03 - CONTAMINATION FINGERPRINTING TECHNIQUES: EXAMINING THE IMPACT OF DOMESTIC WASTEWATER TREATMENT SYSTEMS ON PRIVATE WELLS AND SURFACE WATER

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Abstract

This research compares a range of fingerprinting techniques for identifying sources of contamination in private wells and surface water. In particular, the focus is on quantifying the extent of pollution from domestic wastewater treatment systems (DWWTS) as distinct from agricultural sources. In the course of this research, 212 households across four hydrogeologically-distinct areas dependent on private wells and DWWTS have been evaluated by site assessments and sampling of chemical and microbial parameters. A total of 15% of wells were contaminated with *E. coli* based on a single sampling exercise. Subsequent monthly monitoring of 24 wells found 45% to be contaminated with *E. coli* at least once. In addition, four small catchments, each containing a high density of DWWTSs (16-34 DWWTS/km^2) and underlain by either poorly permeable subsoil or shallow, low permeability bedrock, were selected for study. The primary stream in each catchment was monitored upstream and downstream of the main cluster of DWWTSs for nutrients and indicator bacteria in bi-weekly grab samples.

These wells and streams have also been used to assess the applicability of a range of fingerprinting parameters aimed at identifying chronic and incidental domestic effluent contamination. This range of parameters comprised fluorescent whitening compounds (FWC), faecal sterols, anion ratios (in private wells) and the human-specific *Bacteroidales* faecal source tracking (FST). FST tests of 42 wells targeting regions of *Bacteroidales* 16S rRNA genes found 62% were positive for human specific *Bacteroidales.* However, no wells to date have tested positive for FWCs (determined using fluorometry and UV degradation methods). To date, *Bacteroidales* has been detected at the catchment outlets of two of the surface water study catchments. FWCs and human-specific sterols have also been detected intermittently at the catchment outlets and at mid-catchment sampling points, with high concentrations of these contaminant tracers being associated with highly impacted monitoring points at which *Bacteroidales* was also detected, along with very high concentrations of dissolved and total phosphorus and nitrite and ammonia.

1. INTRODUCTION

Approximately 438,000 households in Ireland depend on domestic wastewater treatment systems (DWWTS) to treat the wastewater onsite (CSO, 2012). Septic tanks and percolation areas are the most commonly used onsite wastewater treatment system in Ireland. The most commonly used DWWTS system comprises a septic tank which provides primary treatment, followed by a percolation area in the soil which provides secondary and tertiary treatment (EPA, 2010).

While the treatment processes are well studied, there can be large variations in DWWTS performance which can lead to the release of wastewater-associated contaminants, in turn posing potential risks to human health and aquatic ecosystems if they reach surface waters or groundwaters (Daly, 2000; Beal *et al.* 2005; Withers *et al.* 2013). Variable and poor treatment efficiency can occur due to infrequent or absence of maintenance and/or poor design and construction (Beal *et al.* 2005; EPA, 2015). While best practices in onsite DWWTS are well studied, approximately 48% of DWWTSs that were inspected to date as part of the National Inspection Plan have failed the assessment (EPA, 2015). This percentage was even higher (52%) for houses that were served by a private well. The most common grounds for failure cited in the National Inspection Plan (EPA, 2015) were the lack of routine desludging of the DWWTS, followed by direct discharge of untreated effluent to surface water, or the lack of an adequate depth of subsoil resulting in discharge to surface water or groundwater. Over 100 incidences of surface ponding of poorly-treated effluent were also recorded (EPA, 2015).

In highly permeable or in very low permeability settings, a DWWTS can pose a contamination risk for different reasons. In highly permeable areas, contaminants can leach through the percolation area and underlying unsaturated zone without adequate attenuation (Beal *et al.* 2005; O'Luanaigh *et al.* 2012). This may lead to episodic leaching of contaminants to groundwater. This concept is reflected in Ireland's groundwater vulnerability classes and mapping scheme, based primarily on the thickness and permeability of the subsoil overlying the bedrock aquifer. While soil clogging and biomat formation are essential in free-draining soils to decrease percolation rates and achieve adequate attenuation of contaminants, a saturated underlying subsoil or a high water table can result in unfavourable attenuation conditions. In areas where there is a likelihood of inadequate percolation to groundwater (LIPTG), saturated subsoils can impede the percolation of effluent and hinder treatment performance. In such areas, surface ponding can occur, potentially resulting in contaminant runoff into surface water bodies and into poorly constructed wells (Siegrist *et al.* 2000; Beal *et al.* 2005). Digital mapping of DWWTS and LIPTG indicates that >140,000 DWWTSs are located in areas of very high LIPTG. In addition to the risk of surface ponding in these areas, direct pipeline connections between the primary settlement tank and a nearby surface water receptor are also common in poorly-drained catchments (McCarthy *et al*, 2011).

