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The effect of secondary treatment and eco-region on the environmental fate of fatty alcohol based surfactants[☆]



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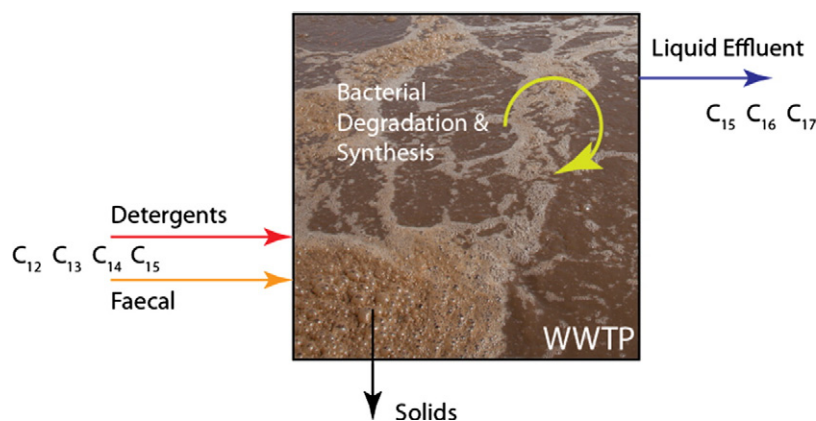
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HIGHLIGHTS

- Secondary treatment technology does not alter the efficiency of removal of fatty alcohols.
- Stable isotope analysis shows fatty alcohols in the effluent are not those from the influent.
- The eco-region affects the influent composition and deuterium content of plant matter.
- Naturally occurring fatty alcohols dominate in the sediments of the receiving waters.

GRAPHICAL ABSTRACT



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ABSTRACT

Samples of influent, effluent and sediments of the receiving waters of eight WWTPs were collected in each of three eco-regions of the USA, a total of 24 facilities. Six different treatment technologies were included to determine the fate of anthropogenic fatty alcohols. The lipids were analysed by compound-specific stable isotope ratio mass spectrometry. There were significant differences in the profiles of the influent among eco-regions, due to differences in the products used within the catchment, the diets of the inhabitants, or in-pipe processes. The sediments of all the receiving waters had similar fatty alcohol profiles, with terrestrial plant matter dominating and secondary contributions from algal and bacterial synthesis. Any contributions from the WWTP liquid effluents were small (<1%) and not from the original fatty alcohols suite in the influent. These compounds might have the same chain lengths, but they have different stable isotopic signatures. The type of secondary treatment did not affect the removal of fatty alcohols and the sediments of the receiving waters were dominated by terrestrial plant inputs; the eco-region may affect the profile of the influents but not the stable isotopes. The ecological risk from the use of these particular chemicals, which are disposed of down the drain, is minimal.

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1. Introduction

Fatty alcohols and their derivatives are widely used as surfactants in many detergents and personal care products. These high-production-

volume chemicals have been assessed under REACH and similar global chemical management programmes, with data requirements driven by the need to understand the environmental sources, fate and effects of these compounds (Belanger et al., 2006, 2009). The principal chemical form of these compounds in product formulations is as ethoxylates with the number of ethoxylate units ranging from 0 to 18 (Morrall et al., 2006), as ethoxysulphates and sulphates (Federle and Itrich, 2006). The global market for these detergent alcohols reach 2.18 million tonnes in 2012 (Gubler and Inoguchi, 2013). The principal disposal route for all of these compounds is down-the-drain with subsequent removal in wastewater treatment plants.

The fate of the detergent fatty alcohols is of particular importance as regulators need to assess the risk to the environment from their use. Recent evaluation of river fauna communities based on risk calculations using alcohol ethoxylates in river water, pore water, sediments and benthic organisms (Sanderson et al., 2013) indicated that factors other than fatty alcohol contributions were responsible for the observed structure.

The fate of these chemicals has been investigated previously; analysis of multiple samples collected from two different catchments – one in the UK (Mudge et al., 2010) and one in the USA (Mudge et al., 2012) – led to the conclusions that fatty alcohols in the effluent from the two wastewater treatment plants (WWTPs) were not the same as the ones in the influent, and that the fatty alcohols in the sediments of the receiving waters were dominated by naturally occurring compounds, not those from the WWTPs. While these results are consistent and confirm laboratory investigations (Federle and Itrich, 2006; Itrich and Federle, 2004; Matthijs et al., 1995), the secondary biological treatments used in both cases were oxidation ditches.

Wastewaters are treated using several technologies and the secondary biological treatment processes may directly affect the degradation of compounds and may induce of different chemical products (McVicar et al., 2012). For instance, it is possible that the microorganisms present in a relatively static lagoon system lead to a different chemical suite in the effluent compared to an oxidation ditch or activated sludge system. Therefore, to ensure that the conclusions drawn from previous studies are valid across and wider geographical area, a range of WWTP technologies were included in the sampling design.

Previous work (Mudge et al., 2010, 2012) used the stable isotopes of carbon (^{12}C and ^{13}C) and hydrogen (^1H and ^2H) to develop chemical signatures of the organic compounds. These naturally occurring isotopes have slightly different ratios depending on the processes that the sources undergo during fatty alcohol synthesis. Therefore, there are measureable differences between the petrochemical and oleochemical surfactants along with many other natural sources. This approach was able to separate the different origins within the mixed source environment of the sewer system and receiving waters.

North America has been divided into 15 ecological regions, ranging from the high arctic to tropical wet forests; these were proposed by Omernik (1987) and developed by the USEPA (2012). Although there are 15 regions, the bulk of the USA is encompassed by just six, with two of these having restricted ranges along the West Coast. The previous USA study (Mudge et al., 2012), conducted in Luray, Virginia, was in ecological region 8.0 (sub-region 8.3, south-eastern USA plains), part of the Eastern Temperate Forests. The bulk of the USA's population live toward the East and West Coasts, with comparatively fewer population centres in the middle.

