where the FA-TMS carbon number is 15; TMS is three and FA is 12. The total number in the 2-dodecanol – TMS ether is 15. The mean measured $\delta^{13}C$ for the nine underivatised 2-dodecanol standards is -35.151‰ and the TMS derivatised version is -35.513‰. Therefore, the TMS group has a mean $\delta^{13}C$ value of -36.960‰ and one standard deviation of 1.335‰.

In the case of the $\delta^{2}H$ content, the number of hydrogen atoms in the whole TMS derivatised molecule is 34 and the original 2-dodecanol has 26 although one is replaced in the derivatisation process. The TMS group adds nine extra to the original molecule. The measured $\delta^{2}H$ values for the final molecule and the original were -102‰ and -89‰. Therefore, the TMS group has a $\delta^{2}H$ value of -137.5‰.

The full table of stable isotope results can be found in the Appendix but summary statistics of the internal standard can be seen in Figure 19.

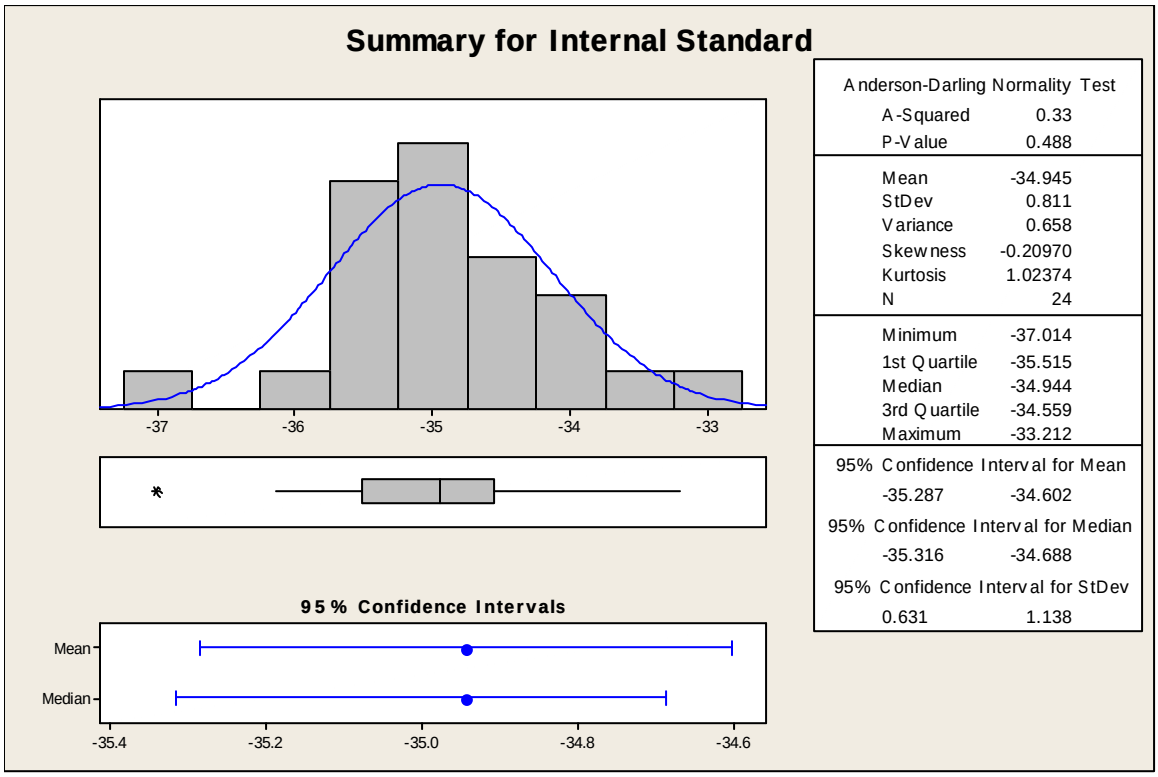

Figure 19. The summary statistics for the $\delta^{13}C$ values for the internal standard (n=24).

A total of 168 compound (fatty alcohol) specific $\delta^{13}C$ values were measured across all samples; this may be a small percentage of the total number of fatty alcohols quantified (25%) but the limits of detection for the stable isotopes are not as good as the compound itself since only a small percentage of the carbon in the molecule is ^{13}C. Accordingly, it is the lower concentration components, notably the odd chain and branched fatty alcohols, which have missing data.

The limits of detection are not as good for the ^{2}H component of the molecule which reduces the number of stable isotope pairs to 91. The data from these analyses are combined with the data from the initial study to demonstrate the appropriateness of the method and the cross plot can be seen in Figure 20.

The data sets from the two different laboratories used for the analyses fit together very well and do not suggest any significant difference between the two sets of values. The data from Figure 1 obtained during Phase I of the project is incorporated into Figure 20; there is a small difference in the $\delta^{13}C$ values to a more negative number but the $\delta^{2}H$ values are almost identical. Key aspects of the results apparent in Figure 20 are:

1. All of the fatty alcohols from the terrestrial environments (soils and leaves) have $\delta^{13}C$ values less than -30‰ which is consistent with the terrestrial carbon fixation pathway.

2. The corresponding $\delta^{2}H$ values are within a broader range from -130 to -330‰ for these terrestrial samples although there is a general trend of the short chain compounds having a less negative value and the longer chains being more negative. The phytol in the soils, a C_{20} branched fatty alcohol, consistently has $\delta^{2}H$ values less than -230‰.

