

Instream coliform gradients in the Holtemme, a small headwater stream in the Elbe River Basin, Northern Germany

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Abstract The Holtemme is a small headwater stream in North Germany's Elbe River Basin. According to German and European legislation, hygienic monitoring is not mandatory for such water bodies which are neither drinking water sources nor categorized as bathing waters. Consequently, relatively little is known about the occurrence of –potentially pathogenic– bacteria and viruses in Germany's streams and rivers. The Holtemme was selected for a case study because it is relatively well monitored for both chemical water quality and aquatic ecology, but not for hygiene. Originating in the mountains of Harz Nature Park, the 47 km long Holtemme is characterized by a strong longitudinal gradient in chemical water quality, which is related to different land uses and the influx of treated wastewater from two urban areas (Wernigerode and Halberstadt). Waste water loads received by the Holtemme are comparatively high when compared to similarly small streams.

In 2015, total coliform concentrations between more than 200 and 77,010 bacteria per 100 mL, and fecal coliform concentrations between 5 and 24,060 bacteria per 100 mL were observed in the Holtemme's main channel. The highest concentrations were typically found below the outlets of the two wastewater treatment plants. The treated wastewater contained total and fecal coliform concentrations of up to 200,500 and 83,100 per 100 mL, respectively; however, there were significant temporal variations. While the observed concentrations are unproblematic from a legal perspective (because no maximum permissible limits are defined for streams in Germany), they would exceed the tolerable limits for bathing waters in the EU, indicating moderate to critical pollution limits.

Keywords surface water, stream, hygiene, instream coliforms

1 Introduction

In Germany, there is no general legal standard for the hygienic quality of natural water bodies. However, the microbiological water quality is of interest when surface water bodies are used as a source for drinking water (e.g., via riverbank filtration, or surface water abstractions from reservoirs), when their water is used for agricultural purposes (i.e., for irrigation or as a drinking water source for farm animals), or when water bodies are used for water sports (Graw and Borchardt, 1995; Kistemann et al., 2002; Kirschner et al., 2009; Schulz and Childers, 2011). Moreover, rivers are relevant as pathways for the transport of pathogenic microorganisms, including antibiotic-resistant bacteria which originate from wastewater inputs containing antibiotics and their metabolites (Schreiber and Kistemann, 2012; Bessa et al., 2014; Reder et al., 2015). Bacteria form an important part of the natural biocoenosis and are present in all natural water bodies. They are a prerequisite for the functioning of river and lake ecosystems because of their relevance for matter turnover and self-purification. However, wastewater discharge and influx via diffuse sources may lead to shifts in the composition of bacterial communities via increased loads of fecal bacteria and potential pathogens (Graw and Borchardt, 1995).

The European Water Framework Directive does not contain any general regulations on surface water hygiene. Two bacteriological thresholds are, however, defined by the German (and European) Bathing Water Ordinances. In bathing waters, at least 95% of the samples tested need to have concentrations of less than 1800 colony-forming units (CFU) per 100 mL of *Escherichia coli* and less than 700 CFU/100 mL of *Enterococcus faecalis* (Kirschner et al., 2009). In lakes and rivers from which water is abstracted for drinking water production, fecal coliform concentrations should be below 20,000 CFU/100 mL even in case of intensive treatment (SLUG, 2002). Only a few

countries worldwide, such as the Russian Federation or Taiwan, China have hygienic norms for river water quality in the absence of bathing or drinking water use (Sorokovikova et al., 2013; Taiwan EDA, 2016). In Germany and worldwide, monitoring-based data on the microbiological quality of river water is comparatively scarce; therefore, loadings with organisms such as coliform bacteria have to be modelled or estimated for larger scale investigations (Reder et al., 2015). In the recent past, systematic and detailed investigations on surface water hygiene have been conducted in only a few river basins, including the Danube (Kirschner et al., 2009), the Ruhr (Mälzer et al., 2015) and the Swist (Kistemann et al., 2007; Schreiber et al., 2016). Both droughts and extreme precipitation events have been found to be a cause for elevated loadings with (pathogenic) microorganisms, particularly in catchments where agricultural land use plays an important role or where combined sewer overflows discharge untreated wastewater into the stream (Karte, 2015).

Hygienically relevant microorganisms, which include protozoans, bacteria and viruses have a few typical characteristics: a) they are excreted by humans or animals in high doses or have low infective doses; b) they can survive in the environment for an extended period of time; c) they may be difficult to inactivate during water treatment (Funari et al., 2012). All these factors increase the likelihood of disease transmission, particularly when such organisms enter drinking water sources.

Typically, the microbiological water quality is assessed via a small set of indicator bacteria such as total/fecal coliforms and fecal enterococci. However, the commonly used methods (culture techniques) have the advantage of being slow, without much potential for automation and poorly suited for the assessment of virological water quality (Schulz and Childers, 2011; Karte et al., 2016).

