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Assessing the impact of inland navigation on the faecal pollution status of large rivers: A novel integrated field approach

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ABSTRACT

The contribution of ships to the microbial faecal pollution status of water bodies is largely unknown but frequently of human health concern. No methodology for a comprehensive and target-orientated system analysis was available so far. We developed a novel approach for integrated and multistage impact evaluation. The approach includes, i) theoretical faecal pollution source profiling (PSP, *i.e.,* size and pollution capacity estimation from municipal *vs.* ship sewage disposal) for impact scenario estimation and hypothesis generation, ii) high-resolution field assessment of faecal pollution levels and chemo-physical water quality at the selected river reaches, using standardized faecal indicators (cultivation-based) and genetic microbial source tracking markers (qPCR-based), and iii) integrated statistical analyses of the observed faecal pollution and the number of ships assessed by satellite-based automated ship tracking (*i.e.,* automated identification system, AIS) at local and regional scales. The new approach was realised at a 230 km long Danube River reach in Austria, enabling detailed understanding of the complex pollution characteristics (*i.e.,* longitudinal/cross-sectional river and up-stream/downstream docking area analysis). Faecal impact of navigation was demonstrated to be remarkably low at regional and local scale (despite a high local contamination capacity), indicating predominantly correct disposal practices during the investigated period. Nonetheless, faecal emissions were sensitively traceable, attributable to the ship category (discriminated types: cruise, passenger and freight ships) and individual vessels (docking time analysis) at one docking area by the link with AIS data. The new innovative and sensitive approach is transferrable to any water body worldwide with available ship-tracking data, supporting target-orientated monitoring and evidence-based management practices.

1. Introduction

Maritime and inland navigation has been an important part of human history, enabling civilizations to explore new lands and establish trade routes (Sanches et al., 2020). Currently, approximately 80–90 % of global cargo is transported by ships and ferries using maritime transportation (Schnurr and Walker, 2019). Freight transportation and the transportation of people, especially cruise tourism, have experienced

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rapid growth within recent decades (Carić and Mackelworth, 2014; Wondirad, 2019). Inland navigation is an important extension for the waterway transportation of goods and persons into intracontinental areas, with the world's busiest rivers being the Yangtze River (cargo traffic volume: 4 360 million tons in 2018), the Rhine River (311 million tons in 2019), the Illinois Inland River (90.5 million tons in 2017) and the Danube River (69 million tons in 2019) (Danube Comission, 2021; David et al., 2021; Farazi et al., 2022; Wu et al., 2022).

Within the European Union, the total inland waterway network has a length of 26 000 km with an approximate annual transportation volume of 558 million tons of goods, accounting for 6 % of all goods being transported in the EU in *2019* (Viadonau, 2019a). River cruise tourism is very popular, with 1.64 million tourists taking a river cruise in Europe in 2018 (Interreg - Danube Transnational Programme, 2019). The advent of the COVID-19 pandemic led to an abrupt decrease in 2020, though a rise towards the prepandemic level has been observed within recent years (Viadonau, 2022, 2021).

Environmental pollution arising from the shipping industry is increasingly in the focus of global research, especially in terms of air emissions and water pollution, such as from wastewater discharges (Cao et al., 2018; Jägerbrand et al., 2019). For maritime navigation, the International Maritime Organization (IMO) adopted the International Convention for the Prevention of Pollution from Ships (MARPOL), which includes regulations concerning the prevention of air pollution as well as pollution from oil, noxious and harmful substances, garbage and sewage (Annex IV) (MARPOL, 1997; Vaneeckhaute and Fazli, 2020). For inland navigation, there are no internationally harmonized regulations concerning pollution arising from ships, though several national or river-specific regulations/roadmaps exist*,* such as the CCNR roadmap for the Rhine River (CCNR, 2022).

Microbial faecal pollution can have a direct impact on human health if the water resource is used for recreational activities, water withdrawal for drinking water production or irrigation due to the possible presence of pathogens (World Health Organization, 2021, 2017). In urban coastal areas or at large rivers, faecal pollution primarily arises from municipal wastewater, though ship wastewater discharges may pose an additional impact source. To date, only a minor number of publications have studied the impact of wastewater discharge from ships on ambient waters. A few studies have been published on the impact of maritime cruise ships in vulnerable maritime regions, such as the Bering Sea, the Baltic Sea or the Adriatic Sea (Huhta et al., 2007; Loehr et al., 2006; Perić et al., 2016). Some studies performed calculations of the generated amount of wastewater and/or discharged general chemical pollutants (COD, BOD, SS, TN) based on ship numbers assessed using inland AIS for the Yangtze River or from interviews with watermen at the Danube River in Serbia, but without investigations of the water quality of the river (Chen et al., 2022; Presburger Ulnikovic et al., 2012). Even fewer studies have investigated the microbial faecal water quality of maritime bays/ports and the local number of smaller pleasure boats or sailing ships, frequently reporting a connection of ships and elevated concentration of faecal coliform bacteria (Maria A Faust, 1982; Koboević et al., 2022; M D Sobsey et al., 2003).

The aim of this study was to develop and evaluate a novel integrated field approach for the impact assessment of ships on the microbial faecal pollution status of navigable river reaches by i) faecal impact scenario estimation/comparison using pollution source profiling (*i.e.,* catchment specific analysis of the produced numbers of *E. coli* from potential sources of faecal pollution) including different wastewater handling scenarios from navigation and their comparison with municipal wastewater disposal, as a basis for subsequent hypothesis formulation and field analysis, ii) high-resolution longitudinal and cross-sectional microbial and chemical water quality analysis at selected river locations, and iii) statistical analysis covering observed faecal pollution patterns and detailed ship traffic activities for the investigated river locations (covering regional river reach *vs.* local ship dock impact considerations). The chosen study region was a 230 km river reach of the Danube River in Austria, as a representative large navigable river with high international importance (ICPDR, 2021; Kirschner et al., 2009). Microbial faecal pollution analysis was based on standardized faecal indicator bacteria enumeration (cultivation-based) and state-of-the-art (qPCR-based) genetic microbial source tracking (Demeter et al., 2023). For precise ship data assessment, raw navigational data from the Donau Riverine Information Service (DoRIS), including inland Automated Identification System (AIS) data, were processed to extract near-real-time ship counts within specific timeframes and areas. The novel approach is suggested to be universally applicable to other large navigable water bodies worldwide if the needed raw navigation data are available.

2. Material and methods

2.1. *The selected Danube River reach, transects and docks*

The investigated river reach stretches from river-km 2111 to 1873 in the upper region of the Danube River, including the large city of Vienna as well as a highly touristic region for cruise ships in the Wachau Valley in the province of Lower Austria (Fig. 1). The catchment of the Danube River reach in Austria has a size of 28.074 km^2 and the sub-catchment of the investigated Danube reach has an area of 14 126 km^2 (Hydrographic Service in Austria www.ehyd.gv.at). A total number of approximately 3 370 000 citizens including average daily tourists were registered in the investigated sub-catchment in the year of 2019 (data from the government of Lower Austria). This vast catchment area is subject to the influence of the alpine regime, which governs hydrological conditions, resulting in highly variable flow patterns and peak water levels, particularly in early summer (Schiemer et al., 1999). Land use in the sub-catchment in Lower Austria is unevenly distributed, with 40 % agricultural land, 40 % forest, and 13 % grassland (Petschko et al., 2014). The areas south of the Danube and the relatively flat northeast region are predominantly agricultural, while the steeper slopes in the south and southwest are mainly covered by coniferous and deciduous forests (Eder et al., 2011). The average discharge of the Danube River in this region is appr. 1800 m^3/s, and the river width ranges from 217 (Wachau Valley) to 330 m in the flatlands. The 7 sampling transects (A to G) were selected with respect to river morphology, settlements/cities, and highly frequented Danube ship docks to sensitively detect potential faecal emission from navigation (further details are given in the legend of Fig. 1). As many as 4 additional transects (*B*+, C+, *D*+, *E*+) were chosen for upstream/downstream sampling of ship docking areas (*B*+/B, C+/C, *D*+/D, *E*+/E), resulting in a total of 11 sampled transects. The touristic region of the Wachau Valley lies between sampling site (B) Melk and site (D) Krems.

2.2. *Water quality and faecal pollution*

2.2.1. *Sampling at selected transects*

To account for possible transversal differences in water composition, each transect consisted of 5 sampling points symmetrically distributed in the cross-profile with orographically left or right: appr. 10–20 m distance from the riverbank, middle: centre of river, and middle-left/right: half-distance of outer and centre point. Sampling was performed in collaboration with local authorities and the Austrian shipping inspectorate with their official ship, enabling precise navigation to the same sampling locations each time. Due to logistics, sampling at the 11 transects was performed on four different days each month, with a 4-week interval, resulting in a total of 48 different sampling days within the monitoring timeframe from March 2019 to March 2020 (**Supplementary Table S1**). Water samples were taken with a sampling rod in approx. 30 cm water depth, stored in the dark in cooling boxes during transportation to the lab, and processed within hours (< 6 h).

