Marine Pollution Bulletin 133 (2018) 762–772

Contents lists available at ScienceDirect

Marine Pollution Bulletin

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

Fecal contamination in shallow temperate estuarine lagoon: Source of the pollution and environmental factors

M. Kataržytė[a,*], J. Mėžinė[a], D. Vaičiūtė[a], S. Liaugaudaitė[a], K. Mukauskaitė[a], G. Umgiesser[b,a], G. Schernewski[c,a]

[a] Marine Research Institute, Klaipėda University, H. Manto 84, LT-92294 Klaipeda, Lithuania
[b] ISMAR-CNR, Institute of Marine Sciences - National Research Council, Castello 2737/F, 30122 Venice, Italy
[c] Leibniz Institute for Baltic Sea Research Warnemünde (IOW), Seestrasse 15, D-18119 Rostock, Germany

ARTICLE INFO

Keywords:
Bathing water quality
Turbidity
Escherichia coli

ABSTRACT

In inner coastal waters such as lagoons, which are very turbid and rich in suspended particles, the survival of fecal pollution microorganisms may find favorable environments. In order to better characterize the sources and dynamics of fecal pollution in a strongly turbid environment, in situ observations were made in the Curonian Lagoon. A combination of traditional monitoring and molecular methods were used. To monitor the water quality, the indicator *Escherichia coli* (EC) was selected as a proxy for fecal contamination. *E. coli* concentration correlated with environmental parameters as pH, oxygen and turbidity. The main pollution sources are the sewage outlets in the lagoon area, while the pollution coming via rivers did not play a significant role. Still the human associated *E. coli* consisted only of 0 up to 20% of analyzed isolates, and did not correlate with the *E. coli* concentrations in the study sites. The role of birds, especially for potentially virulent *E. coli* may be underestimated in the lagoon.

1. Introduction

Transitional waters such as coastal lagoons are important ecosystems from the ecological and economic perspective. They provide many valuable ecosystem goods and services (Newton et al., 2014). However, those ecosystems are exposed to environmental pressures, mainly related with anthropogenic activities. Human populations are concentrated in coastal areas. Urbanization, extensive agriculture, sewage discharge and recreation can result in an increase in the flow of microbial pollutants.

Due to the increase in tourism in coastal areas (Meiner et al., 2013) and local communities' needs for recreational sites, there is a demand to have sanctioned beaches not only along the sea, but also in the inner coastal waters such as lagoons or bays (Schernewski et al., 2017). Still due to special environmental conditions of the inner coastal waters: higher turbidity and algal blooms, weaker dilution of pollutant, shallowness, and higher concentration of particulate organic matters, the microbial (fecal) pollution exposure and related potential risks can be much higher than in the sea environment. Bathing water in officially established beaches according the Bathing Water Directive (2006/7/EC) should be monitored for fecal pollution using two indicators: *E. coli* and *Enterococci*. Different indicator thresholds are used for inland (rivers and lakes), coastal and transitional waters for bathing water quality assessment.

The fecal pollution depends on the interaction between physical forcing, biological and biochemical processes (Pommepuy et al., 2005) and can vary greatly depending of the body of water (Sinton et al., 1999; Campos et al., 2013; Perkins et al., 2016). It is known that comparably high amounts of the organic matter and turbidity can favor the fecal bacteria survival (Clermont et al., 2011; Quero et al., 2015; Tymensen et al., 2015; Perkins et al., 2016). In the turbid systems the role of suspended particles together with other environmental factors could be of significant importance in promoting the growth and distribution of pathogenic bacteria (Amalfitano et al., 2017). Additionally, dissolved organic materials (DOM) can also be an important trigger of enhanced microbial pollution, to suspended solids (Andersson et al., 2015). DOM in aquatic ecosystems also known as yellow substances, gelbstoff or colored dissolved organic matter (CDOM) has a strong absorption in the ultraviolet (UV) portion of the spectrum and protects aquatic biota including bacteria from damaging UV medium wave radiation (Blough and Green, 1995). CDOM itself is eventually destroyed by sunlight, releasing nutrients and trace elements, which sustain the growth of bacteria (Moran and Zepp, 1997). CDOM may be present in large amounts in inner coastal waters like lagoons or estuarine

