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RESEARCH ARTICLE

Evaluation of the distribution of fecal indicator bacteria in a river system depending on different types of land use in the southern watershed of the Baltic Sea

Anna Gotkowska-Płachta[1] · Iwona Gołaś[1] · Ewa Korzeniewska[1] · Józef Koc[2] · Andrzej Rochwerger[2] · Kamil Solarski[2]

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Abstract The aim of the study was to determine the effects of land use management on changes in the fecal contamination of water in the Łyna River, one of the main lowland watercourses in the southern watershed of the Baltic Sea (northern Poland). A total of 120 water samples were collected in different seasons of 2011 and 2012 at 15 sites where the river intersected forest (FA), agricultural (AA), and urbanized (UA) areas. Fecal indicator bacteria (FIB), the counts of *Enterobacteriaceae* and *Escherichia coli*, total bacterial counts (TBCs), and domain *Bacteria* (EUB338) were determined by culture-dependent and culture-independent methods. Temperature, pH, chemical oxygen demand, dissolved oxygen, total dissolved solids, ammonia nitrogen, nitrite nitrogen, nitrate nitrogen, orthophosphate, and total phosphorus were also determined. The lowest bacterial counts were noted in water samples collected in FA, and the highest in samples collected in UA. Statistically significant differences were determined between bacterial populations across the analyzed land use types and in different sampling seasons. Significant correlations were also observed between the populations of FIB and physicochemical parameters. The results indicate that land use type influenced FIB concentrations in river water. The combined use of conventional and molecular methods improves the accuracy of fecal contamination analyses in river ecosystems.

Keywords *Enterobacteriaceae* · *Escherichia coli* · Indicator bacteria · FISH · River water · Type of land use

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✉ Anna Gotkowska-Płachta
aniagp@uwm.edu.pl

[1] Department of Environmental Microbiology, Faculty of Environmental Sciences, University of Warmia and Mazury in Olsztyn, Prawocheńskiego 1, 10-720 Olsztyn, Poland

[2] Department of Land Reclamation and Environmental Management, University of Warmia and Mazury in Olsztyn, Plac Łódzki 2, 10-759 Olsztyn, Poland

Introduction

Rivers are dynamic ecosystems that undergo continuous changes between the source and the river mouth. Those modifications are induced by the main current and river runoffs, both natural and those associated with human activities. The above factors influence the trophic state, microbiological quality, and epidemiological safety of river ecosystems.

As sources of drinking and domestic and recreational water, rivers have to be continuously monitored. Water quality is determined primarily by the type of land use in a catchment area. Potential sources of pollution include surface runoffs from agricultural land, including pastures, fields, and meadows fertilized with liquid manure (Bu et al. 2014; Edge et al. 2009; Wilkes et al. 2011). Rivers are receptacles of domestic and industrial effluents as well as municipal and rural rainwater (Daly et al. 2013; Kacar 2011; Servais et al. 2007). Effluents discharged to rivers contain fecal pathogens such as viruses, bacteria, and protozoa. Those microorganisms can be transported across significant distances, and they pose a serious threat to public health (Gotkowska-Płachta et al. 2013; Harnisz 2013; Harnisz et al. 2011; Korzeniewska and Harnisz 2013; Korzeniewska et al. 2013). The monitoring of water resources and sanitary risk identification are priority tasks in many countries. The European Union has introduced the Water Framework Directive (WFD 2000/60/EC) and the

Bathing Water Directive (2006/7/EC) to protect, monitor, and rationally manage the quality of surface waters. In line with these directives, water quality is evaluated based on various physicochemical and ecological indicators. Sanitary risks in water reservoirs are identified by determining the concentrations of fecal indicator bacteria (FIB). Fecal microbes are easy to detect, and their presence in water samples can be associated with other gut pathogens and the risk of infection (Haller et al. 2009; Kay et al. 2007; Oliver et al. 2009; Shah et al. 2011). *Escherichia coli* can be easily identified under environmental conditions. It is regarded as the most universal, reliable, and mandatory indicator of contamination. The abundance of *E. coli* and other FIB in surface waters is determined by various physicochemical and biological factors, including water temperature, pH, solar radiation, content of mineral and organic compounds, predation, and competition (Huachang et al. 2010; Juhna et al. 2007; Wilkes et al. 2011; Zhu et al. 2011).

The most popular methods for identifying coliform bacteria include relatively rapid methods that rely on specific marker enzymes (beta-D-galactosidase for coliform bacteria and beta-D-glucuronidase for *E. coli*) and chromogenic media (Bonadonna et al. 2007; Rompré et al 2002), as well as molecular methods such as fluorescence in situ hybridization (FISH) (Amann et al. 1996; Garcia-Arminsen and Servais 2004). In the FISH method, specific oligonucleotide probes are used to identify bacterial species under an epifluorescence microscope (Amann et al. 1996). This method supports rapid detection (6–8 h) and identification of viable but non-culturable (VBNC) bacteria which lose the ability to proliferate on standard growth media but remain viable in the environment (Lew et al. 2010; Rompré et al. 2002). The combined use of conventional and molecular techniques improves the accuracy of microbiological contamination analyses in aquatic habitats (Korzeniewska and Harnisz 2012; Servais et al. 2007).

The Łyna, one of the main lowland rivers flowing into the southern Baltic Sea, is exposed to anthropogenic pressure. The sources of water pollution have not been accurately identified in the catchment area of the Łyna. During four seasons between autumn 2011 to summer 2012, water samples were collected for physicochemical and FIB analyses from the headwater section in a forest and from downstream sites in agricultural and urban sections of the catchment area. A combination of conventional and molecular analytical techniques was used to accurately describe FIB contamination levels in the Łyna River. Discharges from urban sections and farm fields were expected to increase chemical indicators and FIB levels in river water. The correlations between physicochemical parameters and microbiological indicators of river water were determined.

Materials and methods

Study area

The study was carried out in the Łyna River, the main watercourse in northeastern Poland (Fig. 1) with a total length of 263.1 km. Łyna feeds into the Pregolya River in the Kaliningrad Region. On the Polish territory, Łyna has the length of 200 km and a catchment area of about 5700 km^2. Łyna intersects a lake region, and it is a slowly flowing and strongly meandering watercourse that locally resembles a mountain stream. The width of the river channel ranges from less than 20 m to around 5 m. Łyna has an average depth of 1.5–2.5 m. The average annual flow volume ranges from 7 to 35 m^3 s^{-1}, and the highest flows are noted in spring (Glińska-Lewczuk and Burandt 2011). The catchment area is occupied by arable land, meadows, and pastures in 52.92 %, and by forests in 26.30 %. The southern and central sections of the catchment are dominated by forests that occupy 68 % of the basin area, whereas the northern part features mostly arable land. Łyna intersects urbanized areas (UA; numerous villages and five towns), including Olsztyn, the capital city of the Region of Warmia and Mazury with the population of 176,000. Treated municipal waste (approximately 41,000 m^3/d from five wastewater treatment plants) is discharged into the river. The source of the Łyna River is situated at an altitude of 153 m above sea level on the territory of the Łyna Spring nature reserve. The Łyna source was formed by headward erosion, a phenomenon that is rarely observed in lowlands. In its upper course, the river intersects the Warmia Forest nature reserve with an area of 1656 ha, the habitat of many protected wildlife species. The reserve is part of the Central Łyna Valley Protected Landscape Area. In its middle course, the Łyna flows through the Sępopol Plain, which is part of the Warmia Bird Refuge of the Natura 2000 network.

Sampling sites

Water samples collected along the 200-km-long Polish section of the Łyna River were subjected to microbiological and chemical analyses. The samples were collected at 15 sampling sites at specific points along the river, situated between the river source and the Polish-Russian border (Fig. 1). Four sampling sites were established in forest areas (FA) and agricultural areas (AA) each. Seven sampling sites were created in urban areas to assess the influence of urban agglomerations on the catchment area. The sites were selected to reflect variations in the microbiological and physicochemical parameters of water, with special emphasis on the varied effects of FA, AA, and UA on the catchment. The characteristic features of 15 sampling sites are presented in Table 1.

