Fatty alcohols in the Terrestrial Environment

A case study around Luray, VA

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Abstract

Fatty alcohols are naturally produced hydrocarbon present in all living organisms. They are also used in a series of detergent and cosmetic formulations to provide surfactant properties. These compounds may be sourced from either petroleum or biological materials and are typically disposed of down the drain entering waste water treatment plants (WWTP). The effluent from these works also contains fatty alcohols and these enter the environment with such discharges.

A key question that may be asked by regulators, operators of WWTPs and alike might be what proportion of the fatty alcohols that are present in the environment has arisen from the anthropogenic sources in the catchment. Due to the use of identical compounds in detergent products as are produced naturally, an approach using stable isotopes is required to answer the question. Stable isotopes of carbon and hydrogen provide a fingerprint for the different sources since the chemicals are produced through different chemical or metabolic pathways where the isotopes may be partitioned. A similar approach was used in the UK study which indicated that the compounds in the influent were not the same as the ones in the effluent which in turn were not the same as the ones in the environment.

This study was conducted on a freshwater catchment within the USA. A selection procedure was used to reduce approximately 10000 candidate WWTPs to a few for consideration. The town of Luray, VA was selected as it satisfied the criteria of having clean headwaters, agricultural and forested zones, a town with surface water runoff and a single discharge from an established WWTP. Samples were collected from across the catchment to incorporate all potential sources of fatty alcohols to the Hawksbill River, a tributary of the Shenandoah River. Sales data was purchased that provided quantitative data on the usage of fatty alcohol containing products in the catchment. This data indicated that ~2kg of fatty alcohols may enter the WWTP from this source every day. Samples of all available products were purchased in the local supermarkets.

Analysis of the detergents showed that the majority of the products had fatty alcohols derived from petroleum sources with a few niche products containing biologically sourced compounds. In some cases, these were marketed as “green” products on the label. Reconstruction of the fatty alcohols in the influent on the basis of the sales data indicated a mix of odd and even chain compounds with the C_{12} being the dominant compound. This profile was strongly influenced by the liquid laundry detergents which made up 69% of the total contribution to the total detergent alcohols.

Analysis was conducted on a wide range of environmental samples including agricultural and suburban soils, woodlands, river sediments, road dusts and different stages within the WWTP. All samples were analysed by GC-MS to quantify the individual compounds present and by stable isotope ratio mass spectrometry to determine the $\delta^{13}C$ and $\delta^{2}H$ signature. The results indicated that the agricultural soils and long chain components in river sediments had the most negative values for the $\delta^{13}C$ and were clearly distinguishable from the algal fatty alcohols produced within the river system. These latter compounds were short chain (C_{14} – C_{16}) and had less negative $\delta^{13}C$ values. The road dusts collected throughout the catchment had similar profiles and isotope values to the agricultural soils indicating that the terrestrial plants are a common source for each.

The influent of the WWTP was a mixture of the faecal and detergent sources in a ratio of 75% to 25%. This was the same proportion seen in the UK study. Some of the fatty alcohols may have a stronger detergent source than others but the 25% is for the total. The fatty alcohols in the effluent had different stable isotopic signatures and chain length profiles to the influent and indicate that these compounds are not the same ones that entered the works. These compounds are likely to be derived from bacterial synthesis and recycling with the oxidation (biological) stage of the WWTP. The total quantity of fatty alcohols leaving the WWTP through the effluent pipe was low compared to the UK study with just 32 g per day entering the river. This compares to ~300 g in the UK system where the static population is >15000.

Analysis of the contributions based on the stable isotopes and profiles suggests that of the fatty alcohols present in the river system downstream of the WWTP, 84% were derived from terrestrial plant production, 14% came from *in situ* algal synthesis and 1% was derived from the effluent of the WWTP. However, it must be remembered that the fatty alcohols in the effluent are not the same ones as in the influent and so when considering the detergent input to the river it could be considered as zero.

Introduction

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

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

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

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

It has been shown during Phase 1 and 2 of the studies on fatty alcohols in the environment, which were conducted in the UK, that stable isotopes of carbon and hydrogen can be used to develop a fingerprint for the different source materials (Figure 1). The carbon-13 content of molecules can vary depending on the synthetic pathway which may preferentially favour the lighter (^{12}C) or heavier (^{13}C) isotope. These

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

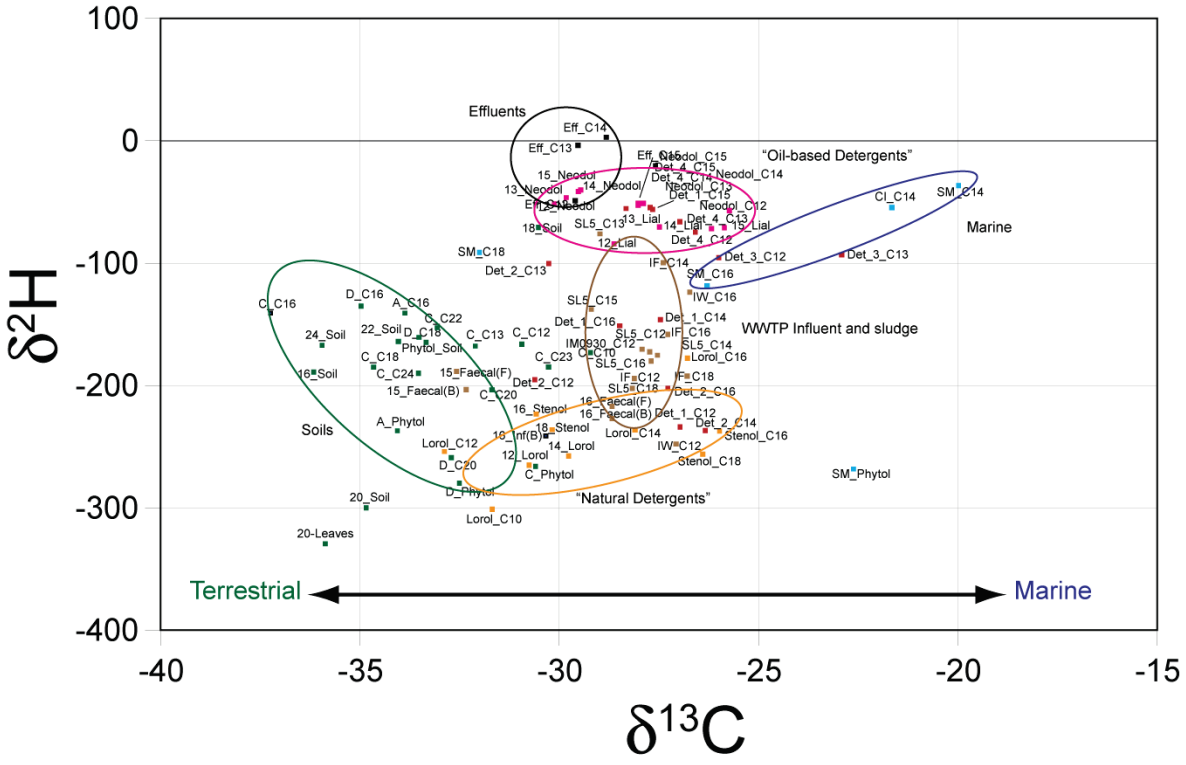

Figure 1. The two dimensional stable isotope ($\delta^{2}H$ and $\delta^{13}C$) signatures for samples from the Phase I and II study. The coloured circles indicate the different sample types – green = terrestrial soils, orange = natural based surfactants and faecal matter, brown = WWTP influent samples and sludge, lilac = oil based surfactants, black = WWTP effluent and blue = marine sediments.

These results shown in Figure 1 demonstrate that the fatty alcohols in the influent to this WWTP are a mixture of faecal matter and naturally derived surfactants on one hand and petroleum based surfactants on the other. The stable isotopes show that effluents have a different signature and are not the same compounds as those that enter the works. The environmental data also indicates that the fatty alcohols in the marine sediments at the effluent discharge point are not the same as those of the effluent or the influent. The implication is that most of the compounds entering the works are degraded or removed with the biosolids and that new ones are synthesised by the bacteria during the secondary oxidation step. These compounds are then diluted and dispersed in the marine system and the compounds in the sediments are from *in situ* marine production.

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

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

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

The Phase 1 and 2 projects were able to discriminate between sources showing that this approach is valid and useable in the context of source apportionment. In Phase 1, the sampling and analysis was conducted to determine if the stable isotopes could distinguish between natural and synthetic fatty alcohols. The fatty alcohol profiles analysed by GC-MS for the synthetic raw materials (Lial and Neodol) are very different from the natural materials as these mixtures are rich in mid-chain branched compounds which are essentially absent from the natural environment and all sewage samples. In all the environmental fatty alcohol analyses reported (Mudge et al., 2008), no mid-chain branched compounds were identified and only the *iso* and *anteiso* moieties were seen. The δ^2H and $\delta^{13}C$ stable isotope values for these two synthetic detergent materials also had different values to each other and samples taken from the WWTP. However, this cannot be said for the two detergent material samples that were derived from palm oil (Lorol and Stenol). Since they have biological precursors, they had similar fatty acid / alcohol profiles as natural materials and also had similar but not identical stable isotope values. Therefore, consumer products made with these formulations will have similar stable isotopic signatures and may be more difficult to distinguish from fatty alcohols in other sample extracts.

This second phase of work provided source apportionment for all the potential sources that may contribute to the marine environment adjacent to the outfall of a WWTP using the stable isotope method and a linear mixing model. The data indicated that the soils were not contributing directly to the WWTP influent although they were to the marine sediments probably through terrestrial runoff. The influent to the WWTP was made of a mixture of faecal matter and natural sourced fatty alcohols on one hand and oil-based surfactants on the other. The most important of the alcohols in terms of surfactant contribution may be the C_{12}. The fatty alcohols in the effluent of the WWTP had different stable isotopic signatures to the other compounds in the analyses and these are probably due to bacterial synthesis based on both their profile and δ^2H values. The marine sediments adjacent to the discharge point did not exhibit fatty alcohols from the WWTP but from natural marine production instead. There was one exception which may be due to outflow from a Combined Sewer Overflow (CSO).

The work reported here was undertaken in the USA were the principal discharge route to the environment is through rivers and streams. These systems differ from the marine environment as the natural inputs have different stable isotopic signatures and the pH of the sea is (usually) more alkaline and the ionic composition is orders of magnitude greater. In many terrestrial environments, the short chain compounds with carbon chains less than 20 are often absent although algae and other aquatic fauna may produce some fatty alcohols in rivers. The detergent range alcohols tend to be in the C_{12} to

C_{18} range and may be distinguishable from natural products by their profile and stable isotopic composition.

Before instigating a sampling campaign, a large number of potential WWTP sites were screened using EPA's Permit Compliance System on the basis of the following criteria:

1. denoted as sewage treatment facilities (SIC code 4952)
2. having flow greater than 1 MGD ("major" facilities)
3. having 10 or less single-event effluent exceedances *e.g.* measured concentration for single effluent parameter greater than permitted value
4. having no formal enforcement actions in the last five years. Formal enforcement actions typically are levelled against facilities having persistent, significant issues with effluent exceedances. Violations that are minor, short in duration or quickly corrected by the facility typically do not warrant formal enforcement action.
5. currently not in significant noncompliance (*e.g.* not having an exaggerated level of effluent exceedances or reporting violations such as a failure to file appropriate paperwork).

Additionally, all chosen sites had to utilise the activated sludge or oxidation ditch processes. This generated a list of ~350 locations across the USA. The site performance data were compared with visual observations using Google Maps and Google Earth to select a short list of sites that fulfil the secondary set of criteria:

1. a silty river as the fine grain sediments have greater surface area and usually a greater lipid loading making measurement of the stable isotopes easier,
2. upland headwaters with no development or only small sewage related inputs to provide a "clean" signature,
3. forests with ideally both coniferous and deciduous trees,
4. agriculture land with upland grazing and lowland arable,
5. a well defined town / city with urban surface water runoff into the river prior to any WWTP facility,
6. a downstream WWTP,
7. close proximity to a laboratory to facilitate extraction of the fatty alcohols obviating the need to ship many kilograms of sediment and water back to the UK.

After reviewing the remaining 60 sites, the town of Luray in Virginia appeared to be as close to the ideal scenario as possible and so a reconnaissance visit was undertaken by EA Engineering, Science, and Technology for the SDA (see Factsheet Luray Site Visit report). Permission was sought and granted by the WWTP operators.

Materials and Methods

System Overview and Sampling Plan

The project site is located in the Hawksbill Creek watershed of Page County, Virginia. The 57,000-acre watershed is rural with forested and agricultural lands making up 62 and 33 percent of the watershed respectively. The remainder of the watershed consists of developed lands (USEPA, 2004). The Hawksbill Creek, a small tributary of the Shenandoah River, extends approximately 17 miles from its headwaters in Shenandoah National Park. The watershed of Hawksbill Creek consists of approximately three miles of undeveloped wooded areas from the headwaters, approximately eight miles of agricultural and low density development areas, approximately two miles of moderately developed areas associated with the Town of Luray, Virginia. The remaining two miles of the Hawksbill Creek receive discharge from a WWTP before terminating at the confluence with the Shenandoah River.

The project site encompasses areas within the Hawksbill Creek watershed starting at the headwaters of Little Hawksbill Creek in Shenandoah National Park. After the confluence of Little Hawksbill Creek and Hawksbill Creek in the town of Stanley, Virginia, the sampling sites follow Hawksbill Creek through agricultural and low-density development areas east of Stanley, Virginia, urban areas associated with Town of Luray, Virginia, and the discharge zone of a WWTP just outside of Luray, Virginia.

Sampling locations for the project were selected in the field for each watershed segment identified during the reconnaissance survey of Hawksbill Creek watershed. The watershed has been divided into six zones that will each be sampled (Figure 2 – Figure 7). These zones and their role in the study are as follows:

- **Little Hawksbill Creek Headwaters Zone:** Samples from this zone characterize the influence of natural sources of fatty alcohols in relatively undeveloped forested areas of Shenandoah National Park.
- **Upper Valley Zone and Middle Valley Zone:** Samples from these zones characterize sources of fatty alcohols associated with agricultural and low-density residential land use.
- **Town of Luray Zone:** Samples from this zone characterize urban and agricultural sources of fatty alcohols.
- **WWTP/Downstream:** Samples of influent, effluent and processes within the WWTP will provide an understanding of the anthropogenic inputs of fatty alcohols through wastewater, transformation processes within the plant, and eventual contributions through effluent.

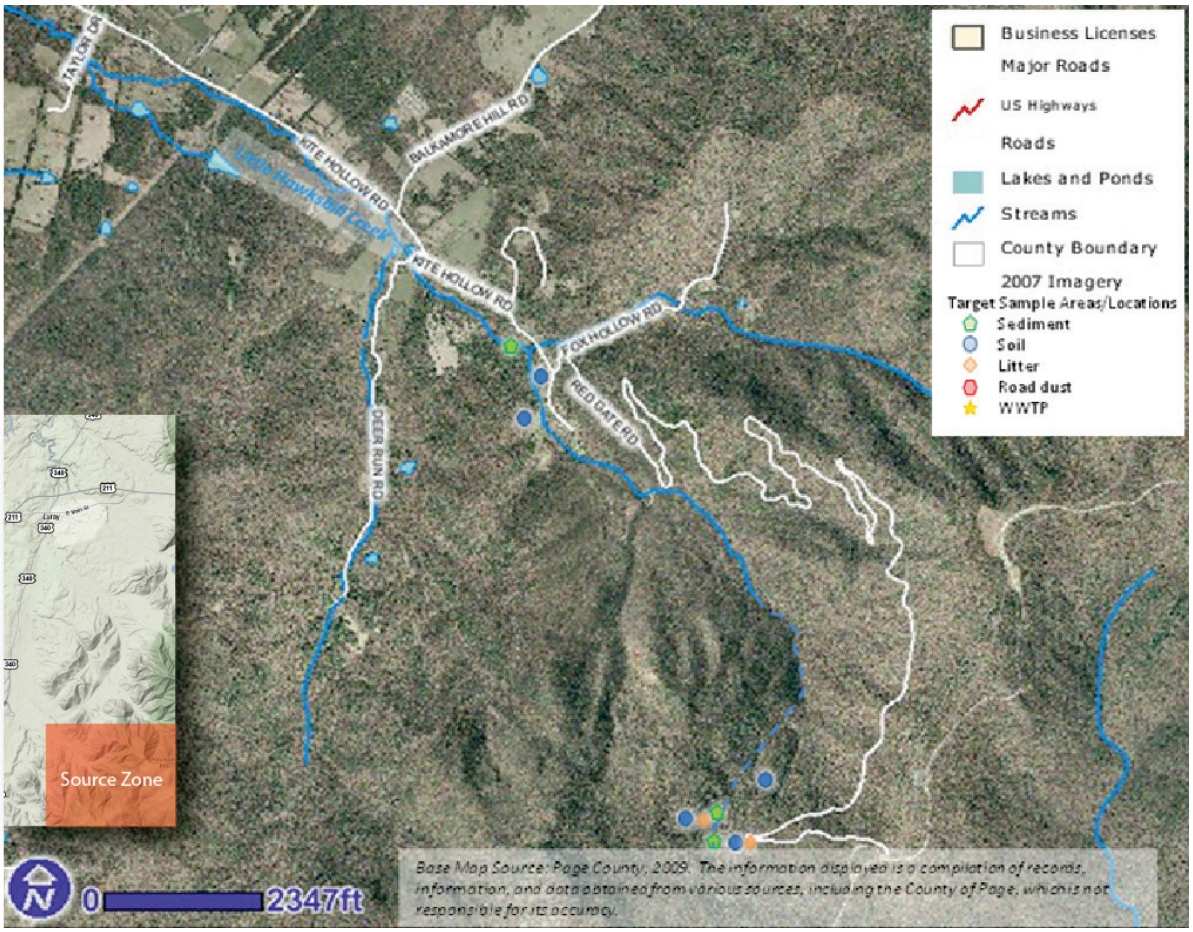

Figure 2. Sampling locations in the headwaters region. This is the source of the Hawksbill Creek in the Shenandoah National Park. The creek flows from South to North.

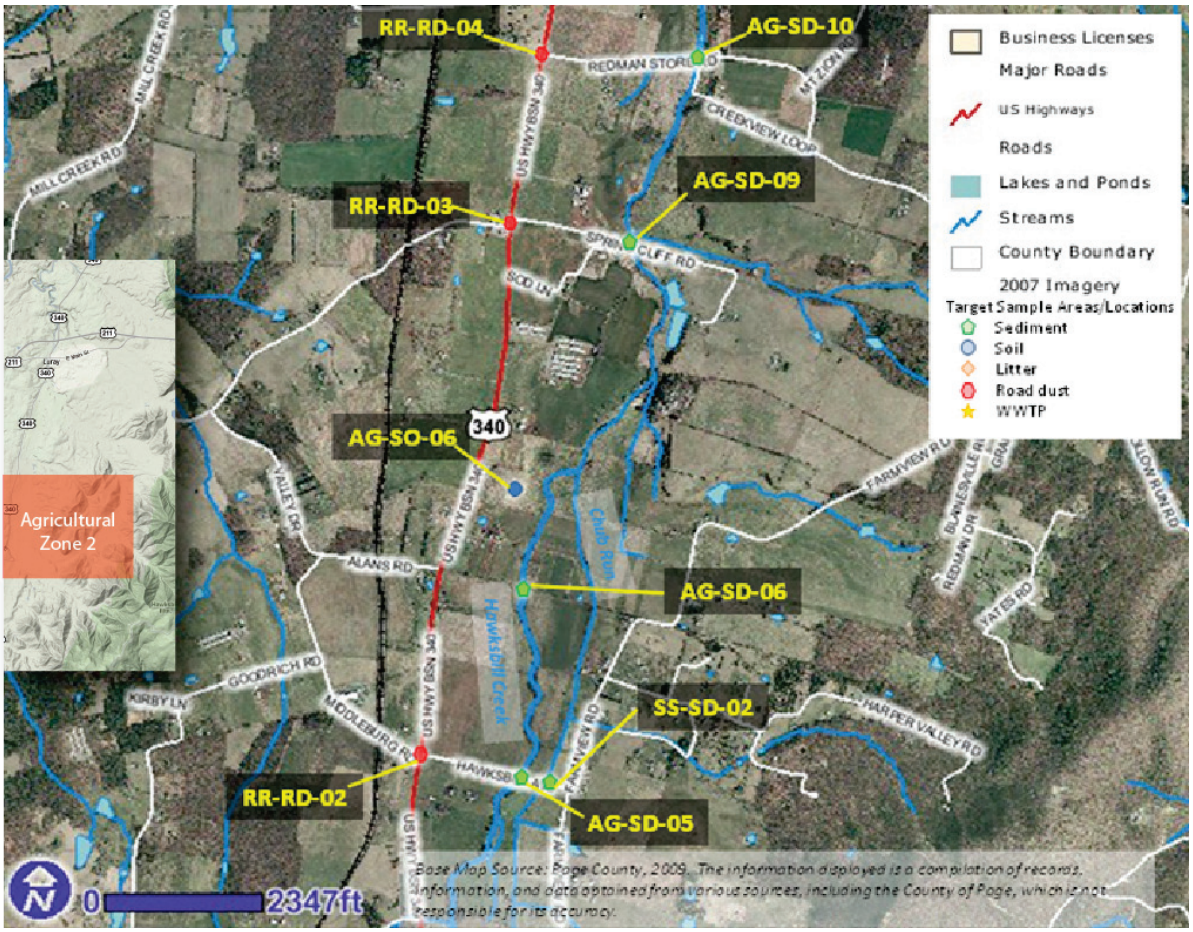

Figure 3. Sample locations in the mid-section of the agricultural catchment zone.

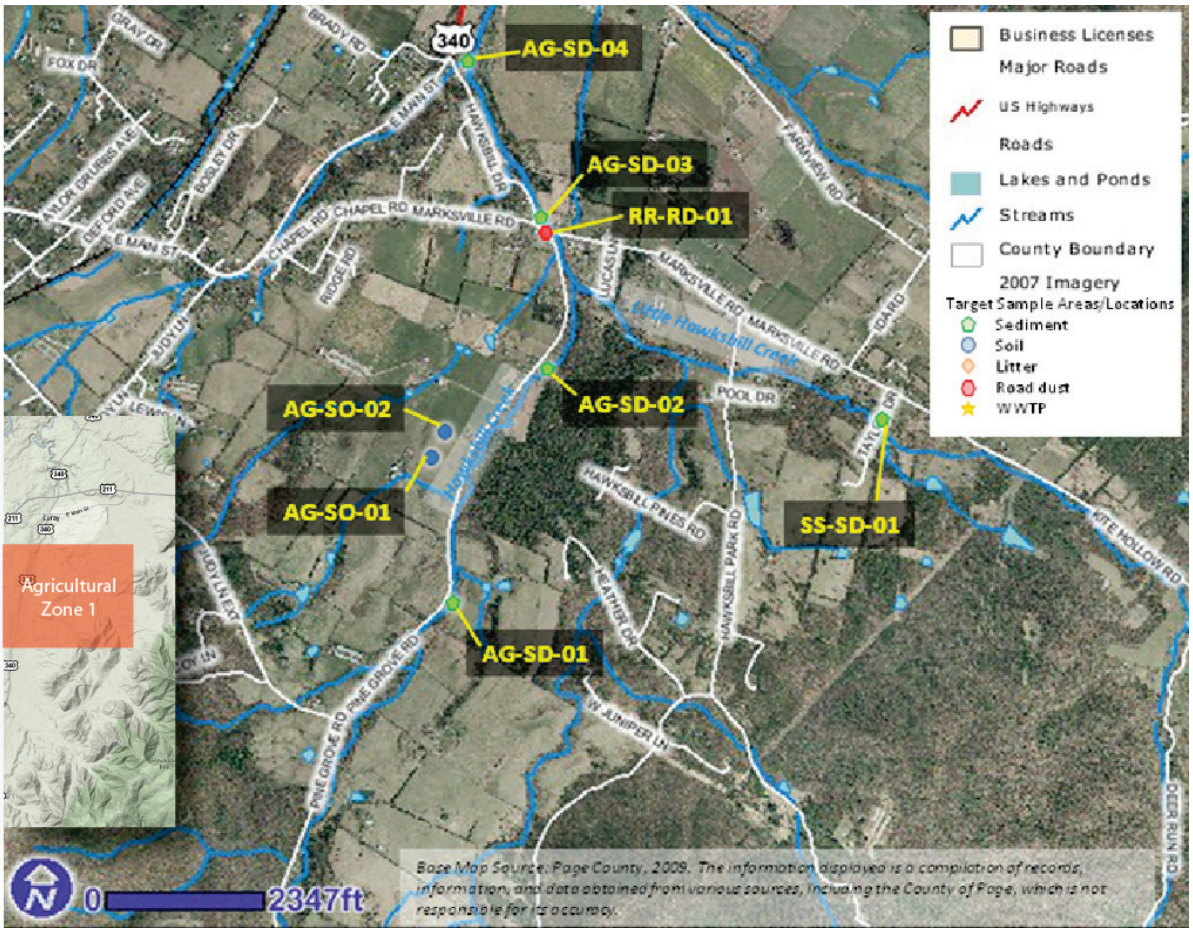

Figure 4. Sample locations in the agricultural zone above the town of Luray.