In the absence of legal norms, criteria for the assessment of surface water hygiene have been proposed by Kirschner et al. (2009), who proposed a classification system for instream coliform and fecal enterococci concentrations that is suitable for central European rivers (see Table 1).

For the Elbe River, and the Saale as one of its main tributaries, Reder et al. (2015) calculated typical instream fecal coliform concentrations between 0 and 2000 CFU/(100 mL) but pointed out that small tributaries may have much higher concentrations. According to the Saxonian State Agency for Environment and Geology, background instream concentrations in the Elbe are around 13,000 CFU/(100 mL) for fecal coliforms and 67,000 CFU/(100

mL) for total coliforms around Dresden; but significant rises were recorded during and directly after a major flood in 2002 (SLUG, 2002; Karrasch et al., 2004) which also led to elevated concentrations in flooded soils, indicating potential risks for the safety of drinking water wells in the floodplains (Strauch et al., 2004; Abraham et al., 2005).

Coliform concentrations in surface water bodies can vary within large ranges. This does not only depend on variations in coliform sources (e.g., wastewater discharge, presence of animal manure; see for example Stapleton et al., 2008; Bougeard et al., 2011), but also on other external influences including precipitation and snowmelt (which affect discharge), temperatures (which control bacterial survival and reproduction), sunlight and water turbidity (which influence UV disinfection rates), water chemistry (e.g., presence of nutrients, oxygen saturation, pH) and river ecology (e.g., bacterial control by predation) (Bagde and Rangari, 1999; Tiefenthaler et al., 2009; St Laurent and Mazumder, 2014; Shelton et al., 2014).

In this paper, the findings of a monitoring campaign conducted on the Holtemme River between April and November 2015 are presented. The main objectives of this study were the assessment of spatial and temporal pattern of instream coliform loads and their determinants. Moreover, it was investigated whether basic water quality parameters such as temperature or electric conductivity would be good predictors of coliform concentrations. Ultimately, such insights could help to determine how much, when and where microbiological monitoring is meaningful in the future.

2 Material and methods

2.1 Study region

The Holtemme is a small stream that has its source in the northeastern Harz Mountains and drains into the Bode River, which itself is a tributary of the Saale. Near Magdeburg, the nearest major city, the Saale drains into the Elbe, the most important river basin in northern Germany.

The Holtemme and its catchment (278 km²) is representative for the Bode River Basin (3284 km²), which drains very distinct landscapes: the mountains of the High and Upper Harz, the hills of the “Harzvorland” and the (highly fertile) plains of the “Magdeburger Börde” which are characterized by an intensive agricultural use (LHW Sachsen-Anhalt, 2012). While there is a major drinking water reservoir on the upper Bode (the Rappbode

Table 1 Classification of surface water hygiene based on Kirschner et al. (2009)

Parameter	Pollution level (classes)				
	I - Little	II - Moderate	III - Critical	IV - Strong	V - Excessive
Total coliforms (per 100 mL)	≤ 500	> 500 – 10,000	> 10,000 – 100,000	> 100,000 – 1,000,000	> 1,000,000
Fecal coliforms (per 100 mL)	≤ 100	> 100 – 1000	> 1000 – 10,000	> 10,000 – 100,000	> 100,000