Water samples were collected from the main river stream at a depth of 0.3–0.5 m, two times in each of the four sampling

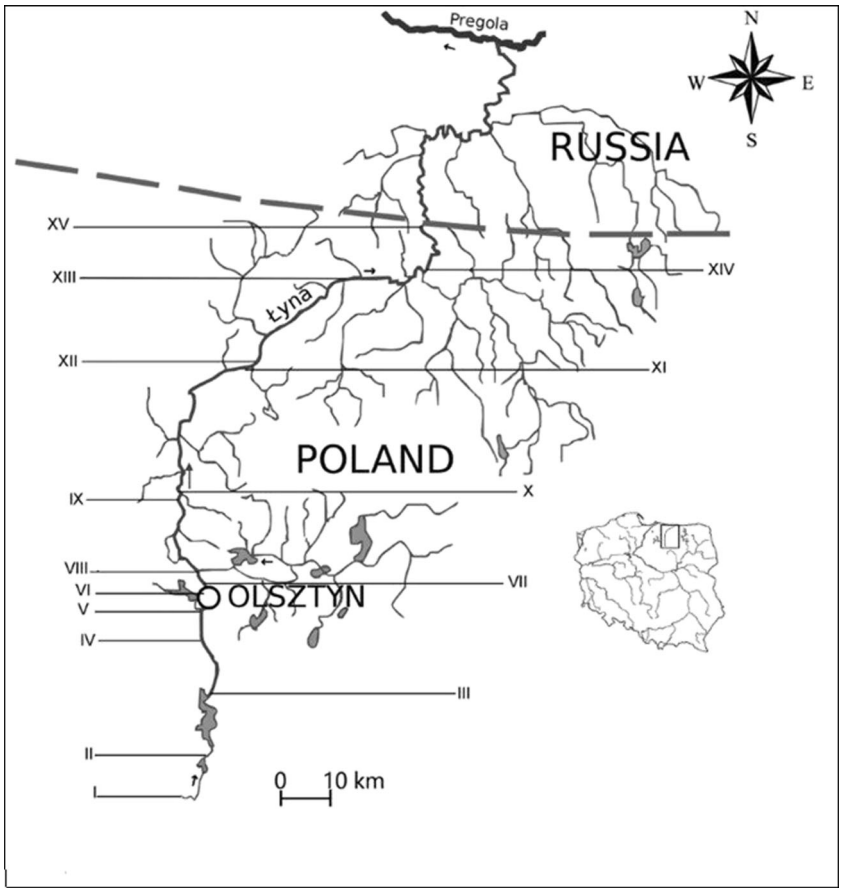

Fig. 1 Location of sampling sites (*I*, *II*,…*XV*) in the Łyna River. A map of Poland showing the Łyna River

Table 1 Characteristic of Łyna River water monitoring sites investigated

No	Site	Length of the river (km)	Catchment area (km^2)	Site description and land use of catchment
I	Headwaters	263.7	5.8	Forest areas - FA
II	Kurki	252.8	358.6	Forest and fallow areas - FA
III	Ustrych	238.0	437.4	Forest and fallow areas - FA
IV	Ruś	226.7	474.4	Forest areas - FA
V	Posorty	218.7	567.7	Grasslands, arable lands - AA
VI	Olsztyn	213.5	578.6	Urban area, city park - UA
VII	Olsztyn	212.5	1785.1	Urban area below the wastewater treatment plant (31,500 m^3/24 h)[a] - UA
VIII	Redykajny	212.3	1790.1	Urban area - UA
IX	Knopin	173.9	2034.9	Pasture and agricultural lands - AA
X	Kosyń	167.3	2056.8	Urban area below the wastewater treatment plant (1,600 m^3/24 h)[a] - UA
XI	Lidzbark	127.9	2426.1	Urban area - UA
XII	Lidzbark	125.3	2449.3	Urban area below the wastewater treatment plant (4,400 m^3/24 h)[a] - UA
XIII	Bartoszyce	94.3	3169.6	Urban area below the wastewater treatment plant (3,400 m^3/24 h)[a] - UA
XIV	Sępopol	72.1	3606.1	Pasture, agricultural lands, fallow areas - AA,
XV	Stopki	60.9	5314.9	Pasture, agricultural lands - AA,

[a] The amount of sewage discharged directly into the river during 24 h

seasons between winter 2011 and autumn 2012. The samples were collected in winter (December, January), spring (March, May), summer (July, August), and autumn (October, November). Three water samples were collected from each site on every sampling date. The samples were combined in equal proportions, and the resulting pool sample was subjected to microbiological analysis. A total of 32, 32, and 56 combined water samples were acquired from FA, AA, and UA, respectively. Overall, 120 samples of river water were collected and analyzed separately. All samples were placed in sterile bottles at 4 °C, transported to the laboratory, and assayed within 12 h of collection.

Physicochemical parameters

The following physicochemical parameters of river water samples were determined: temperature, pH, chemical oxygen demand (COD), dissolved oxygen (DO), total dissolved solids (TDS), ammonia nitrogen (NH_4-N), nitrite nitrogen (NO_2-N), nitrate nitrogen (NO_3-N), orthophosphate (PO_4-P), and total phosphorus (P_T).

All microbiological and physicochemical determinations were carried out in the same water samples. Temperature, pH, and DO (mg O_2 mL^{-1}) were determined with the YSI 556 MPS Handheld Multiparameter Instrument (Multiprobe System) with measurement precision of ±0.1 °C, ±0.01 pH, and ±0.01 mg O_2 L^{-1}. COD, TDS, NH_4-N, NO_2-N, NO_3-N, PO_4-P, and P_T were determined in accordance with the methods described by Hermanowicz et al. (1999), and the results were calculated and expressed in mg L^{-1}.

Microbiological analysis

Determination of Enterobacteriaceae and E. coli counts by the culture-dependent method

Enterobacteriaceae and E. coli counts were determined by membrane filtration. Bacteria were passed through sterile polycarbonate filters (47 mm in diameter, 0.45-μm pore size) (Millipore) into water samples of 10 to 100 mL, depending on the expected microbial concentrations. Membrane filters were transferred to Petri dishes filled with Chromocult coliform agar (CCA) and incubated for 24 h at 35±2 °C. The CCA medium is used to isolate and enumerate *Enterobacteriaceae* and *E. coli* in analyses of contamination with human and animal feces (Finney et al. 2003). After incubation, *Enterobacteriaceae* and E. *coli* colonies were counted based on the color reaction of the CCA medium, and they were identified based on colony morphological parameters, Gram stain results, and the production of indole and cytochrome oxidase. Oxidase-negative bacteria forming pale green or violet colonies (resulting from salmon-galactoside cleavage by β-d-galactosidase) were identified as *Enterobacteriaceae*. Dark blue colonies (resulting from the cleavage of salmon-galactoside and X-glucuronide by β-d-galactosidase and β-d-glucuronidase) were identified as *E. coli*.

Analyses were performed according to the standard microbiological method for testing water quality in Poland and the Standard Methods for the Examination of Water and Wastewater (APHA 1998). All determinations were performed in three replicates in the same water sample. Plate counts were expressed as colony-forming units (CFU) per 100 mL of the sample.

Determination of total bacterial counts (DAPI staining) and in situ hybridization (FISH)

The samples for the quantification of bacterial communities by the FISH method and 4′,6′-diamidino-2-phenyloindole (DAPI) staining were fixed according to the protocol described by Korzeniewska and Harnisz (2012) and Loy et al. (2007). After preliminary analyses, selected hybridization conditions were modified to optimize the results. This approach increased the efficiency of FISH analysis to 75–90 % of total cell counts.