3. In contrast to the terrestrial samples, the short chain fatty alcohols from the marine environment have $\delta^{13}C$ values around -20 to -25‰; again, this is consistent with the source of carbon utilised by marine organisms. The longer chain compounds ($>C_{18}$) tend toward the more negative values of $\delta^{13}C$ consistent with a terrestrial origin.

4. The fatty alcohols used in the manufacture of detergents and the detergents themselves fall into two major groups although at least one shows a mixture of compounds.

    a. The Neodol and Lial fatty alcohols are coincident in a region around -28‰ for the $\delta^{13}C$ and -50‰ for the $\delta^{2}H$. These have the profile of oil derived compounds. Detergents 3 and 4 have a similar stable isotopic signature and fatty alcohol profile.

    b. The Lorol and Stenol have more negative $\delta^{2}H$ values and separate cleanly from the oil based materials.

    c. Detergent 1 is comprised of C_{12} C_{14} C_{15} and C_{16}. If this came from a natural source then the presence of the C_{15} is unusual. If it came from oil based raw materials, the lack of the C_{13} is unusual. The stable isotopic signatures, however, suggest two different sources; the C_{12} is purely derived from a natural source and C_{15} is purely derived from an oil

based raw material. The C_{14} and C_{16} appear to be derived from both sources as they have intermediate δ^2H values.

d. A similar condition exists for detergent 2 although the major fatty alcohol, the C_{12}, also appears to have a small natural component as well as an oil based component.

5. The even chain fatty alcohols in the sewage influent samples have very similar stable isotopic signatures to the C_{16} measured in faecal material. The C_{15} in faecal matter which is of bacterial origin has a more negative $\delta^{13}C$ value indicative of a different carbon source to the even chain length compounds.

6. In one case, the C_{12} of the influent sample (Wednesday) was coincident with the similar chain length fatty alcohols from Stenol and Detergents 1 and 2. In all other cases, the influent fatty alcohol stable isotope signatures were less negative on the δ^2H axis and are consistent with a mixed faecal and detergent source based on the δ^2H values.

7. The fatty alcohols in the sludge are also consistent with mixed sources. The even carbon numbered compounds may be a mixture of faecal matter, naturally derived detergents and oil based detergents while the odd carbon numbered compounds may be a mixture of faecal matter, bacterially derived fatty alcohols with a more terrestrial signature and oil based detergents.

8. The effluent has δ^2H values more positive than any other fatty alcohols and may reflect *de novo* synthesis by bacteria as the profile was rich in odd chain length compounds. There may also be a contribution from the oil-based detergents with similar δ^2H values but different $\delta^{13}C$ values.

9. Although the fatty alcohol profiles for the WWTP influent and effluent look similar (Figure 14), the stable isotopes indicate that these are different chemicals. The effluent fatty alcohols may be synthesised by bacteria from other materials in the liquors such as sugars or they may be "re-cycled" from partly metabolised fatty alcohols and acids.

10. The sediments of the receiving waters do not indicate any fatty alcohols with a faecal matter, bacteria or detergent source and the predominant signature is that of marine production.

The position of the individual compounds in Figure 20 suggests that the ^{13}C is not particularly diagnostic for separating the natural and oil based detergents as the major differences are on the ^2H axis. It is possible to determine the mean projection in the figure for each compound and from this calculate the mixing between potential sources.

The best example of this is the C_{12} as it occurs in both natural and oil based detergents and faecal material. The odd chain compounds are not generally present in the natural materials unless produced by bacteria. The C_{16} and C_{18} are not generally present in the detergents derived from oil sources and so no end-member can be calculated for that source. For the C_{12} derived from the natural materials, the mean δ^2H value is -239.6‰ while for the oil-based detergents it is -72.3‰. By using these values in a

simple two end member mixing algorithm for each of the WWTP influents, values between 0% oil-based detergents to 40% can be calculated with a mean of 26%. It is most likely, however, that the majority of this compound is derived from human faecal material.

In the case of the C_{15} fatty alcohol which either comes from bacterial production or oil-based detergents, the stable isotope values in the sludge (biosolids) indicate a 40% / 60% split. This is in agreement with the C_{12} partitioning for the WWTP influent.

The effluent from the WWTP required concentrating in order to obtain robust stable isotope values. Once achieved, it showed that only the short chain ($<C_{16}$) compounds were present and that the δ^2H values were significantly greater than other samples. The carbon numbers also indicated a different range to that of the influent or the sludge. This observation together with the known degradation rates of fatty alcohols in WWTPs (Battersby et al., 2001) suggests that these compounds are being synthesised within the plant by bacteria. The profile is also rich in odd chain compounds indicative of bacteria. Although the Neodol and Lial samples are nearby in the figure (Figure 20), the difference in the δ^2H values is significant.

The marine samples collected from the receiving waters of the effluent did not have the sample fatty alcohol profile as the effluent (cf. Figure 18).

