Final Report

Fatty Alcohols in the Riverine Environment:
The effect of wastewater treatment type and Eco-region

A study of the fatty alcohols in the influent, effluent and sediments associated with municipal wastewater treatment plants in Oklahoma, Ohio and Oregon, USA.

Prepared for the American Cleaning Institute

by

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August 2012

Abstract

Previous studies on the fate of fatty alcohols passing through wastewater treatment plants (WWTPs) had indicated that the compounds in the influent were settled out and rapidly degraded such that the effluents had fatty alcohols principally derived from de novo bacterial synthesis. These discharges also made small contributions to the receiving waters which were dominated by terrestrial plant and algal compounds. This work was undertaken to widen both the geographical range of the studies and the different technologies that are used in the WWTPs.

Samples of the influent, effluent and sediments of the receiving waters were collected in three different eco-regions of the USA. In each state (OK, OH and OR), eight WWTPs were sampled and a total of six different technologies investigated. Samples were extracted using standard approaches and the concentrated lipids were analysed by conventional GC-MS to obtain concentrations and the profiles present. The lipids were also analysed by stable isotope ratio mass spectrometry to obtain the $\delta^{13}C$ and $\delta^{2}H$ signatures.

No differences could be observed between the efficiencies of removal of the fatty alcohols by the six different treatment technologies. Overall, 98% of the fatty alcohols were removed from the influent by the WWTPs. However, this hides that fact that the fatty alcohols in the effluent had different stable isotopic signatures and those compounds in the effluents were derived from new bacterial synthesis as before. There were significant differences in the profiles of the influent; each eco-region had a different mean profile and although each region was internally consistent, these were different from the other eco-regions. This may be due to differences in the products used within the catchment (still to be determined), differences in the diets of the inhabitants leading to different faecal profiles or through different in-pipe processes.

The sediments of the receiving waters had similar fatty alcohol profiles to each other with terrestrial plant matter dominating. Secondary sources from algal and bacterial synthesis could be seen as well. The long chain and short chain fatty alcohols exhibited different behaviour and were clearly separated in several statistical analyses. Any contributions from the WWTP liquid effluents were small (<1%) and then not from the original fatty alcohols suite in the influent. These compounds might have the same chain lengths but they have different stable isotopic signatures.

Significant differences could be seen in the $\delta^{2}H$ values of the long chain fatty alcohols in the sediments and these varied according to the $\delta^{2}H$ in the precipitation. This effect was not seen in the fatty alcohols of the influent in the different eco-regions.

Overall, the same conclusions must be drawn from this more extensive study that (a) the fatty alcohols of the influent are readily removed with the WWTPs; (b) the type of secondary treatment does not affect the removal; (c) the sediments of the receiving waters are dominated by the terrestrial plant inputs; (d) the eco-region may affect the fatty alcohol profile of the influents but not the stable isotopes while the $\delta^{2}H$ values are effected for the terrestrial plant matter but not the profiles and (e) the ecological risk from the use of these chemicals which are disposed of down-the-drain is small.

Chapter 1. Introduction

The analysis of multiple samples collected from two different catchments, one in the UK (Mudge *et al.* 2010) and one in the USA (Mudge *et al.* 2012), both led to the same conclusions; the fatty alcohols in the effluent from the waste water treatment plants (WWTPs) were not the same as the ones in the influent and that the fatty alcohols in the sediments of the receiving waters were dominated by naturally occurring compounds, not those from the WWTPs. While these results are consistent and confirm laboratory investigations (Itrich and Federle 2004), the secondary biological treatments used in both cases were oxidation ditches. It may be argued that there are differences over the range of climatic conditions that occur in the USA and also between the different secondary treatment processes available. Therefore, this work was undertaken to examine both of these potential factors in determining the fate of fatty alcohols in waste water streams.

The previous work indicated that analysis of the influent, effluent and sediments in the receiving waters would be sufficient to determine the contribution that the different fatty alcohol sources make to the environment. The sampling plan, therefore, was designed to sample the three sites (influent, effluent and sediment) in the different ecological regions across the different secondary treatment techniques.

Ecological Regions in the USA

North America has been divided into 15 Ecological Regions ranging from the high arctic to tropical wet forests; these were proposed by Omernik (Omernik 1987) and developed by the US EPA (http://www.epa.gov/wed/pages/ecoregions.htm). Although there are 15 regions, the bulk of the USA is encompassed by just six with two of these having restricted ranges along the west coast (Figure 1). The previous USA study conducted in Luray, Virginia was in Ecological Region 8.0 (sub-region 8.3, south eastern USA plains), part of the Eastern Temperate Forests (Mudge *et al.* 2012). The bulk of the population in the USA live toward the East and West coasts with comparatively fewer population centres in the middle.

Three different Eco regions were chosen for this study:

- the Great Plains (Region 9) and the sampled zone was further sub-classified as region 9.4, South Central, Semi-Arid Prairies. As the name suggests, the zone has low rainfall, wide open grass plains typically grazed by cattle, cool winter temperatures followed by hot dry summers. The region is also characterised by tornadoes. Although the region extends northward into Canada, the major population centres are in the south.

- the Eastern Temperate Forests (Region 8) and the sampled zone is further sub-classified as regions 8.1, mixed wooded plains; 8.2, central USA plains, and 8.4, Ozark, Ouachita-Appalachian forests. The region is distinguished by a moderate to mildly humid climate, a diverse forest cover and a high density of human inhabitants (approximately 160 million). Activities include standard urban industries, agriculture and some forestry (CEC). In some parts of this region, the forests have been cleared and the land is now used for agriculture, especially corn and soy bean.

- and the Marine West Coast Forests (Region 7.1) which has no further sub-classification. This region is described as highly productive, rain-drenched evergreen forests. The region includes the Willamette Valley, which runs from south to north between the Oregon Coast Range to the west and the Cascades Range to the east. The Eco region is drained mostly by the Willamette River and its tributaries, which flows into the Columbia River straddled by Portland, Oregon.

It may be hypothesised that the different Eco regions will have an effect on the indigenous flora and lead to different chemical signatures in both the material entering the rivers and streams as well as the bacteria in the WWTPs. These differences may lead to a difference of performance between plants in the removal of compounds from the wastewater input and the fate of any subsequent discharge into the streams.

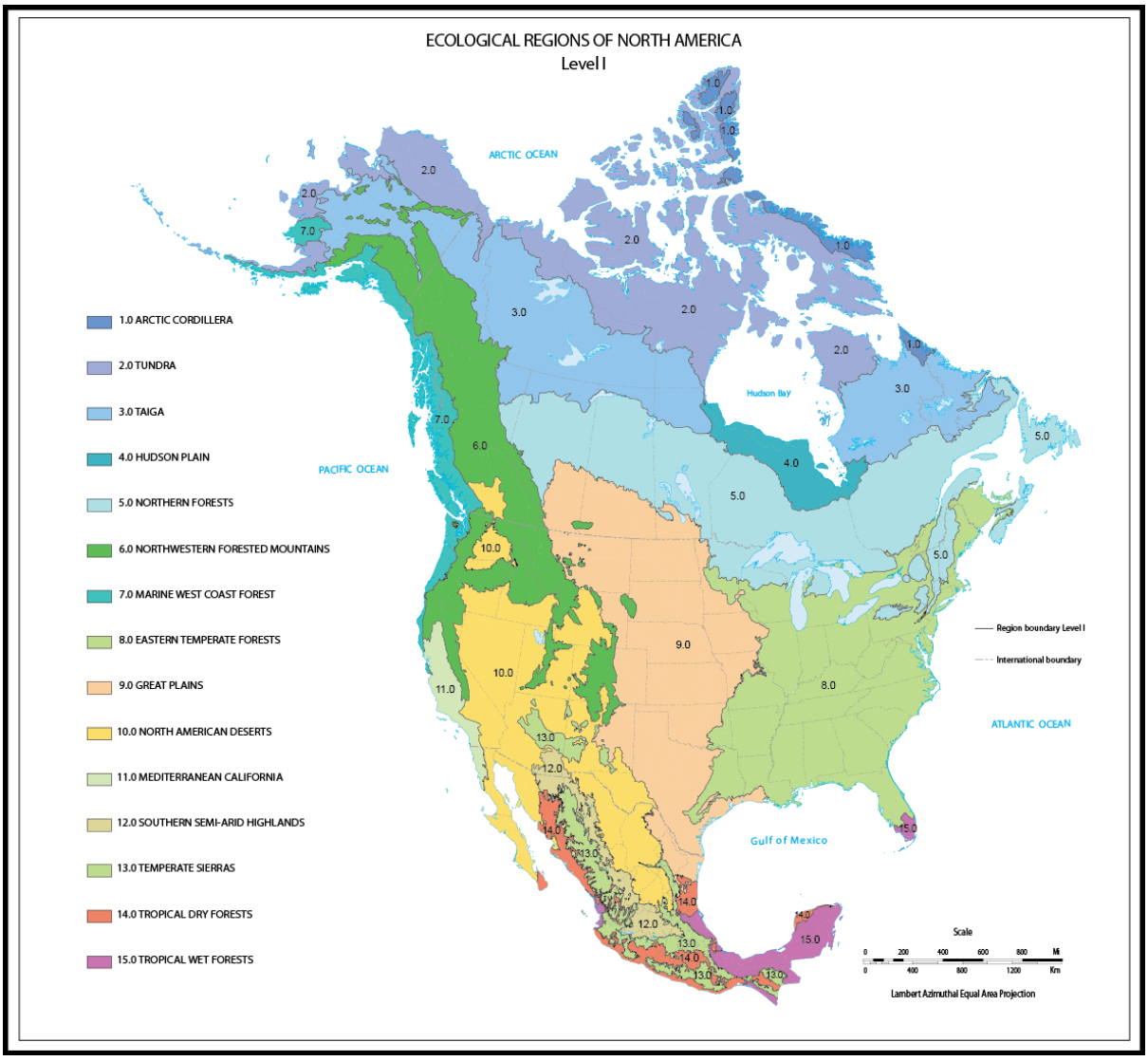

Figure 1. Ecological Regions of North America at level 1. While there are 15 regions across the whole continent, there are only six with a significant population in the USA. Modified from the USEPA.

Waste Water Treatment Processes

Waste waters undergo a series of treatments at WWTPs; these are summarised in Figure 2. The influent is screened to remove grit and other debris which are typically disposed of at a land fill site. In most cases, the solids are principally removed through settlement as a first stage of treatment. The liquid supernatant is then passed forward for secondary biological treatment (see below). In some cases, the effluents receive a tertiary treatment step, usually UV irradiation, to reduce the number of viable bacteria.

The sludges (biosolids) are dewatered, thickened and often spread on agricultural land as a fertiliser. In the past there was a tendency to digest the solids anaerobically to generate methane: this was uncommon in the Oklahoma plants but more common in these Ohio plants where several methane flares were observed. Only two plants in the Oregon sampling digested their sludges.

There are a range of secondary treatment processes some of which are outlined below.

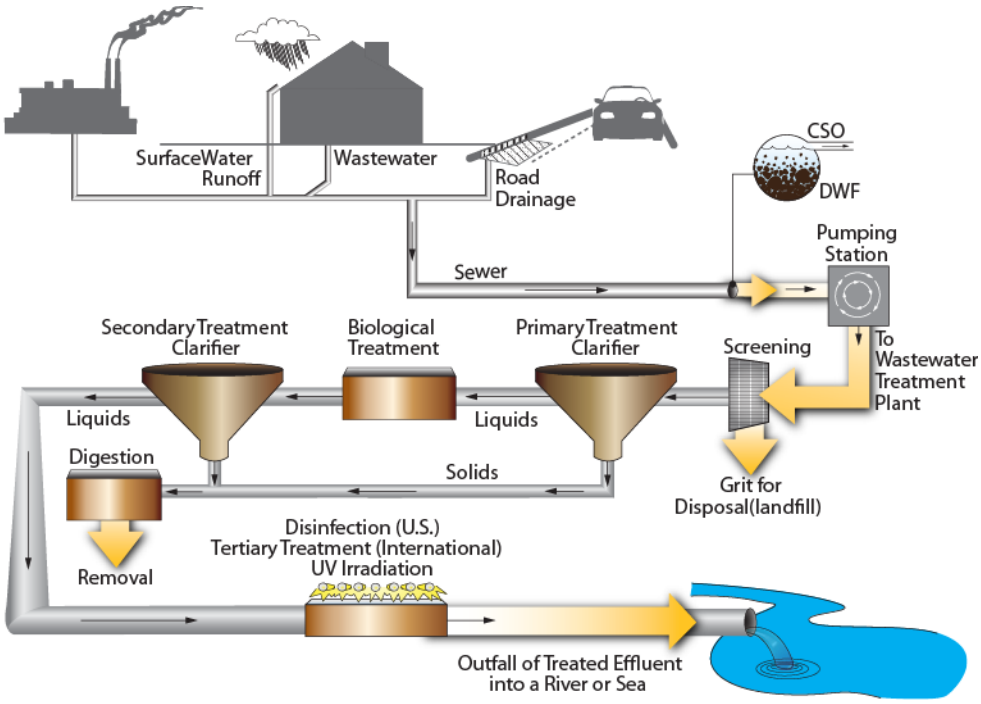

Figure 2. Schematic representation of a typical sewage system (Mudge and Morrison 2010).

Oxidation Ditch and Activated Sludge

Along with all secondary treatment processes, the oxidation ditch is designed to promote bacterial and protozoan growth within the waste water; the growth of these organisms converts organic components and nutrients in the water into biomass and carbon dioxide. The physical layout of an oxidation ditch is a deep channel where the screened and settled waste water is circulated or, occasionally, flows directly through. There is a removal of liquids carrying suspended solids at one end at a rate equal to the inflow. The retention time of the solids and liquids may be different and typically the waters have 24 – 48 hour

retention times while the solids may remain for 8 – 10 days. The high biological activity consumes the oxygen in the water and to prevent the whole system from becoming anoxic, air is introduced either through pumps or brush aerators. An example of a brush aerator system at Stow, OH can be seen in Figure 3.

The difference between oxidation ditches and activated sludge may be down to simple semantics based on size of the installation although residence times have been cited as a distinguishing feature. During periods of elevated rainfall, the WWTPs will try to balance the flow through these ditches to optimise the treatment. However, if the rainfall is sustained, the residence time in this stage may be reduced in order to prevent backup of the sewer system. An alternative approach is the use of combined sewer overflows (CSOs) to relieve the pressure in the upstream network.

Figure 3. Brush aerators as part of the secondary biological treatment process at Stow (Fish Creek), OH.

Percolating or Trickling Bed Filters (TBF)

The biological assemblage in this type of treatment is supported on a solid matrix usually made of stone chippings, coke or some other high surface area material. The liquor is spread over the surface and allowed to percolate or trickle down through the support. In some cases, air may be introduced at the bottom to maintain oxic conditions. The biomass that accumulates within the bed may slough off and this, together with remaining solids, is removed in a humus tank or clarifier similar to the primary settlement tanks. In Massillon, OH, this system was used in conjunction with other processes as well to improve the quality of the final effluent. At Everett, WA, the WWTP treated all influent through a TBF up to 16 million US gallons per day (MGD); flows above 16 MGD were directed through a lagoon system leading to two different effluents from the same influent.

Lagoons

When space is not at a premium, it may be sensible to use lagoons as the secondary treatment stage. These large basins, that may be several hectares in area, can be arranged in sequence or parallel collect the liquid and provide aeration and subsequent BOD removal. Mixing within such systems is not as good as an oxidation ditch and the residence time is substantially longer. Solids are removed by draining the tanks in a rota. Example of lagoons in Danville, OH and Everett, WA are shown in Figure 4.

The two lagoon WWTPs in Ohio both reported issues with ammonia concentrations. One of the plants (New Bremen, OH) had a horizontal percolating bed filter in series with the lagoons before the final discharge to assist in lowering these concentrations.

Rotating Biological Contactor (RBC)

In this arrangement, the solid support that maintains the biological community is held on several closely spaced, large discs that rotate through the waste water. Oxygenation is achieved partly by rotation of the disc but air may be blown into the liquid to help maintain oxic conditions. The community that develops on the disc may comprise an aerobic outer layer with an anaerobic layer adjacent to the disc itself.

Sequencing Batch Reactor (SBR)

Unlike the other technologies outlined above, in this type of plant all of the treatment takes place in a single tank rather than being pumped or gravity-fed around a site. The tank is filled with influent, some initial settlement may be allowed followed by the pumping of air through the liquid to encourage secondary biological degradation of the organic matter. The whole system is allowed to settle again and then the liquid is decanted off and disposed of as the final effluent. The sludge remaining in the tank is then removed for dewatering and land disposal. An example of such as system is shown in Figure 5.

The time between fillings is in the order of several hours which leads to a substantially shorter residence time than most linear treatment plants. Another advantage of this design is the small footprint required and so these plants may be used in towns and cities where land is restricted. No WWTPs of this type were sampled in Ohio (Ecoregion 8).

Figure 4. A lagoon at Danville, OH (top). No aeration is provided in this particular lagoon although systems are in place in the first two lagoons in the series. A large static system at Everett, WA (bottom).

Figure 5. The surface of an SBR at Chehalis, WA with scum accumulating on the surface. The sludge is removed from below after the liquid has been drained off.

Chapter 2. Materials and Methods

Selection of WWTPs

Sampling sites were chosen on the basis of the performance of the WWTPs, the influent volume (less than 40 million gallons per day (MGD = 3.8 million litres per day) but greater than 1 MGD), effluent flow as a proportion of the receiving water flow and the absence of significant industrial contribution to the influent (<10%). This is the same screening process that was used in the selection of the Luray catchment (Mudge *et al.* 2012). One of the objectives of the study was to determine whether the secondary treatment process for a facility influenced the magnitude and distribution of fatty alcohols discharged to the environment. Generally, samples from facilities covering at least four styles of secondary treatment were collected in each Ecoregion (activated sludge, oxidation ditch, lagoon, a fixed film technology either trickling filter or rotating biological contactor). Facilities using fixed film technology and meeting the other criteria were often difficult to locate, so sequencing batch reactor facilities were sampled as an alternative in some cases. In order to include a sufficient number of lagoon facilities, the criterion for the >1 MGD had to be relaxed as the lagoons were only available in rural, small communities with a lower influent flow rate.

Permission was sought from candidate WWTPs and those willing to participate were contacted. The 24 selected WWTPs and their statistics can be seen in Table 1. Contact, permission and statistics for each plant were obtained by Doug Fort (Fort Labs, OK) and EA Engineering (OH and OR) prior to sampling. Fort Labs and EA Engineering also provided the sampling equipment and assistance during the sample collection phase of this work.

Sampling

At each site, ~2.5 litres of liquid influent were collected. All sites provided composite samples of the influent based primarily on flow or time were provided by the WWTP. At each site, a 2.5 litre sample of the effluent was collected from the discharge stream.

Sediments were collected downstream of the effluent discharge point in the rivers. In most cases there was no obvious indication to the proximity to the WWTP. Surface scrapes to 1 cm were collected in pre-cleaned 125 ml glass jars.

Table 1. WWTPs selected for sampling in the three Ecological Regions

WWTP	Secondary Treatment	Influent MGD (litres per day x 10^6)	Population served (in thousand)
Oklahoma Sites			
Winfield (KS)	Oxidation Ditch	1.2 (4.6)	12
Stillwater	Activated Sludge	5.4 (20.5)	48
Edmond (Coffee Creek)	Oxidation Ditch	7 (26.6)	84
Deer Creek	RBC & Activated Sludge	15 (57)	82
Del City	SBR	1.5 (5.7)	25
Ada	SBR	2.5 (9.5)	15
Weatherford	Activated Sludge	1 (3.8)	10
Elk City	Lagoon	1.2 (4.6)	12
Ohio Sites			
East Liverpool	RBC	1.7 (6.5)	11
Alliance	Activated Sludge	4.0 (15.2)	23
Massillon	Oxidation Ditch + TBF	14.8 (56.2)	36
Summit/Stow/Fish Creek	Oxidation Ditch	3.5 (13.3)	40
Strongsville	RBC	1.0 (3.8)	15
French Creek	Activated Sludge	5.8 (22.0)	50
Danville	Lagoon	0.1 (0.4)	1.1
New Bremen	Lagoon + TBF	0.8 (3.0)	3.5
Oregon Sites			
Everett (WA)	TBF and Lagoon	13.5 (51.1)	150
Chehalis (WA)	SBR	1.5 (5.7)	9
Astoria	Lagoon	1.6 (6.1)	10
McMinnville	Oxidation Ditch	3 (11.4)	33
Molalla	Lagoon	1.1 (4.2)	8.1
Silverton*	Activated Sludge	1 (3.8)	8.0
Stayton	SBR	1 (3.8)	10
Corvallis	Activated Sludge	6 (22.7)	55

* Data from the EPA Clean Watersheds Needs Survey for populations and daily flow rates at Silverton.

Sediment Extraction

All samples were returned to the appropriate laboratory (OU in OK, P&G Miami River Innovation Center in OH and OSU in OR) in cool boxes with ice. For the OK and OH samples, ~120 g wet weight was extracted using the following protocol (Mudge and Norris 1997).

1. Approximately 120 g wet weight was weighed accurately to two decimal places and placed in a round bottom flask. An internal standard was added (1.0 ml of a 1.00 $mg \cdot ml^{-1}$ solution of 2-

dodecanol from Sigma Aldrich in methanol[1]) together with 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 0.5 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.

For the OR samples, an Accelerated Solvent Extractor (ASE) was used (Mudge *et al.* 2012).

1. Approximately 100 – 200 g wet weight of each sample was weighed accurately to one decimal place and placed on aluminium foil and air dried for 60 hours. The samples were re-weighed and lightly ground with a glass pestle and mortar. This material was packed into stainless extraction tubes (mean extraction weight of 73 g) and settled with a steel spatula. An internal standard was added (1.00 ml of a 1.00 $mg \cdot ml^{-1}$ solution of 2-dodecanol from Sigma Aldrich in methanol) by pipette to the top of each sample before sealing.

2. Solid samples were extracted in a Dionex accelerated solvent extractor (ASE 300; Sunnyvale, CA) at 100°C and 1500 psi using dichloromethane (DCM) : hexane (1:1), employing two 5 minute extraction cycles with DCM : hexane followed by a 270 s vessel purge.

3. The DCM : hexane extraction solvent was taken to dryness under a stream of N_2 in a water bath at 50°C. When the volume was ~1 ml, the sample was transferred to a small vial, taken to dryness under N_2.

4. The samples were re-dissolved in 8% KOH (w/v) in methanol for two hours with regular agitation to saponify the lipids. After cooling, the free lipids were extracted into hexane twice, the solvents combined and taken to dryness again.

Liquid Sample Extraction

The samples collected within the WWTP were liquids with suspended solids and the fatty alcohols were extracted from the whole sample. The extraction method for the liquid samples followed that developed in the previous studies. The protocol used was as follows:

1. On collection, 30 g of KOH was added to the 2.5 litres of the liquid samples which were kept in cool boxes with ice until return to the laboratory.

[1] The exact concentration of the internal standard varied between extractions although they were approximately 1.00 $mg.ml^{-1}$ and a final added concentration of 1 mg per sample was achieved.

2. Two litres of sample was poured into a 2 litre separating funnel and 1.0 ml of the internal standard was added (1.0 mg added per sample). Approximately 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.

Derivatisation

The final lipids extracts from both the liquid and solid samples were derivatised at 60°C with ~5 drops of BSTFA for 2 h to ensure complete derivatisation of the primary (Figure 6) and secondary alcohols. Excess BSTFA was evaporated under nitrogen and the final samples re-dissolved in 1 ml of hexane. The same batch of BSTFA was used in each case.

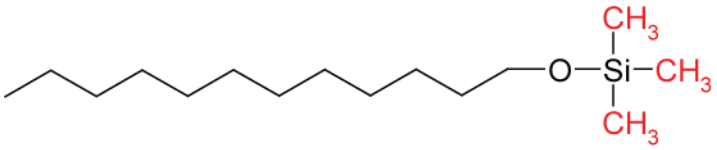

Figure 6. The atoms highlighted in red have been added as part of the TMS group and will contribute to the overall $\delta^{13}C$ and $\delta^{2}H$ values. Therefore, a correction needs to be applied to calculate the original molecule values.

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 batch, different analytical equipment was used but they all achieved the same outcome:

For OK, 1 µl was injected into an Agilent GC (Model 7890A) with a 5975C MS detector. A split - splitless injector was used with the following conditions:

1. DB-5MS (J&W) column, 60 m x 0.25 mm ID x 0.25 µm film thickness.

2. Temperature programme of injection at 40°C, held for 1.5 min, 4°C per min to 300°C with a final hold of 24 min.

3. The mass spectrometer scanned from 50 to 510 *m/z*.

For OH, 1 µl was injected into a Perkin Elmer GC-MS (Model Clarus 680) with Turbomass software v5.4.2. A split - splitless injector was used with the following conditions:

1. VF-5MS HT (Agilent) column, 30 m x 0.25 mm ID x 0.25 µm film thickness.
2. Temperature programme of injection at 60°C, held for 1.0 min, 8°C per min to 350°C with a final hold of 10 min.
3. The mass spectrometer scanned from 40 to 590 *m/z*.

For OR, 1 µl was injected into an Agilent GC (Model HP 6890) with a 5972A MS detector. A split - splitless injector was used with the following conditions:

1. DB-5MS (J&W) column, 30 m x 0.25 mm ID x 0.25 µm film thickness.
2. Temperature programme of injection at 60°C, held for 2 min, 10°C per min to 300°C, 3°C per min to 325°C with a final hold of 2 min.
3. The mass spectrometer scanned from 45 to 525 *m/z*.

All spectra were processed with the AMDIS v2.69 software using the NIST library.

Compound Specific Isotope Ratio Mass Spectrometry

All samples were sent to the James Hutton Institute (formerly known as the Scottish Crop Research Institute) in Dundee, Scotland for analysis on a Thermo Delta V Plus Isotope Ratio Mass Spectrometer. 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 Isotope Ratio Mass Spectrometer (Meier-Augenstein *et al.* 1994; Meier-Augenstein 1995). The GC conditions were: Injector 250°C, splitless for 0.5 min; carrier flow 1.2 $mL \cdot min^{-1}$ (constant flow); oven: 60°C for 2 min, $6°C \cdot min^{-1}$ 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.