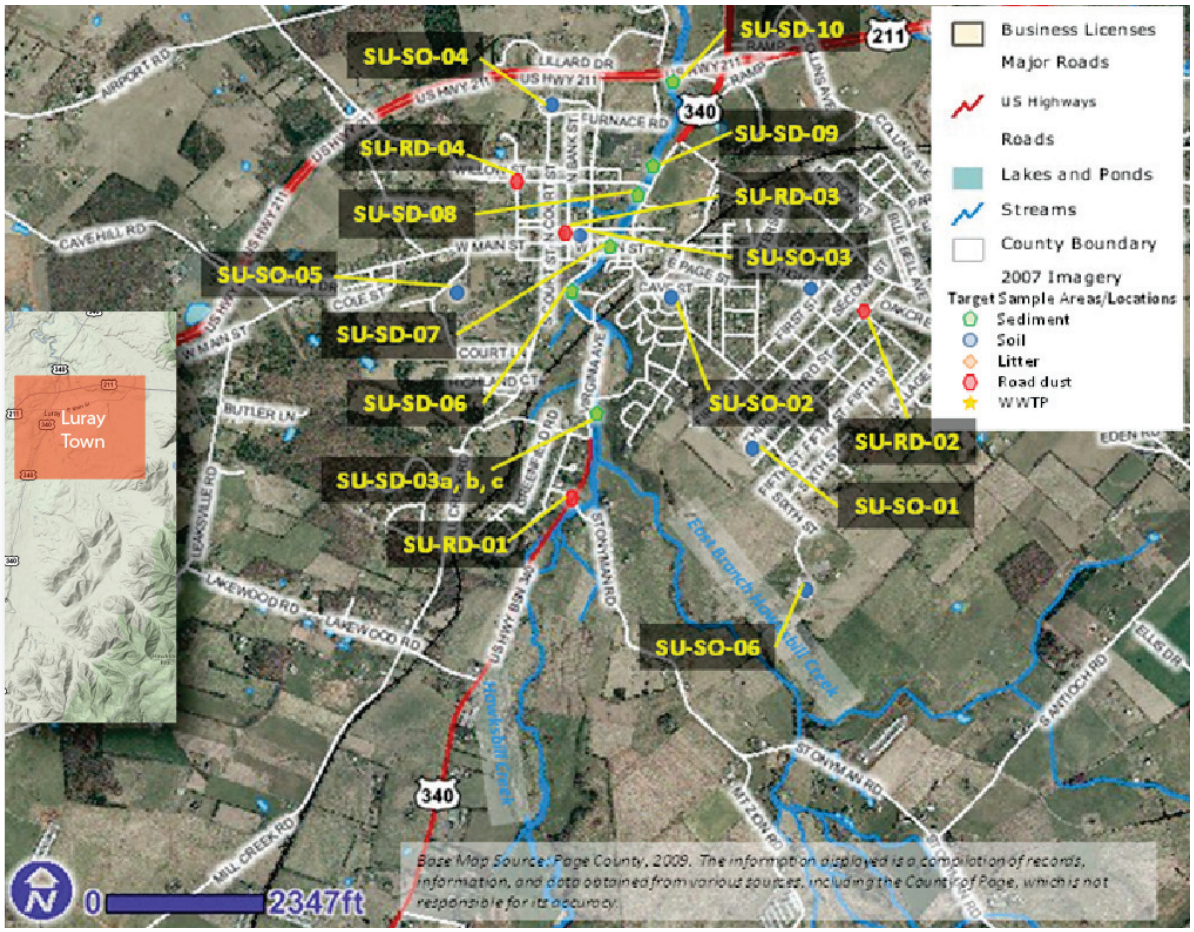

Figure 5. Sampling locations around the town of Luray above the WWTP.

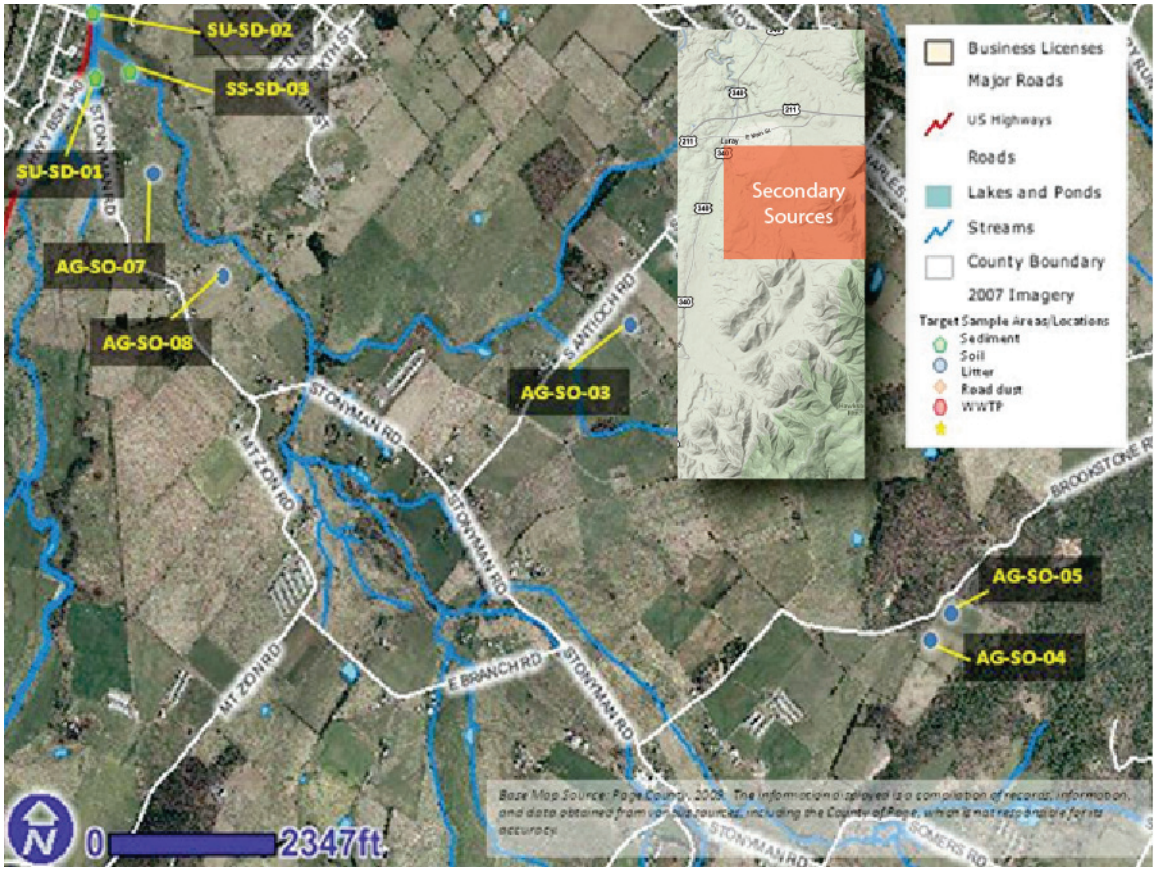

Figure 6. Sampling location for soils on the outskirts of Luray. This zone also includes a secondary stream source.

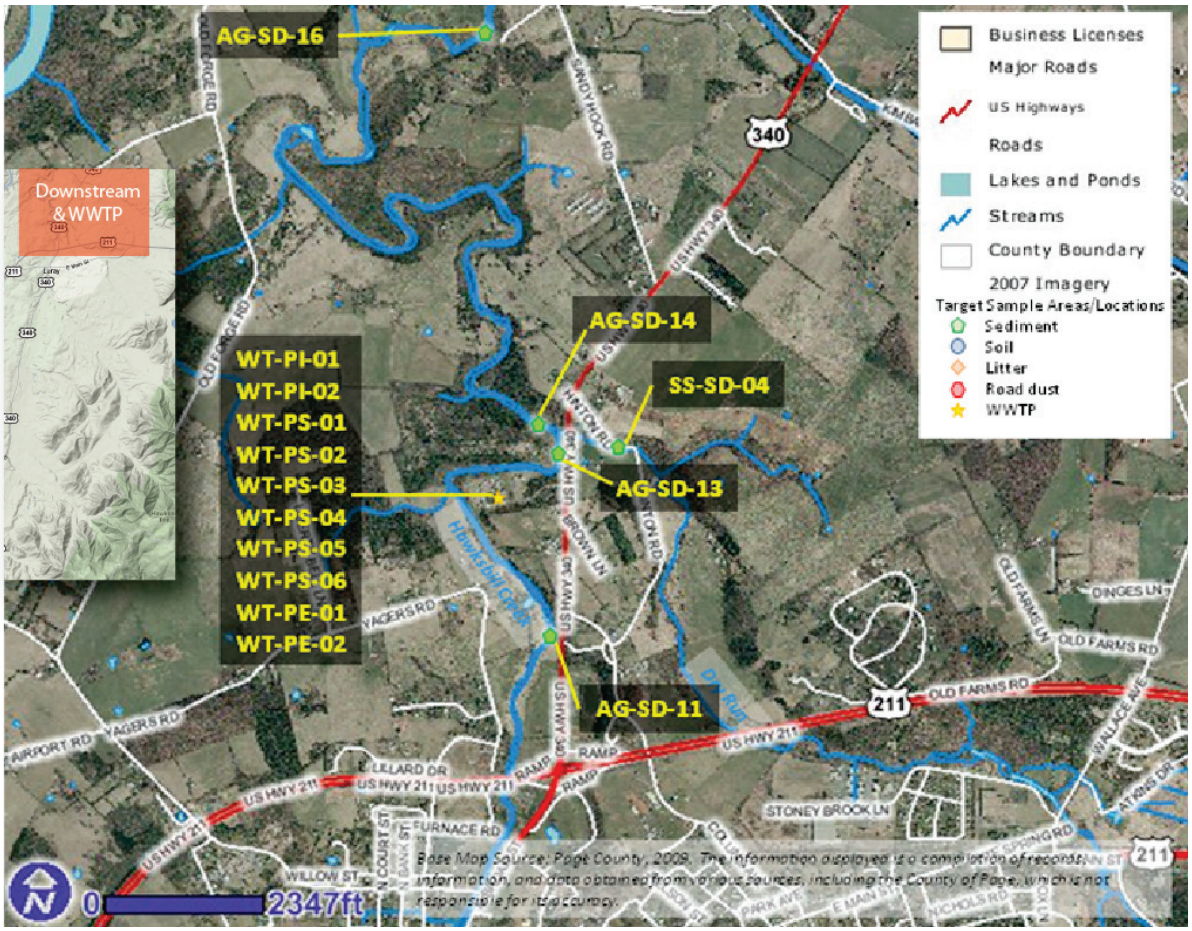

Figure 7. Sampling locations at the WWTP and downstream of the town of Luray.

The target quantity and type of samples collected at each of the Hawksbill Creek watershed zones is shown in Table 1. A few actual sample locations were altered or omitted in the field based on site conditions or access.

Table 1. Distribution and target number of sample types to be taken within the Hawksbill Creek watershed

SOURCE (CATEGORY)	SAMPLE TYPE						
	Forest or Rural Soils	Urban Soils	Sediment	Leaf Litter	Roads	WWTP	Total Samples
Headwaters (HW, WL)	8		4	4			16
Upper Valley (AG, RR, SS)	4		5		1		10
Middle Valley (AG, RR, SS)	4		7		3		14
Luray (SU, SS, RR)	2	6	11		4		23
WWTP & Downstream Zone (WT)			7			10	17
Total Number of Samples	18	6	34	4	8	10	80

Watershed Categories:

HW = Headwater
WL = Woodland
AG = Agricultural
RR = Rural Road
SS = Secondary Source
SU = Suburban and Urban Zone
WT = Wastewater Treatment Plant

Full details of the sampling are provided in the *Field Report* from EA Engineering, Science, and Technology and Mudge (2009).

Potential Source Materials

Final products were selected after a quantitative survey (Information Resources, Inc.) of the different brands of liquid detergents and soaps available in the major supermarkets (*e.g*. Food Lion) in the Page County Region. It was assumed this would also be appropriate for the smaller compartment of Luray, Virginia, although this was verified by searching for the brands on the supermarket shelves. On the basis of these surveys, 34 solid and liquid market-leading formulations containing fatty alcohols were selected, purchased in the stores and blind sub-samples provided for analysis. Only one brand of liquid laundry detergent that was in the top 10 (position 9) of the wider survey was not available in the local stores. The fatty alcohols in these products were extracted as their alkyl iodides using the following methodology:

1. 100 µl of the liquid samples or 100 mg of the solid samples were added to a Reactivial together with 2 ml of 55% hydrogen iodide. The mixture was shaken and heated to 130°C for 100 min with intermittent agitation.
2. After cooling, the mixture was transferred to a 100 ml separation funnel with 2 x 2 ml water and 3 x 3 ml pentane washes to ensure all materials were transferred. The mixture was vigorously shaken and sufficient 0.1 N sodium thiosulphate was added to mop up any free iodine thus making the solution colourless. The mixture was shaken for a further 30 s.

3. The lower aqueous phase was runoff and the upper pentane phase retained and rewashed with 15 ml of sodium thiosulphate.
4. In the case of several detergents, this resulted in a clear pentane phase within a few minutes. This was then separated from the lower aqueous phase, re-washed with water (2 x 15 ml), taken to dryness under a stream of nitrogen and re-dissolved in 1 ml of hexane.
5. For those detergents that produced an emulsion on shaking which did not separate cleanly even after 24 h, the lower aqueous phase was removed and the emulsion layer was transferred to a glass centrifuge tube and spun briefly at 2500 rpm. The now clear pentane phase was removed by pipette and taken to dryness under a stream of nitrogen. The sample was then re-dissolved in 1 ml of hexane.

River Sediments, Road Dusts and Soils

The selected watershed of Hawksbill Creek, Va was divided into distinct geo-morphological sectors on the basis of slope and land use (see Figures 2 – 7). The screening programme identified a number of key locations where fatty alcohols may enter the river and these were selected for sampling although with regard to the agricultural soils, the exact field sampled was not critical.

Soils

Soil samples were collected from land that would potentially contribute to the Hawksbill Creek, Va. Soils were collected as surface scrapes from agricultural fields and woodland. The location of the samples can be seen in Figures 2 – 7 and photographs can are in the Appendix. In each case, ~200 ml of soils was collected. The river sediment samples were collected in glass jars from the bank in a similar fashion. Where possible, fine-grained sediments were targeted as they have higher organic matter content; river gravels have a very low organic matter content making it difficult to identify the compounds present and even harder to generate a stable isotope signature. The moisture content of the samples can be seen in Table 2.

Table 2. Key sample data. One of the soil locations (AG-SO-06) was sampled three times from within the same $1m^2$ and one river sediment site (SU-SD-03) was sampled in the same manner.

Sample Code	Wet wt (g)	Dry wt (g)	Percent water	ASE Extracted wt (g)
Agricultural zone – river sediments				
AG-SD-01	127.3	76.5	39.9	39
AG-SD-02	128.7	32.6	74.7	17.6
AG-SD-03	196.8	88.3	55.1	30.5
AG-SD-04	115.2	38.1	66.9	26.6
AG-SD-05	117.6	32.2	72.6	21.6
AG-SD-06	111	67.2	39.5	33.4
AG-SD-09	120.7	35.6	70.5	23.9
AG-SD-10	160.8	63.1	60.8	25.7
AG-SD-11	181.9	152.3	16.3	54.1
AG-SD-13	134.6	41.6	69.1	26.7
AG-SD-14	198.3	171.4	13.6	51
AG-SD-16	113.7	36.0	68.3	24
Agricultural zone – soils				
AG-SO-01	122.1	90.3	26.0	35.9

AG-SO-02	129.5	103.9	19.8	37.6
AG-SO-03	156.9	127.2	18.9	35
AG-SO-04	129.6	102.6	20.8	43.2
AG-SO-05	139.6	108.9	22.0	34.7
AG-SO-06A	110.9	96.9	12.6	40.7
AG-SO-06B	131.9	116.3	11.8	37
AG-SO-06C	110.1	92.1	16.3	40.2
AG-SO-07	130	101.2	22.2	32.7
AG-SO-08	172.1	130.7	24.1	32
Rural Roads – road dusts				
RR-RD-01	189.7	189.3	0.2	55.6
RR-RD-02	249.3	248.7	0.2	59.2
RR-RD-03	210.6	209.8	0.4	51.2
RR-RD-04	269.3	268.3	0.4	53.6
Secondary Sources – river sediments				
SS-SD-01	290.5	231.1	20.4	48.2
SS-SD-02	129.2	32.2	75.1	21.4
SS-SD-03	221.8	179.7	19.0	54.6
SS-SD-04	160	120.5	24.7	44.7
Suburban Zone – road dusts				
SU-RD-01	122.3	121.3	0.8	40.9
SU-RD-02	177.4	175.6	1.0	46.3
SU-RD-03	240	239.7	0.1	59.7
SU-RD-04	271.1	271.0	0.0	52.9
Suburban Zone – river sediments				
SU-SD-01	228.2	158.4	30.6	43.5
SU-SD-02	244.2	199.1	18.5	56.1
SU-SD-03A	176.4	55.2	68.7	22.4
SU-SD-03B	201.8	122.1	39.5	44
SU-SD-03C	169.6	50.0	70.5	23.9
SU-SD-06	129	97.6	24.3	48.8
SU-SD-07	100.7	25.0	75.2	19.8
SU-SD-08	149.2	112.0	24.9	47.7
SU-SD-09	117.1	48.3	58.8	33.2
SU-SD-10	105.3	28.5	72.9	20.7
Suburban Zone – soils				
SU-SO-01	115.2	85.6	25.7	37.9
SU-SO-02	168.8	143.2	15.2	33.7
SU-SO-03	105.6	86.4	18.2	39.4
SU-SO-04	152.9	123.1	19.5	42.8
SU-SO-05	142.2	125.6	11.7	43.8
SU-SO-06	100	77.4	22.6	31.1
Woodlands – river sediments and soils				
WL-SD-02	238.1	187.8	21.1	46.5
WL-SO-04	79.8	33.8	57.6	15.1
WL-SO-05	130.8	101.4	22.5	35.9
WWTP – sludge				
WT-PS-03	50.1	14.6	70.9	15.7

All samples were kept in a cool box with ice packs for transport to the VIMS laboratory at Gloucester Point, Va. Samples were extracted by Accelerated Solvent Extraction (ASE-200, Dionex). The amount of sample that was extracted (Table 2) was governed in part by the water content of the sample which in

turn is controlled by the grain size distribution. Samples with a high water content were fine grained which led to smaller packed weights in the extraction tube. The relationship for the majority of the samples can be seen in Figure 8.

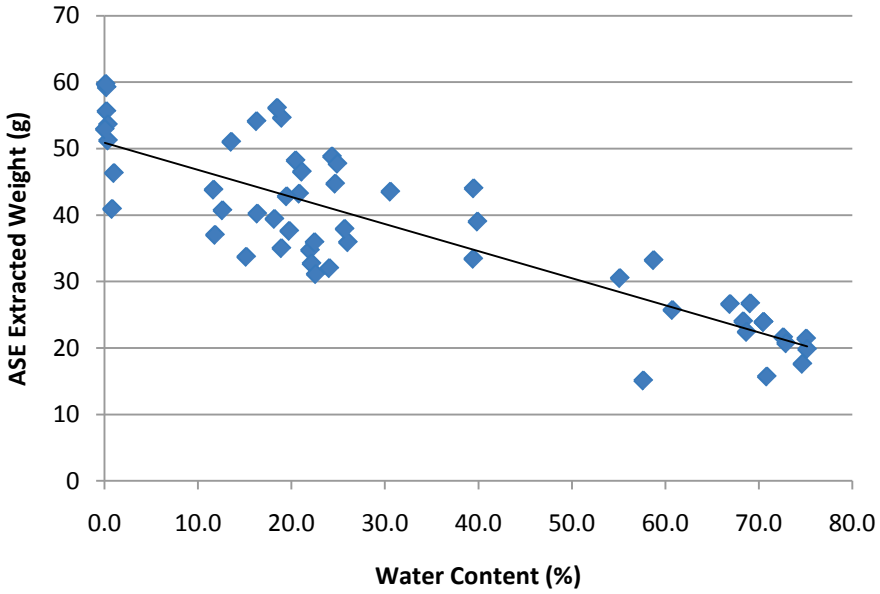

Figure 8. The relationship between water content and extracted weight excluding the headwaters samples analysed by VIMS. Gravelly samples had low water contents and a high mass in the extraction tube; the organic matter content of such samples is likely to be low except for road dust.

1. Approximately 100 – 200 g wet weight of each sample was weighed accurately to one decimal place and placed on aluminium foil and dried at 80°C for 24 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 38 g) and settled with a steel spatula. An internal standard was added (1.00 ml of a 1.00 $mg.ml^{-1}$ 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 200; Sunnyvale, CA) at 100°C and 68 atmospheres using dichloromethane (DCM) : hexane (1:1), employing two 5 minute extraction cycles with DCM : hexane followed by a 60% vessel flush (a solvent flush equivalent to 60% of cell volume is passed through the cell).
3. The DCM : hexane extraction solvent was taken to dryness under a stream of N_2 in a water bath at 60°C. When the volume was ~1 ml, the sample was transferred to a small vial, taken to dryness under N_2 and returned to the UK for subsequent analysis.
4. The samples were re-dissolved in DCM : hexane, transferred to a 14 ml vial, taken to dryness, re-dissolved in 6%KOH in methanol and heated to 60°C for two hours to saponify the lipids. After cooling, the free lipids were extracted into hexane twice, the solvents combined and taken to dryness again.

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

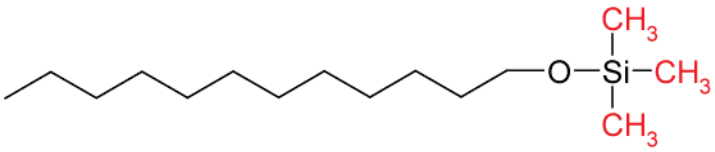

Figure 9. The atoms highlighted in red have been added as part of the TMS group and will contribute to the overall $\delta^{13}C$ and $\delta^{2}H$ values. Therefore, a correction needs to be applied to calculate the original molecule values.