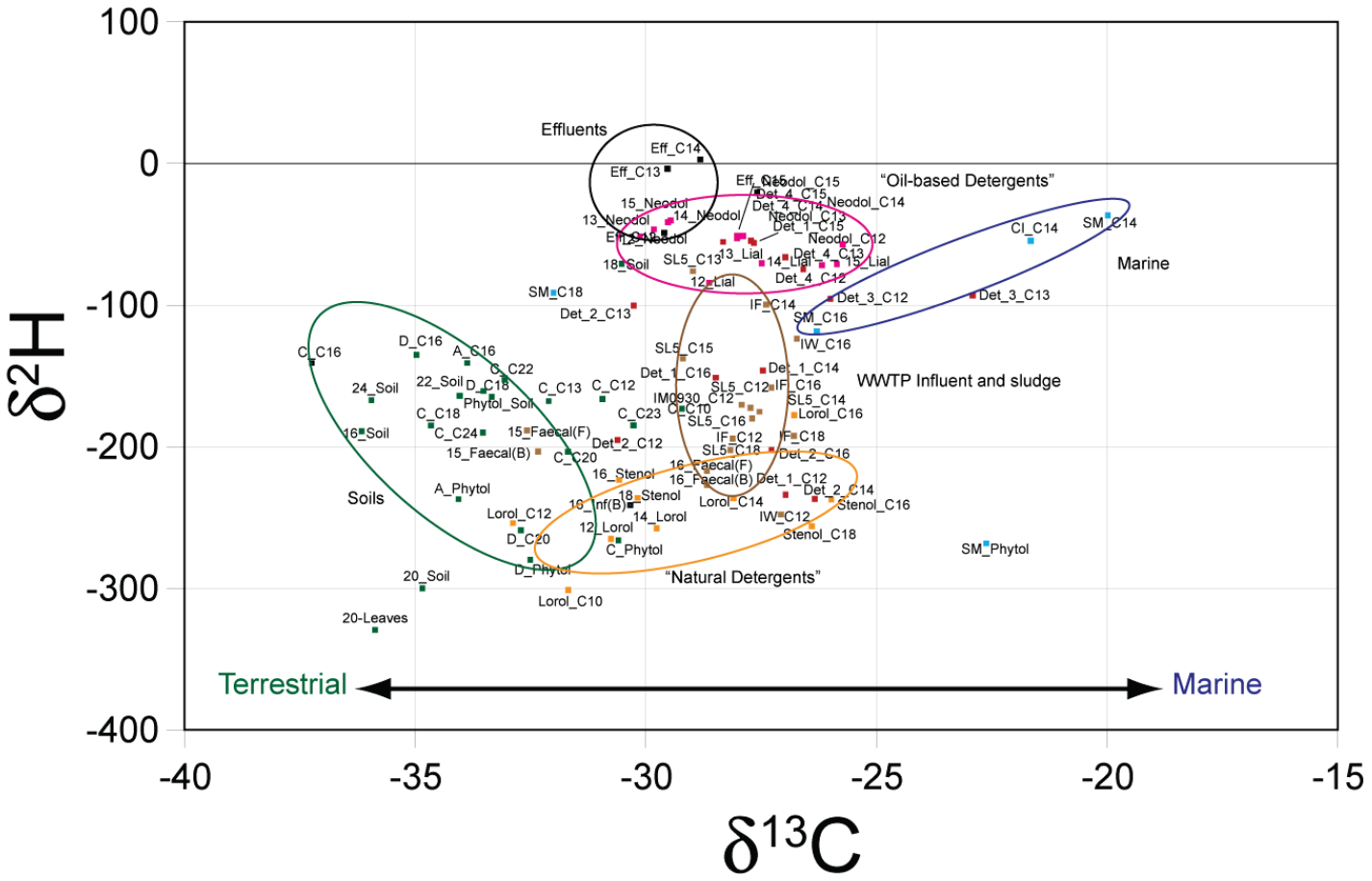

Figure 20. Cross plot of all the stable isotope pairs for all samples. The terrestrial soils are indicated by the green ellipse; the detergents and raw materials based on natural fatty alcohols are indicated by the orange ellipse; the oil-based detergents are within the lilac ellipse; the WWTP influent samples within the brown ellipse fall between the two detergent sources; the effluents have much greater δ^2H values and are located in the black ellipse at the top of the figure. The sediments of the marine receiving waters are shown in blue.

Discussion

The key question that this work focussed on was whether there is evidence of the fatty alcohols used in detergent and cosmetic formulations passing through a WWTP and contributing to the loading in the sediments of the receiving waters. The influent to the WWTP certainly had a mixture of fatty alcohols that were within the detergent range but this also coincides with fatty alcohol profile for faecal material (see report on Phase I), notably the C_{15} and C_{16}. The terrestrial soils which may become a source of organic matter to drains and through combined sewers to the WWTP had similar fatty alcohols to faecal matter and some detergents. From this information alone it would not be possible to determine if either the soils or detergents were making a significant contribution to the influent materials of the WWTP.

It is necessary to consider the fatty alcohol profiles and compositions to understand the sourcing issues. Fatty alcohols used in the manufacture of consumer products may be made from oil in which case the odd chain compounds are just as prevalent as the even chain compounds. Odd chain compounds in environmental samples tend to come from bacterial synthesis and are usually minor constituents of the total fatty alcohol suite. The exception to this would be in areas where bacterial activity and biomass is particularly high such as within faecal matter and sewage treatment plants. Even in these situations, the even chain length compounds usually contribute the majority of the mass.

The detergents made from naturally sourced fatty alcohols will have odd – even chain profiles similar to the initial biological material. The most common source of these raw materials is palm oil and its derivatives. This vegetable oil is rich in the short even chain C_{12} to C_{16} fatty alcohols.

It might be expected, therefore, that the influent to a WWTP would contain fatty alcohols derived from soils, faecal matter, food waste and detergents (including laundry, dish washing and shampoos). However, when viewing the stable isotopes of the fatty alcohols taken from the WWTP influent, the data indicate that soils and terrestrially derived compounds do not make a significant contribution and that the sources appear to be from faecal matter and detergents. This is based on the $\delta^{13}C$ values for the compounds. Terrestrial plants, especially C_3 plants tend to exclude the ^{13}C and have more negative values on the $\delta^{13}C$ axis. In Figure 20, all the terrestrial soils and plant matter have values less than -32‰ which is consistent with their expected values (Boutton, 1991).

