Cross-tracking of faecal pollution origins, macronutrients, pharmaceuticals and personal care products in rural and urban watercourses

Lisa Paruch and Adam M. Paruch

ABSTRACT

This study describes microbial and chemical source tracking approaches for water pollution in rural and urban catchments. Culturable faecal indicator bacteria, represented by *Escherichia coli*, were quantified. Microbial source tracking (MST) using host-specific DNA markers was applied to identify the origins of faecal contamination. Chemical source tracking (CST) was conducted to determine contaminants of emerging concern (CEC) of human/anthropogenic origin, including pharmaceuticals and personal care products (PPCPs) and endocrine-disrupting chemicals (EDCs). In addition, the eutrophication-causing macronutrients nitrogen and phosphorus were studied. MST tests revealed both anthropogenic and zoogenic faecal origins, with a dominance of human sources in the urban stream; non-human/environmental sources were prevalent in the rural creek. CST analyses revealed a higher number of CECs in the urban stream than in the rural watercourse. Positive correlations between PPCPs and both *E. coli* and the human DNA marker were uncovered in the urban stream, while in the rural creek, PPCPs were only highly correlated with the anthropogenic marker. Interestingly, macronutrients were strongly associated with primary faecal pollution origins in both watercourses. This correlation pattern determines the main pollutant contributors (anthropogenic or zoogenic) to eutrophication.

Key words | Bacteroidales 16S rRNA gene markers, *Escherichia coli*, faecal water contamination, microbial and chemical source tracking, nitrogen and phosphorus, pharmaceuticals and personal care products

HIGHLIGHTS

- Microbial source tracking is essential to define the origins of faecal contamination.
- Chemical source tracking supports microbial source tracking faecal pollution results.
- Pharmaceuticals and personal care products correlate with the human DNA marker.
- Dominant faecal origin reveals primary sources of macronutrient pollution in water.

INTRODUCTION

All terrestrial and aquatic ecosystems are directly or indirectly impacted by faecal contamination, which creates serious problems for public and environmental health. Human and animal faeces are the main sources of enteric pathogens responsible for food- and waterborne disease outbreaks (*Paruch & Paruch 2018*). The mortality and morbidity associated with faecal water contamination represent nearly 10% of the total burden of human disease worldwide (*WHO 2020*). Therefore, the identification of faecal origins and tracking the sources of contamination become crucial for water quality.
management (Pascual-Benito et al. 2020), protection strategies for aquatic ecological environments (Paruch et al. 2019), treatment options for point and non-point/ diffuse sources (Chen et al. 2020), the development of remediation regimes (Ahmed et al. 2020) and the potential health risk assessment for humans and the environment (Salim Dantas et al. 2020).

Faecal indicator bacteria (FIB), represented usually by Escherichia coli (E. coli), have been extensively examined to evaluate water quality associated with faecal contamination. The presence of FIB indicates the occurrence but not the origin of faecal pollution largely due to its low host specificity, replication in the environment and geographic and temporal variability (Tran et al. 2015). Therefore, various other microbes, indicators and markers have been selected and validated for microbial source tracking (MST) of faecal pollution. These include host-associated molecular markers derived from Bacteroidales 16S rRNA genes, which exhibit high host specificity and geographical stability; thus, they have been extensively applied worldwide in diverse water-quality-related studies focused on urban and agricultural watersheds (Sowah et al. 2017), natural surface and ground waters (Tran et al. 2015), drinking water sources (Paruch et al. 2020), coastal and marine aquatic environments (Jardé et al. 2018) and recreational beaches (Molina et al. 2014). The MST tests based on quantitative polymerase chain reaction (qPCR) have been proven to achieve rapid detection and accurate quantification of specific microbial genetic markers developed for different hosts, e.g. humans, ruminants, pigs, horses, dogs and others (Paruch et al. 2015; Staley et al. 2016; Sowah et al. 2017; Haramoto & Osada 2018).

In addition to MST, chemical source tracking (CST) has been implemented to determine the origins of faecal water contamination. Various CST identifiers/markers have been used (e.g. caffeine, faecal sterols and stanols, bile acids, laundry brighteners, fragrances and pesticides) and among them, pharmaceuticals and personal care products (PPCPs) are the trace contaminants most frequently detected in close association with anthropogenic activities (Yang et al. 2020). Hence, they have been employed as chemical markers to detect and evaluate human faeces in water bodies. PPCPs are of high concern in terms of the direct or indirect impact on human and environmental health since they and their metabolites constitute substantial sources of contaminants of emerging concern (CEC) in aquatic environments (Su et al. 2020).