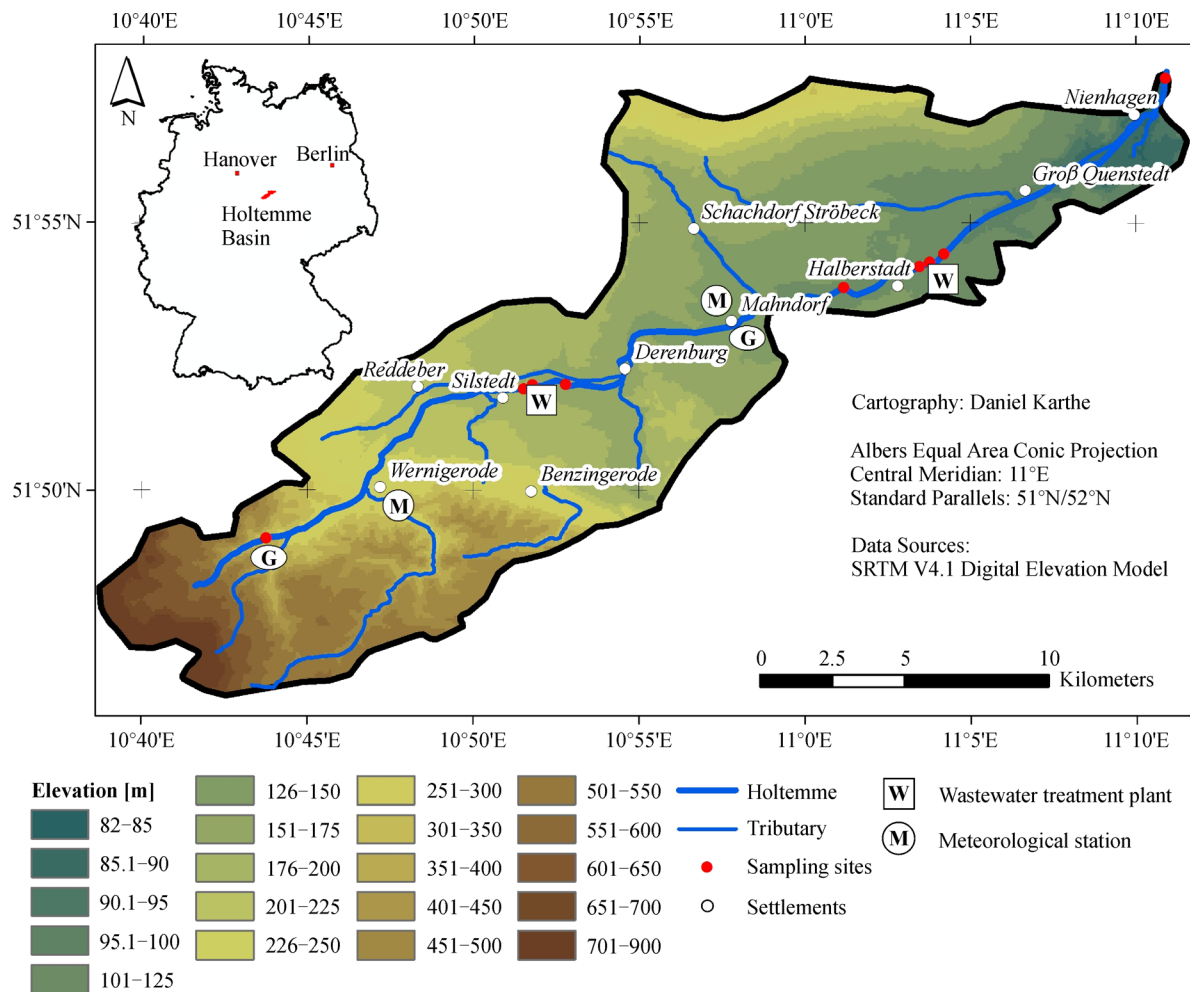


Fig. 1 Topography of the Holtemme River Basin, including gauging and meteorological stations and wastewater treatment plants.

Dam), there is a smaller drinking water reservoir on one of the Holtemme's tributaries, the Zilierbach (a 13 km long right side tributary draining into the Holtemme in Wernigerode, Figs. 1 and 2).

Over a distance of 47 km, the Holtemme covers an elevation range of 659 m (Fig. 1). Its entire catchment is located in the state of Saxony-Anhalt and is characterized by a strong land use gradient that ranges from forest cover in the mountains to agricultural land and urban areas in the mid- and downstream sections (Fig. 2; Kammerad, 2011; Kamjunke et al., 2013). The Holtemme can be classified as a small carbonatic highland river dominated by fine substrate (Lange et al., 2014) according to the German LAWA classification (LAWA, 2003).

In the upstream part, the Holtemme's course is almost without morphological alterations. It flows through a V-shaped valley that features several rocky cascades, which has led to the designation "Steinerne Renne" ("stony run") in this relatively fast flowing section. The water is relatively acidic and therefore without fish. Further down-

stream, the Holtemme passes through Wernigerode (population about 35,000). From here onwards, large parts of its course has been rectified and channelized with dams (Kammerad, 2011). A first wastewater treatment plant (WWTP) discharges into the Holtemme at Wernigerode-Silstedt, and a second one downstream of Halberstadt, the largest town in the catchment (population about 40,000). Even though chemical contamination is not considered a major problem with the exception for elevated phosphorus loads, the ecological state of the Holtemme was classified as "poor" in a recent assessment, largely because of an impaired river morphology (Lange et al., 2014). The inflow of treated wastewater and agricultural activities (mainly fertilizer application on fields; there is no significant amount of livestock along the river) are the most important sources of chemical (mostly nutrient) and bacteriological pollution. In fact, wastewater loads in the Holtemme are very significant, with 88 inhabitants per l*s of discharge, which compares to a maximum recommended limit of 10 inhabitants per l*s of discharge in