It is possible to perform PCA on the $\delta^{13}C$ values for each fatty alcohol in the profile where the concentration was high enough. The scores plot for these data can be seen in Figure 21. Three major groups can be seen in the data; the first is for all of the terrestrial soils which project to the top right as they have similar $\delta^{13}C$ and fatty alcohol profiles. The second is the marine sediments which also group somewhat loosely to the left of the figure. The final group are the remaining samples which include detergents and sewage samples. This indicates a wide grouping with a range of $\delta^{13}C$ values although some very close associations can be seen between Neodol and Detergent 4, for instance. On its own, this would not differentiate between sources.

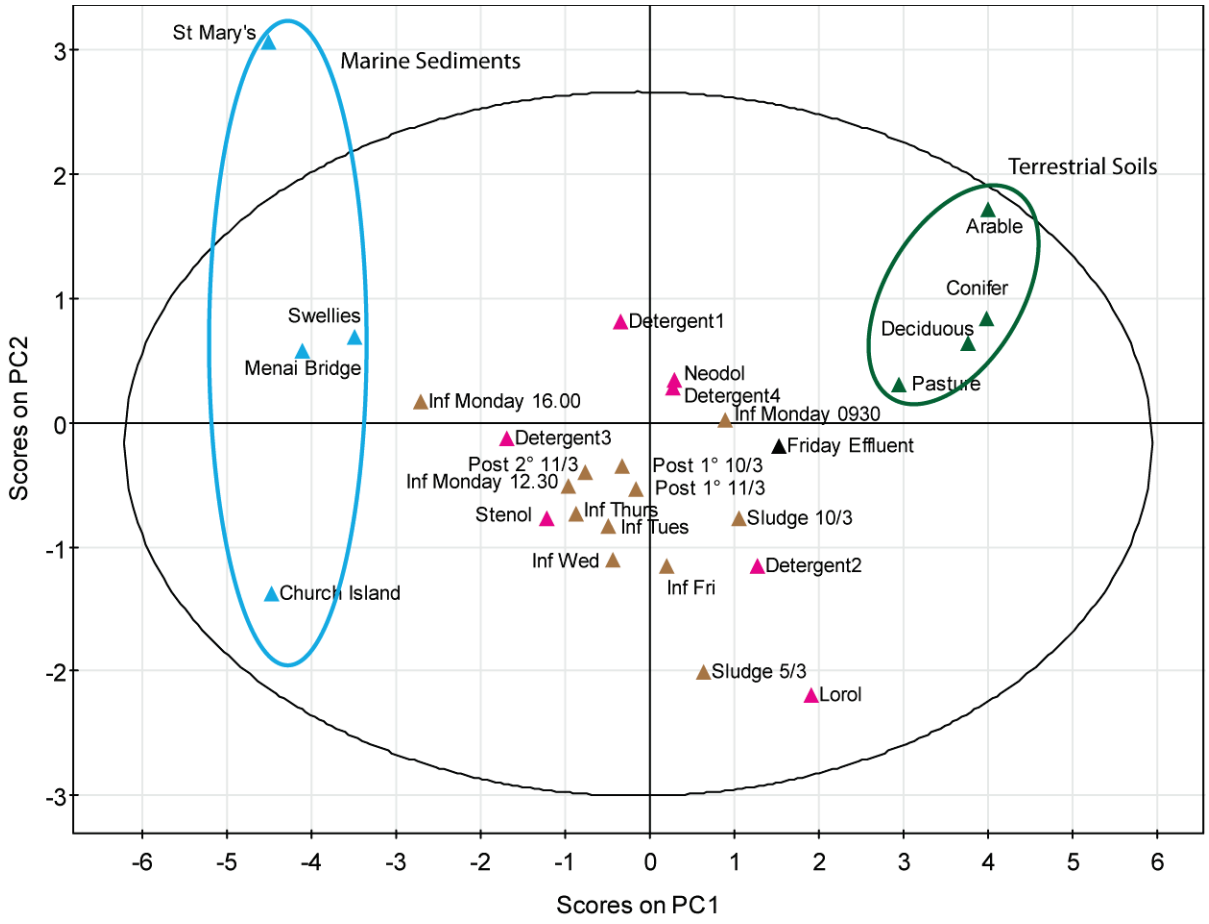

Figure 21. The Scores plot from PCA conducted on the $\delta^{13}C$ values for each fatty alcohol. The data were log transformed after multiplying by -1 to convert the numbers to positive values.

The $\delta^{13}C$ range for WWTP influent samples and detergents ranges between -25 and -30‰ and, in general, it is not possible to unambiguously separate them on this axis. However, there is a big difference in the $\delta^{2}H$ values between the oil-based detergents and the natural, vegetable derived detergents. At one extreme are the oil based compounds with values around -50‰ and at the other are the natural detergents with $\delta^{2}H$ values around -230‰. The faecal matter has similar stable isotope values to these latter detergents. The fatty alcohols in the influent spread themselves between these two extremes and indicate mixing of these sources. Analysis of the real detergent products used in the catchment indicates that the majority of them are oil based with profiles and stable isotope values similar to that of Neodol. However, at least one detergent (labelled Detergent 1) may be a mixture with C_{12} component derived from natural sources rather than oil. This is consistent with the profile PCA (Figure 18).