Different measures based on MST host-specific genetic markers and CST identifiers have been developed and applied in tracking faecal sources in contaminated waters. MST and CST have usually been applied separately as independent methods, but recently, the combined usage of MST and CST has been advocated to support a more robust result (Staley et al. 2016; Devane et al. 2019). However, to date, some important technical questions have not yet been well addressed, e.g.: (i) How, in practice, should MST and CST methods be incorporated, especially under different circumstances? (ii) How can the results from these two methods be reasonably integrated and validated in order to achieve more robust and reliable results?

Therefore, in the current study, we attempted to shed some light on these aspects rather than making side-by-side comparisons of the two methods (i.e. pros and cons), since those have been explored in detail in earlier investigations (Staley et al. 2016; Devane et al. 2019). We implemented both MST and CST methods to address faecal contamination (microbial pollution and PPCP contamination) in two differently representative water types: urban and rural watercourses. Moreover, nutrient levels (mainly nitrogen – N and phosphorus – P) are drastically elevated due to faecal water pollution (e.g. wastewater discharges and agricultural run-off) causing eutrophication and the deterioration of the quality of aquatic ecosystems (Blankenberg et al. 2016); therefore, we also monitored nutrient parameters and linked them to the MST studies. We hypothesised that quantitative microbial source tracking (QMST) can also facilitate the source identification of N and P in water environments. To the best of our knowledge, this is a pioneering study, as it focuses on the integrated cross-tracking of various micro-, macro- and emerging water contaminants; hence, it provides valuable input for better interpreting the results of MST and CST tests, as well as novel insights for defining the origins of faecal pollutants in aquatic environments.

**MATERIALS AND METHODS**

**Study sites and water sampling**

This study was conducted in two watercourses (a rural creek and an urban stream) located in agricultural and urban catchment areas of neighbouring municipalities, Ås and Nordre Follo, on the border of south-east Oslo, Norway.
The rural creek, Grytelandsbekken, is approximately 2.5 km long and runs east of the town of Ås (approximately 11,000 people). It has a catchment (also known as the Skuterud catchment) covered mostly with farmland (60%) and forest/marshland (31%). The first study site was located at Grytelandsbekken (G), which is downstream of this catchment and at the mouth of the creek. The urban stream, Blåveisbekken, runs through the city of Ski (approximately 20,000 residents) and is partially culverted (Figure 1). The second study site was situated at the end of the culvert in Blåveisbekken (B). Water samples were collected at G and B at quarterly intervals for over 2 years (November 2014 to March 2017). In total, 80 samples, 40 per study site (20 for CEC, 10 for macropollutants and 10 for microbial contaminants), were collected during 10 sampling events (Nov. 2014, Feb. 2015, Jun. 2015, Sep. 2015, Dec. 2015, Mar. 2016, Jun. 2016, Sep. 2016, Dec. 2016, Mar. 2017).

Figure 1 | Location of the study sites G (Grytelandsbekken rural creek) in Ås, and B (Blåveisbekken urban stream) in Ski. The satellite image and maps were obtained from NIBIO’s primary map service, Kilden (https://kilden.nibio.no).
Water quality examination

Standard water quality control measures were conducted to determine some of the most common macropollutants, such as organic matter content expressed as chemical oxygen demand (COD), total dissolved solids (TDS) and eutrophication-causing macronutrients (total nitrogen – TN and total phosphorus – TP). All macropollutants and pH were analysed in an accredited laboratory of the ALS Laboratory Group Norway AS according to the respective ISO and national standards for COD (ISO 15705), TDS (CSN 757346, CSN 757347, EN 16192), pH (ISO 10523, EPA 150.1, EN 16192), TN (EN 12260) and TP (ISO 6878, ISO 15681-1).

Chemical source tracking (CST)

The study sites were examined for CEC, particularly PPCPs, including their metabolites, and endocrine-disrupting chemicals (EDCs). These compounds were tested by the Water Management Laboratory Plzeň, Povodi Vltavy, State Enterprise following national, ISO and EPA standards (CSN ISO 20179, CSN ISO 25101, EPA 1694 and EPA 535). All detectable (above limits of quantitation – LOQ) CECs were recorded and used for data analysis in this study.