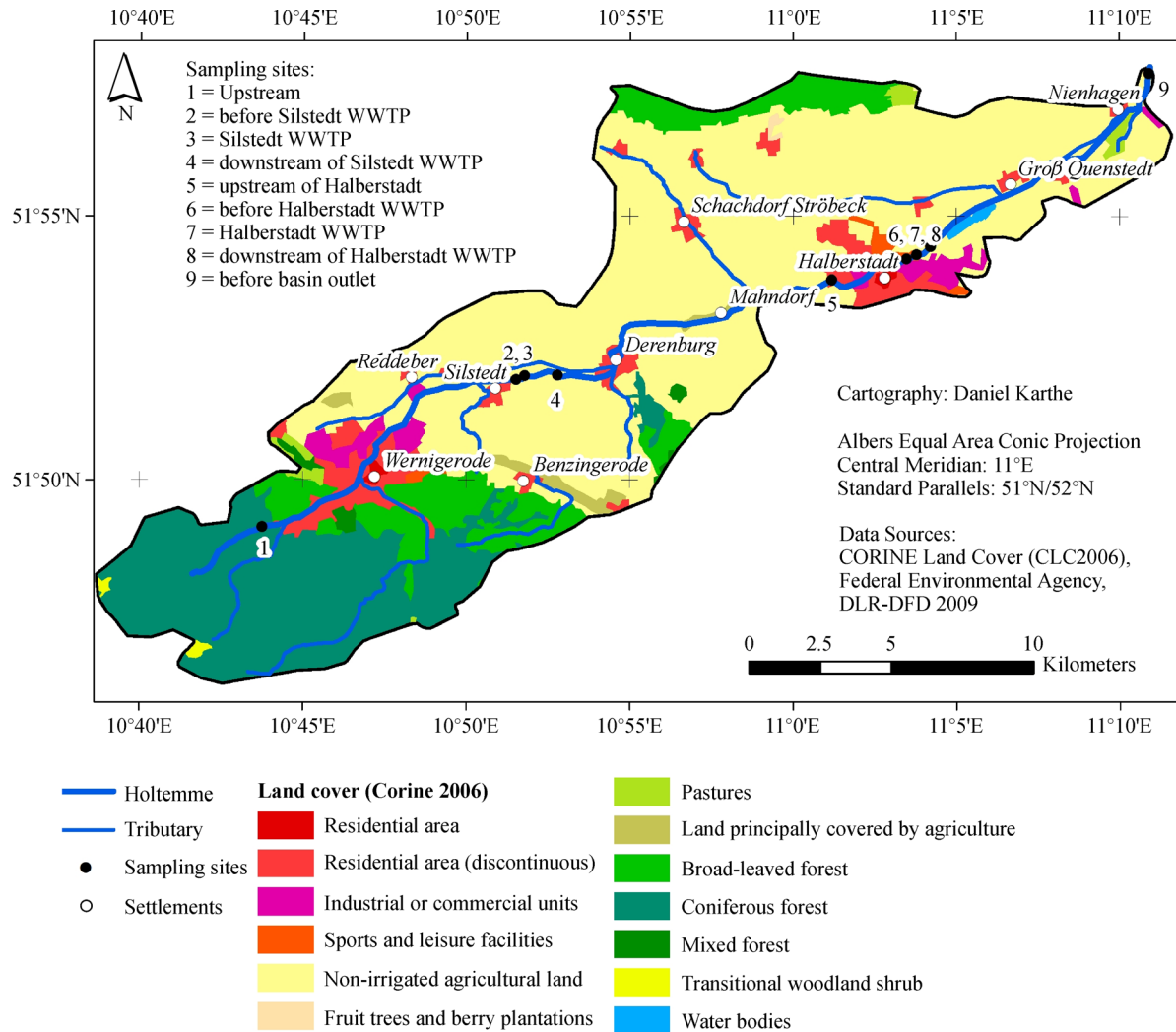


Fig. 2 Land cover and location of sampling sites in the Holtemme Basin.

Germany (Ecosystem Saxonia, 2014; Lange et al., 2014).

There are two gauging stations on the Holtemme river, which are located at Steinerne Renne (subcatchment: 15.7 km²; 41 km from basin outlet; long-term mean discharge MQ = 0.341 m³/s) and at Mahndorf (subcatchment: 168 km²; 19.3 km from basin outlet; MQ = 1.330 m³/s) and operated by the state organization for flood protection and water management Saxony-Anhalt (LHW Sachsen-Anhalt, 2012).

The Holtemme's hydrograph shows two annual peaks: one in January and one in March/April. Typically, the discharge decreases significantly during the summer months (Fig. 3).

2.2 Sample collection and analysis

Water sampling was conducted from April to November 2015 at roughly monthly intervals, typically during dry

periods or with only minor precipitation amounts over two days preceding the sampling. The sampling locations were chosen to represent known water quality gradients (Kamjunke et al., 2013 and unpublished data by colleagues; see Table 2).

Samples were collected in sterile single-use plastic bottles which were sealed directly after sampling and then transported to the laboratory in a cooling box. The transport time to the laboratory did not exceed 4 hours.