Many of these rural areas are not served by a public water supply network, and so approximately 162,000 Irish households rely on private wells for their water supply (CSO, 2012). However private wells in Ireland are largely unregulated and unmonitored, and are often poorly sited and constructed, and therefore can be vulnerable to contamination (Robins and Misstear, 2000; IGI, 2007). As outlined by Hynds *et al.* (2012), the susceptibility of a private well to contamination is a function of more than its hydrogeological setting. The risk of contamination is also likely to be influenced by several localized site specific factors such as the well's location, or specific well construction details – the absence of a proper wellhead cover or sanitary seal around the pump-chamber casing are particular risk factors. Previous sampling studies of private wells for the presence of faecal indicator bacteria have shown that approximately 30% are polluted, at least intermittently, with one of the main sources of microbial pathogens believed to be DWWTS, mainly septic tanks. The most common illness associated with the consumption of microbially-contaminated water is generalized acute gastrointestinal illness (AGI) resulting in symptoms of fever, nausea, diarrhoea, and/or vomiting. Although associated illnesses are often of short duration, some can be fatal (Macler and Merkle, 2000).

Contamination Tracers

As faecal indicator bacteria are not source-specific, the aim of this current research is to compare and evaluate a range of chemical and microbiological fingerprinting techniques in an attempt to identify a robust method for apportioning groundwater and surface water contamination to a specific source such as agriculture or DWWTS. Potential fingerprinting techniques evaluated here include a range of emerging organic compounds (EOC's), sterols, microbial source tracking (MST) and ionic ratios.

EOC's commonly associated with domestic wastewater include pharmaceutical and personal care products (PPCP's), artificial sweeteners, fluorescent whitening compounds and caffeine, all of which are examined in this research. The specific anthropogenic origin of these EOC's has led to several investigations into their potential use as wastewater contamination tracers (Buerge *et al.* 2009; Lapworth *et al.* 2012).

Fluorescent whitening compounds (FWC), also known as optical brighteners (OB) are added to common laundry detergents to make them appear brighter in colour (Hartel *et al.* 2007; Cao *et al.* 2009). Owing to their almost ubiquitous use in modern households, FWC are regularly discharged via wastewater into the treatment network which has led to studies into their use as tracers for domestic wastewater (Geary *et al.* 2014).

Artificial sweeteners are common in modern diets due to their presence in many foods and drinks. Increasingly, they are being used as tracers of wastewater contamination in surface and groundwater systems (Tran *et al.* 2014). Some artificial sweeteners are not metabolised by the human body, and recent studies indicatethat certain artificial sweeteners are also resistant to degradation during wastewater treatment processes. As a result, these compounds may persist in the environment (Buerge *et al*., 2009; Scheurer *et al.* 2009; Van Stempvoort *et al.* 2011). Similarly, of the caffeine consumed by humans a small proportion (approx. 3%) is not metabolised and is excreted in urine (Tang-liu *et al.* 1994), which subsequently enters the household DWWTS. Caffeine has been detected at concentrations of between 100 and 120 µg/L in septic tanks (Seiler *et al.* 1999) and has been used successfully as a tracer of aquatic contamination by wastewater to variable extents (Rabiet *et al.* 2006; Stuart *et al.* 2013). Although potentially useful, this evidence suggests that unlike artificial sweeteners caffeine is not as conservative with particular uncertainty around its environmental persistence (Sieler *et al.* 1999; Gill *et al.* 2009).