Chapter 3. Results and Discussion of the Oklahoma Samples

Photographs of the lipid extracts in variable amounts of solvent can be seen in Figure 7. The concentrations of the fatty alcohols in each sample are presented in the appendix. During the period prior to sampling, OK and other southern states had been experiencing drought and diminished river flows. These are discussed in the final chapter.

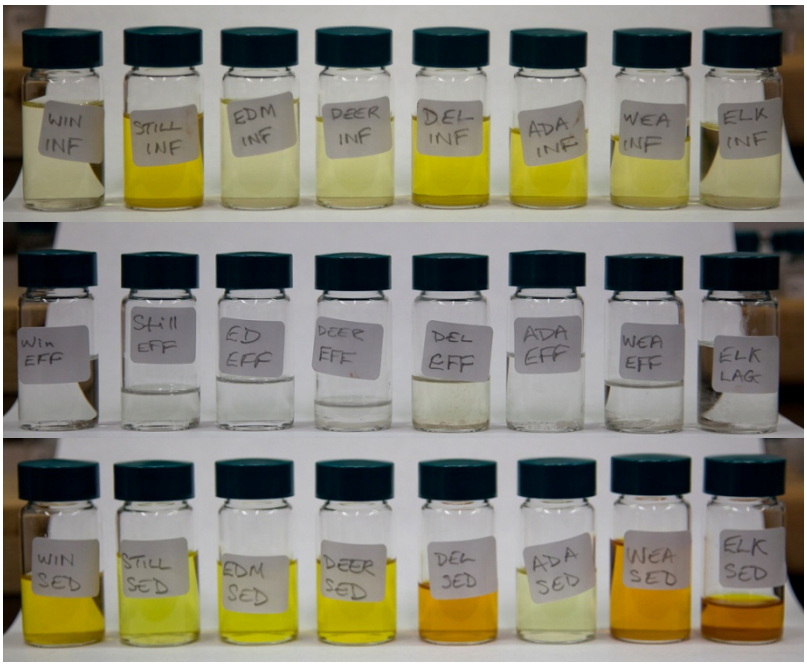

Figure 7. The extracted lipids in hexane for each location; the top row are the influent samples, the middle row are effluents and the bottom row are sediment extracts. The intensity of colour does not necessarily represent the final concentration of fatty alcohols in the extract.

An example of the GC trace can be seen in Figure 8.

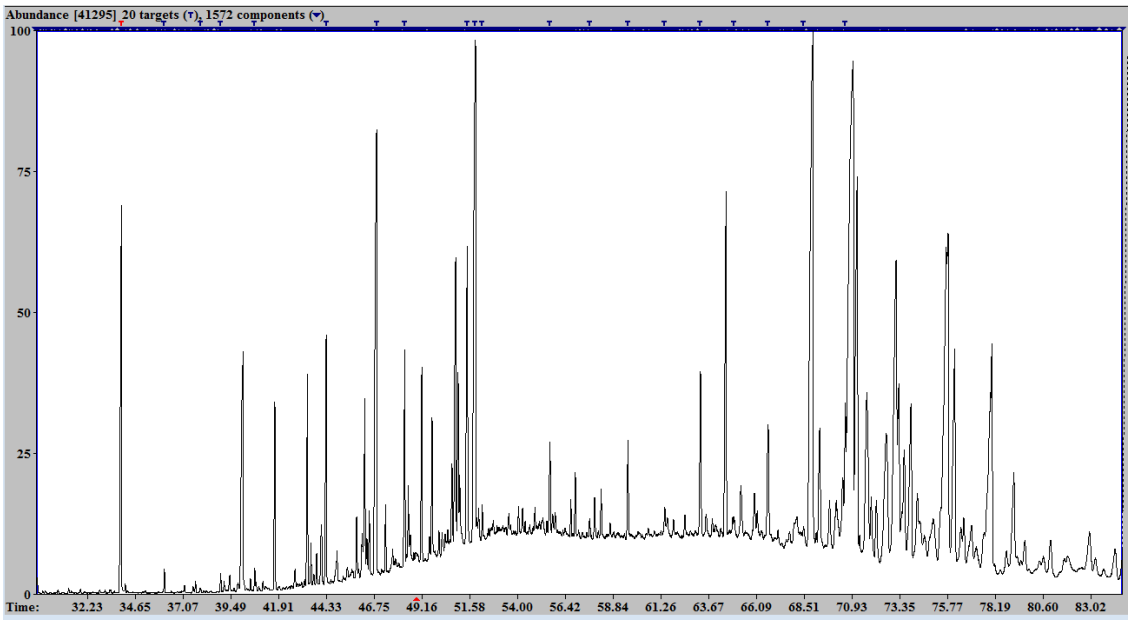

Figure 8. The Total Ion Count (TIC) for Del City sediment. The small T in the upper margin indicates a quantified fatty alcohol. The sterols are to the right of the trace.

Influent

The mean concentration of total fatty alcohols in the influent was ~440 µg·L^{-1} compared to ~200 µg•L^{-1} in the UK study (Mudge *et al.* 2010) and ~600 µg•L^{-1} in the Luray study (Mudge *et al.* 2012). The profile can be seen in Figure 9. In this figure, the 18 carbon compound dominates the profile; this is the same as the influent samples from Luray and the UK study. The 18 carbon compound is not abundant in the detergent formulations (DeLeo *et al.* 2011) and may be formed within the pipe through bacterial action. The second most prevalent fatty alcohol is the 16 carbon moiety which is the major alcohol formed through the fatty acid synthase pathway (Mudge *et al.* 2008). The third most important alcohol is the C_{12} which may include a component from detergents since this is the most prevalent fatty alcohol in detergents used in the Luray catchment and likely to be the same here.

Small amounts of long chain fatty alcohols (>C_{22}) were present derived from terrestrial plants. These may enter the waste stream from ingested plants, food waste or terrestrial plant matter entrained into surface water runoff.

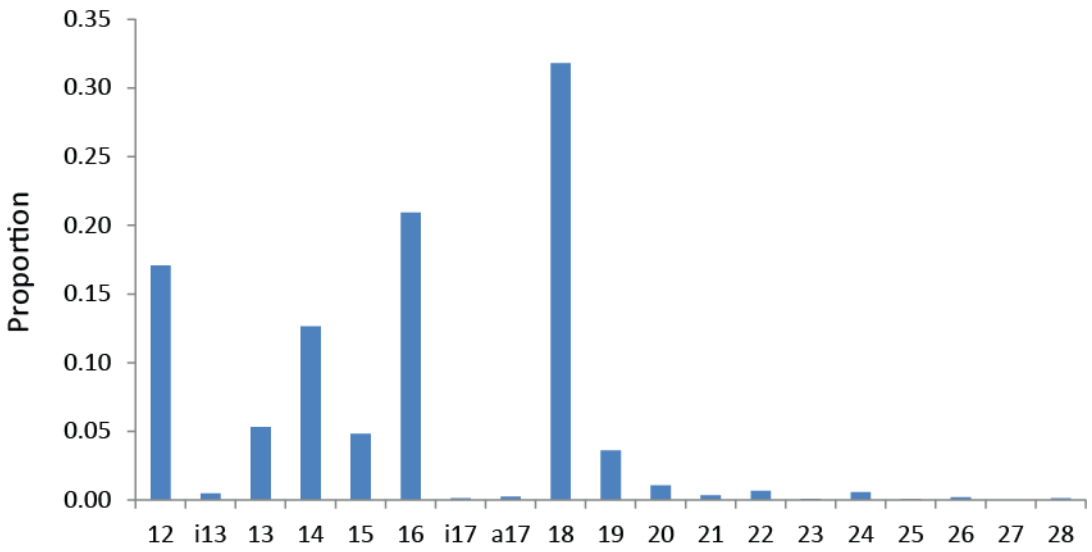

Figure 9. The mean fatty alcohol profile for all influent samples. The mean total concentration was 440 µg•L^{-1}.

Effluent

The concentrations of fatty alcohol in the effluent were significantly less than that of the influent indicating a substantial removal during the treatment process. The mean concentration was 8.3 µg•L^{-1} and the mean of site specific removal factors compared to the influent was 98%. As with previous analyses, there was also a substantial change in the fatty alcohol profile (Figure 10) to C_{12} domination and substantial amounts of odd chain and branched fatty alcohols that are derived from bacteria.

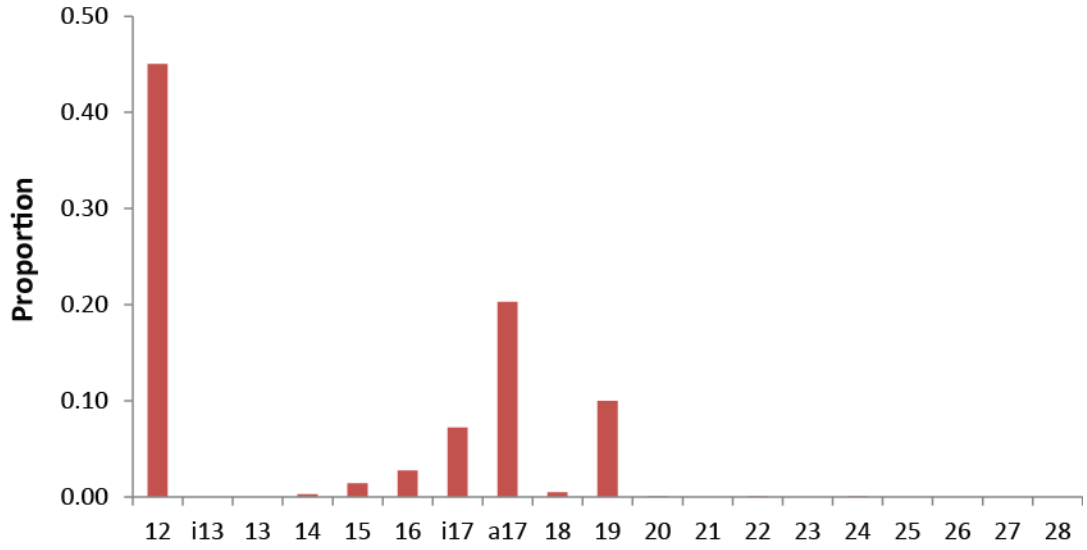

Figure 10. The mean fatty alcohol profile for all effluent samples. The mean total concentration was 8.3 µg•L^{-1}. No long chain alcohols from terrestrial plants were detected in these samples.

The mean carbon preference index (CPI), the ratio between odd and even chain compounds across the C_{12} to C_{18} chain lengths, was 6.0 compared to 0.13 in the influent indicating the importance of bacterial synthesis in these samples.

Sediments

The profile of fatty alcohols in the sediments is different from the WWTP samples since they contain a significant amount of long chain alcohols derived from terrestrial plants (Figure 11). The profile reflects two major sources with terrestrial plants having even chain length compounds in the C_{22} to C_{28} range and algal fatty alcohols centred on C_{16}. The presence of C_{15} and C_{17} fatty alcohols implies there is a contribution from bacteria in these samples and the mean CPI is 0.31 which is less than that measured in the river sediments from the Luray study (0.68, (Mudge *et al.* 2012)).

The algal contribution is greater than that in Luray and is consistent with observed algal mats seen in several rivers which were essentially effluent dominated at the time of sampling.

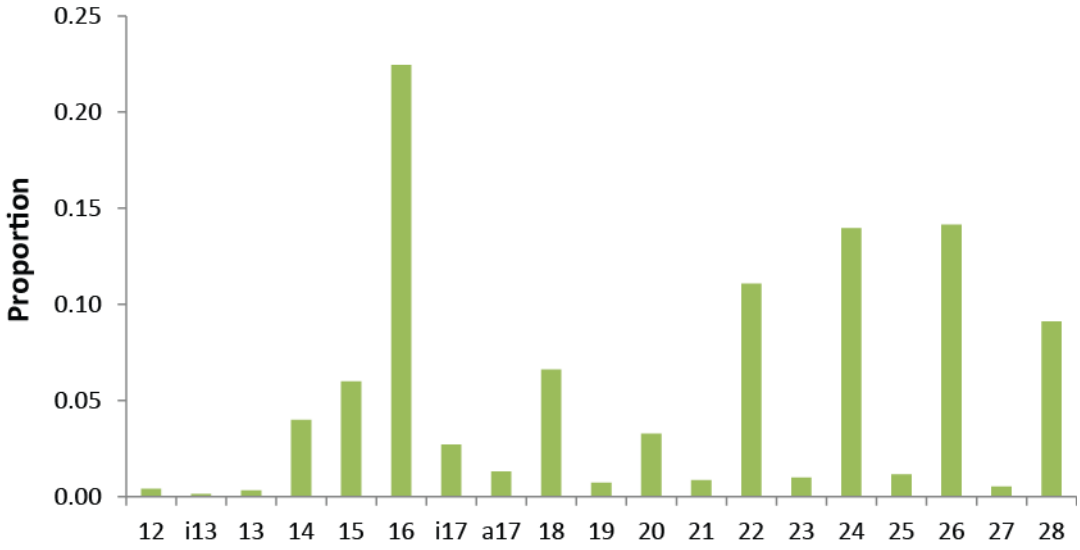

Figure 11. The mean fatty alcohol profile for all sediment samples. The mean total concentration was 107 mg•kg^{-1}.

Principal Component Analysis

One of the best ways to view that data across multiple samples with multiple chemicals is through the use of PCA (Mudge 2007). This projection method allows the composition of all samples to be viewed on just two types of diagram; the loadings and the scores. For these data, the results were converted to proportions to remove the concentration effects and to retain any chemical signature that may be present. PCA first determines the vector within the data matrix that explains the greatest amount of variance within the data. This may be likened to conducting regression analysis in multi-dimensional space. The vector is defined by loadings for each variable (fatty alcohol proportions) and can be used to

calculate a score for each observation (sample). Once the primary vector has been determined, further vectors can be fitted at right angles to the first so there is no component of PC1 in PC2.

The scores for each sample (Figure 12) clearly separate the samples according to their type. Principal Component 1 (PC1) separates the WWTP samples from the sediment samples while PC2 separates the influent from the effluent. The effluent group is tightly clustered in this figure indicating very similar compositions. The other two groups are bigger and indicate some variability within the group but there is no overlap with any other sample type.

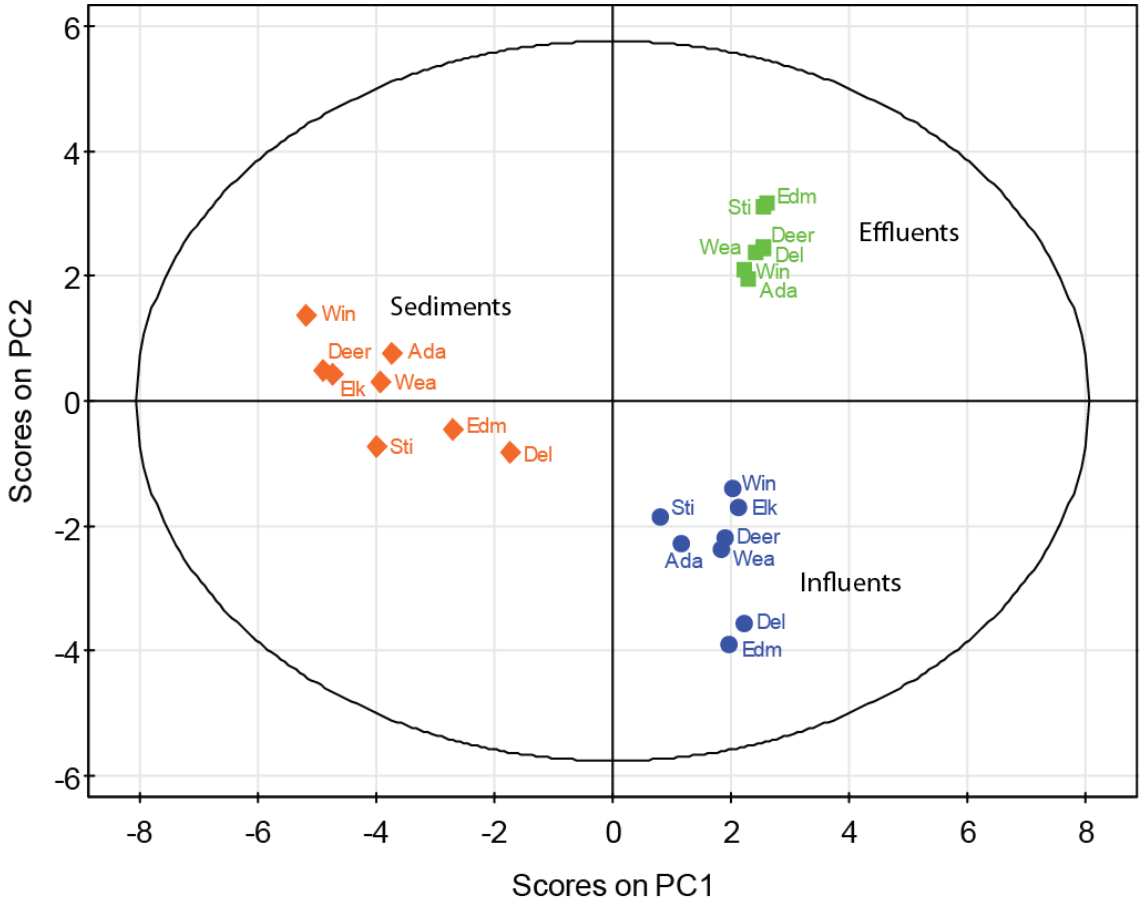

Figure 12. The scores for each sample based on the PCA of all fatty alcohols expressed as proportions.

The loadings on the fatty alcohols associated with the samples (Figure 13) indicate which compounds are deterministic within the sample score groups. The influents are relatively enriched in the C_{13}, C_{14} and C_{18} fatty alcohols (labelled Bacteria Type I) while the effluents have the C_{12}, C_{17} and C_{19} compounds (Bacteria Type II). The sediments are dominated by long chain ($>C_{20}$) fatty alcohols from terrestrial plants.

Within the sample types in the scores plot (Figure 12), there is no grouping associated with the secondary treatment methodology shown in Table 1 and so it is not possible to distinguish between, say, SBR and RBCs on the basis of their fatty alcohol composition in the effluent.

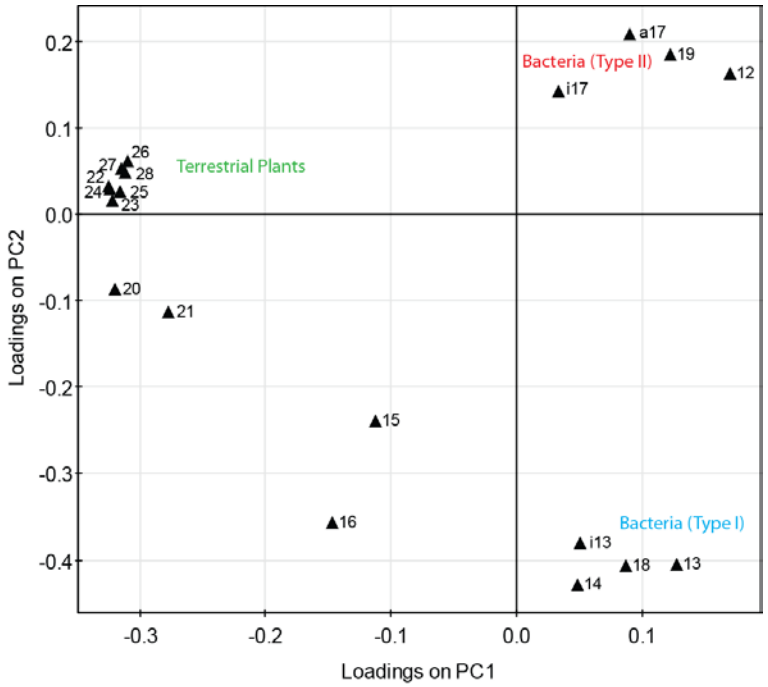

Figure 13. The loadings for each fatty alcohol with the data expressed as proportions.

Efficiencies

The removal efficiencies for the fatty alcohols in each plant can be seen in Table 2. These values are calculated from the total fatty alcohol concentrations in the influent compared to the effluent. Some of these compounds will have been removed through the sludges and disposed of elsewhere.

Table 2. Removal efficiencies by secondary treatment method.

WWTP	2° Treatment	Fatty alcohol removal (%)
Winfield (KS)	Oxidation Ditch	90.5
Stillwater	Activated Sludge	95.2
Edmond (Coffee Creek)	Oxidation Ditch	99.5
Deer Creek	RBC and Activated Sludge	99.9
Del City	SBR	97.1
Ada	SBR	99.4
Weatherford	Activated Sludge	99.9
Elk City	Lagoon	100

Sterols

Sterols are structural components of cells and occur in waste waters. There are several sterols and stanols that are diagnostic of the source of organic matter and processes that matter may have been subjected to (Mudge and Norris 1997). Sewage may be identified through the presence of 5β-coprostanol which is formed in the human (and other higher animals) gut through biohydrogenation of

cholesterol (Mudge *et al.* 1999). Raw human sewage has high 5β-coprostanol / cholesterol ratios (Leeming *et al.* 1996) which can be distinguished from agricultural herbivores that typically produce higher quantities of 24-ethyl coprostanol derived from the terrestrial plant sterol, β-sitosterol (Mudge and Lintern 1999).

In WWTPs, 5β-coprostanol may be converted to epi-coprostanol and the ratio between these two compounds is indicative of sewage treatment. Likewise, cholesterol may be converted into cholestanol in anaerobic reducing environments outside of the gut. It is possible, therefore, to use the sterols and stanols to indicate the source of organic matter in samples. In these samples, the concentrations of fatty alcohols and sterols were linked (Figure 14). The full results are presented in the Appendix. The strong association between these two groups of compounds might be suggestive of a common origin. However, within the total sterols will be compounds derived from both sewage (*e.g.* 5β-coprostanol) and terrestrial plants (*e.g.* β-sitosterol) and so the total sterol concentration might not be diagnostic. In which case, it is better to investigate known ratios which can distinguish between sources if we are looking for a link to the fatty alcohols.

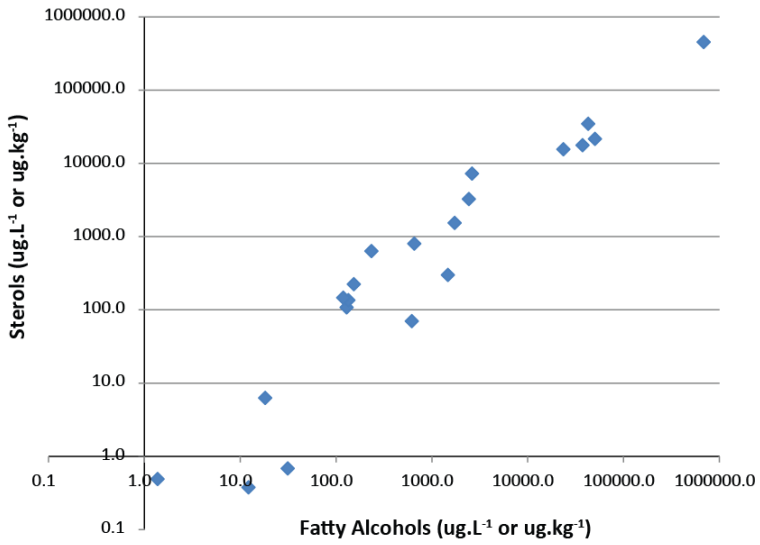

Figure 14. The relationship between the fatty alcohols and sterols in all samples. N.B. the axes are both expressed as logs. A linear regression of the data has the following equation: y = 0.6503x - 156.79 with an R^2 = 0.9987.

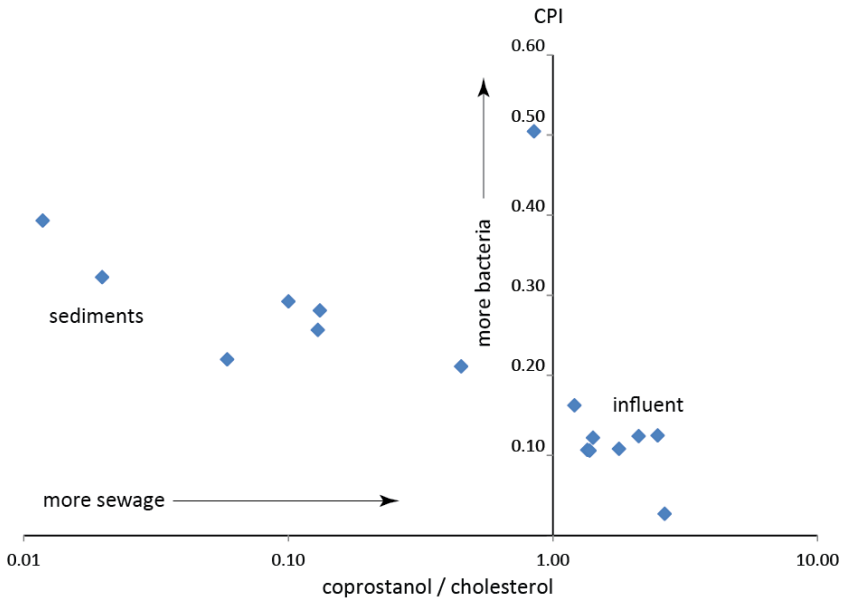

Figure 15. The relationship between the sewage marker 5β-coprostanol / cholesterol and the carbon preference index, CPI (Grimalt and Albaiges 1990).

To investigate the source of the bacterial fatty alcohols in the samples, especially the sediment samples, the CPI (Grimalt and Albaiges 1990) was plotted against the 5β-coprostanol / cholesterol ratio to determine if a link exists. These data (Figure 15) indicate that the influent to the WWTPs have a smaller bacterial signature based on the fatty alcohol CPI compared to the environmental sediments. This is probably due to the presence of human derived fatty alcohols which will have a large amount of the C_{16} and C_{18} and dwarf the bacterial odd chain fatty alcohols despite their significant presence in the waste waters. The even chain compounds are metabolised or settled out of the influent in the WWTP and the effluent is rich in the bacterial signature. The sediments will contain fatty alcohols derived from the WWTP superimposed on a background where natural degraders are present. These degraders include bacteria and fungi.