In the laboratory, samples were screened for total coliforms, *Escherichia coli* (*E. coli*) and fecal coliforms using the Colilert®-18 system (IDEXX Laboratories, Westbrook, Maine, USA). The Colilert® system is an internationally approved standard technique for the microbiological surveillance of both drinking and surface water. Test results are not much affected by the microbial background flora, and while underestimations are possible (as with most other microbiological techniques), Colilert®

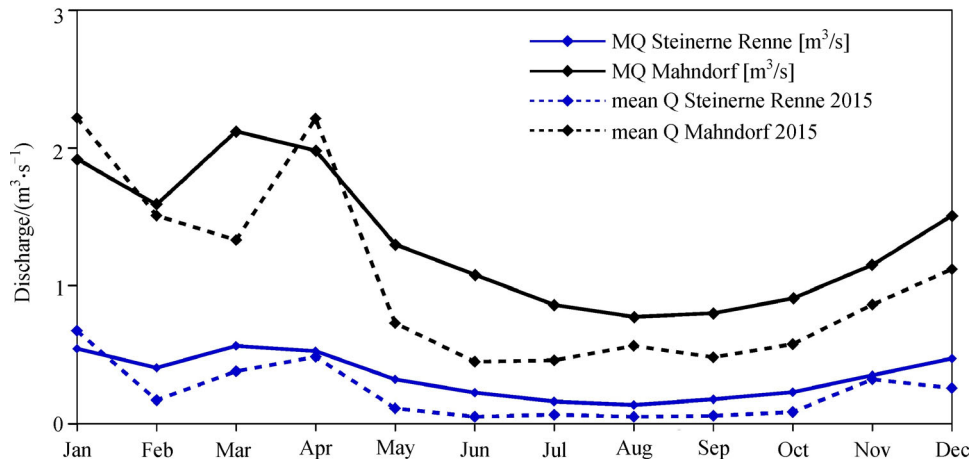


Fig. 3 Hydrograph of the Holtemme, comparing long-term average data from 1971/72 to 2014 to the hydrological situation in 2015. Source: LHW Sachsen-Anhalt (2016).

is suitable for the enumeration of coliforms in different water types such as drinking water, surface water or wastewater (Rompré et al., 2002; Pitkänen et al., 2007).

In the laboratory, samples were diluted as necessary with sterile water and thoroughly mixed with the culture substrates. Samples were then poured into the QuantiTray containers (IDEXX Laboratories, Westbrook, Maine, USA), in which they were incubated for 18 hours at $35\pm 0.5^\circ\text{C}$ (for total coliforms and *E. coli*) and $44.5\pm 0.2^\circ\text{C}$ for fecal coliforms. Subsequently, the most probable number (MPN) for each sample were calculated using interpretation tables provided by the manufacturer and the IDEXX MPN Generator (Version 1.4). In addition to the water samples collected, we measured basic water quality parameters (temperature, pH, EC) in situ with the portable Multiline 2 sensor system (WTW GmbH, Weilheim, Germany) and used hydrological data and precipitation data provided from the monitoring stations operated by Saxony-Anhalt's state organization for flood protection and water management, plus meteorological data from the German weather service (DWD) stations in Wernigerode and Wernigerode-Schierke.

3 Results

Total coliform concentrations (TC) in the Holtemme ranged between MPNs of 200.5 to 77,010 per 100 mL, while fecal coliform (FC) concentrations of 0 to 24,060 MPN per 100 mL were observed in the Holtemme. A synoptic overview of minimum, average and maximum concentrations in the river and in treated wastewater is given in Table 3.

The measured concentrations of total and fecal coliforms in the Holtemme showed a strong longitudinal gradient which was similar for both parameters (Fig. 4(a)). In the upstream section (sampling site 1, which is located upstream of the first larger settlement along the Holtemme), average coliform concentrations were the lowest. Due to the relatively pristine and forested environment, these background concentrations are likely to be mostly of natural sources.

The highest concentrations of TC and FC in the Holtemme were measured at the sampling sites directly downstream of the wastewater treatment plants. On average (and in most but not all cases), concentrations

Table 2 Description of sampling sites. Numbers correspond with the locations shown in Fig. 2

No	Km	Sampling site	Coordinates	
1	2.3	Upstream in relatively pristine and forested environment (at Steinerne Renne gauging station)	51.819° N	10.729° E
2	14.5	Just before Wernigerode-Silstedt WWTP	51.865° N	10.858° E
3	15.0	Treated wastewater, Wernigerode-Silstedt WWTP effluence	51.867° N	10.862° E
4	16.2	Downstream of Wernigerode-Silstedt WWTP	51.867° N	10.879° E
5	28.4	Upstream of Halberstadt	51.897° N	11.019° E
6	31.7	Just before Halberstadt WWTP	51.904° N	11.056° E
7	32.0	Treated wastewater, Halberstadt WWTP effluence	51.905° N	11.062° E
8	32.5	Downstream of Halberstadt WWTP	51.908° N	11.070° E
9	43.3	Outlet of the Holtemme (just before confluence with the Bode river)	51.962° N	11.182° E