Microbial source tracking (MST)

Microbial examination of faecal contamination were carried out using FIB tests, i.e. enumeration of E. coli. These tests were performed in the Laboratory of Aquatic Microbial Biotechnology (LAMB) administrated by the Norwegian Institute of Bioeconomy Research (NIBIO) and were executed using the Colilert 18 method (IDEXX Laboratories, Inc., Westbrook, Maine, USA) constituting the worldwide ISO standard 9308-2:2012. This method determines concentrations of E. coli as the most probable number (MPN) of organisms in 100 ml of examined water. The MPN counts were supported by 95% confidence limits estimated by the IDEXX MPN Generator Ver. 1.4, Quanti-Tray® and Quanti-Tray®/2000 MPN Table Program (©2020 IDEXX Laboratories, Inc., Westbrook, Maine, USA).

The faecally contaminated (positive for E. coli) water samples were further analysed using molecular tools in order to determine the anthropogenic/human and zoogenic/environmental (e.g. pet, livestock and wild animal) sources of faecal pollution. For this purpose, each polluted water sample (200 ml) was concentrated in an ultrafiltration unit and the solid material obtained was subjected to microbial genomic DNA extraction using a DNeasy PowerWater Kit (QIAGEN GmbH, Hilden, GERMANY) following the protocol provided by the manufacturer. The purified DNA material was used in a real-time quantitative polymerase chain reaction (RT-qPCR) for the detection and quantification of host-specific Bacteroidales 16S rRNA genetic markers implemented in the QMST technique. The markers applied and methodology of this technique have been previously described in detail (Paruch et al. 2015, 2017).

Toolkit determining faecal dominant origin and source

The microbial and molecular analyses constitute the integrated components of the testing toolkit developed at NIBIO for defining faecal pollution origins and determining the dominant source of pollution. The established toolkit consists of three interrelated methodological steps: (1) microbial examination for screening faecally contaminated water samples; (2) molecular diagnostic of the contaminated samples, i.e. QMST testing; (3) source apportionment profile of the genetic markers detected in the faecally polluted sample. Based on the QMST outcomes, a faecal source apportionment profile (FSAP) was established. The FSAP presents the percentage distribution of DNA-based markers; thus, it determines the dominant faecal origin and source in the examined water. The scientific basis and methodological procedures of this toolkit have been described in depth elsewhere (Paruch et al. 2020).

Statistical analysis

A redundancy analysis (RDA) was conducted to determine the correlations between biotic (E. coli and the dominant faecal sources) and abiotic components (chemical parameters) based on Pearson’s correlation test, with a statistical significance level above 95% (p < 0.05). This was performed using the MPN counts, faecal source abundance data, amount of total PPCPs (including detected metabolites and bisphenol A – BPA) and indicative PPCPs (i.e. the most frequently detected, such as ibuprofen, gabapentin, paracetamol and caffeine), and content of macropollutants. In addition, the biotic and abiotic factors were applied to generate the Pearson’s correlation heat maps, distinguishing between the positive and negative data (correlation coefficients between 1 and −1). The RDA and the correlation analysis were performed using XLSTAT-ECOLOGY statistical software package version 2019.1.1 (Addinsoft 2020, Boston, USA, https://www.xlstat.com).
RESULTS AND DISCUSSION

The standard water quality control measures exposed higher contents of both organic matter and macronutrients in Grytelandsbekken (study site G; 9–40 mg O₂/l, 0.02–0.23 mg TP/l and 2.56–9.68 mg TN/l; Table 1) than in Blåveisbekken (study site B; 5–30 mg O₂/l, 0.03–0.16 mg TP/l and 1.5–4.07 mg TN/l; Table 2). These outcomes reflect the different characters of the studied catchments (rural and urban, Figure 1) and are associated with agricultural practices (e.g. mineral and organic N/P fertilisers) where agrarian run-off is recognised as the main source of eutrophication-causing macronutrients in freshwater bodies. Firmansyah et al. (2016) revealed that run-off, erosion and leaching in agricultural systems contribute significantly to N/P loss. Blankenberg et al. (2016) reported that run-off from agricultural areas was the main source of nutrients in surface waters. In addition, an earlier study by Paruch et al. (2019) on aquatic microbial community composition in Grytelandsbekken demonstrated a dominance of archaeal populations that were positively correlated with fertiliser use and farmyard manuring.

A side effect of the use of manure is the contamination of soil and water with animal faecal matter. However, this is not the only faecal source in agricultural catchments; wild animals, livestock farming and human settlements can also contribute to faecal pollution (Paruch et al. 2017). The microbial investigations of both study sites demonstrated constant faecal water contamination, with much higher E. coli concentrations (expressed as MPN with 95% confidence limits) in the urban stream than the rural creek (Figures 2 and 3). The molecular tests unveiled that these contaminations were both anthropogenic and zoogenic (Figures 2 and 3).