It is possible that the different climates across the USA may lead to different microbial assemblages in the WWTPs or in the environment. Along with the different secondary treatment processes, this may influence the chemical composition of the final effluents.

2. Materials and methods

It can be argued that performance differences may occur over the range of climatic conditions in the USA, and also between the different

secondary treatment processes available. Therefore, this work was undertaken to examine both of these potential factors in determining the fate of fatty alcohols in wastewater streams. Different eco-regions (climatic zones) were chosen for this study:

- The Great Plains (region 9), with the sampled zone further sub-classified as region 9.4, South Central, Semi-Arid Prairies. As the name suggests, the zone has low rainfall, wide-open grass plains typically grazed by cattle, and cool winter temperatures followed by hot, dry summers. Although the region extends northward into Canada, the major population centres are in the south and samples were collected in Oklahoma.
- The Eastern Temperate Forests (region 8) and the sampled zone are further sub-classified as sub-regions 8.1 (mixed wooded plains), 8.2 (central USA plains), and 8.4 (Ozark, Ouachita–Appalachian Forests). The region is distinguished by a moderate to mildly humid climate, a diverse forest cover, and a high density of human inhabitants (approximately 160 million). Activities include standard urban industries, agriculture, and forestry. In large parts of this region, the forests have been cleared and the land is now used for agriculture, especially corn and soybean farming. Samples were collected in Ohio.
- The Marine West Coast Forests (region 7.1), which has no further sub-classification. This region is described as highly productive, rain-drenched evergreen forests. The region includes the Willamette Valley, which runs from south to north between the Oregon Coast Range to the west and the Cascades Range to the east. The eco-region is drained mostly by the Willamette River and its tributaries, which flows into the Columbia River straddled by Portland, Oregon. The majority of the samples were collected in Oregon, with two sites in Washington (WA).

Previous work (Mudge et al., 2010, 2012) indicated that analysis of the influent, effluent and sediments in the receiving waters would be sufficient to determine the contribution that the different fatty alcohol sources make to the environment. The sampling plan, therefore, was designed to sample the three locations at each WWTP (influent, effluent and sediment) in the three different ecological regions across the four or five different secondary treatment techniques per eco-region depending on finding suitable WWTPs.

2.1. Sampling plan

Sampling sites were chosen on the basis of the performance of the WWTPs, with an influent flow of less than 152 million litres per day (equivalent to 40 million gallons per day, MGD) but greater than 3.8 million litres per day (1 MGD), a low effluent flow as a proportion of the receiving-water flow and <10% industrial contribution to the influent. This is the same screening process that was used in the selection of the Luray catchment in the initial study (Mudge et al., 2012). One of the objectives of the study was to determine whether the secondary treatment process for a facility influenced the magnitude and distribution of fatty alcohols discharged to the environment. Generally, samples from facilities covering at least four styles of secondary treatment were collected in each eco-region (activated sludge, oxidation ditch, lagoon and a fixed-film technology, either trickling filter or rotating biological contactor); these secondary treatment technologies were observed to be among the most common in the US EPA's quadrennial Clean Watersheds Needs Survey (<http://water.epa.gov/scitech/datat/databases/cwns/>). Facilities using fixed-film technology and meeting the other criteria could not be located in some areas, so sequencing batch reactor (SBR) facilities were sampled as an alternative technology that was observed to be prevalent. In order to include a sufficient number of lagoon facilities, the criterion >1 MGD influent flow had to be relaxed, because some lagoons were only available in small, rural communities and had lower influent flow rates. The data associated with the chosen WWTPs can be seen in Table 1.

Table 1

Metadata for the wastewater treatment plant, and site abbreviations.

WWTP	Abbrev.	Secondary treatment	Influent MGD (litres per day $\times 10^6$)	Population served (in thousands)
<i>Oklahoma sites</i>				
Winfield (KS)	WIN	Oxidation ditch	1.2 (4.6)	12
Stillwater	STI	Activated sludge	5.4 (20.5)	48
Edmond (Coffee Creek)	EDM	Oxidation ditch	7 (26.6)	84
Deer Creek	DEE	RBC & activated sludge	15 (57)	82
Del City	DEL	SBR	1.5 (5.7)	25
Ada	ADA	SBR	2.5 (9.5)	15
Weatherford	WEA	Activated sludge	1 (3.8)	10
Elk City	ELK	Lagoon	1.2 (4.6)	12
<i>Ohio sites</i>				
East Liverpool	E.L	RBC	1.7 (6.5)	11
Alliance	ALL	Activated sludge	4.0 (15.2)	23
Massillon	MAS	Oxidation ditch + TBF	14.8 (56.2)	36
Summit/Stow/Fish Creek	FIS	Oxidation ditch	3.5 (13.3)	40
Strongsville	STR	RBC	1.0 (3.8)	15
French Creek	FRE	Activated sludge	5.8 (22.0)	50
Danville	DAN	Lagoon	0.1 (0.4)	1.1
New Bremen	NEW	Lagoon + TBF	0.8 (3.0)	3.5
<i>Oregon sites</i>				
Everett (WA)	EVE	TBF and lagoon	13.5 (51.1)	150
Chehalis (WA)	CHE	SBR	1.5 (5.7)	9
Astoria	AST	Lagoon	1.6 (6.1)	10
McMinnville	MCM	Oxidation ditch	3 (11.4)	33
Molalla	MOL	Lagoon	1.1 (4.2)	8.1
Silverton	SIL	Activated sludge	1 (3.8)	8.0
Stayton	STA	SBR	1 (3.8)	10
Corvallis	COR	Activated sludge	6 (22.7)	55

RBC = rotating biological contactor; TBF = trickling bed filter; SBR = sequencing batch reactor.

