Wastewater disinfection at river Ilz to improve bacteriological water quality: effects and constraints

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Keywords: fecal indicator bacteria; microbial source tracking; bathing water quality; UV irradiation; membrane filtration; ozonization

1 Introduction

Contamination with fecal microorganisms often hampers the use of surface waters for bathing purposes. Exposure to fecally polluted water might cause severe infections like gastroenteritis or else respiratory, eye-, ear-, and skin-related illnesses. Waterborne pathogens frequently involved in gastrointestinal diseases include enteric bacteria (e.g. *Salmonella, Yersinia, Clostridium,* and *Campylobacter* species), enteric viruses (Enteroviruses, Hepatitis A virus, Norwalk virus), and protozoan parasites (*Cryptosporidium* and *Giardia* species) (Stewart et al. 2007).

There are different sources that may deteriorate water quality: Municipal wastewater is one of the major sources and contains pathogenic microorganisms excreted by ill human beings and chronic carriers. Other point sources are storm water and sewer overflows. Moreover, fecal pollution can also originate from non-point sources like agricultural runoff or wildlife. Hence, effective measures to improve bathing water quality imply a detailed evaluation of the contamination origin.

If fecal input mainly results from wastewater treatment plants (WWTP) disinfection of secondary effluents is supposed to noticeably improve microbiological water quality. During wastewater treatment the number of bacteria decreases only marginally. This is even true for secondary treatment, such as precipitation, sand filtration, and polishing ponds (Baumann and Popp 1991). A satisfying reduction of fecal microorganisms – including pathogens – is only achieved by specific disinfection procedures like UV irradiation, membrane filtration, ozonization, or chlorination (Popp 1998).

Within a restoration project initiated by the Bavarian State Ministry of the Environment and Public Health to improve the microbiological quality of the River Ilz – a tributary of the Danube from the Bavarian Forest – a total of five WWTPs has recently been equipped with disinfection systems (UV irradiation; membrane filtration; ultrasound/ozone treatment). To monitor the disinfection efficiencies and the resulting effects on the water quality of the Ilz fecal indicator organisms were determined as prescribed in the Bathing Water Directive (Tab. 1). As these parameters indicate the general contamination of a water body only but do not provide any information about its origin an integrated approach was used for tracing back the sources of the remaining fecal pollution. This approach combined the already mentioned cultivation-based determination of fecal indicators and a quantitative Microbial Source Tracking (MST) method identifying genetic *Bacteroidetes* markers specific to human and ruminant hosts.

Table 1. *Microbiological parameters of the Bathing Water Directive 76/160/EEC*

Parameter	Guide value [per 100 ml]	Mandatory value [per 100 ml]
Fecal coliforms (*E. coli*)	1.0×10^2	2.0×10^3
Intestinal enterococci	1.0×10^2	-

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2 Material and Methods

Sampling: From April to October 2009 water samples were collected fortnightly from six sites along the River Ilz and from four tributaries between Hutthurm and Passau. Effluent samples were taken from the five WWTPs Hutthurm, Ruderting, Salzweg, Straßkirchen, and Neukirchen before and after wastewater disinfection. All samples were collected in sterile glass bottles and stored at 4°C until further processing (maximum 24 hours).

Fecal indicator organisms: Enumeration of *E. coli* and intestinal enterococci was carried out by standardized most probable number methods using microplates (ISO 9308-3 and 7899-1) with 1 (disinfected wastewater), 2 (river samples), or 4 dilutions (non-disinfected wastewater).

Microbial Source Tracking (MST): For quantification of the source-specific genetic markers aliquots of the samples were filtered through 0.2 µm polycarbonate membrane filters and stored at -80 °C until nucleic acid extraction. DNA extraction was performed according to Griffiths et al. (2000). Taq-Man qPCR analyses were carried out as described by Reischer et al. for ruminant- (2006) and human-specific (2007) *Bacteroidetes* markers on a CFX96 Real-Time System (Bio-Rad).

3 Results and Discussion

3.1 Evaluation of disinfection efficiencies

The different disinfection systems at five WWTPs along the River Ilz between Hutthurm and Passau mostly achieved a satisfying reduction of the fecal indicators *E. coli* and intestinal enterococci.