2.2.2. *Chemical and physical water quality parameters*

Temperature (°C), pH (-), conductivity (µS/cm) and oxygen (mg/L)

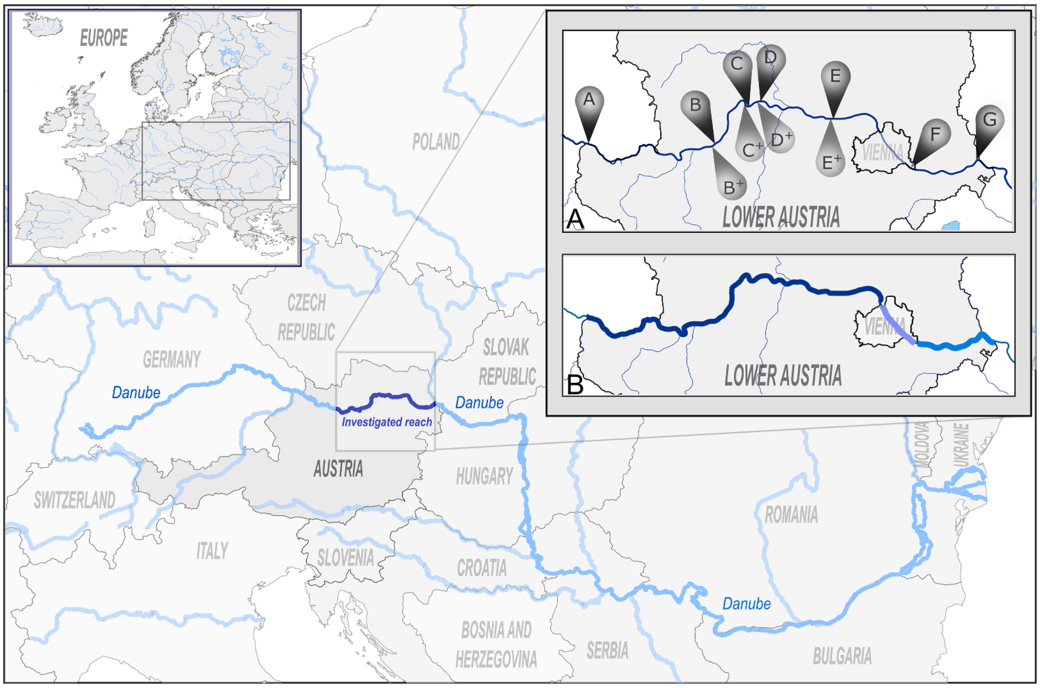


Fig. 1. Overview map of the investigated Danube River reach (dark blue) in the centre of Europe. Upper right map A shows the region of Lower Austria with the sampling locations (A) St Pantaleon - rkm 2108; (B^+) Melk - upstream ship docks - rkm 2036.6; (B) Melk - downstream ship docks - rkm 2035.5; (C^+) Dürnstein - upstream ship docks - rkm 2009; (C) Dürnstein - downstream ship docks - rkm 2008; (D^+) Krems - upstream ship docks - rkm 2003; (D) Krems – downstream ship docks - rkm 2002; (E^+) Tulln – upstream ship docks: rkm 1964.4; (E) Tulln – downstream ship docks - rkm 1963.4; (F) Wien/Vienna - rkm 1915; (G) Hainburg - rkm 1883. The Wachau Valley spans from (B) – Melk to (D) – Krems. Upper right map B: The investigated Danube River reach and sub-reaches i) upstream of Vienna (darkblue), ii) Vienna (light purpleblue) and iii) downstream of Vienna (middle blue) for the theoretical impact estimation, *i.e.*, pollution source profiling (PSP). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

were measured directly on the ship using a multimeter device (HQ40D, Hach U.S.) equipped with the corresponding probes. Chemical oxygen demand (COD), total phosphorus (mg/L), total nitrogen (mg/L) and NH_4-nitrogen (mg/L) were analysed in the laboratory by spectrophotometric methods following the manufacturer's instructions (LCK1414, LCK349, LCK304, Hach U.S.).

2.2.3. *Faecal indicator bacteria and genetic microbial faecal source tracking markers*

All water samples ($n = 665$) were analysed for the standard faecal indicator bacterium *E. coli* following ISO 9308–2 (ISO, 2012) using Colilert-18 (IDEXX, Germany). Analysis of genetic microbial faecal source tracking (MST) markers was performed for water samples ($n = 100$) from three transects (*St. Pantaleon* – A, *Krems* – D and *Hainburg* G). River water samples (500 mL) were filtered through a polycarbonate filter (pore size 0.22 µm, GTTP04700, Merck Millipore) and stored at −80 °C. DNA extraction was performed following a bead-beating and phenol/chloroform protocol (Linke et al., 2021; Mayer et al., 2018; Reischer et al., 2006). DNA was redissolved in 100 µL of 10 mM TRIS at pH 8. Samples were analysed for human-associated genetic faecal markers HF183/BacR287 (Green et al., 2014) and BacHum (Kildare et al., 2007), as well as for the ruminant-associated marker BacR (Reischer et al., 2006) and the pig-associated marker Pig2Bac (Mieszkin et al., 2009). All qPCR reactions were performed in duplicate with a total reaction volume of 15 µL on a Rotor-Gene Q thermocycler (Qiagen, Hilden, Germany) with each including 2.5 µL sample DNA dilution (1:4). The reaction mixtures and cycling parameters were applied as described previously (Linke et al., 2021; Mayer et al., 2018; Reischer et al., 2013). Quality assessment was performed as described previously (Mayer et al., 2018; Reischer et al., 2011, 2006). All qPCR runs in this study revealed calculated qPCR efficiencies between 95 and 100 %, R-square values of the calculated standard curves were ≥ 0.99 and no-template and extraction controls were consistently negative. A previously established threshold of detection (TOD) concept was applied, considering the filtration volume, the DNA extract volume, potential dilutions and the minimal amount of detectable targets per reaction (Reischer et al., 2007, 2006). It is a robust approximation for the sample limit of detection, covering sampling and sample processing and the efficiency of the qPCR analysis (Demeter et al., 2023). The results of the qPCR analysis were expressed as marker equivalents per 100 mL [$\log_{10}$ (ME + 1)/100 mL].

2.3. *Ship data assessment (SDA) using the DoRIS database*

In Europe, standardized River Information Services (RIS) were implemented and harmonized to enable reliable and efficient inland waterway transport via the EU Directive 2005/44/EC (EUROPEAN PARLIAMENT, 2005). The Austrian version Donau RIS (DoRIS) is maintained by via Donau GmbH, operating the 23 land-based AIS stations connected to a central database server storing and processing all ship tracking data.

We developed a Python programming language-based script for ship data assessment (SDA) using raw navigational data from the DoRIS database (Via Donau GmbH), including inland AIS data of all ships navigating the Austrian Danube River reach (rkm: 2223–1873) in the 12-month monitoring timeframe (see **Appendix B** for the script). The resulting database consisted of 101 966 ASCII files (one file per ship per day), including 23 AIS data fields with data records every 15 s, resulting in a total of 405 000 000 data records.

The script was designed to extract data based on two input fields: i) a specific date and timeframe and ii) the start and end distance of a specific river area (in river kilometres) (Fig. 2). The obtained reduced ship datasets for specific areas/timeframes were sorted into three main shipping categories, namely, cruise (with accommodation), passenger (without accommodation), and freight (including working) ships, based

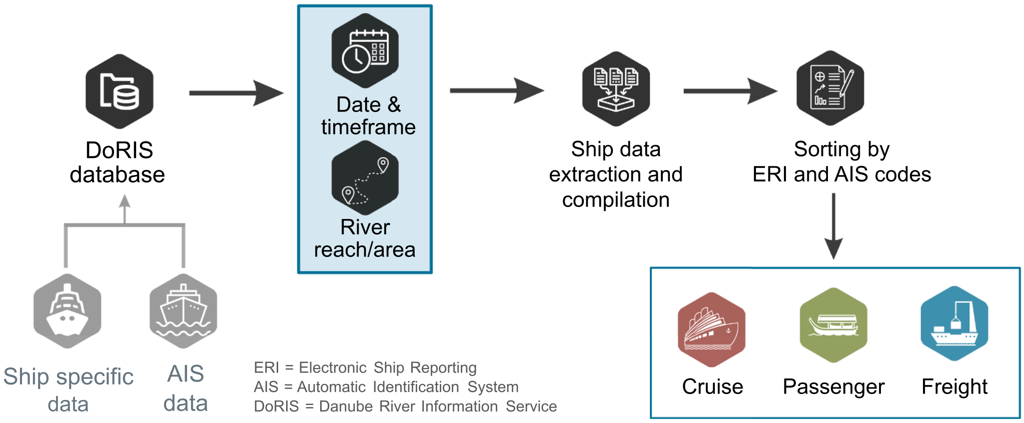


Fig. 2. Scheme of the ship data assessment process: Input data of date/time and river reach for ship data extraction and compilation and subsequent sorting by ERI (Electronic Ship Reporting) and AIS (Automated Identification System) codes for obtaining the number of ships per type in the selected area/timeframe.

on the Electronic Reporting International (ERI) and AIS codes, with small pleasure boats and others not being considered. Three different ship data assessment approaches were performed for subsequent analysis, namely, i) daily shipping activity at different Danube River reaches for pollution source profiling, ii) reverse ship traffic approximation (RSTA) accounting for the flow of the polluted water for association analysis of the general faecal pollution and ships at the entire river reach (regional), and iii) counting of ships in the ship dock areas within the timeframe of sampling for local ship association analysis. For more details on the RIS and the three different ship counting concepts, see **Supplementary 2.3.**

2.4. *Pollution source profiling (PSP) for impact scenario estimation*

PSP was designed for the analysis of faecal pollution sources for a specific catchment/river reach to estimate the amount of faecal bacteria emitted from all considered human and animal faecal pollution sources (Derx et al., 2023; Frick et al., 2020; Reischer et al., 2011). Here, we transferred the concept to estimate human faecal pollution based on the daily *E. coli* load, extending it for inland navigation and investigation of different ship wastewater handling scenarios (correct/incorrect) and comparison with municipal wastewater input (see **Supplementary 2.3.2** for details). PSP was performed for the selected Danube River reach, as well as the sub-reaches: upstream of Vienna, Vienna and downstream of Vienna with high and low season differentiation as performed for ship activity assessment (Fig. 1 **panel B and Supplementary 2.3.2** for details).