In general, the SBRs and RBC had shorter hydraulic residence times in the range of three to eight hours. The activated sludge and oxidation ditch facilities had longer residence times with operators reporting times in the 24–60 hour range. The lagoons had even longer residence times potentially out to 40 days. At Elk City in Oklahoma, the WWTP makes no discharges to the river over the summer months and sells the treated effluent to golf courses for irrigation. In this case, the irrigation water was collected for analysis.

2.2. Sample collection

At each site, a single 2.5-L sample of liquid influent was collected in a pre-cleaned amber glass bottle. All WWTP facilities provided 24 hour composite samples of the influent based primarily on flow. Similarly, at each site a single 2.5-L grab sample of the final effluent was collected from the active discharge stream. Sediments were collected 100–200 m downstream of the effluent discharge point in the rivers. Surface scrapes to 1 cm were collected in pre-cleaned 125-mL glass jars. All samples were stored in cool boxes with ice until extracted in the laboratory. The two liquid samples from each WWTP had 30 g of KOH added immediately after collection to begin the saponification process.

2.3. Extraction and analysis

All samples were extracted using published techniques (Mudge et al., 2010, 2012). Upon receipt in the laboratory, an internal standard (1.0 mL of a 1.00 mg·mL⁻¹ solution of 2-dodecanol from Sigma Aldrich in methanol) was added before all extractions for quantitation.

For the liquid samples, the fatty alcohols were extracted into hexane by liquid–liquid separation; two litres of the shaken sample was added to a separating funnel along with 100 mL of hexane. The extraction was repeated once and the hexane combined. Any water remaining in this phase was removed by anhydrous sodium sulphate and the extracts were taken to dryness under a stream of nitrogen.

The sediment samples (120 g wet weight) were refluxed in 6% KOH in methanol for 4 hours. After removing the solids through centrifugation, the fatty alcohols in the solvent phase were partitioned into hexane by liquid–liquid separation. As with the liquid samples above, any remaining water was removed with anhydrous sodium sulphate and the extracts were taken to dryness under a stream of nitrogen. The moisture content of the sediments was calculated by drying at 40 °C a known amount of the moist sediment until constant weight was achieved. All results are reported per kg dry weight.

All dry extracts from the sediments and liquids were derivatised with the same batch of BSTFA (Sigma Aldrich); the lipids were derivatised at 60 °C with ~5 drops of BSTFA for 0.5 h to ensure complete derivatisation of the secondary alcohol. Excess BSTFA was evaporated under nitrogen and the final samples re-dissolved in 1 mL of hexane and were analysed by Gas Chromatography–Mass Spectrometry to both identify and quantify the fatty alcohols.

The GC was operated with a DB-5MS column (60 m \times 0.25 mm ID \times 0.25 μ m film thickness) with an injection at 40 °C and a steady ramp to 300 °C. The MS was operated in the full scan mode from 45 to 510 *m/z*. Each compound was identified through its characteristic ion (*M*-15 *m/z*) and quantified with the Automated Mass Spectral Deconvolution and Identification System (AMDIS) software from the National Institute of Standards and Technology (NIST).

Additionally, all samples were sent for compound-specific stable-isotope analysis on a Thermo Delta V Plus Isotope Ratio Mass Spectrometer. For each sample, 1 μ L was injected for carbon-13, and 2–3 μ L for hydrogen-2 analysis into a split–splitless port. The GC conditions were the same as above and measurements were made relative to Vienna Pee Dee belemnite for ¹³C and standard marine ocean water (SMOW) for deuterium.

As part of a QA/QC programme, the standard was derivatised and injected to develop a calibration curve. In this case, all samples fell within the linear portion of this curve and all recoveries were greater than 76%. A part derivatised sample was used to determine the $\delta^{13}\text{C}$ and $\delta^2\text{H}$ contribution that the trimethylsilyl (TMS) group made to the

total fatty alcohol molecule. Each stable isotopic value was corrected for the three carbons and nine hydrogens added as part of this TMS group. Repeat injections were made for three samples to determine the coefficient of variation in these pseudo-replicates.

2.4. Statistical treatment

The fatty alcohol profile data from all samples were investigated by principal components analysis (PCA) after conversion of the concentrations to proportions in order to preserve the chemical signature. If the data were significantly not normally distributed, a \log_{10} transformation was used to improve interpretation. The commercial programme SIMCA-P from Umetrics (version 10.0.4.0) was used for this purpose.

3. Results

The three eco-regions sampled in this survey were all different, especially with regard to rainfall. Oklahoma was hot and dry; Oregon was cool and wet, and Ohio was in between and this reflects their climatic region. In the period running up to the sampling in Oklahoma, there had been a sustained period of drought and a reduction in the stream flows (USGS, 2012). This meant that, at some locations, the WWTP liquid discharges made up the majority of the stream flows, so that environmental concentrations of compounds from the municipal wastewater discharge might have been greater than usual. The influents will have been more dilute than normal in Oregon, due to high rainfall, and potentially more concentrated in Oklahoma. The greater influent flow rates in Oregon might have led to shorter residence times in the WWTPs, depending on the technology used. The distribution of total fatty alcohol concentrations in the influents can be seen in Fig. 1. While the Ohio and Oregon samples were similar in range ($<100 \mu\text{g/L}^-$), the sum of the fatty-alcohol concentrations in the dry state of Oklahoma was significantly greater, with an outlier at Weatherford, Oklahoma, of $1477 \mu\text{g/L}^-$.

Some WWTP locations used balancing tanks or pools to regulate the flows where possible. The effluent concentrations might have been greater than normal in Oregon if the hydraulic residence (treatment) time was reduced. However, the sediments in Oregon rivers would potentially contain fewer compounds discharged from the WWTP, because they would remain in suspension and be carried away from the sampling sites. Conversely, the Oklahoma sediments might have experienced higher concentrations, because there was little water in the streams, and in some cases, the stream flow was almost entirely from the WWTPs.