Total bacterial counts were determined in 20–40-mL samples of river water (subject to the sampling site and suspension concentrations) which were homogenized for 2×1.5 min at 7000 rpm (Silent Crusher, Heidolph) to separate bacterial cells. The samples were fixed with freshly prepared paraformaldehyde (PFA, pH 7.4) to a final concentration of 4 % (*v*/*v*) and were stored at the temperature of 4 °C for 12 h. Fixed samples were passed through white polycarbonate filters (0.2-μm pore size, 47-mm in diameter) (GTTP, Millipore) under low negative pressure and with the use of base filters (0.45-μm pore size, Sartorius) to distribute cells evenly on filters. Filters were rinsed with 10 mL of ultrapure water (MQ Millipore) to remove excess paraformaldehyde and were dried for several minutes at room temperature.

Total bacterial counts (TBCs) in samples were determined by staining filtered water samples with 4′,6′-diamidino-2-phenyloindole (DAPI, showing affinity for cellular DNA) with a final concentration of 0.1 ng mL^{-1} (Pernthaler et al. 2001).

The samples fixed on filters were subjected to the FISH procedure with the use of oligonucleotide probes complementary to the conservative region of 16S rRNA or 23S rRNA of the analyzed bacteria. *Enterobacteriaceae* were identified with the ENT183 probe (Friedrich et al. 2003), *E. coli* with the ECO1167 probe (Neef et al. 1995), and the probe specific for the domain Bacteria (EUB338) was applied to identify all bacterial species (Amann et al. 1990). All samples were simultaneously screened with the NON338 probe (Wallner et al. 1993), complementary to EUB338, to control autofluorescence and non-specifically stained cells, the mean value of which did not exceed 5 % for the analyzed samples. All oligonucleotide probes were labeled with Cy3 dye.

For the hybridization process, 2 μL (50 ng μL^{-1}) of EUB338 and ENT183 or ECO1167 probe solution was combined with 18 μL of the hybridization buffer containing 1 mM Tris HCl pH 7.4, 5 M NaCl, 10 % SDS, and formamide. Formamide concentrations varied subject to the applied probe (20 % for ENT183, 35 % for EUB338, and 40 % for ECO1167). The prepared solution (20 μL) was applied to filter pieces and hybridized at 46–50 °C for 1.5–3 h, subject to the applied probe. The probe was cleaned with a warm rinse buffer (48 °C) whose composition was identical to that of the hybridization buffer with the exception of formamide, which was replaced with 0.5 M EDTA, pH 8. Filters were rinsed with ultrapure water; they were dried and stained with the DAPI solution (50 μL) for 3 min at room temperature in dark. Stained filters were thoroughly rinsed with 80 % ethanol for more than 10 s to remove non-specific bonds; after which, they were rinsed with sterile distilled water and dried in air. Filter pieces were attached to slides with Citifluor (Agar Scientific, Essex, UK) and Vectashield (Vector Laboratories, Burlingame, CA) immersion oils, applied 4:1, and covered with coverslips.

The specimens were viewed under a fluorescent microscope (Olympus BX61) equipped with a×100 oil immersion lens, UV lamp, DAPI and CY3 filters, and a CCD camera (Olympus). Images were analyzed in the Cell F application (Olympus). More than 100 hybridized bacterial cells (FISH) and 1000 to 10,000 DAPI-stained bacterial cells were identified per every evaluated sample.

Mean values and standard deviation were calculated from 20 random fields of view in every filter section and expressed in terms of 100 mL of water. Additionally, the ENT183 and ECO1167 probe-specific cell counts were presented as the number and percentage of cells visualized by DAPI.

Statistical analysis

The significance of differences in bacterial counts and physicochemical parameters of water samples from the Łyna River between sampling sites and seasons were determined by one-way analysis of variance (ANOVA). Leven's test was used to assess the homogeneity of variance. When Leven's test produced statistically significant results, the verified hypothesis was rejected. The Kruskal-Wallis test, a non-parametric version of classical one-way ANOVA (Stanisz 2006), was then applied. The correlations between the counts of the analyzed bacterial groups and physicochemical parameters of water samples were determined by Spearman's non-parametric rank correlation test ($p<0.05$, $p<0.01$). Statistical analyses were performed in the Statistica 10.0 (StatSoft, Poland) application.

Results

Physicochemical parameters of river water

The mean values and standard deviation of the evaluated physicochemical parameters of water samples (temperature, pH, COD, DO, TDS, NH_4-N, NO_2-N, NO_3-N, PO_4-P, and P_T) are presented in Table 2. These parameters varied during the seasons and land use management. Seasonal fluctuations ($p<0.05$, $p<0.01$, or $p<0.001$) in the analyzed parameters over the entire period of the study (2011–2012) were confirmed by the Kruskal-Wallis test, except for NO_2-N and NO_3-N. No significant differences (ANOVA, $p<0.05$) in values of temperature, pH, and NO_3-N were observed between land use throughout the experimental period.

Surface water temperature ranged from 3.8 °C in winter to 25.6 °C in summer (not shown in the table), and the observed differences were statistically significant ($p<0.001$). The lowest pH was noted in AA samples collected in winter, whereas the highest pH was observed in FA samples collected in summer. The observed differences in pH were statistically significant ($p<0.001$) only between seasons. Significant differences in COD and DO values of water samples collected in the upper and lower course of the Łyna River were noted between land use types ($p<0.05$) and seasons ($p<0.001$). COD concentrations were highest (25.7 mg O_2 L^{-1}) in summer samples from UA, whereas the highest DO levels (11.3 mg O_2 L^{-1}) were observed in winter samples from FA (not shown in the table). Significant differences in TDS concentrations were observed between the analyzed samples, subject to land use type ($p<0.05$) and season ($p<0.01$). The lowest TDS values were noted in samples from FA or UA and the highest in samples obtained from AA in summer and spring, respectively. The concentrations of NH_4-N and NO_2-N ($p<0.05$ or $p<0.001$) varied significantly between sites characterized by different land use types and between seasons, respectively. The lowest NH_4-N and NO_2-N values were reported in samples obtained from FA in winter and autumn, respectively. The highest NH_4-N and NO_2-N concentrations were noted in water samples collected from UA in summer and spring, respectively. The concentrations of NO_3-N tended to decrease along the river, but no statistically significant differences were observed between samples collected in sites with different land use types and in different seasons. The concentrations of PO_4-P and P_T varied between sites characterized by different land use types. Statistically significant differences ($p<0.01$, $p<0.001$) between PO_4-P and P_T levels were observed between seasons. The concentrations of PO_4-P and P_T differed significantly ($p<0.05$, $p<0.001$) between sampling sites. The lowest concentrations of both forms of phosphorus were determined in spring in FA samples, whereas water sampled from AA and UA in autumn was most abundant in PO_4-P and P_T.