Waste Water Treatment Plant Samples

The majority of the samples collected within the WWTP were liquids with suspended solids. The only exception was the sludge sample (WT-PS-03) collected and processed in a similar manner to the river sediments and soils detailed above. Samples were collected at several points within the WWTP and at different times to assess the temporal variability. A schematic showing the position of samples within the treatment cycle at the WWTP can be seen in Figure 10.

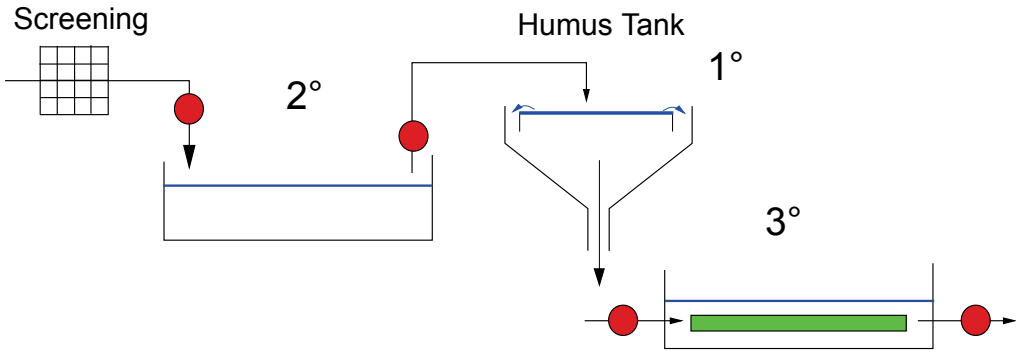

Figure 10. Schematic layout of the Luray WWTP. The layout is unconventional without primary settlement before the oxidation ditch. The residence time in the oxidation ditch is in the order of 48 hours. The majority of the solids are removed after this stage and before UV irradiation. The process has been modified over time due to the changing nature of the influent. The red spots indicate the sampling locations. *N.B. the WWTP is currently undergoing improvement works to increase the removal of nutrients from the final effluent.*

The Luray WWTP has evolved over the years due to the changing nature of the influent. A large denim jeans manufacturer has premises in the catchment and they used to cut the cloth on site and wash out the starch before distribution. This led to very high Biochemical Oxygen Demands (BOD) and the operators of the WWTP had to add nutrients to encourage degradation of these materials. The cutting of the cloth has now been transferred to a different location and the premises are used solely for distribution (Tom Brown, Town of Luray WWTP, *pers. comm.*).

An aerial view (from Google Earth) showing the treatment steps and the sample locations is shown in Figure 11. Sub-samples of the influent were collected from the time-weighted aggregate sample taken by the WWTP staff for other analyses. This combined feed to the oxidation ditch was taken on Tuesday, Wednesday and Thursday at 07.00 and representing the previous 24 hours. Exact dates can be seen in Table 3.

Post oxidation ditch and post settlement samples were taken on two consecutive days from the locations indicated on Figure 10. Final effluent was collected on Tuesday, Wednesday and Thursday at 09.00 from the effluent pipe discharging into the Hawksbill Creek. One sludge samples was collected on the Tuesday. At this works, the sludge samples are only processed when the accumulated volume demands treatment and the dewatered dried materials are usually applied directly to agricultural land as a fertiliser. Photographs of the key plant stages can be seen in the appendix.

Table 3. Key sample data for WWTP. Three replicates of the final effluent samples collected in September were taken. The sludge samples are expressed in dry weight.

Sample	Date	Volume (ml) or *Dry Weight (g)
Influent	19th May 2009	980
Influent	20th May 2009	970
Influent	21st May 2009	980
Effluent	19th May 2009	960
Effluent	20th May 2009	960
Effluent	21st May 2009	940
Sludge*	19th May 2009	15.7
Post Oxidation	19th May 2009	960
Post Oxidation	20th May 2009	940
Post Clarifier	19th May 2009	950
Final Effluent (am)	14th September 2009	2 x 40000 1 x 45000
Final Effluent (noon)	15th September 2009	2 x 40000 1 x 48000
Final Effluent (pm)	16th September 2009	2 x 40000 1 x 48000

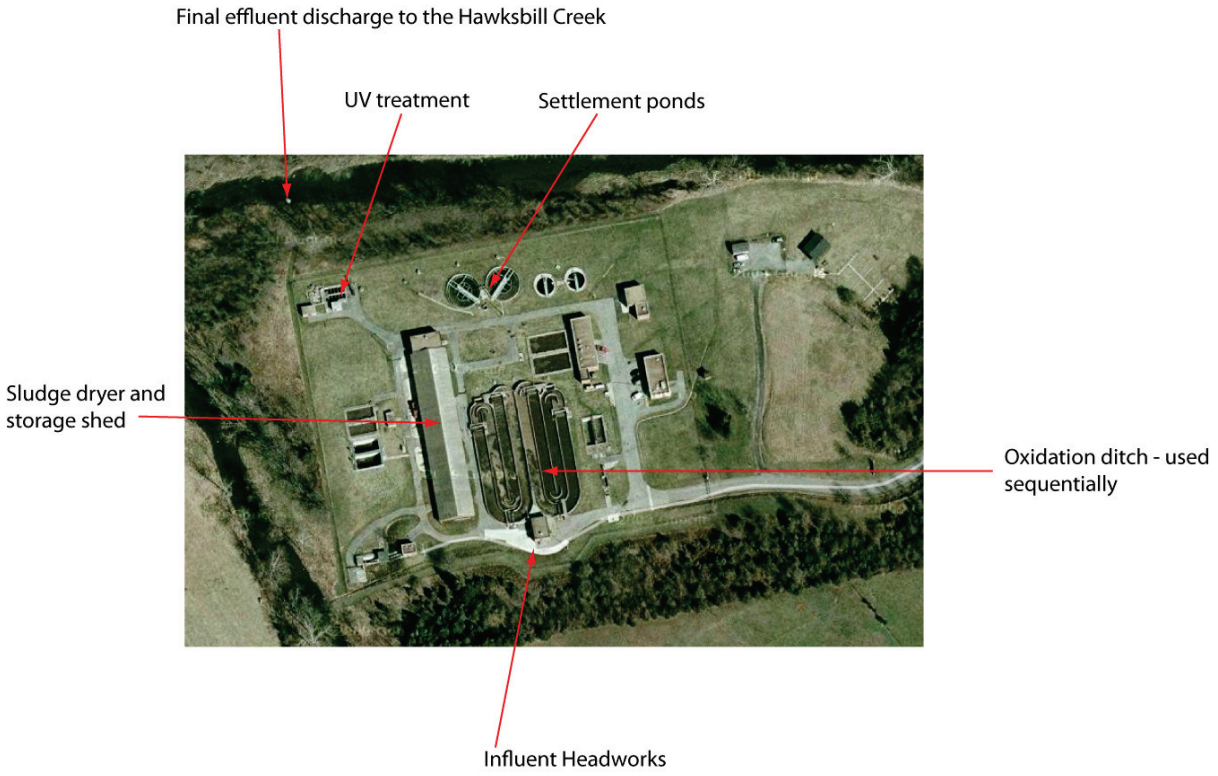

Figure 11. An aerial view of the Luray WWTP with the location of the different processes.

The extraction method for the liquid samples required a different approach to that of the solid samples. The protocol used was as follows:

1. On return to the laboratory, 15 g of KOH was added to one litre of the liquid samples together with 1 ml of the internal standard. The mixture was shaken and left for 24 h at room temperature to allow an *in situ* saponification to occur. The sample was shaken periodically throughout the 24 h period.
2. The whole sample was poured into a 1 l separating funnel and ~100 ml of hexane was added. The sample was shaken, allowed to settle and the lower aqueous phase drawn off. The hexane phase was collected and the aqueous phase returned to the separating funnel.
3. A further 100 ml of hexane was added to sample and re-extracted. The hexane phases were combined.
4. The samples were reduced to <5 ml through rotary evaporation and finally taken to dryness under a stream of nitrogen.
5. The lipids were derivatised with BSTFA for 2 h at 60°C, taken to dryness again before being re-dissolved in 1 ml of hexane.

After analysis of the liquid effluent samples (see Results), further sampling was needed to characterise the discharge from the WWTP into the Hawksbill Creek as all compounds were below the limit of detection. A method using a deep torturous path fibre filter stack was used. These have routinely been used for collecting particulates at low concentrations in large volumes of water and for clarifying

drinking water sources. This system was tested prior to deployment by mixing a small volume of fine grain marine mud into water and then retrieving the particles in the filter. The filtrate was clear to the naked eye indicating substantial removal of the particles by the fibrous filter.

Larger sample volumes were processed using this filtration technique (Figure 12). In this arrangement, at least 40 litres of final effluent after UV irradiation was collected in a pre-cleaned eight litre bucket and poured slowly through a deep nylon filter and a 20 µm membrane sheet. The filtrate was discarded and the solid materials retained in both filters were sealed in zip-lock bags, refrigerated and returned to the UK for extraction. In this case, the filters were cut up, placed in round bottom flasks, 1 ml of internal standard was added and refluxed in 6% KOH in methanol for four hours. The final liquor was allowed to cool, centrifuged to remove any solid particles and hexane extracted as previously. The concentrated lipid fraction was derivatised with BSTFA as detailed above.

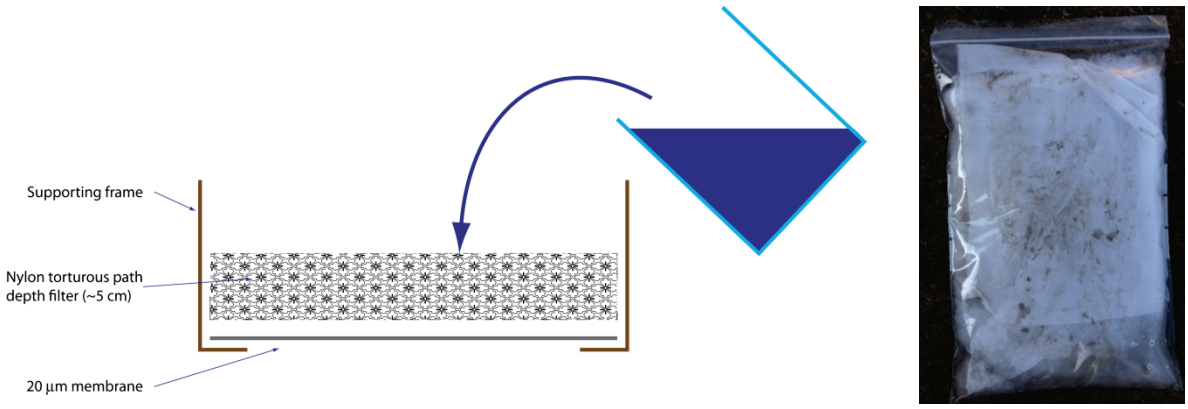

Figure 12. Filtration arrangement for final effluent samples during the September sampling. An exposed sample can be seen after collection in the right-hand photograph.

Analysis

Gas Chromatography – Mass Spectrometry

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

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

Compound Specific Stable Isotope Ratio Mass Spectrometry

All samples were taken to the Scottish Crop Research Institute in Dundee, Scotland for analysis on a Thermo Delta V Plus Stable Isotope Ratio Mass Spectrometer. For each sample, 1 µl was injected for carbon-13 and 3 µl for hydrogen-2 analysis into a split – splitless port under the following conditions:

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

Multivariate Statistics

Where appropriate, the data were investigated by Principal Components Analysis (PCA) using the SIMCA-P software from Umetrics. Data were used as proportions to remove any concentration effect and may be log transformed if the distributions were skewed.

Results

General

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

Fatty Alcohol Profiles and Concentrations

Standards and Waste Water Treatment Plant

The GC-MS trace for the standard and one of the samples can be seen in Figure 13. In the standard, the underivatised 2-OH dodecanol can be seen together with the TMS derivatised form. The mass spectrum of the TMS form is also included showing the principal fragment at 117 *m/z* due to the loss of the TMS – O – $CHCH_3$ fragment. The M^+ - 15 (loss of a methyl group) is also a diagnostic ion and usually used in quantification.

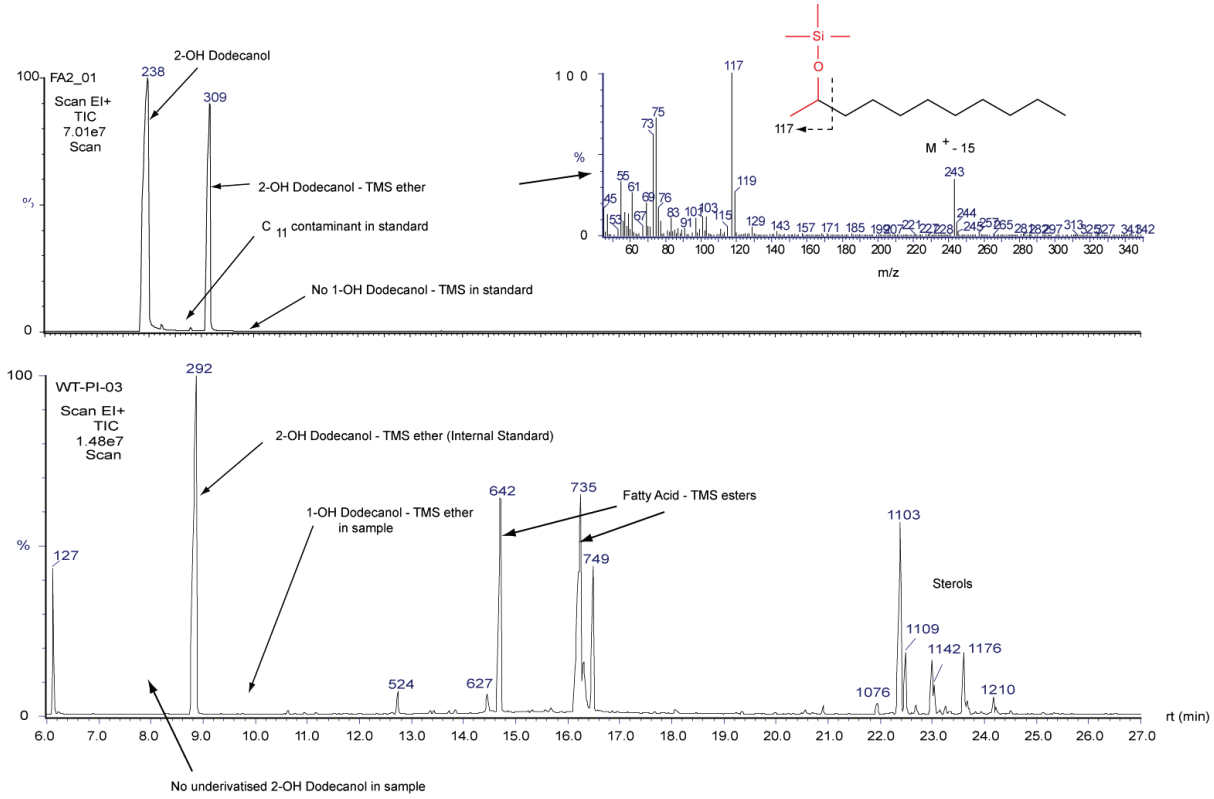

Figure 13. The GC trace of the part derivatised standard in the upper panel together with the mass spectrum of the TMS ether. The lower panel shows one of the samples including the derivatised standard.

As well as the expected C_{12} compounds in the standard, there was also a C_{11} fatty alcohol (1-undecanol as its TMS ether) and another unidentified compound which may be 3-dodecanol. There was no 1-dodecanol as its TMS ether in the standard and so no contribution to any that may be in the environmental samples. The environmental sample shown in the lower pane indicates no underivatised

2-dodecanol indicating complete derivatisation of the standard, a hindered secondary alcohol. There is also a good separation in time between the primary and secondary alcohols and, therefore, no interference making calculation of the areas relatively easy.

The fatty alcohols of interest elute between 9 and 27 min with the sterols eluting between 22 and 25 min. In the sample shown, peaks of 5β-coprostanol (1103), the main faecal stanol of humans, cholesterol (1142) and 24-ethyl coprostanol (derived from plant matter in the human gut) at scan number 1176 can be clearly seen. A small peak of the terrestrial plant sterol sitosterol (24-ethyl cholesterol) can be identified at scan number 1210 (24.25 min). In these samples, fatty acids could also be identified including the 16:0 (palmitic acid) and 18:1ω9 (oleic acid).

The initial effluent samples collected in May had no discernable compounds above the background. The internal standard was present indicating a good recovery. Therefore, a second sampling round for the effluent was undertaken in September involving filtration of 40+ litres through a 20µm filter. A comparison of the two effluents can be seen in Figure 14. In at least three of these samples, an indigo colour could be discerned suggesting a contribution from denim dyes.

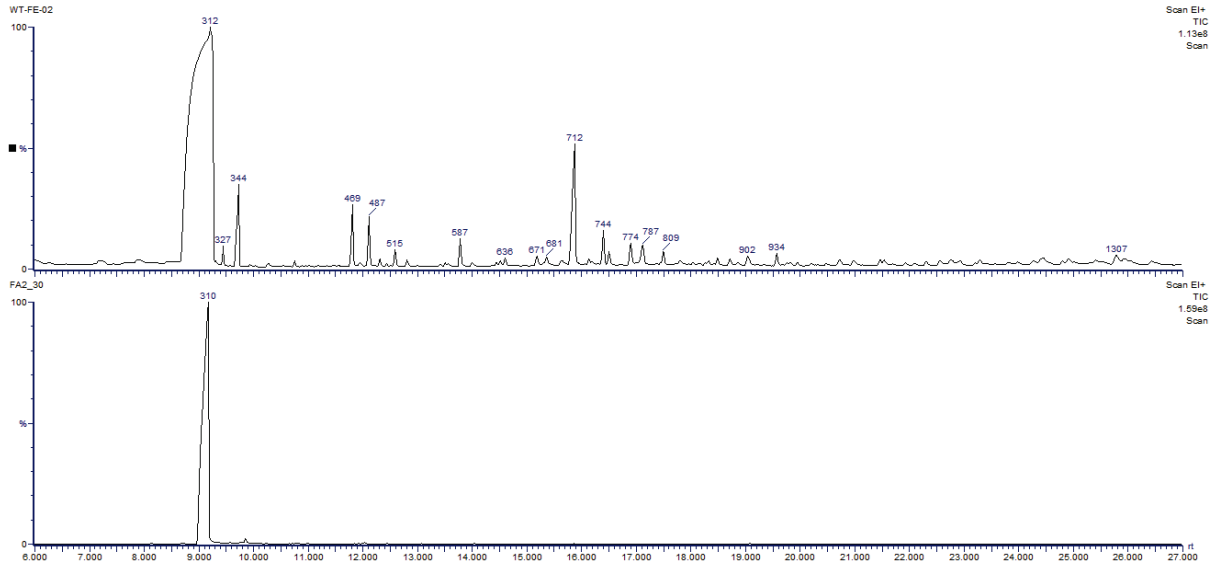

Figure 14. A comparison between the initial sample results based on a liquid sample (1 litre, lower trace) and a reflux of the suspended solids retained on a 20µm filter (40+ litre, upper trace). The final volume of the upper trace has been reduced to ~50 µl to aid in the quantification of the fatty alcohols. This represents a concentration factor of 800000x.

The mean fatty alcohol profiles for influent samples collected from the WWTP can be seen in Figure 15. The GC-MS traces were confounded by the presence of fatty acids, notably 16:0 and 18:1ω9, making quantification of the smaller odd chain compounds more difficult. There are few long chain compounds in these influent samples and a significant amount of bacterial derived compounds such as odd chain or branched fatty alcohols. However, the traces are dominated by the 18 carbon fatty alcohol which was

>40% of the total fatty alcohols present. This is different to the case of the influents to the UK WWTP where the C_{12} dominated (Mudge, 2009).

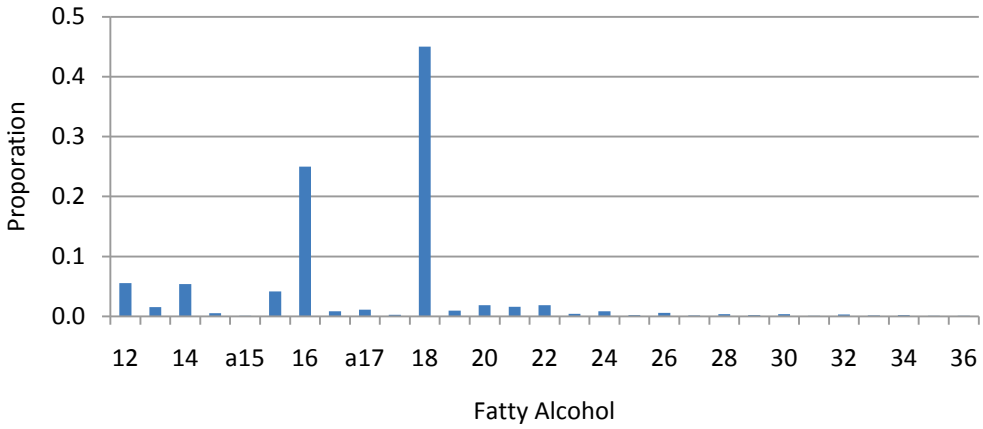

Figure 15. The mean fatty alcohol profile for the three influent samples. There are few long chain compounds present in these samples suggesting little soil entrainment at the time of sampling.

The fatty alcohol profiles of the WWTP influent are dominated by the short chain compounds. The profile is weighted towards the even carbon short chain compounds with relatively few plant derived long chain compounds present. This is typical of animal and bacterial derived material. The total concentration of fatty alcohols in the samples was variable across the WWTP and may reflect the concentration of suspended solids in each sample. The breakdown of concentrations for each fatty alcohol in each sample may be found in the Appendix.

The effluent profile (Figure 16) is different from the influent; the main fatty alcohol in these samples was phytol which was derived from algae growing within the discharge stream and sloughing off into the sample bucket. Of the remaining straight chain alcohols, the dominant alcohol was the C_{12} followed by the C_{14} and C_{16}. This is very similar to the effluent from the UK WWTP (Mudge, 2009) and suggests a similar mechanism of formation (bacterial synthesis) although in this case the odd chain components are not as prevalent.

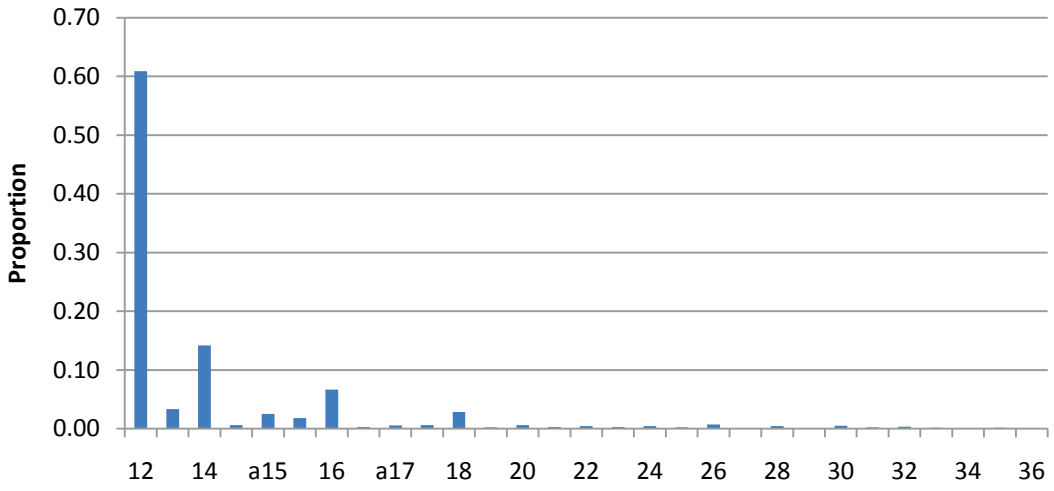

Figure 16. The fatty alcohol profile for the effluent from the WWTP. This is the mean of nine samples. The dominant peak was phytol (not included here) is most likely to be derived from the algae sloughing off the walls of the discharge channel.