Notwithstanding these climatic differences, there was less variation with respect to the WWTPs and their operation. The influent flow was

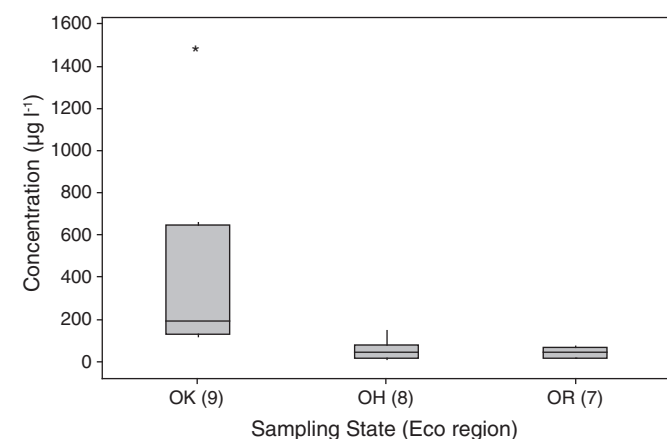


Fig. 1. A box-and-whisker plot of the concentrations in the influent to eight WWTPs in each eco-region.

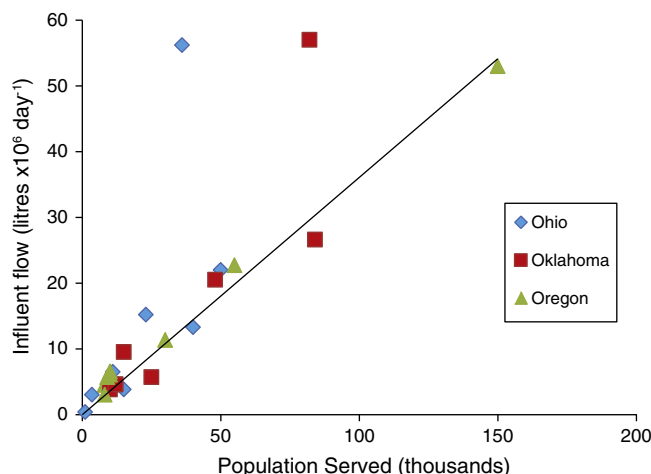


Fig. 2. Influent flow measured at the WWTPs compared to the population served. The line is a regression line fitted to the data excluding the two high flow sites; the intercept was forced to zero and the slope represents the *per capita* water usage rate (366 L/day , $R^2 = 0.946$).

directly related to the population served, with only two exceptions (Fig. 2). In this figure, a significant correlation can be seen between the flow and the population at most sites enabling those with unusual flows to be identified. One Ohio WWTP can be seen that has a considerably elevated flow relative to the population served; this is Massillon, Ohio, where 60% of the influent was from “the county” and the remainder from “the city.” In their summary statement, the WWTP indicated that 40% of their influent was described as “industrial,” and this added input was from food processors and a paper mill; this information was not available during the initial screening process. The other large flow relative to its population is Deer Creek, Oklahoma, which appeared to take twice as much influent than the population might suggest. The reason for this is not readily apparent, although the location was described by WWTP operators as relatively affluent.

If these two points are removed from the calculation, the linear regression between the two is strongly significant ($R^2 = 0.946$) and indicates a *per capita* contribution to the wastewater system of 366 L/day , although the iSTREEM® model suggests a national median of 440 L/day . This does not vary by region, including in Oklahoma, where water scarcity might be expected.

With regard to the composition of the influent, there are measurable regional differences. These can be seen in the PCA of the influents alone (Fig. 3). The data were converted to proportions to remove the concentration effects (Mudge, 2007). The influents collected from the Ohio Eco-region were significantly enriched in the C_{12} fatty alcohols compared to the other two regions. The Oklahoma sites had higher proportions of the longer chain fatty alcohols (C_{20} – C_{25}) compared to the Oregon region and imply greater amount of terrestrial plant matter in the influent. The fatty alcohol composition of the influent is determined, in part, by the local diet, the sequestration of surface waters and the chemical composition of the detergents and personal care products used within the catchment. The location of the samples in the PCA figure indicates a significant spread for each eco-region, although every eco-region is distinctly different from the others. The vectors from the loadings data are added to indicate the compounds which drive the distribution.

The stable isotopes provide the definitive source apportionment in these cases, because both the carbon-13 and the deuterium content of the fatty alcohols are dependent on the mechanisms by which the compounds are formed. The individual plots for each eco-region indicated that there were significant differences between the stable isotopic content of the fatty alcohols in each of the three sample types (influent, effluent and sediments). These data can be combined and presented on a single cross plot, together with the compound-specific stable-isotope values for the major detergent fatty alcohols derived

