Norwegian study on microbial source tracking for water quality control and pollution removal in constructed wetland treating catchment run-off

Lisa Paruch, Adam M. Paruch, Anne-Grete Buseth Blankenberg, Ketil Haarstad and Trond Mæhlum

ABSTRACT

This study describes the first Norwegian microbial source tracking (MST) approach for water quality control and pollution removal from catchment run-off in a nature-based treatment system (NBTS) with a constructed wetland. The applied MST tools combined microbial analyses and molecular tests to detect and define the source(s) and dominant origin(s) of faecal water contamination. Faecal indicator bacteria *Escherichia coli* and host-specific *Bacteroidales* 16 s rRNA gene markers have been employed. The study revealed that the newly developed contribution profiling of faecal origin derived from the *Bacteroidales* DNA could quantitatively distinguish between human and non-human pollution origins. Further, the outcomes of the MST test have been compared with the results of both physicochemical analyses and tests of pharmaceutical and personal care products (PPCPs). A strong positive correlation was discovered between the human marker and PPCPs. Gabapentin was the most frequently detected compound and it showed the uppermost positive correlation with the human marker. The study demonstrated that the NBTS performs satisfactorily with the removal of *E. coli* but not PPCPs. Interestingly, the presence of PPCPs in the water samples was not correlated with high concentrations of *E. coli*. Neither has the latter an apparent correlation with the human marker.

Key words | catchment run-off, constructed wetland, microbial source tracking, nutrients, personal care products, pharmaceuticals

Lisa Paruch
Adam M. Paruch (corresponding author)
Anne-Grete Buseth Blankenberg
Ketil Haarstad
Trond Mæhlum
Division of Environment and Natural Resources,
NIBIO – Norwegian Institute of Bioeconomy Research,
Pb 115,
Aas NO-1431,
Norway
E-mail: *adam.paruch@nibio.no*

INTRODUCTION

Various sources (point and diffuse) and origins (human and non-human) of pollution run-off affect catchment water quality. Although point source pollution can be somewhat localised and defined (normally as industrial and/or municipal/domestic wastewater discharge), the diffuse/non-point sources of water pollution (usually characterised by storm and urban water run-off as well as agricultural run-off with faecal contamination from humans, livestock, pets and wild animals) cannot be entirely distinct. The diffuse sources of pollution are very often characterised based on some presumptive observations and anticipated data, but their individual contributions to water pollution have been proven very rarely by appropriate techniques. Since the multiple sources and origins of water contamination cannot be completely controlled, it is quite challenging to implement a tool of adequate measure for water quality protection (Blankenberg *et al.* 2015; Paruch *et al.* 2015a).

To select and apply highly efficient measures (e.g. on-site purification systems for point source pollution or water protective buffers against diffuse contamination) at the most relevant spots (e.g. where the primary origin of water pollution has been definitely proven), identification of the dominant contributor(s) to water contamination is quite significant. For this reason, various pollution source tracking techniques have been applied worldwide (Edge *et al.* 2010; Gourmelon *et al.* 2010; Keegan *et al.* 2014). In Norway, microbial source tracking (MST) tools for environmental water investigations have been recently implemented (Paruch *et al.* 2015b) with a particular focus on faecal contamination of aquatic ecosystems, as this influences significantly human and environmental health (WHO 2011). The MST methods along with molecular biology tests applying real-time quantitative polymerase chain reaction (RT-qPCR) for the detection of host-specific 16S

doi: 10.2166/wst.2017.303

rRNA genetic markers have provided a vital tool in both detecting and quantifying the involved faecal polluting sources (Layton *et al.* 2006; Reischer *et al.* 2007; Shanks *et al.* 2008; Tambalo *et al.* 2012).

A number of faecal indicators have been applied in water pollution investigations and one of the most frequently employed was *Escherichia coli* bacteria (Paruch & Mæhlum 2012). Yet, none of these indicators can definitely identify the origin(s) of faecal pollution since they cannot satisfactorily fit the criteria of a source identifier due to the low host specificity, replication in the environment, and geographic and temporal variability (US EPA 2005; Field & Samadpour 2007). Therefore, another group of Gram-negative bacteria belonging to the phylum *Bacteroidetes*, and in particular species of the order *Bacteroidales*, have been recommended as indicators for MST studies determining the origins of faecal pollution (Dick *et al.* 2005; Tambalo *et al.* 2012; Paruch *et al.* 2015b). These bacteria are one of the most abundant in the intestine of host humans and other warm-blooded animals. For instance, species of the genus *Bacteroides* normally comprise about one-third of total faecal bacteria (Layton *et al.* 2006), but they can constitute up to 52% of human faecal flora (Dick *et al.* 2005) and occur at concentrations of up to 10^{11} organisms per gram of faeces (McQuaig *et al.* 2012). Furthermore, they are highly host-specific, enabling identification between the hosts (Layton *et al.* 2006), and have little potential for growth in the environment because of their strictly anaerobic physiology (Dick *et al.* 2005; US EPA 2005).

In the past decade, a number of host-specific *Bacteroidales* DNA markers have been developed and successfully applied in MST worldwide to determine water pollution sources and distinguish between human and non-human faecal origins (Dick *et al.* 2005; Layton *et al.* 2006; Reischer *et al.* 2007; Shanks *et al.* 2008; Tambalo *et al.* 2012). Yet, there are still quite limited published data on MST approaches identifying sources and origins of faecal pollution of water in Norway. For this reason, the objective of this study was to present a practical implementation of a molecular diagnostic in a catchment water quality control.