* Corresponding author.
E-mail address: marija.katarzyte@jmtc.ku.lt (M. Kataržytė).

https://doi.org/10.1016/j.marpolbul.2018.06.022
Received 12 October 2017; Received in revised form 5 June 2018; Accepted 8 June 2018

ecosystems (Harvey et al., 2015; Vaičiūtė et al., 2015) and play an understudied role for microbial pollution (Wikner and Andersson, 2012).

Of no less importance for pollution prevention in the aquatic environment is the understanding of the sources of pollution. Different molecular methods are recently used for discriminating between sources of indicator bacteria in natural waters. Genetic fingerprinting and repetitive extragenic palindromic-PCR (rep-PCR) methods are quite popular, and are used as a tool to assess the genetic diversity of *E. coli* populations from different sources (Mohapatra et al., 2007). They are considered an effective tool for monitoring freshwater environments (Baldy-Chudzik and Stosik, 2005). This technique uses primers complementary to repetitive sequences distributed throughout the genome of the bacterium and can differentiate between closely related strains (Versalovic et al., 1991). Some recent studies also are using genetic markers which allow the identification of host-specific regions in the environmental *E. coli* DNA (Gomi et al., 2014; Warish et al., 2015). None of the above mentioned methods, to our knowledge, have been used in the Baltic Sea coastal waters for source tracking of *E. coli*.

The main sources of pollution in the south-eastern part of the Baltic Sea are the effluents of industrial and domestic wastewater and agriculture runoff into rivers (Štukova, 2004; Schippmann et al., 2013). During the last decade, in countries surrounding the Baltic Sea, much effort was given to increase the quality of the waters threatened by nutrient input, as well as renovating and implementing wastewater sewage systems. Thus could have an effect on the reduction of fecal pollution, too.

In one of the biggest temporally brackish water bodies in Europe, the Curonian Lagoon, in the past pollution has reached thresholds (Štukova, 2004). Despite > 10 years of monitoring along the Baltic Sea coastline, where the beaches have obligatory monitoring, the last data about fecal bacteria pollution in the Curonian Lagoon is from the year 2004 (except one official beach where the monitoring is performed since 2011). Currently, intensive recreational activities such as kitting, fishing and bathing are observed in the lagoon and there are often high risks of people being exposed to fecal pollutants.

The lack of knowledge about the recent situation in the lagoon area, and potential pollution sources in the light of pollution mitigating circumstances requires deeper insight. Our research focus was to evaluate the recent water quality situation based on *E. coli* evaluation and to identify the potential sources of pollution based on the microbial source tracking using the molecular techniques. We also analyzed how external drivers and internal processes influence fecal pollution in the turbid lagoon environment.

2. Study site and methods

2.1. Study site and period

The Curonian Lagoon is situated in the southeastern part of the Baltic Sea (Fig. 1) and it is the largest lagoon (surface area 1584 km^2) in Europe. The lagoon is shallow (mean depth 3.8 m) and hypereutrophic, almost entirely restricted from the Baltic Sea (Žaromskis, 1996). The Klaipeda Strait provides the only narrow connection (width of 0.4 km) to the Sea, while in the eastern part of the lagoon the Nemunas River (one of the largest rivers in the Baltic region) enters the lagoon. Therefore, the Curonian Lagoon is always influenced by mixing masses of brackish and fresh-riverine waters. The inflow of brackish-waters depends on wind speed and its direction, whereas the largest part of the lagoon consists of fresh water, which is mainly influenced by discharge from the Nemunas River. Intrusions of brackish waters usually occur in the northern part of the Curonian Lagoon, but sometimes they were recorded 40 km away from the Klaipeda Strait (Zemlys et al., 2013).