The QMST tests determined that zoogenic faecal sources dominated in the rural site G, as demonstrated by FSAP ranging from approximately 80% to 99% (Figure 2). Only one case showed a prevalence of an anthropogenic faecal impact, though under the lowest E. coli concentration. This could have been a consequence of some sporadic leakage from decentralised/on-site wastewater treatment systems, which have been previously localised in nearby scattered rural settlements in the catchment of Grytelandsbekken (Blankenberg et al. 2016).

In contrast to the rural water at site G, a high proportion of anthropogenic faecal sources was detected in the urban water at site B (Figure 3). This finding is in line with a general opinion that urban faecal water contamination is anthropogenic. It derives principally from sanitary and/or combined sewer systems (sewage leakages, wastewater discharges and overflows from pumping stations and storm and drainage systems). However, this opinion is not entirely

| Parameter                        | Nov. 2014 | Feb. 2015 | Jun. 2015 | Sep. 2015 | Dec. 2015 | Mar. 2016 | Nov. 2016 | Dec. 2016 | Mar. 2017 |
|----------------------------------|----------|----------|----------|----------|----------|----------|----------|----------|----------|
| Chemical oxygen demand – COD (mg O₂/l) | 28       | 15       | 22       | 40       | 26       | 24       | 9        | 29       | 27       |
| Total dissolved solids – TDS (mg/l)      | 139      | 107      | 185      | 133      | 140      | 124      | 177      | 194      | 153      |
| Total phosphorus – TP (mg P/l)        | 0.05     | 0.03     | 0.23     | 0.06     | 0.05     | 0.02     | 0.03     | 0.06     | 0.12     |
| Total nitrogen – TN (mg N/l)         | 6.19     | 3.06     | 3.37     | 4.42     | 9.68     | 2.56     | 3.98     | 3.91     | 7.48     | 3.83     |
| pH                                | 7.34     | 7.53     | 7.43     | 7.09     | 6.91     | 7.46     | 7.67     | 7.45     | 7.35     | 7.01     |

| Parameter                        | Nov. 2014 | Feb. 2015 | Jun. 2015 | Sep. 2015 | Dec. 2015 | Mar. 2016 | Nov. 2016 | Dec. 2016 | Mar. 2017 |
|----------------------------------|----------|----------|----------|----------|----------|----------|----------|----------|----------|
| Chemical oxygen demand – COD (mg O₂/l) | 25       | 6        | 20       | 29       | 22       | 5         | 6         | 28       | 11       | 30       |
| Total dissolved solids – TDS (mg/l)      | 148      | 228      | 187      | 120      | 144      | 200       | 181       | 188      | 174      | 161      |
| Total phosphorus – TP (mg P/l)        | 0.04     | 0.05     | 0.04     | 0.16     | 0.03     | 0.03      | 0.03      | 0.15     | 0.06     | 0.06     |
| Total nitrogen – TN (mg N/l)         | 3.69     | 3.05     | 1.50     | 2.85     | 3.91     | 1.72      | 2.56      | 4.07     | 3.70     | 2.88     |
| pH                                | 7.68     | 7.63     | 8.40     | 7.40     | 7.70     | 7.60      | 7.66      | 7.63     | 7.74     | 7.39     |
Figure 2 | Concentrations of *E. coli* as MPNs with 95% confidence limits and FSAP in water samples from the rural creek Grytelandsbekken.

Figure 3 | Concentrations of *E. coli* as MPNs with 95% confidence limits and FSAP in water samples from the urban stream, Blåveisbekken.
true, especially nowadays with the ‘blue-green’ development trend of urban areas, which are favoured and inhabited by various wildlife species (Gallo & Fidino 2018; Partridge & Clark 2018). The urban fauna, represented mostly by birds (e.g. pigeons, crows, ravens and gulls), in particular waterfowl (swans, geese and ducks), and mammals (e.g. rats, bats, raccoons, foxes and beavers), contribute directly and/or indirectly (e.g. via urban run-off) to faecal water contamination (Paruch & Paruch 2018). The city sewerage harbours a flow of pollution generated by a variety of urban life (i.e. human and animal); hence, not all that comes from sewerage has an anthropogenic origin. Therefore, it is time to update the fact that faecal pollution in urban catchments has also substantial footprints of zoogenic origins, as documented at study site B (Figure 3). To this end, we implemented QMST to distinguish the major (human or non-human) faecal pollution source in urban water.