2.4.1. *PSP parameters and scenarios*

Estimates of faecal emissions from ships and from municipal wastewater treatment plants (WWTP) were based on the number of people contributing to the given source, the amount of *E. coli* shed per person and day, and the reduction in *E. coli* concentrations due to treatment (depending on the investigated scenario and ship type). The amount of *E. coli* shed per person and day was calculated using 10^8 CFU *E. coli* per gram faeces and 150 g faeces per person and day according to Reischer et al. (2011). For municipal wastewater input calculation, the number of citizens and tourists in the catchment and the three sub catchments were obtained from the government of Lower Austria for the year 2019. A median reduction of $\log_{10}$ 2.3 for biological wastewater treatment was assumed as reported by Mayer et al. (2016). Summer tourism in Austria (May to September) significantly builds around outdoor recreation activities, with the peak holiday season occurring in July and August (Falk, 2014; Pröbstl-Haider et al., 2021), while in winter (December to February), tourism industry predominantly focuses on ski sports (Steiger and Scott, 2020). These tourism patterns are also related with seasonal variation in ship numbers in Austria (2019 – 2022, (Viadonau, 2022, 2021, 2019b)). Based on these patterns, we selected the two months January and February for low season (LS) and July and August for the high season (HS) investigation. Information about the anticipated number of passengers was obtained from the local authorities (government of Lower Austria expert judgement: Günther Konheisner) with: i) cruise (with accommodation): 185 persons (both seasons), ii) passenger (day trip – without accommodation): high season on weekdays: 250 persons; on weekend: 500 persons; low season weekend/weekday: 20 persons; and iii) freight: 5 persons (both seasons). The treatment of ship wastewater and therefore the reduction in emissions is dependant on the type of ship as well as the assumed scenario of correct or incorrect ship wastewater handling. Therefore, two different scenarios with i) the correct handling of wastewater (scenario 1) and ii) with incorrect handling of wastewater (scenario 2) were assumed for theoretical load calculation for both seasons:

Scenario 1. - `Correct Wastewater Handling Ship Emission`: assuming, i) cruise ships with an on-board WWTP, achieving a $\log_{10}$ 1.9 reduction of *E. coli* (referring to the 25th percentile reduction by mechanical biological wastewater treatment according to Mayer et al. (2016)), as no specific reduction data for on board WWTPs was available, ii) passenger ships equipped with sewage tanks, assuming no emissions to the waters if correctly handled (*i.e.,* transfer to municipal WWTPs), and iii) freight ships with no wastewater treatment or storage facilities, hence direct emission (no reduction).

Scenario 2. - `Maximum Potential Ship Emission`: in the case of incorrect wastewater handling there is no reduction of any of the emissions from cruise, passenger or freight ships assumed (no treatment, no storage).

2.5. *Statistical analysis and data visualization*

Statistical analysis and data visualization were performed using R and RStudio (R Core Team, 2022; RStudio Team, 2021) with the support of Microsoft Excel (Microsoft Corporation, 2019). For more details on the RStudio packages used, specific functions and additional software used for graphic design, see **Supplementary 2.5**. For all statistical tests the level of significance was set to $p \leq 0.05$ and in case of multiple testing, correction of probability was applied (Bonferroni or false discovery rate (fdr)).

2.5.1. *Statistical analysis of the water quality data*

Analysis of variance (ANOVA) was used for the analysis of the microbial faecal pollution data *i.e.,* differences in the decadic logarithm of the concentration of *E. coli* at the different transects and sampling points. Post-hoc Tukey test was additionally performed to obtain more information of the specific group differences. Non-parametric Spearman rank correlation was used for the investigation of the correlation of *E. coli* concentrations and the other physico-chemical parameters assessed in the water samples. For specific information on the used R functions see **Supplementary 2.5**.

2.5.2. Associations of faecal pollution and ship activity: correlation and regression analysis

For the entire selected river reach association analysis (regional analysis), Spearman rank correlation was performed with the *E. coli* concentrations (decadic logarithm) from transects A, B, C, D, E, F, and G and i) environmental/hydrological parameters such as the 3-day sum of precipitation and river/tributary discharge, ii) wastewater treatment plant discharge data, and iii) the number of ships counted with respect to river flow, sampling time and date (see RSTA **Supplementary 2.3.2, Figure S1**). For *E. coli* concentrations, median values of the cross-section samples were used for correlation analysis, resulting in a total of 84 individual sample sets (11, 11, 13, 13, 12, 12, and 12 for transects A to G, respectively). For detailed information on the environmental parameters, WWTP discharge data and the used functions, see **Supplementary 2.5, Table S2** and **Table S3**.

For the ship dock analysis (local analysis), the change in *E. coli* concentration of samples on the respective side of the ship docks (orographically right river side: *B*+/B, *E*+/E; left: C+/C, *D*+/D) was calculated as the ratio of increase (downstream divided by upstream, see **Supplementary Figure S4**) or the positive difference (downstream minus upstream) of *E. coli* concentrations (see Fig. 10) and plotted against the number of ships between the upstream/downstream sampling transects during sampling. Spearman rank correlation as well as a multiple linear regression analysis was performed to assess relations amongst the number of ships and hydrological parameters (precipitation/rain, river discharge) on the increase on *E. coli* concentrations (dependant variable) at the dock C+/C. For detailed information on the used functions see **Supplementary 2.5.**

3. Results

3.1. Establishing AIS-based shipping activities for the selected Danube reaches

The inland AIS data-based SDA script was used to analyse the ship traffic volume in the investigated Danube River reach, revealing frequent activity with seasonal differences. Especially for cruise and passenger ships seasonal fluctuations were observed, in line with ship schedules and elsewhere reported seasonal patterns (Viadonau, 2022, 2021, 2019b). The daily median ship number at the entire Danube River reach (river-km: 2111–1873) in the high season (HS, summer months) was 159 ships in total, with approx. 44 % of cruise ships ($n = 69$), 5 % of passenger ships ($n = 8$) and 46 % of freight ships ($n = 73$) (Fig. 3, left panel) during the analysed period. In the low season (LS, winter months), the median daily ship number decreased to 114, with the highest drop in cruise ships ($n = 31$), though the daily number of passenger ships ($n = 5$) and freight ships ($n = 73$) remained almost the same.

Sub-reaches of the river reach were analysed to obtain detailed information, revealing a higher number of cruise ships for the reach in Vienna (HS/LS: $n = 44/6$) and upstream of Vienna (HS/LS: $n = 44/26$) in comparison to the downstream Vienna reach (HS/LS: $n = 27/1$) (Fig. 3 right panels). No passenger ships were observed in the LS upstream and downstream of Vienna, which is appropriate, as passenger ferries did not operate in winter months. Freight traffic is highest for the Danube reach upstream of Vienna (HS/LS: $n = 50/48$), followed by the reach in Vienna (HS/LS: $n = 35/37$) and the downstream of Vienna reach (HS/LS: $n = 26/24$)) (Fig. 3 right panels).

3.2. PSP reveals high local faecal pollution potential from the shipping industry

For the PSP, we estimated the theoretical daily emitted number of *E. coli* from of ships, with correct or incorrect ship wastewater handling scenarios, *versus* the daily emitted number of *E. coli* from municipal WWTPs. The scenario of correct ship wastewater handling for the entire Danube River reach resulted in a theoretical daily faecal load of 12.9 $\log_{10}$ CFU *E. coli* during HS and 12.8 $\log_{10}$ CFU *E. coli* during LS (Fig. 4 left panel, 'correct handling ship emission') for the investigated period of 03/2019–03/2020. In the case of incorrect ship wastewater handling, the daily faecal load was considerably higher, with 14.4 $\log_{10}$ (HS) or 13.9 $\log_{10}$ (LS) CFU *E. coli,* which in HS is in the same order of magnitude as the input from mechanically biologically treated municipal wastewater (14.4 $\log_{10}$ CFU *E. coli*) (Fig. 4 left panel, 'max. potential ship emission'). The results for sub-reaches gave analogous results: For two of the three sub-reaches, the theoretical daily emitted load in the case of