In addition, this research is also examining the potential use of simple ionic ratios (potassium/sodium and chloride/bromide) to apportion private well contamination to a specific source. Potassium levels in Irish groundwater are generally less than 3.0 mg/l, with the potassium/sodium ratio (K/Na) in most Irish groundwaters generally less than 0.4 (Daly and Daly, 1982). In contrast, effluents from known sources of contamination such as farmyards often have potassium levels or K/Na ratios distinctly elevated with respect to those of natural uncontaminated groundwaters and septic tank effluent. Such different ratios can be used to identify sources of decaying organic matter, such as farmyards, as sources of contamination. Similarly, the specific ratio of Cl/Br in groundwater generally remains relatively unchanged over time (Davies *et al.* 1998; Vengosh and Pankratov, 1998). In contrast to the K/Na ratio, the Cl/Br ratio of DWWTS effluent is elevated with respect to most natural groundwaters. Any subsequent increases in this ratio have been used as a diagnostic tool to identify an impact from domestic wastewater (Jagucki and Darner, 2001; Katz *et al.* 2011).

Finally, the *Bacteroidales* order of bacteria have also been examined as an appropriate MST method to attempt to detect and quantify contamination of water bodies from human wastewater inputs. Research into MST by Kildare et al. (2007) has resulted in the development of a human-specific assay, one which enables the enumeration of the human-specific 16S rRNA *Bacteroidales* genetic markers in environmental samples.

2. METHODOLOGY

Catchment Selection Strategy

The first objective of the private well and surface water study was to identify potentially suitable study sites at which to carry out the research. The most important characteristics in assessing catchment suitability for the surface water study were high density of DWWTSs, a permanently flowing first-order stream in a small catchment, low subsoil permeability and the absence of major industry or an urban wastewater treatment works. In selecting the areas for the private well research the criteria in order of decreasing importance were: groundwater vulnerability (two areas should be of extreme vulnerability based on the delineation outlined in DELG (1999)), permission from site owners, contrasting bedrock types and a high density of private wells, followed by a high density of DWWTS and an avoidance of gley soils.

The sites selected for the surface water research were located in Co. Wicklow (Catchment 1, C1), Co. Wexford (Catchment 2, C2), Co. Cavan (Catchment 3, C3) and Co. Longford (Catchment 4, C4) – see Figure 1. Table 1 highlights relevant characteristics of these catchments. Private well research was carried out at sites C2 and C3, as well extreme groundwater vulnerability sites in Co. Kilkenny (Study Site 5, C5) and in Co. Wexford (Study Site 6, C6) – Figure 1.

Each surface water catchment contained two stream monitoring stations – one upstream of the main cluster of DWWTSs and one downstream. These sites are denoted as 'Upper' (U) and 'Lower' (L) throughout the study, e.g. the upstream study site in Catchment one is denoted as 'C1U'. Each monitoring point was instrumented with an OTT Hydromet CTD (conductivity, temperature and depth) probe in a stilling well. Stream discharge was recorded twice weekly throughout the monitoring period using an OTT Hydromet electromagnetic flowmeter. These discharge data, coupled with corresponding depth readings from the CTD probe, were used to establish flow rating curves such that continuous records of stream discharge were constructed for each site. In addition to the upstream and downstream monitoring sites, a number of mid-catchment grab-sampling points were also selected at strategic points along the river channel.

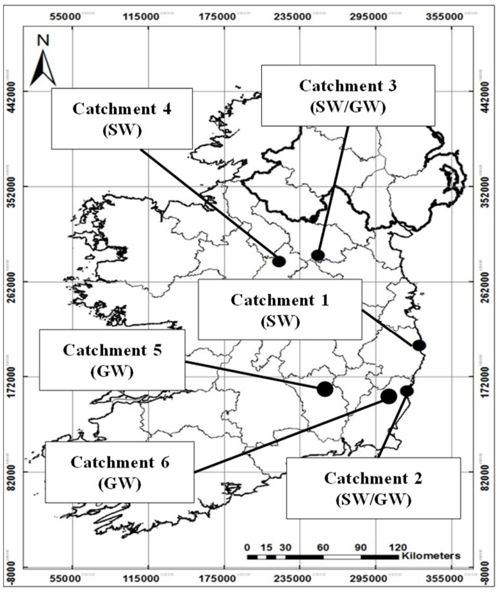

Figure 1 Location of Surface Water (SW) and Groundwater (GW) study sites

Table 1 Catchments 1 - 4 selected for the study on impacts of DWWTSs on surface water bodies. Note: 'Upstream Catchment' refers to the area upstream of the Upper monitoring point. 'Downstream Catchment' refers to the whole catchment area.