Source tracking and discrimination between anthropogenic and zoogenic sources of faecal water contamination is of vital importance in safeguarding and mitigating public health risks and sustaining functional aquatic microbial ecology (Paruch et al. 2019, 2020). The major health threats are incurred by enteric pathogens, especially pathogens of emerging concern (emerging pathogens). These are primarily responsible for severe waterborne zoonoses because zoonotic pathogens comprise 75% of emerging infectious diseases (Bolin et al. 2004). In addition, impaired human and environmental health effects are caused by emerging chemical pollutants derived primarily from anthropogenic practices. Therefore, urban watersheds are highly vulnerable to CEC contamination; wastewater, sewage and sludge are the major reservoirs of PPCPs and EDCs in the environment (Su et al. 2020; Yang et al. 2020). Similarly, in our study, the CST tests revealed that the highest levels of CEC were detected in the urban stream (Table 3). These included a range of PPCPs (ibuprofen, gabapentin, paracetamol and caffeine being the most frequently detected), their metabolites,
and BPA (a known endocrine disruptor). Much lower amounts of CEC were recorded in the rural creek (Table 4); ibuprofen, gabapentin, paracetamol and caffeine were also the PPCPs most often found in water.

Regardless of the primary faecal contamination origin (Figures 2 and 3), all detected CECs in both rural and urban watercourses were solely correlated with anthropogenic faecal pollution (Figures 4 and 5). As revealed by the RDA,

| Table 4 | Concentrations of emerging contaminants (ng/l) along with their LOQ in water samples from the rural creek, Grytelandsbekken |
|-----------------|---------------------------------------------------|---------------------------------------------------|---------------------------------------------------|---------------------------------------------------|---------------------------------------------------|---------------------------------------------------|---------------------------------------------------|---------------------------------------------------|---------------------------------------------------|---------------------------------------------------|---------------------------------------------------|
| Compound        | LOQ      | Nov. 2014 | Feb. 2015 | Jun. 2015 | Sep. 2015 | Dec. 2015 | Mar. 2016 | Jun. 2016 | Sep. 2016 | Dec. 2016 | Mar. 2017 |
| Ibuprofen       | 20       | 270       | <20       | <20       | <20       | 2,500     | 160       | 140       | 46        | <20       |
| Gabapentin      | 10       | 30        | 18        | 40        | 21        | 37        | 79        | <10       | 13        | <10       | 14        |
| Paracetamol     | 10       | <10       | 28        | 24        | <10       | <10       | 10        | 10        | 60        | 89        | 12        |
| Caffeine        | 100      | <100      | <100      | 280       | <100      | <100      | 390       | 280       | 160       | 220       | <100      |
| Saccharin       | 50       | <50       | <50       | 80        | <50       | <50       | 140       | <50       | 70        | <50       |
| Naproxen        | 50       | <50       | <50       | <50       | <50       | <50       | 100       | <50       | 50        | <50       | <50       |
| Tramadol        | 10       | <10       | <10       | <10       | <10       | <10       | 86        | 24        | 24        | <10       |
| Chloramphenicol | 20       | <20       | <20       | <20       | <20       | <20       | <20       | <20       | 20        | <20       | <20       | 890       |
| Carboxyibuprofen| 20       | n.t.      | n.t.      | n.t.      | <30       | <30       | 76        | 1,100     | 430       | 110       | 68        |
| 2-hydroxyibuprofen| 30    | n.t.      | n.t.      | n.t.      | <30       | <30       | 76        | 1,100     | 430       | 110       | 68        |

In the periods where some compounds were not tested, the concentrations are marked n.t.

**Figure 4** | RDA map of significant (p < 0.05) relationships between biotic (E. coli) and the dominant faecal sources, i.e. zoogenic and anthropogenic) and abiotic (chemical parameters including PPCPs) components in the rural creek, Grytelandsbekken.
strong statistically significant ($p < 0.05$) relationships were established between faecal contamination from anthropogenic sources and the total and most frequently detected PPCPs. This supports the application of PPCPs as chemical indicators for anthropogenic source tracking. Furthermore, Pearson’s coefficient matrices indicated positive correlations between the total and most frequent PPCPs with both *E. coli* and a human DNA marker in the urban stream (Figure 6). The same trend was observed in the rural creek; however, *E. coli* was positively correlated only with the total PPCPs. Interestingly, the correlation patterns between the individual PPCPs and the anthropogenic DNA marker were much stronger in the rural creek (e.g. coefficient 0.949 for ibuprofen, 0.938 for gabapentin and 0.667 for caffeine) than in the urban stream, where the highest positive correlation (coefficient 0.85) was between *E. coli* and paracetamol (Figure 6). Yet, in the urban stream, the correlation patterns were stabilised among the faecal anthropogenic pollution origin and the total and most frequent PPCPs.
We also discovered that the main eutrophication-causing macronutrients (TN and TP) and TDS were significantly (p < 0.05) associated with the primary faecal pollution origins in the studied watercourses studied, i.e. with animal/environmental contamination sources in the zoogenic dominated rural creek (Figure 4) and human pollution sources in the anthropogenic dominated urban stream (Figure 5). This trend was also supported by Pearson’s correlation matrices, which indicated the strongest links between TDS and E. coli, and TN and the zoogenic DNA marker in the rural creek, and between TP/TN and E. coli, and TDS and the anthropogenic DNA marker in the urban stream (Figure 7).