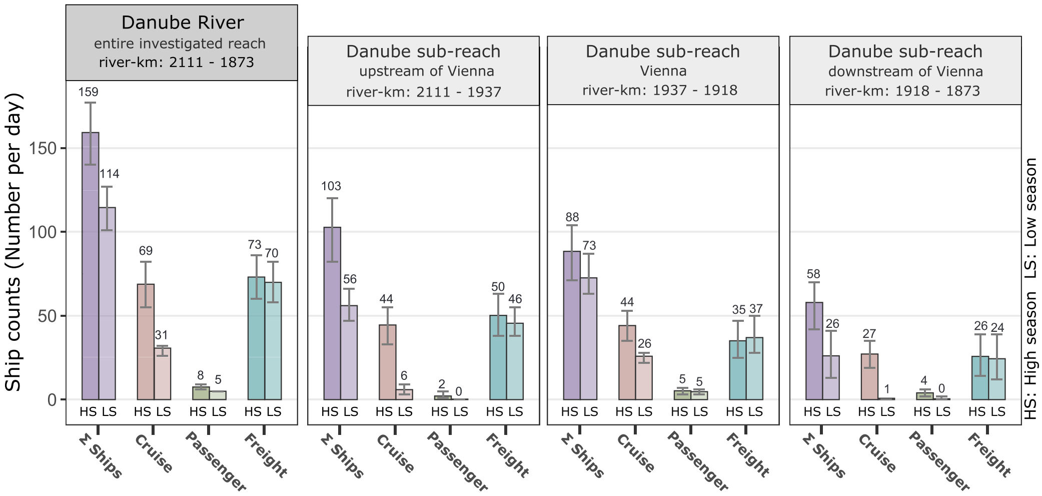


Fig. 3. Bar chart of the daily shipping activity with seasonal (HS: high season; LS: low season) differentiation for all ships ($\sum$ ships) and ships per type (cruise, passenger, and freight) for the river reaches and investigated period 03/2019–03/2020 i) entire investigated reach of the Danube River in Lower Austria (river-km: 2111–1873), and sub-reaches of the Danube River in Lower Austria: ii) upstream of Vienna (river-km: 2111–1937), iii) in Vienna (river-km: 1937–1918) and iv) downstream of Vienna (river-km: 1918–1873). Median value given; lower/upper error bars: smallest/largest value within 1.5 times interquartile range below/above 25th/75th percentile. HS: high season (summer months, 2019); LS: low season (winter months, 2020).

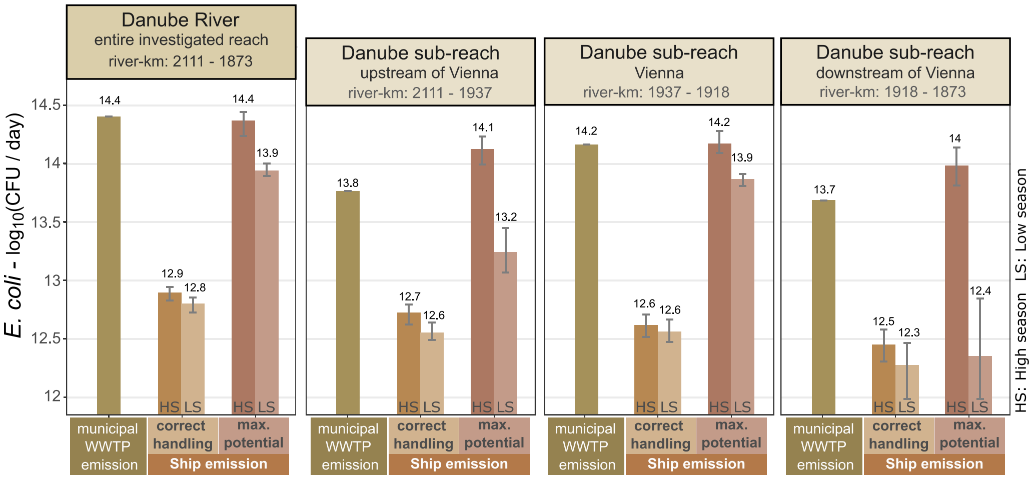


Fig. 4. Bar chart of the estimated E. coli load ($\log_{10}$ CFU per day) discharged into i) the entire reach of the Danube River in Lower Austria (river-km: 2111–1873) and the sub-reaches ii) upstream of Vienna (river-km: 2111–1937), iii) Vienna (river-km: 1937 to 1918) and iv) downstream of Vienna (river-km: 1918–1873) for emissions from municipal WWTP emission, from ships with correct treatment of ship wastewater (correct handling ship emission) and for ships with incorrect/no ship wastewater treatment giving the maximum potential load/worst-case scenario (max. potential ship emission). Median value given; lower/upper error bars: smallest/largest value within 1.5 times interquartile range below/above 25th/75th percentile. HS: high season (summer months, 2019); LS: low season (winter months, 2020).

incorrect ship wastewater handling was slightly higher than the input from treated municipal wastewater in the high season (Fig. 4 right panels). In general, the scenario assuming correct ship wastewater handling resulted in a lower input by 1 to 2 orders of magnitude for all sub-reaches compared to incorrect wastewater handling.

Hypothesis formulation based on PSP scenarios. Even under the incorrect wastewater handling scenario from entire navigation activities (*i.e.,* 100 % raw wastewater direct emission input to the river reach), the PSP indicates that the pollution capacity from the shipping industry does not exceed municipal faecal wastewater emissions (mechanical biological treatment without disinfection). We assume that the realistic situation ranges somewhere in between the highly unrealistic worst case and the optimum situation of correctly handled emissions, amounting to a few percent (3–6 %) in comparison to treated municipal wastewater emissions. Considering the long mixing stretches between the selected transects (A to G, Fig. 1) and the inherent statistical uncertainty observed in microbiological quantification, we hypothesized that faecal emissions from shipping cannot be detected at the regional scale for the current situation of wastewater disposal at the investigated Danube River reach (*i.e.,* regional-scale faecal pollution hypothesis). In contrast, especially for spatially aggregated ships with incorrect wastewater handling, we hypothesize that navigation sources have the highest faecal pollution capacity locally (*i.e.,* local-scale faecal pollution hypothesis), such as observed downstream of docks (*i.e.,* pairs of *B*+/B, C+/C, *D*+/D, *E*+/E, Fig. 1). These two hypotheses formed the foundation for the subsequent field investigations and statistical analyses (see chapter **2.5.2** for details on performed statistics).

3.3. Realizing water quality assessment at high spatial resolution

3.3.1. Overall faecal pollution, classification and chemical and physical parameters

Water quality analysis for the entire selected river reach during 03/2019–03/2020 ($n = 665$) revealed an overall concentration range of *E. coli* from $\log_{10}$ 0.9 to $\log_{10}$ 3.38 (MPN+1) per 100 mL, with a median concentration of $\log_{10}$ 2.08 (MPN+1) per 100 mL (Table 1). The variation of Danube River discharge on the sampling days was representative for the annual variation within the monitoring year, which was in the same range as the years before (**Supplementary Figure S2**). Descriptive statistics of the assessed key faecal indicator parameter *E. coli* as well as of the two human-associated faecal MST markers and chemical and physical parameters are given in Table 1.

According to the faecal pollution classification of Kavka et al. (2006), the majority (94 %) of water samples ranged within low and moderate

Table 1
Descriptive statistics of analysed water quality parameters: faecal indicator bacteria E. coli, human-associated microbial faecal genetic source tracking markers (HF183/BacR287 and BacHum) and chemo-physical parameters. n… number of samples, $n >$ LOD (TOD for MST markers) number of samples with concentrations higher than the limit of detection (LOD) or threshold of detection (TOD), median, arithmetic mean, and range (minimum value to maximum value).

parameter	n	$n >$ LOD*	% > LOD*	median	mean (arithm.)	range (min-max)		unit
E. coli	665	665	100	2.08	2.16	0.90	3.38	$\log_{10}$ (MPN+1) per 100 mL
HF183/BacR287 (human-associated)	100	75	75	3.36	3.49	2.32	4.83	$\log_{10}$ (ME+1) per 100 mL
BacHum (human-associated)	100	84	84	3.91	3.95	2.78	5.39	$\log_{10}$ (ME+1) per 100 mL
temperature	665	665	100	11.4	12.2	3.3	21.9	°C
pH	665	665	100	8.2	8.2	6.8	8.7	–
conductivity	665	665	100	312	315	164	494	µS/cm
oxygen	665	665	100	10.7	10.7	8.1	15.0	mg/L
COD	665	414	62.3	6.0	6.9	< 5	21.0	mg/L
nitrogen (total)	665	620	93.2	2.0	2.0	< 1	5.0	mg/L
phosphorus (total)	665	74	11.10	0.06	0.06	< 0.05	0.09	mg/L
ammonium	665	495	74.40	0.03	0.04	< 0.015	0.40	mg/L

*TOD (Threshold of Detection) for MST markers faecal pollution levels. Only 6 % of the samples showed critical levels of faecal pollution, though no strong or excessive faecal pollution events were observed (Fig. 5 left panel). Spearman rank correlation analysis of *E. coli* and the chemical and physical parameters revealed significant positive correlations between *E. coli* concentration and chemical oxygen demand - COD (rho $= 0.20$, adjusted $p = 1.6 \times 10^{-5}$), total phosphorous - P total (rho $= 0.23$, adj.$p = 2.8 \times 10^{-7}$) and ammonium - NH_4—N (rho $= 0.32$, adj.$p = 5.7 \times 10^{-16}$) concentrations (Fig. 5 right panel).