Table 3 Daily discharge, total coliform (TC) and fecal coliform (FC) concentrations along the Holtemme for the monitoring period (April – November 2015; $n = 7$ per location)

Sampling site	Discharge/($\text{m}^3 \cdot \text{s}^{-1}$)			TC/(MPN per 100 mL)			FC/(MPN per 100 mL)		
	Min	Avg	Max	Min	Avg	Max	Min	Avg	Max
Upper reach (sampling sites 1,2; discharge at Steinerne Renne)	0.019	0.103	0.405	200.5	3366	12,098	5	664	1936.5
Middle and lower reach (4–6, 8–9; discharge at Mahndorf)	0.298	0.677	1.96	1920	17,150	77,010	0	6362	24,060
WWTP Silstedt (3)	-	-	-	17,890	40,286	200,500	10,170	28,506	83,100
WWTP Halberstadt (7)	-	-	-	23,800	49,079	129,970	15,075	37,712	77,010

below the outlet of the first WWTP in Wernigerode-Silstedt (site 4) were higher than those below the second WWTP in Halberstadt. Coliform concentrations showed a decreasing trend further downstream of both WWTPs; the decrease is more pronounced downstream of the first WWTP, with only a little reduction towards the river basin outlet.

Measurements of basic water quality parameters (Figs. 4 (b) and 4(c)) showed that between the first two sampling sites, electrical conductivity (EC) rises sharply, indicating a significant change in water quality. Further downstream, EC continues to rise, particularly due to wastewater influx.

There is also a clear temperature gradient along the Holtemme, with average temperatures (for the sampling events) rising from 11.3°C at the first sampling site to 17.1°C at the final sampling site near the river basin outlet. By contrast, pH along the river is relatively stable, with non-acidic conditions along its entire course.

Basic water quality parameters such as temperature, pH and electric conductivity were found to be only weakly correlated with coliform concentrations (temperature to instream TC 0.25, temperature to instream FC 0.45; EC to instream TC 0.38, EC to instream FC 0.42; pH to instream TC 0.40, pH to instream FC 0.47). Typically, these