	Catchment 1	Catchment 2	Catchment 3	Catchment 4
Area (km^2)				
Upstream Catchment	0.43	1.18	0.29	0.40
Downstream Catchment	3.07	2.96	3.85	2.07
DWWTS Count	71	96	60	40
DWWTS Density (DWWTS/km^2)	23	32	15	19
Likelihood of Inadequate Percolation to Groundwater				
% Very High LIPTG	35	95	98	56
% High LIPTG	4	5	0.9	30
% Moderate LIPTG	61	0	1.1	14
% Low LIPTG	0	0	0	0
Primary Landuse	Pasture (40%), Arable (11%), Forest (25%)	Pasture (84%)	Pasture (90%)	Pasture (96%)

Multi-parameter weather stations were set up close to the upper monitoring point in each catchment. Isco autosamplers were installed and programmed to collect a surface water sample for water quality analysis every seven hours, over one week in each month of the study, an adaptation of the '24/7' monitoring strategy described by Jordan and Cassidy (2011).

A site assessment survey was carried out on the private wells in each of the four groundwater study sites. The survey assessed each individual sampling site in terms of general site details, well details, surrounding land use, DWWTS variables and other potential contamination hazards. All surveyed wells were subsequently sampled and analysed for microbiological and chemical quality indicators as well as for trace elements. Samples were taken from a cold water tap fed directly from the well and purged for a set period of five minutes. After interpretation of the sampling results and the site assessment surveys, 24 wells (six in each of the four groundwater study sites) were selected for monthly monitoring, over a 14-month period, to evaluate temporal scale variation in private well water quality.

Surface water samples were analysed for dissolved reactive phosphorus (DRP), total phosphorus (TP) using a HACH DR2800 spectrometer. A *Merck Spectroquant Nova 60 spectrophotometer* was used for Nitrate (NO_3), nitrite (NO_2), and ammonia (NH_3) analysis. Total nitrogen (TN) was measured in a Shimadzu TOC/TN Analyser. Microbiological parameters (Total Coliforms, *E.coli* and Enterococchi) were analysed in fresh grab samples, using the IDEXX Colilert and Enterolert systems.

All private well samples were tested onsite for pH, electrical conductivity (EC) and temperature using a *Hanna Instruments HI-98129 pH & Water Analysis Meter*. In the Environmental Engineering laboratory at Trinity College Dublin, alkalinity was determined by titration to an end point pH in accordance with method 2320b of APHA/AWWA/WEF (2005). Nitrate, chloride, ammonium and sulphate were analysed using a *Merck Spectroquant Nova 60* and associated accredited reagents. Cation analysis was completed by Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) using a *Varian-Liberty AX Sequential AES*. Bromide analysis was done by City Analysts Limited using UKAS accredited ion chromatography. Total *Coliforms* and *E. coli* were analysed using the aforementioned IDEXX Colilert system.

Tracer techniques for detecting human-sourced contamination in water samples referred to in Section 1 were also developed, applied and evaluated. The analysis for fluorescent whitening compounds was carried out at a Perkin Elmer LS55 Fluorescence Spectrometer using a modified photo decay method developed after the principles outlined in Hartel *et al.* (2007) and Cao *et al.* (2009). Microbial source tracking (MST) using 16S rRNA-based assays and real-time PCR for the detection of human-specific *Bacteroidales* in surface water and private well samples was carried out by the School of Natural Sciences at the National University of Ireland, Galway, in line with the methodology developed by Kildare et al. (2007). Quantification of artificial sweeteners, caffeine and EOCs was carried out in the Teagasc laboratory in Ashtown, Dublin using the Agilent Technologies 6460 Triple Quad LC-MS system. Sterols/stanols were extracted from samples according to the method described by Shah et al. (2006). Samples were analysed for 10 different sterols/stanols using a Thermo Scientific ITQ 900 Ion Trap GC-MS system at the analytical chemistry laboratory in DIT Kevin Street. Sterol profiles were evaluated and human and/or herbivore contribution in the sample were estimated (ESR, 2016).