The plot of CPI against the ergosterol concentration (not shown) indicates that the higher the fungal biomarker concentration, the higher the CPI, indicating more bacteria. Therefore, it may be concluded that the bacterial fatty alcohol markers are related to the natural degraders in the environment. Ergosterol was not present in the effluent or influent except in one sample.

Stable Isotopes

Not all fatty alcohols that were detected in the GC-MS were present at sufficiently high concentrations to enable both the ^{13}C and ^{2}H to be determined. A cross plot of those that were measured can be seen in Figure 16. On this figure are placed labels indicating the most likely chemical signature for the fatty alcohols. Towards the top left are compounds with $\delta^{13}C$ values smaller than -30‰ that are indicative of terrestrial plant matter. In the upper right is the location of petroleum based detergent fatty alcohols based on results from the Luray study (Mudge *et al.* 2012). No compound measured in this study exhibited a pure petroleum based detergent signature.

In the lower right of the figure would be compounds that have an oleochemical fatty alcohol signature. These compounds are derived from terrestrial plant oils such as palm kernel oil and have a distinct stable isotope signature. Algal plant matter tends to have a signature that sits between the two detergent sources and these compounds are synthesised in rivers by micro-organisms. To the left of this signature would be the typical signature for faecal matter and the majority of short chain compounds in this study fell into this bracket.

There are no samples that indicate a high proportion of petroleum based detergents and the ones that come closest are for influents from Stillwater and Elk City. None of the environmental samples had values that came close and these data indicate, in common with the previous studies, that none of the fatty alcohols entering the WWTP are reaching the sediments. The concentrations in the effluents were too low despite concentrating the sample to yield any compound specific data.

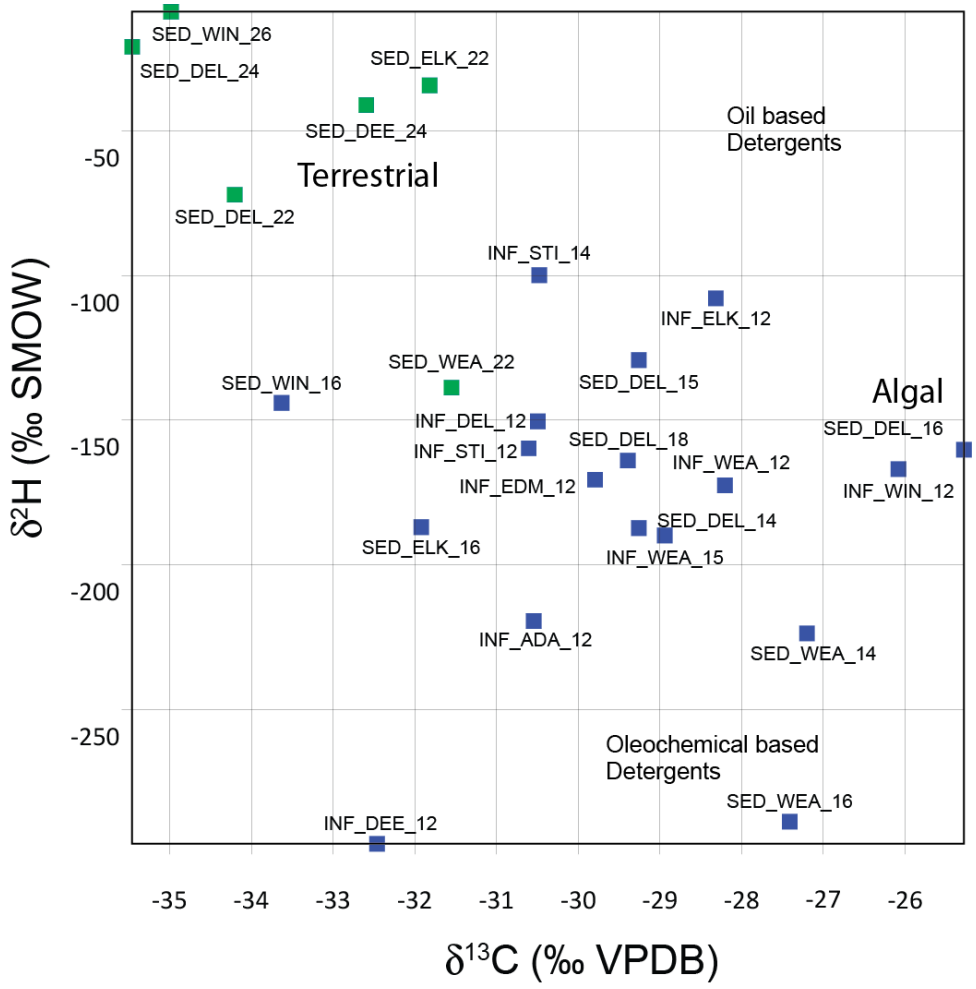

Figure 16. A cross plot of the carbon-13 and hydrogen-2 in individual compounds. The first three letters of the label indicate the sample type (sediment or influent; no effluents satisfied the criteria of having both $\delta^{13}C$ and $\delta^{2}H$ values): the second three letters indicate the sample location and the final number is the chain length of the fatty alcohol.

Fatty alcohols, principally as ethoxylates, sulphates and ethoxysulphates, are present in many household cleaning products; there is a wide range of reported concentrations in the formulations and this may vary according to the market (*e.g.* EU vs. USA). An accepted mean of 3% has been used (Veenstra *et al.* 2009; Sanderson et al. 2012) although values up to 24% have been reported (HERA 2009) by the industry. The disposal route for these products in typically down the drain into the main sewer means that the compounds have to pass through a WWTP before they can enter the environment as part of the liquid discharges. It is possible that some materials could enter rivers and coastal waters through septic tank systems and through combined sewer overflows during periods of high rainfall. However, although it has not been quantified, this route probably makes a small contribution overall.

From previous data, it is known that the majority of the fatty alcohols are removed during the treatment stages in the WWTP. Most are incorporated with the solids that go for sludge disposal. Since a large proportion of these wastes are spread on agricultural land, this might form a route by which some compounds may re-enter the aqueous environment as non-point source runoff. Again, the magnitude of this route is not known.

Notwithstanding the above caveats, the fatty alcohols measured in the river sediments are overwhelmingly from natural sources as determined by the GC-MS profiles and the stable isotopes. The distribution of chain lengths is bimodal with a large proportion arising from terrestrial plant waxes (C_{22+} with $\delta^{13}C$ values around -33‰). The second major component comes from algal synthesis through the fatty acid synthase system; this produces an acid (that subsequently may become an alcohol) with a change length of C_{16} and typically has a $\delta^{13}C$ value between -25 and -30‰. The absence of data for the effluent highlights the low concentrations present in these liquid discharges and the small contribution they make to the river sediment inventory.

The influent samples contain elevated C_{18} fatty alcohols which do not obviously arise from either detergents or plant matter (algal or terrestrial). This has been observed in the previous samples and it is likely that they arise from in-pipe processes. The effluents have a different signature with substantial amounts of odd chain fatty alcohols arising from bacterial synthesis. The samples do have C_{12} fatty alcohols in them although the removal relative to the influent is 97.7%. The majority of the compounds will be removed in the sludges and through microbial degradation. The sediment samples do contain C_{12} but at 0.4% of the total fatty alcohols. Therefore, even if all of the C_{12} in the sediments did arise from the effluent rather than *in situ* production, it is only a small (<1%) component.

The PCA scores plot (Figure 12) clearly shows how the three sample types (influent, effluent and sediments) have widely different fatty alcohol profiles. These profiles are significantly different from each other while the individual locations are not. The influents are all similar to each other while the effluents are even more closely related to each other. The sediment samples have a greater range of compositions than the other sample types but are all distinctly different from the WWTP samples. The different treatment processes used at the WWTP do not have a dramatic effect on the final effluent composition. The efficiencies of fatty alcohol removal are all above 90% and all but one are above 95%. There is no consistent variation by treatment method. The lagoon at Elk City did achieve the highest

efficiency as no fatty alcohols were detected in the effluent. This may be due to the long residence time allowing the suspended materials to drop out leaving a clear effluent.

The stable isotopes confirm the apportionment established with the profiles; the fatty alcohols in the sediments were derived from algal or terrestrial plant sources and there was no evidence of a substantial detergent contribution.

Conclusions for Oklahoma Samples

1. The concentrations in each of the three sample types (influent, effluent and sediments) are in broad agreement to those concentrations established in previous studies.

2. The fatty alcohol profiles in the influent (rich in C_{18}) suggest some in-pipe bio-transformations are occurring before the wastewater reaches the WWTP. There is a small contribution from terrestrial plants in these wastewaters that may come from food waste or surface water entrainment. The short chain compounds suggest a mixture of faecal matter, bacterial matter and detergents.

3. The effluent has a substantially different profile to the influent and indicates the majority of the compounds have been removed through sorption, settlement and biodegradation in the WWTP. The C_{12} fatty alcohol dominates the effluent but the concentrations are too small to establish the stable isotopic signature for this compound.

4. The sediments have a signature that is dominated by terrestrial plant matter (long chain alcohols from C_{22}). The second contribution to the sediments is from algal matter synthesised *in situ*. There may be a small bacterial contribution although even if all the C_{12} in the sediments is due to WWTP effluent, it is still less than 0.5% of the total in the sediments.

These data confirm the observations seen in the previous studies that the fatty alcohols in the influent are not passed through to the effluent of the WWTP. The river sediments are dominated by terrestrial plant matter and the effluents do not make a substantial contribution.

Chapter 4. Results and Discussion of the Ohio Samples

The concentrations of the fatty alcohols in each sample are presented in the appendix. The concentrations at all of these sites are lower than those found in the Oklahoma survey. This is somewhat difficult to explain especially since the recovery of the internal standard was marginally greater in the samples from Ohio than those from Oklahoma. An example of the GC trace can be seen in Figure 17.

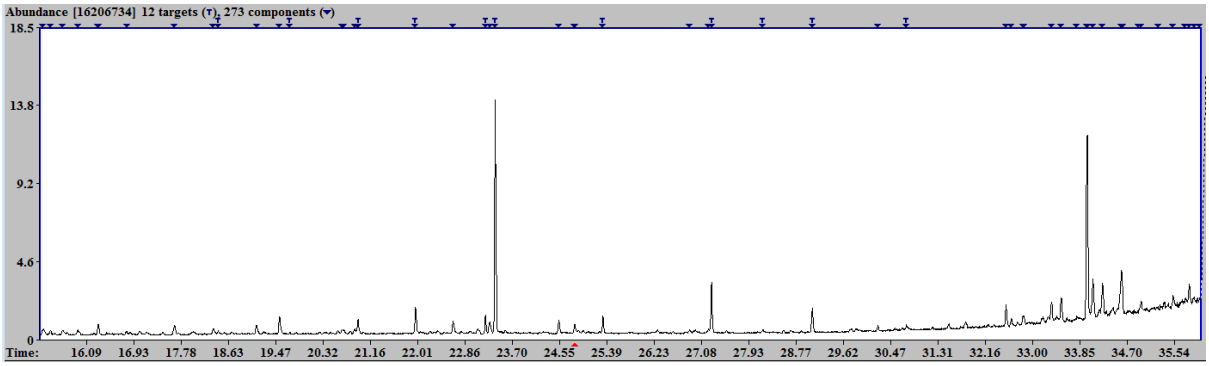

Figure 17. The Total Ion Count (TIC) for East Liverpool sediment. The small T in the upper margin indicates a quantified fatty alcohol. The largest peak is phytol (standard is not shown in this case) and the larger peaks to the right are the sterols.

Influent

The mean concentration of total fatty alcohols in the influent was 54.5 µg•L^{-1} compared to ~200 µg•L^{-1} in the UK study (Mudge *et al.* 2010); ~600 µg•L^{-1} in the Luray study (Mudge *et al.* 2012) and 440 µg•L^{-1} in the Oklahoma samples. The profile can be seen in Figure 18. In this figure, the 12 and 18 carbon compounds dominates the profile. The 18 carbon compound is not abundant in the detergent formulations (DeLeo *et al.* 2011) and may be formed within the pipe through bacterial action. This is consistent with observations at the other locations. The most important alcohol is the C_{12} which may include a component from detergents since this is the most prevalent fatty alcohol in detergents used in the Luray catchment and likely to be the same here. The *iso*C_{17} is the third most abundant compound and will be derived from bacteria in the wastewater.

No long chain fatty alcohols (>C_{18}) derived from terrestrial plants were present in these samples. These may enter the waste stream from ingested plants, food waste or terrestrial plant matter entrained into surface water runoff although they were not observed here.

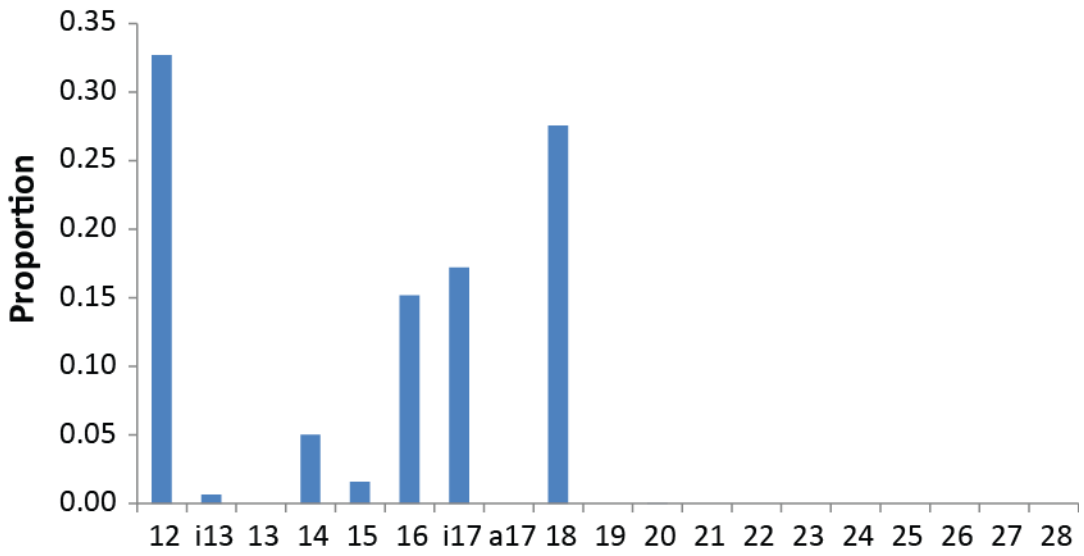

Figure 18. The mean fatty alcohol profile for all influent samples. The mean total concentration was 54.5 $\mu g \bullet L^{-1}$.

Effluent

The concentrations of fatty alcohol in the effluent were significantly less than that of the influent indicating a substantial removal during the treatment process. The mean concentration was only 0.24 $\mu g \bullet L^{-1}$ and the mean of site specific removal factors compared to the influent was >99%. As with previous analyses, there was also a substantial change in the fatty alcohol profile (Figure 19) to leave only C_{18}. This type of profile has not been seen before but was consistent between samples in Ohio.

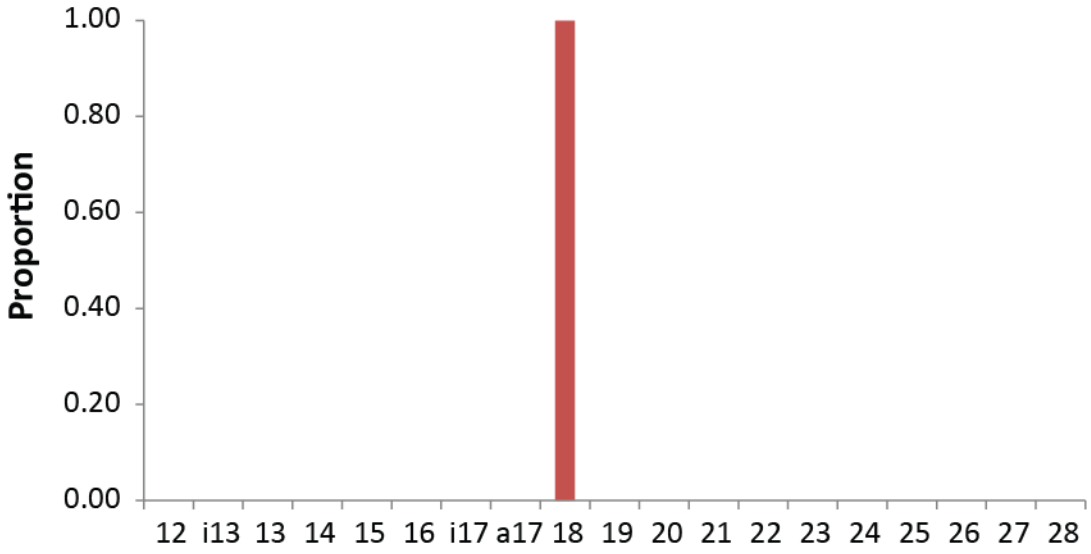

Figure 19. The mean fatty alcohol profile for all effluent samples. The mean total concentration was 0.24 $\mu g \bullet L^{-1}$. No long chain alcohols from terrestrial plants were detected in these samples.

Sediments

The profile of fatty alcohols in the sediments is different from the WWTP samples since they contain some long chain alcohols derived from terrestrial plants (Figure 20) although the profile is much less dominated by these compounds compared to the Oklahoma sites. The profile reflects two major sources with terrestrial plants having even chain length compounds in the C_{20} to C_{28} range and algal fatty alcohols centred on C_{16}, the dominant source in these samples. The presence of C_{15} and C_{17} fatty alcohols implies there is a contribution from bacteria in these samples and the mean CPI is 0.13 which is less than that measured in the river sediments from the Luray study (0.68, (Mudge *et al.* 2012) and Oklahoma (0.31).

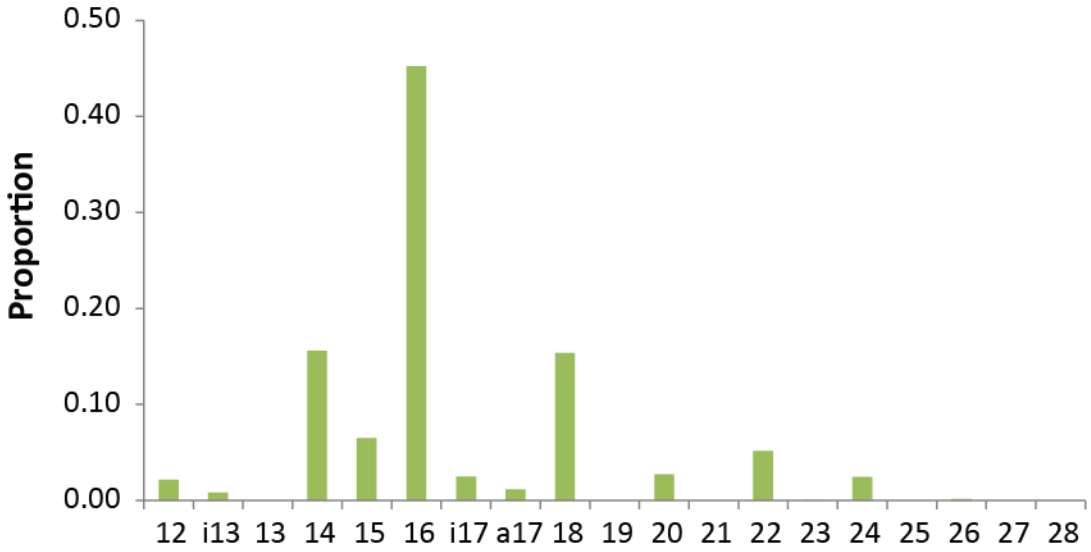

Figure 20. The mean fatty alcohol profile for all sediment samples. The mean total concentration was 2257 µg.kg^{-1}.

The algal contribution is greater than that in Oklahoma and Luray; the streams and rivers sampled did not have a significant amount of surrounding trees in most cases. Although the Eco-region is described as the Eastern Temperate Forests, relatively few woodlands were encountered during the sampling and grasslands were more common.

Principal Component Analysis

One of the best ways to view that data across multiple samples with multiple chemicals is through the use of PCA (Mudge 2007). This projection method allows the composition of all samples to be viewed on just two diagrams; the loadings and the scores. For these data, the results were converted to proportions to remove the concentration effects.

The scores for each sample (Figure 21) clearly separate the samples according to their type. Principal Component 1 (PC1) separates the East Liverpool sediment sample for the rest while PC2 separates all the other samples. This is an unusual pattern and highlights how different East Liverpool sediment is from all the other samples analysed. The driver for this difference is the presence of long chain terrestrial fatty alcohols in the former sediment and their absence in the remainder. The effluent group

show no variability as they only contain C_{18}. The other two groups are bigger and indicate some variability within the group but there is not as clear a separation between groups as was seen in the Oklahoma samples. These data and the distributions will be discussed in the combined report including the Oregon data as well.

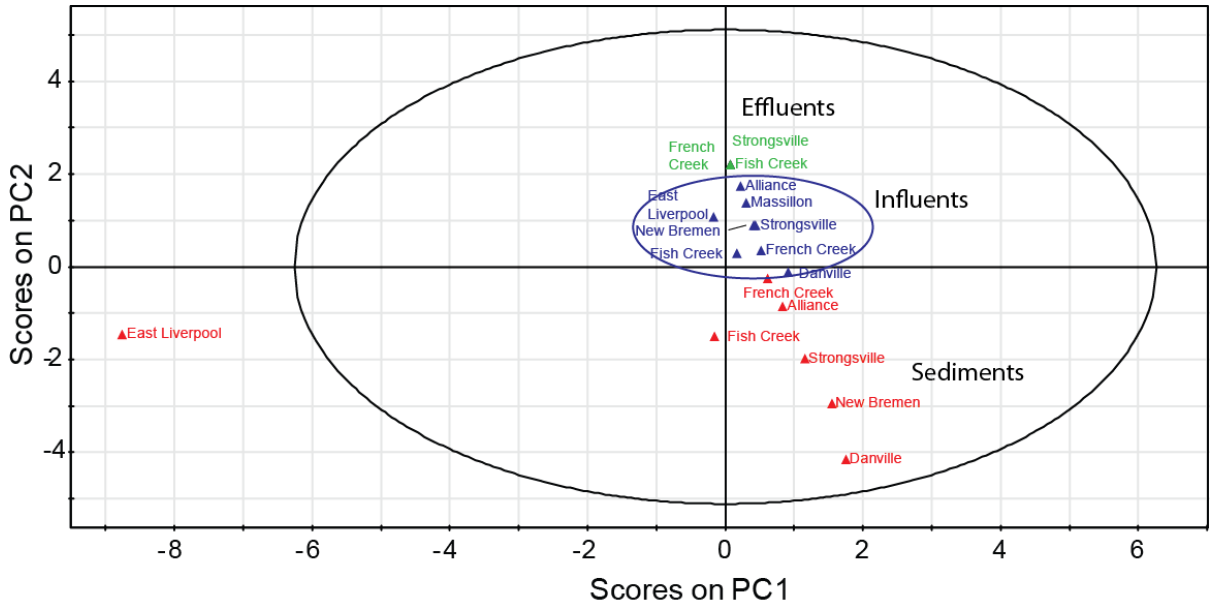

Figure 21. The scores for each sample based on the PCA of all fatty alcohols expressed as proportions. The oval is the circular 95% confidence limit (Hotelling's T^2).

The loadings on the fatty alcohols associated with the samples (Figure 22) indicate which compounds are deterministic within the sample score groups. In these samples, the sediments are dominated by the short chain algal and bacterial fatty alcohols and not the long chain compounds seen in the Oklahoma samples. Only one sample had substantial long chain fatty alcohols (East Liverpool) and in this case is considered unusual. The effluents were only C_{18} in these samples while the influents contained a mixture of alcohols in the C_{12} to C_{18} range.

Within the sample types in the scores plot (Figure 21), there is no grouping associated with the secondary treatment methodology shown in Table 1 and so it is not possible to distinguish between, say, Lagoons and Oxidation Ditches on the basis of their fatty alcohol composition in the effluent.

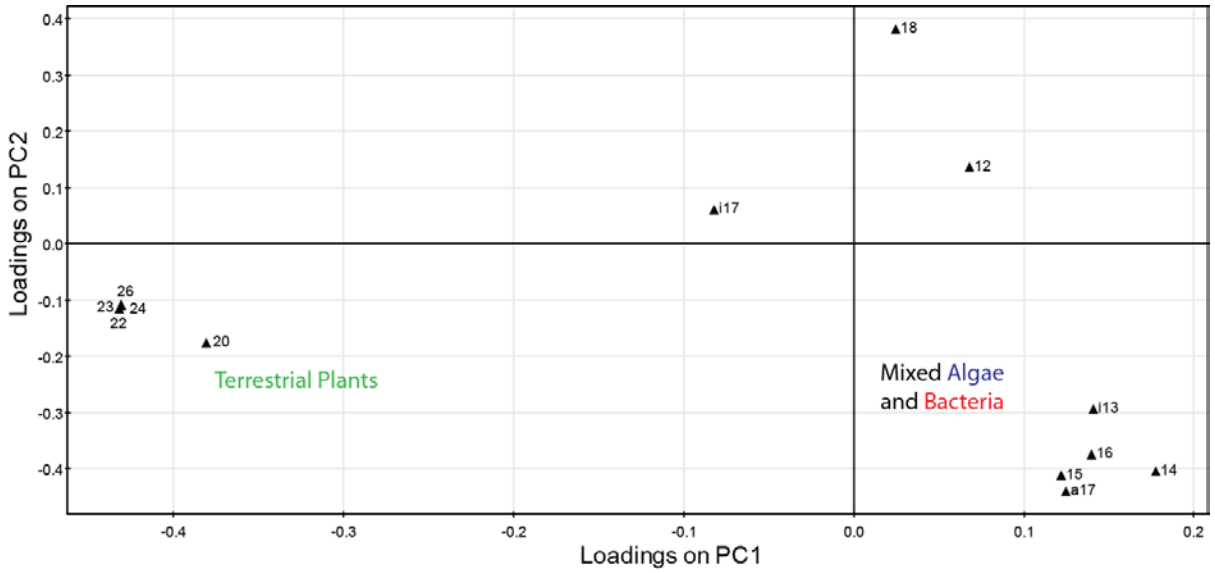

Figure 22. The loadings for each fatty alcohol with the data expressed as proportions.