Table 2 Mean (±SD) values of physicochemical parameters of water in relation to land use in the Łyna River catchment

Parameter	Land use			Differences (p) between	
	FA (32)[a] Mean±SD	AA (32)[a]	UA (56)[a]	Land use	Seasons
Temp.	13.59±7.95	13.83±5.68	14.40±5.30	0.641	0.000***
pH	7.47±0.47	7.24±0.32	7.33±0.32	0.408	0.000***
COD	16.75±6.83	23.18±6.97	22.46±6.51	0.040*	0.000***
DO	9.42±1.62	8.91±0.83	8.83±1.28	0.026*	0.000***
TDS	0.25±0.06	0.29±0.06	0.25±0.04	0.036*	0.001**
NH_4-N	0.07±0.05	0.09±0.05	0.10±0.06	0.043*	0.041*
NO_2-N	0.01±0.00	0.03±0.01	0.03±0.02	0.000***	0.247
NO_3-N	0.68±0.44	0.43±0.34	0.23±0.21	0.057	0.075
PO_4-P	0.13±0.10	0.34±0.14	0.29±0.14	0.000***	0.001**
P_T	0.44±0.20	0.65±0.27	0.62±0.25	0.019*	0.000***

All units are given in mg L^{-1} except for temperature (°C) and pH

FA forested areas, *AA* agriculture areas, *UA* urban areas, *SD* standard deviation, *COD* chemical oxygen demand, *DO* dissolved oxygen, *TDS* total dissolved solids, P_T total phosphorus

*Statistically significant differences at $p<0.05$; **statistically significant differences at $p<0.01$); ***statistically significant differences at $p<0.001$

[a] Number of samples

Bacterial counts in water samples

The mean values and standard deviation of total *Enterobacteriaceae* and *E. coli* counts determined by the culture-dependent method, as well as TBC, EUB338, ENT183, and ECO1167 values determined in a culture-independent analysis of water samples, are presented in Table 3. Bacterial counts differed by 7 orders of magnitude, subject to the applied analytical method, the analyzed bacterial species, sampling site, land use type, and season. Significant differences ($p<0.05$, $p<0.01$, or $p<0.001$) in the counts of the analyzed bacterial species were observed between land use type and seasons. Throughout the entire experimental period, the lowest TBC values were noted in water samples collected in FA. Somewhat higher bacterial concentrations were reported in samples from AA, whereas the highest TBC values were observed in water samples collected in UA.

In water samples collected in FA, *Enterobacteriaceae* and *E. coli* counts were determined by the culture-dependent method at 2×10^3 to 9×10^3 CFU 100 mL^{-1} and 0.1×10^3 to 0.2×10^3 CFU 100 mL^{-1}, respectively. The abundance of *Enterobacteriaceae* and *E. coli* bacteria in water samples from AA reached 12×10^3 to 68×10^3 CFU 100 mL^{-1} and 0.4×10^3 to 2.5×10^3 CFU 100 mL^{-1}, respectively. The size of *Enterobacteriaceae* and *E. coli* populations in water samples collected in UA was determined at 79×10^3 to 163×10^3 CFU 100 mL^{-1} and 1.7×10^3 to 5.3×10^3 CFU 100 mL^{-1}, respectively.

The counts of *Enterobacteriaceae* (ENT183) and *E. coli* (ECO1167) determined by the fluorescence method were approximately 10^2–10^3 times higher than the size of those bacterial populations determined by the culture-dependent method. The lowest bacterial counts were noted in water samples collected in FA. The highest bacterial concentrations were determined in samples collected in UA. Despite the above, similar differences in bacterial counts were noted between samples collected in sites characterized by different types of land use. In samples obtained from FA, TBC, and the counts of bacteria hybridized with probes EUB338, ENT183, and ECO1167 did not exceed 5.3×10^8, 1.9×10^8, 0.03×10^8, and 0.009×10^8 cells 100 mL^{-1}, respectively. The maximum abundance of the analyzed bacteria, TBC, and bacteria hybridized with probes EUB338, ENT183, and ECO1167 in water sampled from AA was determined at 7.3×10^8, 3.6×10^8, 0.08×10^8, and 0.019×10^8 cells 100 mL^{-1}, respectively. Water samples collected in UA were characterized by the highest TBC and the highest counts of bacteria hybridized with probes EUB338, ENT183, and ECO1167 at 10.1×10^8, 4.5×10^8, 0.71×10^8, and 0.058×10^8 cells 100 mL^{-1}, respectively.

Significant fluctuations ($p<0.05$ or $p<0.001$) in the abundance of the evaluated bacteria were noted between seasons. The counts of *Enterobacteriaceae* and *E. coli* determined by the culture-dependent method and FISH technique were lowest in winter (FA) when water temperature reached 3.8–9.0 °C, and they were determined at 0.11×10^4 and 0.1×10^3 CFU 100 mL^{-1}, and 0.1×10^7 and 0.2×10^6 cells 100 mL^{-1}, respectively. The highest *Enterobacteriaceae* and

Table 3 Mean (±SD) of studied groups of bacteria in the Łyna River water samples in 2011–2012

Land use	Number of site (120)[a]	Bacteria determined by					
		Cultivated methods		Fluorescence methods			
		Enterobacteriaceae ($\times 10^3$ CFU 100 mL^{-1})[b] (mean±SD)	*E. coli*	TBC ($\times 10^8$ cell 100 mL^{-1}) (mean±SD)	EUB338	ENT183	ECO1167
FA	I	2.0±1.0	0.1±0.05	3.1±1.0	1.8±0.8	0.02±0.01	0.003±0.001
	II	9.0±5.0	0.1±0.04	3.6±1.8	1.7±1.1	0.02±0.01	0.006±0.002
	III	3.0±1.0	0.1±0.03	4.6±3.8	1.9±0.5	0.02±0.01	0.007±0.003
	IV	6.0±2.0	0.2±0.12	5.3±3.2	1.7±1.1	0.03±0.02	0.009±0.003
AA	V	12.0±4.0	0.4±0.21	5.8±3.6	2.3±0.7	0.04±0.02	0.016±0.001
	IX	68.0±47.0	2.5±1.5	7.0±3.5	3.6±2.8	0.04±0.01	0.014±0.009
	XIV	29.0±13.0	1.1±0.7	5.9±2.4	2.9±1.7	0.08±0.05	0.017±0.012
	XV	33.0±19.0	1.3±0.9	7.3±4.0	2.8±1.2	0.05±0.03	0.019±0.015
UA	VI	80.0±36.0	2.2±1.4	8.3±2.7	3.7±1.3	0.17±0.04	0.049±0.023
	VII	163.0±51.0	5.3±1.2	10.1±6.5	4.5±3.9	0.71±0.17	0.058±0.011
	VIII	94.0±56.0	4.6±1.9	10.0±2.6	3.8±2.9	0.10±0.05	0.051±0.033
	X	102.0±77.0	2.3±1.3	6.3±3.2	3.7±2.9	0.27±0.20	0.034±0.032
	XI	79.0±29.0	1.9±1.0	8.7±2.1	3.9±0.5	0.28±0.18	0.046±0.032
	XII	121.0±96.0	3.3±1.7	8.5±1.7	2.9±1.4	0.09±0.03	0.048±0.030
	XIII	21.0±10.0	1.7±1.1	6.3±3.5	2.7±1.8	0.08±0.03	0.040±0.020
Kruskal-Wallis test							
Differences (p) between:							
Land use		0.000***	0.000***	0.000***	0.000***	0.000***	0.000***
Seasons		0.025*	0.047*	0.003**	0.000***	0.000***	0.000***

FA forested areas, *AA* agriculture areas, *UA* urban areas, *SD* standard deviation, *TBC* total bacterial count

*Statistically significant differences at $p<0.05$; **statistically significant differences at $p<0.01$; ***statistically significant differences at $p<0.001$

[a] Number of samples

[b] *CFU* colony forming units per 100 mL in cultivation methods (the number of samples was performed in triplicate)

E. coli counts were noted in summer (UA) when water temperature ranged from 19.4 to 25.6 °C. They were determined at 2.2×10^5 and 6.5×10^3 CFU 100 mL^{-1}, and 9.0×10^7 and 6.9×10^6 cells 100 mL^{-1}, respectively (Fig. 2).

The percentage of DAPI-stained bacteria visualized by probes ENT183 and ECO1167 did not exceed 0.65 and 0.17 % of TBC, respectively, in water samples from FA, 1.30 and 0.32 % of TBC, respectively, in samples from AA, and 7.0 and 0.68 % of TBC, respectively, in samples from UA (Fig. 3). The percentages of the evaluated bacteria varied subject to the type of land use, and they increased with a rise in the concentrations of P_T and NH_4-N.