The summary data for the total fatty alcohols at each treatment stage are presented in Figure 17. In this works, the settlement stage after the oxidation ditch is particularly effective at removing the fatty alcohols which presumably were associated with the solid phase in suspension. These data are different to the UK WWTP where there was a slight increase in the final effluent concentration of fatty alcohols probably due to bacterial synthesis within the works. This was also indicated by the different stable isotope signature for these compounds (Mudge, 2009).

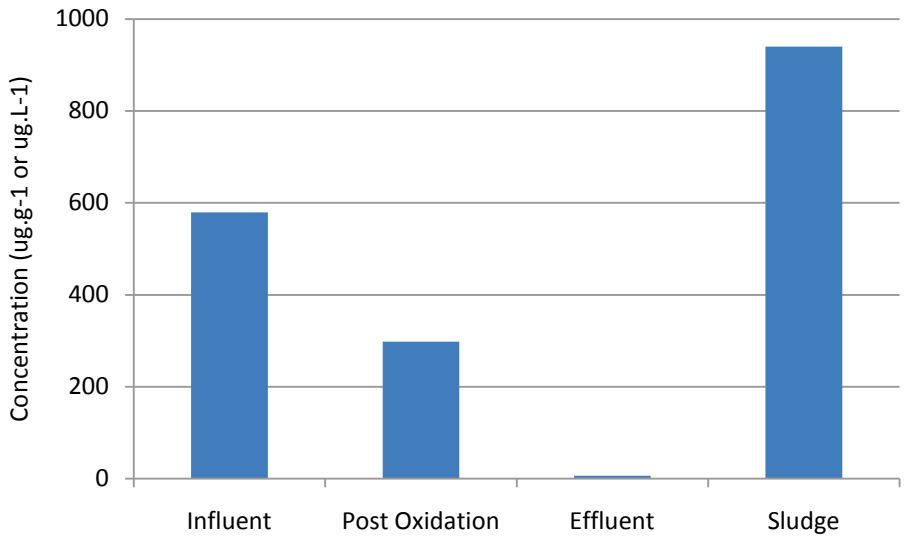

Figure 17. The mean fatty alcohol profiles for the different stages within the WWTP. The sludge data are presented as $\mu g.g^{-1}$ DW while the others are all $\mu g.l^{-1}$.

Agricultural Soils

The compounds present in an agricultural soil sample are shown in Figure 18. A full suite of straight chain odd and even carbon numbered alcohols are present although the majority of the compounds with a chain length less than 22 are not visible in this figure. Small quantities of branched odd carbon chain compounds are also present (not labelled).

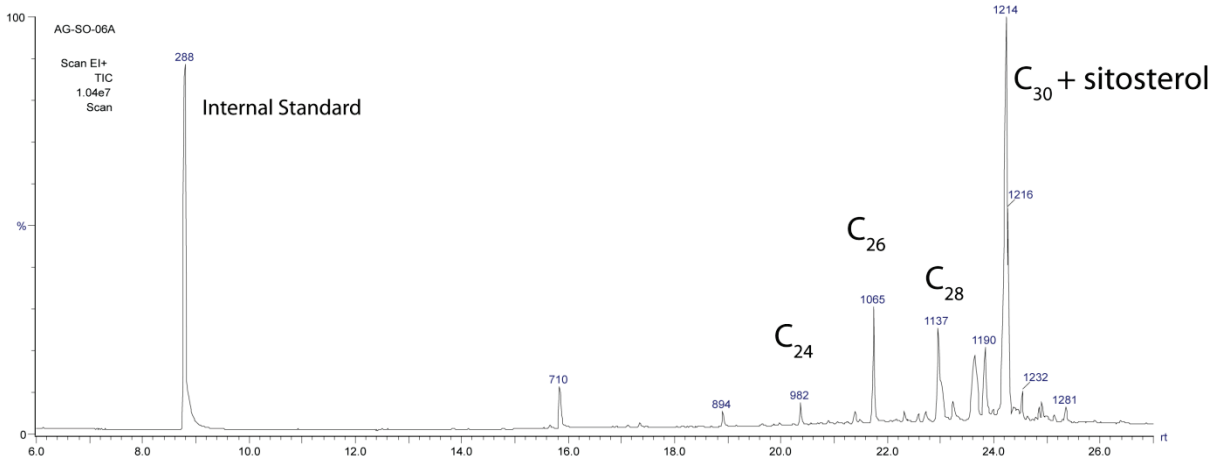

Figure 18. Fatty alcohols and sterols present in an agricultural soil sample. Short chain compounds $<C_{22}$ are generally absent from these samples but are present in river sediment samples.

The mean fatty alcohol proportion for all soils sampled, including agricultural and urban soils, can be seen in Figure 19. One standard deviation averaged 58% of the mean value; these SD values were higher for the odd chain compounds which were present at lower concentrations. The major fatty alcohol is C_{26} with long chain moieties extending out to at least C_{36}. An isolated peak at C_{16} is probably indicative of the synthetic pathway for these compounds (Mudge et al., 2008). The high proportion of long chain compounds is what might be expected from terrestrial plants in a situation where water conservation can be important.

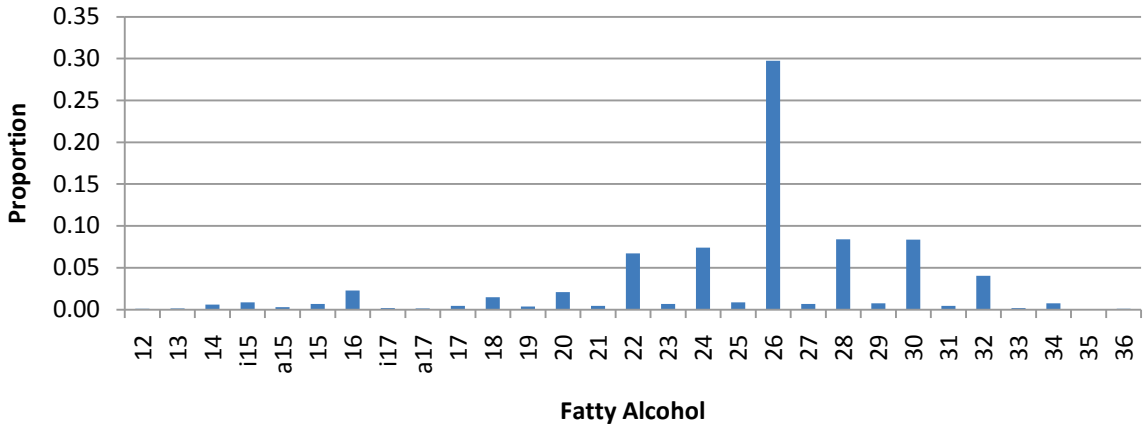

Figure 19. Mean proportion of fatty alcohols (excluding phytol) in the agricultural soils (*n*=21).

River Sediments

The short chain compounds were present in higher concentrations in the river sediments. In Figure 20, a series of compounds including short chain moieties (C_{14} – C_{18}) potentially derived from aquatic algae can be seen together with phytol, the side chain of chlorophyll. There is a bimodal distribution with several long chain compounds peaking at C_{26}; these are most likely to be derived from terrestrial plants and may be washed into the river.

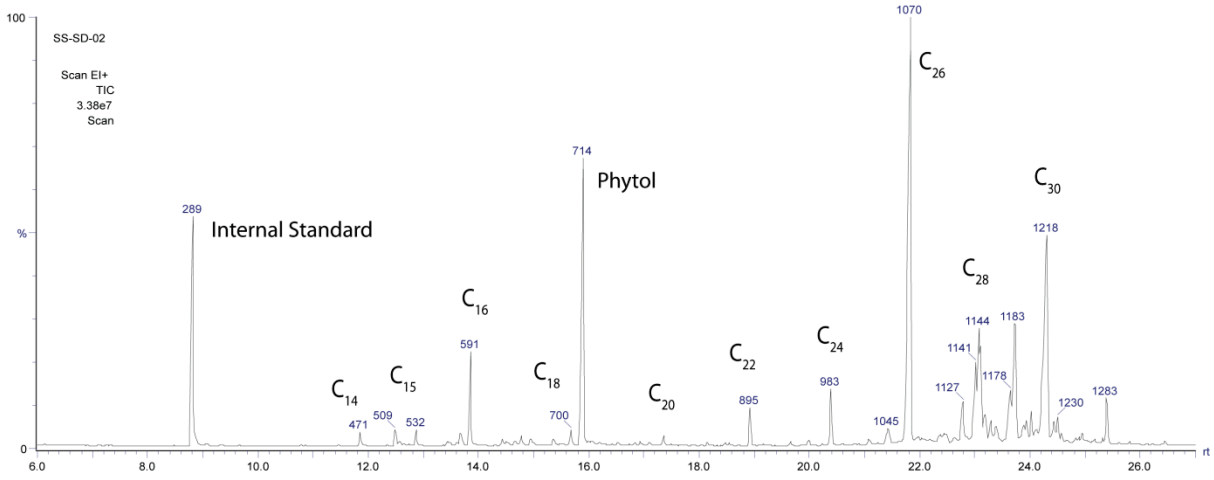

Figure 20. Fatty alcohols present in a river sediment sample.

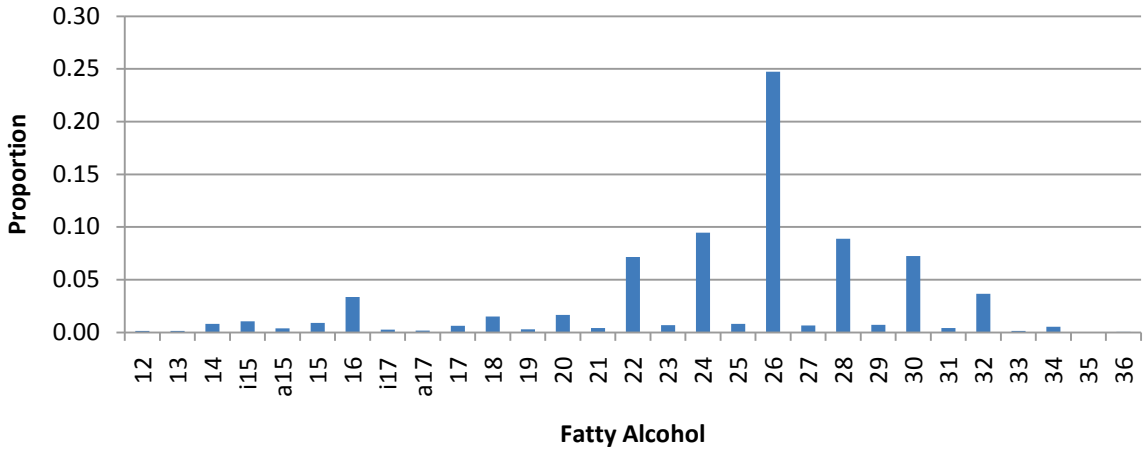

Figure 21. Mean fatty alcohol (excluding phytol) profile for the river sediments (*n*=27).

The mean profile (Figure 21) for the river sediments is very similar to that of the agricultural soils (Figure 19) and suggests a common source for the majority of these compounds.

Road Dusts

The road dust fatty alcohol profiles mimic that of the terrestrial plant matter (Figure 22). This is not surprising as a significant proportion of the dusts will be comprised of local soils and windblown plant

matter. The most significant difference to the other profiles is the increased contribution from the C_{18} fatty alcohol.

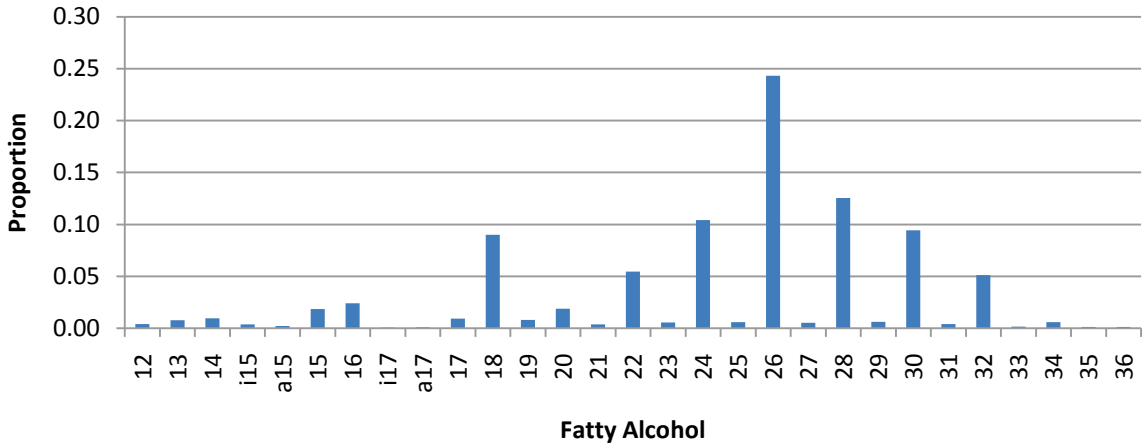

Figure 22. The mean profile of fatty alcohols in road dusts (*n*=7).

Detergents and Other Products

Of the 34 supplied products, only 22 had measureable fatty alcohols. The breakdown of the presence can be seen in Table 4. In some cases, the manufacturer / formulator of the products may switch between fatty alcohols and olefin sulphates and this was apparent here.

Table 4. Fatty alcohols in the products used within the catchment.

Product Type	Product Code	Number analysed	Number with Fatty Alcohols (percentage)
Liquid Hand Soaps	LHS	4	3 (75%)
Liquid Laundry Detergents	LLD	10	8 (80%)
Hand Dish Detergents	HDD	5	5 (100%)
Shampoo	SHA	1	1 (100%)
Deodorant	DEO	2	2 (100%)
Powdered Laundry Detergent	PLD	3	3 (100%)
Automatic Dish Detergent	ADD	4	0 (0%)
Liquid Fabric Softeners	LFS	5	0 (0%)

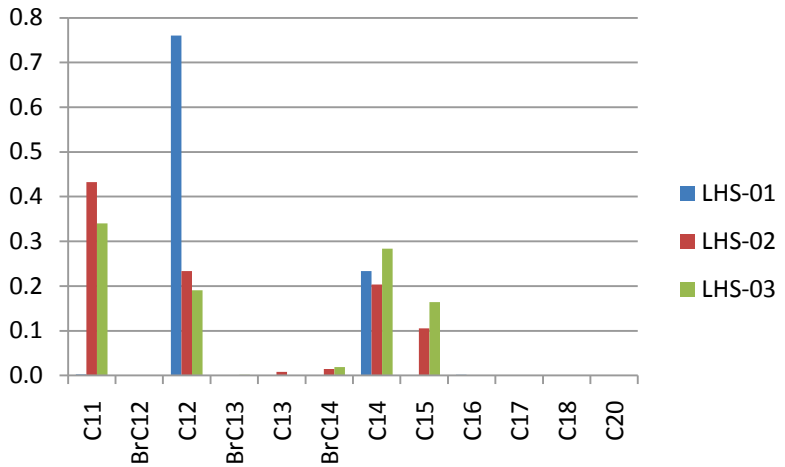

Figure 23. The fatty alcohol profile for the three Liquid Hand Soaps (LHS). The presence of the C_{11} and C_{15} in two of the formulations suggests the raw fatty alcohol source materials were derived from oil rather than a natural source.

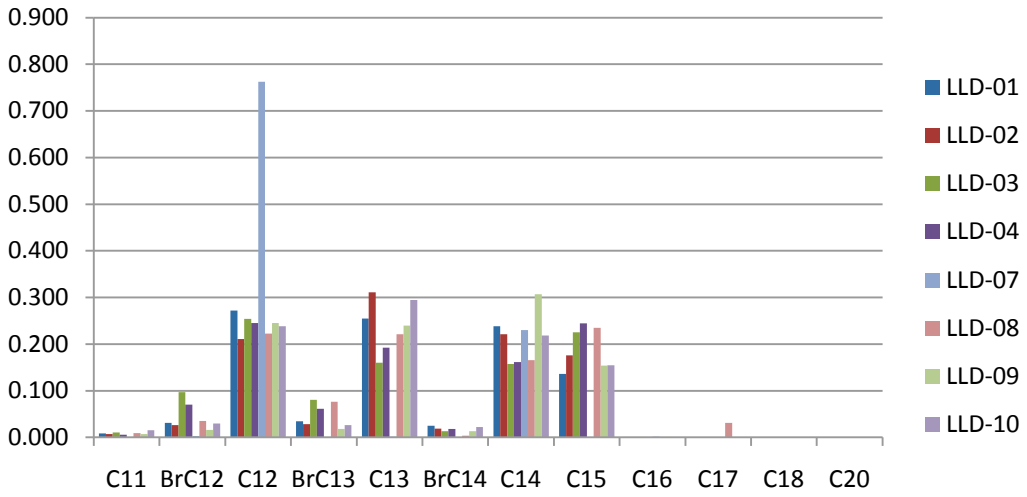

Figure 24. The fatty alcohol profile for eight Liquid Laundry Detergents (LLD). The presence of the C_{13} and C_{15} in most of the formulations suggests the raw fatty alcohol source materials were derived from oil rather than a natural source. Only LLD-07 had a profile that was indicative of a natural source (with high C_{12} and C_{14} and no odd chain components).

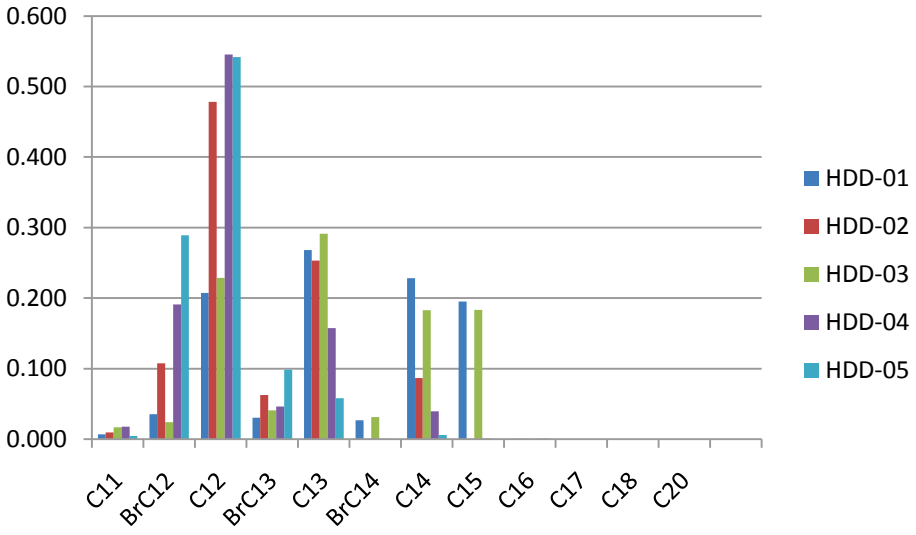

Figure 25. The fatty alcohol profile for the Hand Dish Detergents (HDD). The presence of the C_{13} in all of the formulations suggests the raw fatty alcohol source materials were derived from oil rather than a natural source.

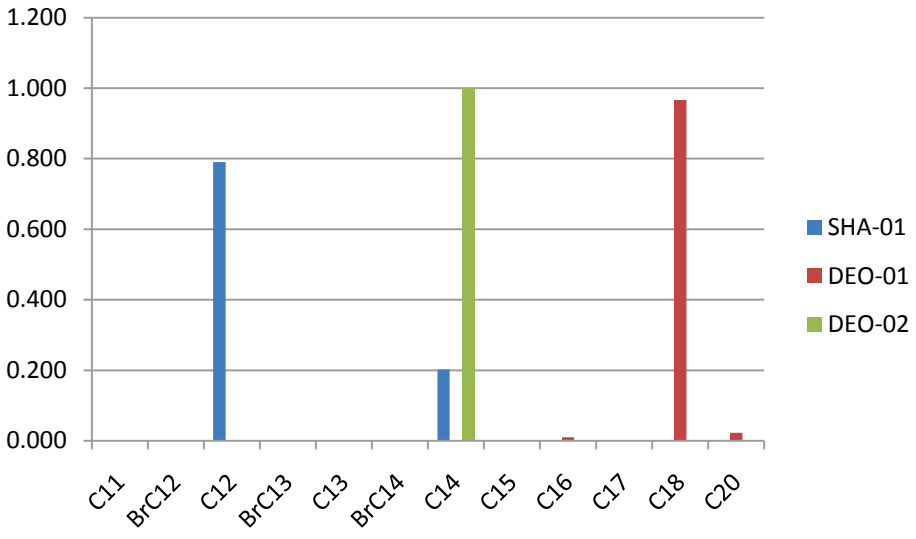

Figure 26. The fatty alcohol profile for the three other products (Shampoo and Deodorant). The lack of odd chain compounds may also suggest natural source of the fatty alcohols. DEO-01 has longer chain components than other products.

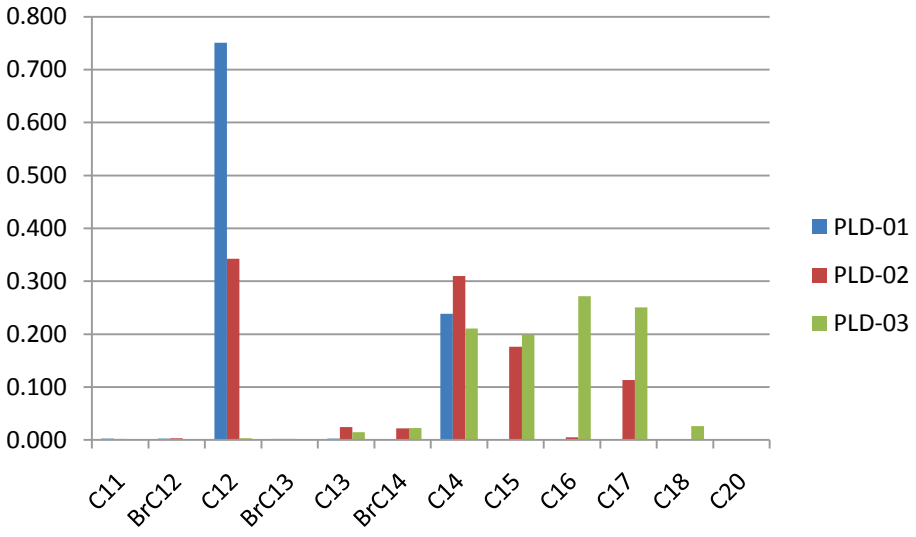

Figure 27. The fatty alcohol profile for three solid products (Powdered Laundry Detergent). PLD-01 only has C_{12} and C_{14} compounds with no odd chain fatty alcohols.

The raw fatty alcohol components used for manufacturing consumer products fall into two major groups; those synthesised from oil and those synthesised from natural plant or animal materials. This sourcing may be reflected in the fatty alcohol product profiles as the synthetic materials have high proportions of odd chain length fatty alcohols not usually seen in the natural products which are

essentially restricted to even carbon numbered compounds. Within these major groups are several sub-groups as the fatty alcohols may include branched chains, be principally short around C_{12} or be longer around C_{14} – C_{15}. The easiest way to visualise these separations is to conduct Principal Components Analysis (PCA) on the products profiles. This can be seen in Figure 28 with typical profiles overlaid to indicate the source type.

