Water Research 112 (2017) 248–253

Contents lists available at ScienceDirect

Water Research

journal homepage: www.elsevier.com/locate/watres

Mobilisation of microbial indicators, microbial source tracking markers and pathogens after rainfall events

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ARTICLE INFO

Article history:
Received 28 November 2016
Received in revised form
31 January 2017
Accepted 1 February 2017
Available online 2 February 2017

Keywords:
Microbial source tracking
Global change
Pathogen ecology
Growth and survival
Modelling

ABSTRACT

Climate change is expected to affect the Mediterranean region by causing an increase in the number of heavy rainfall events. The aim of this study was to assess the effect of extreme river flow variations due to rainfall on the persistence and mobilisation of various microorganisms. These included faecal pollution indicators (*Escherichia coli* (EC), somatic coliphages (SOMCPH) and sulphite reducing clostridia spores (SRC)), microbial source tracking indicators (*Bacteroides thetaiotaomicron* GA17 strain phages (GA17PH) and sorbitol fermenting bifidobacteria (SFBIF)), and two pathogens (*Salmonella* spp and *Enterovirus*). Water and sediment samples were taken at different distances from the river before and after heavy rainfall events. The microbial load was higher in sediment samples closer to the river course. The concentration of some faecal indicators (EC and SFBIF) increased in sediments and river water after rainfall events, whereas the most conservative parameter (SRC) showed almost no variation. After rainfall, the indicators persisted at a different rate. *Salmonella* spp and *Enterovirus* were detected in some samples but always at lower concentrations than the microbial indicators. In conclusion, sediments are reservoirs of faecal and MST indicators and pathogens and could therefore pose a risk of pathogen dissemination.

1. Introduction

Heavy rainfall events have frequently been associated with outbreaks linked to drinking water obtained from surface water, groundwater, bathing waters and shellfish consumption (MacKenzie et al., 1994; Curriero et al., 2001; Hrudey et al., 2003; Thomas et al., 2006; Nichols et al., 2009; Drayna et al., 2010; Chen et al., 2012; Tornevi et al., 2013). Increased amounts of faecal-oral waterborne pathogens and microbial indicators have even been reported in finished tap water after rainfall events. The cause of this increase is not clear; some authors have related it to mobilisation of pathogens previously entrapped in sediments (Jamieson et al., 2005), while others have suggested that wastewater treatment plant (WWTP) failures after heavy rainfall events are the main source of this faecal contamination (Curriero et al., 2001). Although water treatment plants are designed to protect human health, they can become overloaded after heavy rainfall events and unable to cope with the subsequent increase in the number of pathogens (Curriero et al., 2001). For example, the 1993 cryptosporidiosis outbreak in Milwaukee was attributed to post-rainfall runoff containing high levels of *Cryptosporidium* which the treatment plant could not remove (MacKenzie et al., 1994), while the 2000 outbreak in Walkerton (Canada) was linked to *E. coli* O157 contamination of the water supply after a heavy rainfall event (Hrudey et al., 2003). Research on this topic is therefore of extreme importance to develop predictive models that help reduce the overall associated risk to humans within the future scenario predicted by climate change experts, namely an increased number of heavy rainfall events in the Mediterranean region (Confalonieri et al., 2007).

The Mediterranean climate is characterised by irregular distribution of rainfall periods, with a higher concentration of heavy rainfall events in autumn, and to a lesser extent, in spring. This uneven distribution of precipitation leads to significant flow regime variations in Mediterranean rivers over the course of the year, typically ranging from 5 m^3/s up to 1000 m^3/s after rainfall events or snowmelt, according to data from the Catalan Water Agency. Mediterranean rivers thus present suitable environments for studying microorganism mobilisation in order to develop predictive models within the future climate change scenario.

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http://dx.doi.org/10.1016/j.watres.2017.02.003
0043-1354/

It is too expensive to identify individual faecal pathogens for water quality monitoring, and therefore global practice is to detect microbial indicators instead. In recent years, some countries have incorporated new microbial indicators such as bacteriophages in their regulations, including Colombia (Republica de Colombia, 2014), Australia (Queensland Government, 2005; Australian Government, 2006; Western Australia Government, 2012), Canada (Anonymous, 2001b) and the USA (USEPA, 2006; North Carolina Administration, 2011). Many studies have assessed the usefulness of the main bacterial indicators such as *E. coli* or enterococci after heavy rainfall events, but little is known about the dynamics of these "alternative" indicators in such events. In addition, alternatives to traditional indicator detection methods, such as quantification of enterococci by qPCR, have also recently been accepted for recreational water monitoring in the USA (USEPA, 2012), but little information is available on the detection of indicators by qPCR to predict the associated human risk in rainfall events.

The aim of this research was to study the dynamics of different microbial indicators, including *E. coli* (EC), somatic coliphages (SOMCPH) and sulphite reducing clostridia spores (SRC), and two pathogens, *Salmonella* spp (SAL) and *Enterovirus* (ENTV), in the presence of rainfall events in a Mediterranean river used as model to assess microorganism mobilisation after these events. The use of a molecular target, EC detection by qPCR, was also assessed. In addition, two microbial source tracking indicators (*Bacteroides thetaiotaomicron* GA17 strain phages (GA17PH), and sorbitol fermenting bifidobacteria (SFBIF)) were assessed in order to evaluate their behaviour in heavy rainfall events. The data obtained on the persistence and mobilisation of these microorganisms will be useful for microbial risk assessment in the future.