Efficiencies

The removal efficiencies for the fatty alcohols in each plant can be seen in Table 3. These values are calculated from the total fatty alcohol concentrations in the influent compared to the effluent. Most of these compounds will have been removed through the sludges and disposed of elsewhere, principally to agricultural land although some may go to landfill.

Table 3. Removal efficiencies by secondary treatment method.

WWTP	2° Treatment	Fatty alcohol removal (%)
East Liverpool	RBC	100
Alliance	Activated Sludge	100
Massillon	Oxidation Ditch + TBF	100
Summit / Stow / Fish Creek	Oxidation Ditch	99.1
Strongsville	RBC	96.6
French Creek	Activated Sludge	98.9
Danville	Lagoon	100
New Bremen	Lagoon + TBF	100

Stable Isotopes

Not all fatty alcohols that were detected in the GC-MS were present at sufficiently high concentrations to enable both the ^{13}C and ^{2}H to be determined. The samples were concentrated for stable isotope analysis and a few extra compounds were detected in the effluent fractions after reduction to ~10 µl. A cross plot of those that were measured can be seen in Figure 23. On this figure are placed labels

indicating the most likely chemical signature for the fatty alcohols. In the region towards the top left are compounds with $\delta^{13}C$ values smaller than -30‰ that might be indicative of terrestrial plant matter if the chain length was longer. In this case, however, these are mainly effluent samples which have distinguished themselves from the influent compounds of the same chain length. In the upper right is the location of petroleum based detergent fatty alcohols based on results from the Luray study (Mudge *et al.* 2012). No compound measured in this study exhibited a pure petroleum based detergent signature although there may be contributions to the influent samples (orange squares) which tend to lie in the centre of this figure with $\delta^{13}C$ values between -26 and -31‰.

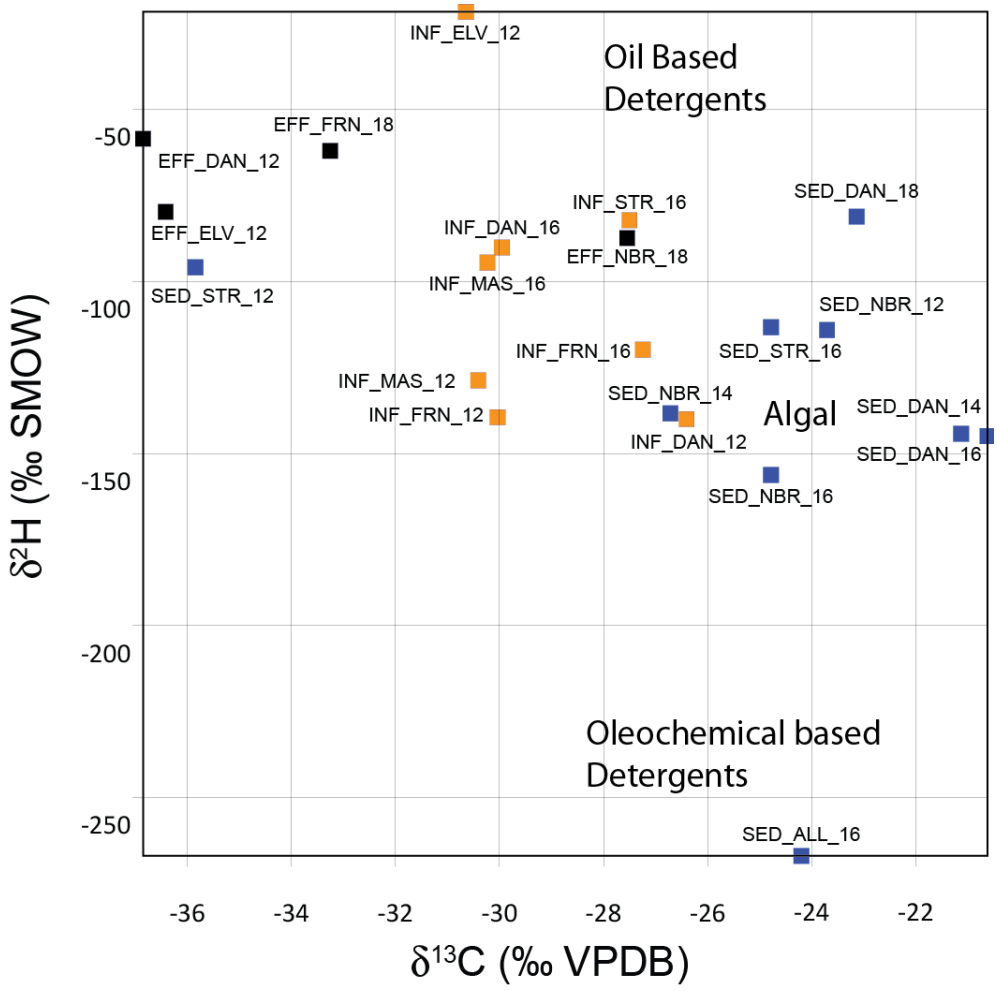

Figure 23. Cross plot of the stable isotopes detected in the Ohio samples. The effluent samples are black squares; the influent samples are orange squares and the sediments are blue.

All of the detectable fatty alcohols in the sediments, with two exceptions, are both the correct chain length and have the right stable isotopic signature to be from unicellular algal synthesis. The two exceptions are a C_{12} which plots in the same location as the effluent C_{12} fatty alcohols. This sample was from the Strongsville plant, an RBC. No C_{12} was measureable in the effluent but it is possible that at this location, a component of the C_{12} measured in the sediments was derived from effluent of the WWTP.

The other unusual compound is the C_{16} in the Alliance sediment which has a stable isotopic signature that reflects an oleochemical based detergent source. This is not likely and may be an artefact.

There is some overlap between the influent stable isotopic signature and some fatty alcohols measured in the sediments. In this case, it is possible that a proportion of the sediment fatty alcohols are derived from the influent through sewer overflows or from septic tank systems. There are no data available to quantify the magnitude of this input in these systems. The compounds in the sediments, however, are not the same as the ones that are in the effluent as the stable isotopic signature is different.

Discussion

The majority of detergents used in domestic properties in this study are disposed of down the drain and enter a WWTP. These data suggest that the type of WWTP has little effect on the overall fate of the fatty alcohols which are present in the formulations principally as ethoxylates, sulphates and ethoxysulphates. There is consistent evidence across several studies of some in-pipe processes which lead to the formation of C_{18} in the influent to the WWTP. The effluent contains fatty alcohols with a different stable isotopic signature which may be indicative of production by bacteria within the biological treatment stage.

The fatty alcohols in the sediments have a different stable isotopic signature to either the influent or the effluent and suggest *in situ* algal production as their source. This is consistent with their chain length profile (C_{14} – C_{16}, mainly even chain length). At one site, however, there is some evidence of a C_{12} fatty alcohol with a similar signature to that of the effluent. There may also be some overlap between the influent and sediment signatures although this would suggest a non-WWTP source. This latter route might be through Combined Sewer Overflows or septic tank systems making direct discharges to the rivers. The contribution this may make is not quantified here.

There may also be a contribution to the river sediments from the runoff of agriculturally applied sewage sludge. The majority of sites stated that their sludges were routinely applied to soil. Most of the fatty alcohols are removed from a WWTP through the solid wastes (Mudge *et al.* 2012) and a route exists through surface water runoff to the rivers. In some places (*e.g.* the European Union) there are regulations regarding the method by which the sludges can be applied (sub-surface, no rain forecast) although it has been suggested that the US regulations are not as stringent (Harrison *et al.* 1999). The EPA Part 503 Biosolids Rules suggest surface application is possible and this increases the potential for runoff during periods of rainfall.

Notwithstanding the above caveats, the fatty alcohols measured in the river sediments are overwhelmingly from natural sources as determined by the GC-MS profiles and the stable isotopes. This is consistent with the other studies. The separation on the PCA (Figure 21) is not as strong as in the Oklahoma study and this is due to the unusual lack of long chain fatty alcohols in the sediments. The GC traces were examined from two separate injections on different machines and both were consistent. At the time of sampling, the algae may be dominating due to favourable light and nutrient conditions and leaf litter may be absent as this was before the main leaf fall.

Conclusions

1. The concentrations in each of the three sample types (influent, effluent and sediments) are slightly lower than those concentrations established in previous studies.

2. The fatty alcohol profiles in the influent (rich in C_{18}) suggest some in-pipe bio-transformations are occurring before the wastewater reaches the WWTP. The short chain compounds suggest a mixture of faecal matter, bacterial matter and detergents.

3. The effluent has a substantially different profile to the influent and indicates the majority of the compounds have been removed through sorption, settlement and biodegradation in the WWTP. The C_{12} and C_{18} fatty alcohols dominate the effluent but the stable isotopic signature is different to that of the influent indicating that these are not the same compounds as the ones that enter the system through down the drain disposal.

4. The sediments have a signature that is dominated by algal matter synthesised *in situ*. In one instance (Strongsville) there may be evidence that the C_{12} in the sediments may have come from the WWTP effluent although this is an isolated case and cannot be confirmed at this time. There is a small degree of overlap between the WWTP influent and the sediments which may suggest that fatty alcohols from Combined Sewer Overflows, septic tank systems or agricultural runoff may also contribute to the total load in this region.

5. These data confirm the observations seen in the previous studies that, in general, the fatty alcohols in the influent are not passed through to the effluent of the WWTP. These river sediments are dominated by algal matter and the effluents do not make a substantial contribution.

Chapter 5. Results and Discussions of the Oregon Samples

The full data for these samples can be seen in the Appendix. During the sampling in March 2012, there was significant rainfall and the rivers were higher than normal with corresponding high flows for the influent into the WWTPs. An example of the elevated river flow can be seen in Figure 24 where the effluent from the Molalla WWTP discharges. It was initially thought that the erosion of the sediments by the river flow might have removed any observable effect of the WWTP discharges. However, the faecal sterol marker, 5β-coprostanol, was measureable in the sediments indicating an input of human waste probably from the WWTP.

Figure 24. Eroded river bank at the discharge point from the Molalla, OR WWTP.

The net effect of the higher than normal influent flows, according to the WWTP operators, is that the retention time in the secondary treatment stage was less than normal. On some plants there were "balancing tanks" or storm flow tanks which were used to regulate the influent flows through the plant to keep them within specification. It was also suggested that the CSOs may have been operating and untreated effluents were reaching the rivers as well.

In many cases the pastures were completely sodden with further rainfall ponding on the top or running off to the nearest water course. In these circumstances, surface applied sludge from the WWTPs might also be washed off at the same time. This was not observed directly during the sampling as no fields that receive sludge were visited.

An example of a river sediment trace can be seen in Figure 25. The profile is similar to those of the other two sampling campaigns although more terrestrial plant compounds could be seen in these samples compared to those of Ohio.

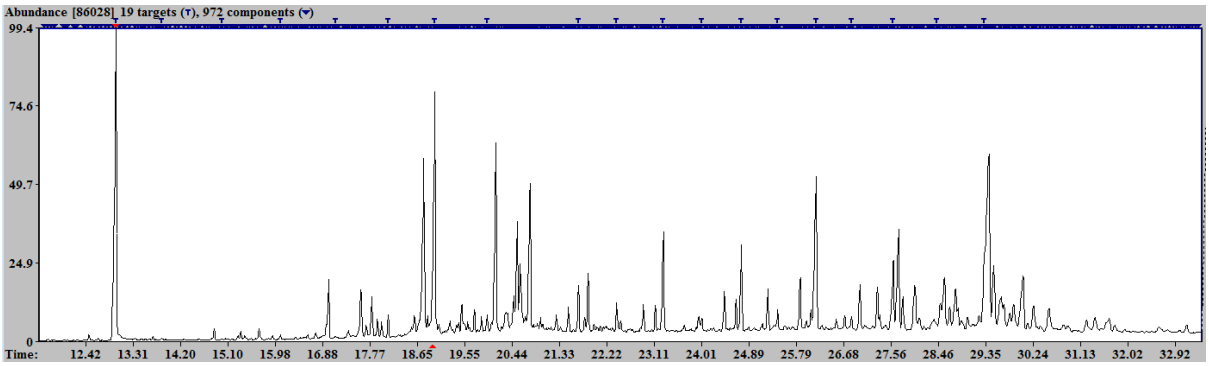

Figure 25. GC trace of Everett Sediment. The internal standard is the major peak to the left with the alcohols and sterols to the right.

Influent

The mean profile of fatty alcohols in the influent to the Oregon WWTPs can be seen in Figure 26. The C_{19} fatty alcohols was excluded as they overlapped with TMS esters of a fatty acid in these analyses and as a small component was not readily distinguishable. The profile is similar to those seen at many other sites with a high C_{18} potentially derived from in pipe processes. In this case, there were traces of terrestrial plant fatty alcohols (C_{22+}) in the influent which may have arisen from food waste or sequestration of plant matter due to the high rainfall.

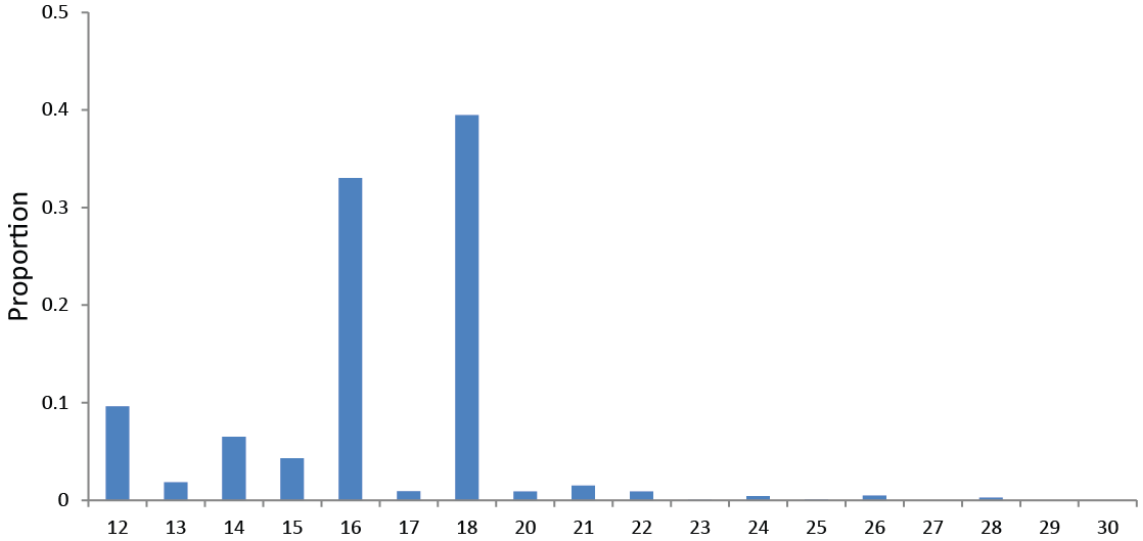

Figure 26. The mean fatty alcohol profile for all influent samples. The mean total concentration was 42.7 $\mu g \bullet L^{-1}$. The C_{19} fatty alcohol was excluded as it overlapped a fatty acid in these analyses. The mean CPI was 0.08.

The profile of the influent does not mirror that of the reconstructed detergent profile determined for the Luray, VA catchment. This suggests that in this case the contribution from the detergents may be less than at the Virginia site.

Effluents

Unlike in the previous investigations, the fatty alcohol profile for the effluents (Figure 27) is quite similar to that of the influent. The major differences are the great contribution of the odd chain (bacterial) C_{15} and more of the terrestrial long chain alcohols. Whether these compounds are the same as the ones in the influent can only be determined by their stable isotopic signature. The CPI for the effluent was 0.39 which is greater than the influent (0.09) and suggests greater bacterial contributions. The shorter than usual residence times in the WWTPs due to the rainfall may also lead to poorer performance overall. However, there has been a reduction in the total concentration from 42.7 $\mu g \bullet L^{-1}$ to 1.4 $\mu g \bullet L^{-1}$ during the treatment. The "missing" fatty alcohols will have been degraded within the plant and also removed with the sludges.

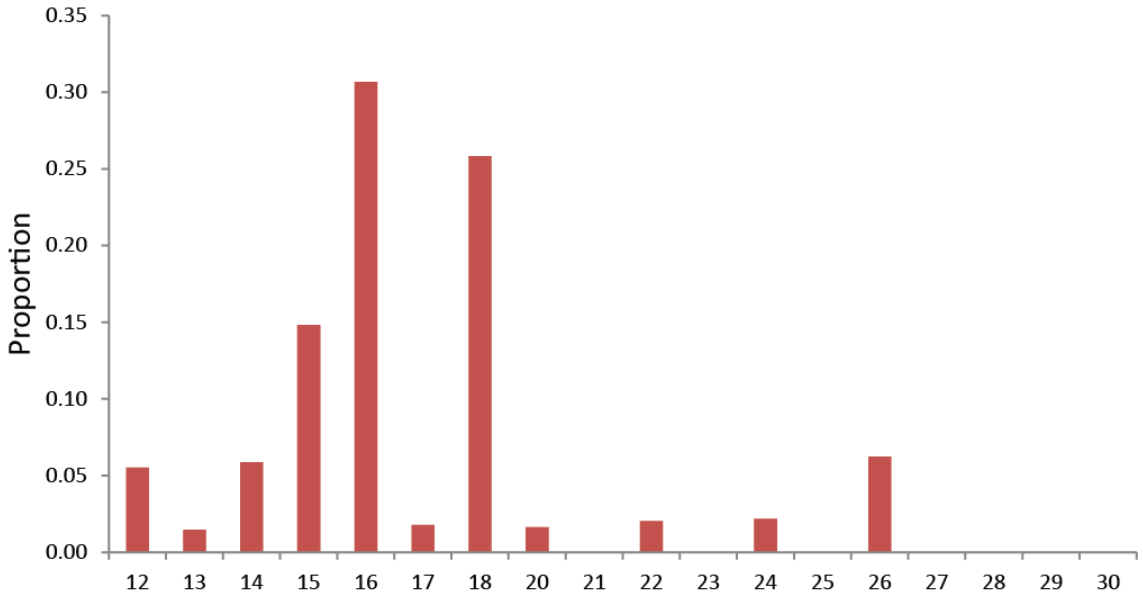

Figure 27. The mean fatty alcohol profile for all effluent samples. The mean total concentration was 1.4 $\mu g \bullet L^{-1}$. A few long chain alcohols from terrestrial plants were detected in these samples unlike previous samples. The mean CPI was 0.38, substantially greater than the influent.

Sediments

The fatty alcohol profile in the environmental sediments collected downstream of the discharge points for the WWTPs can be seen in Figure 28. Despite the high river flows experienced during the sampling campaign, there was some evidence of faecal matter present in the form of 5β-coprostanol. The profile is skewed towards the long chain terrestrial fatty alcohols on this occasion with little evidence of the algal alcohols centred on C_{16}. This is not surprising due to the collection of samples higher up the river channel sides where aquatic algae are less likely to grow. These samples were also collected early in the

season (March) and the day length and temperatures might not have reached suitable values to encourage algal growth.

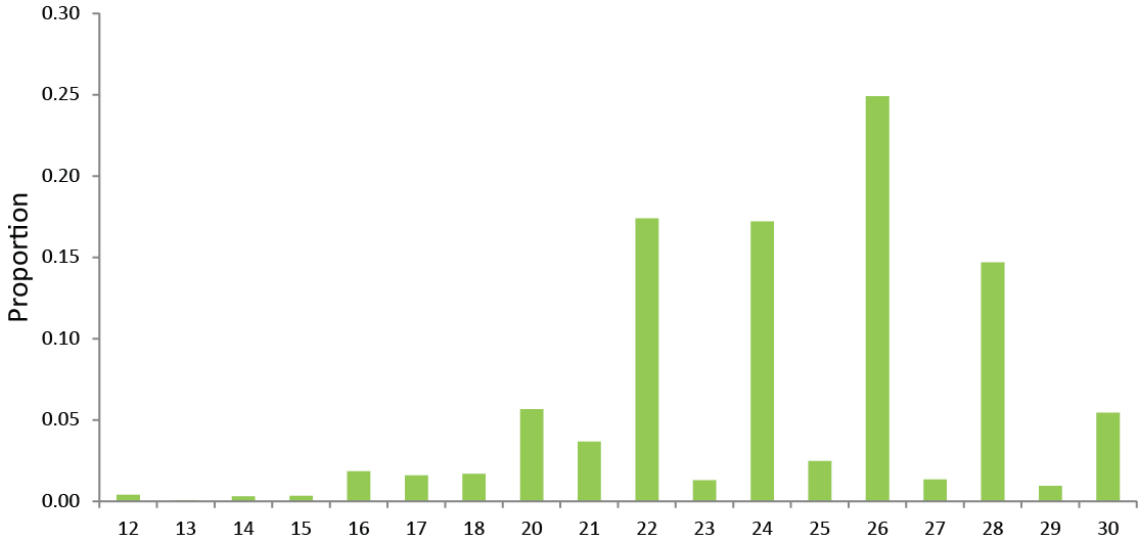

Figure 28. The mean fatty alcohol profile for all sediment samples. The mean total concentration was 46 $mg \bullet kg^{-1}$. Few short chain algal alcohols were measured in these samples. The mean CPI is 0.11.

Principal Component Analysis

One of the best ways to view that data across multiple samples with multiple chemicals is through the use of PCA (Mudge 2007). This projection method allows the composition of all samples to be viewed on just two diagrams; the loadings and the scores. For these data, the results were converted to proportions to remove the concentration effects and the data were log_{10} transformed to improve the separation between points. The scores can be seen in Figure 29. In this case, in common with the data from Oklahoma, the three sample types (influent, effluent and sediment) clearly separate from each other on the basis of their fatty alcohol profile.

The different wastewater treatment processes do not seem to form any type of separation as the major differences are between influent and effluent and may mask any differences. Further investigation of the effluent composition in relation to the secondary treatment processes are considered below.

The loadings associated with the scores can be seen in Figure 30. As expected, the prime separation on PC1 is due to chain length with all the long chain terrestrially derived alcohols loading to the left while the short chain compounds potentially due to algae, bacteria or detergents load to the right. The occurrence of the C_{27}, C_{29} and C_{30} in the centre of this axis is possibly due to their absence in the OK and OH samples.

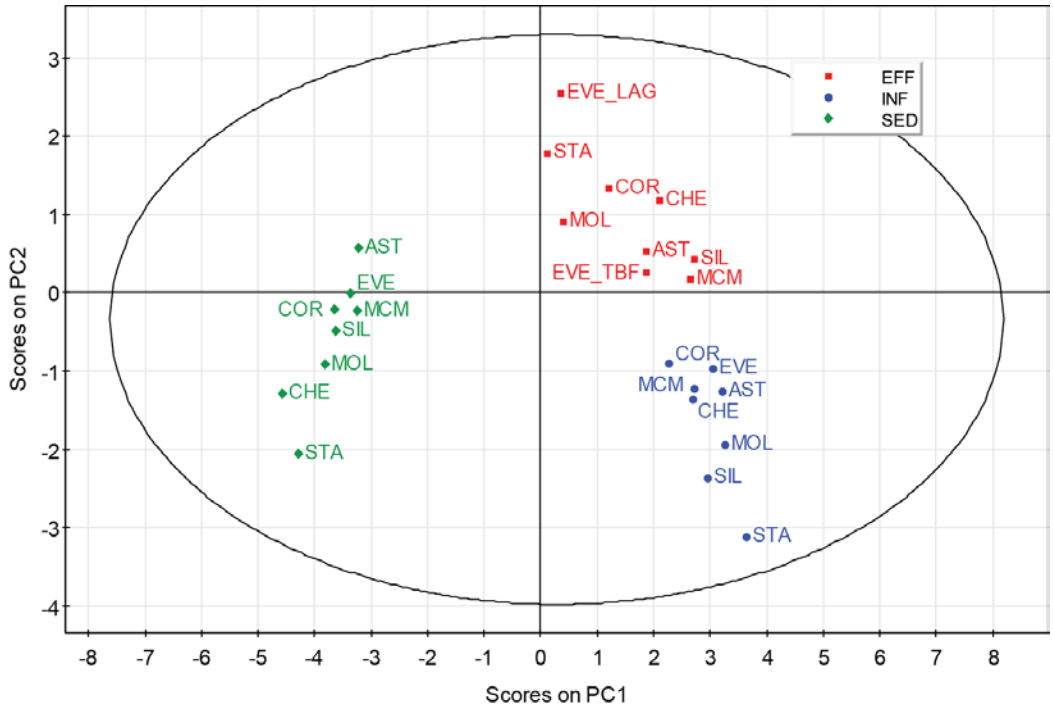

Figure 29. The scores for each sample based on the PCA of all fatty alcohols expressed as proportions after $\log_{10}$ transformation.