Based on this data, it appears as if the products analysed are principally oil based and this was confirmed anecdotally by members of the APAG fatty alcohols group. Therefore, the influent fatty alcohols can be considered as a mixture of oil detergents and faecal matter. On this basis, the influent mixture is around 25% from the detergents and 75% from faecal matter. An important aspect that we

currently do have any data for is the amount of each detergent used within the catchment. This aspect is being incorporated into the study currently underway in the USA funded by the SDA.

Previous experimental work (Battersby et al., 2001) has indicated that fatty alcohol ethoxylates are rapidly degraded within a WWTP and as part of that degradation the compounds will become less water soluble as the length of the ethoxylate chain is reduced. The resulting compounds will accumulate in the sludge and the concentrations in the sludge were many orders of magnitude greater than in the aqueous phase (see Figure 15).

The effluent samples had short chain fatty alcohols only and the stable isotopes were indicative of new (bacterial?) production although there may be a small component of the C_{12} derived from oil based detergents. The consent to discharge from this works is in the order of 8000 $m^3.day^{-1}$; if the concentration of the C_{12} was entirely derived from the oil based detergents, which is unlikely, it would contribute ~300 g to the receiving waters per day.

Analysis of the sediments in the vicinity of the discharge point in the Menai Strait indicate around 1 $\mu g.g^{-1}$ of the C_{12} fatty alcohol in the dry sediment and this was below the limit of detection for the δ^2H stable isotope analysis. However, the more prevalent C_{14} and C_{16} had $\delta^{13}C$ values that were considerable greater than any of the WWTP or detergent samples indicating an entirely marine production. The longer chain compound did show $\delta^{13}C$ values that tended towards those of the terrestrial soils and this would indicate a degree of runoff into these waters. This was also confirmed by the presence of β-sitosterol, a sterol produced by terrestrial vascular plants and not produced by marine organisms. The $\delta^{13}C$ value for the C_{12} in the marine sediment samples was around -20‰ for two samples (see the data in the appendix) including the site closest to the WWTP outfall. This suggests the WWTP discharges were not concentrating in these sediments. One sample at Church Island, approximately 1.7 km from the discharge site did have $\delta^{13}C$ values for the C_{12} that were co-incident with the detergent values. This site may also be receiving terrestrial discharges and local runoff due to its proximity to Combined Sewer Overflows (CSOs).

Conclusions

1. Two dimensional stable isotope analysis (^{13}C and ^{2}H) is a suitable analytical tool to separate the different sources of fatty alcohols that may exist in a WWTP and in the receiving waters from that WWTP. The ^{13}C alone may be good enough to separate terrestrial from marine sources but it does not separate faecal sources from either natural or oil-based detergents.

2. Natural plant based detergents have $\delta^{13}C$ values between -26 and -32‰ while oil-based detergents occupy a range between -25 and -30‰. The corresponding δ^2H values are -250‰ for natural sourced materials and -50‰ for oil-based detergents which enables these two sources to be separated.

3. Of the supplied detergents which are typical of the local catchment, samples 3 and 4 appear to exclusively derived from oil-based raw materials while detergents 1 and 2 have C_{12} and C_{14} components from natural sources combined with some oil-based longer chain fatty alcohols.

4. The influent to the WWTP contains fatty alcohols which originate from mainly faecal sources (75%) with a smaller amount potentially derived from oil-based detergents (25%). This mixture is compound specific and only realistically applies to the C_{12} and may be the C_{14} fatty alcohols. Longer chain compounds do not appear commonly in the influent.

5. The effluents from the WWTP contain mainly short chain compounds with a chain length less than C_{16}. Their δ^2H (and to a lesser extent the $\delta^{13}C$) stable isotope signature is different to the other potential sources examined and suggests bacterial synthesis during the treatment processes. Therefore, the alcohols in the effluent are not the same ones that entered *via* the influent. They are most likely to arise from the recycling of other lipids (partial chain shortening) or *de* novo synthesis from other organic matter such as sugars in the liquor. On the basis of the mean discharge rates and the mean C_{12} concentration in the effluent, this WWTP would contribute up to 300 g•day^{-1} to the receiving waters although these alcohols are not from the influent. The total alcohol discharge of all chain lengths was ~640 g•day^{-1}.

6. The sludge produced from the WWTP had relatively high concentrations of fatty alcohols as would be expected from their low water solubility. The exact production rate is hard to quantify but the majority of fatty alcohols that enter through the influent works will leave the WWTP in this form. The stable isotopic signatures were consistent with a mixture of faecal and detergent sources although this again was variable depending on the particular compound examined. For instance, the C_{18} does not appear to have any detergent influence although the C_{13} might have but this compound is also synthesised by bacteria within the WWTP.

7. The marine sediment samples had fatty alcohols that are typical of marine production (short chain) and with stable isotope values that indicate exclusive marine production for the C_{14} with potentially mixed terrestrial for the C_{16} and C_{18} compounds. Therefore, the fatty alcohols in the marine environment are not from the WWTP effluent which, in turn, are not directly from the fatty alcohols in the influent. Only the $\delta^{13}C$ value is available for the C_{12} and this might indicate some detergent contribution to this system for that compound alone at one site. It is also possible the CSOs might contribute direct surface water runoff to that location.