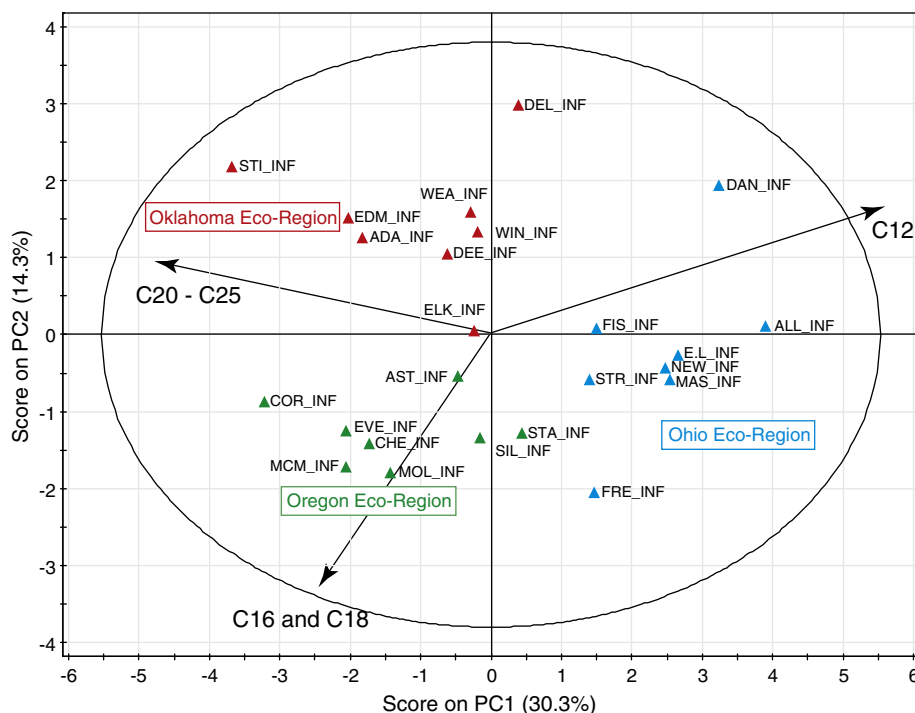


Fig. 3. PCA scores plot of the municipal WWTP influents with the fatty alcohols that drive the distribution indicated as vectors. The three different eco-regions are clearly separated on the basis of their fatty alcohol composition. Location name abbreviations are expanded in Table 1. The first principal component (PC1) explains 30.3% of the variance and PC2 explains 14.3%.

from the Luray study (DeLeo et al., 2011; Mudge et al., 2012). These colour-coded results are presented in Fig. 4.

4. Discussion

The concentrations of fatty alcohols were similar to previously measured values in the USA (Mudge et al., 2012), however, the profiles for each eco-region had distinct differences. There are several reasons why separation of the influents (Fig. 3) was observed, including contributions from each of the following:

1. Human diet – Differences in the diets among the populations in each eco-region. It is possible that the proportion of meat and vegetables varies among the regions. However, the most prevalent fatty alcohol, C₁₂, is not a great contributor to the human diet and tends to come from other sources.
2. Detergent use – Differences among catchments in the detergent product preferences and usage. Data from Luray (DeLeo et al., 2011) indicated that liquid laundry detergents would be the greatest contributor of C₁₂ to the influent. Luray is in the same broad eco-region as Ohio. However, it is unlikely that the chemical composition of the detergents used was substantially different when compared to the other two regions; the proportion that each product contributes toward total product sales may be different, reflecting local preferences. This would alter the composition of the influent surfactant load although the profile is not expected to vary considerably.
3. In-sewer alterations – Differences in the in-pipe processes due to different environmental conditions such as ambient temperature. Sampling in Oklahoma was conducted in spring, when the weather was warm and dry. For Ohio, sampling was done in the autumn, when the weather was cool and mostly dry. In comparison, sampling in Oregon was conducted in March, when the weather was cold and wet. As the influent passes through the pipe, it may be cooled substantially before it reaches the treatment facility, which may lead to altered biochemical transformations. In this case, the Oregon samples were relatively rich in the C₁₈ and C₁₆ fatty alcohols.

In the UK data (Mudge et al., 2010), the C₁₈ was also elevated, although in this case, the C₁₂ component was the major contributor. The C₁₈ is not a significant compound in the detergents (DeLeo et al., 2011), and the UK initial study (Mudge et al., 2010) indicated that the C₁₈ was between 20% and 25% of the C₁₆ concentration in human faecal matter.

4. Surface-water contribution – Significant sequestration of surface waters containing long-chain terrestrial fatty alcohols in the Oklahoma and Oregon eco-regions. All the long-chain compounds locate to the left of the centre line for PC1 in Fig. 3. The two regions that occupy this location are both the wettest and the driest, so this condition may come down to the integrity of the infrastructure.

If the fatty alcohol profiles for all samples (wastewater influent, effluent and receiving water sediments) are considered for the three eco-regions, significant patterns emerge. The scores plot from a PCA after log₁₀ transformation can be seen in Fig. 5. It is clear from this figure that the samples are distributed according to their sample type, with the influent samples clustering together; the effluents occupy a separate location in the figure between the influent samples and the sediments. The sediments are dominated by the longer chain terrestrial fatty alcohols, while the influents and effluents have greater proportions of the short-chain compounds, notably the C₁₂. This distribution is also confirmed through multivariate analysis of variance (MANOVA, not shown) using sample type (influent, effluent and sediment), treatment type (lagoon, oxidation ditch, SBR, activated sludge, trickling bed filter and RBC) and eco-region (Region 7, 8 or 9) as potentially controlling variables. Significant differences could be seen only in the sample type when all samples are included.

These PCA and MANOVA results point to significant differences between the sample types (influent, effluent and sediment) and can clearly be seen in the mean fatty alcohol profiles of individual samples. There are also significant differences among the eco-regions for the influent samples only (Fig. 3) which may be due to a diversity of factors which contribute to the influent profile such as biochemical processes and local vegetation. In contrast, the different wastewater treatment