2. Material and methods

2.1. Sampling

The sampling site was located in the Spanish Mediterranean basin, near the end of the Llobregat river course and close to the catchment point of a drinking water treatment plant. The Llobregat river mainly receives contamination of human origin in its lower transect (Casanovas-Massana et al., 2015), and has an annual mean river flow of 12 m^3/s, although this can vary from 3 m^3/s to 1200 m^3/s during a heavy rainfall event and/or during snowmelt from the mountains.

Samples (n = 52) of river water and sediment (1 cm deep), were taken after heavy rainfall (wet sampling campaign) and during a period of at least 15 days without rainfall events (dry sampling campaign), over one year. Sediment samples were divided into two categories according to contact with river water: A, sediments in permanent contact with river water; and B, sediments which were occasionally (rainfall event) covered by river water. Samples were taken aseptically in sterile containers, transferred to the laboratory at 4 °C and analysed within 4 h. Particle size was analysed with a Beckman Coulter LS Particle Size Analyser.

2.2. Enumeration of microorganisms by culturable methods

Enumeration of the bacterial indicators was performed as follows. Briefly, sediment samples were mixed at a 1:10 ratio in phosphate buffered saline (pH 7.0) (PBS), homogenised by shaking for 30 min at room temperature and centrifuged at 300 × g for 3 min. The supernatant was collected and used for subsequent analyses. In the case of river water samples, the water was analysed without any pre-treatment. EC were enumerated using the pour plate method in Chromocult agar (Merck, Germany) (Astals et al., 2012). SRC were enumerated using mass inoculation in SPS agar and incubated for 24 h at 44 °C, as previously described (Ruiz-Hernando et al., 2014). TBIF and SBIF were enumerated using HBSA selective medium, which enables differentiation of sorbitol fermenting bifidobacteria, some of which may have a human origin (Mara and Oragui, 1983). SAL was quantified by the MPN method using presence/absence tests on 10, 1 and 0.1 g of sediment as previously described (Guzman et al., 2007a).

SOMCPH and GA17PH were extracted from sediments as described previously (Guzman et al., 2007b). In the case of river water samples, they were filtered through 0.22 μm pore size polyethersulphone non-protein binding membrane filters (Millipore, USA). Bacteriophages were counted using the double layer agar method (Anonymous, 2000, 2001a).

2.3. Enumeration of microorganisms by molecular methods

Total EC were enumerated by quantification of a fragment of 16S rRNA gene by a qPCR TaqMan® assay using the TaqMan® Environmental Master Mix 2·0 (Applied Biosystems, Barcelona, Spain) as previously described (Pascual-Benito et al., 2015). Briefly, DNA was extracted from 0.25 g of sediment sample using a PowerSoil® DNA isolation kit (Mo Bio Laboratories, Inc., Carlsbad, CA, USA) and eluted in 100 μl elution buffer provided in the kit. In the case of river water samples, DNA was extracted from 1 ml of sample using a QIAamp DNA blood minikit (Qiagen GmbH, Hilden, Germany) and eluted in 200 μl elution buffer provided in the kit. Samples were analysed in duplicate and the absence of PCR inhibitors in the samples was assessed by diluting the samples at a 1:10 ratio in PCR-grade water.

For ENTV detection, 2 L of river water samples was filtered through 3 μm pore size cellulose nitrate membrane filters. Viral particles were released by agitation with 0.25 M glycine +3% beef extract solution (pH 9.5). After adjusting pH to 7, samples were filtered through 0.22 μm pore size low protein binding polyethersulphone membrane filters. Sediment samples were mixed at a 1:10 ratio in 0.25 M glycine solution (pH 9.5) and homogenised by shaking at room temperature. Samples were then clarified by centrifugation at 1800 × g. Subsequently, two ultracentrifugation cycles at 80,000 × *g* for 1 h were applied and viral particles were recovered in 140 μl PBS. ENT were quantified by RT-qPCR using a previously described method (Martin-Diaz et al., 2016). All the quality and inhibition controls described in Martín-Díaz et al. (2016) were followed.

2.4. Statistical analyses

The values obtained were $\log_{10}$ transformed to perform statistical analysis. The Kruskal Wallis test was performed to assess indicator distribution differences. Kendall's Tau coefficient was used to study the correlation between cultivable *E. coli* (cEC) and total *E. coli* (tEC).

3. Results

3.1. Descriptive characteristics of the samples

The characteristics of the analysed samples are shown in Table 1. The dry weight content of the samples increased significantly with distance to the river (B > A, $p = 0.0283$ and $p=0.0055$ in dry and wet sampling campaign samples, respectively). The river flow presented a maximum of 120 m^3/s, with average values ranging from 5.6 m^3/s to 37.0 m^3/s in dry and after rainfall measurements, respectively. Turbidity increased up to 1994.0 NTU after rainfall, with an average of 38.4 NTU and 503.6 NTU in dry and wet