To the best of our knowledge, this study describes the first multidisciplinary approach in assessing the performance of a constructed wetland (CW) treating catchment run-off in Norway. The approach combined a complex of microbiological, molecular, physical and chemical analyses for the detection and source tracking of water contamination. Furthermore, it is also the first time the outcomes of the implemented MST studies have been compared with results of physicochemical analyses of nutrients, organics, and pharmaceutical and personal care products (PPCPs) in order to strengthen the findings on the principal source(s) and origin(s) of water pollution in Norway.

METHODS

The study site is located in a catchment of the Gryteland stream (known also as Skuterud catchment), south-east Norway, approximately 30 km south-east of Oslo (Figure 1). The catchment has an area of about 4.5 km^2 divided between agricultural lands (2.7 km^2), forest/marshlands (1.4 km^2) and settlements (0.4 km^2). The Gryteland stream receives pollution from multiple sources and origins – point (scattered settlements) and diffuse (agricultural areas with livestock and grain production), human and non-human – transported with water run-off from the entire catchment. To reduce the transport of this pollution further down the Gryteland stream, in 2000 a nature-based treatment system (NBTS) was constructed downstream of the catchment (Figure 1).

The NBTS has two components, a sedimentation pond (50 m long, 10 m wide and 2 m deep) and a CW, which is the main section of the entire system. This section consists of two wetland filters; the first filter is 100 m long, while the second is 75 m long and both are 8 m wide and 0.5 m deep (Figure 2). Both filters are planted with local wetland

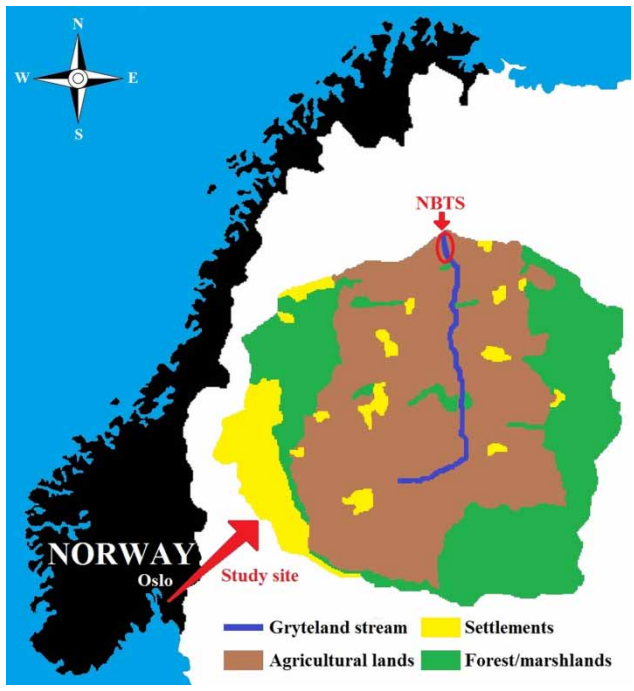

Figure 1 | Location of the study site and nature-based treatment system (NBTS).

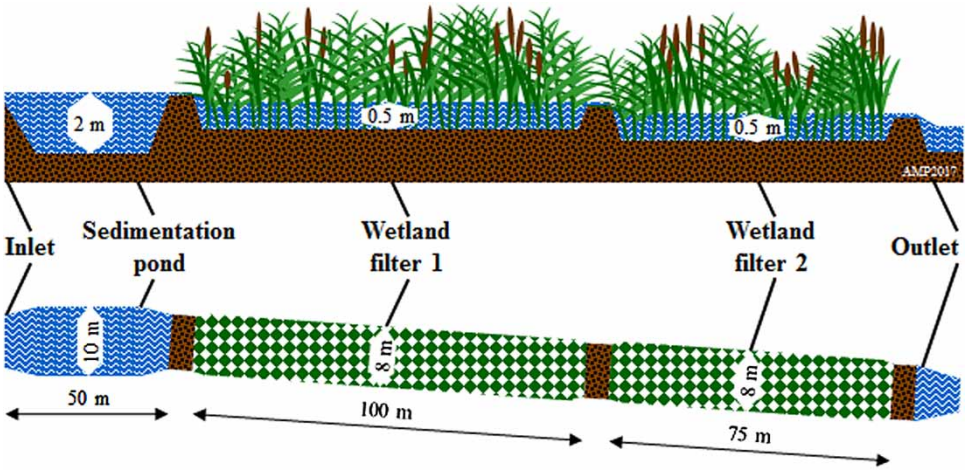

Figure 2 | Layout and cross-section of the nature-based treatment system in the Gryteland stream.

vegetation such as *Glyceria fluitans*, *Iris pseudacorus*, *Phalaris arundinacea*, *Sparganium erectum* and *Typha latifolia*. The water surface of the entire CW is approximately 2,300 m^2 covering about 0.09% of the catchment's agricultural lands and 0.05% of the total catchment area. Retention time in the CW varies with water flow, but is on average approximately 5 hours.

The study was conducted on water samples collected from the Gryteland stream before and after its passing through the entire NBTS, i.e. at the inlet and outlet site of the treatment system (Figure 2). Water grab samples were collected monthly from November 2014 to April 2015 and thereafter quarterly until June 2016. All the collected samples were examined through a complex of microbiological, molecular, physical and chemical tests for catchment water quality control and source tracking of contamination.