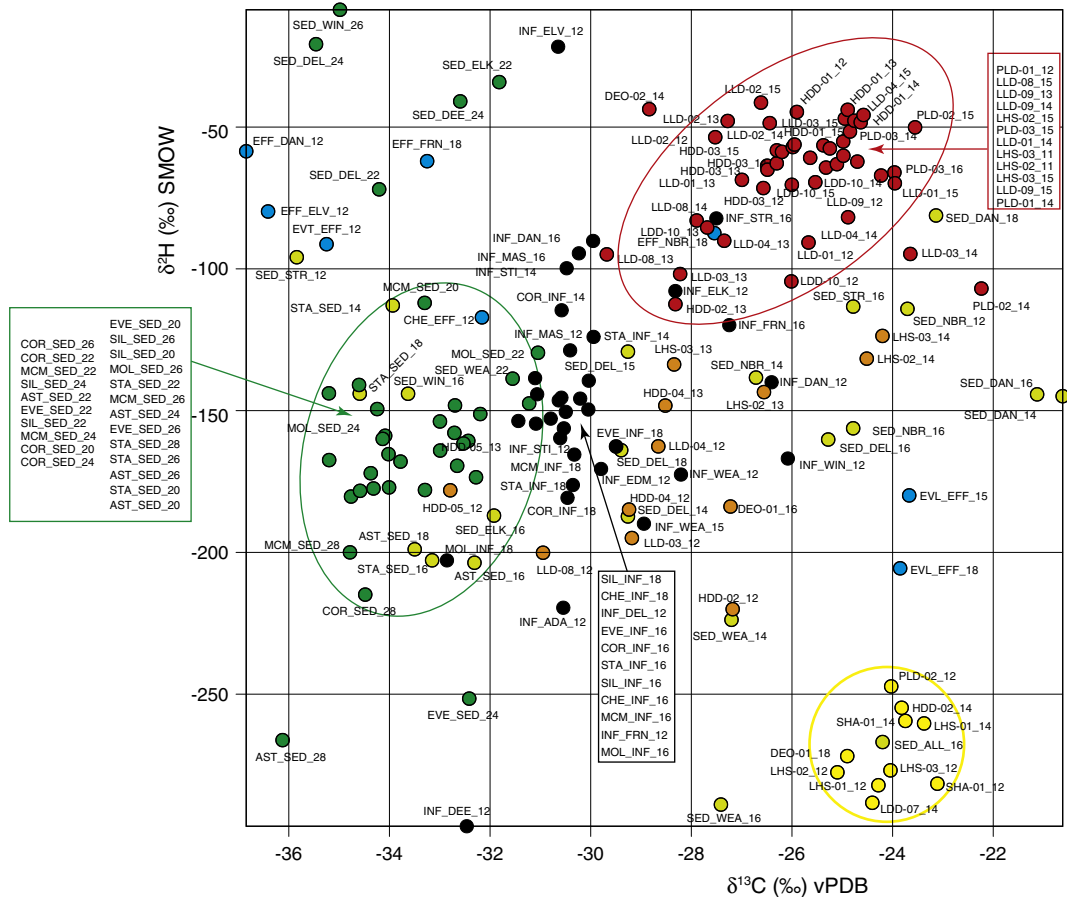


Fig. 4. The stable-isotope cross plot for all fatty alcohols measured in the three eco-regions, together with the detergent-derived fatty alcohols from the Luray study (DeLeo et al., 2011; Mudge et al., 2012). The data are colour-coded according to their likely source. Dark green circles indicate long-chain (C₂₀₊) compounds from terrestrial plants. Pale green/yellow circles indicate short-chain compounds, typically from algal synthesis. Blue circles are used for all effluent compounds. Black circles denote fatty alcohols in influent samples. Red circles indicate the petroleum-derived detergent fatty alcohols from the Luray study. Yellow circles are the oleochemical fatty alcohols in detergents from the same Luray study. Orange circles are used for detergent-derived fatty alcohols that have a stable isotopic signature that suggests a blend of both petrochemical and oleochemical sources.

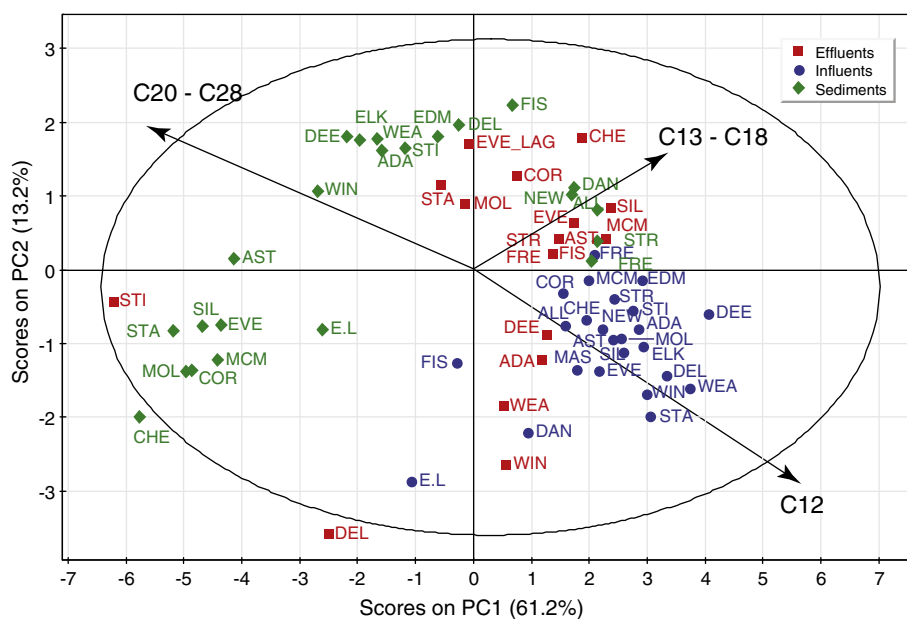


Fig. 5. A scores plot of the fatty alcohol profile data from all sites and samples as proportions after log₁₀ transformation. The data are colour-coded according to their sample type. Sample locations are expanded in Table 1. The first principal component (PC1) explains 61.2% of the variance and PC2 explains 13.2%.

Table 2
Mean apparent efficiency of fatty alcohol removal across all three eco-regions.