The principle of **membrane filtration** is based upon the separation of solids (particles, bacteria, viruses) suspended in an aqueous solution by means of a pressure difference. This technique yielded high reduction rates at WWTP Hutthurm. The effectivity of membrane filtration to remove microorganisms from wastewater has already been demonstrated in various pilot and technical plants in Germany and other countries (Wintgens et al. 2005; Shang et al. 2005; Bleisteiner et al. 2006). Membrane fouling, however, turned out to be a problem at WWTP Hutthurm causing severe flux decline and requiring intense cleaning efforts with chemicals harmful to the environment.

UV irradiation led to reduction rates of up to four log-units. At WWTP Ruderting more than 90 % of the disinfected effluent samples complied with the guide values of the Bathing Water Directive. Technical problems currently existing at WWTPs Salzweg and Strasskirchen led to considerable fluctuations of the fecal indicator concentrations in the effluent. These should, however, be resolved in the near future. Surveys at the River Isar already proved UV disinfection to be an environmentally sound and economically competitive method (Popp et al. 2004).

The third system for advanced sewage treatment combines **ultrasound irradiation and ozone treatment**. The applicability of this new technique was investigated in a commercial scale reactor installed at WWTP Neukirchen. Besides wastewater disinfection this technique is supposed to also be suitable for removal of micro-pollutants, i.e. pharmaceuticals or endocrine disruptors. For optimizing reactor performance different operation conditions were tested. Thus, reduction rates of fecal indicators showed considerable fluctuations due to the varying conditions. Ozone dosage of 8 g/h, for example, yielded indicator concentrations below the guide values of the Bathing Water Directive.

The results indicate that secondary effluents no longer account for a considerable fecal pollution in the investigated stretch.

3.2 Monitoring of the microbiological water quality

The effects of the advanced wastewater treatment on the microbiological water quality of the Ilz were monitored by determination of *E. coli* and intestinal enterococci. Under dry weather conditions fecal indicator concentrations never exceeded the mandatory values of the Bathing Water Directive at the six sampling sites along the Ilz during operation of the disinfection systems (Fig. 1). *E. coli* and enterococci levels varied between $4.3 \times 10^1 – 1.5 \times 10^3$ MPN per 100 ml and $1.0 \times 10^1 – 2.9 \times 10^2$ MPN per 100 ml, respectively. Compliance with the more stringent guide values, however, was only achieved in two cases at the sampling site “Triftsperre”. Intense rain events led to a significant impairment of the microbiological water quality. Mandatory values were continuously exceeded (Fig. 1) with maximum concentrations of 2.8×10^4 and 1.7×10^4 MPN per 100 ml for *E. coli* and enterococci, respectively.

A slight improvement of the water quality was observed at the sampling sites “Unterilzmühle” and “Triftsperre”. Heavy rain falls also caused a significant increase of fecal indicator concentrations in the four investigated tributaries (Fig. 1). Median values were about 1 log-unit higher during rain events.

The investigations revealed a considerable persistent contamination in the stretch between Hutthurm and Passau, although secondary effluents – as described – no longer contribute to fecal pollution. Noticeably *E. coli* and enterococci concentrations did not comply with the guide (dry weather) and mandatory values (rain events) even at the first sampling site “Pegel Kalteneck”. This refers to high rates of fecal input in the catchment area of the Upper Ilz (upstream “Pegel Kalteneck”). Hence, a quantitative MST method was applied to specify the sources of the still existing fecal contamination.