3. RESULTS

Private Water Wells

Of the 212 wells sampled initially, 66% (n=139) tested positive for *Total Coliforms,* while 15% tested positive for *E.coli*. The proportion of wells that tested positive for faecal indicator bacteria did not differ appreciably between the four study areas. A range of statistical techniques were employed to examine the relationships between faecal contamination occurrence and variables recorded in the site assessment surveys as well as antecedent rainfall. Bivariate statistics have been carried out between each of the independent variables (site specific variables) and outcome variable (*E. coli* occurrence) for each individual study areas as well as for an amalgamated dataset of all wells. For the amalgamated dataset a total of seven variables were statistically associated with *E. coli* at the 0.05 significance level. Several of these variables are related to the specific construction of the well and well head finish, including the presence or absence of a well cap, concrete surface apron and secure boundary. For instance, wells not fitted with a cap were 2.7 times more likely to be contaminated with *E.coli* than those that were adequately capped (OR 0.361 95% CI 0.157 - 0.831). Additionally, wells situated within 100 m of a point agricultural contaminant source were 6.1 times more likely to be contaminated with *E. coli* (OR 6.1 95% CI 2.73 – 13.42). This association was highly statistically significant ($X^2 = 22.8$, $p < 0.01$). Another significant variable was well age, i.e. the older the well the more likely it was to have tested positive for *E. coli* ($t = -2.13$, $p < 0.05$). All factors must, however, be considered collectively to examine how each variable is not only related to other variables, but also how these variables in combination can influence the susceptibility of a private well to faecal contamination. Therefore, the results from these simple tests are currently being used to inform and develop a multivariate logistic regression model, and build on work outlined previously by Hynds *et al.* (2012); this modelling will provide greater insights into private well contamination.

Nitrate concentrations varied notably between the four sites, with higher concentrations found in areas of extreme groundwater vulnerability, and a larger proportion of samples from the areas with inadequate percolation found to have concentrations below the detection limit of the method. A statistically significant difference exists between the topsoil classes when they are classed as either well of poorly drained (U=1580.5, p <0.01), with highest concentrations observed in areas of shallow well drained (AminSW) soil. Consistent with this, a general trend is apparent of significantly higher nitrate concentrations in the extreme and *X* (an area with a rock outcrop and subsoil less than 1m thick) vulnerability categories than in the low, medium and high vulnerability categories (U=993, p <0.01; U=366, p <0.01 and U=344, p <0.01).

Considerable temporal variation is seen in the monthly water quality data from the 24 monitoring wells. The percentage of wells that tested positive for *E.coli* in a single month varied from 17% to as much as 46%. Over the 14 months of monitoring, 67% (n=16) of the wells tested positive for *E.coli* at least once.

With regards to contamination tracer methods chloride/bromide ratio was evaluated for all 24 wells for two separate monitoring events, in which the same four wells tested positive for *E.coli.* Cl/Br ratios were compared to ranges of elevated ratios from previous studies used to indicate an impact from wastewater (Davies *et al.* 1998; Vengosh and Pankratov, 1998; Jagucki and Darner, 2001; Panno *et al*. 2007; and Katz *et al.* 2011). Due to natural variations in groundwater chemistry no definitive statements can be made regarding anthropogenic effects on waters that have Cl/Br ratios between 200 and 400 (Jagucki and Darner, 2001). This range includes the Cl/Br ratio measured for two of the wells that tested positive for *E.coli*, which indicates there was no apparent impact from a DWWTS. These two wells did, however, show consistently high concentrations of potassium and K/Na ratios, indicating an impact from decaying organic matter. This is consistent with their location within working beef farmyards. The two remaining *E.coli* positive wells did exhibit elevated Cl/Br ratios indicating an impact from the DWWTS in proximity to the well.

Fluorescent whitening compounds were not detected in well samples using the photo decay method. Initial fluorescence concentrations did differ between the four study areas; however, concentrations were low in general. Subsequent exposure to UV light did not yield a significantly meaningful degradation in fluorescence and so positive identification of FWC was not possible. Total sterol concentrations were detected in all well samples tested during the two monitoring events. However, very low concentrations close to detection limit were observed, making it difficult to discern sterol origin and draw conclusions on the contamination source. Plant sterols were, however, detectable indicating the presence of at least these sterols in groundwater. A broad range of EOC's were analysed for in well samples from the two monitoring events; however, results are still preliminary with analysis still ongoing. Artificial sweetener acesulfame has been widely detected, with the exception of area C2. Artificial sweetener cyclamate has been detected to a lesser extent. However, caffeine, carbamazepine, sucralose, sulfamethoxazole and saccharin have not been detected. Microbial source tracking tests on an expanded subset of monitoring wells (n = 42), targeting regions of *Bacteroidales* 16S rRNA genes, found 62% were positive for human specific *Bacteroidales.* MST results from the two monitoring events are pending.