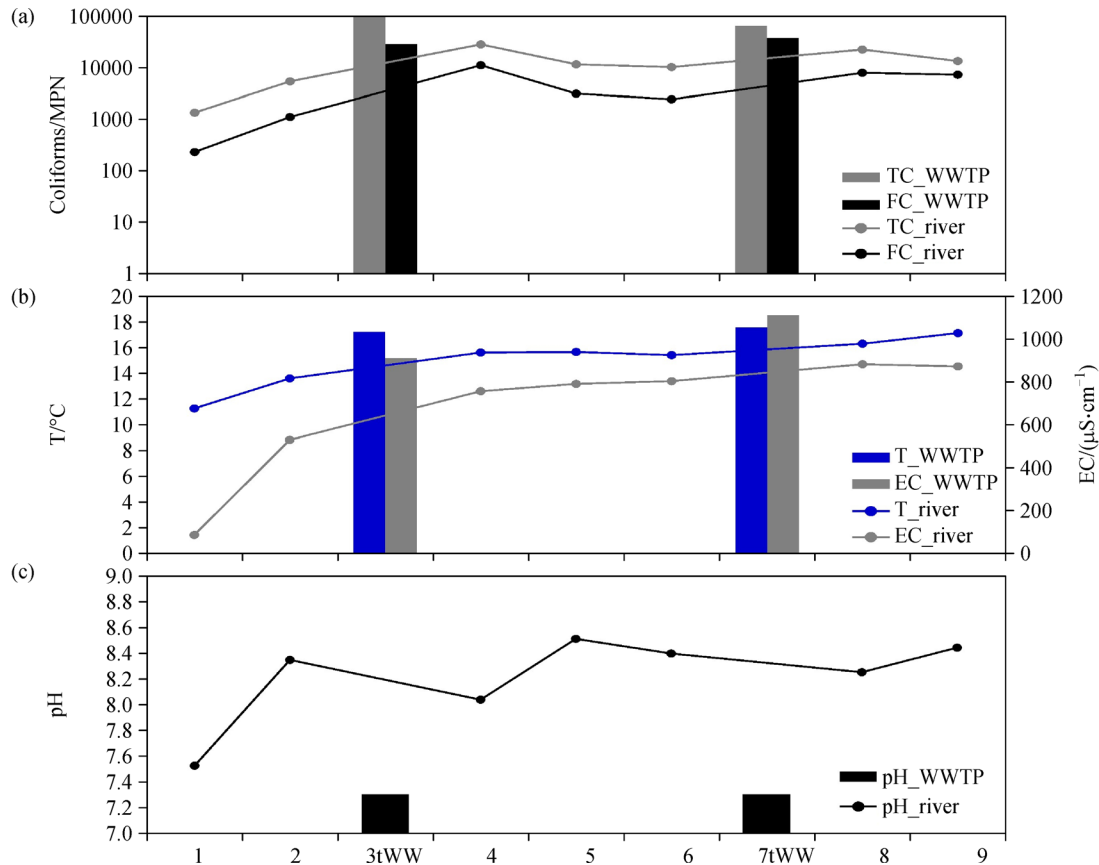


Fig. 4 Average instream coliform concentrations (a), temperature, electric conductivity (b) and pH (c) along the Holtemme and in the treated wastewater (tWW) effluent (sites 3 and 7). EC = electric conductivity; FC = fecal coliforms; MPN = most probable number; TC = total coliforms; WWTP = wastewater treatment plant.

indicators showed a comparable spatial (particularly regarding wastewater influx; Fig. 4), while the temporal pattern of instream coliforms were less comparable to the variations of temperature, pH and EC.

When considering temporal variations in water quality, it is important to note that discharge was comparable to longterm MQ only during the first sampling in April 2015. In all subsequent months, discharge of the Holtemme and adjacent rivers was below long term means (Fig. 3). In the upstream section of the Holtemme, the lowest total and fecal coliform concentrations were measured in April and October, under two very different discharge situations (1960 L/s and 367 L/s, respectively). The highest fecal coliform concentration (945/(100 mL)) was recorded in June, the highest total coliform concentration in July (3106/(100 mL)). Both values imply a moderate pollution level (Fig. 5(a)).

Further downstream, in the midstream section below Silstedt WWTP (Fig. 5(b)), instream concentrations were at least one order of magnitude higher and in general more stable. The lowest concentrations were recorded in April, and the peak for both total and fecal coliforms occurred in August (TC 55,995/(100 mL); FC 21,760/(100 mL)). At

that time, not only the highest temperatures in the mid- and downstream sections were recorded (20.6°C to 23.2°C), but also the lowest discharge (298 L/s) during the observation period. Both the low dilution factor (i.e., high wastewater discharge vs. streamflow) and warm temperatures are plausible reasons for the high microbial loads, signifying a critical or even strong pollution level.

Coliform concentrations are only one side of the medal, and do not correlate well with coliforms loads. Low discharge situations can result in comparatively high coliform concentrations (because of little dilution), but not necessarily in very high coliform loads (because of limited discharge). Conversely, relatively low concentrations measured during high discharge situations can actually represent high coliform loads transported by the stream (Table 4). Nevertheless, rising pollution levels along the river result in a significant increase in coliform loads.