Recommendations and Future Possibilities

The results of this research have indicated that the fatty alcohols in the influent are not the same as those in the effluent as they have a different stable isotopic signature. It is suggested that these new fatty alcohols may be synthesised *de novo* by bacteria or may be recycled from the partial degradation of existing compounds. The use of radio-labelled fatty alcohols to determine their environmental half-life has been used in a laboratory representation of a WWTP; it may be possible to use stable isotope

approaches to confirm the metabolic pathways and origins of the effluent fatty alcohols without the need for radioactive compounds. This could be accomplished in "real" WWTPs without much problem.

The nature of the fatty alcohols in the influent may need further investigation to determine their speciation – waxes, lipid bound, ethoxylates, sulphates *etc*. The same approach could also be considered on the effluent to establish the partitioning between these classes in the source and discharge. It is likely that they will have completely different profiles.

This Phase II work has given a maximum input for the fatty alcohols to the receiving waters at this location. It has shown that the influent fatty alcohols are not the same as the effluent ones and that they do not concentrate in the sediments near the outfall. What is not known is how representative this situation is of other WWTP with different processes or from different catchments. Therefore, a good follow on to this work would be a series of samples from a range of WWTPs types and locations.

The Luray study in the USA had access to survey data regarding how many units of each detergent product was used within a geographical area. These data were considered by the SDA steering group and the most appropriate products which contain fatty alcohols were purchased in the catchment of the Luray WWTP. The products were analysed to determine their fatty alcohol profiles and subsequently their stable isotopic signatures. Combination of these data allows mass contributions to be made to the influent of the WWTP. The profile of fatty alcohols to the influent can be reconstructed from these data. This might aid on the mass balance in the UK situation if such data could be gathered.

The four marine samples collected from the Menai Strait told different stories. The sample collected closest to the discharge point for the WWTP had fatty alcohol profiles similar to those of the effluent but the stable isotope signature showed that these compounds had been synthesised by marine organisms and were not derived from the WWTP effluent. However, one sample near a Combined Sewer Overflow (CSO) did have a C_{12} fatty alcohol with the same $\delta^{13}C$ value as the oil-based surfactants or sewage effluent. It is not known of this is an isolated case or if there is some "leakage" of selected fatty alcohols to the environment through CSOs. A wider study based on the proximity to CSOs may improve our knowledge on the contribution this route may have on the fatty alcohol inputs to the environment.

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Appendix

Photographs of soil sampling locations

Pasture Field

Deciduous (oak) woodland soil

Coniferous woodland soil

Arable field

Photographs of sediment sampling locations with distances from the outfall location

St. Mary's Church with discharge on opposite side of the Menai Strait (250 m)

Between the Bridges, The Swellies, with Menai Bridge in the background (1000 m)

Church Island (1700 m)

Menai Bridge (2700 m)

Photographs of the WWTP at Treborth

Influent samples were collected from the central feed to this primary settlement tank

Post primary settlement samples were collected at the start of the oxidation ditch

Post secondary treatment samples were collected as they crossed the final weir

Final effluent samples were collected at the designated Environment Agency sampling point

Sludge samples were collected from the holding bays after lime treatment

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Fatty Alcohol	Pasture	Deciduous	Conifer	Arable	Swellies	Menai Bridge	Church Island	St. Mary's	Post 1° 10/3	Sludge 5/3
Sample reference	FA2_02	FA2_03	FA2_04	FA2_05	FA2_06	FA2_07	FA2_08	FA2_09	FA2_10	FA2_11
C10	0.07	0.05	99.37	0.01	0.22	0.00	0.04	0.17	14.13	10.76
C11	0.27	0.05	13.09	0.01	0.43	0.01	0.03	0.47	5.76	4.14
C12	0.27	0.14	58.24	0.09	2.87	0.03	1.17	1.00	11.10	367.25
iC13	0.03	0.01	0.00	0.01	0.05	0.00	0.01	0.07	0.08	0.24
aC13	0.09	0.00	39.07	0.01	0.03	0.00	0.00	0.04	0.23	0.28
C13	0.12	0.07	8.96	0.02	0.27	0.01	0.00	1.30	0.74	42.13
brC14	0.28	0.01	1.41	0.02	0.21	0.00	0.00	0.69	0.21	0.28
C14	0.68	0.17	23.10	0.00	3.11	0.04	0.64	3.43	1.39	264.16
iC15	0.96	0.07	2.15	0.16	0.47	0.00	0.00	0.96	0.00	1.21
aC15	0.24	0.02	4.79	0.02	0.42	0.00	0.00	0.69	0.13	1.21
C15	0.40	0.11	3.32	0.05	0.84	0.01	0.00	2.27	0.34	21.91
C16	5.14	18.00	42.54	0.85	1.94	0.02	0.17	3.90	4.13	300.62
iC17	0.11	0.00	0.00	0.00	0.04	0.00	0.00	0.26	0.09	0.00
aC17	0.06	0.01	0.01	0.00	0.08	0.00	0.00	0.42	0.02	1.52
C17	0.20	0.01	0.27	0.00	0.03	0.00	0.00	0.56	0.10	0.04
C18	3.03	48.77	75.86	0.05	0.64	0.01	0.02	4.04	6.37	127.98
C19	0.09	0.02	0.39	0.00	0.01	0.00	0.00	0.05	0.06	0.01
C20	2.34	2.09	8.40	0.10	0.07	0.00	0.00	1.02	0.09	0.02
C21	0.18	0.01	0.09	0.00	0.01	0.00	0.00	0.03	0.04	0.00
C22	2.06	0.07	8.70	0.01	0.03	0.01	0.00	0.24	0.15	0.01
C23	0.13	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.05	0.00
C24	1.01	0.01	0.02	0.00	0.01	0.00	0.00	0.02	0.06	0.01
C25	0.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.08	0.00
C26	3.05	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.06	0.00
C27	0.09	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.09
C28	0.21	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.77
TOTAL (ug / g DW)	21.20	69.67	389.79	1.42	11.78	0.16	2.11	21.65		1144.66
or (ug / L) for liquid samples									45.47	