These findings imply that the QMST technique could open a new avenue of source identification of macronutrient pollution in water. It provides more ‘real-time’ and accurate information than currently applied statistics-based methods for point source pollution and model-based systems for non-point/diffuse source pollution (Strokal et al. 2015; Yi et al. 2017). Some studies have also implemented a stable isotope approach in nutrient source tracking (Zhang et al. 2014), but due to the high cost, sophisticated techniques, limited analysis capacities and challenges when multiple nutrient sources are present, this method is still under development and improvement (Grimmeisen et al. 2017; De Bondt et al. 2018). In comparison, QMST is a well-established methodology that can provide more biologically characterised information on the primary pollution source, i.e. anthropogenic or zoogenic, rather than merely geographical localisation of the sources. In this regard, QMST demonstrates a practical potential for rapid tracking of eutrophication-causing macronutrients in aquatic ecosystems.

CONCLUSIONS

Overall, there are limited published records revealing an integrated cross-tracking of various faecal water contaminants and aligning them with primary anthropogenic or zoogenic pollution origins. We have integrated the outcomes of MST and CST tests and discovered that CEC (represented by PPCPs, their metabolites and BPA) correlates significantly with both E. coli and the human DNA marker. However, CST could not identify the primary pollution source of faecal water contamination. Source attribution was only attained through the QMST analysis together with derived FSAP, which enabled the pinpointing of the dominant faecal origin in the examined waters. The CST method, albeit supportive in validating QMST findings, is relevant for cases with apparent anthropogenic impact. The identified primary faecal pollution origins were significantly associated with the main eutrophication-causing macronutrients. Such findings underpin the key role of QMST in monitoring and targeted mitigating of water-related health risks and deterioration of aquatic environments. The results of our study highlight the need to prioritise QMST for regular water quality control, not only to track down faecal origins but also to aid the source attribution of macropolutants in water. In this context, further research with regard to the apportionment profile of various zoogenic sources and their associations with waterborne contaminants would be interesting.

FIGURES

Figure 1 was generated from a satellite image and topographical map of Norway provided by the NIBIO. The Institute's geographical data is archived in its primary map service, Kilden (https://kilden.nibio.no). All maps and images stored in Kilden are open source and can be saved or printed (https://www.nibio.no/en/subjects/soil/national-land-resource-map?locationfilter = true).
Figures 2–7 were created using tools in the Addinsoft (2019) XLSTAT statistical and data analysis solution (Boston, USA. https://www.xlstat.com).

ACKNOWLEDGEMENTS

This work was supported by the Norwegian Financial Mechanism [grant number CZ09-0013] under the project ‘Assessing water quality improvement options concerning nutrient and pharmaceutical contaminants in rural watersheds’ [ID number 7F14341].

DATA AVAILABILITY STATEMENT

The datasets generated during this study are available from the corresponding author upon reasonable request.

REFERENCES

Ahmed, W., Payyappat, S., Cassidy, M., Harrison, N. & Besley, C. 2020 Sewage-associated marker genes illustrate the impact of wet weather overflows and dry weather leakage in urban estuarine waters of Sydney, Australia. Science of The Total Environment 705, 155390. https://doi.org/10.1016/j.scitotenv.2019.135390.

Blankenberg, A.-G. B., Paruch, A. M., Paruch, L., Deelstra, J. & Haarstad, K. 2016 Nutrients tracking and removal in constructed wetlands treating catchment runoff in Norway. In: Natural and Constructed Wetlands (J. Vymazal, ed.). Springer International Publishing Switzerland, pp. 23–40. https://doi.org/10.1007/978-3-319-38927-1_2.