ALS Laboratory Group Norway AS performed the physicochemical analyses in all water samples in accordance with the ISO and national standards for the following parameters respectively: chemical oxygen demand (COD_{Cr}: ISO 15705), total suspended solids (TSS: CSN EN 872, NS 4733), phosphate phosphorus (PO_4-P: ISO 6878 SM 4500-P), total phosphorus (TP: ISO 6878, ISO 15681-1), ammonium nitrogen (NH_4-N: ISO 11732, ISO 13395), nitrite nitrogen (NO_2-N: ISO 10304-1), nitrate nitrogen (NO_3-N: CSN EN ISO 11732, CSN EN ISO 13395, CSN EN 16192, CSN EN 12506), total nitrogen (TN: EN 12260), total organic carbon (TOC: EN 1484), dissolved organic carbon (DOC: EN 1484), electrical conductivity (EC: EN 27 888, SM 2520B, EN 16192), total dissolved solids (TDS: CSN 757346, CSN 757347, EN 16192) and power of hydrogen (pH: ISO 10523, EPA 150.1, EN 16192).

Water Management Laboratory Plzeň, Povodi Vltavy, State Enterprise performed tests with contaminants of emerging concern (CECs), including PPCPs, their metabolites and endocrine disrupting chemicals (EDCs) in water samples collected on six occasions, i.e. in November 2014, February, June, September and December 2015, and March 2016. In total, 46 compounds were tested, each according to the EPA, ISO and national standards (EPA1694, EPA 535, CSN ISO 20179, CSN ISO 25101). These compounds were separated and detected by the combined methods of liquid chromatography with mass spectrometry (LC-MS/MS). A 1200 Ultra High-Performance Liquid Chromatograph tandem with a 6410 Triple Quad Mass Spectrophotometer was employed. The LC-MS/MS protocol has been described in greater detail elsewhere (Vymazal *et al.* 2017). Not all the compounds are mentioned in this paper, as some of them had concentrations below their limits of quantitation (LOQ) in all water samples tested; therefore only those CECs with at least one concentration above their LOQ have been presented in the results of this study.

Both the faecal water contamination and its source tracking were tested in the microbial and molecular laboratories of the Norwegian Institute of Bioeconomy Research (NIBIO). The faecal contamination, reported in terms of faecal indicator bacteria (*E. coli*) and coliform bacteria concentrations, was tested in 100 ml of sampled water and expressed as the most probable number (MPN)/100 ml with a detection limit of <1 MPN/100 ml. The samples were analysed by using the Colilert 18/Quanti-Tray®2000 method (IDEXX Laboratories Incorporated, Westbrook, Maine, USA) according to a four-step procedure described

in greater detail elsewhere (Paruch *et al.* 2015a). To detect and define the source(s) and dominant origin(s) of faecal water contamination, namely to distinguish between human and non-human (livestock, pets and wild animals) faecal origins, an MST with molecular diagnostics using RT-qPCR for the detection and quantification of host-specific *Bacteroidales* 16S rRNA genetic markers was implemented. The scientific background and procedures of the MST technique applied in water testing have been described in greater detail elsewhere (Paruch *et al.* 2015b).

Selected data were subjected to a statistical analysis using the XLSTAT statistical software package version 2014.01.02 (Addinsoft™, Paris, France). The statistical study and analysis outputs are described further along with the results achieved.

RESULTS AND DISCUSSION

The outcomes of the physicochemical analyses (Table 1) revealed, in general, lower values of the basic parameters measured in the outflow water grab samples of the NBTS (outlet from the second wetland filter) than in the inflow water grab samples (inlet to the sedimentation pond). Yet, some exceptions have also been noticed in particular for mean and maximum values of ammonium nitrogen (NH_4-N), EC and organic matter (expressed by COD_{Cr}), respectively (Table 1). Similar variations, especially in the content of organic matter, were observed in earlier studies reporting a possible transport of sediments and accumulated pollutants (e.g. organic matter and TP) out of the wetland filters (Blankenberg *et al.* 2013). The content of organics and nutrients, especially TP, was assumed to be elevated with potential pollution from wastewater or other faecally contaminated matter, as the Gryteland stream receives run-off from both scattered settlements and agricultural areas including livestock. This assumption was positive, because faecal contamination with *E. coli* was detected in water samples collected from the Gryteland stream. Yet, the origin of this contamination was never entirely proven until this study, described herein, was undertaken.

The established contribution profile of the host-specific *Bacteroidales* DNA markers in faecal contamination derived from the MST study clearly distinguished between human and non-human pollution origins (Figures 3 and 4). It revealed that human-originated faecal contamination increases and becomes dominant in cold seasons and relatively dry periods, particularly in winter and spring. This, however, is related neither to the concentrations of coliforms nor to *E. coli* counts. Such uncorrelated phenomena were also observed and reported by Tambalo *et al.* (2012).

Although the NBTS in the Gryteland stream was designed for run-off purification (mainly sediments and nutrients) and not for wastewater treatment, it could still reduce the concentrations of *E. coli*. In general, lower numbers of these bacteria were detected in water grab samples collected

Table 1 | The range (min = minimum, max = maximum and st.d. = standard deviation) of contaminants (mg/l, and mS/m for EC) in water samples collected from the Gryteland stream before (inlet content) and after (outlet content) passing through the nature-based treatment system