Choosing the sampling sites in the Curonian Lagoon were based on two criteria: a) potential pollution *source* (sewage outlet, river entering lagoon, agriculture, numerous bird activity) b) and *sinks*, where recreational activities are observed in the water (bathing, boating, kitting) or official bathing site (Table S1).

2.2. Quantification and sampling of Escherichia coli and total coliforms

The samples for the *E. coli* and coliform analysis were sampled monthly in 2015 and 2016 from May to August throughout the whole study area (Fig. 1). Samples were taken 30 cm below the water surface in one-meter depth. 250 ml sterile polypropylene bottles were used for sampling. Water samples were protected from exposure to light and kept in a cool box until arrival at the laboratory and analyzed on the same working day. Membrane filtration method (ISO 9308-1:2014) was used for evaluation of *E. coli* concentration: the results were expressed as the colony forming units (cfu) of total coliforms and *E. coli* present in 100 ml of water. According to the Bathing Water Directive (2006/7/EC) the thresholds for *E. coli* specifically in coastal and transitional waters is 500 cfu 100 ml^{-1} (based upon a 90-percentile evaluation) for classifying the bathing water as poor (insufficient) quality.

Additionally, data (year 2011–2016) of *E. coli* and *Enterococci* from two study points (K4 and K1) were taken from Silute municipality administration data.

A single *E. coli* colony was selected from each plate (up to 10 from each sampling site per sampling date). Isolates showing the *E. coli* phenotypic characteristics were incubated overnight at 37 °C in Luria-Bertani agar plates and used for further molecular analysis. Since the initial isolated *E. coli* number varied, different numbers of isolates were used for molecular analysis.

2.3. Molecular E. coli analysis

For fingerprint analysis of *E. coli* only samples from 2015 year were used. Rep-PCR fingerprints have been obtained (Fig. 2) with the use of the primer: BOX A1R.

For the same isolated strain of *E. coli* (2015 and additionally 2016) a) two specific genetic markers of human wastewater-associated *Escherichia coli* and b) Shiga-toxigenic *E. coli* associated virulence genes were used (Table S2). The amplification reactions (Table S3) have been carried out in “Mastercycler® pro” (Eppendorf AG, Germany). Gel images were obtained using a Molecular Imager (DNR Bio-Imaging Systems Ltd., Israel).

2.4. Environmental variables

Temperature, salinity and dissolved oxygen (DO) were measured in situ during field campaigns using YSI 460 multiple probe. Additionally, surface water samples were collected in 2015 monthly from May to August to investigate the absorption of colored dissolved organic matter (aCDOM), organic (SPOM) and inorganic (SPIM) fractions of suspended particulate matter (SPM) and chlorophyll *a* (Chl-*a*) analysis. Water samples for Chl-*a* measurement were filtered through glass fiber GF/F filters with a nominal pore size 0.7 μm and extracted into 90% acetone for 24 h at 4 °C. Chl-*a* was analyzed by spectrophotometry (Jeffrey and Humphrey, 1975; Parsons et al., 1984). Water samples for SPM measurements were filtered through pre-combusted glass fiber GF/F filters (nominal pore size 0.7 μm) and assessed gravimetrically using the method proposed by Strickland and Parsons (1972). Organic and inorganic fractions were determined after combustion of filters at 550 °C for 4 h. CDOM was measured spectrophotometrically after filtration through 0.22 μm membrane filters. The CDOM absorption coefficient at 440 nm (aCDOM) was derived according to Kirk (2011). Turbidity was measured with a portable turbidity meter Eutech Instruments TN-100 (Landsmeer, The Netherlands) in Nephelometric Turbidity Unit (NTU).

Missing salinity and temperature data have been obtained from the SHYFEM model simulation results. The model calibration results and input data are presented in Umgiesser et al. (2016). Solar radiation, wind and precipitation data were obtained from the analysis of the