4 Discussion

Even though the Holtemme is not an excessively polluted

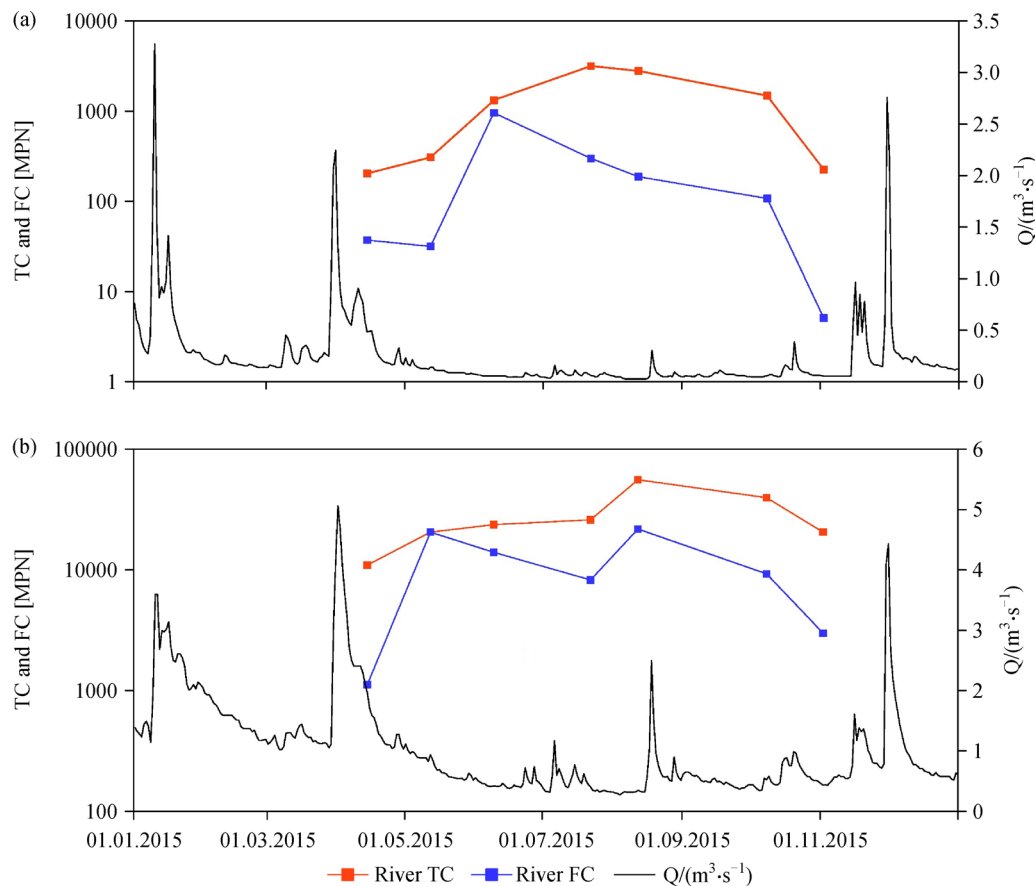


Fig. 5 Temporal pattern of instream coliform concentrations and discharge at the uppermost sampling site, Steinerne Renne (a) and in the midstream section below the wastewater treatment plant in Silstedt (b). Discharge data based on LHW Sachsen-Anhalt 2016. FC = fecal coliforms, MPN = most probably number; Q = discharge; TC = total coliforms.

Table 4 Total coliform (TC) concentrations and loads during different discharge situations. MPN = most probably number. Upstream refers to site 1 (Steinerne Renne), midstream to site 4 (downstream of Wernigerode). High and low discharge refers to the highest and lowest discharge situations recorded during the sampling period

Item	Upstream high discharge	Upstream low discharge	Midstream high discharge	Midstream low discharge
Discharge/(m ³ ·s ⁻¹)	0.479	0.019	1.96	0.331
TC concentration/ (MPN per 100 mL)	200.5	2,737.5	10,910	55,995
Pollution level according to Kirschner et al. (2009)	Little	Moderate	(Moderate to) critical	Critical
TC load/(bacteria·s ⁻¹)	960,395	520,125	213.8 mill.	185.3 mill.

water body, instream concentrations of total coliforms on average indicate a moderate pollution before the first WWTP (sampling sites 1 and 2), and a critical pollution in all sampling sites further downstream. According to the classification scheme mentioned above (Kirschner et al., 2009), only a small fraction of the samples taken in the upstream section fell into the ‘little pollution’ category; similarly, only few samples downstream of the first WWTP fell into the ‘moderate pollution’ category; the majority of samples showed a higher degree of pollution. The same picture is true for fecal coliforms, for which average pollution levels downstream of the first WWTP would even have to be classified as ‘excessive’.

A clear gradient in surface water hygiene can be observed along the Holtemme. Increases in both total and fecal coliform concentrations occur below the influx points of treated wastewater, which itself contains coliform concentrations that are about one order of magnitude greater than surrounding instream concentrations. Not surprisingly, wastewater discharge by the municipal treatment plants is therefore a major driver of hygienic water quality. However, it is apparently not the only driver of water hygiene in the Holtemme: between sampling sites 1 and 2, there is a notable increase in both total and fecal coliform concentrations. Downstream of the WWTPs, coliform concentrations decrease by factors of 3 to 4, indicating a self-purification of the river. While bacterial biodegradation (e.g., of nutrients) is considered to be one of the most important forms of river self-purification (Edeline and Lambert, 1979), less is known about the fate of bacteria such as coliforms in the river. However, there is compelling evidence that sunlight (via by UV sterilization) and temperatures play an important role for the inactivation of bacteria in surface waters (Noble et al., 2004; Schultz-Fademrecht et al., 2008). Moreover, tributaries not impacted by wastewater which enter the Holtemme downstream of the two wastewater treatment plants are likely to dilute the coliform concentrations.

5 Conclusions

Even though there is no legal mandate for a hygienic

monitoring in small streams like the Holtemme, our results show that contamination levels are critical, albeit with large ranges of spatial and temporal variation that require a better understanding. While influx of treated wastewater could be identified as the major determinant of instream coliform concentrations, there appear to be additional sources. Even though electric conductivity and temperature showed the same general (i.e., increasing) trend along the river, these routinely monitored water quality indicators were only poorly correlated with coliform concentrations. Therefore, they are not well suited as predictors for microbiological water quality. However, the limited data collected at roughly monthly intervals between April and November 2015 mean that additional samplings would be helpful, particular for situations that reflect moister conditions than the below average streamflow which prevailed during most of the year 2015. Moreover, further research into a) the identification of coliform sources as well as causes for decreases and b) correlations between coliforms and other water quality parameters would help to better understand the spatio-temporal dynamics of water hygiene.

A better understanding of instream coliform sources and inactivation could in the future not only help to assess public health risks associated from bacteriological pollution in surface waters (e.g., Mälzer et al., 2015), but also improve models of coliform transport in river systems (e.g., Reder et al., 2015) which are important tools for the assessment of coliform loads transported on larger scales.

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