	Inlet content				Outlet content			
Parameter	**Min**	**Mean**	**St.d.**	**Max**	**Min**	**Mean**	**St.d.**	**Max**
COD_{Cr}	9.0	23.0	7.6	38.0	11.0	22.8	8.2	40.0
TSS	5.6	19.1	26.2	92.3	6.2	18.7	20.1	71.0
PO_4-P	0.01	0.03	0.03	0.09	0.01	0.03	0.02	0.09
TP	0.02	0.09	0.08	0.23	0.03	0.07	0.06	0.22
NH_4-N	0.00	0.12	0.26	0.90	0.03	0.14	0.22	0.77
NO_2-N	0.02	0.04	0.02	0.07	0.02	0.03	0.02	0.05
NO_3-N	1.8	2.6	0.7	3.9	1.8	2.5	0.7	3.9
TN	2.6	5.2	2.4	9.7	2.5	5.0	2.1	8.3
TOC	3.1	7.7	2.7	12.4	3.7	7.6	2.5	11.6
DOC	3.0	7.3	2.6	12.0	3.6	7.3	2.4	11.5
EC	11.8	18.6	5.2	31.6	13.3	19.2	5.6	31.6
TDS	107.0	148.1	34.2	225.0	105.0	148.1	32.4	215.0
pH	6.9–7.7				7.1–7.5			

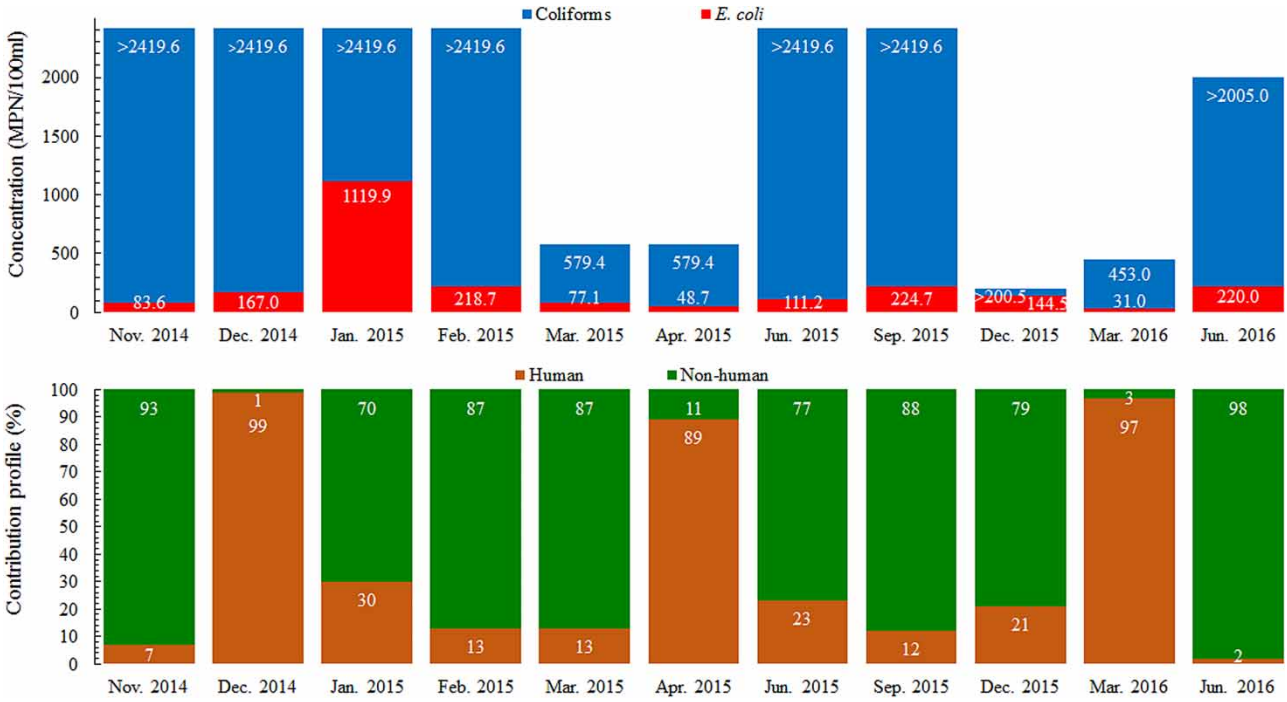

Figure 3 | Inlet concentrations of coliforms and *E. coli* (upper chart) along with the contribution profile of genetic markers in faecal contamination (lower chart) of water samples collected from the Gryteland stream before passing through the nature-based treatment system.

at the outlet of the NBTS in comparison to *E. coli* counts in the inlet water samples (Figures 3 and 4). Yet, two substantial exemptions with higher *E. coli* concentrations at the outlet of the system were observed in the two spring seasons of 2015 and 2016, i.e. April and March, respectively. Both cases reflect a complex of factors affecting the purification of