Correlations between bacterial counts and physicochemical parameters of river water

The correlations between the counts of the analyzed bacterial groups identified in samples of river water during the entire period of the study and selected physicochemical parameters of river water are shown in Table 4. Spearman's test revealed statistically significant ($p<0.05$, $p<0.01$) positive correlations between most bacterial groups. Significant ($p<0.05$, $p<0.01$) negative and positive correlations were noted between all bacterial groups versus water temperature, COD, DO, NH_4-N, NO_2-N, and P_T concentrations. Correlations were also observed between PO_4-P levels versus *Enterobacteriaceae* counts determined by the culture-dependent method, TBC, and the counts of EUB338-visualized bacteria, and between water pH versus the counts of EUB338-visualized bacteria, *Enterobacteriaceae*, and *E. coli* determined by the culture-independent method.

Discussion

The monitoring of water resources and sanitary risk identification are priority tasks in many countries around the world. The abundance, type, and origin of microbial pollutants may be very difficult to determine in aquatic habitats (Haller et al. 2009; Huachang et al. 2010;

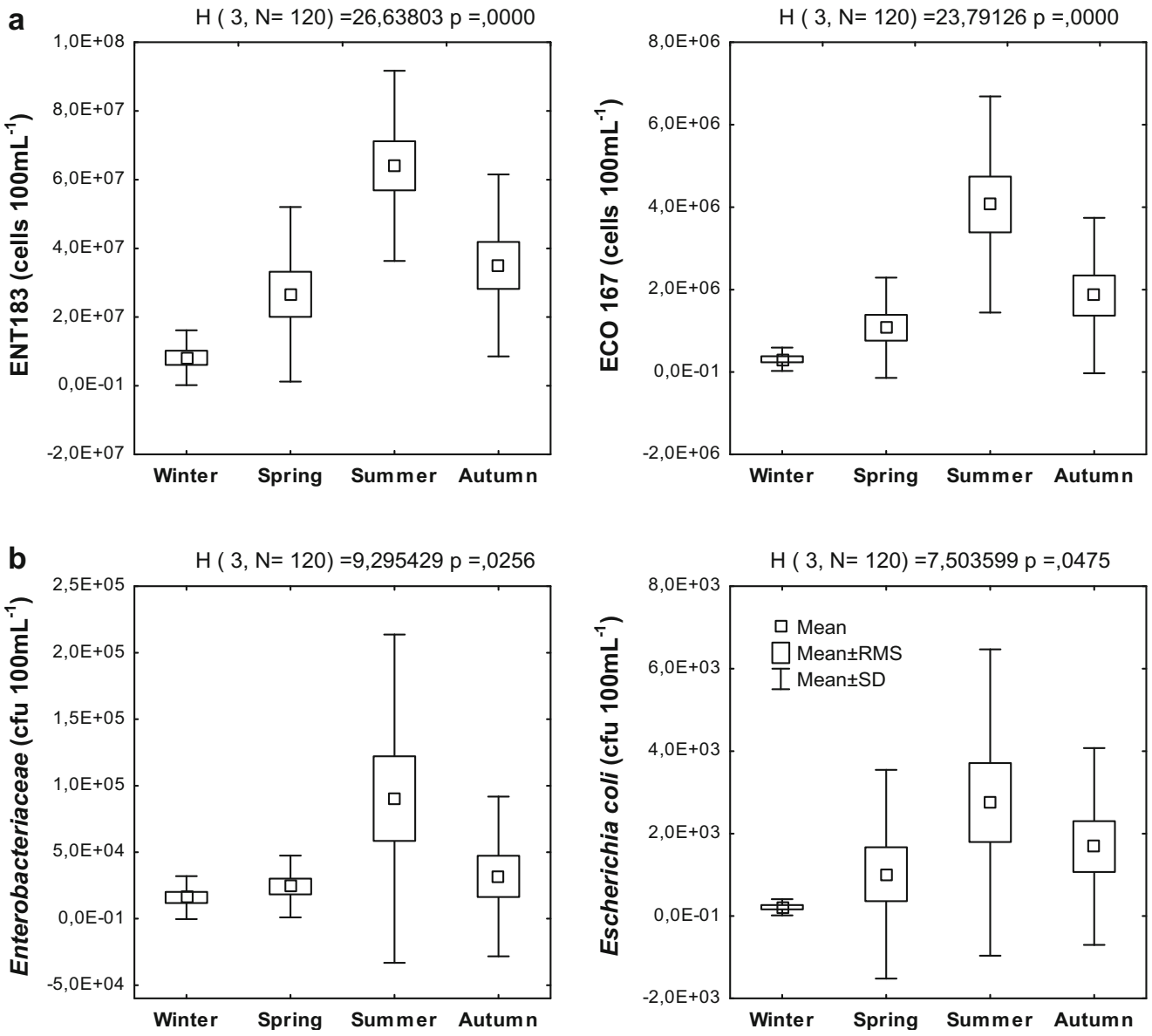

Fig. 2 Counts of *Enterobacteriaceae* and *Escherichia coli* determined by **a** the FISH technique (bacteria hybridized with probes ENT183 and ECO1167) and **b** the culture-dependent method in water samples collected at different sites throughout the study. Independent variable (assembling): season. *RMS* random mean square, *SD* standard deviation, *N* number of samples, *p* significance level

Łuczkiewicz et al. 2013; Oliver et al. 2009). The counts of *Enterobacteriaceae* and *E. coli* indicator bacteria are determined to assess epidemiological risks in water ecosystems (Korzeniewska and Harnisz 2012). An increase in the abundance of indicator bacteria could point to the presence of other potentially pathogenic microorganisms (Kacar 2011; Olapade and Weage 2010).

In this study, the concentrations of indicator bacteria in water samples from the Łyna River differed between sampling sites and experimental seasons. The observed variations were attributed to different types of land use in the surveyed parts of the catchment area as well as environmental factors. Statistical analyses revealed significant ($p<0.05$, $p<0.01$, $p<0.001$) differences in the abundance of indicator bacteria in samples collected in different seasons from sites characterized by different types of land use. Statistically significant ($p<0.05$, $p<0.01$) positive and negative correlations were also observed between the analyzed bacterial groups, and between bacterial counts and the analyzed physicochemical parameters of river water.

Enterobacteriaceae and *E. coli* concentrations determined by the culture-dependent method

Statistical analyses revealed significant differences between the counts of indicator bacteria (*Enterobacteriaceae* and

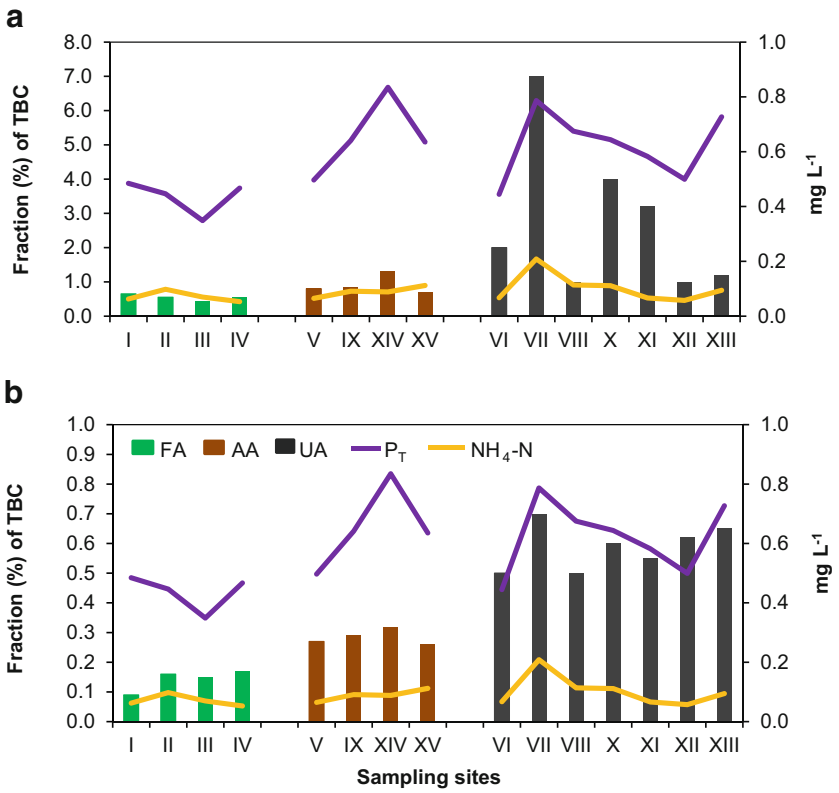

Fig. 3 Average percentages of *Enterobacteriaceae* and *Escherichia coli* bacteria hybridized with probes ENT183 (**a**) and ECO1167 (**b**) compared with TBC at different concentrations of P_T and NH_4-N in water samples collected at different sites throughout the study