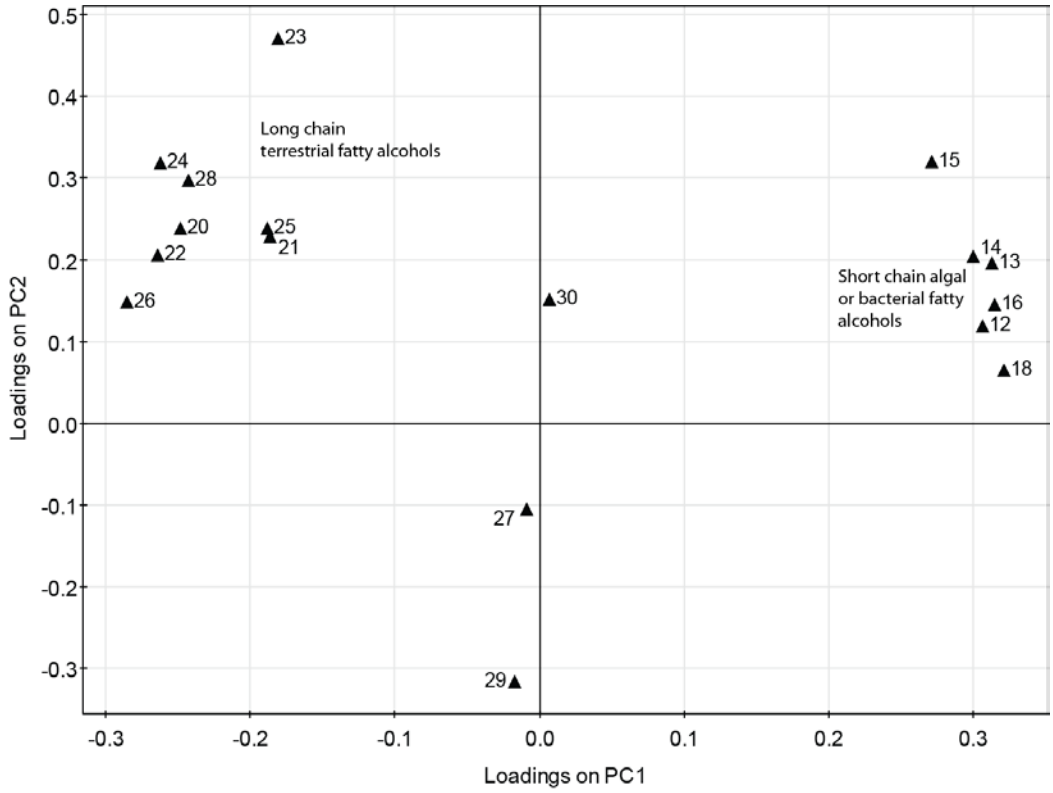

Figure 30. The loadings for each fatty alcohol showing the main separation on PC1 is due to chain length.

Efficiencies

The removal efficiencies for the fatty alcohols in each plant can be seen in Table 4. These values are calculated from the total fatty alcohol concentrations in the influent compared to the effluent. Some of these compounds will have been removed through the sludges and disposed of elsewhere. The Everett plant has two effluents as flow above 16 MGD is directed to the lagoon treatment while everything under this is treated through a trickling bed filter.

Table 4. Removal efficiencies by secondary treatment method.

WWTP	2° Treatment	Fatty alcohol removal (%)
Everett (WA)	TBF	98.3
Everett (WA)	Lagoon	99.9
Chehalis (WA)	SBR	98.9
Astoria	Lagoon	84.6
McMinnville	Oxidation Ditch	94.0
Molalla	Lagoon	97.0
Silverton	Activated Sludge	95.3
Stayton	SBR	95.9
Corvallis	Activated Sludge	93.8

In general, the removal efficiencies are high with most above 95% removal for the fatty alcohols. However, the Astoria lagoon system was the least efficient of all sampled with only 84.6% of the total alcohols removed. This may be due to a lower concentration in the influent due to rain than an inefficient site in this case. Several operators did comment that they may not be able to make compliance on their overall WWTP efficiency, not due to the failure of the works, but the dilution of the influent.

Sterols

Sewage may be identified through the presence of 5β-coprostanol which is formed in the human (and other higher animals) gut through biohydrogenation of cholesterol (Mudge *et al.* 1999). The mechanism of formation passes through a ketone as shown in Figure 31. In the influent samples from Oregon, both the cholestenone and cholestanone could be clearly identified although no internal standard was added to enable quantification. However, raw counts from the GC-MS traces can be used to determine ratios between compounds. Raw human sewage has high 5β-coprostanol / cholesterol ratios (Leeming *et al.* 1996) which can be distinguished from agricultural herbivores that typically produce higher quantities of 24-ethyl coprostanol derived from the terrestrial plant sterol, β-sitosterol (Mudge and Lintern 1999). A table of the ratios can be seen in Table 5.

These data show that half of the sediment samples had measureable 5β-coprostanol although the ratio with cholesterol did not indicate significant contamination with faecal matter. None of the sediment samples had measureable 24 ethyl coprostanol, the environmental indicator of herbivores rather than human sources for the faecal matter in the sediments. All sediments did contain some 5α-cholestanol, the product of anaerobic reduction from cholesterol. All of the sediment samples, bar one, had high

terrestrial plant markers from the β-sitosterol. The only one that had a ratio of β-sitosterol / cholesterol less than one was Silverton which coincided with the greatest faecal matter indicator.

As expected, the faecal matter markers were much more visible in the WWTP influents and effluents. The ratio that indicates the presence of faecal matter was greater than one in most cases confirming its suitability to indicate the presence of WWTP discharges in the environment. Similarly, the 24 ethyl coprostanol was relatively low and the ratios all indicate human rather than herbivore waste. The marker for the terrestrial matter was low except in the case of the Everett lagoon effluent. This may reflect the propensity for these systems to support both algae and terrestrial plants. The sterols data confirm the observations and conclusions regarding the source of organic matter from the fatty alcohol results.

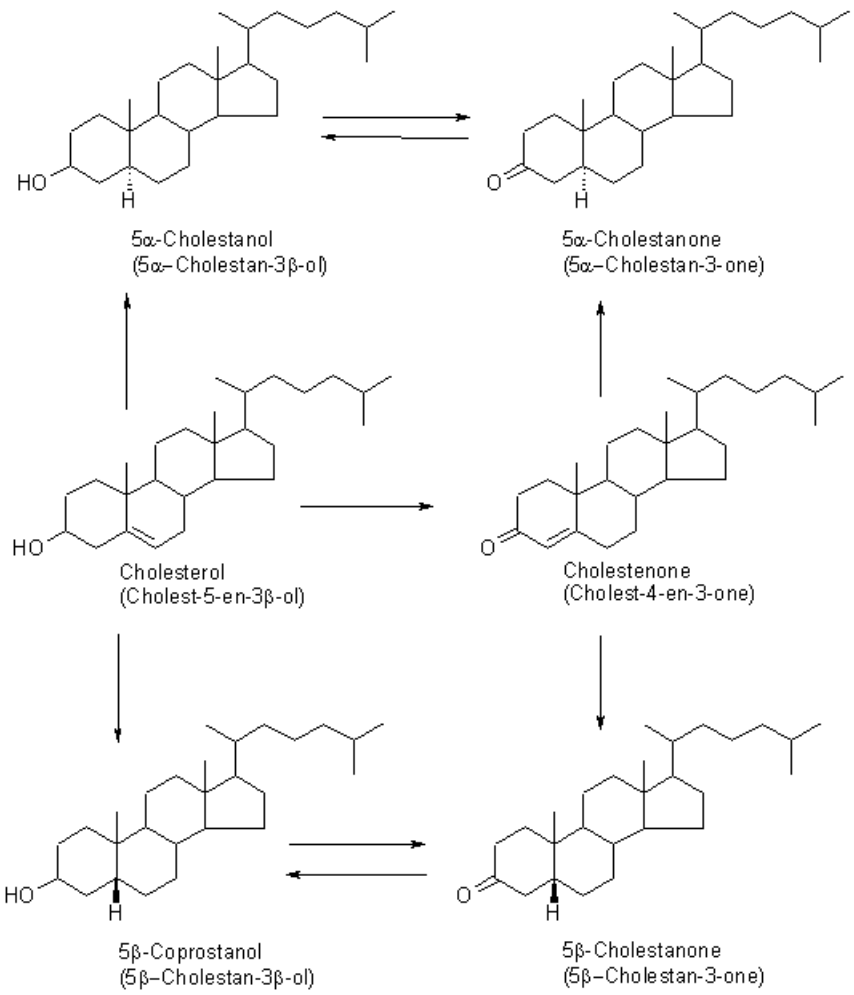

Figure 31. The mechanism of formation of 5b-coprostanol from cholesterol through cholestenone and cholestanone (redrawn from Grimalt *et al.* 1990).

Table 5. Key diagnostic ratios used to apportion the source from stanols and sterols. The raw area counts from the GC-MS can be seen in the Appendix.

	Everett	Everett	Chehalis	Astoria	McMinnville	Molalla	Silverton	Stayton	Corvallis
SEDIMENT									
5β/chol	0.013			0.044			0.145	0.038	
5β/24ethyl									
5α/chol	0.233		0.549	0.117	0.120	0.259	0.067	0.416	0.217
sito/chol	2.367		5.941	1.248	8.317	7.554	0.523	6.614	16.880
INFLUENT									
5β/chol	1.409		1.678	1.429	1.600	0.917	1.377	1.588	1.340
5β/24ethyl	3.031		3.376	3.435	3.292	3.247	3.120	3.372	2.781
5α/chol									
sito/chol	0.278		0.076	0.270	0.278	0.231	0.344	0.280	0.390
EFFLUENT	(TBF)	(LAG)							
5β/chol	2.047	0.732	1.475	1.376	1.682	0.087	0.713	1.725	1.220
5β/24ethyl	9.867	2.962	5.803	3.589	3.496	11.984	3.905	7.497	2.947
5α/chol		0.127	0.169	0.182		0.127			0.237
sito/chol	0.583	1.120	0.286	0.426	0.278	0.108	0.024	0.311	0.340

5β/chol = 5β–coprostanol /cholesterol (an indicator of faecal matter, usually human)

5β/24ethyl = 5β–coprostanol /24 ethyl coprostanol (an indicator to differentiate between human and animal faecal matter)

5α/chol = 5α–cholestanol /cholesterol (an indicator of environmental reducing processes)

sito/chol = β-sitosterol/cholesterol (an indicator of the relative magnitude of the terrestrial matter contribution)

Stable Isotopes

The compound specific stable isotope values can be found in the appendix and seen as a cross plot in Figure 32. The samples separate on this plot according to their source. The longer chain fatty alcohols (C_{18} and longer) entirely measured in the sediments, occupy a region of this figure to the left of the centre with $\delta^{13}C$ values less than -30‰. This is entirely consistent with the other sediment and soil samples and indicates the terrestrial origin of these compounds. In general, the longer chain compounds with 26 and 28 carbons had $\delta^{13}C$ values less than -33‰. The $\delta^{2}H$ values had a wider range but were concentrated between -140 and -180‰.

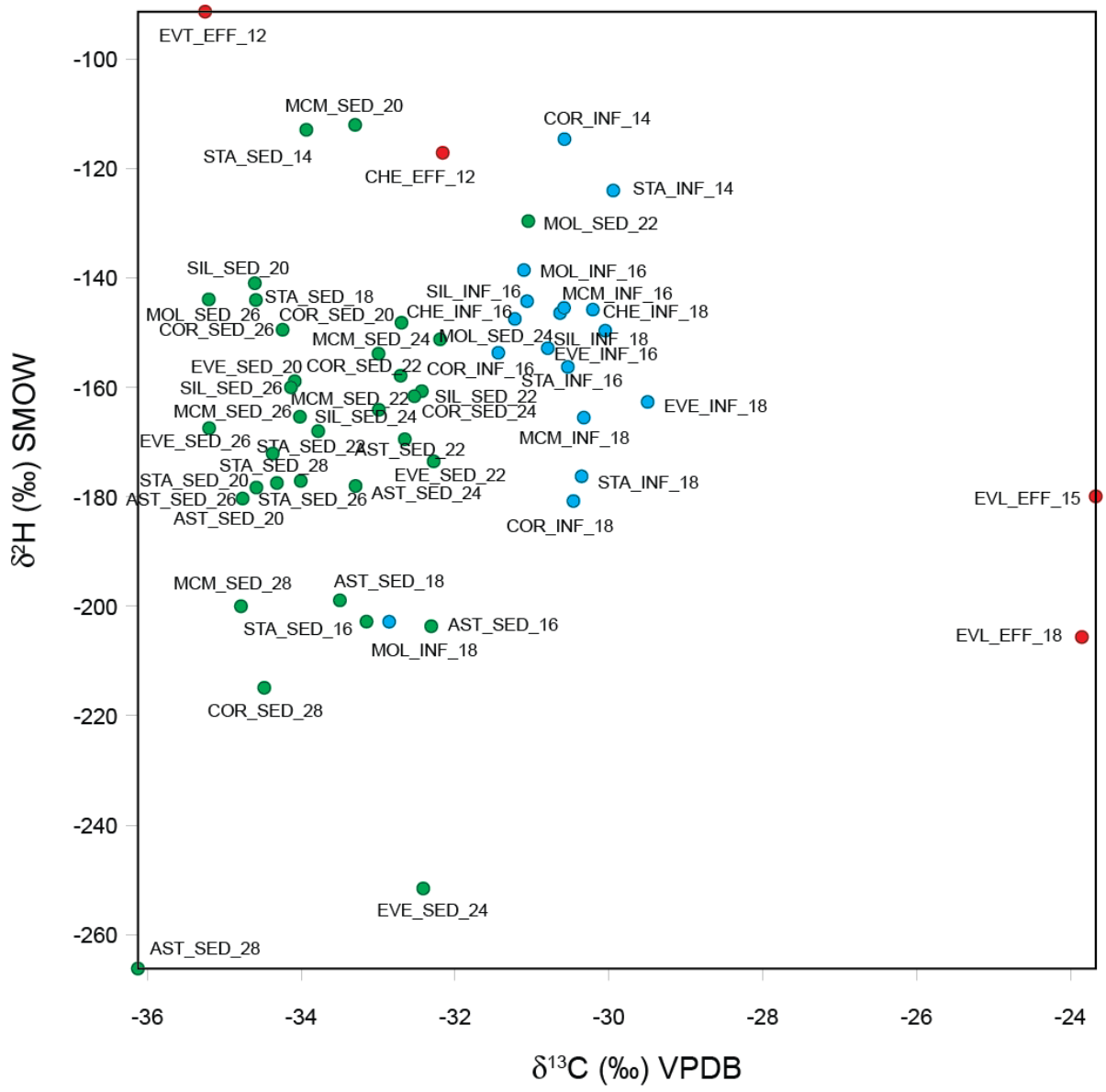

Figure 32. A cross plot of the stable isotopes for each fatty alcohol. The influent samples are in blue; the effluent in red and the sediments in green. The suffix denotes the chain length of the fatty alcohol.

The stable isotopes from fatty alcohols in the influent to the WWTPs occupied a narrow band of $\delta^{13}C$ values around -30‰. There was very little overlap between the terrestrial (sediment) fatty alcohols and the influent fatty alcohols. Again, the $\delta^{2}H$ values were of a broader range and considering the initial source investigations indicate a mixture of faecal material (smaller $\delta^{2}H$ values towards the bottom of the figure) and detergent surfactants.

The effluent samples indicated in red on the figure are remote from both the sediments and the influent samples indicating a different source. This is consistent with the other sampling locations and suggests that bacterial synthesis of these alcohols is occurring in the WWTP. This is also indicated by the presence of the C_{15} fatty alcohol which is typical of bacterial biomass (Mudge *et al.* 2008).

The samples in Figure 32 do not indicate any clustering by site or by treatment technology *e.g.* the activated sludges plants do not co-locate in this figure. The terrestrial plant and influent data are consistent with all previous studies and do not suggest any regional variability. The position of the effluent samples may be location dependent and this will be investigated further when all three eco-regions are compared.

Discussion

The data from the Oregon study are entirely consistent with the previous studies in the USA and UK. In this regard, the fatty alcohol profiles are markedly different between influent, effluent and river sediment. The influent samples are dominated by the short chain alcohols with C_{16} and C_{18} present in the greatest proportion. Very few long chain alcohols are present in the influent and suggest that there is no entrainment of surface runoff (soils *etc*.) into the sewerage system. This is somewhat surprising given the amount of rainfall at the time.

The liquid effluents discharged from the Oregon WWTPs have similar fatty alcohol profiles to the influent although there are greater proportions of odd chain compounds indicative of bacterial synthesis. This is not surprising given the active biological stages present in all works sampled. The data do not indicate a significant or consistent difference between the different treatment technologies. In terms of efficiencies, the lagoon at Astoria was the least efficient at removing the fatty alcohols from the influent but this may be an artefact of the prevailing weather conditions. At the time of sampling, there was substantial rainfall and the influent may be more dilute than normal. The long residence time that lagoon system have may reflect that an influent from several weeks earlier where the fatty alcohol concentrations were “normal”. The concentration of the fatty alcohols in the influent at the time of sampling was the lowest of the eight sampled in Oregon while the effluent was the third highest.

The presence of the faecal sterol markers in the sediments confirm that these sediments are receiving wastes from the WWTPs even though the fatty alcohol content is low. Once again, it must be concluded that the fatty alcohols entering the WWTPs are rapidly and completely biodegraded within the works by the bacteria in the secondary (biological treatment) stages. This process occurs in all of the treatment types investigated in this study. The fatty alcohols in the effluent are significantly reduced in concentration, have an altered profile relative to the influent and different stable isotopic contents. This implies that these compounds are newly synthesised within the works, probably from a non-lipid base.

The sediments in the river contain sterol markers for faecal matter although the fatty alcohol profile is that of terrestrial plants in the main. In this case, the algal signature is relatively small; this may be due to the time of year when the samples were taken (March) as the sunlight induced growth phase would be yet to start. Water temperatures were also still too cold to enhance unicellular algal growth.

The data also do not suggest a significant WWTP contribution to the sedimentary fatty alcohols. The odd chain component is small along with the short chain (detergent) range.

The solids generated from the WWTPs are principally disposed of on agricultural land where they act as an organic fertiliser. The data show that the vast majority of the fatty alcohols leave the works *via* this route as the concentrations are several orders of magnitude greater than the liquid effluent (Mudge *et al.* 2012). At this time, it must also be concluded that this disposal route does not pose a significant input to the riverine sediments as the profile is not showing up in these sediment samples. There may be opportunities for the sludges to be washed-off the land during rainfall events although this was not seen during this sampling programme. It may be that sludges had not been spread for several weeks due to the rainfall and at other times of the year, there may be a greater contribution.

Conclusions

1. The data from the study in Oregon collected in March 2012 are consistent with the samples collected in other eco-regions. The mean concentration in the influent was 42.7 $\mu g \bullet L^{-1}$ which was reduced to 1.4 $\mu g \bullet L^{-1}$ in the effluent. The overall efficiencies of removal were high (>95%) although at Astoria the value was lower due to dilution of the influent with rainwater and the long residence time in the lagoon system.

2. Both the influent and the effluent had relatively high C_{18} content which may be due to in-pipe / WWTP processes by the bacteria as this fatty alcohol generally does not contribute significantly to either faecal matter or surfactants.

3. The mean concentration of fatty alcohols in the sediments was 46 $mg \bullet kg^{-1}$ with a profile and stable isotopic signature that denotes a strong terrestrial plant source. This may be due to the time of year when the samples were taken as freshwater algae may not have started their growth phase by that time. There was little to no fatty alcohol contribution from the WWTPs despite there being faecal sterols present in the samples.

4. Once again, the stable isotopes clearly separate the influent from the effluent indicating a different, non-surfactant, source while the sediments have a different signature both in terms of the profile and the stable isotopes indicating that the liquid discharges are not contributing significantly.

5. The principal disposal routes for fatty alcohols offsite will be through the spreading of the solids (sludges) on agricultural land. The WWTPs indicated this was their usual action. The lack of this signature in the riverine sediments suggests that this is also not forming a significant indirect route to the rivers.

Chapter 6. Comparisons between the three Eco-Regions

The three eco-regions sampled in this survey were all different, especially with regard to rainfall. OK was hot and dry; OR was cool and wet and OH was in-between. In the period running up to the sampling in OK, there has been a sustained period of drought and a reduction in the stream flows (Figure 33). This meant that at some locations, the WWTP liquid discharges made up the majority of the stream flows and so environmental concentrations of compounds in the discharge might have been greater than usual.

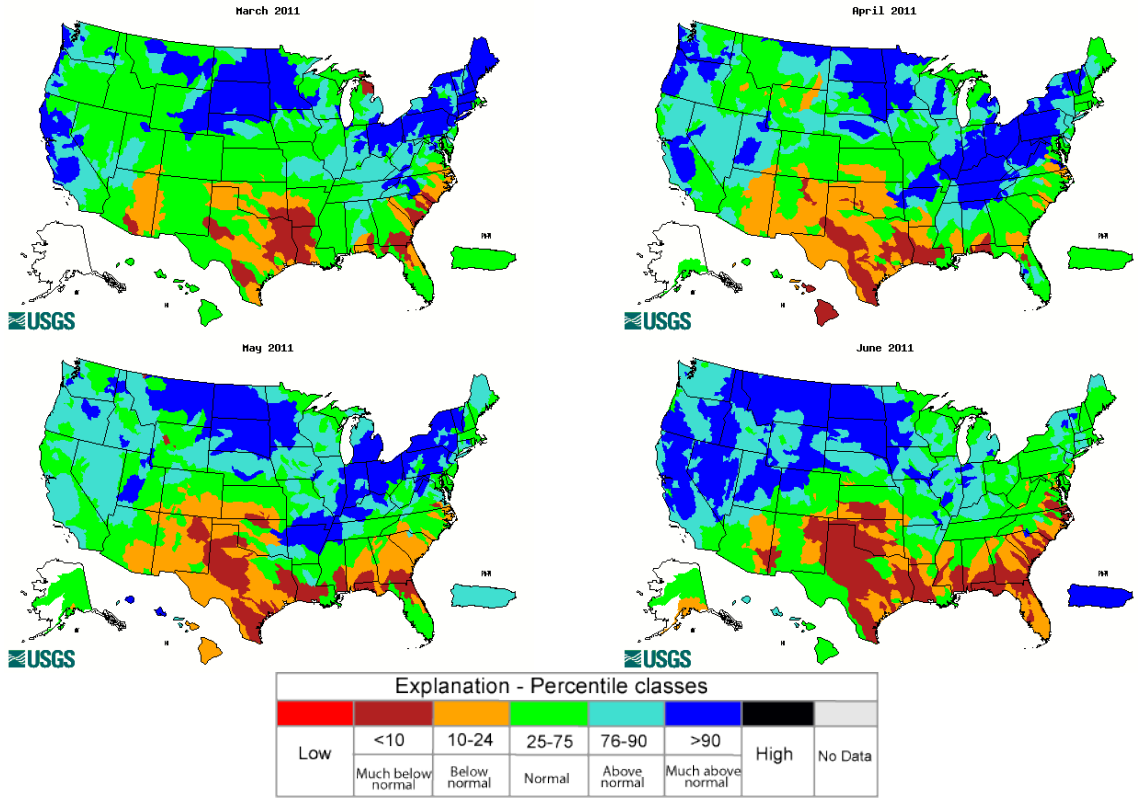

Figure 33. River flow conditions relative to the long term average. A significant deficit can be seen in the southern states over the sampling period in 2011. Data from the USGS.

Similarly, the river flows and rainfall were significantly greater than the long term average over the sampling period in OR where river banks had been eroded (see Figure 24). This was occurring at the beginning of spring (March 2012) when there was snow melt in the rivers as well. Due to the high river flows (Figure 34), there was a substantial suspended sediment load visible in the river, the majority of which would have been eroded river sediments and terrestrial soils.

These local climatic conditions may have led to differences in the concentration of fatty alcohols in all of the samples. The influents will have been more dilute than normal in OR and potentially more concentrated in OK. The greater influent flow rates might have led to shorter residence times in the WWTPs, depending upon the technology used. Some location used balancing tanks to regulate the flows where possible. The effluents might have been more concentrated than normal in OR if the treatment

time was reduced. However, the sediments in the rivers of OR would potentially contain less compounds discharged from the WWTP as they would remain in suspension and be carried away from the sampling sites. Conversely, the sediments in OK might have experienced higher concentrations as there was little water in the streams and in some cases, this was almost entirely from the WWTP.

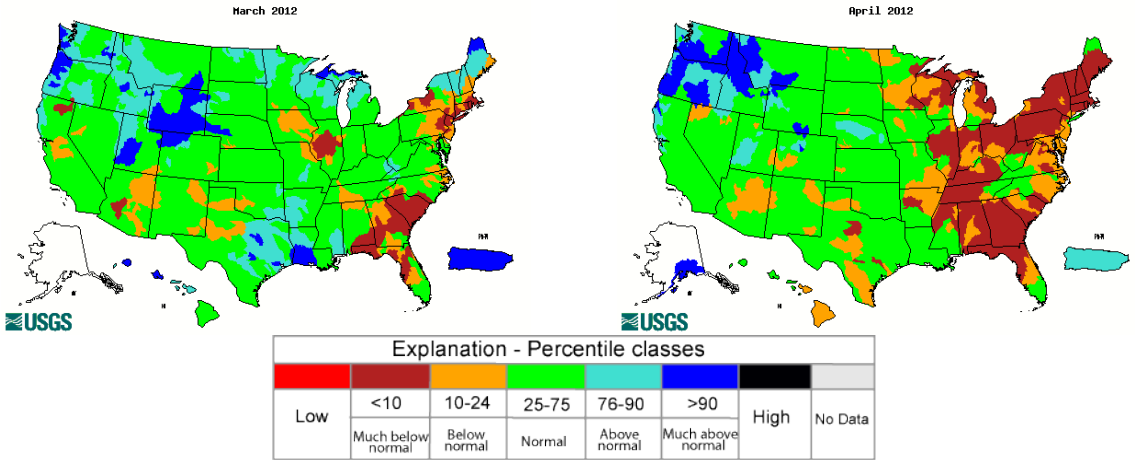

Figure 34. River flow conditions relative to the long term average. A significant excess can be seen in OR and WA over the sampling period in 2012. Data from the USGS.

Notwithstanding these climatic differences, there was less variation with respect to the WWTPs and their operation. The influent flow relative to the population served for all three regions can be seen in Figure 35. In this figure, a weak but significant correlation can be seen between the flow and the population enabling sites with unusual flows to be identified.

One Ohio WWTP with a considerably elevated flow relative to the population served can be seen; this is Massillon, OH who reported that 60% of their influent was from “the county” and the remainder from “the city”. In their summary statement, that 40% was also described as “industrial” and this added input from food processors and a paper mill may account for the flow. The other large flow relative to its population is Deer Creek, OK which appeared to take twice as much influent than the population might suggest. The reason for this is not readily apparent although the location was described by WWTP staff as relatively affluent.

If these two points are removed from the calculation, the linear regression between the two is strongly significant (R^2>0.95) and indicates a per capita water contribution to the waste water system of 340 litres per day. This does not vary by region including OK where water scarcity might be expected.