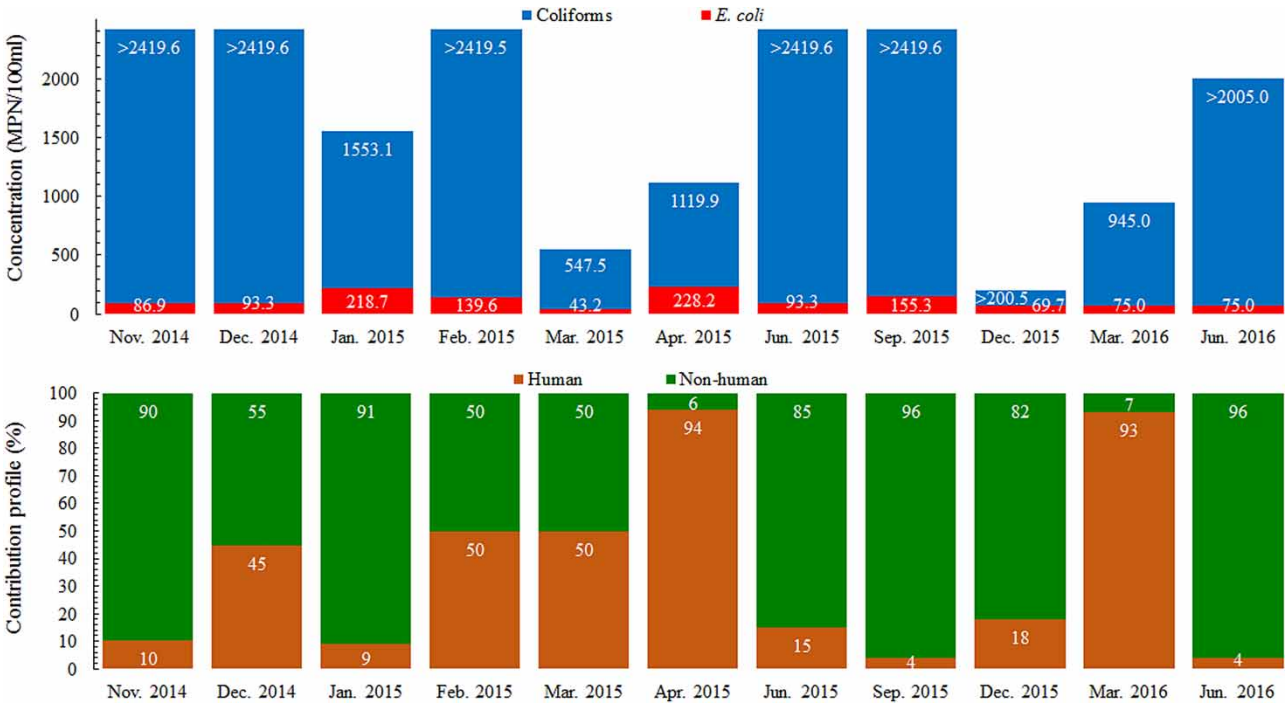

Figure 4 | Outlet concentrations of coliforms and *E. coli* (upper chart) along with the contribution profile of genetic markers in faecal contamination (lower chart) of water samples collected from the Gryteland stream after passing through the nature-based treatment system.

multi-polluted run-off in the NBTS. The first rational cause of the elevated *E. coli* concentrations might have derived from a direct discharge of wastewater, which was strongly revealed by human dominance in both inlet and outlet water samples (respectively, 89% and 94% in April 2015 and 97% and 93% in March 2016). The other factors are rather indirect and can be generally defined by: (i) resuspension of faecally polluted sediments under yearly development of wetland vegetation (e.g. expansion of roots and shoots during growing season), causing higher concentrations of *E. coli* bacteria in water (Sanders *et al.* 2005; Brinkmeyer *et al.* 2015); (ii) ambient turbulence, hindering settling of solids and flocculation in wetlands and further distribution of solids over the entire water depth (Tchobanoglous 1993); (iii) survival and possible multiplication of faecal indicator bacteria in wetland sediments (Sanders *et al.* 2005).

The implemented MST tools were specifically validated in a previous Norwegian study on faecal water contamination in an agricultural catchment of the Mørdre stream – a tributary of the Glåma River, the longest and greatest watercourse in Norway (Paruch *et al.* 2015b). The origin of faecal contamination was quantitatively distinguished between human, horse and ruminant based on the RT-qPCR detection of three *Bacteroidales* host-associated markers. Furthermore, tracking of the human faecal pollution demonstrated its dominance in areas where potential water contamination with domestic wastewater occurred (Paruch *et al.* 2015b). In the present study, the contaminating spots were not tracked down, but evidence of contamination with wastewater, which was demonstrated by human dominance, was supported by the detection of CECs, which can be tracked in sewage. Among the CECs, one of the most representative groups of organic chemicals is PPCPs. These compounds emerge because of their continuous disposal route from sewage/wastewater to the environment and their health risk issues for humans and the environment (in particular aquatic life), which are not fully understood yet (Petrie *et al.* 2015). Various PPCPs are commonly used nowadays (e.g. drugs, cosmetics, household cleaning products and chemicals, and nutritional supplements); thus their presence in wastewater cannot be neglected. These agents were determined as being ubiquitous in the wastewater and surface water of European and North American countries (Ternes *et al.* 2007; Aga 2008). They are hardly removed in conventional wastewater treatment plants and are quite persistent substances in the aquatic environment (Miao *et al.* 2005; Rodarte-Morales *et al.* 2011). It has been reported that the removal of PPCPs has been attributed to a combination of sorption, partial biodegradation, phytoremediation and plant uptake (Matamoros *et al.* 2009; Dordio *et al.* 2010).

Eight different CECs, representing mostly PPCPs, were detected in water grab samples collected from the Gryteland stream (Table 2). Seven chemicals within PPCPs and their metabolites were quantitated at the inlet of the NBTS. The same compounds and one EDC (bisphenol A) were quantitated at the outlet of the NBTS. Since PPCPs prevailed in the detected chemicals, the general content of PPCPs is referred to further in this study. These chemicals were detected on

Table 2 | Concentrations of emerging contaminants (ng/l) along with their LOQ in water samples collected from the Gryteland stream at the inlet (upper values) and the outlet (lower values) of the nature-based treatment system