Treatment type	Minimum efficiency	Maximum efficiency	Mean efficiency	Number of WWTPs
Oxidation ditch	0.91	1.00	0.97	5
Activated sludge	0.94	1.00	0.98	7
SBR	0.96	0.99	0.98	4
Lagoon	0.85	1.00	0.97	6
RBC	0.97	1.00	0.98	2
TBF	1.00	1.00	1.00	1

processes do not appear to exhibit any control on the fatty alcohol profiles of the effluent. The operators of the WWTPs report the removal efficiency as a simple ratio between the influent and effluent concentrations for any particular constituent. However, the influent and effluent are generally taken at the same time and do not represent a parcel of water passing through the plant which would be a true removal efficiency value. When there is a long residence time within the WWTP, this may lead to inaccurate results as the influent concentration may have changed with time. If the mean *apparent* efficiency of fatty alcohol removal is considered using concentrations of fatty alcohols in influents and effluents collected at essentially the same time, remarkable similarity is observed, indicating that all types of treatment are equally effective (Table 2). These figures also underestimate the true removal efficiency because the fatty alcohols in the effluent are not a small portion of the influent that remains, but rather, are a new suite of compounds formed within the treatment plant (see below).

With regard to the stable isotopes (Fig. 4), clear separations between many of the major source types can be seen. The petroleum-derived fatty alcohols used in the manufacture of most surfactant components in the USA occupy a region centred on -26% and -60% for the $\delta^{13}\text{C}$ and $\delta^2\text{H}$ respectively. In comparison, the oleochemical-derived fatty alcohols (most likely to be from palm products) are positioned at the bottom of this figure, with a mean projection at -24% and -270% for the $\delta^{13}\text{C}$ and $\delta^2\text{H}$, respectively. All of the compounds in this latter region are of even carbon chain length indicative of their biological synthesis. The stable isotopes reflect the differences in the initial carbon and hydrogen used during the synthesis of the fatty alcohols (chemically or biochemically). These values were obtained from the analysis of products purchased in the Luray catchment (DeLeo et al., 2011). However, it is a reasonable assumption that the same products are available across the country as production is centralised.

As well as these two clearly separated source groups, a number of other specific detergent-associated fatty alcohols are positioned between the petrochemical and oleochemical groups. These are coded in orange and are likely to indicate a mixing of alcohols from different sources, oleochemical and petrochemical, blended during the formulation and manufacturing processes.

The dark green circles indicate fatty alcohols with a chain length $>C_{19}$ that were quantified in the sediment samples. These will be derived from terrestrial plants, either directly as leaf litter in the sediments, or from secondary sources such as soils and dusts initially derived from the terrestrial plants. Their mean projection is at -33% and -160% for the $\delta^{13}\text{C}$ and $\delta^2\text{H}$, respectively. However, there is a greater spread of the data compared to the fatty alcohols in detergents. Some of the sediments from Oklahoma contained fatty alcohols with stable isotope signatures that are significantly lighter with respect to the hydrogen, even though the carbon signature is consistent with the other sediment samples. These compounds are located in the top left of Fig. 4. Likewise, there are terrestrial fatty alcohols (long chain, with $\delta^{13}\text{C}$ values between -32% and -36%), which have $\delta^2\text{H}$ values between -200% and -275% . These samples were collected from eco-region 7 in Oregon.

There is some evidence that the terrestrial plant isotopic ratios vary in response to precipitation and humidity (Waterhouse et al., 2002) and references therein. The spread of $\delta^2\text{H}$ values can be seen more clearly in Fig. 6. Here, the deuterium values are shown for the sediment fatty alcohols with the eco-region. While there is a spread of values in each eco-region, there is a general trend toward less negative values in the drier environments. In a study of deuterium in precipitation (Dansgaard, 1964), samples collected close to the poles had $\delta^2\text{H}$ values around -220% , which become close to zero near the equator. These results are consistent with these observations and indicate that the stable isotopic composition of the rainfall is mirrored to some extent in the fatty alcohols synthesized by the terrestrial plants (Hoefs, 2009).

The bulk of the WWTP influent samples occupy a very narrow range with regard to the $\delta^{13}\text{C}$ values around -31% . These are shown in Fig. 4 as black circles. However, there is a wider spread of corresponding $\delta^2\text{H}$ values from -20% to -300% , although there is no eco-region trend as with the terrestrial plant fatty alcohols. The position of these influent fatty alcohols coincides closely with the values measured for faecal material as part of the initial phase of these investigations. The mean projection of the free and bound faecal fatty alcohols was -30% and -200% for $\delta^{13}\text{C}$ and $\delta^2\text{H}$, respectively. The differences in the $\delta^2\text{H}$ values may be due to (a) differences that may be ascribed to eating habits or climatic (rainfall) patterns, and (b) contributions from petrochemical-derived surfactants in detergents and personal care products. There is very little overlap between the influent stable isotopic signatures and the other samples in this cross plot. There are, however, a small number of influent fatty alcohol samples that may be considered to be enriched in the surfactant fatty alcohols (three samples from Ohio and one from Oklahoma). None of the influent samples had a signature that indicated a significant contribution from oleochemical-derived surfactants, which are shown in yellow in Fig. 4. This is consistent with the usage statistics on source materials used in fatty alcohol production in the USA (Gubler and Inoguchi, 2013).

The effluent samples are shown in blue in Fig. 4, and they are fewer in number than the other sample types because the concentrations in the effluents were about two orders of magnitude lower than for the influent, based on the removal efficiencies. There is no consistent pattern and no overlap with the known sources, except for the case of one sample (New Bremen, Ohio, C_{18}) that co-locates with the petrochemical-derived surfactants. However, there are few C_{18} fatty alcohols in detergent products, and the Luray review suggests that this compound contributes less than 1% of the total in detergents (DeLeo et al., 2011). Therefore, it is likely that this is not a surfactant from a detergent product but a newly synthesized compound from bacterial activity in the WWTP.