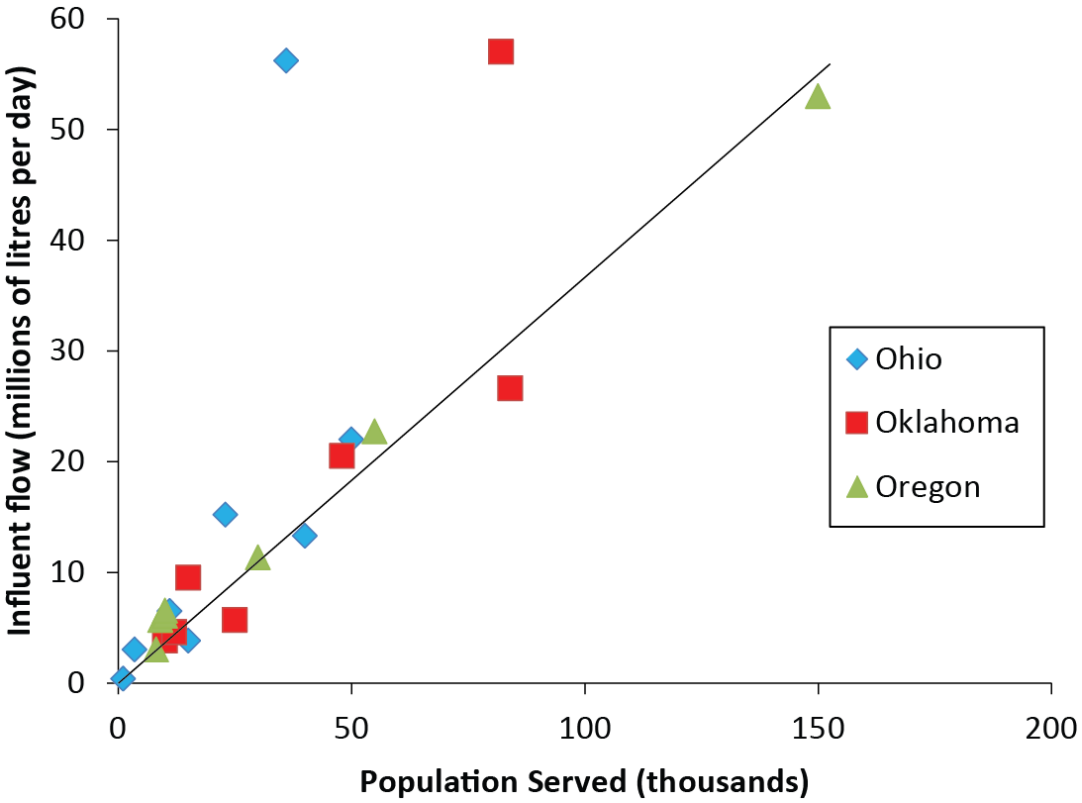

Figure 35. The influent flow measured at the WWTPs compared to the population served. Data from Table 1. The line is a trend line for illustration purposes and not a regression line.

With regard to the composition of the influent, there are measureable regional differences. These can be seen in the PCA of the influents alone (Figure 36). The composition of the influent will be determined, in part, by the local diet, the sequestration of surface waters and chemical composition of the detergents and personal care products used. The location of the samples in the PCA figure indicates a significant spread for each eco-region although each eco-region is distinctly different from each other. The driver for these differences can be seen in the loadings plot (Figure 37).

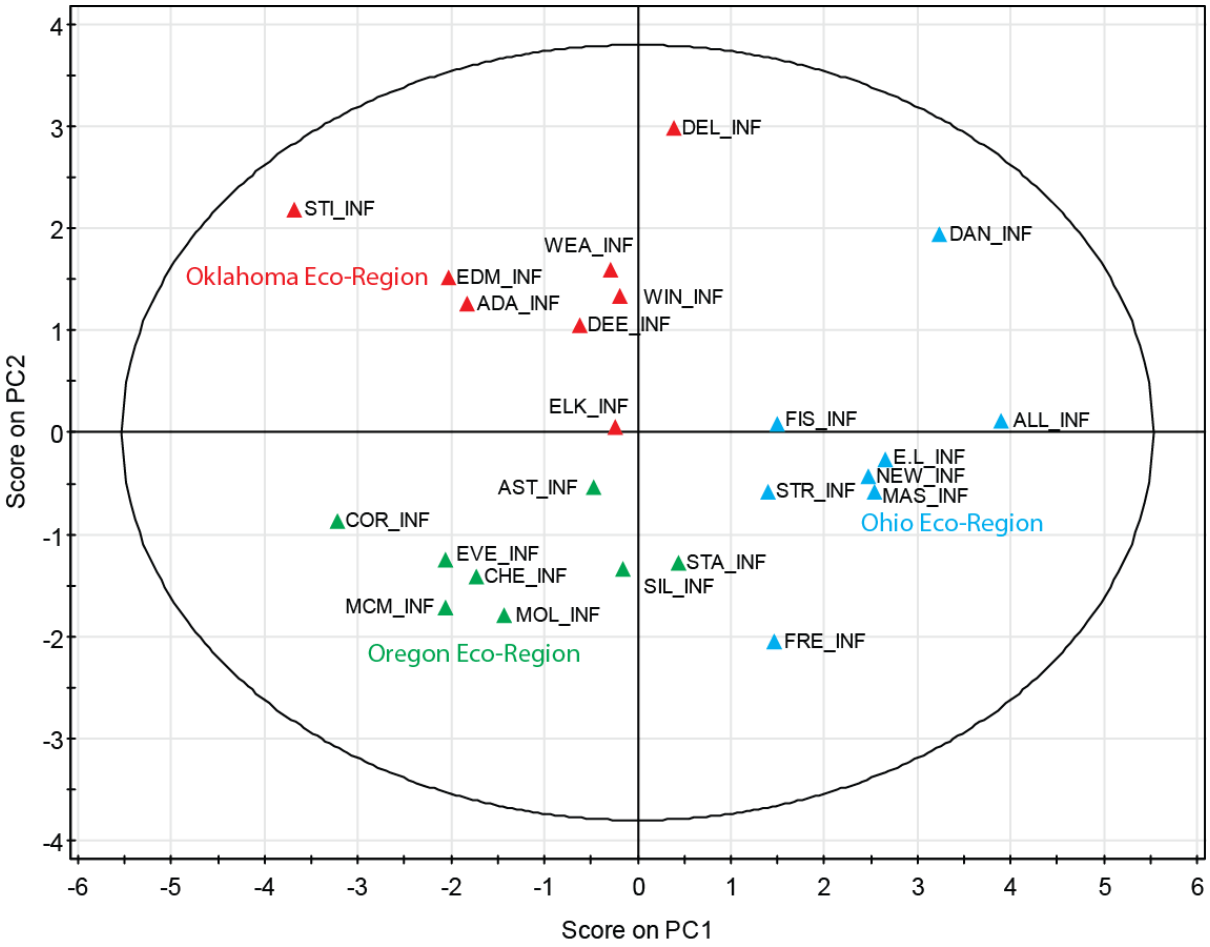

Figure 36. The PCA for the *influent* samples only for the three eco-regions.

The samples collected from the OH region have a significantly greater C_{12} contribution than the other two regions. This leads to all of the WWTP influents from OH being to the right of the centre line on PC1. The position of the OK samples are driven by a suite of chemicals but are typified by the short chain C_{13} and C_{14} compounds. In contrast, the OR samples are dominated by the C_{16} and C_{18}.

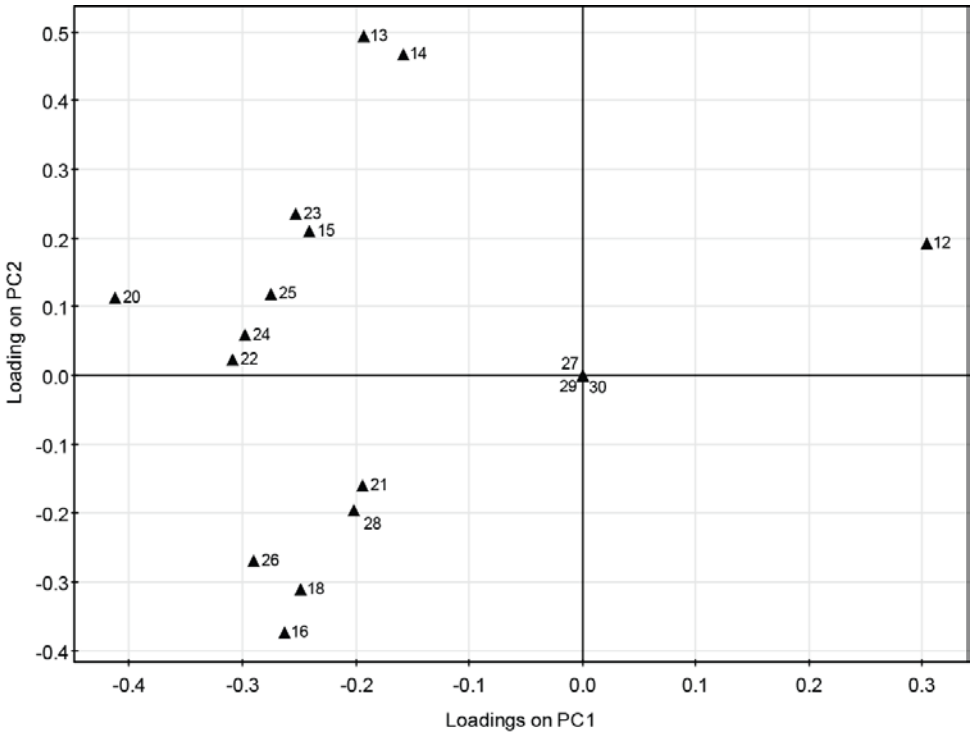

Figure 37. The loadings plot for the influent samples. All points in Figure 36 (the scores plot) that load to the right are strongly influenced by the presence of the C_{12} fatty alcohol. Those towards the top of the figure are influenced by the C_{13} and C_{14} while the C_{16} and C_{18} dictate the position of those samples in the lower left.

The reasons behind this separation may be due to:

1. Differences in the diets between the populations in each eco-region. It is possible that the proportion of meat and vegetables varies between the regions. However, the C_{12} fatty alcohol is not a great contributor to the human diet and tends to come from other sources.

2. Differences in the detergents used between catchments. Data will be obtained in the near future to enable reconstruction of the influent profiles based solely on detergent and personal care product usage. The data from Luray (DeLeo *et al.* 2011) indicated that the detergents would contribute C_{12} in the greatest proportion to the influent. Luray is in the same broad eco-region as Ohio. However, it is unlikely that the chemical composition of the detergents used was substantially different when compared to the other two regions; the proportion that each product contributes towards the total sales may be different with local preferences. This would alter the composition of the influent surfactant load.

3. Differences in the in-pipe processes due to different environmental conditions such as ambient temperature. Sampling in OK was conducted in spring and was warm and dry. For OH, sampling was in the autumn and the weather was cool and mostly dry. In comparison, sampling in OR was conducted in March and the weather was cold and wet. The temperature of the influent as it passes through the pipe may have been cooled substantially before it reached the works and

may have led to altered biochemical transformations. In this case, the OR samples were relatively rich in the C_{18} and C_{16} fatty alcohols. The UK data (Mudge *et al.* 2010), the C_{18} was also observed although in this case the C_{12} component was the major contributor. The C_{18} is not a significant compound in the detergents (DeLeo *et al.* 2011) and the UK Phase 1 data (unpublished report to ERASM) indicated that the C_{18} was between 20 and 25% of the C_{16} concentration in human faecal matter.

4. Significant sequestration of surface waters containing long chain terrestrial fatty alcohols in the OK and OR eco-regions. All the long chain compounds load to the left of the centre line for PC1 in Figure 35. The two regions that occupy this location in Figure 34 are both the wettest and the driest and so this may be down to the integrity of the infrastructure.

It is likely that each of the above factors will have some contribution to the final condition.

If all the fatty alcohol profile data are considered for the three eco-regions, significant patterns emerge. The scores plot from a PCA after log transformation can be seen in Figure 38. The same data are presented in Figure 39 but colour-coded according to their location rather than sample type.

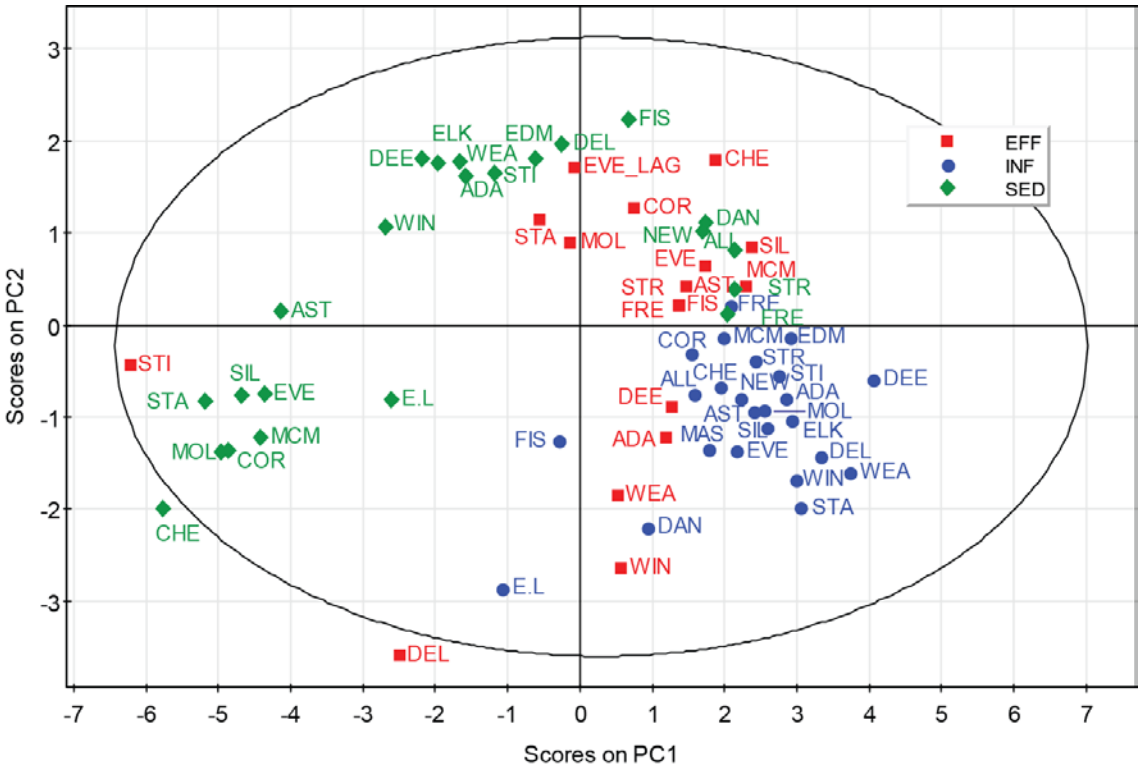

Figure 38. A scores plot of the fatty alcohol profile data from all sites and samples as proportions after $\log_{10}$ transformation. The data are colour-coded according to their sample type.

In Figure 38 the samples can be seen to separate according to their type: the sediment samples tend towards the left of the figure while the influent samples tend towards the right. The effluents occupy a more central location but are more closely related to the influents than the sediments. The separation is

not completely clean as several of the OH sediments are intermingled with the effluents. This is due to the unusual relative absence of long chain fatty alcohols in these samples.

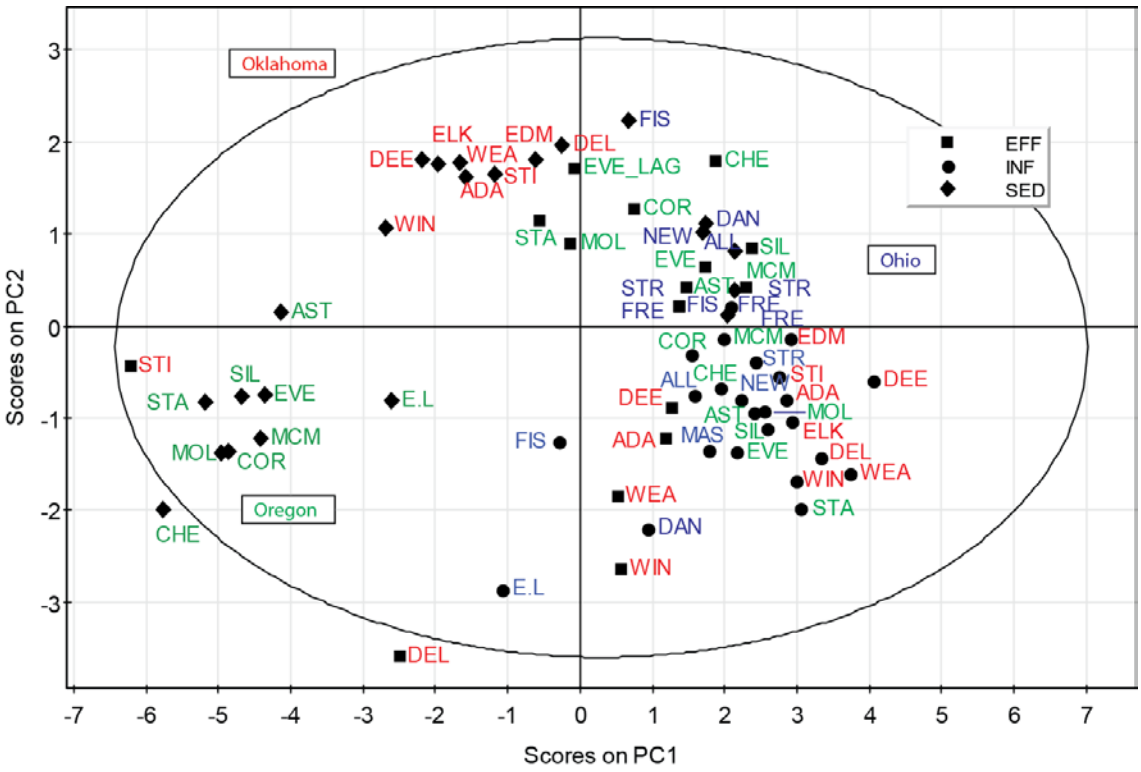

Figure 39. A scores plot of the fatty alcohol profile data from all sites and samples as proportions after $\log_{10}$ transformation. The data are colour-coded according to their location.

The score data can also be presented colour-coded according to the eco-region (Figure 39). In this case, it is clear that the samples are not clustering together by location and that the sample type is the major factor controlling the location on the sample in this figure.

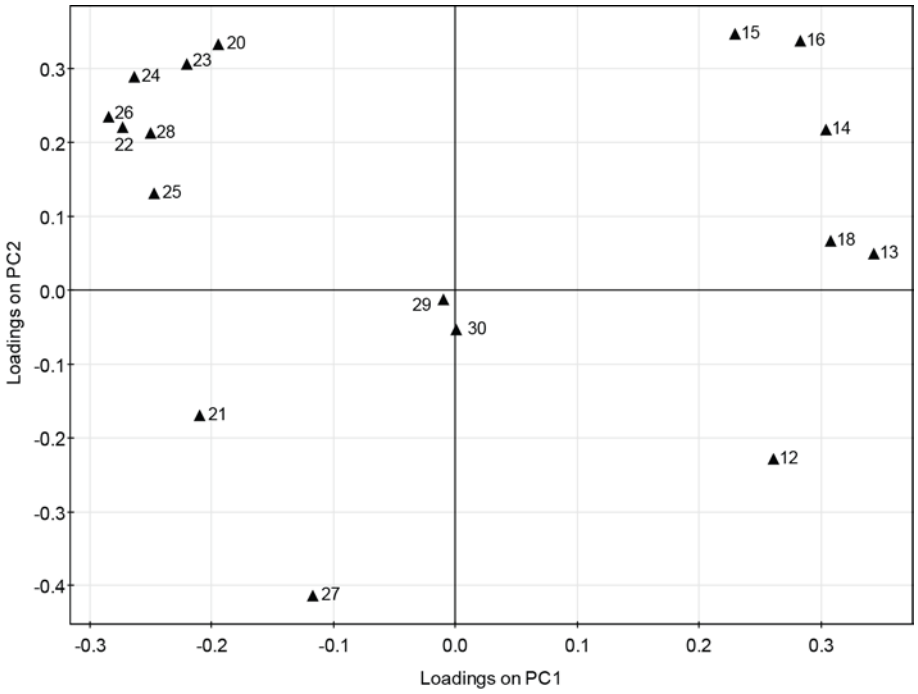

Figure 40. A loadings plot of the fatty alcohols from all samples after $\log_{10}$ transformation.

The drivers for the position of the samples in the scores plots (Figures 38 and 39) can be seen in the loadings plot for these samples (Figure 40). Short chain compounds are positioned to the right and the long chain compounds to the left; the C_{29} and C_{30} occupy a central location (near zero) and have little influence on the samples.

Another approach to investigate the differences / similarities between samples is through multivariate analysis of variance (MANOVA). In this case, three controlling variables were assigned:

- Sample type (influent, effluent and sediment)
- Treatment type (lagoon, oxidation ditch, SBR, activated sludge, trickling bed filter, and RBC)
- Eco-region (Regions 7, 8 and 9).

The multivariate data to be tested were the fatty alcohol profiles normalised as proportions to remove any concentration effect that may exist. The results can be seen in Table 6.

Table 6. Summary results from MANOVA of the normalised fatty alcohols profiles. The analysis was conducted with Minitab v15.1.1.0.

MANOVA for Sample Type
s = 2 m = 7.0 n = 19.5

Criterion	Test Statistic	F	DF Num	DF Denom	P
Wilks'	0.03949	9.725	34	82	0.000
Lawley-Hotelling	14.19280	16.697	34	80	0.000
Pillai's	1.36056	5.257	34	84	0.000
Roy's	13.43898				

MANOVA for Treatment
s = 5 m = 5.5 n = 19.5

Criterion	Test Statistic	Approx F	DF Num	DF Denom	P
Wilks'	0.26184	0.761	85	202	0.924
Lawley-Hotelling	1.58903	0.737	85	197	0.946
Pillai's	1.14392	0.785	85	225	0.901
Roy's	0.52917				

MANOVA for Ecoregion
s = 2 m = 7.0 n = 19.5

Criterion	Test Statistic	F	DF Num	DF Denom	P
Wilks'	0.05929	7.493	34	82	0.000
Lawley-Hotelling	6.56798	7.727	34	80	0.000
Pillai's	1.49200	7.256	34	84	0.000
Roy's	4.50321				

The data in Table 6 clearly show that there are statistically significant differences between the sample types (all the probabilities of exceeding the null hypothesis [that all the samples are the same] are zero). The same is true of the Eco-region results. However, the treatment type data are all non-significant indicating that there is no significant difference between them.

All of these results, PCA and MANOVA, point to significant differences between the sample types (influent, effluent and sediment) and that can clearly be seen in the mean fatty alcohol profiles of each in the individual chapters above. There are also significant differences between the eco-regions which may be due to a diversity of factors contributing to the influent profile, biochemical processes and local vegetation. In contrast, the different wastewater treatment processes do not appear to exhibit any control on the fatty alcohol profiles. If the mean efficiency of removal of the fatty alcohols is considered, bearing in mind that the samples of influent and effluent were taken at the same time and do not represent a parcel of water passing through the plant, there is remarkable similarity indicating all types of treatment are equally effective (Table 7).

Table 7. The mean efficiency of fatty alcohol removal across all three eco-regions.

Treatment Type	Mean efficiency	Number of WWTPs
Oxidation Ditch	0.97	5
Activated Sludge	0.98	7
SBR	0.98	4
Lagoon	0.97	6
RBC	0.98	2
TBF	0.98	1

Another approach to identification of similarities between samples from different locations is through Cluster Analysis. A simplistic explanation of the approach might be that all samples are plotted in multi-dimensional space with a number of axes equivalent to the number of variables (fatty alcohols in this case). The distance between each sample can then be "measured" and those that are close together have a high degree of similarity while those that are spatially distant, have a low similarity. The same approach can be used with a clustering of the variables to see which chemicals are behaving the same.

The fatty alcohol profiles from all samples were used in a cluster analysis (Minitab v15.1.1.0). The linkages between samples (observations) can be seen in Figure 41. In general, the influent samples are located to the left of the figure and are clustered quite closely (90% similarity); the sediments occupy a position in the centre of the figure with a similar degree of similarity. In contrast, the effluents are more diverse and have a lower degree of similarity. These results confirm the differences that exist between the sample types. No clustering was seen based on the eco-region or water treatment type in this figure.

A similar type of analysis can be undertaken with the variables to determine the degree of similarity between the different chain lengths. The results of this analysis can be seen in Figure 42. As expected, and confirmed by the PCA and other analyses, the short chain compounds behaved differently from the long chain compounds; this reflects the different origins for the fatty alcohols. The long chain compounds are produced principally by terrestrial plants and these compounds exhibited a high degree to similarity reflecting this common source. The short chain compounds may arise from detergents, bacteria and algae. This may explain the wider spread among these compounds.