Fatty Alcohol	Post 2° 10/3	Inf Mon 16.00	Inf Mon 12.30	Inf Mon 09.00	Inf Tues	Inf Thurs	Inf Fri	Post 1° 11/3	Post 2° 11/3	Sludge 10/3
Sample reference	FA2_13	FA2_14	FA2_15	FA2_16	FA2_17	FA2_19	FA2_20	FA2_22	FA2_25	FA2_29
C10	1.18	7.97	0.15	0.37	0.27	1.08	0.75	6.32	4.30	5.82
C11	0.59	31.67	0.53	0.37	0.67	4.91	1.30	2.05	2.81	3.89
C12	2.69	57.76	1.72	137.99	33.10	60.18	313.97	11.41	9.16	31.55
iC13	0.06	0.39	0.04	0.08	0.06	0.50	0.25	0.00	0.04	1.33
aC13	0.08	0.38	0.00	0.08	0.06	0.34	0.26	0.14	0.04	0.89
C13	0.39	8.07	0.13	1.00	0.76	7.53	7.42	1.19	0.64	82.82
brC14	0.17	0.63	0.04	0.06	0.02	0.17	0.04	0.25	0.15	0.72
C14	0.77	20.70	0.36	1.09	2.04	18.71	25.53	2.22	1.14	163.10
iC15	0.14	0.58	0.03	0.04	0.01	0.32	0.17	0.26	0.17	3.81
aC15	0.00	0.49	0.00	0.03	0.06	0.32	0.07	0.26	0.00	13.70
C15	0.24	6.14	0.10	0.12	0.13	3.33	3.20	0.90	0.42	46.32
C16	0.89	59.95	1.21	1.27	2.70	48.97	79.82	25.87	11.63	194.98
iC17	0.02	0.20	0.01	0.05	0.02	0.43	0.05	3.63	1.11	4.00
aC17	0.02	0.62	0.00	0.02	0.04	0.43	0.06	6.44	0.23	1.87
C17	0.03	0.71	0.21	0.03	0.04	0.89	22.26	0.51	0.41	0.71
C18	1.04	54.86	0.37	1.45	1.68	48.91	87.61	77.26	19.23	78.61
C19	0.12	0.12	0.03	0.03	0.02	0.16	0.10	0.09	0.06	0.05
C20	0.07	0.81	0.04	0.03	0.05	0.49	0.31	0.55	0.49	0.23
C21	0.08	0.08	0.03	0.03	0.03	0.06	0.05	0.00	0.05	0.00
C22	0.11	0.72	0.01	0.02	0.03	0.01	0.02	0.06	0.54	0.10
C23	0.09	0.04	0.02	0.01	0.04	0.05	0.07	0.18	0.00	0.01
C24	0.04	0.01	0.01	0.04	0.03	0.17	0.13	0.07	0.15	0.04
C25	0.04	0.03	0.02	0.04	0.02	0.03	0.02	0.08	0.00	0.00
C26	0.06	0.02	0.01	0.04	0.02	0.14	0.00	0.12	0.26	0.04
C27	0.02	0.01	0.00	0.03	0.05	0.01	0.03	0.22	0.01	0.16
C28	0.15	0.02	0.01	0.03	0.03	0.05	0.01	0.07	0.17	0.55
TOTAL (ug / g DW)										635.31
or (ug / L) for liquid samples	9.10	252.98	5.08	144.36	41.97	198.17	543.50	140.17	53.22	

Fatty Alcohol	Eff Wed	Eff Fri	Eff Mon	Inf Wed
Sample reference	FA2_30	FA2_31	FA2_32	
C10	0.08	0.11	0.18	0.00
C11	0.78	4.83	9.62	0.00
C12	12.93	39.85	57.96	65.90
iC13	0.15	0.28	1.09	0.00
aC13	0.08	0.84	1.12	0.00
C13	0.84	3.77	8.04	11.62
brC14	0.15	0.83	0.00	0.00
C14	2.05	7.62	14.10	37.10
iC15	0.05	0.05	0.19	0.00
aC15	0.13	0.05	0.27	0.00
C15	0.02	1.37	2.86	8.59
C16	1.02	2.62	5.09	80.25
iC17	0.01	0.01	0.02	0.00
aC17	0.01	0.01	0.00	0.00
C17	0.00	0.02	0.01	16.02
C18	0.71	1.45	2.61	65.23
C19	0.00	0.01	0.02	0.00
C20	0.02	0.00	0.05	1.60
C21	0.01	0.00	0.01	0.00
C22	0.02	0.01	0.00	2.90
C23	0.01	0.01	0.01	0.00
C24	0.01	0.01	0.01	0.78
C25	0.01	0.01	0.00	0.00
C26	0.01	0.00	0.01	1.24
C27	0.01	0.01	0.01	0.00
C28	0.01	0.01	0.00	0.00
TOTAL (ug / g DW)				
or (ug / L) for liquid samples	19.11	63.75	103.26	291.23