Compound	LOQ	Nov. 2014	Feb. 2015	Jun. 2015	Sep. 2015	Dec. 2015	Mar. 2016
Ibuprofen	20	270	120	<20	<20	<20	2,500
		73	<20	<20	<20	<20	35
2-hydroxy-ibuprofen	30	Not tested	Not tested	Not tested	<30	<30	76
					<30	<30	45
Carboxy-ibuprofen	20	Not tested	Not tested	Not tested	<20	<20	34
					<20	<20	47
Gabapentin	10	30	18	40	21	37	79
		24	21	25	22	36	87
Paracetamol	10	<10	28	24	<10	<10	<10
		21	17	<10	<10	<10	32
Caffeine	100	<100	<100	280	<100	<100	390
		<100	<100	440	<100	120	640
Saccharin	50	<50	<50	80	<50	<50	<50
		<50	<50	74	<50	<50	<50
Bisphenol A	50	Not tested	Not tested	Not tested	<50	<50	<50
					<50	160	<50

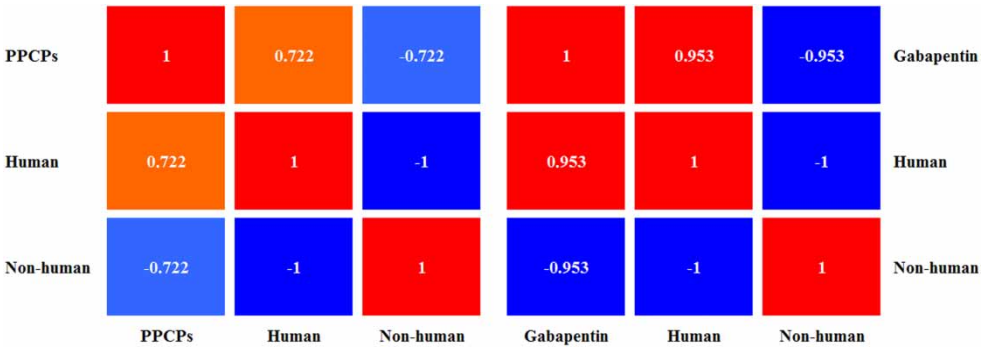

Figure 5 | Pearson's correlation heat map of PPCPs (left matrix) and gabapentin (right matrix) with genetic markers detected in the inlet water samples collected from the Gryteland stream before passing through the nature-based treatment system. The correlation map uses a red-blue (hot-cold) scale to display the correlation close to 1 (red colour) and correlation close to −1 (blue colour). Please refer to the online version of this paper to see this figure in colour: http://dx.doi.org/10.2166/wst.2017.303.

different occasions over the investigated period, but interestingly they were not correlated with high concentrations of indicator *E. coli* bacteria. Thus, faecal contamination cannot prove or disprove the presence of PPCPs in water.

The highest concentrations among the tested PPCPs were found for ibuprofen in the inlet water samples (Table 2). This compound, defined as an analgesic, anti-pyretic and non-steroidal anti-inflammatory drug, is among the most widely used pharmaceuticals in the world and is found in sewage and seawater in Norway (Weigel *et al.* 2004). Its highest content was accompanied by the greatest concentrations of four other PPCPs detected in inlet water samples collected in March 2016. Correspondingly, the highest numbers of PPCPs (six different compounds) were detected in the outlet water samples (Table 2). During the same period, the dominance of humans in faecal contamination was identified based on the contribution profile of the *Bacteroidales* DNA markers in both water samples (Figures 3 and 4). The most frequently detected compound among the PPCPs tested was gabapentin (Table 2), an anticonvulsant drug used widely to relieve neuropathic pain (Petrie *et al.* 2015). Its concentrations varied along with human contributions in the faecal water pollution and reached the highest content in March 2016, when human dominance and the highest contents of the other PPCPs in water samples were defined.

The observations on human contributions in the faecal water pollution and variations in PPCP contents advocated conducting a statistical test to confirm/reject any relationships between the PPCPs detected and human/non-human origin of faecal water contamination. For this purpose, a total pool of the quantitated PPCPs was applied and the compounds detected on each occasion were weighted by their percentage in the pool. A Pearson correlation matrix was employed for testing both PPCP percentage and actual concentrations of gabapentin, as it was the only compound detected on each sampling occasion (Table 2). The results of the statistical test indicated a relatively strong correlation between the occurrence of the PPCPs with gabapentin in water samples and the human contribution in faecal contamination of these samples (Figures 5 and 6). Pearson's correlation coefficients showed a positive

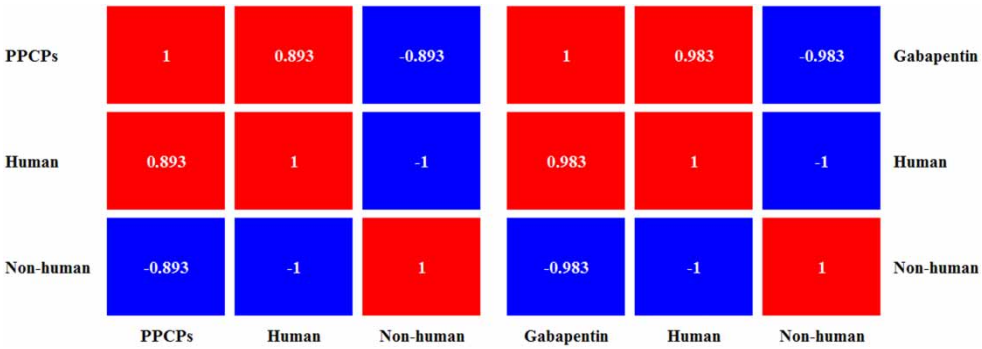

Figure 6 | Pearson's correlation heat map of PPCPs (left matrix) and gabapentin (right matrix) with genetic markers detected in the outlet water samples collected from the Gryteland stream after passing through the nature-based treatment system. The correlation map uses a red-blue (hot-cold) scale to display the correlation close to 1 (red colour) and correlation close to −1 (blue colour). Please refer to the online version of this paper to see this figure in colour: http://dx.doi.org/10.2166/wst.2017.303.

correlation between the PPCPs and human contribution in the water samples tested, with a higher coefficient in the outlet water (0.893) than in the inlet water (0.722). In contrast, the correlation was negative for non-human faecal contamination sources in both water samples (Figures 5 and 6). An even stronger positive correlation was exposed in the case of gabapentin, where the coefficient approached 1 (0.983 and 0.953 in the outlet and inlet water, respectively).