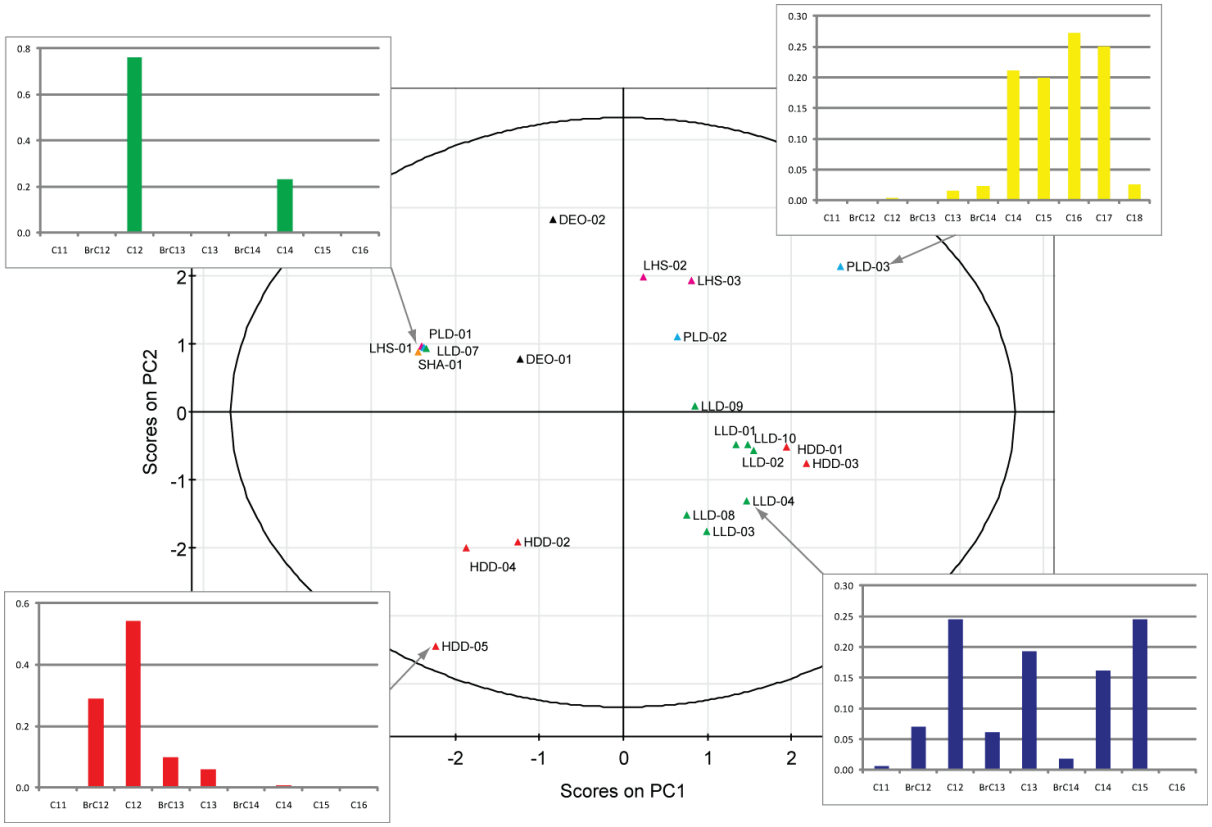

Figure 28. The Scores plot from the PCA of the fatty alcohol profiles of the detergents and other products (n=22). The profile is overlaid to show typical compositions in each quadrant.

The upper left quadrant of the scores plot (Figure 28) has a group of diverse products made solely from C_{12} and C_{14} fatty alcohols. These may have a natural origin initially (*e.g.* Lorol) or be a component of an oil based formulation. To determine the most appropriate category, stable isotope analysis will be needed. The other three groups in the figure all indicate oil based formulations due to the high abundance of branched or odd chain fatty alcohols in the profile. To the lower left are hand dish detergents made with short and branched chain C_{12} and C_{13} fatty alcohols; to the upper right are longer even and odd chain fatty alcohols. Those to the lower right, principally liquid laundry detergents and some hand dish detergents, are made from the mid-length even and odd chain compounds. These products may be made from mixed source materials as in the case of one of the detergent products from the UK study. In that case (Mudge, 2009), the stable isotopes indicated that the C_{12}, C_{14} and C_{16} came from a natural source and were mixed with C_{14}, C_{15} and C_{16} materials from an oil based source.

Contributions to the WWTP Influent

Using the data from the product survey, it is possible to calculate the contribution that the fatty alcohol containing detergents and associated products make to the influent of the Luray WWTP. The overall data can be seen in Table 5. These data do not include one of the major supermarkets within the town of Luray and the total contribution calculated (~2 kg per day) may be modified upwards by between 50 and 100% to account for their sales. Consideration of this is made in the discussion.

Table 5. The contribution of fatty alcohols to the influent of the Luray WWTP based on survey data of detergent usage, population and mean alcohol content.

Product	Market Volume (Units)	Unit	Conversion (g/16 oz.)	Mean Alcohols Content (%)	Mass Alcohols (grams)	Survey Period (weeks)	Survey Population	Per capita daily contribution (g)	Luray STP Resident Population Receiving Treament (2004 CWNS)	Daily alcohols contribution (g)
Liquid Laundry Detergent	5,069,518	16 oz. eq	453.6	3.0%	68,986,001					
Powdered Laundry Detergent	1,286,893	16 oz. eq	453.6	3.0%	17,512,040					
Hand Dish Detergent	1,285,168	16 oz. eq	453.6	3.0%	17,488,566					
Liquid Hand Soap	244,884	16 oz. eq	453.6	3.0%	3,332,381					
Body Wash	289,575	16 oz. eq	453.6	3.0%	3,940,537					
Shampoo	541,257	16 oz. eq	453.6	3.0%	7,365,425					
Subtotal contribution					118,624,950	24	2,291,845	**0.31**	6,586	2,029
Deodorant	489,960	unit	75	17.5%	6,430,725	24	2,291,845	**0.02**	6,586	110

The mean fatty alcohol content has been taken from (Modler, 2004). The unit size is specified in fluid ounces and a density of 1.0 has been assumed when converting to grams. Powdered laundry detergents are sold by mass.

It is possible to reconstruct the fatty alcohol profile of the influent using the fatty alcohol composition taken from the analyses of the products and the sales data within the catchment.

The contribution that each product makes can be seen through the marketing data for each detergent in the survey (Table 6).

Table 6. The contributions from each of the detergent products with their coding used in the blind analyses.

Product	Volume Sales (16 oz. units)		Product	Volume Sales (16 oz. units)
Liquid Laundry Detergent			**Liquid Hand Soap**	
LLD-01	454,609		LHS-01	66,806
LLD-02	489,466		LHS-02/03	114,101
LLD-03	130,779		LHS-04	82,010
LLD-04	518,007		**Subtotal**	262,917
LLD-05/06	622,908		Remainder of market	22,979 (8%)
LLD-07	539,720			
LLD-08	1,304,989		Total Liquid Hand Soap Market	285,896
LLD-09	262,735			
LLD-10	781,404			
Subtotal	5,104,617			
Remainder of market	696,774 (12%)		**Body Wash**	
			Total Body Wash Market	289,575
Total Liquid Laundry Market	5,801,392			
Powdered Laundry Detergent			**Shampoo**	
PLD-01	137,482		SHA-01	141,132
PLD-02	262,954		**Subtotal**	141,132
PLD-03	474,493		Remainder of Market	400,125 (74%)
Subtotal	874,929			
Remainder of market	411,964 (32%)		Total Shampoo Market	541,257
Total Powdered Laundry Market	1,286,893			
Hand Dishwashing Detergent			**Deodorant**	
HDD-01	187,594			
HDD-02	228,596		DEO-01	53,630
HDD-03	96,738		DEO-02	35,001
HDD-04/05	226,281		**Subtotal**	88,632
Subtotal	739,209		Remainder of Market	401,328 (82%)
Remainder of market	545,959 (42%)			
			Total Deodorant Market	489,960
Total Hand Dish Detergent Market	1,285,168			

Using a combination of the usage data (Table 6) and the profiles of fatty alcohols in each product, it is possible to reconstruct the profile of the fatty alcohols entering the waste water system and becoming the influent to the WWTP at Luray. This can be broken down by class of product and then combined according to usage volume. The contribution profiles for the six product classes can be seen in Figure 29.

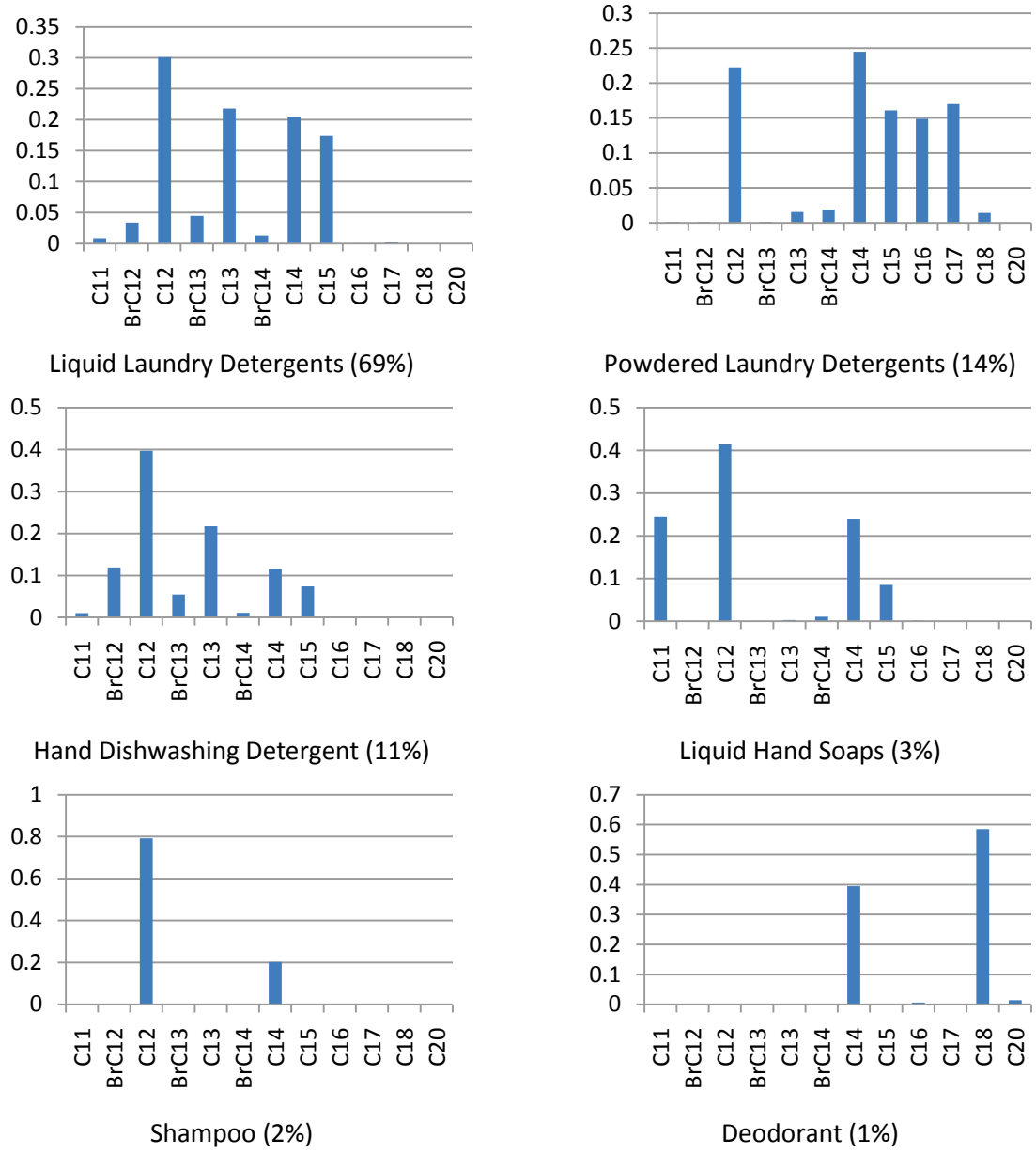

Figure 29. The combined contributions from each detergent product class together with the proportion of the total fatty alcohol contribution made from that class.

These figures indicate that the liquid laundry detergents dominate the overall market for fatty alcohols and contribute almost 70% of the total input to the WWTP. When these profiles and contributions are

combined to reconstruct the detergent contribution to the influent, the liquid laundry products will have the greatest influence. This combined effect can be seen in Figure 30.

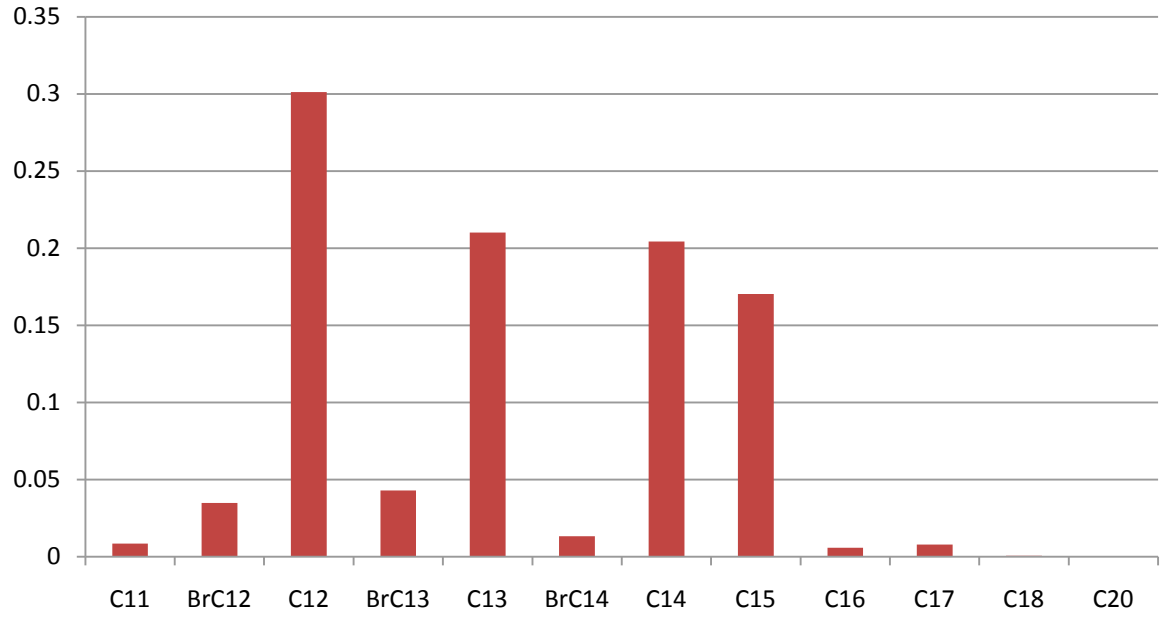

Figure 30. The final detergent product contributions to the WWTP influent based on the usage of each individual product.

The profile has relatively high proportions of the odd chain compounds (C_{13} and C_{15}) enabling this to be distinguished from non-bacterial sources where these compounds are minor constituents.

This contribution can be compared to that of faecal material analysed as part of the Phase I study conducted in the UK. There may be regional differences in the profile of the faecal material based on the differing diets between the countries but there should be a reasonable degree of similarity between the UK and USA. Figure 31 shows the mixing between the two potential sources in comparison to the actual WWTP influent profile. Visually, the closest match is with the 75:25 mixture of faecal matter and detergents although the C_{18} cannot be explained by either source and must have a different origin. Coincidently, the stable isotope analyses in the UK study also indicated a 75:25 mixture between faecal matter and natural based detergents on one hand and oil based detergents on the other (Mudge, 2009).

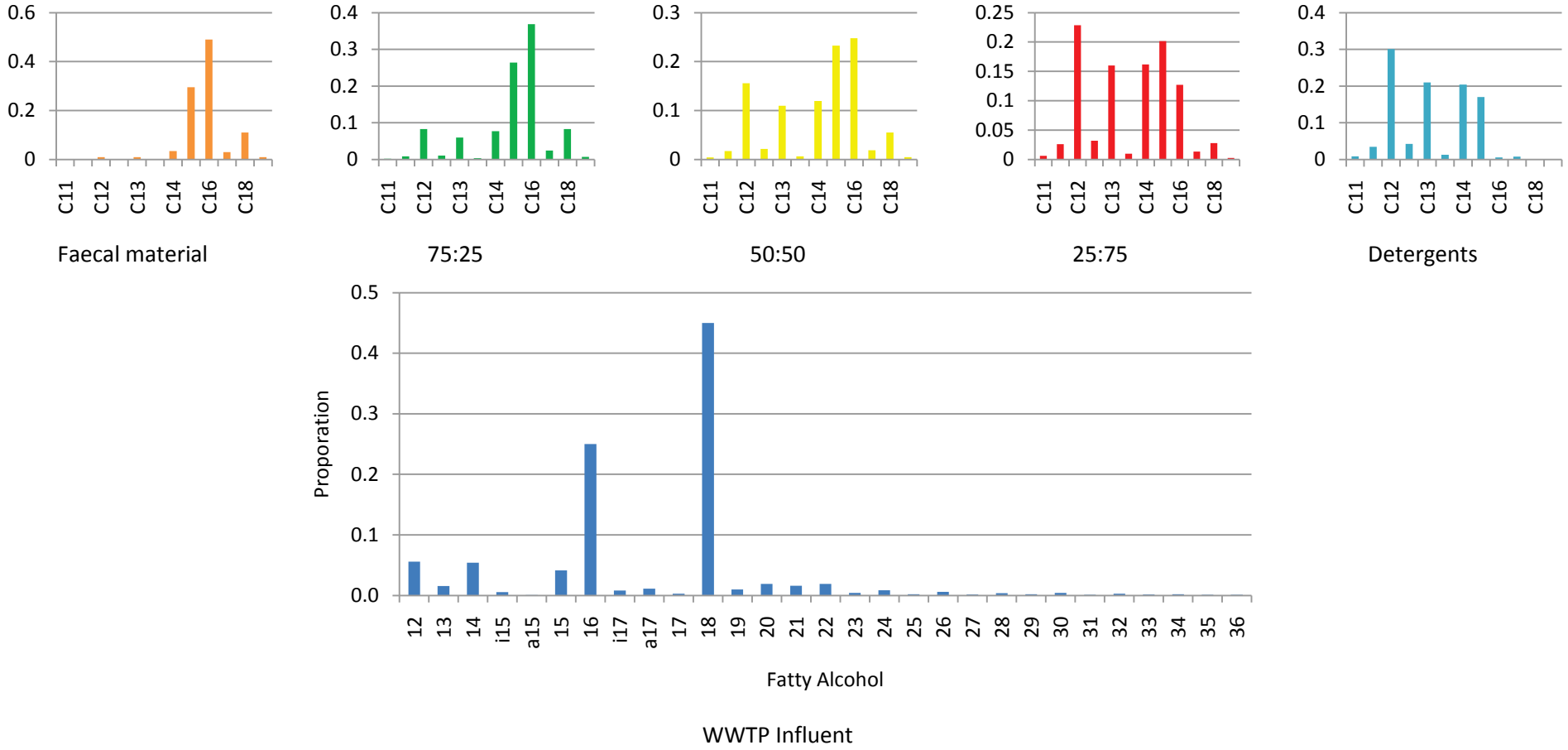

Figure 31. The mixing between 100% faecal material on the left through mixtures to 100% reconstructed detergent profiles. The actual influent profile is shown below.

Principal Components Analysis of Environmental Samples

In a similar manner to the analysis of the products above, PCA can be undertaken on the fatty alcohol profiles of the environmental and WWTP samples to establish which sample types have similar compositions. The scores plot can be seen in Figure 32. The associated loadings plot indicating the composition of the samples is shown in Figure 33.

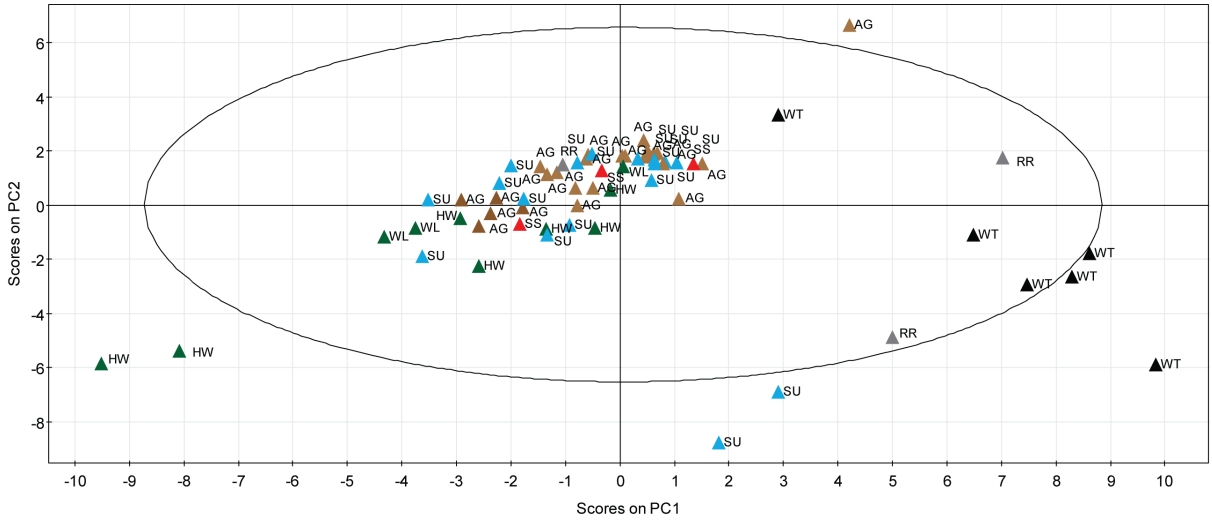

Figure 32. PCA of the environmental samples coded according to the sample locations. Green = Head Waters and Woodlands (HW and WL); blue = Suburban (SU); brown = Agricultural (AG); red = Secondary Sources (SS); grey = Rural Roads (RR) and black = WWTP (WT).

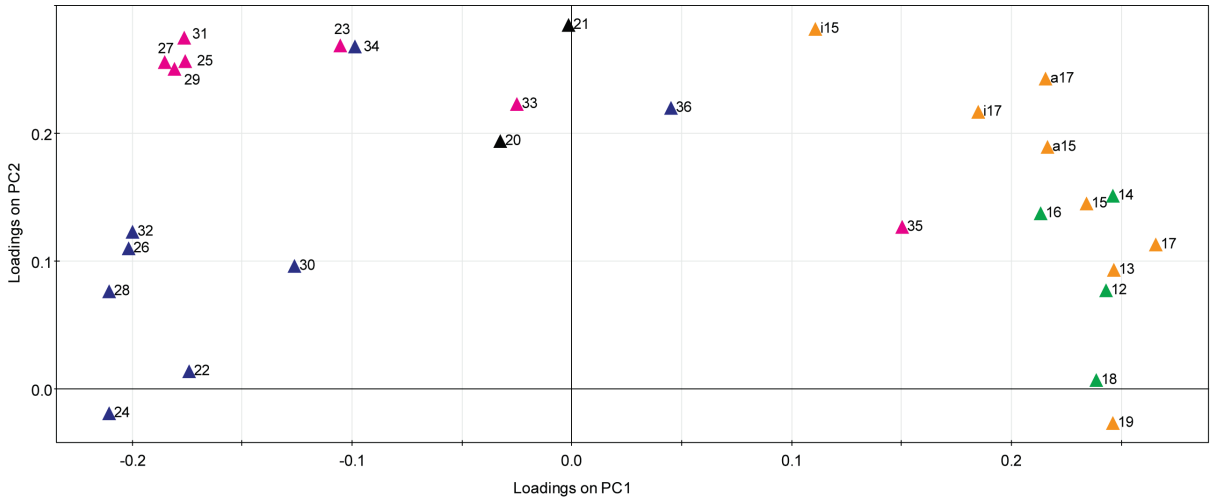

Figure 33. The fatty alcohol loadings associated with Figure 32. All short chain compounds of either bacterial (orange) or algal (green) origin load to the right hand side while the majority of the odd chain (pink) and even chain (purple) long fatty alcohols (C_{22}+) load to the left. These are typically of terrestrial plant origin.