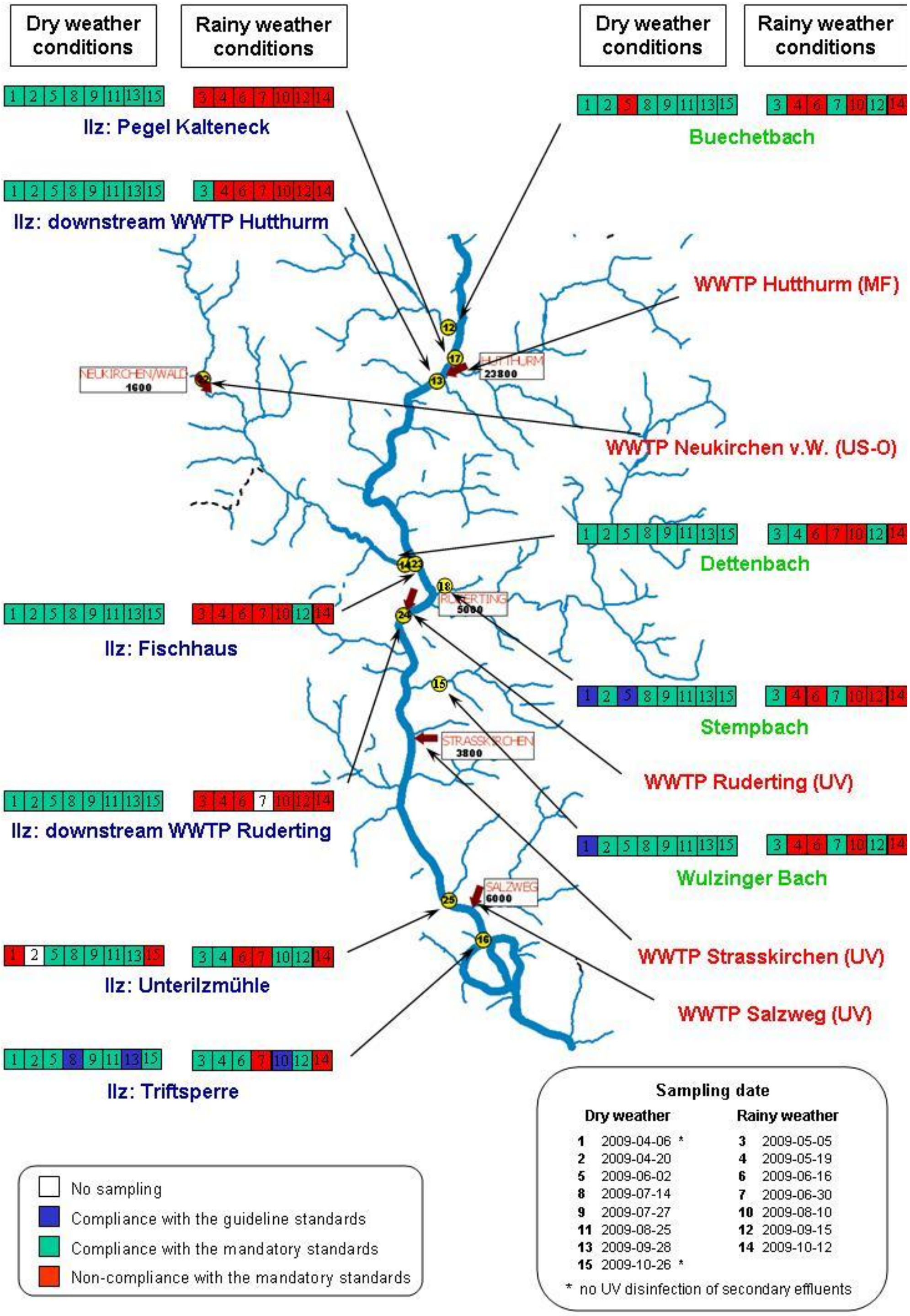

Figure 1. *Microbiological water quality at six sampling sites along the Ilz and of four tributaries between Hutthurm and Passau under dry weather (left column) and rainy weather conditions (right column). Water quality was assessed by estimation of fecal indicator organisms according to the Bathing Water Directive. MF: membrane filtration; US-O: ultrasound/ozone treatment; UV: ultraviolet irradiation.*

3.3 Microbial Source Tracking

Genetic *Bacteroidetes* markers specific to human (BacH) and ruminant (BacR) hosts were detectable at all sampling sites along the Ilz with almost equal frequency. No clear dominance could be observed. The occurrence of these markers in the environment and their temporal genetic stability has already been proved in previous surveys (Reischer et al. 2008). Under dry weather conditions median concentrations of around 1 x 10^4 BacH and 4 x 10^3 BacR marker equivalents (ME) per 100 ml were detected (Fig. 2). Both marker concentrations increased after heavy rain falls to median concentrations of 3.4 x 10^4 -5.4 x 10^5 ME per 100 ml for BacH and 1.5 x 10^3 – 1.4 x 10^5 ME per 100 ml for BacR (Fig. 2). In some cases this increase was more distinct for the human-specific marker and yielded BacH levels up to 2.5 log-units higher than BacR (e.g. at "Unterilzmühle" and "Triftsperre"). In the investigated tributaries the markers revealed considerable fluctuations concerning their dominance. Whereas in the Dettenbach the human-specific BacH marker was detected at levels far exceeding BacR throughout the study and irrespective of the weather conditions (BacH median: 2.9 x 10^4 ME per 100 ml; BacR median: 4.2 x 10^2 ME per 100 ml), no clear dominance of one of both markers was observed in the other tributaries Buechetbach, Stempbach, and Wulzinger Bach. For BacH and BacR both equal marker concentrations were found as well as varying dominance at different sampling times.