CONCLUSIONS

This research study described the first Norwegian MST approach for water quality control and pollution removal from the Gryteland stream passing through the NBTS designed solely for treatment of catchment run-off. The outcomes of this complex investigation conducted through the physical, chemical, microbiological and molecular tests revealed that catchment water quality is influenced by a multi-polluted run-off. The range of catchment pollution includes, among others, nutrients, organics, microbes and emerging contaminants that originate from different sources. The identification of contaminating source(s) and/or origin(s) is vital for an optimal implementation of adequate treatment measures at accurate contaminating spot(s). The applied MST tools (combining microbial analyses and molecular tests) detected and defined the source(s) and dominant origin(s) of faecal water contamination in the investigated catchment. The study demonstrated that the newly developed contribution profiling of faecal origin derived from the qPCR-based host-specific *Bacteroidales* DNA distinguished quantitatively between human and non-human pollution origins. The human dominance in faecal contamination was observed in cold seasons and relatively dry periods, particularly in winter and spring. Moreover, a strong positive correlation was discovered between the human marker and the PPCPs detected. Out of eight PPCPs quantitated, gabapentin was the most frequently detected compound in all water samples tested and it demonstrated the uppermost positive correlation with the human marker.

Although the NBTS (with CW as the main system component) was designed originally for the removal of nutrients and sediments from the Gryteland stream, continuously loaded with catchment run-off influenced by both point (scattered settlements) and diffuse (agricultural areas with livestock) source pollution, it could not consistently achieve complete removal of these pollutants throughout the course of the study.

In general, the NBTS performed satisfactorily with the removal of faecal indicator bacteria (*E. coli*) but not PPCPs. Interestingly, the presence of these chemicals in the water samples tested was not correlated with high concentrations of *E. coli*. Neither has the latter an apparent correlation with the human marker. It can therefore be stated that faecal contamination (expressed by *E. coli*) cannot prove or disprove the presence of PPCPs in water. An additional reflection could be that a high concentration of *E. coli* does not necessarily indicate human-originated faecal contamination of water.

The data presented in this research study suggest that greater emphasis should be placed on catchment water quality control and source tracking of contamination. Further investigations that expand upon this research are required to address the impact of other host-specific *Bacteroidales* 16S rRNA genetic markers on the contribution profile in faecal water contamination.

ACKNOWLEDGEMENTS

The research leading to these results has received funding from the Norwegian Financial Mechanism 2009–2014 under Project Contract no. 7F14341 'Assessing water quality improvement options concerning nutrient and pharmaceutical contaminants in rural watersheds'.

REFERENCES

Aga, D. S. 2008 *Fate of Pharmaceuticals in the Environment and in Water Treatment Systems*. CRC Press, Taylor & Francis Group, Boca Raton, FL, USA.

Blankenberg, A.-G. B., Deelstra, J., Øgaard, A. F. & Pedersen, R. 2013 Phosphorus and sediment retention in a constructed wetland. In: *Agriculture and Environment – Long Term Monitoring in Norway* (M. Bechmann & J. Deelstra, eds). Akademika Publishing, Trondheim, Norway, pp. 299–314.

Blankenberg, A.-G. B., Haarstad, K. & Paruch, A. M. 2015 Agricultural runoff in Norway: the problem, the regulations, and the role of wetlands. In: *The Role of Natural and Constructed Wetlands in Nutrient Cycling and Retention on the Landscape* (J. Vymazal, ed.), Springer International Publishing, Switzerland, pp. 137–147.

Brinkmeyer, R., Amon, R. M. W., Schwarz, J. R., Saxton, T., Roberts, D., Harrison, S., Ellis, N., Fox, J., DiGuardi, K., Hochman, M., Duan, S., Stein, R. & Elliott, C. 2015 Distribution and persistence of *Escherichia coli* and Enterococci in stream bed and bank sediments from two

urban streams in Houston, TX. *Science of the Total Environment* **502**, 650–658.

Dick, L. K., Bernhard, A. E., Brodeur, T. J., Santo Domingo, J. W., Simpson, J. M., Walters, S. P. & Field, K. G. 2005 Host distributions of uncultivated fecal *Bacteroidales* bacteria reveal genetic markers for fecal source identification. *Applied and Environmental Microbiology* **71** (6), 3184–3191.

Dordio, A., Carvalho, A. J. P., Teixeira, D. M., Dias, C. B. & Pinto, A. P. 2010 Removal of pharmaceuticals in microcosm constructed wetlands using *Typha* spp. and LECA. *Bioresource Technology* **101** (3), 886–892.

Edge, T. A., Hill, S., Seto, P. & Marsalek, J. 2010 Library-dependent and library-independent microbial source tracking to identify spatial variation in faecal contamination sources along a Lake Ontario beach (Ontario, Canada). *Water Science and Technology* **62** (3), 719–727.

Field, K. G. & Samadpour, M. 2007 Fecal source tracking, the indicator paradigm, and managing water quality. *Water Research* **41** (16), 3517–3538.