The majority of the agricultural soils and other samples within the catchment formed a wide cluster near the origin of the figure. The two extremes on PC1 were the WWTP samples which the loadings plot (Figure 33) suggest have principally short and odd chain compounds dominating, and the head waters up in the wooded area above the valley. These latter samples would be rich in the long chain fatty alcohols typical of plant waxes. This association can be seen to the left of the loadings plot. Another way of viewing these compositions is the contributions plots which show the content of a sample relative to the mean projection on the loadings figure. Two examples, one for the WWTP and one for the headwaters leaf litter can be seen in Figure 34.

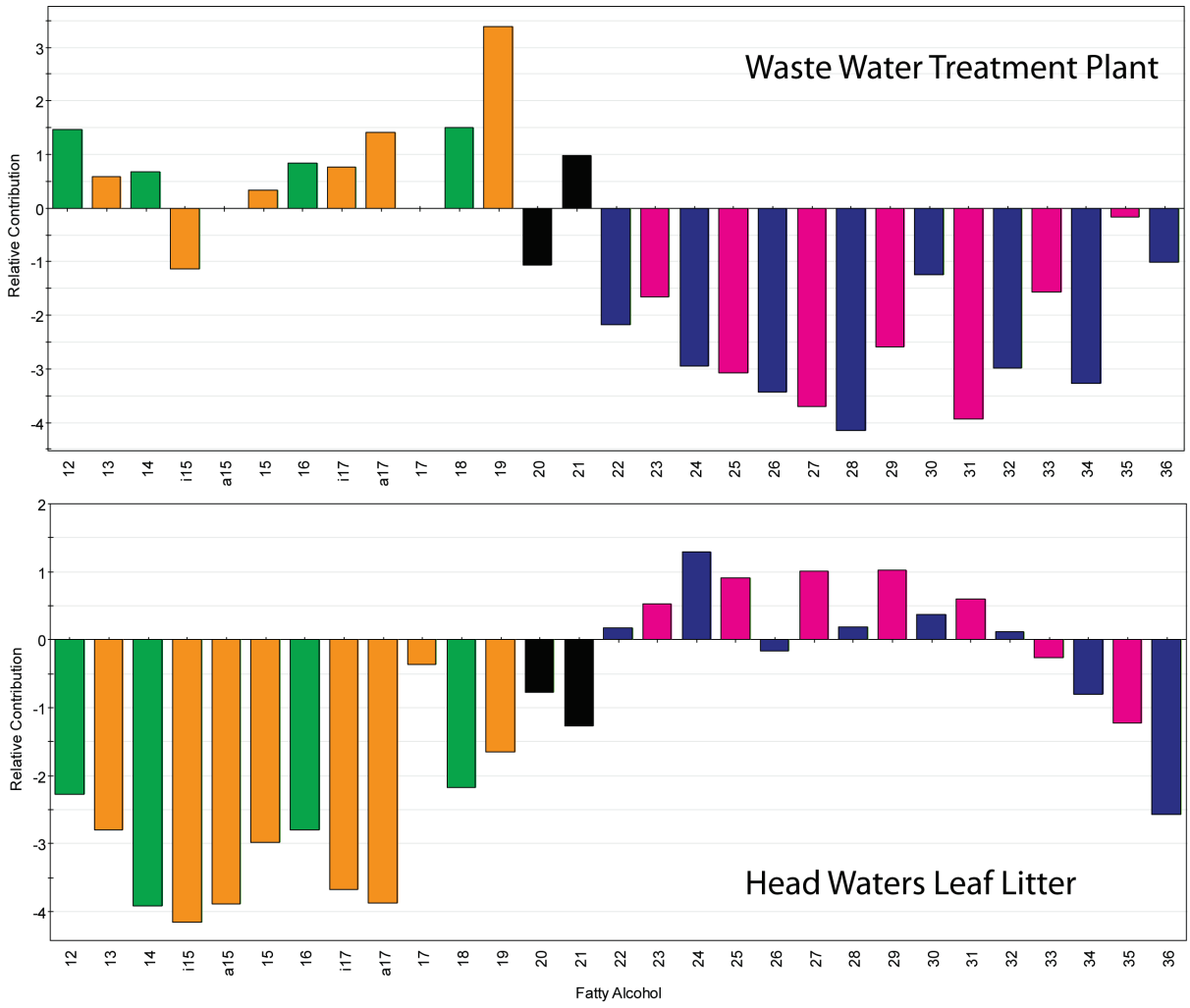

Figure 34. The Contributions plot for a WWTP sample (from the right of the Scores plot, Figure 29) and a leaf litter sample from the Head Waters (from the left of the Scores plot, Figure 32). The compounds are colour coded as in the Loadings Plot (Figure 33).

The two contributions plots indicate the two opposite sample types according to the first PC. The WWTP sample is enriched in the short chain compounds, especially the bacterial markers, and is depleted in the long chain terrestrial plant markers. In contrast, the leaf litter sample (Figure 34) is depleted in all the short chain compounds and enriched in the longer chain moieties. The two compounds with 20 and 21

carbons fall between the two source types and are not diagnostic in this case. The majority of the sample sites fall in the middle of the scores plot (Figure 32) and suggest a mixed source between the two extremes. These samples have contributions plot that are essentially flat with no significant enrichment or depletion. However, there is a gradient between those to the left of the group and those to the right (Figure 35).

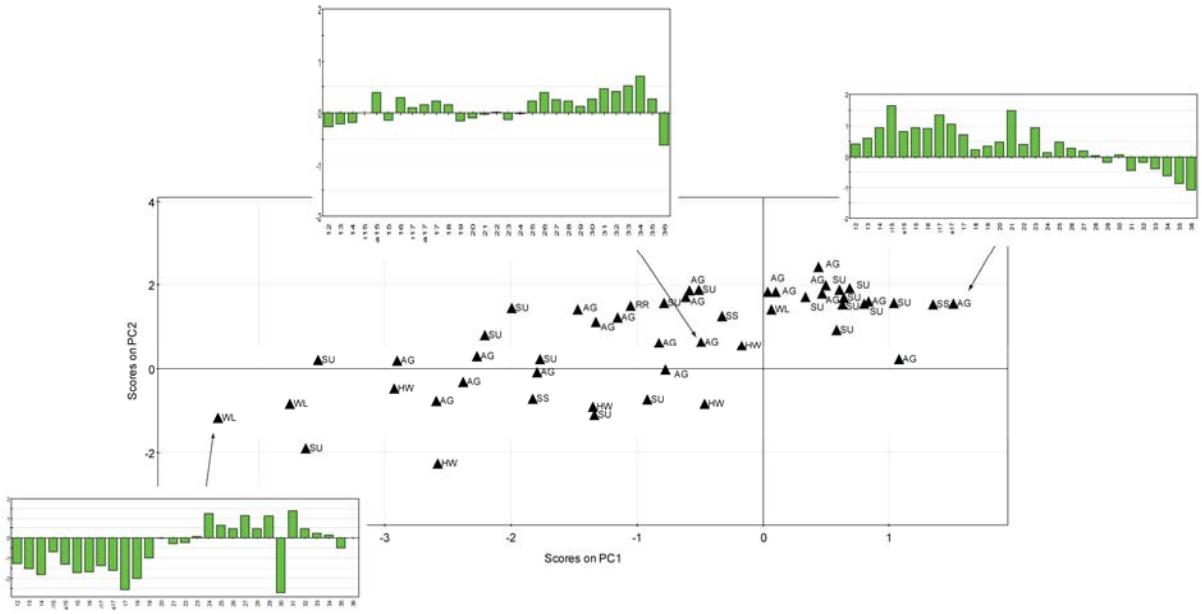

Figure 35. The expanded central portion of the Scores plot (Figure 32) showing the change in relative contribution in each sample from the long chain rich, depleted short chain fatty alcohols on the left to the opposite at the top right. Samples near the centre have a neutral mix.

Stable Isotopes

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

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

where the FA-TMS carbon number is 15; TMS is three and FA is 12. The total number in the 2-dodecanol – TMS ether is 15. In this case, the TMS group has a $\delta^{13}C$ value of -36.69‰.

In the case of the $\delta^{2}H$ content, the number of hydrogen atoms in the whole TMS derivatised molecule is 34 and the original 2-dodecanol has 26 although one is replaced in the derivatisation process. The TMS

group adds nine extra to the original molecule. The TMS group has a δ^2H value of -137.5‰. The full table of stable isotope results can be found in the Appendix.

The results are best viewed as a single cross plot of the stable isotopes, δ^{13}C versus δ^2H for all samples. A colour coded and labelled plot can be seen in Figure 36.

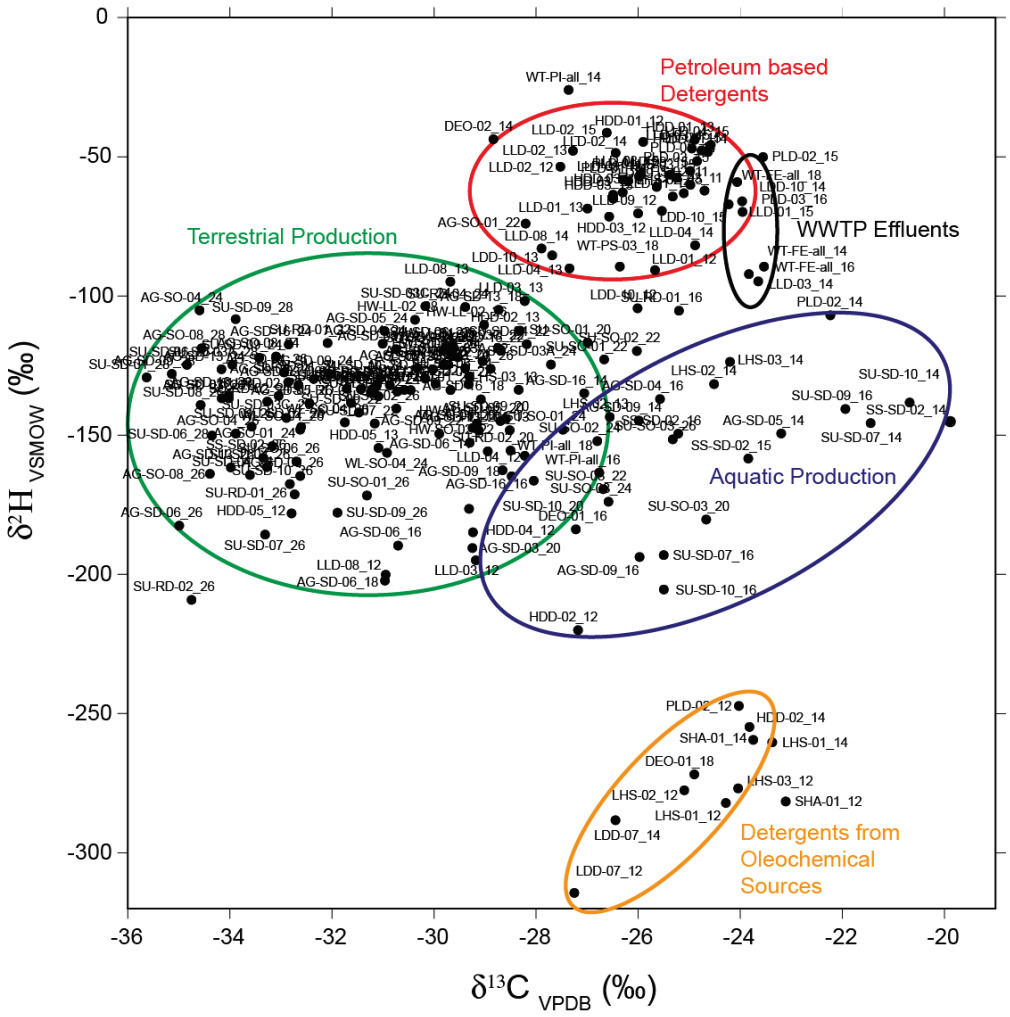

Figure 36. Cross plot of the stable isotopes of carbon and hydrogen for each sample and each fatty alcohol. The different sources or locations are indicated by the clusters.

The key observations that can be made from this figure are as follows:

1. The general pattern of the different sources is almost identical to those seen in the UK study shown in Figure 1. The terrestrial plant samples are located to the left of the figure with $\delta^{13}C$ values around -30 to -36‰. This is due to their carbon source coming from atmospheric CO_2. In contrast, the algal fatty alcohols in the river sediments have $\delta^{13}C$ values between -20 and -28‰ which is consistent with the marine production in the UK study. This is due to the carbon source in this case being bicarbonate (HCO_3^-) dissolved in the water.

2. The fatty alcohols in the terrestrial group are long chain with lengths of C_{20} and greater. The fatty alcohols in the aquatic production group (algal synthesis) are shorter with chain lengths from C_{14} to C_{20}. The shorter chain compounds were probably synthesised by algae within the river either in the water column or in the riverbed. A summary of the mixing can be seen in Figure 37.

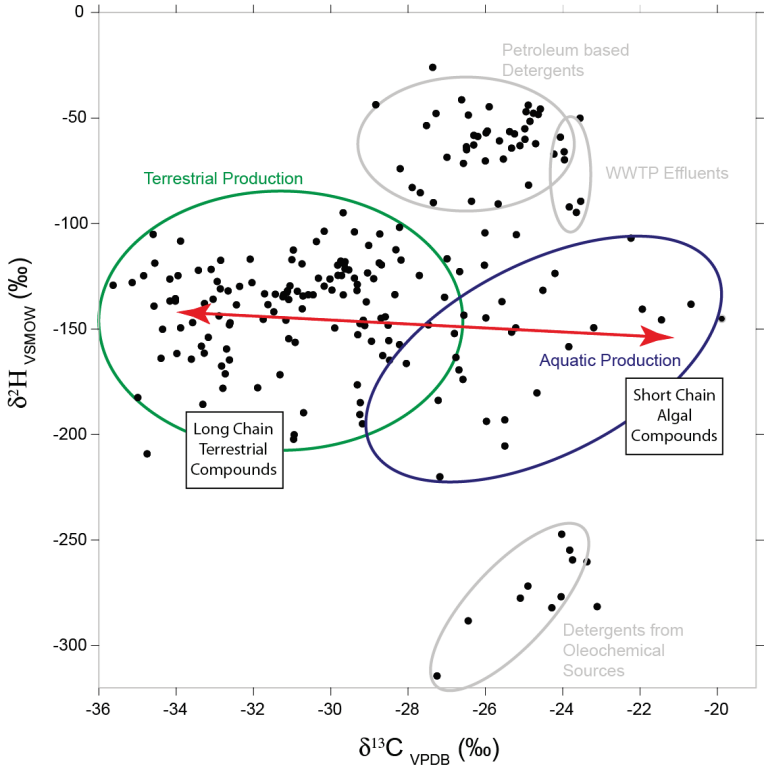

Figure 37. Mixing line between short and long chain fatty alcohols in the environmental samples.

3. The petroleum source fatty alcohols used in the manufacture of detergent products are present in a group towards the top of the figure with $\delta^{13}C$ values between -24 and -28‰. Their $\delta^{2}H$ values are more positive than almost all other samples with a mean projection around -50‰. This group consists of a mix of odd chain and even chain compounds consistent with a synthetic source pathway.

4. The detergent products that were marketed as having naturally sourced fatty alcohols all present stable isotope values that isolate themselves as a separate group. The $\delta^{2}H$ values are more negative than the petroleum sources and are entirely consistent with the precursor fatty alcohols and products from the UK study (see Figure 1). The compounds in this group are all

even chain only consistent with their biological production. The location of this group relative to the terrestrial and aquatic groups suggests that the raw materials did not come from the local environment and may be derived from palm products.

5. Some of the fatty alcohols in the detergent products are located within the terrestrial group. This suggests that, in this case, these raw materials may have a similar terrestrial plant origin or be mixtures of two or more sources. This group consists principally of even chain compounds but not exclusively. A few detergent compounds also exist within the aquatic cluster and may represent mixed petroleum and oleochemical sources in the formulation; this was also seen in one or two of the UK sourced detergent products.

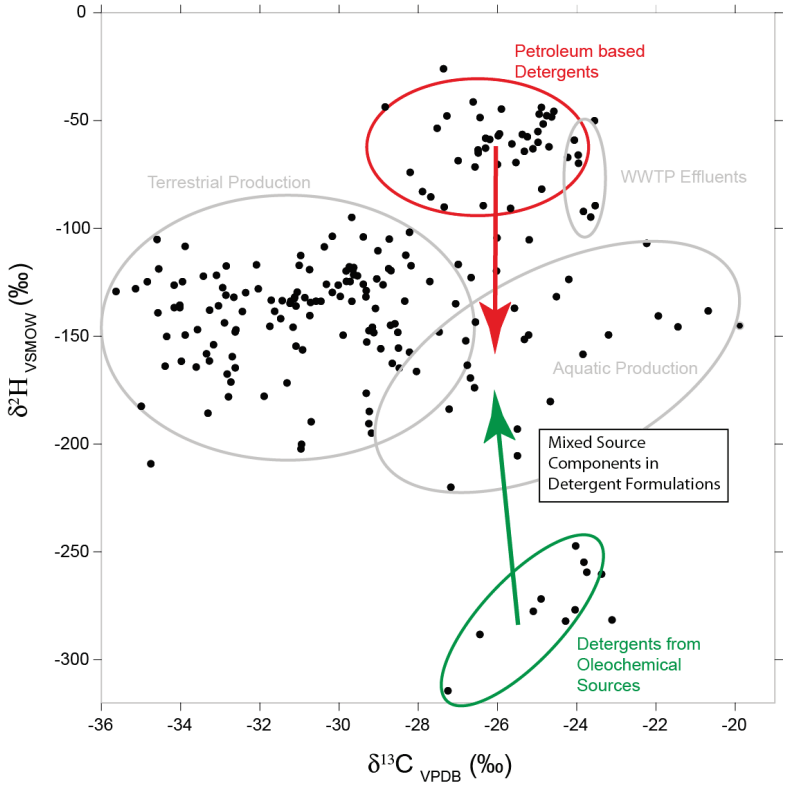

Figure 38. Mixing between the petroleum and oleochemical surfactant components in the detergent products. Selected chain lengths in selected products have intermediate stable isotopic signatures.

6. Two of the fatty alcohols associated with the influent to the WWTP fit into the aquatic and terrestrial plant overlap. These compounds, the C_{16} and C_{18} may be a mixture of faecal and detergent compounds. Faecal material was not specifically sampled within this catchment but in the UK analyses, the stable isotope values were almost coincident at -29‰ for the $\delta^{13}C$ and -

200‰ for the δ^2H. The C_{14} from this sample locates to the top of the figure with the largest δ^2H value of the sampling set. This compound may have a strong detergent source influence and also contain bacterially synthesised contributions. None of the odd chain compounds were present in sufficient concentration in the influent to measure the stable isotopes.

7. The fatty alcohols present in the sludge (biosolids) collected at the WWTP had a stable isotope signature close to that of the influent and petroleum based detergent samples. This is as expected since most of these chemicals are settled out from the influent within the works although there has been some modification as the waste waters entering the site have undergone biological treatment before settlement. This is due to the unusual design of this particular WWTP.

8. The effluent signature is significantly different to the influent with less negative $\delta^{13}C$ values. The chain length of the compounds has also changed with more short chain compounds present which were not in such high concentrations in the influent. These samples co-locate with some of the petroleum based fatty alcohols present in the powdered laundry detergents.

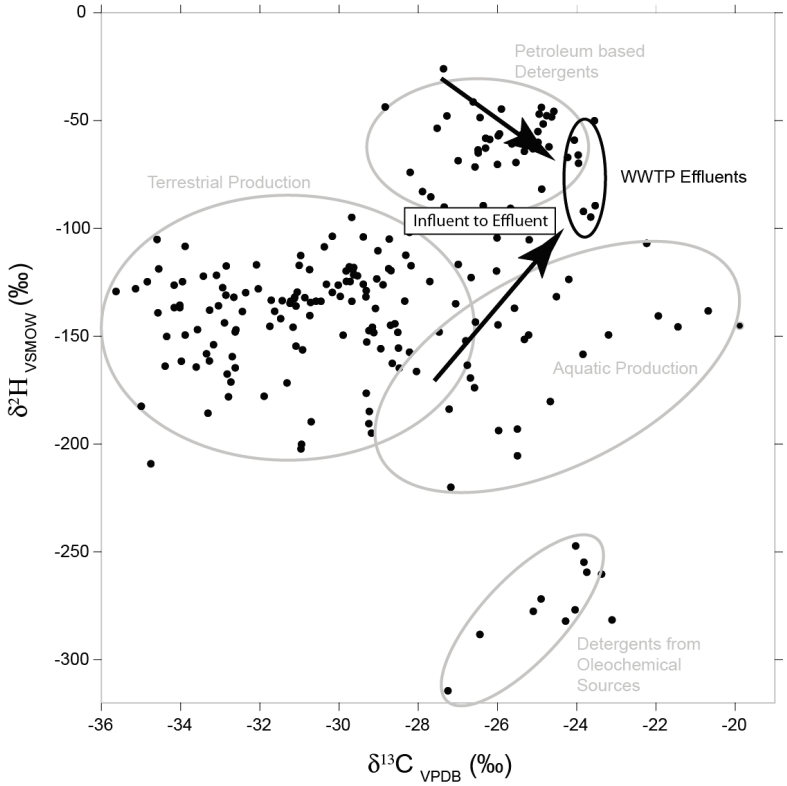

Figure 39. Change in the isotopic signature as WWTP influent components pass into the effluent.

9. The Secondary Sources (SS) and Sub-Urban (SU) samples were not significantly different from the Agricultural (AG) samples; what did control the differentiation within these samples was the sample type. Sediments (SD) tended to co-locate with the short chain compounds positioning close together in the aquatic cluster while the long chain compounds were all in the terrestrial group. The same was apparent for the Road Dusts (RD) which behaved similarly to the Soils (SO) and Sediments (SD); the long chain compounds positioned these samples in to the terrestrial group. This suggests that there was a commonality of sources between all of these samples and it was only the chain length of the fatty alcohols that differentiated between them.

Discussion

These results from this study are consistent with the results obtained from the UK study. The stable isotope values are appropriate and similar detergent profiles can be seen.

The influent to the WWTP contained fatty alcohols at a higher concentration than the UK study (600 $\mu g.L^{-1}$ compared to 200 $\mu g.L^{-1}$) although the profile was somewhat similar as they were both enriched in the short chain compounds. There was a relatively small presence of the odd chain compounds compared to the detergents and the mean reconstructed profile (Figure 30) indicates that the detergents were not the major contributor of fatty alcohols in the influent. The short chain profile indicates that there was little to no entrainment of terrestrial plant matter or soils into the influent stream at the time of sampling.