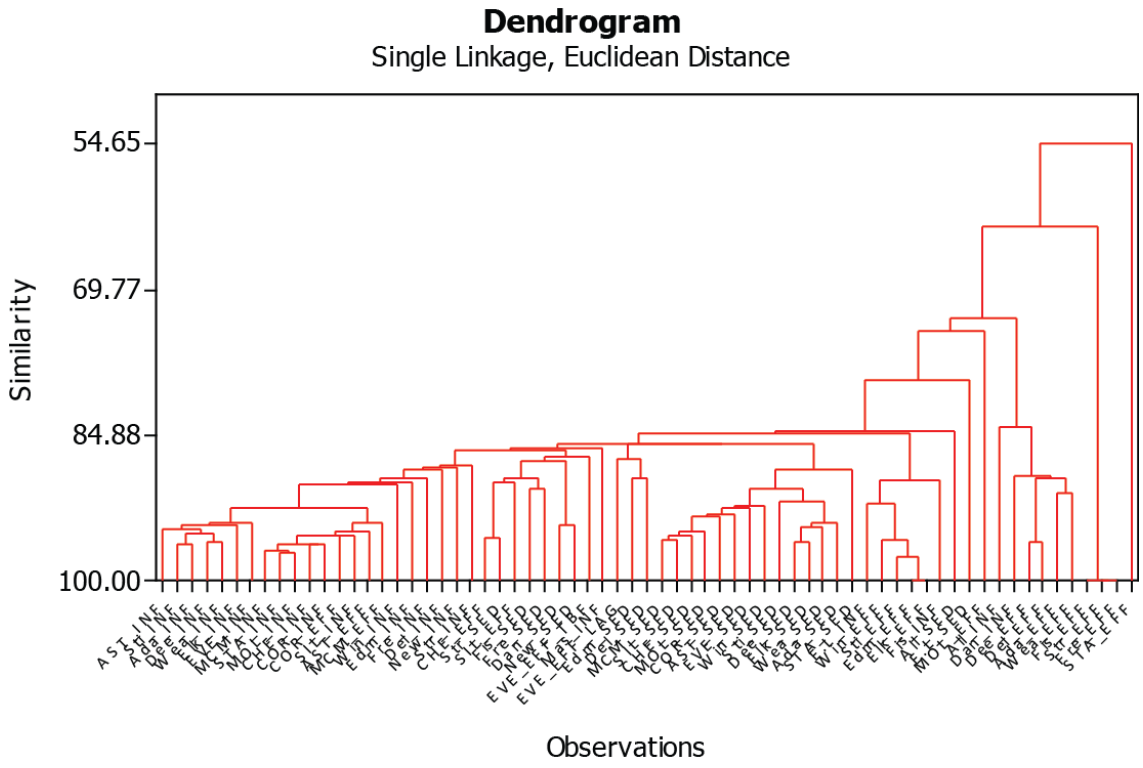

Figure 41. The similarity between samples based on a cluster analysis of their fatty alcohol profiles.

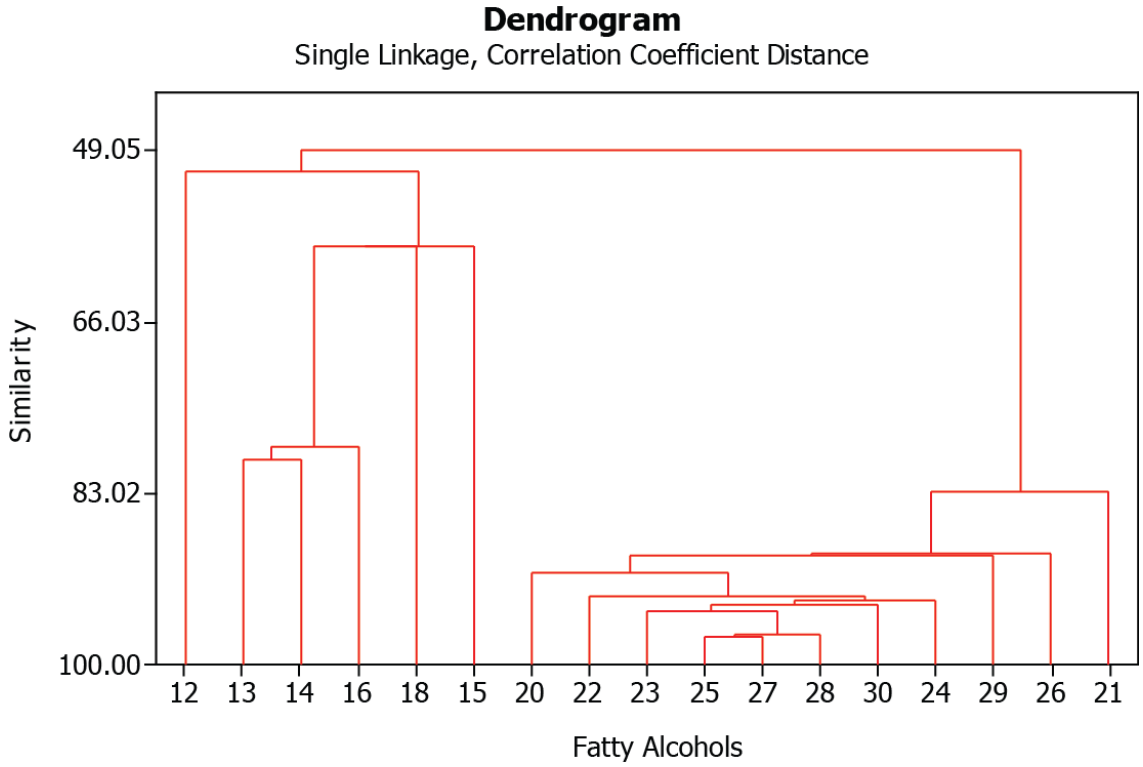

Figure 42. A cluster analysis of the variables (fatty alcohols) indicates a similarity between the long chain compounds (C_{20+}) but less similarity among the short chain compounds.

The stable isotopes provide the definitive source apportionment in these cases since both the carbon-13 and deuterium content of the fatty alcohols are dependent on the mechanisms by which the compounds are formed. The individual plots for each eco-region indicated that there were significant differences between the stable isotopic content of the fatty alcohols in each of the three sample types (influent, effluent and sediments). These data can be combined and presented on a single cross plot together with the compound specific stable isotopic values for the major detergent fatty alcohols derived from the Luray study (DeLeo *et al.* 2011; Mudge *et al.* 2012). These colour-coded results are presented in Figure 43.

There are clear separations between many of the major sources types in this figure. The petroleum-derived fatty alcohols used in the manufacture of detergents components occupy a region centred on -26 and -60‰ for the $\delta^{13}C$ and $\delta^{2}H$ respectively. In comparison, the oleochemical-derived fatty alcohols (most likely to be from palm products) are positioned at the bottom of this figure with a mean projection at -24 and -270‰ for the $\delta^{13}C$ and $\delta^{2}H$ respectively. This reflects the differences in the initial carbon and hydrogen sources used during the synthesis of the fatty alcohols (chemically or biochemically).

As well as these two clearly separated groups, there are a number of specific detergent fatty alcohols that are positioned between the petrochemical and oleochemical groups. These are coded in orange are likely to indicate a mixing of alcohols from different sources (blending) during the formulation and manufacturing processes. Although all these detergent and personal care products were purchased in the Luray catchment, it is likely that these are the same products that are available across the whole country.

The dark green circles indicate those fatty alcohols that have a chain length $>C_{19}$ and occurred in the sediment samples. These will be derived from terrestrial plants either directly as leaf litter in the sediments or secondary sources such as soils and dusts derived from the terrestrial plants. The mean projection is at -33 and -160‰ for the $\delta^{13}C$ and $\delta^{2}H$ respectively. However, there is a greater spread of the data compared to the detergent fatty alcohols. Some of the sediments from OK contained fatty alcohols with stable isotopic signatures which are significantly lighter with respect to the hydrogen even though the carbon signature is consistent with the other sediment samples. These compounds are located in the top left of Figure 43. Likewise, there are terrestrial fatty alcohols (long chain, $\delta^{13}C$ values between -32 and -36‰) which have $\delta^{2}H$ values between -200 and -275‰. These samples were collected from eco-region 7 in Oregon.

There is some evidence that these isotope ratios vary in response to precipitation and humidity (Waterhouse *et al.* 2002) and references within. The spread of $\delta^{2}H$ values can be seen more clearly in Figure 44. Here, the deuterium values are shown for the sediment fatty alcohols with the eco-region. While there is a spread of values in each eco-region, there is a general trend towards less negative values in the drier environments. In a study of deuterium in precipitation (Dansgaard 1964), samples collected close to the poles had $\delta^{2}H$ values around -220‰ which become close to zero near the equator. These results are consistent with these observations and indicate that the stable isotopic composition of the rainfall is mirrored to some extent in the fatty alcohols synthesised by the terrestrial plants.

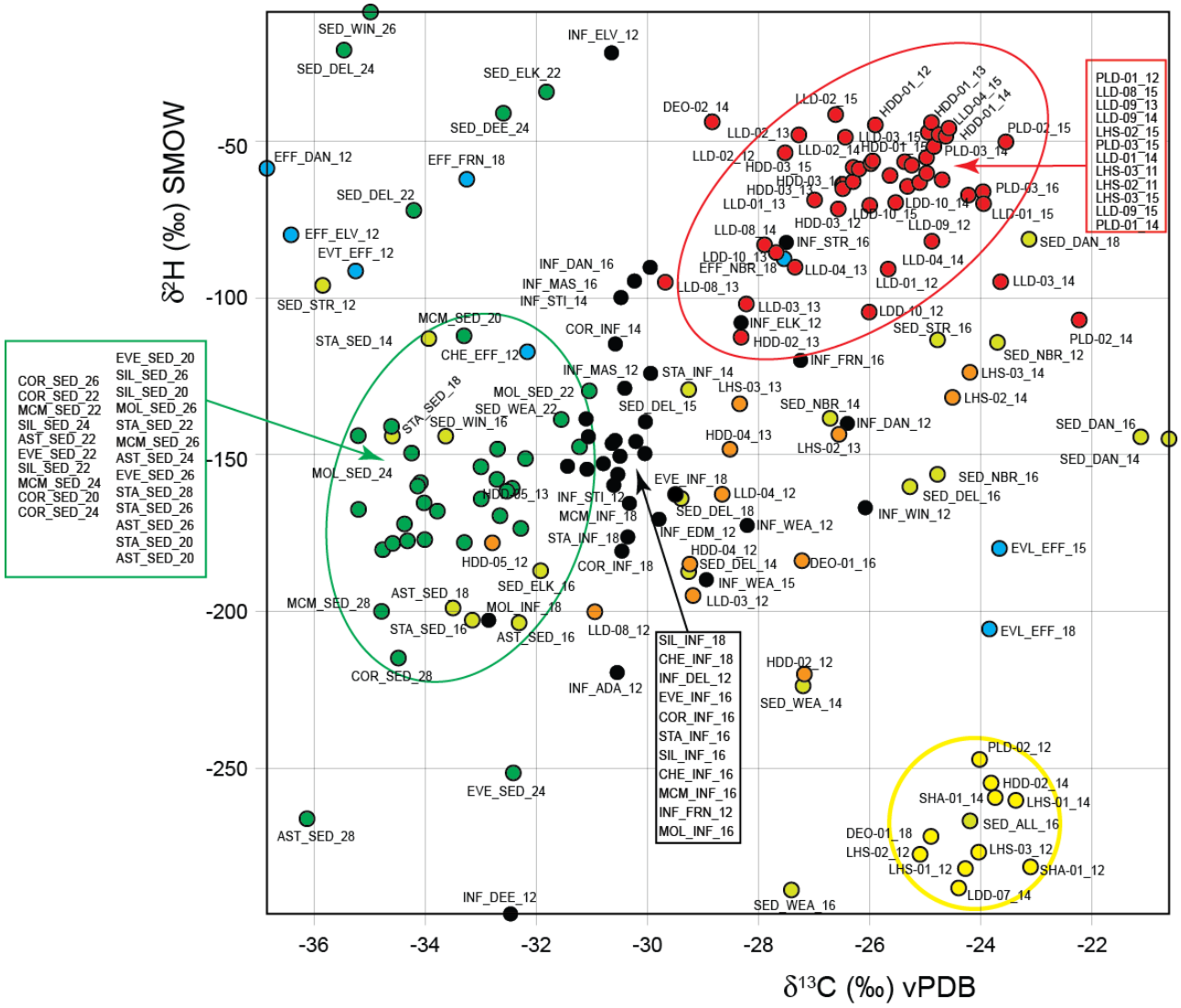

Figure 43. The stable isotope cross plot for all fatty alcohols measured in the three eco-regions together with the detergent derived fatty alcohols from the Luray study (DeLeo *et al.* 2011; Mudge *et al.* 2012). The data are colour-coded according to their likely source.

Dark green circles indicate long chain (C20+) compounds from terrestrial plants found in sediment samples.
Pale green/yellow circles indicate short chain compounds typically from algal synthesis found in sediment samples.
Blue circles are used for all effluent compounds.
Black circles denote fatty alcohols in influent samples.
Red circles indicate the petroleum derived detergent fatty alcohols from the Luray study.
Yellow circles are the oleochemical fatty alcohols in detergents from the same Luray study.
Orange circles are used for detergent derived fatty alcohols that have a stable isotopic signature that suggests a blending from both petrochemical and oleochemical sources.

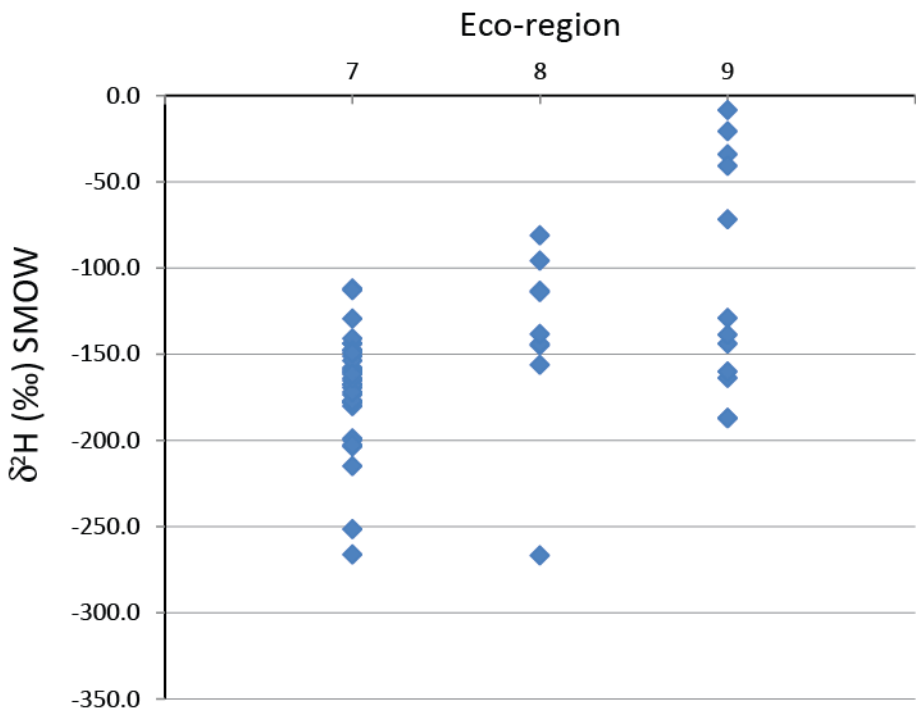

Figure 44. The spread of sedimentary fatty alcohol δ^2H values for the three eco-regions.

These observations are entirely consistent with the mean δ^2H values in precipitation (Figure 45) suggested by (Hoefs 2009). The samples from OR were taken from the region where the tongue of polar air reaches the Pacific coast.

The bulk of the WWTP influent samples occupy a very narrow range with regard to the δ^{13}C values around -31‰. These are shown in Figure 43 as black circles. There is a wide spread of δ^2H values from -20 to -300‰ although there is not the same eco-region trend as with the terrestrial plant fatty alcohols. The position of these influent fatty alcohols coincides closely with the values measured for faecal material undertaken as part of the initial phase of these investigations. The mean projection of the free and bound faecal fatty alcohols was -30 and -200‰ for δ^{13}C and δ^2H respectively. The differences in the δ^2H values may be due to (a) eco-region differences that may be ascribed to eating habits or climatic (rainfall) patterns and (b) contributions from petrochemical-derived surfactants in detergents and personal care products. There is very little overlap between the influent stable isotopic signatures and the other samples in this cross plot. There are, however, a couple of influent fatty alcohols that may be considered to be enriched in the surfactant fatty alcohols (three samples from OH and one from OK). None of the influent samples had a signature that indicated a significant contribution from oleochemical-derived surfactants which are shown in yellow in Figure 43.

It has been shown in several studies (Liu *et al.* 2006; Fraser and Meier-Augenstein 2007; Ehleringer *et al.* 2008; Bowen *et al.* 2009; Sachse *et al.* 2010; Thompson *et al.* 2010; Yang *et al.* 2011) that the stable isotopic composition of the food consumed in the USA is relatively homogeneous due to the distribution through supermarkets. Only a few communities which eat only locally grown produce exhibit distinctive

isotopic signatures. This means there is a smearing of the geographic effects in the bulk of the food consumed and so in the faecal matter produced.

Figure 45. Values of δ^2H in precipitation across the USA redrawn from (Hoefs 2009).

The effluent samples are shown in blue in Figure 41 and they are fewer in number than the other sample types as the concentrations in the effluents were about two orders of magnitude lower than for the influent based on the removal efficiencies. There is no consistent pattern and no overlap with the known sources except for the case of one sample (New Bremen, OH, C_{18}) that co-locates with the petrochemical-derived surfactants. However, there are very few C_{18} fatty alcohols in detergent products and the Luray review suggests that this compound contributes less than 1% of the total (DeLeo *et al.* 2011). Therefore, it is likely that this is not a surfactant from a detergent product but a newly synthesised compound from bacterial activity in the WWTP.

The remaining compounds in Figure 41 and typical of algal synthesis with δ^{13}C values in the -21 to -27‰ range. These compounds are short chain (C_{12} to C_{16}) with an even number of carbons indicating a unicellular algal source. This is consistent with previous observations (Mudge *et al.* 2012). There are one

or two compounds which nominally have the correct chain length to be algal in origin but the stable isotopes indicate a terrestrial plant source. These may be degradation products or compounds "recycled" by bacteria in the sediments.

Conclusions

The conclusions that can be drawn from the entire study reinforce the conclusions that can be developed for the individual eco-region studies. While the data may differ between regions, there is a consistent message that comes through in the results.

1. The fatty alcohols that are present in the influent to the WWTPs are derived from faecal matter, waste food and surfactants. The majority of the fatty alcohols have a carbon-13 and deuterium signature that is consistent with a primary source of faecal matter (mean projection at -30 and -200‰ for $\delta^{13}C$ and $\delta^{2}H$, respectively). A similar result with a 75% faecal matter and 25% petroleum-derived surfactant mixture was observed in the Luray catchment (Mudge *et al.* 2012). The fatty alcohol profiles clearly show short chain compounds ($<C_{20}$) with almost no contribution from terrestrial plant matter. This implies there was little or no surface water entrainment or capture into the sewerage system. This is despite considerable rainfall at the time of sampling in the OR catchment.

   The presence of the C_{18} fatty alcohol in the influent samples indicates in-pipe biochemical processes as the surfactants used in the manufacture of detergents have less than 1% C_{18} based on the analysis of data from the marketing survey in the Luray catchment (DeLeo *et al.* 2011). Faecal matter contains relatively little of the C_{18} fatty alcohol. Several WWTPs also produced this compound as part of the treatment process and so it may also be seen in the effluents.

2. There were statistically significant differences between the fatty alcohol profiles in the influents for the three eco-regions. The data clustered together for each eco-region but each eco-region was distinctly different on the basis of the composition. This may be due to the diet of the local populations, differences in the amounts and types of detergents used in the catchment or differences in the in-pipe processes. The stable isotopes did not show a consistent difference across the eco-regions, just the profiles. The role of the products used in the catchment could be investigated in the future when more complete marketing data, including sales at Walmart, becomes available.

3. The effluents from the WWTPs were significantly depleted in fatty alcohols with removal efficiencies of ~98% although the real value may be even better as the fatty alcohols in the effluent were not derived from the influent. The bulk of the compounds would have been lost through sorption and settling of solids during the primary stage or final clarification, and biodegradation. There was no difference for the removal efficiencies or the profiles between the six different secondary treatment technologies investigated. One WWTP had a removal efficiency around 84% but this was during a period of relatively heavy rainfall which dilutes the influent. The plant used a lagoon system and the residence times were fairly long meaning the effluent sampled at the same time as the influent came from a different rainfall condition.

The stable isotopic signatures for the effluents were not the same as those of the influents and indicate a different source. In this case, this source is likely to be the bacteria within the biological treatment stage. The bacteria are activity degrading all the organic matter in the waste stream and may use carbon derived from carbohydrates to synthesise new lipids. In that case, the new lipids would have a different stable isotope signature and reflect the carbon and hydrogen sources from the original materials.

4. The sedimentary fatty alcohols were similar in profile to those measured in the Luray catchment; the major components of the suites were long chain compounds typical of terrestrial plant matter. These compounds with a chain length of >20 carbons had a $\delta^{13}C$ value around -33‰. Their $\delta^{2}H$ values spanned a wide range from 0 to -270‰. There was a systematic variation in this value which was related to the eco-region. The $\delta^{2}H$ values of the rainfall across the USA (Hoefs 2009) has a very close linkage to the $\delta^{2}H$ values of the terrestrial fatty alcohols with the most negative values present in the OR catchment and least in the OK region. Within each eco-region, there was a spread of numbers for the $\delta^{2}H$ values that may be due to the diversity of routes by which water and hydrogen are acquired by the plants.

5. Along with the terrestrial plant fatty alcohols in the sediments, there was a suite of compounds indicative of both algal and bacterial synthesis. Statistical analyses of the fatty alcohol profiles indicate that the short chain and long chain compounds behave differently and have different origins. These short chain compounds may have the same chain lengths as surfactants used in detergents and personal care products but the stable isotopes indicate a different source. The $\delta^{13}C$ values around -24‰ are consistent with the values measured for these chain lengths in both Luray (a freshwater system) and the Menai Strait (a marine system). These compounds are clearly attributable to micro-organism metabolism obtaining carbon from dissolved CO_2 species, most likely as HCO_3^-.

6. There were no apparent differences between the different WWTPs investigated and each of the secondary (biological) stages was as efficient in the removal of fatty alcohols as any other one. Having said that, the SBRs visually appeared to have the largest number of solids in the effluent and it is possible that the bacteria had insufficient time to removal all the compounds. However, several of the WWTP had extra stages after the SBR to improve the quality of the final effluent. The lagoons were as effective probably due to the long residence times. The large lagoon in OK did not discharge liquid effluents to the receiving waters between May and November each year and the waters were sold on to golf courses for irrigation.

   The major export route for the fatty alcohols from the WWTPs will be with the solids. Although these were not quantified in these studies, in Luray the solids contained ~900 $\mu g.g^{-1}$ although each plant treats their solids in a different way, some adding lime, other preforming a digestion, leading to potentially different contributions. Studies on the transport of personal care product components after land application (Karnjanapiboonwong *et al.* 2011) have shown that water solubility is an important factor although fatty alcohols were not expressly studied in this paper.

7. As with previous studies, it can be concluded that fatty alcohols are not making a substantial (or, in most cases, quantifiable) contribution to the liquid effluents from WWTPs. Overall, the type of secondary treatment in a well-functioning plant does not alter the removal of fatty alcohols from the system. While the eco-regions may have different influent profiles (due to the usage of different products?), these differences are small compared to the differences between the influent and effluent profiles.

   As before, the sediments of the receiving waters are dominated by the terrestrial plant signatures both in terms of the profiles and the stable isotopes. Any contribution from the WWTPs is small at best. Even then, these effluent fatty alcohols are not anthropogenically derived but come from biochemical processes in the secondary treatment stages of the WWTPs. This work answers the specific question posed by Dyer and colleagues (Dyer *et al.* 2006) regarding the apportionment of fatty alcohols between the natural and anthropogenic sources. These results confirm that the environmental risk associated with fatty alcohols from detergents is negligible from the traditional (down-the-drain) disposal route.

Recommended Follow-up Studies

Although this work has convincingly answered the big question about how much of the fatty alcohols in the influent make it into the environment through a range of different WWTPs, there are a number of additional items that could be followed-up.

1. When the marketing data from Information Resources becomes available, a reconstruction of the influents in each eco-region could be made; these profiles could be compared to the ones measured in these studies and with the one from Luray (DeLeo *et al.* 2011). The formulation of the products is unlikely to vary too much across the USA and there is evidence of homogenisation of the food constituents. With more accurate data on the exact composition of each product (which may become available), a more refined contribution might be possible.

   It would also be possible to investigate the likely stable isotope contributions from these compounds. This should help explain why there are differences across these eco-regions and it would also be possible, depending on the correlations in the results, to expand the interpretation across the whole of the USA to determine the magnitude of this effect. This would depend upon the availability and cost of the datasets.

2. The major removal mechanism for the fatty alcohols from WWTPs is through the export of the sludges. Since these chemicals are not water soluble, they are associated with the solid phase and settle out in the primary treatment stage. These solids which contained up to 900 $\mu g.g^{-1}$ fatty alcohols in the Luray catchment (Mudge *et al.* 2012) may undergo some form of additional treatment (*e.g.* addition of lime, aerobic or anaerobic digestion) before being spread / injected into agricultural land where they act as a fertiliser. This is a common disposal mechanism around the developed world. What is not known is the extent to which these solids are then washed off and make it back into the riverine environment. It is also possible that repeat addition of the sludges will lead to enrichment in the soils of the less degradable components.

Acknowledgements

Many people have assisted during this extensive sampling and analytical campaign.

The candidate list of which WWTPs to investigate was produced by Scott Dyer and his team at Procter and Gamble.

Samples from OK were extracted in OU and thanks must go to Paul Philp, Coralie Biache and the rest of the team. Doug Fort provided the sampling equipment and arranged the WWTP visits in OK.

The samples were collected in OH and OR with the assistance of Kristen Rigney and Mike Ciarlo at EA Engineering. Kristen arranged all the WWTP visits. Samples were extracted in P&G's Miami River Innovation Center and thanks must go to Scott Dyer, Scott Belanger, Monica Lam, Quinn for their help.

The OR samples were extracted at OSU and Staci Simonich and Jill Schrlau provided assistance in the laboratory.

Thanks must go to all the WWTP operators and staff who willingly provided their time and samples across all eco-regions.

All stable isotope analyses were conducted by Wolfram Meier-Augenstein at The James Hutton Institute, Dundee.

Finally, all the members of the ACI assisted in the development of the project but none of this would have been possible without the interest and dedication of Paul DeLeo at the ACI. Many thanks.

The financing of this project was through the ACI and their members.