3.3.2. Microbial source tracking uncovers dominating human faecal pollution

Genetic microbial source tracking analysis was performed to further characterize the observed faecal pollution: human (including human wastewater from municipal and ship origin) and suspected animal sources (ruminant and pig/boar) on 100 out of the 665 samples taken in the 12-month sampling timeframe. The results revealed human faecal pollution to be dominant with a positive detection rate of 75 %, a median of $\log_{10}$ 3.18 and a range from $\log_{10}$ 2.32 to 4.83 for HF183/BacR287 and 84 %, a median of $\log_{10}$ 3.63 and a range from $\log_{10}$ 2.78 to 5.39 (ME+1) per 100 mL for BacHum, respectively (range min to max of positive detects). The animal-associated markers showed a considerably lower occurrence, with only 23 % for the ruminant-associated marker BacR and 7 % for the pig-associated marker Pig2Bac, both with a median value below the TOD, *i.e.,* not detectable (Fig. 6). Association with Danube River discharge showed that especially at base flow (< 1800 m^3/s), human-associated faecal pollution was dominant, and animal-associated markers were predominantly detected at higher river discharge, where the concentration of human-associated markers was also increased (Fig. 6 right panel).

3.3.3. Longitudinal faecal pollution analysis at selected river transects

The performed water quality analysis allowed for a detailed investigation of the faecal pollution at the investigated Danube River reach, as of the high resolution of sampling (for the faecal indicator bacteria (FIB) *E. coli* see **Supplementary Figure S3**). Longitudinal concentrations of *E. coli* at the eleven sampling transects ranged from a median of 1.91 $\log_{10}$ to log 2.29 $\log_{10}$ (MPN+1) per 100 mL (all five cross-profile samples gathered; Fig. 7). Performed ANOVA revealed that there was no significant difference in the annual median *E. coli* concentrations between the transects ($p = 0.1$, $n = 55$ - 65). Descriptive statistics of all analysed water quality parameters for each individual transect are given in **Supplementary Table S4**. The faecal pollution dataset at the principal sampling locations A to G (dark blue) was used for the regional river reach association analysis in 3.4.1.

3.3.4. Cross-sectional high-resolution faecal pollution analysis

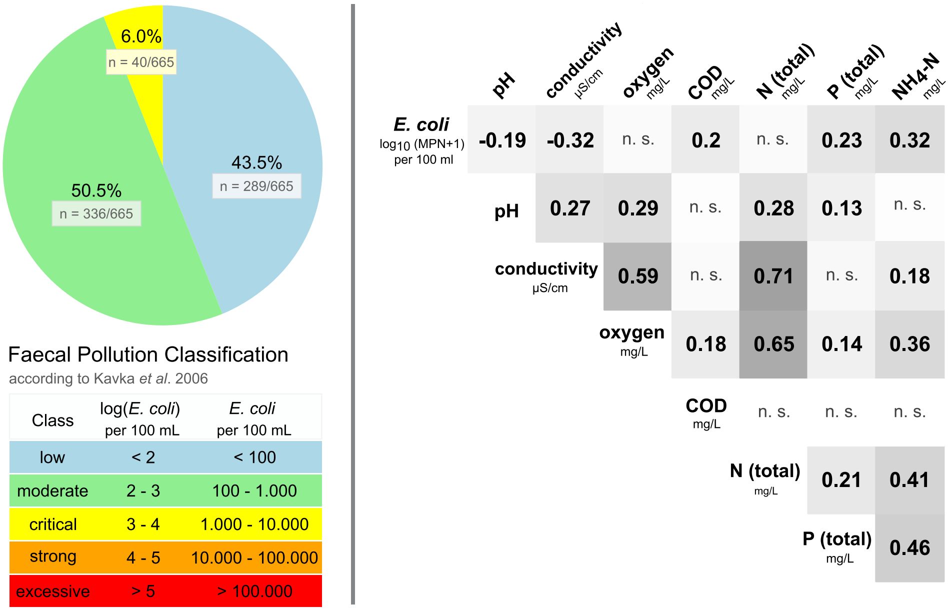
Cross-profile analysis showed strikingly homogeneous *E. coli* concentrations for the majority of the transects (Fig. 8). Only for transect F (Wien/Vienna) a statistically significant difference of *E. coli* concentrations across the profile, with increased concentrations at the orographic right river side, was obtained (ANOVA, $p < 0.001$, $n = 11$ - 13). A plausible reason is the inflow of the Danube channel at the right river side, which receives wastewater from the Vienna WWTP 6.5 km upstream of the sampling transect, wastewater from smaller WWTPs as well as combined sewer overflows (CSOs) of the Viennese sewer.

Additionally, an upstream/downstream ship dock comparison of *E. coli* concentrations with respect to the upstream/downstream and river cross-profile position and the sampling date was performed. Only one significant upstream–downstream difference in concentrations of the orographically left sample of the dock C+/C (Dürnstein) was revealed, whereas for the other, no significant differences were obtained. These upstream/downstream pairs were used for investigation of the local ship dock area association analysis in chapter **3.4.2**.

3.4. Associations of faecal pollution with ship activity, WWTP discharge and hydrological parameters

3.4.1. Correlation analysis at the investigated river reach (regional scale)

To obtain insight into influential factors on the observed faecal pollution on the regional scale (covering the entire investigated river reach), a Spearman rank correlation analysis with hydrological parameters (precipitation, river discharge), municipal WWTP discharges and the number of ships assessed by the RSTA approach was performed. Data from the principal sampling transects (A to G, $n = 84$) were used, as



Faecal Pollution Classification
according to Kavka *et al.* 2006

Class	log(*E. coli*) per 100 mL	*E. coli* per 100 mL
low	< 2	< 100
moderate	2 - 3	100 - 1.000
critical	3 - 4	1.000 - 10.000
strong	4 - 5	10.000 - 100.000
excessive	> 5	> 100.000

	pH	conductivity µS/cm	oxygen mg/L	COD mg/L	N (total) mg/L	P (total) mg/L	NH_4-N mg/L
E. coli $\log_{10}$ (MPN+1) per 100 ml	-0.19	-0.32	n. s.	0.2	n. s.	0.23	0.32
pH		0.27	0.29	n. s.	0.28	0.13	n. s.
conductivity µS/cm			0.59	n. s.	0.71	n. s.	0.18
oxygen mg/L				0.18	0.65	0.14	0.36
COD mg/L					n. s.	n. s.	n. s.
N (total) mg/L						0.21	0.41
P (total) mg/L							0.46

Fig. 5. Left panel: Faecal pollution classification of all water samples ($n = 665$) based on E. coli concentration according to Kavka et al. 2006. Pie chart giving the proportion of samples as well as number and percentage of samples within each pollution class. Right panel: Spearman rank correlation table of all E. coli concentration values ($n = 665$) and physical (pH, conductivity) and chemical (chemical oxygen demand (COD), total nitrogen, total phosphorus and ammonium) water quality parameters. Rho values are given only if the p value is below the level of significance (alpha ≤ 0.05) after p value adjustment using the Bonferroni method. n. s.: not significant.

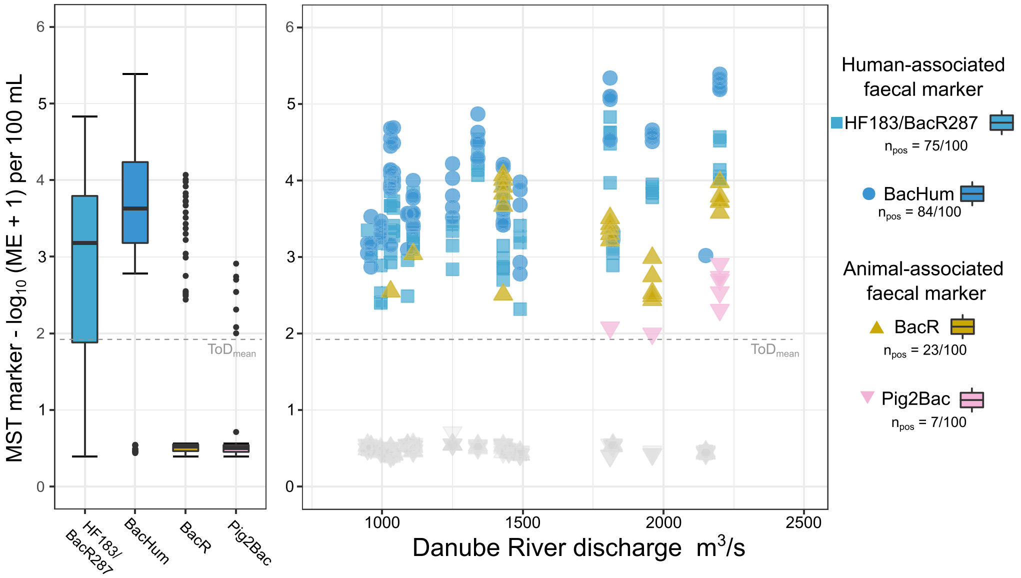


Fig. 6. Left panel: Boxplot of genetic faecal marker concentrations: human-associated HF183/BacR287, BacHum and animal-associated BacR (ruminant) and Pig2Bac (pigs). For nondetects, the sample-specific ½ ToD was taken as the value for plotting; the mean ToD is highlighted with a dotted line. Right panel: Scatterplot of the microbial source tracking marker concentrations (y axis) and the Danube River discharge, $n = 100$, positive detects: HF183/BacR287 n(pos) = 75, BacHum n (pos) = 84, BacR n(pos) = 23, Pig2Bac n(pos) = 7.