Surface Water

Each of the four surface water bodies monitored during this study displayed relatively high concentrations of TP averaged across the year (see Figure 2): the threshold for good water quality with respect to dissolved P (the DRP fraction) is ≤ 0.035mg/L P, which appears to have been consistently exceeded in Wexford catchment (C2) and in the Cavan catchment (C3) during the monitoring period. In the Longford catchment (C4), the mean DRP value lies on the threshold between 'Good' and 'Poor' water quality status with respect to DRP, and DRP in C1 appears to be below the threshold, with occasional exceedances.

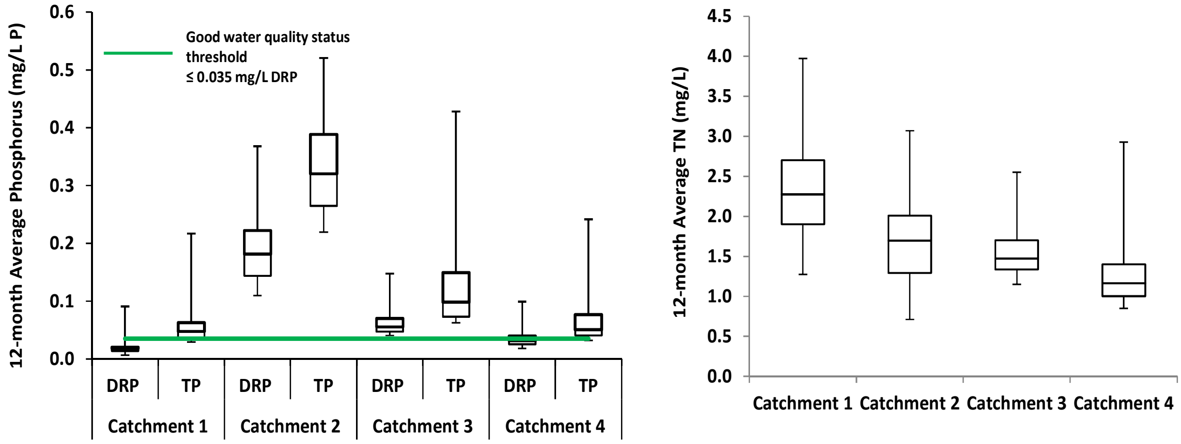

Figure 2: Twelve-month average water quality in the four surface water catchments for dissolved reactive phosphorus (DRP), total phosphorus (TP) and total nitrogen (TN) measured at the Lower monitoring site in each catchment. Note, the Good Water Quality threshold level for P (annual mean ≤ 0.035 mg/L MRP) is referenced from the Surface Water Regulations S.I. 202 of 2009. DRP is equivalent to MRP in filtered samples. There is not currently a water quality threshold with respect to TP and TN for rivers in Ireland.

Positive signals for FWC were detected at the Wicklow upper (C1U) station on three occasions, and once at the Wicklow lower (C1L) station. They were also detected on three separate occasions at a mid-catchment point, where the odour of domestic wastewater was also noted on several site visits. This point is located close to a cluster of four houses. FWC was also detected in the Wexford catchment (C2), although never at the Wexford lower (C2L) monitoring site. Instead, it was detected at the Wexford upper (C2U) site, and at a mid-catchment point with a number of houses in close proximity. FWCs were only detected on one occasion in the Cavan catchment (C3) – at the upper point. Over the entire sampling period, FWC was not detected at the upper or lower sites, or at the mid-catchment points in the Longford catchment (C4).

A strong human-specific sterol signal (indicating 96% human contribution versus herbivore contribution) was detected at C1L on 10th of June 2015. The same sample returned a DRP of 0.06mg/L P and a TP of 0.144mg/L P, both of which were unusual results for the C1L site (see Figure 2) given that DRP is generally below the 0.035mg/L 'Good Status' dissolved P threshold at this point. On the 15th June 2015, a human sterol signal was also detected at C1L, but the results indicated a mixed human/herbivore contribution. On this occasion, DRP was 0.02mg/L P and TP was 0.062mg/L. At the mid-catchment point in C1, where the FWC was detected on numerous occasions, positive signals for human sterols were also obtained on the same dates as the positive FWC readings. Again, these sterol readings indicated mixed herbivore and human contributions.

Human-specific sterol signals were consistently detected throughout the C2 catchment. The average percentage of human contribution versus herbivore contribution was 67% at the C2U site and 47% at the C2L site. In general, the results indicate a mixed human and herbivore source. Human-specific sterols were detected in mid-catchment sites, with very high concentrations detected at the same site where the FWCs were detected in C2.