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Appendix

Table A1. Fatty alcohol concentrations in the INFLUENT samples from OKLAHOMA

ug/litre	INFLUENT							
	Winfield	Stillwater	Edmond	Deer Creek	Del City	Ada	Weatherford	Elk City
Sample Vol (l)	2	2	2	2	2	2	2	2
C12-O-TMS	39.58	96.40	2.04	29.87	109.57	33.52	326.65	19.69
isoC13-O-TMS	0.54	1.01	0.98	0.65	3.81	0.64	6.14	1.02
nC13-O-TMS	7.78	19.81	11.10	6.76	46.58	10.12	82.85	4.05
C14-O-TMS	13.02	70.50	21.66	18.01	132.17	26.97	197.63	7.81
nC15-O-TMS	3.77	35.45	5.98	6.72	54.65	12.52	63.66	3.55
C16-O-TMS	18.40	121.90	32.16	37.05	152.50	51.09	296.84	24.40
isoC17-O-TMS	0.09	0.00	0.21	0.23	0.00	0.00	0.00	0.78
anteC17-O-TMS	0.00	3.00	0.00	0.00	2.42	1.42	5.95	0.00
C18-O-TMS	28.86	266.62	56.79	51.09	119.17	86.69	459.22	35.02
C19-O-TMS	14.21	0.44	0.15	0.12	0.04	1.29	1.66	20.18
C20-O-TMS	0.60	15.07	1.46	1.33	0.72	4.13	14.48	1.04
C21-O-TMS	0.66	4.34	0.28	0.03	0.00	1.77	5.85	0.15
C22-O-TMS	0.38	11.41	0.94	1.01	0.00	2.04	7.31	0.58
C23-O-TMS	0.00	2.16	0.12	0.12	0.00	0.09	0.16	0.08
C24-O-TMS	0.48	8.63	0.85	0.82	0.00	1.88	5.94	0.55
C25-O-TMS	0.23	0.95	0.00	0.04	0.00	0.29	0.80	0.00
C26-O-TMS	0.51	0.42	0.45	0.22	0.00	0.38	1.79	0.35
C27-O-TMS	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C28-O-TMS	0.32	0.16	0.40	0.21	0.00	0.00	0.00	0.39
TOTAL	**129.43**	**658.27**	**135.56**	**154.31**	**621.63**	**234.84**	**1476.93**	**119.63**

NOTE: Although the concentrations of non-detects are reported as zeros in these tables, they are really less than the limit of detection. This varies between analytical dataset generated. The inclusion of the zero here has been made so that the data can be readily converted to proportions in signature analysis and PCA.

Table A2. Fatty alcohol concentrations in the EFFLUENT samples from OKLAHOMA

ug/litre	EFFLUENT							
	Winfield	Stillwater	Edmond	Deer Creek	Del City	Ada	Weatherford	Elk City
Sample Vol (l)	2	2	2	2	2	2	2	2
C12-O-TMS	0.11	0.23	0.00	0.09	17.35	1.20	1.60	0.00
isoC13-O-TMS	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
nC13-O-TMS	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C14-O-TMS	0.25	0.09	0.00	0.00	0.00	0.00	0.00	0.00
nC15-O-TMS	0.98	1.05	0.00	0.00	0.00	0.00	0.00	0.00
C16-O-TMS	0.38	0.07	0.00	0.00	0.08	0.18	0.10	0.00
isoC17-O-TMS	0.00	18.13	0.00	0.00	0.00	0.00	0.00	0.00
anteC17-O-TMS	7.47	0.00	0.57	0.00	0.48	0.00	0.37	0.00
C18-O-TMS	0.28	0.10	0.00	0.00	0.22	0.00	0.00	0.00
C19-O-TMS	2.70	11.75	0.14	0.00	0.20	0.00	0.00	0.00
C20-O-TMS	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C21-O-TMS	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C22-O-TMS	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C23-O-TMS	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C24-O-TMS	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C25-O-TMS	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C26-O-TMS	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C27-O-TMS	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C28-O-TMS	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
TOTAL	**12.23**	**31.41**	**0.70**	**0.09**	**18.34**	**1.38**	**2.08**	**0.00**

Table A3. Fatty alcohol concentrations in the SEDIMENT samples from OKLAHOMA. The sample weight is the dry weight extracted.

ug/kilogram	SEDIMENT							
	Winfield	Stillwater	Edmond	Deer Creek	Del City	Ada	Weatherford	Elk City
Sample wt (g)	64.37	110.46	99.99	63.54	7.12	110.45	84.57	62.28
C12-O-TMS	50.5	22.2	5.8	33.6	6296.5	4.3	224.6	125.6
isoC13-O-TMS	61.4	9.9	0.0	49.3	1276.9	0.0	51.8	52.1
nC13-O-TMS	61.4	9.9	11.8	49.3	5079.7	0.0	159.9	97.0
C14-O-TMS	738.7	188.0	108.1	832.8	5725.5	54.2	2773.7	1840.0
nC15-O-TMS	1194.5	153.5	141.0	1007.9	87756.9	127.2	2165.2	1895.5
C16-O-TMS	3761.9	502.5	629.3	4385.1	207712.6	533.9	11994.3	8707.1
isoC17-O-TMS	866.9	58.2	0.0	704.2	72602.4	0.0	1011.1	711.7
antC17-O-TMS	0.0	0.0	86.3	0.0	0.0	146.2	0.0	0.0
C18-O-TMS	1006.1	189.7	108.4	945.5	110779.6	255.9	1073.7	1873.2
C19-O-TMS	189.1	9.0	14.7	163.7	9442.6	15.7	204.9	464.4
C20-O-TMS	1305.2	88.4	46.7	850.4	16097.2	80.7	1867.4	1632.5
C21-O-TMS	210.9	35.8	10.0	238.5	4954.9	16.4	501.5	460.8
C22-O-TMS	6141.2	321.8	118.6	3731.9	29346.7	287.1	4660.4	5233.9
C23-O-TMS	413.5	24.1	17.2	394.3	3721.7	26.4	390.8	453.0
C24-O-TMS	7195.9	482.3	128.8	3940.7	56587.6	378.2	5716.0	6419.5
C25-O-TMS	515.7	26.3	9.3	326.2	6884.7	25.9	487.2	868.8
C26-O-TMS	9302.0	197.4	131.6	3604.7	35165.6	350.3	10437.1	7959.7
C27-O-TMS	364.6	13.6	9.8	125.7	1397.0	16.1	211.9	216.6
C28-O-TMS	4244.1	110.6	160.6	2267.5	30510.5	312.2	6620.0	3873.5
TOTAL	**37623.7**	**2443.2**	**1738.1**	**23651.3**	**691338.8**	**2630.6**	**50551.4**	**42884.8**

Table A4. Sterol and stanol concentrations in the INFLUENT samples.

ug/litre	INFLUENT							
	Winfield	Stillwater	Edmond	Deer Creek	Del City	Ada	Weatherford	Elk City
Epicoprostanol	0.00	47.52	0.00	0.00	0.00	6.56	0.00	0.00
Cholesterol	25.40	168.22	35.37	57.45	3.91	110.25	57.47	33.47
Cholestanol	0.00	28.45	4.26	0.00	0.00	22.22	9.19	0.00
Stigmasterol	1.57	13.73	1.75	3.51	0.00	7.25	3.20	1.65
Stigmastanol	2.13	15.36	2.57	3.37	0.00	8.87	4.33	2.53
Sitosterol	17.66	123.18	20.29	35.53	4.17	73.77	34.89	17.43
Coprostanol	36.06	227.17	42.74	79.17	24.63	274.47	121.40	59.52
Coprostanone	2.87	0.00	0.00	0.00	16.86	0.00	7.56	0.00
5α-cholest-7-en-3β-ol	1.12	6.06	1.25	1.61	0.00	3.72	1.84	1.33
Ergosterol	0.00	3.36	0.00	0.00	0.00	0.00	0.00	0.00
24-ethylcoprostanol	12.83	90.24	16.01	26.83	11.13	87.74	38.44	18.86
Cholest-4-ene-5-one	0.00	0.00	1.60	0.00	8.84	5.32	2.85	2.13
Campesterol	5.73	44.25	6.60	13.38	0.00	25.43	12.73	6.89
Campestanol	1.77	28.13	1.81	2.28	0.00	6.47	2.71	1.80
TOTAL	**107.13**	**795.67**	**134.24**	**223.13**	**69.54**	**632.06**	**296.62**	**145.62**

Table A5. Sterol and stanol concentrations in the EFFLUENT samples.

ug/litre	EFFLUENT							
	Winfield	Stillwater	Edmond	Deer Creek	Del City	Ada	Weatherford	Elk City
Epicoprostanol	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cholesterol	0.37	0.68	0.00	0.00	0.82	0.00	0.00	0.00
Cholestanol	0.00	0.00	0.00	0.00	0.98	0.00	0.00	0.00
Stigmasterol	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Stigmastanol	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sitosterol	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Coprostanol	0.00	0.00	0.00	0.00	2.16	0.49	0.00	0.00
Coprostanone	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5α-cholest-7-en-3β-ol	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ergosterol	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
24-ethylcoprostanol	0.00	0.00	0.00	0.00	2.28	0.00	0.00	0.00
Cholest-4-ene-5-one	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Campesterol	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Campestanol	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
TOTAL	**0.37**	**0.68**	**0.00**	**0.00**	**6.24**	**0.49**	**0.00**	**0.00**

Table A6. Sterol and stanol concentrations in the SEDIMENT samples.

ug/kilogram	SEDIMENT							
	Winfield	Stillwater	Edmond	Deer Creek	Del City	Ada	Weatherford	Elk City
Epicoprostanol	58.47	86.11	40.31	98.22	9416.09	0.00	206.30	128.89
Cholesterol	1572.07	943.79	295.99	2793.03	87529.25	3765.13	4413.57	4915.91
Cholestanol	243.74	165.69	53.29	436.84	31309.91	68.57	1058.07	629.29
Stigmasterol	775.68	162.18	77.10	1126.93	13795.07	171.49	967.16	1313.27
Stigmastanol	626.80	87.38	46.65	460.11	19734.59	38.20	655.17	1050.43
Sitosterol	10697.36	1044.33	463.97	6638.98	96874.62	750.45	7468.23	20001.63
Coprostanol	18.54	122.15	38.98	279.91	74246.57	74.60	1986.75	289.00
Coprostanone	0.00	0.00	0.00	0.00	3078.73	0.00	106.40	0.00
5α-cholest-7-en-3β-ol	58.98	26.63	18.91	181.78	3853.49	20.16	174.04	227.72
Ergosterol	1373.00	121.38	203.21	1137.52	30041.36	646.50	978.97	1358.22
24-ethylcoprostanol	0.00	93.16	53.15	269.33	44413.64	57.79	1125.37	812.92
Cholest-4-ene-5-one	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Campesterol	1968.71	291.61	194.95	1763.16	24701.79	1549.58	1842.98	2889.57
Campestanol	137.45	80.35	38.61	256.65	11212.20	33.52	370.01	745.85
TOTAL	**17530.81**	**3224.77**	**1525.13**	**15442.46**	**450207.32**	**7175.99**	**21353.04**	**34362.71**

Table A7. Stable isotopic signatures from OKLAHOMA.

Sample	$\delta^{13}C$	$\delta^{2}H$
INF_DEE_12	-32.46	-296.6
SED_WEA_16	-27.41	-288.932
SED_WEA_14	-27.20	-223.8
INF_ADA_12	-30.545	-219.5
INF_WEA_15	-28.94	-189.9
SED_DEL_14	-29.26	-187.3
SED_ELK_16	-31.92	-187.015
INF_WEA_12	-28.21	-172.5
INF_EDM_12	-29.794	-170.6
INF_WIN_12	-26.08	-166.9
SED_DEL_18	-29.39	-163.951
SED_DEL_16	-25.28	-160.187
INF_STI_12	-30.61	-159.7
INF_DEL_12	-30.5	-150.4
SED_WIN_16	-33.63	-144.0
SED_DEL_15	-29.26	-129.197
INF_ELK_12	-28.32	-107.8
INF_STI_14	-30.48	-99.8
SED_WEA_22	-31.55	-138.7
SED_ELK_22	-31.82	-34.1
SED_DEL_22	-34.20	-72.0
SED_DEL_24	-35.46	-20.8
SED_WIN_26	-34.98	-8.6
SED_DEE_24	-32.59	-40.9

Table A8. Fatty alcohol concentrations in the INFLUENT samples from OHIO.

ug/litre	INFLUENT							
	East Liverpool	Alliance	Massillon	Fish Creek	Strongsville	French Creek	Danville	New Bremen
Sample Vol (l)	2	2	2	2	2	2	2	2
C12-O-TMS	1.38	6.51	18.93	2.22	1.64	9.73	28.05	14.66
isoC13-O-TMS	0.45	0.00	0.00	0.08	0.00	1.03	1.40	0.00
nC13-O-TMS	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C14-O-TMS	1.30	0.00	2.46	0.93	1.74	0.00	4.16	2.71
nC15-O-TMS	1.54	0.00	0.00	8.29	0.00	0.00	0.47	0.00
C16-O-TMS	9.25	0.00	4.65	7.68	1.95	33.20	3.43	8.22
isoC17-O-TMS	116.20	0.00	0.00	42.91	0.00	2.45	0.00	0.00
anteC17-O-TMS	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C18-O-TMS	15.41	1.94	18.88	16.60	6.52	24.39	2.75	9.78
C19-O-TMS	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C20-O-TMS	0.08	0.00	0.00	0.11	0.00	0.00	0.00	0.00
C21-O-TMS	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C22-O-TMS	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C23-O-TMS	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C24-O-TMS	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C25-O-TMS	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C26-O-TMS	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C27-O-TMS	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C28-O-TMS	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
TOTAL	**145.61**	**8.45**	**44.92**	**78.82**	**11.84**	**70.79**	**40.26**	**35.37**

Table A9. Fatty alcohol concentrations in the EFFLUENT samples from OHIO.

ug/litre	EFFLUENT							
	East Liverpool	Alliance	Massillon	Fish Creek	Strongsville	French Creek	Danville	New Bremen
Sample Vol (l)	2	2	2	2	2	2	2	2
C12-O-TMS	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
isoC13-O-TMS	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
nC13-O-TMS	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C14-O-TMS	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
nC15-O-TMS	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C16-O-TMS	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
isoC17-O-TMS	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
anteC17-O-TMS	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C18-O-TMS	0.00	0.00	0.00	0.73	0.41	0.75	0.00	0.00
C19-O-TMS	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C20-O-TMS	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C21-O-TMS	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C22-O-TMS	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C23-O-TMS	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C24-O-TMS	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C25-O-TMS	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C26-O-TMS	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C27-O-TMS	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C28-O-TMS	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
TOTAL	**0.00**	**0.00**	**0.00**	**0.73**	**0.41**	**0.75**	**0.00**	**0.00**

Table A10. Fatty alcohol concentrations in the SEDIMENT samples from OHIO. The sample weight is the dry weight extracted.

ug/kilogram	SEDIMENT							
	East Liverpool	Alliance	Massillon	Fish Creek	Strongsville	French Creek	Danville	New Bremen
Sample wt (g)	119.12	91.29	94.73	91.54	87.55	107.54	48.93	82.72
C12-O-TMS	0.00	0.00		14.03	96.50	0.00	166.02	193.79
isoC13-O-TMS	0.00	0.00		0.00	45.92	0.00	137.90	50.40
nC13-O-TMS	0.00	0.00		0.00	0.00	0.00	0.00	0.00
C14-O-TMS	3.16	295.80		81.19	493.71	43.42	1709.70	632.36
nC15-O-TMS	2.10	0.00		137.83	306.07	23.12	865.91	192.93
C16-O-TMS	51.58	725.00		774.52	1341.11	337.24	3019.52	1045.71
isoC17-O-TMS	130.77	0.00		0.00	0.00	0.00	0.00	0.00
antC17-O-TMS	0.00	0.00		17.38	41.19	0.00	251.41	39.70
C18-O-TMS	82.50	153.68		338.16	687.28	167.12	350.89	80.74
C19-O-TMS	0.00	0.00		0.00	0.00	0.00	0.00	0.00
C20-O-TMS	84.49	0.00		99.79	12.59	0.00	48.78	0.00
C21-O-TMS	0.00	0.00		0.00	0.00	0.00	0.00	0.00
C22-O-TMS	255.06	0.00		33.56	0.00	0.00	0.00	0.00
C23-O-TMS	0.83	0.00		0.00	0.00	0.00	0.00	0.00
C24-O-TMS	128.84	0.00		0.00	0.00	0.00	0.00	0.00
C25-O-TMS	0.00	0.00		0.00	0.00	0.00	0.00	0.00
C26-O-TMS	8.53	0.00		0.00	0.00	0.00	0.00	0.00
C27-O-TMS	0.00	0.00		0.00	0.00	0.00	0.00	0.00
C28-O-TMS	0.00	0.00		0.00	0.00	0.00	0.00	0.00
TOTAL	**747.86**	**1174.49**		**1496.48**	**3024.39**	**570.90**	**6550.11**	**2235.62**

Table A11. Stable isotopic signatures from OHIO.

Sample	$\delta^{13}C$	$\delta^{2}H$
SED_ALL_16	-24.20	-266.9
SED_STR_12	-35.84	-95.9
SED_STR_16	-24.78	-113.3
SED_DAN_14	-20.62	-144.9
SED_DAN_16	-21.13	-144.3
SED_DAN_18	-23.14	-81.2
SED_NBR_12	-23.71	-114.10
SED_NBR_14	-26.72	-138.34
SED_NBR_16	-24.78	-156.24
INF_ELV_12	-30.65	-21.7
INF_FRN_12	-30.04	-139.4
INF_FRN_16	-27.25	-119.9
INF_DAN_12	-26.41	-140.0
INF_DAN_16	-29.95	-90.2
INF_MAS_12	-30.41	-128.7
INF_MAS_16	-30.24	-94.5
INF_STR_16	-27.50	-82.2
EFF_ELV_12	-36.41	-79.8
EFF_FRN_18	-33.25	-62.0
EFF_DAN_12	-36.85	-58.5
EFF_NBR_18	-27.54	-87.4

Table A12. Fatty alcohol concentrations in the INFLUENT samples from OREGON.

ug/litre	INFLUENT							
	Everett	Chehalis	Astoria	McMinnville	Molalla	Silverton	Stayton	Corvallis
Sample Vol (l)	2	2	2	2	2	2	2	2
C12-O-TMS	7.72	3.66	2.64	1.81	6.52	0.98	6.54	2.57
C13-O-TMS	1.69	1.19	0.42	0.36	0.82	0.26	0.70	0.57
C14-O-TMS	6.15	4.13	1.27	0.90	4.87	1.00	2.71	2.20
C15-O-TMS	3.60	3.17	0.85	0.74	2.31	0.72	1.92	1.35
C16-O-TMS	21.20	17.94	4.59	5.86	25.81	6.62	19.66	13.24
C17-O-TMS	0.46	0.88	0.25	0.47	0.00	0.00	0.20	0.18
C18-O-TMS	21.80	21.27	5.09	7.64	28.37	8.88	23.56	16.94
C20-O-TMS	0.38	0.34	0.08	0.16	0.74	0.22	0.23	0.74
C21-O-TMS	4.72	0.99	0.16	0.05	0.28	0.00	0.23	0.45
C22-O-TMS	0.00	0.73	0.21	0.00	0.20	0.01	0.36	1.36
C23-O-TMS	0.00	0.03	0.00	0.00	0.06	0.00	0.00	0.00
C24-O-TMS	0.24	0.00	0.00	0.40	0.15	0.00	0.01	0.18
C25-O-TMS	0.13	0.00	0.00	0.00	0.00	0.00	0.00	0.09
C26-O-TMS	0.18	0.58	0.04	0.15	0.40	0.04	0.06	0.19
C27-O-TMS	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C28-O-TMS	0.45	0.00	0.06	0.07	0.48	0.00	0.00	0.00
C29-O-TMS	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C30-O-TMS	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
TOTAL	**68.71**	**54.91**	**15.65**	**18.62**	**71.02**	**18.73**	**56.17**	**40.06**

Table A13. Fatty alcohol concentrations in the EFFLUENT samples from OREGON.

ug/litre			EFFLUENT						
	Everett LAG	TBF	Chehalis	Astoria	McMinnville	Molalla	Silverton	Stayton	Corvallis
Sample Vol (l)	2	2	2	2	2	2	2	2	2
C12-O-TMS	0.10	0.00	0.01	0.28	0.09	0.07	0.07	0.10	0.09
C13-O-TMS	0.00	0.00	0.02	0.03	0.03	0.02	0.03	0.01	0.03
C14-O-TMS	0.00	0.00	0.09	0.00	0.10	0.19	0.08	0.08	0.19
C15-O-TMS	0.06	0.00	0.08	0.12	0.13	0.06	0.18	1.63	0.11
C16-O-TMS	0.44	0.04	0.26	0.65	0.31	0.41	0.31	0.08	0.76
C17-O-TMS	0.09	0.00	0.03	0.24	0.04	0.08	0.02	0.00	0.06
C18-O-TMS	0.19	0.03	0.11	0.94	0.42	0.25	0.20	0.09	0.97
C20-O-TMS	0.09	0.00	0.01	0.05	0.00	0.00	0.00	0.00	0.07
C21-O-TMS	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C22-O-TMS	0.10	0.00	0.00	0.05	0.00	0.00	0.00	0.09	0.09
C23-O-TMS	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C24-O-TMS	0.09	0.00	0.00	0.05	0.00	0.03	0.00	0.20	0.00
C25-O-TMS	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C26-O-TMS	0.03	0.00	0.00	0.01	0.00	1.05	0.00	0.00	0.12
C27-O-TMS	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C28-O-TMS	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C29-O-TMS	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C30-O-TMS	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
TOTAL	**1.18**	**0.07**	**0.63**	**2.41**	**1.12**	**2.15**	**0.89**	**2.28**	**2.49**

Table A14. Fatty alcohol concentrations in the SEDIMENT samples from OREGON. The sample weight is the dry weight extracted.

ug/kg	INFLUENT							
	Everett	Chehalis	Astoria	McMinnville	Molalla	Silverton	Stayton	Corvallis
Sample wt (g)	67.8	83.4	44.7	78.8	114.1	73.9	39.8	82
C12-O-TMS	0.05	0.02	0.05	0.33	0.02	0.06	0.13	0.05
C13-O-TMS	0.04	0.00	0.01	0.01	0.00	0.01	0.08	0.00
C14-O-TMS	0.14	0.02	0.18	0.06	0.01	0.08	0.50	0.03
C15-O-TMS	0.06	0.02	0.33	0.05	0.01	0.12	0.48	0.05
C16-O-TMS	0.68	0.18	1.30	0.27	0.05	0.80	1.97	0.25
C17-O-TMS	0.14	0.00	0.71	0.06	0.01	0.21	0.28	0.04
C18-O-TMS	0.48	0.12	0.98	0.31	0.05	0.87	2.19	0.34
C20-O-TMS	1.47	0.71	2.09	0.71	0.18	1.56	31.70	0.56
C21-O-TMS	1.02	0.00	1.86	0.60	0.21	1.44	2.96	1.33
C22-O-TMS	3.92	3.65	4.97	3.00	1.18	5.46	47.47	2.94
C23-O-TMS	0.37	0.16	0.54	0.29	0.05	0.50	1.91	0.30
C24-O-TMS	3.46	4.06	4.35	3.63	0.96	6.64	11.92	5.83
C25-O-TMS	0.64	0.93	0.48	0.46	0.09	0.89	4.76	0.47
C26-O-TMS	9.26	5.65	5.63	3.79	1.50	9.00	50.41	4.41
C27-O-TMS	0.39	0.32	0.32	0.26	0.06	0.45	3.04	0.29
C28-O-TMS	3.29	3.35	4.69	2.28	0.88	4.57	43.80	2.31
C29-O-TMS	0.14	0.18	0.15	0.17	0.05	0.34	4.83	0.12
C30-O-TMS	1.69	1.50	1.13	1.38	0.24	1.88	5.38	1.30
TOTAL	**27.25**	**20.86**	**29.76**	**17.66**	**5.53**	**34.89**	**213.80**	**20.63**

Table A15. Stable isotopic signatures from OREGON.

Sample	$\delta^{13}C$	$\delta^{2}H$
EVE_SED_20	-34.09	-158.9
EVE_SED_22	-32.28	-173.5
EVE_SED_24	-32.41	-251.6
EVE_SED_26	-35.20	-167.4
AST_SED_16	-32.31	-203.6
AST_SED_18	-33.50	-198.9
AST_SED_20	-34.76	-180.3
AST_SED_22	-32.65	-169.5
AST_SED_24	-33.29	-178.0
AST_SED_26	-34.58	-178.3
AST_SED_28	-36.12	-266.2
MCM_SED_20	-33.30	-112.0
MCM_SED_22	-32.71	-157.8
MCM_SED_24	-32.20	-151.2
MCM_SED_26	-34.02	-165.4
MCM_SED_28	-34.78	-200.0
STA_SED_14	-33.93	-112.9
STA_SED_16	-33.15	-202.8
STA_SED_18	-34.59	-144.0
STA_SED_20	-34.32	-177.5
STA_SED_22	-33.78	-168.0
STA_SED_26	-34.01	-177.1
STA_SED_28	-34.37	-172.1
MOL_SED_22	-31.05	-129.6
MOL_SED_24	-31.22	-147.5
MOL_SED_26	-35.20	-143.9
SIL_SED_20	-34.60	-140.9
SIL_SED_22	-32.43	-160.7
SIL_SED_24	-32.99	-164.1
SIL_SED_26	-34.13	-160.0
COR_SED_20	-32.70	-148.2
COR_SED_22	-33.00	-153.8
COR_SED_24	-32.53	-161.6
COR_SED_26	-34.24	-149.4
COR_SED_28	-34.48	-214.9
EVE_INF_16	-30.80	-152.8
EVE_INF_18	-29.50	-162.6
CHE_INF_16	-30.64	-146.4
CHE_INF_18	-30.21	-145.8
MCM_INF_16	-30.58	-145.4
MCM_INF_18	-30.33	-165.5

STA_INF_14	-29.94	-124.0
STA_INF_16	-30.53	-156.2
STA_INF_18	-30.36	-176.2
MOL_INF_16	-31.11	-138.5
MOL_INF_18	-32.86	-202.8
SIL_INF_16	-31.06	-144.2
SIL_INF_18	-30.05	-149.6
COR_INF_14	-30.58	-114.6
COR_INF_16	-31.44	-153.6
COR_INF_18	-30.46	-180.8
EVL_EFF_15	-23.67	-179.9
EVL_EFF_18	-23.85	-205.6
EVT_EFF_12	-35.25	-91.3
CHE_EFF_12	-32.16	-117.1