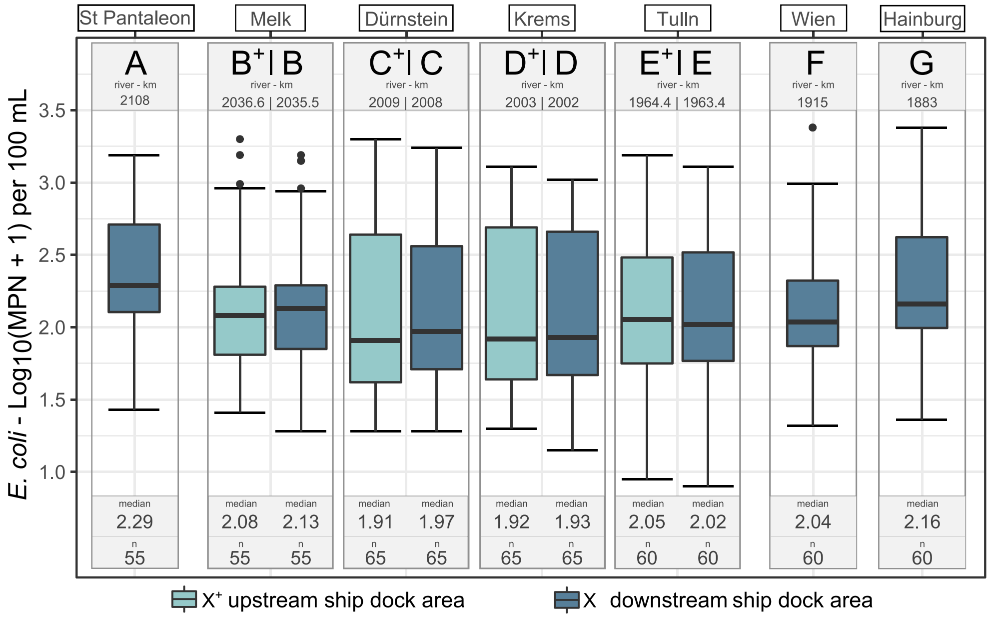


Fig. 7. Boxplots of the observed E. coli concentrations ($\log_{10}$(MPN + 1) per 100 mL) in longitudinal display for all transects A to G for the annual data. n = total number of samples (including all individual samples of each cross-profile at each sampling day). Median values given; lower/upper error bars: smallest/largest value within 1.5 times interquartile range below/above 25th/75th percentile, outliers are given as dots.

described in chapter 2.5. and **Supplementary 2.3.2.** Positive significant correlations of *E. coli* concentrations after p-value adjustment using fdr were observed for all hydrological parameters: i) 3-day sum of local precipitation (rho = 0.33, adj.$p = 5 \times 10^{-3}$), ii) Danube River discharge (rho = 0.48, adj.$p = 1.2 \times 10^{-5}$) and iii) discharge of tributaries with a confluence point < 36 rkm upstream (rho = 0.30, adj.$p = 1.9 \times 10^{-2}$; Fig. 9 upper panel). For WWTP discharge, no significant correlation with WWTPs situated directly at the Danube, but a significant correlation with WWTPs at tributaries (< 90 rkm, rho 0.30, adj.$p = 1.9 \times 10^{-2}$) was obtained. The analysis with the number of ships, including a differentiation of the ship types (cruise, passenger, and freight), showed no significant correlation, irrespective of which metric (1 m/s, 2 m/s river velocity; < 2 h, < 8 h, < 16 h water flow time) was used (Fig. 9 lower panels). Hence, on the regional scale (entire investigated river reach), the number of ships did not have a measurable influence on the *E. coli* concentration in the monitoring timeframe, and faecal pollution was mainly triggered by rainfall and higher river and municipal WWTP discharge.

3.4.2. *Correlation analysis at the ship docks (local scale)*

In contrast to the regional faecal pollution analysis, we observed a significant correlation of the increase in *E. coli* concentrations and the number of ships in the ship dock area at one of the four investigated docks, namely, Dürnstein (C+/C). The disintegration of ships per type

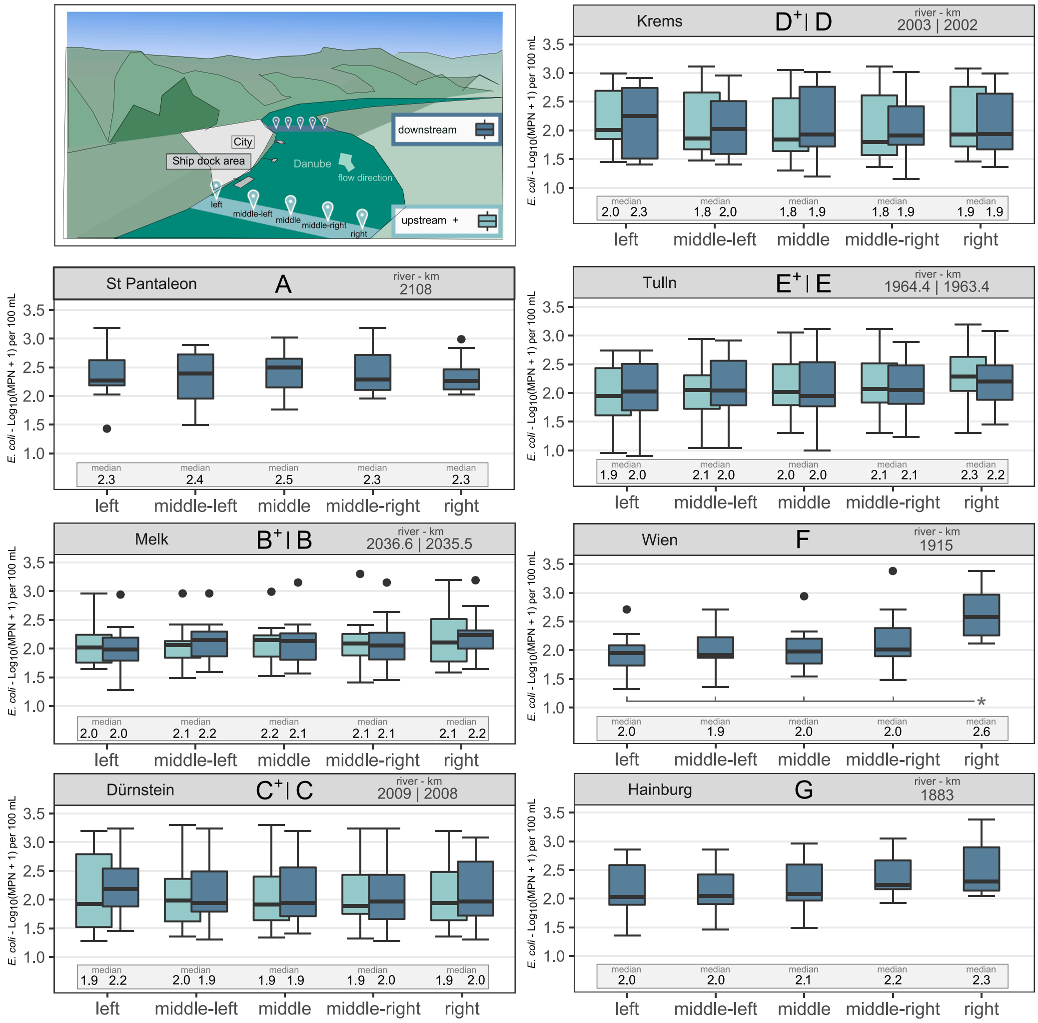


Fig. 8. Boxplots showing the E. coli concentrations in the cross-profile with upstream/downstream samples being paired. Median value given; lower/upper error bars: smallest/largest value within 1.5 times interquartile range below/above 25th/75th percentile, outliers are given as dots. Asterisk * indicates a significant difference of E. coli $\log_{10}$(MPN+1)/100 mL at the cross-profile position to all the others at the sampling transect assessed by ANOVA and a post-hoc Tukey test ($p < 0.05$, $n = 11 - 13$).

revealed that cruise ships accounted for the high correlation (rho = 0.828 and $p = 0.02$, Fig. 10), with a maximum difference in *E. coli* (downstream minus upstream) of 233 MPN per 100 mL. Multiple linear regression analysis revealed that precipitation and river discharge were insignificant parameters for the increase in *E. coli* concentrations at dock Dürnstein, but the number of cruise ships was significant ($p = 0.02$). Note that no WWTP discharge point and no confluence point of a river is situated between transects C+ and C. At all the other ship docks (*B*+/B), (*D*+/D) and (*E*+/E), no association with the number of ships and increase in pollution was observed (**Supplementary Figure S4**).