Gourmelon, M., Caprais, M. P., Le Mennec, C., Mieszkin, S., Ponthoreau, C. & Gendronneau, M. 2010 Application of library-independent microbial source tracking methods for identifying the sources of faecal contamination in coastal areas. *Water Science and Technology* **61** (6), 1401–1409.

Keegan, M., Kilroy, K., Nolan, D., Dubber, D., Johnston, P. M., Misstear, B. D. R., O'Flaherty, V., Barrett, M. & Gill, L. W. 2014 Assessment of the impact of traditional septic tank soakaway systems on water quality in Ireland. *Water Science and Technology* **70** (4), 634–641.

Layton, A., McKay, L., Williams, D., Garrett, V., Gentry, R. & Sayler, G. 2006 Development of *Bacteroides* 16S rRNA gene TaqMan-based real-time PCR assays for estimation of total, human, and bovine fecal pollution in water. *Applied and Environmental Microbiology* **72** (6), 4214–4224.

Matamoros, V., Arias, C., Brix, H. & Bayona, J. M. 2009 Preliminary screening of small-scale domestic wastewater treatment systems for removal of pharmaceutical and personal care products. *Water Research* **43** (1), 55–62.

McQuaig, S., Griffith, J. & Harwood, V. J. 2012 Association of fecal indicator bacteria with human viruses and microbial source tracking markers at coastal beaches impacted by nonpoint source pollution. *Applied and Environmental Microbiology* **78** (18), 6423–6432.

Miao, X.-S., Yang, J.-J. & Metcalfe, C. D. 2005 Fate of carbamazepine and its metabolites in a municipal wastewater treatment plant. *Environmental Science and Technology* **39** (19), 7469–7475.

Paruch, A. M. & Mæhlum, T. 2012 Specific features of *Escherichia coli* that distinguish it from coliform and thermotolerant coliform bacteria and define it as the most accurate indicator of faecal contamination in the environment. *Ecological Indicators* **23**, 140–142.

Paruch, A. M., Mæhlum, T. & Robertson, L. 2015a Changes in microbial quality of irrigation water under different weather conditions in Southeast Norway. *Environmental Processes* **2** (1), 115–124.

Paruch, L., Paruch, A. M., Blankenberg, A.-G. B., Bechmann, M. & Mæhlum, T. 2015b Application of host-specific genetic markers for microbial source tracking of faecal water contamination in an agricultural catchment. *Acta Agriculturae Scandinavica, Section B – Soil & Plant Science* **65** (S2), 164–172.

Petrie, B., Barden, R. & Kasprzyk-Hordern, B. 2015 A review on emerging contaminants in wastewaters and the environment: current knowledge, understudied areas and recommendations for future monitoring. *Water Research* **72**, 3–27.

Reischer, G. H., Kasper, D. C., Steinborn, R., Farnleitner, A. H. & Mach, R. L. 2007 A quantitative real-time PCR assay for the highly sensitive and specific detection of human faecal influence in spring water from a large alpine catchment area. *Letters in Applied Microbiology* **44** (4), 351–356.

Rodarte-Morales, A. I., Feijoo, G., Moreira, M. T. & Lema, J. M. 2011 Degradation of selected pharmaceutical and personal care products (PPCPs) by white-rot fungi. *World Journal of Microbiology and Biotechnology* **27** (8), 1839–1846.

Sanders, B. F., Arega, F. & Sutula, M. 2005 Modeling the dry-weather tidal cycling of fecal indicator bacteria in surface waters of an intertidal wetland. *Water Research* **39** (14), 3394–3408.

Shanks, O. C., Atikovic, E., Blackwood, A. D., Lu, J., Noble, R. T., Santo Domingo, J., Seifring, S., Sivaganesan, M. & Huagland, R. A. 2008 Quantitative PCR for detection and enumeration of genetic markers of bovine fecal pollution. *Applied and Environmental Microbiology* **74** (3), 745–752.

Tambalo, D. D., Fremaux, B., Boa, T. & Yost, C. K. 2012 Persistence of host-associated *Bacteroidales* gene markers and their quantitative detection in an urban and agricultural mixed prairie watershed. *Water Research* **46** (9), 2891–2904.

Tchobanoglous, G. 1993 Constructed wetlands and aquatic plant systems: research, design, operational, and monitoring issues. In: *Constructed Wetlands for Water Quality Improvement* (G. A. Moshiri, ed.). CRC Press, Boca Raton, FL, USA, pp. 23–34.

Ternes, T. A., Bonerz, M., Herrmann, N., Teiser, B. & Andersen, H. R. 2007 Irrigation of treated wastewater in Braunschweig, Germany: an option to remove pharmaceuticals and musk fragrances. *Chemosphere* **66** (5), 894–904.

US EPA 2005 *Microbial Source Tracking Guide Document*. United States Environmental Protection Agency, Office of Research and Development, EPA-600/R-05/064, Washington, DC, USA.

Vymazal, J., Dvořáková Březinová, T., Koželuh, M. & Kule, L. 2017 Occurrence and removal of pharmaceuticals in four full-scale constructed wetlands in the Czech Republic – the first year of monitoring. *Ecological Engineering* **98**, 354–364.

Weigel, S., Berger, U., Jensen, E., Kallenborn, R., Thoresen, H. & Hühnerfuss, H. 2004 Determination of selected pharmaceuticals and caffeine in sewage and seawater from Tromsø/Norway with emphasis on ibuprofen and its metabolites. *Chemosphere* **56** (6), 583–592.

WHO 2011 *Guidelines for Drinking-Water Quality*, 4th edn. World Health Organization, Geneva, Switzerland.

First received 10 November 2016; accepted in revised form 8 May 2017. Available online 19 May 2017