Human-specific sterols (mixed human/herbivore in origin) signal were found in C3 at both the upper and lower sites and in a small drain feeding into the main stream. A strong human sterol signal was detected at the Cavan Lower (C3L) site on the 8th of July 2016. DRP and TP in the same sample were 0.06mg/L and 0.105mg/L respectively. As shown on Figure 2, this TP figure is outside of the mean and upper quartile value; however, the DRP value is not unusual for the site.

Human-specific sterols were detected at the Longford Upper (C4U) and the Longford Lower (C4L) sites, though at a lower frequency and at lower concentrations at C4U than at C4L. They were also found in mid-catchment sampling points in this catchment, with strong signals associated with a site directly downstream of a DWWTSs cluster located close to the stream boundary.

Significant differences in microbiological parameters and BacHum were observed between C1 and C2, which also reflect the differences in P levels between these two catchments, as illustrated in Figure 2. At C1L, average *E.coli* and BacHum were 2,386MP/L and 8,916GCC/L, respectively. At C2L, average *E.coli* and BacHum were 18,798MPN/L and 20,309GCC/L. In addition, samples taken on the 15th of June 2015 at both C2U and C2L indicate that BacHum at the C2U site was lower than at the C2L site (8.88 x10^3 GCC/L versus 1.55 x10^4 GCC/L).

At C1L, concurrent spikes in BacHum and *E.coli* were detected on the 25th of March 2015 at 05:00 (Figure 3). The next sample, taken at 07:00, indicates that both parameters had returned to previous levels. Examination of the DRP record also indicated a concurrent rise in dissolved P from approximately 0.01mg/L to 0.03mg/L – an unusual reading for this site. This peak in DRP appears to have receded more slowly than the BacHum and the *E.coli* spikes. There was also a slight drop in Conductivity from 294 to 259µS/cm; however, this occurred between 13:00 and 15:00. Stream discharge did not alter significantly.

BacHum data for study sites C3 and C4 are currently being analysed.

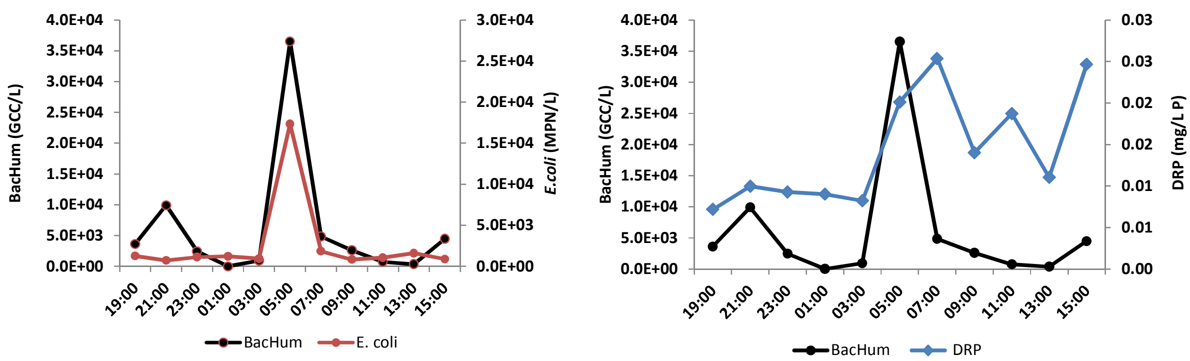

Figure 3: BacHum, E.coli and DRP recorded on the 24-25th March 2016 at the downstream monitoring point of Catchment 1 (C1L).

Preliminary results from the analysis of samples collected in C1, C2, C3 and C4 at the upper and lower monitoring sites and at mid-catchment points appear to indicate that the sweetener, caffeine and EOC tracers of domestic wastewater are detectable at all of the sites except for C1U where none of these tracers has yet been detected. However, they were all detected at C1L and at the mid-catchment points with the highest values found at the same mid-catchment point where FWCs and human-specific sterols were detected. At C2U they were detected in very high concentrations, but conversely, they were detected in lower concentrations at C2L.