4. Discussion

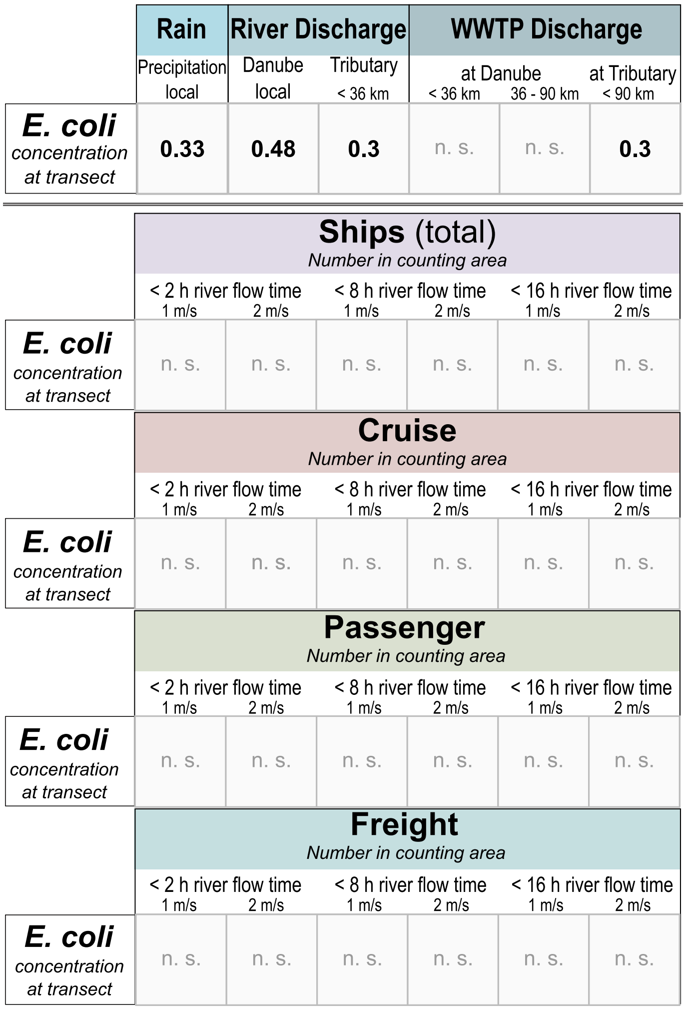
4.1. Realization of a novel integrated approach for assessing the impact of inland navigation by an interdisciplinary toolbox

The impact of inland navigation on the microbial faecal water quality of a large river was assessed using a new, integrated, highly interdisciplinary approach. It is based on three pillars: (1) pollution source profiling (PSP, *i.e.,* estimates of theoretically emitted numbers of *E. coli)* to assess the theoretical impact of various ship faecal emission scenarios, (2) PSP-targeted longitudinal and transversal water quality analyses combined with tailor-made ship-traffic data extraction, and (3) statistical analysis between pollution source patterns on regional and local scales to test the formulated pollution hypotheses.

The approach combined field-data from A) satellite-based automatic identification system (AIS) used for targeted ship traffic assessment and B) highly resolved water quality analysis based on chemical data and cultivation-based standard faecal indicator enumeration (ISO 9308–2 (ISO, 2012)) in combination with state-of-the-art quantitative genetic microbial source tracking (also referred to as genetic faecal pollution diagnostics, GFPD (Demeter et al., 2023; Steinbacher et al., 2021)).

Internationally, there have been two main approaches to assess the impact of navigation on water quality thus far. In one approach, the studies estimated the pollution potential of vessels by calculating nutrient loads or sewage volumes, often incorporating complex ship traffic data, but without any water quality investigations (Chen et al., 2022; Huhta et al., 2007; Loehr et al., 2006; Parks et al., 2019; Perić et al., 2016; Presburger Ulnikovic et al., 2012). In the other approach, the studies focused rather on the microbiological water quality around ports, without having any ship data (Dheenan et al., 2016; Luna et al., 2019). We found only three studies on the association of the occurrence of ships and microbiological-faecal pollution (Maria A. Faust, 1982; Koboević et al., 2022; M. D. Sobsey et al., 2003), all performed at maritime ports.

However, to the best of our knowledge, no study has yet investigated



	Rain	River Discharge		WWTP Discharge		
	Precipitation local	Danube local	Tributary < 36 km	at Danube < 36 km	at Danube 36 - 90 km	at Tributary < 90 km
E. coli *concentration at transect*	**0.33**	**0.48**	**0.3**	n. s.	n. s.	**0.3**

	Ships (total) *Number in counting area*					
	< 2 h river flow time 1 m/s	< 2 h river flow time 2 m/s	< 8 h river flow time 1 m/s	< 8 h river flow time 2 m/s	< 16 h river flow time 1 m/s	< 16 h river flow time 2 m/s
E. coli *concentration at transect*	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.

	Cruise *Number in counting area*					
	< 2 h river flow time 1 m/s	< 2 h river flow time 2 m/s	< 8 h river flow time 1 m/s	< 8 h river flow time 2 m/s	< 16 h river flow time 1 m/s	< 16 h river flow time 2 m/s
E. coli *concentration at transect*	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.

	Passenger *Number in counting area*					
	< 2 h river flow time 1 m/s	< 2 h river flow time 2 m/s	< 8 h river flow time 1 m/s	< 8 h river flow time 2 m/s	< 16 h river flow time 1 m/s	< 16 h river flow time 2 m/s
E. coli *concentration at transect*	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.

	Freight *Number in counting area*					
	< 2 h river flow time 1 m/s	< 2 h river flow time 2 m/s	< 8 h river flow time 1 m/s	< 8 h river flow time 2 m/s	< 16 h river flow time 1 m/s	< 16 h river flow time 2 m/s
E. coli *concentration at transect*	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.

Fig. 9. Spearman rank correlation coefficients (rho) of *E. coli* concentrations at the transects with meteorological and hydrological parameters (upper panel) and the numbers of ships in the upstream counting area for i) ships (total), ii) cruise liners, iii) passenger ferries and iv) freight vessels considering 1 m/s or 2 m/s river flow velocity and a water flow time < 2 h, < 8 h and < 16 h before sampling (lower panels). Hydrological parameters (upper panel): Sum of precipitation during 3 days before sampling, Danube River discharge, tributary river discharge as well as WWTP discharge at the Danube River for WWTP < 36 rkm, or from 36 – 90 rkm, or WWTP at tributaries up to < 90 rkm upstream of the sampling point on the same day of sampling. n.s. not significant ($p \geq 0.05$) after p-value adjustment using fdr.

the microbiological water quality of rivers in relation to local and regional ship traffic and no PSP considering all major wastewater sources (including ships) has yet been combined with a multiparametric water quality investigation.

Here, we propose to integrate the state-of-the art of these assessment types into a common framework. The approach is directly transferrable to any other water body (and not limited to lotic/river systems), especially given the increasing global coverage of RIS, including inland AIS (Creemers, 2023; Trivedi et al., 2021). It can also support evidence-based water management decisions of local authorities.

4.2. The local impact of navigation was unexpectedly low but sensitively traceable

As hypothesized by the PSP, river sections directly downstream of ports and docks would be crucially affected by wastewater discharges from ships. In our study area, assuming all ships illegally discharge raw wastewater (see **chapter 3.2** and Fig. 4 PSP scenario 'Maximum Potential Ship Emission'), the total faecal pollution load from navigation would equal the sum of the total municipal wastewater load entering the considered Danube River reach. The local consequences of such a scenario would be serious, given the extremely high concentrations of faecal microorganisms in raw wastewater (log 7 to log 8 MPN/100 mL *E. coli* (Harwood et al., 2019)), demonstrating the local pollution capacity of navigation on beaches, recreation zones and other types of local usages.

Our investigation found only one site, at dock C+/C, where a statistical increase in *E. coli* concentrations was indicated downstream (Fig. 8). Strikingly, this increase also matched the identified significant correlation with the number of cruise ships extracted from the big-AIS-data near-real-time ship traffic monitoring system (Fig. 10). Furthermore, using the developed SDA algorithm, we were able to trace back to the individual ships causing this correlation as well as to analyse the average time of the ships in the dock area. In fact, cruise ships spent up to 1 to 3 h at dock C+/C, with seasonal differences, with highest average docking times from May to October and shorter times in the area in the winter months (see **Supplementary Figure S5**). In contrast, freight ships all over the year only spent approx. 15 min in the area, passing the dock C+/C (see **Supplementary Figure S5**). The used AIS data were "blinded" before being handed over by the authorities for analysis, not intended to identify any specific ship or company involved. However, these results impressively demonstrate the capacity of this suggested approach for analysis and monitoring (including aspects of sensitivity and specificity) by combining "field" water quality data with "post hoc" extraction of AIS stored near-real-time ship trafficking data. It should also be mentioned that the detectability of changes in water quality

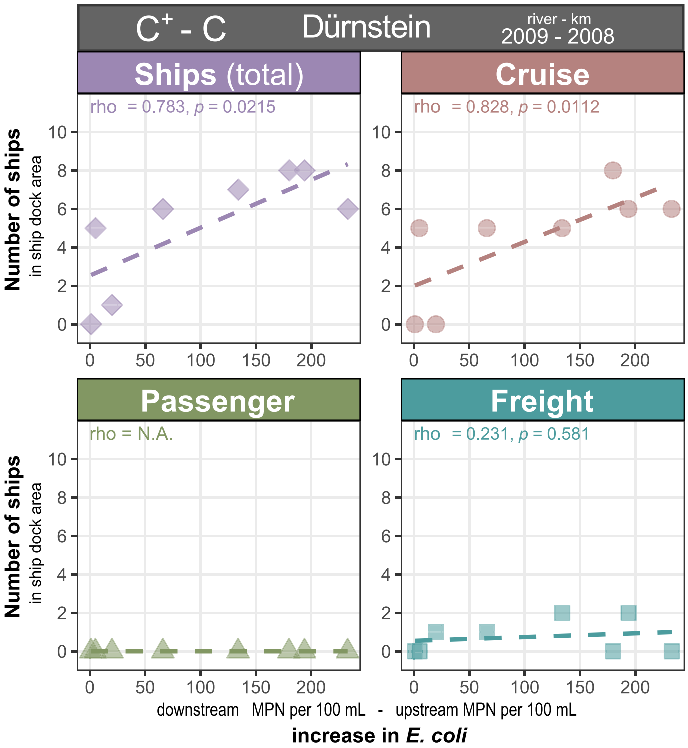


Fig. 10. Scatterplot of the number of ships in the ship dock area and the increase in E. coli concentrations of the downstream (C) and upstream (C+) sample pairs at the left river side at the ship dock area of Dürnstein. Spearman rank correlation coefficients rho and p values are given in the plot. The dotted line gives a fitted linear model.

could even be improved by replacing grab sampling with automated time series sampling, triggered by passing ships (*e.g.,* comparable to event sampling (Stadler et al., 2008).