The presence of the C_{18} in the influent is interesting as this compound is not in high concentrations in either the detergent formulations nor the faecal matter; elevated concentrations were seen in the road dust samples (Figure 22) and there may be some entrainment of this material with newly synthesised compounds present.

The effluent from the WWTP indicated the new synthesis of fatty alcohols as the C_{12} component increased considerably (Figure 16). This was also the dominant fatty alcohol in the effluent in the UK study. These compounds are likely to be derived from *de novo* synthesis by bacteria or the recycling of longer chain fatty alcohols after degradation in the oxidation ditch.

The whole WWTP was very effective in removing the fatty alcohols and suspended matter generally from the influent stream. This is an unexpected finding given the somewhat unusual plant design. From observations, the system was more effective than the UK plant studied as the suspended load in the effluent was much lower necessitating the collection of significantly larger sample volumes. As expected, the major export route for fatty alcohols from the WWTP was through the treated sludges (Figure 17). Both the UK and USA plant had concentrations of 900 $\mu g.g^{-1}$ total fatty alcohols. These materials will be spread on agricultural land and may find their way back into the river but there was no evidence of that happening in the downstream samples in the Hawksbill River.

The agricultural soils contained fatty alcohols derived from plant synthesis with long chain lengths reaching out beyond C_{36}. The principal fatty alcohol in all of these samples was C_{26}. The $\delta^{13}C$ stable isotopic signature for these compounds was consistent with previous observations and ranged from -30 to -36‰; the $\delta^{2}H$ stable isotopic signature ranged between -100 and -200‰. The samples collected from the woodland soils and headwater also had similar signatures. Road dust samples had the same signature since the fatty alcohols present on the roads were derived from windblown soils.

The detergents presented four major fatty alcohol groups based on their profiles (Figure 28). These included a cluster of nominally biologically sourced compounds with only even chain compounds. These compounds also had distinct stable isotope signatures (Figure 36) separating them from all other samples analysed. Interestingly, several individual compounds also fall into this group although on the basis of their profile they are not exclusively biologically sourced. For example, the liquid hand soaps (LHS-02 and LHS-03) have a profile suggesting petroleum based sources but the C_{12} and C_{14} components have exclusively biologically sourced stable isotopes. This indicates a blending of source materials at the formulation stage and this was also seen in the UK study.

The stable isotopes indicate a small suite of detergents with fatty alcohols consistent with local terrestrial plant origins such as corn or soy (see EA Engineering, Science, and Technology Factsheet on the Luray Site Visit). These were relatively short chain (12 – 15 carbons) compared to the rest of the cluster with chain lengths greater than C_{20}. These may be fatty alcohols sourced from local oil seed crops with some chain shortening although these data do not confirm the exact nature of these materials. Further details should be sought from formulators if this is of importance.

Not all of the detergent products that were expected to have fatty alcohols did and this may be due to switching between olefins and alcohols depending on market conditions of price and availability. The market share data indicates that the biggest source of fatty alcohols to the WWTP through the drain and sewage system is the liquid laundry detergents (LLD). This provided 69% of the total detergent fatty alcohols to the influent (Figure 29). Since the large majority of these products are comprised of fatty alcohols derived from petroleum, the reconstructed profile will be rich in odd chain compounds. The slight enrichment of C_{12} in this profile may be due to addition of this as a single compound potentially with a degree of biologically sourced material. This view is supported by the presence of these compounds in the agricultural soil cluster and oleochemical sourced detergents in Figure 36.

A simple linear mixing model indicates that the best description of the fatty alcohols entering the WWTP through the influent is 75% faecal matter with 25% from the detergents. This is the same as was found in the UK study. The presence of the C_{18} in the influent may be due to *in situ* synthesis or transformation prior to reaching the plant.

The fatty alcohols in the WWTP influent are not the same ones as those in the effluent. This is confirmed by both the change in profile and the change in stable isotopic signature. The effluent fatty alcohols which were present in low concentrations compared to the UK study (7 $\mu g.L^{-1}$ compared to 62 $\mu g.L^{-1}$) but the profile was similar with C_{12} dominating. This is likely to come from bacterial synthesis or recycling of compounds within the works.

The low concentration of fatty alcohols in the effluent will automatically mean that any contribution that they make will be small. The fact that the signatures are different to the influent also indicates that they are not the same ones that entered the plant and so could not be considered as detergent derived. However, as a worst case risk assessment, if it was assumed that all of the fatty alcohols leaving the plant through the liquid effluent were detergent derived, the contribution is small compared to the UK system (based on 4542 m^3 flow per day determined during the screening study, only 32 grams of fatty alcohols from all sources). The UK value for this worst case scenario is ~300 grams. This may be modified downwards since only 25% of the influent alcohols were detergent derived which leads to a net daily contribution of 8 grams.

The key river sediment sampled downstream of the WWTP (AG-SD-16) only presented fatty alcohols with either aquatic production (short chain) or terrestrial production (long chain) signatures based on the stable isotopes. This implies that the effluent fatty alcohols were not making a measureable impact on these environmental samples.

Source apportionment for the downstream samples suggests that 84% of the fatty alcohols are derived from terrestrial plant production through addition of agricultural soils and leave litter and the remaining 15% is derived from *in situ* algal production in the river itself. There is no measureable detergent component from the WWTP although ~1% may be assumed to be at the adjacent sites from all fatty alcohols sources in the effluent. Some of the terrestrial plant production may have entered the river through the road runoff route but the ultimate source of the fatty alcohols in this case is still the terrestrial plants.

Conclusions

1. The results of this study are consistent with those from the UK study both in terms of concentrations observed, fatty alcohol profiles and stable isotopic signatures. The general conclusions regarding the sources of fatty alcohols in this system are also similar.
2. The major source of fatty alcohols in the river is terrestrial plant matter (84%). This may enter the water through soil wash off, leaf litter or road dust. There was no evidence of substantial inputs of terrestrial plant matter into the WWTP and thence into the river. The direct routes are the major routes. A summary of the contributions and apportionment can be seen in Figure 40.

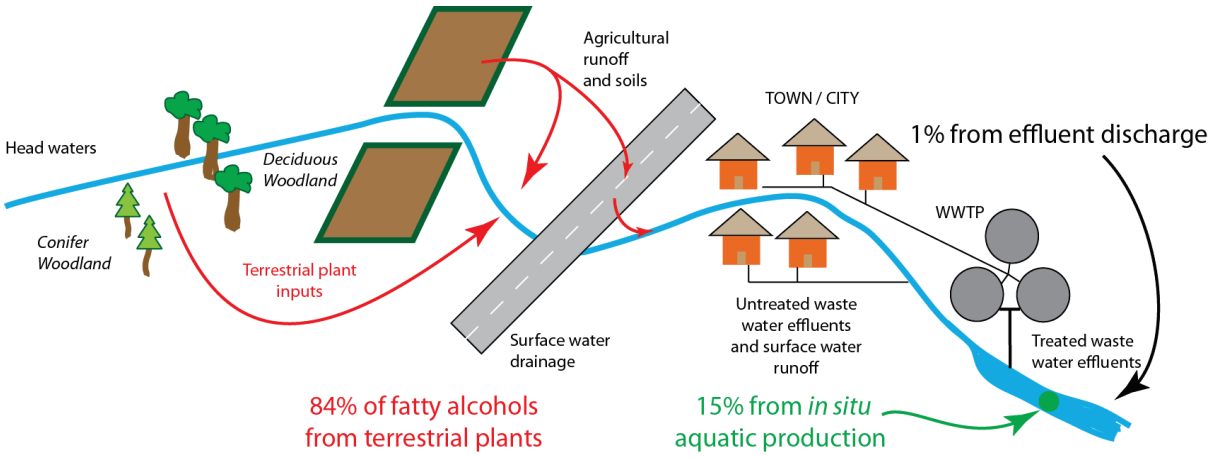

Figure 40. Summary of contributions to a downstream site in the Hawksbill River.

3. *In situ* algal production within the river contributes 15% of the total fatty alcohols; these are restricted to the short chain compounds of C_{14} to C_{18}.
4. The detergent products used within the catchment contribute 2 kg of fatty alcohols to the influent of the WWTP per day. The profile of these compounds suggests the C_{12} will be the dominant fatty alcohol with substantial amounts of the odd chain compounds present. Approximately 69% of all the fatty alcohols from these sources are derived from the liquid laundry products.
5. The majority of the fatty alcohols used in the manufacture of the detergent products are derived from petroleum. A few products contain compounds with a biological signature and are likely to be derived from palm fatty acids. A few products contain fatty alcohols from more than one source with the C_{12} and C_{14} having a palm signature while the longer chain and odd chain compounds have a petroleum signature. Some of the detergents may incorporate fatty alcohols for local terrestrial plant source such as an oil seed crop.
6. Within the influent, 25% of the fatty alcohols are derived from detergents with the remaining 75% coming from faecal and food sources. This is consistent with the results of the UK study.
7. The change in stable isotope signature between the influent and effluent from the WWTP demonstrates that the compounds may at first appear to be the same but in reality are not the same compounds. This supported by the change in profiles to be dominated by the C_{12}, a short chain compound easily synthesised by bacteria and the change in stable isotopes. The same result was observed in the UK study as well.
8. A worst case scenario with all the effluent fatty alcohols being derived from detergents (which is not supported by the evidence) would indicate a daily addition of 32 g of fatty alcohols to the river. This is ~10x less than the UK case where the flow rates were greater and the removal of the solids less effective than in the Luray WWTP.

Acknowledgements

I would like to thank many people for their assistance at all stages of this project; the staff at the Luray WWTP were very generous and accommodating; all at EA Engineering who helped with the sampling and access, especially Anita Struzinski and Mike Ciarlo; Rob Hale and colleagues at VIMS for allowing me to extract samples at their laboratory; Wolfram Meier-Augenstein at SCRI for conducting the stable isotope analyses and all SDA, now ACI participants. I am particularly indebted to Paul DeLeo for his enthusiasm and faith at all stages of the work.

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Appendix

Photographs of soil sampling locations

Corn field soil sampling; three replicates were collected at several different sites.

Pasture land adjacent to the hawksbill Creek.

A hay field in the middle of the agricultural zone.

Photographs of river sediment sampling locations

Hawksbill Creek below the town of Luray. Notice the turbid nature of the waters.

Hawksbill Creek entering Luray from the South. This is the beginning of the Riverside Walk.

Hawksbill Creek in the middle reaches surrounded by agricultural land.

A secondary source cutting across agricultural land before joining the Hawksbill Creek.

Photographs of the WWTP at Luray

After initial screening, the influent enters a series of oxidation ditches.

Samples were taken as the liquor left the oxidation ditch with a residence time of 48 h.

Settlement tanks and weirs to remove solids

UV treatment stage for the clarified liquid effluent.

Final effluent discharge into Hawksbill Creek.

Biosolids (sludge) after drying. The plant is a tomato germinated from a pip that has passed through the system.

Appendix Table 1. Fatty alcohol concentrations in AGRICULTURAL SEDIMENTS

(ug/g DW)	AG-SD-01	AG-SD-02	AG-SD-03	AG-SD-04	AG-SD-05	AG-SD-06	AG-SD-09	AG-SD-10	AG-SD-11	AG-SD-13	AG-SD-14	AG-SD-16
12	0.27	0.12	0.49	0.36	0.84	0.77	1.20	0.99	0.06	0.92	0.07	1.58
13	0.36	0.11	0.49	0.38	1.23	0.98	1.90	1.08	0.07	0.76	0.11	1.67
14	1.90	7.11	3.82	2.03	8.08	7.53	12.15	5.82	0.30	3.39	0.05	11.68
i15	1.93	6.53	5.38	3.68	11.23	11.57	12.02	8.43	0.47	5.54	0.07	9.49
a15	0.89	10.30	1.14	0.89	2.86	2.25	5.31	2.03	0.29	1.72	0.03	10.26
15	1.66	8.74	4.02	2.22	9.31	10.10	11.22	5.86	0.35	5.80	0.11	10.87
16	7.40	30.57	14.51	8.27	39.50	43.24	51.64	21.18	1.96	16.65	0.01	42.28
i17	0.48	2.18	1.35	0.58	3.40	3.65	3.92	2.03	0.10	1.36	0.01	0.00
a17	0.44	2.75	0.58	0.44	2.31	2.10	2.38	0.86	0.08	0.90	0.00	6.36
17	1.16	5.89	2.37	1.48	7.21	5.99	10.97	2.69	0.41	4.00	0.02	6.32
18	4.59	20.86	4.50	3.54	11.81	19.56	17.28	7.98	1.84	8.29	0.09	10.89
Phytol	31.86	468.98	67.22	80.75	221.95	111.79	131.46	56.50	19.47	221.65	12.39	368.64
19	0.52	3.15	0.79	0.77	2.35	3.23	3.27	2.57	0.22	1.55	0.18	1.75
20	7.46	43.95	7.14	6.49	20.81	6.01	15.09	8.86	1.20	10.44	0.14	11.78
21	1.12	9.91	1.62	0.66	4.65	2.17	4.14	3.19	0.30	2.85	0.05	3.20
22	26.56	209.04	32.98	54.02	87.52	14.34	53.91	33.51	4.43	41.76	0.02	44.08
23	2.10	20.51	3.09	2.65	6.20	3.00	5.53	3.94	0.41	4.84	0.06	5.01
24	39.52	340.81	39.75	47.79	74.36	22.89	63.28	28.41	5.49	54.40	0.02	60.63
25	2.38	21.30	3.36	2.70	6.04	5.75	6.87	3.24	0.62	6.86	0.06	6.81
26	23.61	617.69	82.24	101.84	187.01	446.68	307.53	91.73	29.54	209.99	0.31	210.56
27	1.68	26.09	3.21	2.46	4.76	4.66	6.00	2.02	0.52	6.78	0.02	6.88
28	12.15	468.82	47.06	45.07	65.96	67.56	93.25	22.51	8.04	80.79	0.03	85.83
29	1.44	24.43	2.79	1.97	5.13	6.43	7.51	1.64	0.53	7.49	0.05	6.92
30	12.59	303.18	28.60	35.73	72.22	122.08	103.59	17.24	8.15	81.56	0.03	83.48
31	0.90	17.54	1.56	1.11	3.25	3.14	5.16	0.80	0.42	4.68	0.04	5.20
32	6.78	137.18	13.30	10.83	35.59	27.83	51.12	7.82	4.73	40.88	0.03	45.58
33	0.35	6.72	0.64	0.50	1.37	0.99	1.56	0.29	0.18	2.06	0.04	2.79
34	0.89	23.92	2.54	2.03	4.74	4.15	5.70	0.87	0.68	8.97	0.04	11.42
35	0.03	0.51	0.08	0.05	0.12	0.17	0.20	0.02	0.03	0.27	0.04	0.03
36	0.08	0.96	0.30	0.16	0.47	0.78	0.80	0.07	0.03	1.29	0.01	0.95

Appendix Table 2. Fatty alcohol concentrations in AGRICULTURAL SOILS

(ug/g DW)	AG-SO-01	AG-SO-02	AG-SO-03	AG-SO-04	AG-SO-05	AG-SO-06A	AG-SO-06B	AG-SO-06C	AG-SO-07	AG-SO-08
12	0.40	0.03	0.13	0.11	0.06	0.06	0.01	0.11	0.42	0.21
13	0.47	0.07	0.18	0.08	0.23	0.05	0.16	0.10	0.31	0.20
14	2.38	0.31	0.58	0.06	0.78	0.43	0.39	0.34	0.90	0.57
i15	2.56	0.66	1.34	0.00	2.57	0.88	1.20	0.77	2.31	1.71
a15	0.65	0.35	0.21	0.00	0.82	0.33	0.31	0.24	0.90	0.44
15	2.88	0.31	0.43	1.28	0.75	0.27	0.38	0.23	0.70	0.38
16	7.10	1.53	2.52	0.14	2.40	1.18	1.88	0.58	0.87	1.13
i17	0.62	0.07	0.09	0.06	0.33	0.10	0.20	0.09	0.19	0.09
a17	0.35	0.11	0.12	0.03	0.38	0.13	0.19	0.10	0.25	0.09
17	2.24	0.29	0.43	0.25	0.79	0.35	0.51	0.23	0.51	0.46
18	3.85	2.24	4.29	3.38	3.98	2.00	3.74	1.33	2.04	1.94
Phytol	36.72	8.14	13.92	14.21	9.37	25.17	46.27	44.77	15.09	10.17
19	1.23	0.70	0.71	0.79	1.51	0.54	0.88	0.46	0.70	0.89
20	6.59	6.39	6.58	6.84	6.54	2.90	4.61	1.96	2.67	4.84
21	2.05	0.92	1.31	1.28	1.21	0.56	0.79	0.39	0.78	0.87
22	15.81	14.72	15.38	17.06	16.73	6.93	10.46	5.39	9.35	8.56
23	2.57	1.16	1.63	1.41	2.32	0.82	1.41	0.64	1.19	1.07
24	14.44	10.36	15.27	13.74	23.25	8.23	11.96	6.18	10.29	11.27
25	2.80	1.85	2.36	2.31	4.04	1.01	1.88	1.01	1.61	2.40
26	85.67	99.33	59.55	86.18	216.26	36.73	51.17	40.85	57.77	142.39
27	1.74	1.42	1.07	1.35	3.13	0.85	1.57	0.99	1.22	2.01
28	15.12	14.18	8.30	12.10	46.28	11.73	16.76	11.96	18.79	26.90
29	1.83	1.96	0.91	1.05	4.84	1.06	1.86	1.17	1.85	2.00
30	15.79	18.73	5.53	8.34	75.14	11.46	14.07	11.86	24.11	19.80
31	1.08	1.27	0.47	0.89	3.73	0.60	0.87	0.60	1.12	1.47
32	8.59	6.38	3.56	7.29	37.87	5.38	6.65	5.92	14.59	8.69
33	0.35	0.39	0.15	0.10	1.53	0.17	0.29	0.23	0.45	0.51
34	1.30	1.08	0.64	1.18	9.26	1.11	1.56	1.47	2.09	2.41
35	0.05	0.05	0.02	0.04	0.20	0.03	0.04	0.05	0.08	0.09
36	0.17	0.13	0.11	0.15	1.39	0.19	0.29	0.31	0.52	0.50

Appendix Table 3. Fatty alcohol concentrations in HEADWATERS and SECONDARY SOURCES

(ug/g DW)	HW-BLANK	HW-LL-01	HW-LL-02	HW-SD-01	HW-SD-02	HW-SO-01	HW-SO-02	HW-SO-03	SS-SD-01	SS-SD-02	SS-SD-03	SS-SD-04
12	0.00	0.66	1.48	0.11	0.06	1.45	0.19	0.23	0.31	2.11		0.04
13	0.00	1.89	1.55	0.07	0.08	0.23	0.19	0.16	0.06	1.79		0.03
14	0.00	3.15	1.72	0.21	0.22	1.07	0.40	0.48	0.38	24.16		0.22
i15	0.00	2.75	3.41	0.64	0.62	1.40	0.54	0.55	0.43	21.42		1.07
a15	0.00	0.48	0.70	0.64	0.00	0.25	0.11	0.29	0.34	5.71		0.00
15	0.00	5.74	6.96	0.14	0.24	0.46	0.29	0.55	0.20	23.22		0.73
16	0.00	8.34	10.02	0.79	0.63	1.18	0.16	1.54	0.23	89.23		2.58
i17	0.00	1.58	0.32	0.05	0.06	0.13	0.09	0.04	0.09	7.21		0.13
a17	0.00	0.50	0.72	0.06	0.06	0.06	0.04	0.05	0.09	3.32		0.10
17	0.00	3.02	133.65	0.27	0.24	0.63	0.66	0.58	0.22	15.88		0.49
18	0.00	320.57	85.83	1.00	1.55	4.67	9.56	5.16	0.73	24.95		2.87
Phytol	0.00	5023.21	2563.41	9.30	5.75	26.46	15.69	32.60	22.51	386.62		21.52
19	0.00	7.81	13.03	0.11	0.10	0.79	0.87	0.53	0.13	4.53		0.21
20	0.00	282.46	284.69	1.28	1.61	9.30	14.87	7.11	1.30	14.12		2.03
21	0.00	50.66	64.14	0.25	0.30	1.99	2.28	1.36	0.26	5.34		0.41
22	0.00	3383.49	3154.75	7.22	9.52	60.16	56.56	24.93	5.79	44.30		7.82
23	0.00	514.26	437.04	0.55	0.71	4.81	5.13	2.65	0.43	7.31		0.78
24	0.00	19441.26	16563.96	12.60	15.01	82.21	69.74	33.39	6.96	60.31		13.37
25	0.00	1094.14	764.39	0.57	0.67	4.82	3.97	2.20	0.49	11.40		1.58
26	0.00	9114.10	6538.36	7.13	7.16	50.50	30.54	27.47	12.03	450.54		78.62
27	0.00	813.22	614.93	0.45	0.49	3.83	1.96	1.77	0.42	8.67		1.11
28	0.00	5954.70	3988.45	5.73	5.76	42.34	18.20	14.33	6.57	111.66		21.09
29	0.00	875.89	771.23	0.57	0.61	4.77	2.15	1.63	0.36	9.44		1.19
30	0.00	7573.54	6026.00	0.09	0.12	37.36	14.85	0.07	4.44	96.25		14.12
31	0.00	393.80	288.39	0.20	0.21	1.87	0.64	0.62	0.19	4.97		0.67
32	0.00	4204.74	1684.95	1.27	1.39	11.22	2.74	4.03	1.74	50.67		6.50
33	0.00	83.48	45.52	0.02	0.01	0.55	0.01	0.01	0.09	1.41		0.25
34	0.00	155.46	96.54	0.08	0.01	1.20	0.27	0.36	0.31	5.39		0.90
35	0.00	0.56	2.00	0.02	0.01	0.01	0.01	0.01	0.04	0.19		0.04
36	0.00	9.17	1.16	0.01	0.01	0.15	0.04	0.01	0.02	0.80		0.08

Appendix Table 4. Fatty alcohol concentrations in SUBURBAN SEDIMENTS

(ug/g DW)	SU-SD-01	SU-SD-02	SU-SD-03A	SU-SD-03B	SU-SD-03C	SU-SD-06	SU-SD-07	SU-SD-08	SU-SD-09	SU-SD-10
12	0.32		1.12	0.25	1.03	0.16	1.99	0.22	0.34	1.99
13	0.51		1.41	0.32	1.33	0.13	2.62	0.14	0.35	2.29
14	3.41		10.78	1.98	10.04	0.53	27.61	1.13	1.74	16.65
i15	4.95		10.77	2.34	12.74	0.55	23.48	0.94	3.03	22.20
a15	1.78		4.41	0.82	3.73	0.17	8.48	0.43	0.84	4.89
15	4.46		8.86	2.61	9.61	0.78	23.15	1.51	3.51	18.52
16	15.02		36.05	6.23	36.00	2.23	147.67	5.95	14.52	82.94
i17	1.44		2.59	0.52	3.29	0.14	9.80	0.37	0.97	7.20
a17	0.71		1.50	0.33	1.63	0.10	4.62	0.20	0.42	3.47
17	2.81		5.55	1.58	5.33	0.50	20.15	1.05	2.96	12.18
18	5.31		10.04	2.68	10.92	0.97	28.31	1.74	3.03	22.75
Phytol	39.21		200.30	75.55	174.54	18.77	381.05	42.31	67.68	454.61
19	1.13		2.00	0.55	3.03	0.22	5.08	0.23	0.62	3.90
20	5.71		10.77	3.59	11.26	0.84	21.04	1.36	4.13	29.35
21	1.65		3.10	0.90	3.72	0.24	6.39	0.33	1.29	6.39
22	17.79		40.99	11.25	39.34	3.33	93.28	4.97	19.15	105.45
23	2.16		4.67	1.26	5.49	0.37	10.89	0.54	2.26	12.07
24	19.29		44.10	13.22	47.25	4.60	116.41	6.97	23.63	164.56
25	2.56		6.00	1.58	7.04	0.48	15.78	0.78	2.20	20.54
26	119.40		224.41	61.43	239.69	20.07	513.35	31.62	76.85	744.22
27	1.86		5.31	1.39	6.38	0.40	12.34	0.71	1.40	26.50
28	26.22		64.54	18.09	83.77	6.76	150.54	9.72	17.03	314.73
29	2.02		5.93	1.62	6.65	0.39	11.71	0.88	1.38	32.60
30	25.42		62.25	17.85	71.52	5.86	157.13	10.59	15.96	469.35
31	1.42		4.13	1.06	3.93	0.19	6.67	0.57	0.90	20.99
32	12.23		31.62	9.49	32.94	2.93	57.75	5.57	7.04	257.61
33	0.48		1.33	0.35	1.28	0.07	2.16	0.19	0.29	9.44
34	1.65		4.15	1.17	4.13	0.29	8.63	0.93	1.20	42.85
35	0.06		0.14	0.04	0.15	0.01	0.26	0.02	0.05	1.27
36	0.21		0.50	0.12	0.56	0.03	1.28	0.12	0.20	6.17