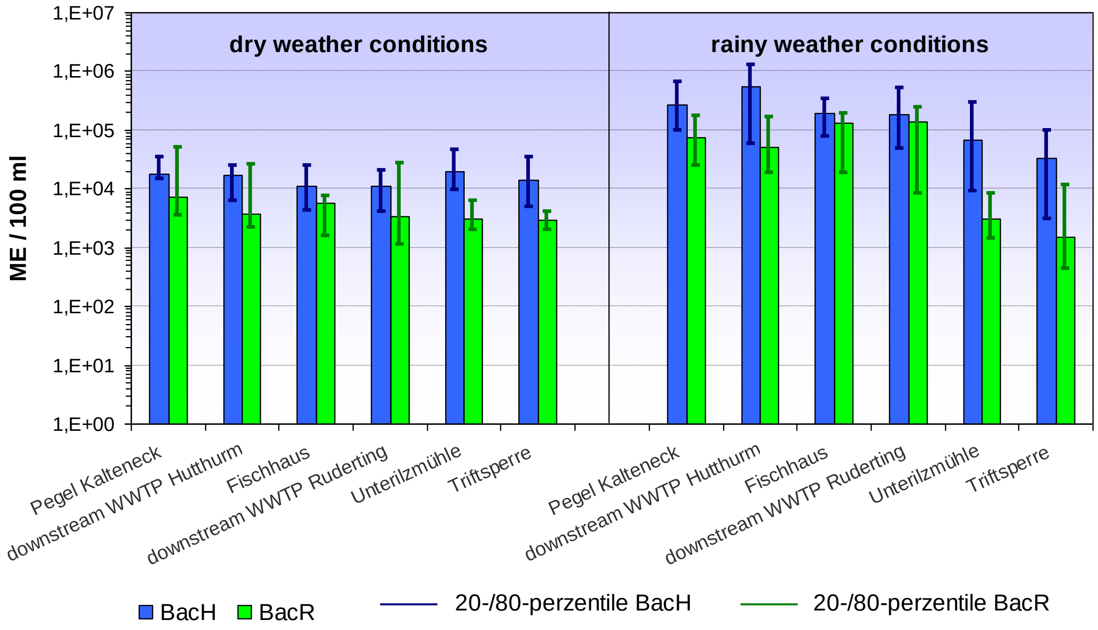

Figure 2. *Median values of genetic Bacteroidetes markers specific to human (BacH) and ruminant (BacR) hosts at six sampling sites along the Ilz. Error bars indicate the 20- and 80-percentile values. ME: marker equivalents.*

The results indicate both an agricultural and a human impact on the microbiological water quality. Whereas BacR markers presumably derive from surface runoff from agricultural fields, high BacH marker concentrations are probably due to the numerous small WWTPs in the catchment area of the Upper Ilz and to rural communities missing wastewater management infrastructure. Moreover, sewer and storm water overflows may be responsible for elevated levels of the human-specific marker, especially after heavy rain falls. Further investigations will be carried out in the bathing season 2010 to obtain enough data for statistical correlation analyses.

4 Conclusions

- Wastewater disinfection at five WWTPs along the River Ilz between Hutthurm and Passau mostly revealed satisfying reduction rates of fecal indicator bacteria. Thus secondary effluents no longer account for a considerable pollution.
- During the bathing season 2009 fecal indicator concentrations complied with the mandatory values of the Bathing Water Directive under dry weather conditions. Intense rain events, however, caused a

significant impairment of the microbiological quality of the Ilz and of four investigated tributaries. Mandatory values were then constantly exceeded.

- Microbial Source Tracking was applied to specify the sources of fecal contamination. MST analyses revealed both human and ruminant impact. The high human-specific marker concentrations, especially after heavy rain falls, suggest that sewer and storm water overflows as well as rural communities missing a wastewater management infrastructure are important causes for the high fecal loads.

Acknowledgements

Financial support by the Bavarian State Ministry of the Environment and Public Health is gratefully acknowledged. We wish to thank Monika Hanf and Julia Weinberger for expert technical assistance.

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