Bolin, C., Brown, C. & Rose, J. 2004 Emerging zoonotic diseases and water. In: Waterborne Zoonoses: Identification, Causes, and Control (J. Cotruvo, A. Dufour, G. Rees, J. Bartram, R. Carr, D. O. Cliver, G. F. Craun, R. Fayer & V. P. J. Gannonp, eds). WHO, London, UK, pp. 21–26.

Chen, L., Zhang, X., Zhi, X., Dai, Y., Zhang, P., Xiao, Y. & Shen, Z. 2020 Tracking faecal microorganisms using the qPCR method in a typical urban catchment in China. Environmental Monitoring and Assessment 192 (3), 158. https://doi.org/10.1007/s10660-020-8130-8.

De Bondt, K., Sevono, F., Petrucci, G., Rodriguez, F., Joannis, C. & Claeys, P. 2018 Potential and limits of stable isotopes (δ18O and δD) to detect parasitic water in sewers of oceanic climate cities. Journal of Hydrology: Regional Studies 18, 119–142. https://doi.org/10.1016/j.ejrh.2018.06.001.

Devane, M. L., Moriarty, E. M., Robson, B., Lin, S., Wood, D., Webster-Brown, J. & Gilpin, B. J. 2009 Relationships between chemical and microbial faecal source tracking markers in urban river water and sediments during and post-discharge of human sewage. Science of The Total Environment 651 (1), 1588–1604. https://doi.org/10.1016/j.scitotenv.2018.09.258.

Firmansyah, I., Spiller, M., de Ruijter, F. J., Carsiens, G. J. & Zeeman, G. 2016 Assessment of nitrogen and phosphorus flows in agricultural and urban systems in a small island under limited data availability. Science of The Total Environment 574, 1521–1532. https://doi.org/10.1016/j.scitotenv.2016.08.159.

Gallo, T. & Fidino, M. 2018 Making wildlife welcome in urban areas. eLife 7, e41548. https://doi.org/10.7554/eLife.41548.

Grimmeisen, F., Lehmann, M. F., Liesch, T., Goeppert, N., Klinger, J., Zopf, J. & Goldscheider, N. 2017 Isotopic constraints on water source mixing, network leakage and contamination in an urban groundwater system. Science of The Total Environment 583, 202–213. https://doi.org/10.1016/j.scitotenv.2017.01.054.

Haramoto, E. & Osada, R. 2018 Assessment and application of host-specific Bacteroidales genetic markers for microbial source tracking of river water in Japan. PLoS One 13 (11), e0207727. https://doi.org/10.1371/journal.pone.0207727.

Jardé, E., Jeanneau, L., Harrault, L., Quenot, E., Solecki, O., Petijean, P., Lozach, S., Chemé, J. & Gourmelon, M. 2018 Application of a microbial source tracking based on bacterial and chemical markers in headwater and coastal catchments. Science of The Total Environment 610–611, 55–63. https://doi.org/10.1016/j.scitotenv.2017.07.235.

Molina, M., Hunter, S., Cyterski, M., Peed, L. A., Kelty, C. A., Sivaganesan, M., Mooney, T., Prieto, L. & Shanks, O. C. 2014 Factors affecting the presence of human-associated and fecal indicator real-time quantitative PCR genetic markers in urban-impacted recreational beaches. Water Research 64, 196–208. https://doi.org/10.1016/j.watres.2014.06.036.

Partridge, D. R. & Clark, J. A. 2018 Urban green roofs provide habitat for migrating and breeding birds and their arthropod prey. PLoS One 13 (8), e0202298. https://doi.org/10.1371/journal.pone.0202298.

Paruch, L. & Paruch, A. M. 2008 Contributors to faecal water contamination in urban environments. In: Water Management and the Environment: Case Studies, Vol 86 (M. Zelenakova, ed.). Water Science and Technology Library, Springer, Cham, pp. 215–230. https://doi.org/10.1007/978-3-319-79014-5_10.

Paruch, L., Paruch, A. M., Blankenberg, A.-G. B., Bechmann, M. & Mæhlum, T. 2015 Application of host-specific genetic markers for microbial source tracking of faecal water contamination in an agricultural catchment. Acta Agriculturae Scandinavica 65 (S2), 164–172. https://doi.org/10.1080/09064710.2014.914392.

Paruch, L., Paruch, A. M., Blankenberg, A.-G. B., Haarstad, K. & Mæhlum, T. 2017 Norwegian study on microbial source tracking for water quality control and pollution removal in constructed wetland treating catchment run-off. Water Science & Technology 76 (5), 1158–1166. https://doi.org/10.2166/wst.2017.303.