4. DISCUSSION

A complex and varied range of factors have been shown in past studies to be related to private well contamination (Hynds *et al.* 2012). This is seen here in the significant associations between *E.coli* presence and various site specific variables, especially aspects of well design. Also of note is the absence of an association with DWWTS variables and a positive association with point sources of agricultural contamination. A better insight should, however, be provided by current work on logistic regression analysis. The differences in nitrate concentrations between the sites are however consistent with recent work by Tedd *et al.* (2014) that highlights how pathway factors can be more important than source factors in relation to determining groundwater nitrate concentrations. This is supported by the statistically significant differences in nitrate concentrations between different topsoil, subsoil and groundwater vulnerability classes.

Results have indicated significant variation in the performance of the tracers tested to date in attributing private well contamination to a specific source. FWC and sterol analysis have so far yielded negative results. Previous studies have shown conflicting results in FWC detection in water wells, raising questions about their potential use as tracers (Close *et al.* 1989; Alhajjar *et al.* 1990). However, it is important to note the analytical techniques used to obtain these results: while negative results may indeed mean that they are not present in the sampled wells, they could also indicate the unsuitability of the analytical method. Further research is needed to assess the performance of other techniques (e.g. HPLC and fluorescent whitening compounds). Ionic ratios have shown promising results, with both K/Na and Cl/Br potentially complementing each other in discerning DWWTS impacts from decaying organic effluent associated with agricultural contaminant sources. These results, in combination with the relatively low cost and ease of analysis, could indicate their potential use as a preliminary investigative tool. The detection of acesulfame in private wells offers a new and promising avenue for research into determining sources of contamination. Consistencies are seen between these preliminary results and previous studies. For example, a European study outlined in Buerge *et al.* (2009) found that wastewater treatment processes had little effect on the artificial sweetener acesulfame. In the analysis of 100 wells sucralose, cyclamate and saccharin were not detected. However, acesulfame was detected in 65 of the 100 wells, sometimes at high concentrations of up to 4.7 µg/L. Similar detection rates in wells was observed in Canada with acesulfame detected in 65.2% and 73.3% of wells, respectively, in two separate sites indicating an impact from DWWTS effluent and the potential usefulness of acesulfame as a tracer (Van Stempvoort *et al.* 2013). Although early results from MST tracing in our study have indicated the presence of human specific *Bacteroidales*, further results are pending and required to evaluate their significance.

In determining the degree to which surface water in a given catchment is impacted by DWWTS, long term monitoring at a high sampling frequency is necessary. In particular, trying to determine the impact from an individual on-site system would require a high frequency (e.g. hourly) monitoring protocol over short time periods. Applying the domestic effluent tracer techniques to the river samples is more challenging for surface waters than for private wells. Rivers naturally exhibit a higher state of flux and, therefore, impacted water quality may not be detected on the basis of a small number of grab samples collected over a year, due to variations in the hydrological regime.

The DRP and TP data illustrated in Figure 2 represent averaged water quality data at the outlet of these four densely populated rural catchments. C2 displayed the highest levels of DRP and TP, followed by C3, C1 and C4. C2 also has the highest number of DWWTSs (96) and the highest DWWTS density (32/km^2) in a catchment of just 2.96km^2. It also has one of the highest proportions of catchment area underlain by highly inadequate percolation conditions, and the highest concentrations of BacHum, with significant differences in average concentrations between the Upper and Lower sites. It is also known that there is at least one direct connection between a primary settlement tank and the stream (*Pers. Comm.*) in this catchment.

Water quality data – particularly with respect to ammonia - at C2U indicated a highly impacted stream, and the use of the contamination tracers indicated that this impact was a result of DWWTS inputs close to the monitoring point, rather than diffuse or agricultural sources of pollution. This highlights the potential suitability of these compounds for use in a multi-tiered approach to detecting instances of DWWTS impacts. However, the FWC test appears to be only suitable for detecting very high levels of DWWTSs inputs to surface water, such as direct connections, as there were relatively few exceedances of the confidence threshold throughout the project compared to the other tracer techniques. The concurrent spikes in *E.coli*, BacHum and DRP (Figure 3) appear to indicate a relationship between these parameters. No concurrent rise in stream discharge was detected at this time, and this potentially indicates that a point source discharge may have caused this spike. Further analysis of the data generated by this research is required in order to establish whether the tracers examined can be used predict a P load attributable to DWWTSs in each catchment.

The results of the groundwater and surface water studies are highly relevant with respect to water quality management policies such as the Water Framework Directive and the on-going National Inspection Plan. Continued outputs from this research project will aim to assist in directing future investigative methodologies which can be employed by environmental enforcement agencies in mitigating the impacts of DWWTSs on surface and groundwater bodies.

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