Both the minor increase in faecal pollution at dock C/C+ and the lack of increase in pollution at the other three sites indicate that ship wastewater was most likely treated and handled adequately (*e.g.,* ship emissions from correctly managed on-board wastewater treatment) for the selected Danube reach and time period. Since the investigation was triggered by citizen complaints and considerable media attention, the navigation industry probably had a high awareness of the importance of correct wastewater handling. Hence, the approach also proved very useful to analyse and highlight the obviously correct realization of the intended management practice. Although a statistically significant but only moderate increase was detected, the formulated local-scale faecal pollution hypothesis had to be rejected, as the potential pollution capacity was not realized at all.

4.3. *Regional impacts of navigation were not detectable, but this could change in the future*

On the regional scale, we hypothesized that the impact of navigation on Danube River faecal pollution patterns would not be discernible under the current state-of-the-art municipal sewage disposal (secondary treatment and no disinfection), irrespective of the assumed type of ship wastewater handling and PSP scenario. Indeed, the recovered results showed no statistically significant increase in the longitudinal development of faecal pollution levels between the selected transects (A to G, Fig. 7), and no correlation between ship counts in any of the ship classes (and RSTA calculation schemes) *versus* the *E. coli* concentrations was detectable (Fig. 9). The regional-scale faecal pollution hypothesis was thus clearly supported by our field data.

However, these proportions may shift in the future, bringing the impact of inland navigation more to the forefront. Proposed changes in European Union law regarding urban wastewater management (EU Regulation 2020/741, 2022) are expected to lead to a reduction in faecal microorganism loads from both treated municipal wastewater and combined sewer overflows in the coming years. If the proposed changes are implemented across the Danube River basin, it is reasonable to assume that the faecal pollution level in the river attributable to municipal wastewater will decrease considerably (*e.g.,* up to 10 000-fold for viral particles (Demeter et al., 2021)), leading to an increase in the relative importance of faecal input from inland navigation.

On the other hand, cruise tourism on the Danube River has great potential to increase (Jászberényi and Miskolczi, 2020). In fact, before the COVID-19 pandemic, during the investigation period, cruise ship tourism on the Danube in Austria grew by 15 % in 2019 compared to 2018 (Viadonau, 2019b). Assuming the same trend in the next decades, ship activity and therefore faecal pollution load from inland navigation could substantially increase. If these two potential changes occurred concurrently, the relationship between municipal wastewater loads and ship discharges could flip, making the navigation sector a critical potential emission source on both the local and regional scales.

4.4. *Large rivers are subject to multiple pressures, and water quality varies on longitudinal and cross-sectional scales*

Faecal pollution can change over time and along the investigated river reach because of changing river morphology, confluence with tributaries and point and nonpoint sources. Therefore, longitudinal investigations are frequently performed in river water quality analyses (Ballesté et al., 2019; Fernández et al., 2022; Kirschner et al., 2017, 2009; Ouattara et al., 2014); however, cross-sections are seldom investigated (Kirschner et al., 2017). In contrast in coastal regions, several studies have been performed to study water quality variations at high spatial resolution (along the shore and in transects (Ahn et al., 2005; Amorim et al., 2014; Manini et al., 2022)).

Our spatially highly resolved water quality investigation at the Danube River showed that there were neither significant longitudinal differences in *E. coli* concentrations along the 223 km Danube River reach nor significant cross-sectional variations at most of the transects (see Figs. 7, and 8). The observed concentrations of *E. coli*, indicative of general faecal pollution, ranged from $\log_{10}$ 0.9–3.38 MPN/100 mL, with a median of 2.10 MPN/100 mL for the Danube River in the monitoring timeframe, consistent with former surveys of the Danube River (Demeter et al., 2021; Kirschner et al., 2017, 2009). This is a typical concentration range for a moderately polluted water body with input from state-of the-art WWTPs. Other navigable rivers show similar *E. coli* concentration levels, for example, the Rhine River with concentrations between $<$ $\log_{10}$ 1.18 and $\log_{10}$ 4.10 and an average of $\log_{10}$ 2.91 MPN/100 mL (Herrig et al., 2019), or the upper Mississippi River with mean values of $\log_{10}$ 1.89 in 2011 and $\log_{10}$ 1.18 CFU/100 mL in 2012 (Staley et al., 2014). However, many rivers are considerably more polluted, such as the rivers of the Bogotá basin in Columbia, with maximum concentrations up to log 6 and log 7 *E. coli* MPN/100 mL at many of the sites (Fernández et al., 2022). Although there are approaches towards global and freely available data concerning the water quality of surface water, such as the GEMStat Water Quality Dashboard project by the UN (https://gemstat.org/), profound and standardized microbial faecal pollution analysis of large rivers on a global scale is scarce. Additionally, in the EU, there is data scarcity, as microbial faecal pollution monitoring is not included in the Water Framework Directive (European Parliament, 2000); hence, we can conclude that the herein published highly resolved multiparametric water quality dataset is one of its kind.

4.5. Increasing use of AIS and RIS in research

The use of AIS data as a big data source for ship-associated research purposes has been increasing in recent decades (Chen et al., 2022; Yang et al., 2019). Especially for maritime research, this data source is used for investigations, *e.g.,* ship behaviour analysis for the detection of illegal fishing (Iacarella et al., 2023; Kurekin et al., 2019). AIS and inland AIS were already used for an environmental impact evaluation of exhaust gas emissions from ships in maritime waters (Goldsworthy and Goldsworthy, 2015; Toscano et al., 2021) and from inland navigation at the Yangtze River (Huang et al., 2022; Zhang et al., 2023). Despite providing comprehensive navigational information, AIS data-based research must consider that only vessels equipped with turned-on AIS transponders are incorporated in the database and that manually edited information might be wrong or not up to date (Harati-Mokhtari et al., 2007).

Inland AIS is one essential tool of RIS, beginning in 1998 in Europe and implemented increasingly globally, such as in India (Trivedi et al., 2021) and the United States of America (Creemers, 2023). RIS is an integral part of the trend towards digitalization in the navigational and water sector, *i.e.,* Intelligent Transport Systems (ITS) and e-Navigation (e-Nav) (Creemers, 2023); hence, inland AIS/RIS data will be increasingly available for research purposes in the future.

5. Conclusions

- We developed and applied a novel integrated approach for evaluating the impact of inland navigation on the microbial faecal water quality of navigable rivers, based on the three pillars (1) pollution source profiling (PSP), allowing for theoretical (*in silico*) impact scenario analysis, (2) highly resolved and advanced field-analysis (*in situ*) of faecal pollution levels, chemo-physical water quality and ship traffic and (3) statistical analysis between pollution patterns and ships on regional and local scales.
- Ship tracking data was extracted from inland AIS data by a specifically developed script, enabling retrospective back tracing of the individual ships per type (*i.e.,* cruise, passenger, freight) on regional and local scale.
- High resolution multi-parametric water quality analysis at the investigated Danube River reach revealed low longitudinal and cross-sectional pollution gradients, from dominant human origin (demonstrated by genetic faecal pollution diagnostics, GFPD), hence identifying *E. coli* as a suitable proxy for human wastewater from municipal WWTPs or ships.
- PSP highlighted a high theoretical faecal pollution potential from ships (with highest pollution capacity locally), which was not detected during the investigated period, indicating correct management of ship wastewater.
- Minor local impact was detectable and attributable to the associated docked cruise ships, highlighting the sensitivity of the approach, and showing its possible extensions towards investigation of *e.g.,* the docking times of individual ships.
- The approach is transferrable to any other water body with available AIS data over the world, enabling detailed understanding of local and regional navigational faecal pollution characteristics, with highest potential for research and authorities worldwide, heading towards a digitalization of the water sector to support environmental quality and human health management.

CRediT authorship contribution statement

Sophia D. Steinbacher: Writing – original draft, Visualization, Investigation, Formal analysis, Data curation. **Ahmad Ameen:** Writing – original draft, Visualization, Software, Investigation, Data curation. **Katalin Demeter:** Writing – original draft, Writing – review & editing, Funding acquisition. **David Lun:** Validation, Formal analysis. **Julia Derx:** Writing – review & editing, Funding acquisition. **Gerhard Lindner:** Investigation. **Regina Sommer:** Methodology, Funding acquisition. **Rita B. Linke:** Validation, Methodology, Investigation, Data curation. **Claudia Kolm:** Writing – review & editing. **Karen Zuser:** Resources, Project administration. **Martina Heckel:** Investigation. **Andrea Perschl:** Investigation, Conceptualization. **Günter Blöschl:** Writing – review & editing. **Alfred P. Blaschke:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization. **Alexander K.T. Kirschner:** Writing – review & editing, Methodology, Funding acquisition, Data curation, Conceptualization. **Andreas H. Farnleitner:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The authors do not have permission to share data.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.watres.2024.122029.

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