The remaining compounds in Fig. 4 are typical of algal synthesis, with $\delta^{13}\text{C}$ values in the -21% to -30% range. These compounds are short chain (C_{12} to C_{16}), with an even number of carbons indicating a unicellular algal source. This is consistent with previous observations (Mudge et al., 2012). There are one or two compounds that nominally have the correct chain length to be algal in origin, but the stable isotopes indicate a terrestrial plant source. These may be

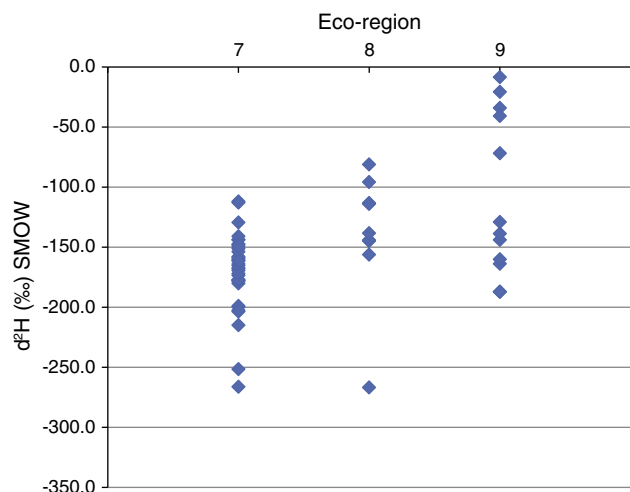


Fig. 6. The spread of sedimentary fatty alcohol $\delta^2\text{H}$ values for the three eco-regions. The deuterium measurements are relative to standard marine ocean water (SMOW).

degradation products or compounds “recycled” by bacteria in the sediments.

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

It is worth noting that there may be two alternative routes by which these detergent-derived surfactants might reach river sediments; the bulk of the fatty alcohols are removed from WWTPs with the sludges (biosolids), and these are frequently applied to agricultural land. The potential exists for these sludges to wash off into adjacent ditches and streams. The second route is through combined sewer overflows operating at times of increased rainfall. On these occasions, diluted untreated influents would enter the rivers, and the fatty alcohols may contribute to the total inventory. It is unlikely that fatty alcohols from detergents contribute to riverine concentrations, with the exception of high-flow-induced overflows.

5. Conclusions

There were statistically significant differences between the fatty alcohol influent profiles for the three eco-regions sampled. This may be due to the diet of the local populations, differences in the amounts and types of detergents used in the catchment, or differences in the in-pipe processes. The stable isotope distributions did not show a consistent difference across the eco-regions.

The data presented here suggest that the fatty alcohols present in the influent to the WWTPs are derived from a mixture of faecal matter, waste food, and surfactants. The majority of the fatty alcohols have a ^{13}C and deuterium signature that is consistent with a primary source of faecal matter. The fatty alcohol profiles clearly show short-chain compounds ($< \text{C}_{20}$) with almost no contribution from terrestrial plant matter. This implies that there was little or no surface water entrainment or capture into the sewer system.

The presence of the C_{18} fatty alcohol in the influent samples indicates the occurrence of in-pipe biochemical processes, because the surfactants used in the manufacture of detergents have less than 1% C_{18} based on the analysis of data from the marketing survey in the

Luray catchment (DeLeo et al., 2011). Faecal matter also had a low C_{18} content.

The effluents from the WWTPs were substantially depleted in fatty alcohols, with apparent removal efficiencies of $\sim 98\%$. The bulk of the compounds would have been lost through biodegradation and settlement of the solids during the primary stage or final clarification. There were no differences in the removal efficiencies or the profiles among the six different secondary treatment technologies investigated.

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

The sedimentary fatty alcohols were similar in profile to those measured in the Luray catchment; the major components of the suites were long-chain compounds typical of terrestrial plant matter. Their $\delta^2\text{H}$ values spanned a wide range, from 0 to -270% . There was a systematic variation in this value, which was related to the eco-region and rainfall.

Along with the terrestrial plant fatty alcohols in the sediments, a suite of compounds was present that indicated both algal and bacterial synthesis. Statistical analyses of the fatty-alcohol profiles indicate that the short-chain and long-chain compounds behave differently and have different origins. These short-chain compounds may have the same chain lengths as surfactants used in detergents and personal care products, but the stable isotopes indicate a different source.

No differences were apparent between the different WWTPs investigated, and the secondary stages were equally efficient in the removal of fatty alcohols. Having said that, the SBRs appeared visually to have the largest number of solids in the effluent, and it is possible that the bacteria had insufficient time to remove all the compounds. However, several of the WWTP had extra stages after the SBR to improve the quality of the final effluent. The lagoons were as effective as the more intensive methods, probably due to the longer residence times.

As with previous studies, it can be concluded that detergent fatty alcohols are not making a substantial (or, in most cases, quantifiable) contribution to the liquid effluents from WWTPs. Overall, the type of secondary treatment in a well-functioning plant does not alter the removal efficiencies of fatty alcohols from the system. While the eco-regions may have different influent profiles, these differences are small compared to the differences between the influent and effluent profiles. The sediments of the receiving waters are dominated by the terrestrial plant signatures, both in terms of the profiles and the stable isotopes. Any contribution from the WWTPs is small at best. Even then, these effluent fatty alcohols are not anthropogenically derived but come from biochemical processes in the secondary treatment stages of the WWTPs.

When determining the risk from these compounds to the biota, it has generally been assumed that all of the fatty alcohols in the sediments (and by implication, the pore water) have been derived from anthropogenic sources (Dyer et al., 2006). However, these analyses indicate that the majority of fatty alcohols are terrestrially derived, and the vast majority in the C_{12} to C_{18} chain-length range, those typically used in detergents and alike, are from natural algal or bacterial synthesis. These compounds would be present in the river even if there were no WWTP discharges at all, as was the case in the samples from Luray (Mudge et al., 2012).

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