Appendix Table 5. Fatty alcohol concentrations in SUBURBAN and WOODLAND SOILS

(ug/g DW)	SU-SO-01	SU-SO-02	SU-SO-03	SU-SO-04	SU-SO-05	SU-SO-06	WL-SD-02	WL-SO-04	WL-SO-05
	37.9	33.7	39.4	42.8	43.8	31.1	46.5	15.1	35.9
12	0.23	0.36	0.08	0.05	0.15	0.15	0.07	0.59	0.18
13	0.22	0.18	0.06	0.04	0.12	0.12	0.10	0.61	0.19
14	0.75	0.40	0.13	0.08	0.47	0.39	0.33	1.64	0.69
i15	1.85	0.76	0.44	0.13	0.78	1.14	0.31	4.94	1.05
a15	0.33	0.51	0.11	0.18	0.18	0.15	0.31	1.06	0.26
15	0.40	0.41	0.09	0.09	0.45	0.27	0.24	2.10	0.45
16	1.63	1.30	0.35	0.30	1.15	1.09	0.83	3.76	1.37
i17	0.13	0.18	0.03	0.02	0.11	0.11	0.04	0.35	0.09
a17	0.14	0.15	0.03	0.01	0.08	0.08	0.07	0.37	0.11
17	0.51	0.67	0.07	0.08	0.56	0.30	0.43	1.44	0.36
18	1.52	1.33	0.79	0.47	1.06	1.44	1.30	8.29	3.47
Phytol	18.76	6.09	0.65	3.86	7.67	4.13	14.48	211.87	90.08
19	0.76	0.59	0.07	0.13	0.47	0.38	0.30	2.72	0.53
20	3.46	2.86	2.28	2.11	2.51	3.87	1.46	38.39	8.19
21	3.69	0.74	0.21	0.26	0.62	0.65	0.18	5.77	1.42
22	11.34	10.37	9.48	9.00	5.52	7.10	5.53	103.92	53.64
23	1.38	0.84	0.46	0.42	0.68	0.85	0.40	15.23	3.64
24	12.45	17.35	20.97	5.59	5.21	8.01	9.84	600.72	72.22
25	2.06	0.99	0.45	0.42	0.71	1.53	0.41	34.55	4.77
26	73.71	97.52	8.17	45.18	23.41	80.59	7.49	782.89	115.24
27	1.87	0.65	0.12	0.39	0.55	1.48	0.37	42.17	4.89
28	26.95	13.11	1.55	13.41	8.08	19.83	5.16	272.53	95.49
29	3.57	1.27	0.17	0.81	0.84	2.11	0.33	52.48	4.12
30	49.94	23.09	2.64	12.76	8.96	25.44	4.59	0.04	55.53
31	2.41	0.86	0.14	0.56	0.63	1.85	0.35	39.08	2.75
32	33.50	9.88	1.69	15.98	9.02	15.55	2.25	130.93	20.29
33	0.81	0.06	0.05	0.27	0.22	0.67	0.10	5.50	1.30
34	4.20	1.07	0.16	1.14	0.77	3.27	0.27	14.26	4.20
35	0.16	0.13	0.01	0.05	0.03	0.10	0.05	0.33	0.17
36	1.03	0.28	0.02	0.11	0.11	1.31	0.05	1.72	0.42

Appendix Table 6. Fatty alcohol concentrations in WWTP and RURAL ROAD samples

(ug/g DW)	WT-PI-01	WT-PI-02	WT-PI-03	WT-PS-01	WT-PS-02	WT-PS-03	WT-PS-04	RR-RD-01	RR-RD-02	RR-RD-03	RR-RD-04
	0.98	0.97	0.98	0.96	0.95	15.7	0.94	55.6	59.2	51.2	53.6
12	22.22	9.59	13.95	9.39	0.00	15.07	18.64	0.27	0.04		0.13
13	7.70	2.97	2.88	2.97	0.00	7.12	4.29	0.24	0.07		0.25
14	28.80	7.29	11.79	13.20	0.00	26.06	30.32	0.37	0.08		0.46
i15	0.55	1.44	1.72	1.64	0.00	49.78	1.60	0.25	0.02		0.17
a15	0.00	0.50	0.00	0.00	0.00	11.69	0.55	0.00	0.02		0.16
15	19.34	7.96	8.14	7.02	0.00	21.13	3.13	0.45	0.19		0.50
16	93.00	57.09	51.82	8.84	0.00	99.52	11.62	0.96	0.17		0.88
i17	3.06	0.44	3.17	1.11	0.00	8.88	0.68	0.11	0.01		0.01
a17	3.05	1.09	4.20	0.18	0.00	6.02	0.00	0.00	0.01		0.12
17	0.00	1.80	0.00	0.00	0.00	8.72	0.00	0.39	0.06		0.48
18	190.15	100.54	84.26	18.35	0.00	68.24	21.67	0.74	1.21		1.06
Phytol	48.19	21.63	16.92	28.70	0.00	116.36	27.18	15.19	0.06		0.90
19	10.56	0.76	0.00	200.81	0.00	2.55	154.45	0.32	0.06		0.35
20	9.29	3.16	3.94	2.51	0.00	15.24	1.63	1.04	0.10		0.86
21	0.97	0.61	8.86	0.53	0.00	4.22	0.38	0.46	0.03		0.02
22	9.20	3.26	3.89	3.19	0.00	45.53	4.26	3.59	0.00		2.34
23	1.80	0.49	1.11	0.39	0.00	7.89	0.97	0.64	0.04		0.01
24	3.91	1.40	2.07	1.55	0.00	45.87	1.31	6.53	0.10		4.25
25	1.16	0.44	0.25	0.32	0.00	6.27	0.23	1.00	0.01		0.00
26	2.37	1.21	1.19	2.52	0.00	56.48	0.95	18.04	0.01		9.67
27	0.85	0.37	0.17	0.48	0.00	4.93	0.11	1.00	0.01		0.01
28	0.77	1.74	0.17	0.61	0.00	106.30	0.67	10.11	0.01		4.70
29	0.45	0.69	0.44	0.46	0.00	7.98	0.32	1.23	0.00		0.00
30	2.47	0.29	0.94	1.32	0.00	88.09	0.38	7.20	0.00		0.01
31	0.27	0.40	0.10	0.11	0.00	3.05	0.12	0.60	0.02		0.02
32	0.19	1.17	0.59	0.79	0.00	82.51	1.03	4.62	0.01		0.01
33	0.87	0.25	0.18	0.23	0.00	1.67	0.41	0.22	0.01		0.02
34	1.83	0.19	0.14	0.36	0.00	20.09	0.30	0.61	0.03		0.03
35	0.31	0.36	0.22	0.22	0.00	0.42	0.43	0.01	0.02		0.01
36	0.42	0.18	0.18	0.09	0.00	2.22	0.27	0.01	0.01		0.01

Appendix Table 7. Fatty alcohol concentrations in SUBURBAN ROADDUSTS (µg.L^{-1}) and WWTP FINAL EFFLUENT (ng.L^{-1})

(ug/g DW)	SU-RD-01	SU-RD-02	SU-RD-03	SU-RD-04	WT-FE-01	WT-FE-02	WT-FE-03	WT-FE-04	WT-FE-05	WT-FE-06	WT-FE-07	WT-FE-08	WT-FE-09
12	0.16	0.54	0.03	0.04	2760.15	3176.13	1502.65	755.23	129.85	383.21	4354.78	723.06	1956.75
13	0.28	0.46	0.23	0.06	215.43	301.84	65.25	37.27	6.46	43.26	122.05	34.69	69.95
14	0.73	1.13	0.12	0.16	1767.02	2016.14	170.97	118.12	7.68	115.92	1128.29	122.49	338.15
i15	0.70	1.31	0.04	0.14	56.97	88.21	10.39	7.19	2.45	3.40	31.43	0.00	7.68
a15	0.00	0.00	0.00	0.00	20.30	36.73	1.20	3.20	4.48	112.82	338.30	0.00	17.85
15	1.08	0.73	0.31	0.37	251.53	355.84	25.98	8.42	1.39	16.95	147.78	10.56	16.90
16	3.36	2.20	0.57	0.50	1498.40	1421.30	79.97	44.10	5.57	19.51	355.09	25.75	50.27
i17	0.13	0.01	0.03	0.01	24.51	21.55	1.69	1.01	1.73	2.97	25.09	0.00	2.25
a17	0.14	0.25	0.00	0.01	96.77	108.39	5.87	2.17	1.73	3.39	0.00	2.88	2.25
17	1.12	0.94	0.02	0.48	45.56	35.43	43.61	10.95	1.32	1.68	25.09	2.32	0.00
18	3.65	4.60	0.84	0.92	545.67	339.42	17.90	15.69	3.55	36.50	279.75	12.64	13.30
Phytol	29.21	86.36	1.56	0.01	2761.42	6184.26	428.38	584.74	4351.11	7355.15	5149.64	2064.96	266.34
19	0.43	0.06	0.25	0.25	16.98	12.52	2.30	3.75	1.14	3.14	0.00	1.67	0.00
20	2.88	7.10	0.00	1.05	63.30	20.22	2.89	4.73	3.11	7.87	57.09	4.41	2.72
21	1.10	2.47	0.01	0.01	7.49	32.44	0.68	6.05	1.72	1.70	19.01	1.31	1.61
22	12.73	32.94	2.01	2.69	57.39	10.75	2.14	0.41	2.40	1.07	97.53	3.78	1.17
23	1.77	4.21	0.00	0.00	9.24	21.21	0.42	1.43	2.31	3.52	11.11	3.20	1.43
24	22.29	45.21	3.70	6.16	43.07	17.12	0.49	6.02	1.77	6.82	52.89	2.70	2.47
25	2.86	6.73	0.00	0.01	7.28	7.09	1.21	0.40	2.21	1.22	21.87	2.39	2.07
26	79.21	133.24	5.15	16.50	112.22	14.14	0.71	12.67	3.33	2.89	119.92	1.84	0.83
27	1.89	6.08	0.00	0.01	0.44	2.06	0.58	0.42	0.46	1.63	22.24	0.48	0.43

28	24.34	69.28	3.64	10.26	69.15	0.49	0.87	10.92	0.59	1.13	127.69	1.22	0.57
29	2.10	9.02	0.01	0.00	5.47	1.17	0.43	0.60	1.18	0.87	22.21	0.93	0.49
30	19.38	97.04	3.25	9.11	24.42	4.85	0.45	5.67	1.37	7.14	107.17	5.37	1.15
31	1.10	3.86	0.00	0.01	0.64	1.14	0.36	0.55	2.80	0.84	6.26	1.08	1.28
32	10.04	34.78	1.92	5.64	9.29	2.42	0.89	3.98	1.23	7.75	23.95	2.54	2.81
33	0.40	1.64	0.02	0.01	0.81	0.00	1.17	0.41	1.43	2.26	26.54	2.28	0.37
34	1.55	5.91	0.07	0.01	2.22	1.07	0.54	1.09	0.21	0.50	13.95	3.04	1.48
35	0.17	0.20	0.01	0.01	0.30	0.00	1.14	0.75	1.57	1.98	28.07	0.62	0.71
36	0.24	0.63	0.02	0.01	0.89	0.52	1.09	0.63	0.93	1.17	9.55	1.69	0.80

Sample ID	$\delta^{13}C$	$\delta^{2}H$
AG-SD-03_20	-29.2481	-190.531
AG-SD-03_22	-30.1702	-129.7
AG-SD-03_24	-30.7444	-119.122
AG-SD-03_26	-32.6557	-131.939
AG-SD-04_16	-25.5731	-137.022
AG-SD-04_20	-28.0924	
AG-SD-04_22	-29.3225	-131.748
AG-SD-04_24	-31.0065	-117.154
AG-SD-04_26	-32.9379	-127.482
AG-SD-05_14	-23.1985	-149.394
AG-SD-05_20	-28.7007	-144.908
AG-SD-05_22	-30.3202	-125.927
AG-SD-05_24	-30.9683	-112.582
AG-SD-05_26	-32.4405	-138.547
AG-SD-06_14	-28.9469	-155.8
AG-SD-06_16	-30.7043	-189.664
AG-SD-06_18	-30.961	-202.245
AG-SD-06_20	-29.8151	-124.583
AG-SD-06_22	-31.2373	-134.772
AG-SD-06_24	-32.856	-130.965
AG-SD-06_26	-34.9913	-182.486
AG-SD-09_14	-25.9889	-144.739
AG-SD-09_16	-25.9733	-193.757
AG-SD-09_18	-28.0455	-166.341
AG-SD-09_20	-29.2447	-147.426
AG-SD-09_22	-29.8975	-149.515
AG-SD-09_24	-32.0381	-128.029
AG-SD-09_26	-32.6212	-164.654
AG-SD-09_28	-35.1365	-127.987
AG-SD-13_18	-28.7327	-104.968
AG-SD-13_20	-29.1228	-148.34
AG-SD-13_22	-30.0214	-126.295
AG-SD-13_24	-34.1633	-126.322
AG-SD-13_26	-33.9808	-161.613
AG-SD-16_14	-27.0575	-134.946
AG-SD-16_16	-28.4789	-164.768
AG-SD-16_18	-29.0815	-137.14
AG-SD-16_20	-27.7485	
AG-SD-16_22	-28.6884	-119.602
AG-SD-16_24	-32.847	-117.444
AG-SD-16_26	-34.0198	-135.613
AG-SO-01_22	-28.2055	-74.0206
AG-SO-01_24	-33.1688	-153.948
AG-SO-01_26	-34.1604	-136.736
AG-SO-04_24	-34.593	-105.16
AG-SO-04_26	-33.8849	-149.459
AG-SO-06C_22	-31.2839	
AG-SO-06C_24	-35.0801	
AG-SO-06C_26	-38.1289	

AG-SO-08_22	-29.7202	-124.649
AG-SO-08_24	-33.0945	-121.754
AG-SO-08_26	-34.3913	-163.888
AG-SO-08_28	-34.552	-118.784
DEO-01_16	-27.2213	-183.816
DEO-01_18	-24.9013	-271.838
DEO-02_14	-28.8364	-43.6847
HDD-01_12	-25.9025	-44.6684
HDD-01_13	-24.8923	-43.9029
HDD-01_14	-24.6338	-48.3031
HDD-01_15	-24.7596	-47.7487
HDD-02_12	-27.1788	-220.025
HDD-02_13	-28.3163	-112.443
HDD-02_14	-23.8202	-254.791
HDD-03_12	-26.5688	-71.5043
HDD-03_13	-26.4874	-65.0266
HDD-03_14	-26.4929	-63.6184
HDD-03_15	-26.3028	-62.774
HDD-04_12	-29.2375	-184.886
HDD-04_13	-28.5169	-148.217
HDD-05_12	-32.7913	-178.103
HDD-05_13	-31.088	-154.599
HW-LL-01_22	-29.1589	-145.831
HW-LL-01_24	-30.7196	-134.344
HW-LL-01_26	-28.8902	-126.149
HW-LL-01_28	-29.6307	-118.146
HW-LL-02_22	-29.9702	-131.563
HW-LL-02_24	-30.5835	-133.777
HW-LL-02_26	-29.0196	-110.378
HW-LL-02_28	-30.3725	-108.561
HW-SO-02_22	-29.3009	-152.708
HW-SO-02_24	-29.3876	-125.906
HW-SO-02_26	-29.6319	-121.505
LDD-07_12	-27.252	-314.42
LDD-07_14	-26.4455	-288.302
LDD-10_12	-26.0125	-104.422
LDD-10_13	-27.6849	-85.4095
LDD-10_14	-24.2282	-67.0805
LDD-10_15	-25.536	-69.4553
LHS-01_12	-24.2825	-282.113
LHS-01_14	-23.3746	-260.32
LHS-02_11	-24.9785	-60.1038
LHS-02_12	-25.0975	-277.561
LHS-02_13	-26.5563	-143.457
LHS-02_14	-24.5136	-131.662
LHS-02_15	-25.1064	-63.0833
LHS-03_11	-24.7011	-62.1736
LHS-03_12	-24.0425	-276.869
LHS-03_13	-28.3422	-133.716
LHS-03_14	-24.2015	-123.656

LHS-03_15	-25.2468	-57.5233
LLD-01_12	-25.6713	-90.7073
LLD-01_13	-26.9945	-68.6086
LLD-01_14	-25.3235	-64.2821
LLD-01_15	-23.9556	-69.8087
LLD-02_12	-27.525	-53.5708
LLD-02_13	-27.2775	-47.8491
LLD-02_14	-26.4419	-48.6057
LLD-02_15	-26.616	-41.3673
LLD-03_12	-29.1813	-194.929
LLD-03_13	-28.2228	-101.827
LLD-03_14	-23.6478	-94.7499
LLD-03_15	-24.9492	-47.014
LLD-04_12	-28.6563	-162.597
LLD-04_13	-27.3465	-90.0937
LLD-04_14	-24.8851	-81.7919
LLD-04_15	-24.5784	-45.7313
LLD-08_12	-30.9475	-200.112
LLD-08_13	-29.68	-94.8914
LLD-08_14	-27.8941	-82.9401
LLD-08_15	-25.95	-56.1593
LLD-09_12	-26.0025	-70.354
LLD-09_13	-26.3003	-58.2088
LLD-09_14	-25.638	-60.8309
LLD-09_15	-25.3752	-56.4367

PLD-01_12	-25.9838	-57.0487
PLD-01_14	-26.1978	-58.7381
PLD-02_12	-24.0275	-247.26
PLD-02_14	-22.2356	-106.941
PLD-02_15	-23.5536	-50.0873
PLD-03_14	-24.8475	-51.5751
PLD-03_15	-24.9816	-55.098
PLD-03_16	-23.964	-65.9946
SHA-01_12	-23.11	-281.57
SHA-01_14	-23.7461	-259.435
SS-SD-02_14	-19.8871	-145.142
SS-SD-02_15	-23.844	-158.383
SS-SD-02_16	-25.2156	-149.445
SS-SD-02_26	-33.3462	-158.158
SU-RD-01_14	-26.2621	
SU-RD-01_16	-25.2014	-105.3
SU-RD-01_20	-29.6771	-133.816
SU-RD-01_22	-32.085	-116.904
SU-RD-01_24	-29.535	-121.991
SU-RD-01_26	-32.7305	-171.245
SU-RD-02_14	-20.3474	
SU-RD-02_16	-24.3381	
SU-RD-02_20	-28.5006	-155.503
SU-RD-02_22	-33.0441	-135.892
SU-RD-02_24	-33.2666	-137.919

SU-RD-02_26	-34.7504	-209.157
SU-RD-04_24	-29.3876	-103.971
SU-RD-04_26	-31.6251	-138.451
SU-SD-01_22	-31.0873	-136.031
SU-SD-01_24	-32.3576	-129.805
SU-SD-01_26	-33.5737	-146.954
SU-SD-01_28	-35.6303	-129.259
SU-SD-02_26	-31.7121	-133.316
SU-SD-03A_22	-29.0486	-123.411
SU-SD-03A_24	-27.7091	-124.636
SU-SD-03A_26	-31.4344	-133.524
SU-SD-03A_28	-33.9519	-124.708
SU-SD-03C_22	-28.7486	-118.698
SU-SD-03C_24	-30.1695	-103.677
SU-SD-03C_26	-32.8922	-143.808
SU-SD-03C_28	-34.0194	-136.765
SU-SD-04_20	-25.59	
SU-SD-04_22	-28.1827	-117.311
SU-SD-04_24	-33.6064	-164.297
SU-SD-04_26	-33.2737	-161.457
SU-SD-06_22	-29.7407	-117.831
SU-SD-06_24	-29.3134	-128.884
SU-SD-06_26	-34.8397	-124.734
SU-SD-06_28	-34.3505	-150.134
SU-SD-07_14	-21.4463	-145.669
SU-SD-07_16	-25.4994	-193.075
SU-SD-07_20	-30.4568	-133.808
SU-SD-07_22	-31.1611	-145.83
SU-SD-07_24	-31.2214	-133.767
SU-SD-07_26	-33.3093	-185.704
SU-SD-07_28	-32.6023	-147.035
SU-SD-08_22	-29.8214	-119.753
SU-SD-08_24	-31.1314	-132.587
SU-SD-08_26	-34.572	-139.15
SU-SD-09_16	-21.9417	-140.604
SU-SD-09_18	-29.0617	
SU-SD-09_20	-28.5903	-144.218
SU-SD-09_22	-31.4736	-141.861
SU-SD-09_24	-33.4241	-122.166
SU-SD-09_26	-31.8917	-177.838
SU-SD-09_28	-33.8855	-108.337
SU-SD-10_14	-20.6825	-138.263
SU-SD-10_16	-25.4983	-205.433
SU-SD-10_20	-29.3137	-176.45
SU-SD-10_22	-30.8645	-132.109
SU-SD-10_24	-31.056	-129.549
SU-SD-10_26	-32.8242	-167.542
SU-SO-01_16	-26.8378	
SU-SO-01_20	-26.9918	-116.71
SU-SO-01_22	-26.6668	-122.792

SU-SO-01_24	-27.474	-148.047
SU-SO-01_26	-31.3139	-171.665
SU-SO-02_20	-26.2558	
SU-SO-02_22	-26.0259	-119.762
SU-SO-02_24	-26.8013	-152.133
SU-SO-02_26	-30.7384	-140.437
SU-SO-03_20	-24.6677	-180.286
SU-SO-03_22	-26.685	-169.386
SU-SO-03_24	-26.5819	-173.891
SU-SO-03_26	-25.3221	-151.487
SU-SO-06_24	-31.119	-131.856
SU-SO-06_26	-32.6959	-159.461
WL-SO-04_22	-29.6486	-119.291
WL-SO-04_24	-30.921	-156.335
WL-SO-04_26	-32.6223	-148.016
WL-SO-04_28	-31.7465	-145.419
WT-FE-all_14	-23.5349	-89.453
WT-FE-all_16	-23.831	-92.1514
WT-FE-all_18	-24.0613	-59.0788
WT-PI-all_12	-18.495	
WT-PI-all_14	-27.3635	-25.9957
WT-PI-all_16	-26.7629	-163.45
WT-PI-all_18	-28.2217	-157.405
WT-PS-03_12	-18.13	
WT-PS-03_18	-26.3597	-89.4439