E. coli) in water samples obtained in different sites and seasons. Significant correlations were noted between the populations of *Enterobacteriaceae* and *E. coli* and between the concentrations of indicator bacteria versus TBC, EUB338, ENT183, and ECO1167 counts determined by the fluorescence technique. In this study, the concentrations of

Table 4 Statistic estimation by Spearman's correlation between numbers of studied microorganisms determined by cultured method and fluorescence techniques (DAPI and FISH) recovered from the water of River Łyna and physicochemical parameters

	Bacteria determined by					
	Cultivated method		Fluorescence methods			
	Enterobacteriaceae	*E. coli*	TBC	EUB338	ENT183	ECO1167
Enterobacteriaceae		0.534**	0.561**	0.548**	0.382**	0.462**
E. coli			0.576**	0.611**	0.355**	0.435**
TBC				0.946**	0.864**	0.852**
EUB338					0.911**	0.894**
ENT183						0.963**
Temperature	0.386**	0.319*	0.467**	0.547**	0.688**	0.664**
pH				0.286*	0.454**	0.453**
COD	0.371**	0.411**	0.413**	0.415**	0.437**	0.512**
DO	−0.324*	−0.323*	−0.308*	−0.372**	−0.394**	−0.379**
TDS					−0.264*	
NH_4-N	0.319*	0.333*	0.343*	0.315*	0.365*	0.489*
NO_2-N	0.522**	0.445**	0.700**	0.656**	0.531**	0.577**
PO_4-P	0.417**		0.337**	0.295*		
P_T	0.462**	0.407**	0.449**	0.509**	0.534**	0.561**

TBC total bacterial count, *COD* chemical oxygen demand, *DO* dissolved oxygen, *TDS* total dissolved solids, P_T total phosphorus

*Statistically significant correlation at $p<0.05$; **statistically significant correlation at $p<0.01$

Enterobacteriaceae and *E. coli* did not exceed 220×10^5 and 10×10^5 CFU 100 mL^{-1}, respectively, and were similar to or different from the values noted in other river ecosystems, subject to the degree and type of river contamination, type of land use in the catchment area, and the applied analytical method. The populations of indicator bacteria were approximately 10-fold lower in the Czarna Hańcza River in the Wigry National Park (Gotkowska-Płachta et al. 2005). In the Vistula River, *Enterobacteriaceae* and *E. coli* counts were estimated in the range of 10^2 to 10^4 CFU 100 mL^{-1} (Donderski and Wilk 2002a, b). In the Kalamazoo River in the USA, the size of indicator bacteria populations was determined in the range of 10^4 to 10^5 CFU 100 mL^{-1} by Olapade and Weage (2010). The abundance of indicator bacteria in the Seine River (France) polluted with effluents was estimated at 10^6 to 10^8 CFU 100 mL^{-1} by Savichtcheva and Okabe (2006). In a study of more than 10 Canadian rivers, Wilkes et al. (2009) determined the counts of indicator bacteria at 10^7 to 10^8 CFU 100 mL^{-1}. In the Łyna River, significant seasonal variations were observed in the size of *Enterobacteriaceae* and *E. coli* populations. Seasonal differences in the counts of indicator bacteria were also noted by Kacar (2011) in five rivers in western Turkey and by Okeke et al. (2011) in the Tallapoosa River in the USA.

In our study, the minimum and maximum counts of *Enterobacteriaceae* and *E. coli* bacteria in the analyzed sampling sites and seasons could be attributed to the stimulating or inhibiting effects of environmental factors as well as differences in the type of land use in the catchment area, which contributed to the inflow of organic and mineral substances from external sources. This conclusion is corroborated by the results of our statistical analysis showing significant positive or negative correlations between the populations of *Enterobacteriaceae* and *E. coli* versus water temperature, COD, DO, NH_4-N, NO_2-N, and P_T. Increased concentrations and loads of P_T, NH_4-N and FIB in stream water are considered to be universal indicators in urban streams, even at low levels of catchment urbanization (Hatt et al. 2004; Tryland et al. 2002). The counts of *Enterobacteriaceae* and *E. coli* were 10- to 50-fold higher in water samples collected in AA and UA than in samples obtained from FA, which indicates that the type of land use influences the microbiological quality of water in the Łyna River. Due to higher levels of bacterial contamination, the quality of water samples from AA and UA were classified as "poor," whereas the quality of water from FA was classified as "excellent" in line with the criteria set out by the Bathing Water Directive (2006/7/EC). Higher concentrations of *E. coli* in urban catchments were also reported by Bu et al. (2014) and Daly et al. (2013). Similar correlations between the type of land use in the catchment area and the physicochemical and/or microbiological parameters of river water were observed by Economou et al. (2013), Nnane et al. (2011), and Wilkes et al. (2009, 2011).

Bacterial concentrations determined by DAPI staining and the FISH method

Total bacterial counts in the Łyna River were determined at 10^6–10^8 cells 100 mL^{-1}, and they were similar to or insignificantly different from the most recent values reported in other rivers around the world. Somewhat lower microbial counts (0.13×10^7 to 330×10^7 cells 100 mL^{-1}) were observed in the Rouge River (USA) by Tiquia (2010), in whose study microbial populations differed significantly between water samples collected in the analyzed seasons. Total bacterial counts of $0.27–8.1\times10^9$ cells 100 mL^{-1} were determined by Bouvy et al. (2010) in the Senegal River and its 13 tributaries, and similar microbial concentrations were observed by Araújo and Godinho (2008) in the Pitimbu River in Brazil. In our study, the differences in TBC values between seasons and sites confirm that aquatic microorganisms are highly sensitive to environmental changes. The physicochemical parameters of water and the size of heterotrophic bacterial populations in water bodies are determined by the type of land use, degree of urbanization, and the intensity of human activities in the catchment area (Glińska-Lewczuk 2006; Gołaś et al. 2008a, b, 2009). In the Łyna River, TBC values were severalfold higher in samples collected from AA and UA than from FA, which clearly indicates that the type of land use in the catchment area influences the microbiological quality of water reservoirs.