Paruch, L., Paruch, A. M., Eiken, H. G. & Sorheim, R. 2019 Faecal pollution affects abundance and diversity of aquatic
microbial community in anthropo-zoogenically influenced lotic ecosystems. *Scientific Reports* 9, 19469. https://doi.org/10.1038/s41598-019-56058-x.

Paruch, L., Paruch, A. M. & Sørheim, R. 2020 DNA-based faecal source tracking of contaminated drinking water causing a large *Campylobacter* outbreak in Norway 2019. *International Journal of Hygiene and Environmental Health* 224, 113420. https://doi.org/10.1016/j.ijheh.2019.113420.

Pascual-Benito, M., Nadal-Sala, D., Tobella, M., Ballesté, E., García-Aljaro, C., Sabaté, S., Sabater, F., Martí, E., Gracia, C. A., Blanch, A. R. & Lucena, F. 2021 Modelling the seasonal impacts of a wastewater treatment plant on water quality in a Mediterranean stream using microbial indicators. *Journal of Environmental Management* 261, 110220. https://doi.org/10.1016/j.jenvman.2020.110220.

Salim Dantas, M., Cordova de Oliveira, J., Cristiane Pinto, C. & Corrêa Oliveira, S. 2020 Impact of fecal contamination on surface water quality in the São Francisco River hydrographic basin in Minas Gerais, Brazil. *Journal of Water & Health* 18 (1), 48–59. https://doi.org/10.2166/wj.2019.153.

Sowah, R. A., Habteselassie, M. Y., Radcliffe, D. E., Bauske, E. & Risse, M. 2017 Isolating the impact of septic systems on fecal pollution in streams of suburban watersheds in Georgia, United States. *Water Research* 108, 330–338. https://doi.org/10.1016/j.watres.2016.11.007.

Staley, Z. R., Grabuski, J., Sverko, E. & Edge, T. A. 2016 Comparison of microbial and chemical source tracking markers to identify fecal contamination sources in Humber River (Toronto, Ontario, Canada) and associated storm water outfalls. *Applied and Environmental Microbiology* 82(21), 6557–6566. https://doi.org/10.1128/AEM.01675-16.

Strokal, M., Kroezec, C., Li, L., Luan, S., Wang, H., Yang, S. & Zhang, Y. 2015 Increasing dissolved nitrogen and phosphorus export by the Pearl River (Zhujiang): a modeling approach at the sub-basin scale to assess effective nutrient management. *Biogeochemistry* 125, 221–242. https://doi.org/10.1007/s10533-015-0124-1.

Su, C., Cui, Y., Liu, D., Zhang, H. & Baninla, Y. 2020 Endocrine disrupting compounds, pharmaceuticals and personal care products in the aquatic environment of China: which chemicals are the prioritized ones? *Science of The Total Environment* 720, 137652. https://doi.org/10.1016/j.scitotenv.2020.137652.

Tran, N. H., Gin, K. Y. & Ngo, H. H. 2015 Fecal pollution source tracking toolbox for identification, evaluation and characterization of fecal contamination in receiving urban surface waters and groundwater. *Science of The Total Environment* 538, 38–57. https://doi.org/10.1016/j.scitotenv.2015.07.155.

WHO 2020 Mortality and Burden of Disease From Water and Sanitation. Available from: http://www.who.int/gho/phe/water_sanitation/burden/en/index2.html (accessed 31 July 2020).

Yang, L., Zhou, Y., Shi, B., Meng, J., He, B., Yang, H., Yoon, S. J., Kim, T., Kwon, B. O., Khim, J. S. & Wang, T. 2020 Anthropogenic impacts on the contamination of pharmaceuticals and personal care products (PPCPs) in the coastal environments of the Yellow and Bohai seas. *Environment International* 135, 105306. https://doi.org/10.1016/j.envint.2019.105306.

Yi, Q., Chen, Q., Hu, L. & Shi, W. 2017 Tracking nitrogen sources, transformation, and transport at a basin scale with complex plain river networks. *Environmental Science & Technology* 51 (10), 5396–5403. https://doi.org/10.1021/acs.est.6b06278.

Zhang, Y., Li, F., Zhang, Q., Li, J. & Liu, Q. 2014 Tracing nitrate pollution sources and transformation in surface- and groundwaters using environmental isotopes. *Science of The Total Environment* 490, 213–222. https://doi.org/10.1016/j.scitotenv.2014.05.004.

First received 8 September 2020; accepted in revised form 11 December 2020. Available online 21 December 2020.