$\delta^{13}C$ Stable Isotope Data

	Pasture	Deciduous	Conifer	Arable	Swellies	Menai Bridge	Church Island	St Mary's	Neodol	Lorol	Stenol
C10			-29.13							-31.60	
C11											
C12			-30.86	-30.58	-21.14		-25.18	-19.73	-25.66	-32.80	
iC13											
aC13											
C13			-32.02					-18.92	-27.58		
brC14											
C14		-29.84	-31.62	-28.60	-24.19	-20.19	-21.58	-19.90	-27.81	-28.02	
iC15			-29.83					-22.56			
aC15			-32.54					-25.18			
C15		-28.64	-30.34	-32.00			-17.12	-20.32	-27.78		
C16	-35.01	-34.89	-37.16	-33.79	-23.96	-24.13	-21.98	-26.21		-26.70	-25.90
iC17											
aC17											
C17		-31.32						-17.83			
C18	-30.67	-33.44	-34.58	-36.72				-31.92			-26.32
C19											
C20	-33.79	-32.63	-31.61	-35.17				-33.18			
C21											
C22	-33.05	-33.92	-32.98	-34.70							
C23			-30.19								
C24	-35.02	-33.31	-33.46								
C25											
C26	-35.97										
C27											
C28											
Phytol	-30.12	-32.42	-30.52	-33.99	-22.32	-21.06	-19.79	-22.54			

	InfMonday 0930	InfMonday 12.30	InfMonday 16.00	Inf Tues	Inf Wed	Inf Thurs	Inf Fri	Detergent1	Detergent2	Detergent3	Detergent4
C10											
C11											
C12	-27.65	-26.04	-23.27	-26.47	-26.99	-26.40	-28.04	-26.89	-30.54	-25.92	-26.51
iC13											
aC13											
C13				-27.36	-27.44		-28.36	-22.30	-30.19	-22.83	-26.10
brC14											
C14	-28.14			-26.46	-25.62	-26.79	-27.31	-27.39	-26.26		-28.25
iC15											
aC15											
C15					-26.27		-26.09	-27.81			-27.64
C16		-25.69	-24.97	-25.61	-26.65	-26.32	-27.20	-28.40	-27.20		
iC17											
aC17											
C17		-24.93			-24.94	-22.85					
C18		-27.39	-26.68	-27.18	-26.06	-26.52	-26.71				
C19											
C20											
C21											
C22											
C23											
C24											
C25											
C26											
C27											
C28											
Phytol											

	Sludge 5/3	Sludge 10/3	Post 1° 10/3	Post 2° 10/3	Post 1° 11/3	Post 2° 11/3	Friday Effluent
C10							
C11							
C12	-27.84	-28.01			-27.43	-26.60	-29.37
iC13		-29.54					
aC13							
C13	-28.90	-30.23					-29.40
brC14							
C14	-27.46	-27.44					-28.64
iC15	-28.17	-28.50					
aC15		-27.99					
C15	-29.12	-29.49					-27.26
C16	-27.62	-27.66	-27.58		-27.04	-26.09	
iC17		-26.71					
aC17		-28.05					
C17	-27.97	-27.29					
C18	-28.09	-28.32	-27.63		-27.83	-27.78	
C19							
C20	-27.11						
C21							
C22	-27.55						
C23							
C24							
C25							
C26							
C27							
C28							
Phytol							

δ^2H Stable Isotope Data

	Pasture	Deciduous	Conifer	Arable	Swellies	Menai Bridge	Church Island	St Mary's	Neodol	Lorol	Stenol
C10			-175.64							-303.73	
C11			-174.35								
C12			-136.63								
iC13			-168.78						-59.68	-256.52	
aC13											
C13											
brC14			-170.33						-58.69		
C14											
iC15							-56.95	-39.23	-53.77	-238.87	
aC15											
C15											
C16									-53.55		
iC17		-137.69	-143.37	-143.39				-120.92		-180.25	-239.67
aC17											
C17											
C18											
C19		-163.15	-187.53					-93.71			-258.49
C20											
C21		-261.49	-206.04								
C22											
C23			-155.40								
C24			-187.44								
C25			-192.54								
C26											
C27											
C28											
Phytol											

	InfMonday 0930	InfMonday 12.30	InfMonday 16.00	Inf Tues	Inf Wed	Inf Thurs	Inf Fri	Detergent1	Detergent2	Detergent3	Detergent4
C10											
C11											
C12	-106.08				-96.70		-102.16				
iC13	-174.90				-250.25		-196.80	-236.40	-197.70	-98.10	-77.20
aC13											
C13											
brC14									-102.90	-95.70	-74.20
C14											
iC15							-102.26	-148.60	-239.40		-57.80
aC15											
C15											
C16								-53.10			-57.00
iC17					-126.26		-160.70	-153.70	-204.90		
aC17											
C17											
C18											
C19							-194.84				
C20											
C21											
C22											
C23											
C24											
C25											
C26											
C27											
C28											
Phytol											

	Sludge 5/3	Sludge 10/3	Post 1° 10/3	Post 2° 10/3	Post 1° 11/3	Post 2° 11/3	Friday Effluent
C10							
C11							
C12	-172.84						-51.40
iC13							
aC13							
C13	-78.60						-6.26
brC14							
C14	-177.77						0.40
iC15							
aC15							
C15	-140.40						-22.86
C16	-182.48						
iC17							
aC17							
C17							
C18	-204.86						
C19							
C20							
C21							
C22							
C23							
C24							
C25							
C26							
C27							
C28							
Phytol							