The abundance of *Enterbacteriaceae* and *E. coli* determined by the FISH method in the Łyna River was approximately 10^2–10^3 higher than that determined with the use of a culture-dependent method (Table 1). The above results indicate that the analyzed river is a substantial reservoir of non-culturable fecal bacteria. Fecal bacteria may cease to proliferate under stress. Intestinal microorganisms that reach river waters with evacuated effluents are particularly exposed to changes in habitat conditions, such as temperature, oxygen concentrations, pH, and nutrient availability, and such bacterial cells could become non-culturable. The above poses a significant problem when pathogenic and opportunistic bacteria are concerned, because such microorganisms are generally characterized by higher virulence than cells not exposed to environmental stressors (Rowan 2004). In many cases, *E. coli* bacteria carry drug resistance genes that can be transferred to other microorganisms in water, soil, and air (Korzeniewska and Harnisz 2013; Korzeniewska et al. 2013). The concentrations of indicator bacteria in aquatic habitats should be accurately determined to eliminate epidemiological and public health risks in rivers. Fluorescent in situ hybridization supported the determination of the actual contamination of the Łyna River with *Enterobacteriaceae* and *E. coli*. The highest *Enterobacteriaceae* and *E. coli* concentrations were noted in water samples from UA, and they were approximately 10-fold lower in FA samples. Bacterial counts were positively correlated ($p<0.01$) with COD,

NH_4-N, NO_2-N, and P_T levels which are indicative of water pollution associated with domestic, municipal, and land use activities. The correlations between *Enterobacteriaceae* and *E. coli* levels versus P_T and NH_4-N (Fig. 3) values suggest that effluents discharged by wastewater treatment plants in urban areas and runoffs from farm fields fertilized with phosphate and ammonia were the combined sources of pollution. Our results indicate that urban areas (UA) situated along the Łyna River significantly contributed to the deterioration in downstream water quality.

Conclusions

The concentrations of fecal indicator bacteria in the Łyna River varied significantly subject to the type of land use in the catchment area and environmental factors. The populations of all analyzed bacterial groups were higher in water samples collected in UA where treated municipal waste was discharged directly to the Łyna River. Microbial concentrations were also higher in water samples collected in AA. At sampling sites characterized by high levels of bacterial contamination, the quality status of water samples was reclassified from excellent to poor in line with the criteria set out by the Bathing Water Directive (2006/7/EC).

The concentrations of fecal indicator bacteria (*Enterobacteriaceae* and *E. coli*) in the Łyna River were most highly correlated with water temperature and COD, DO, NH_4-N, NO_2-N, and P_T concentrations, which could adversely influence self-purification processes in the studied ecosystem. The above contributes to eutrophication and poses greater risk to public health. In the future, the results of microbiological analyses could constitute a valuable reference point for evaluating the rate and directions of changes in the bacteriological profile of the Łyna River.

The combination of conventional and molecular analytical techniques supported accurate determinations of contamination with *Enterobacteriaceae* and *E. coli* indicator bacteria in the Łyna River. The FISH technique successfully identified bacteria that had not been determined by the culture-dependent method. The results of the FISH analysis contributed to a reliable assessment of fecal contamination in the studied river and an evaluation of the suitability of river water for drinking, recreational, and domestic use. Our results indicate that the combined use of conventional and molecular methods improves the accuracy of microbiological contamination analyses in river ecosystems, eliminates the risk of underestimation of pollution levels, and contributes to epidemiological and public health safety.

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Conflict of interest The authors declare that they have no conflict of interest.

References

Amann RI, Binder BJ, Olson RJ, Chisholm SW, Devereux R, Stahl DA (1990) Combination of 16S rRNA-targeted oligonucleotide probes with flow cytometry for analyzing mixed microbial populations. Appl Environ Microbiol 56(6):1919–1925

Amann R, Snaidr J, Wagner M, Ludwig W, Schleifer KH (1996) In situ visualization of high genetic diversity in a natural microbial community. J Bacteriol 178(12):3496–3500

APHA (American Public Health Association) (1998) Standard method for the examination of water and wastewater, 19th edn. American Public Health Association, Washington DC

Araújo MFF, Godinho MJL (2008) Seasonal and spatial distribution of bacterioplankton in a fluvial-lagunar system of a tropical region: density, biomass, cellular volume and morphologic variation. Braz Arch Biol Technol 51(1):203–212, ISSN 1516–8913

Bonadonna L, Cataldo C, Semproni M (2007) Comparison of methods and confirmation tests for the recovery Escherichia coli in water. Desalination 213(1–3):18–23

Bouvy M, Arfi R, Bernard C, Carré C, Got P, Pagano M, Troussellier M (2010) Estuarine microbial community characteristics as indicators of human-induced changes (Senegal River, West Africa). Estuar Coast Shelf Sci 87(6):573–582

Bu H, Meng W, Zhang Y, Wan J (2014) Relationships between land use patterns and water quality in the Taizi River basin. Chin Ecol Indic 41:187–197

Daly E, Kolotelo P, Schang C, Osborne CA, Coleman R, Deletic A, McCarthy DT (2013) *Escherichia coli* concentrations and loads in an urbanised catchment: the Yarra River, Australia. J Hydrol 497: 51–61

Directive 2006/7/EC of the European Parliament and of the Council of 15 February 2006 concerning the management of bathing water quality. Off J Eur Commun L64: 37–51

Donderski W, Wilk I (2002a) Bacteriological studies of water and bottom sediments of the Vistula River between Wyszogród and Toruń. Pol J Environ Stud 11(1):33–40

Donderski W, Wilk I (2002b) The sanitary state of water in the River Vistula between Wyszogród and Toruń. Pol J Environ Stud 11(5): 509–515

Economou V, Gousia P, Kansouzidou A, Sakkas H, Karanis P, Papadopoulou C (2013) Prevalence, antimicrobial resistance and relation to indicator and pathogenic microorganisms of *Salmonella enterica* isolated from surface waters within an agricultural landscape. Int J Hyg Environ Health 216(4):435–444

Edge TA, Droppo I, El-Shaarawi A, Gannon V, Hewitt M, Kent R, Khan I, Koning W, Lapen D, Marcogliese D, Marvin C, Miller J, Neumann N, Phillips R, Robertson W, Schreier H, Shtepani I, Topp E, van Bochove E (2009) An evaluation of *Escherichia coli* as a potential agri-environmental waterborne pathogen standard. Environment Canada, Gatineau, Quebec, Synthesis report 14. National Agri- Environmental Standards Initiative, p 48

Finney M, Smullen J, Foster HA, Brokx S, Storey DM (2003) Evaluation of chromocult coliform agar for the detection and enumeration of Enterobacteriaceae from faecal samples from healthy subjects. J Microbiol Methods 54(3):353–358

Friedrich U, Van Langenhove H, Altendorf K, Lipski A (2003) Microbial community and physicochemical analysis of an industrial waste gas biofilter and design of 16S rRNA-targeting oligonucleotide probes. Environ Microbiol 5(3):183–201

Garcia-Arminsen T, Servais P (2004) Enumeration of viable *E. coli* in rivers and wastewaters by fluorescent in situ hybridization. J Microbiol Method 58:269–279

Glińska-Lewczuk K (2006) Effect of land use and lake presence on chemical diversity of the Lyna river system. Pol J Environ Stud 15(2):259–269

Glińska-Lewczuk K, Burandt P (2011) Effect of river straightening on the hydrochemical properties of floodplain lakes: observations from the Łyna and Drwęca Rivers, N Poland. Ecol Eng 37:786–795

Gołaś I, Zmysłowska I, Harnisz M, Korzekwa K, Skowrońska A, Teodorowicz M, Górniak D, Gros M, Brzozowa S (2008a) Nitrogen cycle bacteria in the waters of the Drwęca River. Pol J Environ Stud 17(2):215–225

Gołaś I, Zmysłowska I, Harnisz M, Korzekwa K, Skowrońska A, Teodorowicz M, Górniak D, Dudziec E (2008b) Anthropogenic impact on quantitative differentiation of nitrogen cycling bacteria in waters of the River Drwęca. Pol J Nat Sci 23(3):667–680

Gołaś I, Korzekwa K, Harnisz M, Zmysłowska I, Teodorowicz M, Terech-Majewska E, Rodziewicz W, Bieńkowska M (2009) Influence of fishery management and environmental factors on occurrence of heterotrophic, hemolytic and mesophilic bacteria and *Aeromonas hydrophila* in waters of Drwęca River, Poland. Arch Environ Protect 35(2):27–40

Gotkowska-Płachta A, Niewolak S, Korzeniewska E (2005) Pollution degree and sanitary state indicator bacteria as the indicators of the purity of Lake Hańcza waters. Arch Environ Protect 31(2):53–68

Gotkowska-Płachta A, Filipkowska Z, Korzeniewska E, Janczukowicz W, Dixon B, Gołaś I, Szwalgin D (2013) Airborne microorganisms emitted from wastewater treatment plant treating domestic wastewater and meat processing industry wastes. CLEAN Soil Air Water 41(5):429–436

Haller L, Pote' J, Loizeau J-L, Wildi W (2009) Distribution and survival of faecal indicator bacteria in the sediments of the Bay of Vidy, Lake Geneva, Switzerland. Ecol Indic 9:540–547

Harnisz M (2013) Total resistance of native bacteria as an indicator of changes in the water environment. Environ Pollut 174:85–92

Harnisz M, Gołaś I, Pietruk M (2011) Tetracycline-resistant bacteria as indicators of antimicrobial resistance in protected waters—the example of the Drwęca River nature reserve (Poland). Ecol Indic 11: 663–668

Hatt BE, Fletcher TD, Walsh CJ, Taylor SL (2004) The influence of urban density and drainage infrastructure on the concentrations and loads of pollutants in small streams. Environ Manag 34:112–124

Hermanowicz W, Dożańska W, Dojlido J, Koziorowski B (1999) Physicochemical methods of water and wastewater examination. (in Polish). Arkady, Warsaw

Huachang H, Jianwen Q, Liang Y (2010) Environmental factors influencing the distribution of total and fecal coliform bacteria in six water storage reservoirs in the Pearl River Delta Region. Chin J Environ Sci 22(5):663–668

Juhna T, Birzniece D, Rubulis J (2007) Effect of phosphorus on survival of *Escherichia coli* in drinking water biofilms. Appl Environ Microbiol 73(11):3755–3758

Kacar A (2011) Analysis of spatial and temporal variation in the levels of microbial fecal indicators in the major rivers flowing into the Aegean Sea, Turkey. Ecol Indic 11:1360–1365

Kay D, Edwards AC, Ferrier RC, Francis C, Kay C, Rushby L, Watkins J, McDonald AT, Wyer M, Crowther J, Wilkinson J (2007) Catchment microbial dynamics: the emergence of a research agenda. Prog Phys Geogr 31(1):59–76

Korzeniewska E, Harnisz M (2012) Culture-dependent and culture-independent methods in evaluation of emission of *Enterobacteriaceae* from sewage to the air and surface water. Water Air Soil Pollut 223:4039–4046

Korzeniewska E, Harnisz M (2013) Extended-spectrum beta-lactamase (ESBL)-positive *Enterobacteriaceae* in municipal sewage and their emission to the environment. J Environ Manag 128:904–911

Korzeniewska E, Korzeniewska A, Harnisz M (2013) Antibiotic resistant *Escherichia coli* in hospital and municipal sewage and their emission to the environment. Ecotoxicol Environ Saf 91:96–102

Lew S, Lew M, Mieszczyński T, Szarek J (2010) Selected fluorescent techniques for identification of the physiological state of individual water and soil bacterial cells—*review*. Folia Microbiol 55(2):107–118

Loy A, Maixner F, Wagner M, Horn M (2007) ProbeBase—an online resource for rRNA-targeted oligonucleotide probes: new features 2007. Nucleic Acids Res 3(1):00–804

Łuczkiewicz A, Felis E, Ziembińska A, Gnida A, Kotlarska E, Olańczuk-Neyman K, Surmacz-Górska J (2013) Resistance of *Escherichia coli* and *Enterococcus spp*. to selected antimicrobial agents present in municipal wastewater. J Water Health 11(4):600–612

Neef A, Amann R, Schleifer KH (1995) Detection of microbial cells in aerosols using nucleic acid probes. Syst Appl Microbiol 18(1):113–122

Nnane DE, Ebdon JE, Taylor HD (2011) Integrated analysis of water quality parameters for cost-effective faecal pollution management in river catchments. Water Res 45(6):2235–2246

Okeke BC, Thomson MS, Moss EM (2011) Occurrence, molecular characterization and antibiogram of water quality indicator bacteria in river water serving a water treatment plant. Sci Total Environ 409(23):4979–4985

Olapade OA, Weage EA (2010) Comparison of fecal indicator bacterial populations in surface waters of the Kalamazoo River, USA. Microbes Environ 25(1):41–44

Oliver DM, Fish RD, Hodgson CJ, Heathwaite AL, Chadwick DR, Winter M (2009) A cross-disciplinary toolkit to assess the risk of faecal indicator loss from grassland farm systems to surface waters. Agric Ecosyst Environ 129(4):401–412

Pernthaler J, Glöckner FO, Schönhuber W, Amann R (2001) Fluorescence *in situ* hybridization with rRNA-targeted oligonucleotide probes. In: J Paul (ed), London: Academic Press Ltd. Methods in Microbiology 30: 207–226

Rompré A, Servais P, Baudart J, De Roubin MR, Laurent P (2002) Methods of detection and enumeration of coliforms in drinking water a review. J Microbiol Methods 49(1):31–54

Rowan NJ (2004) Viable but non-culturable forms of food and waterborne bacteria: Quo vadis? Trends Food Sci Technol 15:462–467

Savichtcheva O, Okabe S (2006) Alternative indicators of fecal pollution: relations with pathogens and conventional indicators, current methodologies for direct pathogen monitoring and future application perspectives. Water Res 40(13):2463–2476

Servais P, Garcia-Armisen T, George I, Billen G (2007) Fecal bacteria in the rivers of the Seine drainage network (France): sources, fate and modeling. Sci Total Environ 375(1–3):152–167

Shah AH, Abdelzaher AM, Phillips M, Hernandez R, Solo-Gabriele HM, Kish J, Scorzetti G, Fell JW, Diaz MR, Scott TM, Lukasik J, Harwood VJ, McQuaig S, Sinigalliano CD, Gidle ML, Wanless D, Ager A, Lui J, Stewart JR, Plano LRW, Fleming LE (2011) Indicator microbes correlate with pathogenic bacteria, yeasts and helminthes in sand at a subtropical recreational beach site. J Appl Microbiol 110(6):1571–1583

Stanisz A (2006) A straightforward course in statistics based on the STAT ISTICA PL program as applied in medicine. Volume Basic statistics . (In Polish). StatSoft Poland. Cracow, 2006. ISBN 83-88724-18-5

Tiquia SM (2010) Metabolic diversity of the heterotrophic microorganisms and potential link to pollution of the Rouge River. Environ Pollut 158(5):1435–1443

Tryland I, Surman S, Berg JD (2002) Monitoring faecal contamination of the Thames estuary using semiautomated early warning system. Water Sci Technol 46:25–31

Wallner G, Amann R, Beisker W (1993) Optimizing fluorescent in situ hybridization with rRNA-targeted oligonucleotide probes for flow cytometric identification of microorganisms. Cytometry 14(2):136–143

WFD, (2000) Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy. J Eur Union

Wilkes G, Edge T, Gannon V, Jokinen C, Lyautey E, Medeiros D, Neumann N, Ruecker N, Topp E, Lapen DR (2009) Seasonal relationships among indicator bacteria, pathogenic bacteria, *Cryptosporidium oocysts*, *Giardia* cysts, and hydrological indices for surface waters within an agricultural landscape. Water Res 43(8):2209–2223

Wilkes G, Edge TA, Gannon VPJ, Jokinen C, Lyautey E, Neumann NF, Ruecker N, Scott A, Sunohara M, Topp E, Lapen DR (2011) Associations among pathogenic bacteria, parasites, and environmental and land use factors in multiple mixed-use watersheds. Water Res 45(18):5807–5825

Zhu X, Wang JD, Solo-Gabriele HM, Fleming LE (2011) A water quality modeling study of non-point sources at recreational marine beaches. Water Res 45(9):2985–2995