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Microbial and chemical quality assessment of the small rivers entering the South Baltic. Part I: Case study on the watercourses in the Baltic Sea catchment area

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Abstract: The area of the Coastal Landscape Park (CLP) due to its location is extremely attractive touristic area. In the summer season, a significant increase in population density is observed, which influences surface water quality. Large numbers of tourists generate an increased amount of municipal wastewater, being treated in local treatment plants and discharged into rivers and streams. The paper presents preliminary research from summer 2016 on three watercourses ending in the Baltic Sea: Piaśnica, Karwianka and Czarna Wda rivers. It is a part of a long-term project conducted in CLP to assess surface waters quality. The scope of research included measurements of *in situ* parameters (temperature, conductivity, pH, dissolved oxygen). Chemical Oxygen Demand was determined using a spectrophotometer. Ion chromatography was used to determine ions concentrations (including biogenic compounds). Sanitary state of watercourses was assessed based on fecal coliforms abundance, which number was determined by the cultivation method. The determination of microbiological parameters such as: prokaryotic cell abundance expressed as total cells number (TCN), prokaryotic cell biovolume expressed as average cell volume (ACV), the prokaryotic biomass (PB) and prokaryotic cell morphotype diversity was determined using epifluorescence microscopy method. Results showed that water quality of Piaśnica and Czarna Wda rivers were affected by discharged treated wastewater. In the case of Karwianka River, the main pollution source could be surface runoff from fields and unregulated sewage management in this area. The conducted research confirmed the urgent need for better protection of this area to conserve both its ecosystem and value for tourism.

Introduction

In many places in the world there are areas that are extremely important for the ecosystem, and which should be particularly protected due to their exceptional nature (Olson and Dinerstein 1998). At the same time, these areas are very popular tourist destinations, attracting thousands of people every year (Curr et al. 2000). In such a situation a compromise has to be made between nature conservation and tourism development, which is not always beneficial for the nature we should protect.

Usually, wastewater treatment plants (WWTPs) operate in fixed systems and under unchanging conditions almost all year round (Munksgaard and Young 1980). They are designed for daily fluctuations in the inflow of pollutants, which does not significantly affect their operation. However, one of the major problems of wastewater treatment associated with tourist resorts is connected to the uneven inflow of the wastewater to the treatment plant (Bugajski and Satora 2009). It is also related to load variability in time, which must be reduced before the wastewater can safely enter the receiver. The efficiency of the wastewater treatment plant is reduced due to high inflow unevenness. It is caused by difficulties in adapting the existing technology to changes in the load supply (Kaczor 2011). A load of pollutants may be two to even four times bigger, which is a serious problem for the WWTP, and for the ecosystem (which is the recipient of potentially under-treated wastewater). Studies have shown that tourism puts pressure on coastal areas around the world (Gössling et al. 2018). In Europe, similar studies are currently being carried out, demonstrating the need for continuous and wider monitoring of watercourses that flow into protected areas (Grabic et al. 2018). Global change and anthropogenic activities put a heavy strain on marine ecosystems. Scientists aim to answer the questions of how to protect the environment and biodiversity without inhibiting socio-economic development and increasingly popular tourism, for example in the Doñana National Park in Spain, very well described in the literature (García-Llorente et al. 2018). Researchers from Germany and Denmark who conducted research in the area of the Wadden Sea (Sylt-Rømø Bight, Norderaue tidal basin and Jade Bay), also proved that it is crucial to develop relevant management plans to protect and benefit from ecosystems (de la Vega et al. 2018).

Wastewater entering the treatment plant is a habitat for various types of microorganisms, including pathogens (Cai and Zhang 2013). As a result of proper WWTP functioning, the physical and chemical quality of the effluent is higher than that of the influent, however, it is usually not subjected to any degree of disinfection before it is discharged into the receiver (Michałkiewicz 2018). This means that significant numbers of microorganisms can be released to the ecosystem in case of inappropriate WWTP operation, e.g., insufficient sludge settling or increased hydraulic load.

The presence of various bacteria and other microorganisms is an important contribution to global biodiversity, and it is crucial to the functioning of ecosystems (Caruso et al. 2016). Different types of prokaryotic microorganisms can be found in different niches, and their development, growth or division possibilities are closely linked to the conditions offered by a particular ecosystem. It has been proven that prokaryotic cells can survive in extreme climate using the pollutants contained in glacial waters as a source of food (Kosek et al. 2018). Other studies show how microorganisms can develop in the oceanic depths or hydrothermal vents, depending on depth, temperature and pressure (la Ferla et al. 2012). Publications confirm that the prokaryotic communities can adapt and survive in various environmental conditions, and colonize even nutrient-poor or extremely hostile, acidic or boiling hot niches (Amin et al. 2017). Therefore, a question arises whether human-related bacteria, possibly released with the treated wastewater, can survive and develop in the receiver significantly deteriorating its quality. While many microorganisms in the ecosystem play an important role in the biodegradation of pollutants (Ostroumov 2017), which can originate from human activity, there is still a risk that this microbial community may contain pathogenic microorganisms that may have an adverse effect on the health and life of animals and people living in the vicinity of such reservoirs.

Hel Peninsula and beaches of the Baltic Sea are example of such extraordinary places in Poland. Coastal Landscape Park (CLP) is located in the northern part of Poland and covers an area of almost 19 ha – over a half is covered by the waters of the Inner Puck Bay and marine coastal areas. It is one of the oldest (established in 1978) areas in Poland covered by this form of nature protection (Majdak 2008). Tens of thousands tourists visit the Park annually, and the number of accommodations increase each year (Borkowski 2019). During the summer season, over 500 thousand people visit the CLP and the nearby area. The population density in Puck County increases from about 150 people per sq. km to over 850 people per sq. km (Statistics Poland 2016a). Every year in the summer season, the Park authorities face the extremely difficult task to ensure that tourists visiting this area leave the place intact. Of course, despite all efforts, this does not always work. Apart from vandalism, illegal garbage disposal, lack of sanitary connections of seasonal accommodation spots, there are other factors that can have a negative impact on the ecosystem, which result only from such seasonal popularity of this area, e.g., the fluctuating amount of released treated wastewater. One of the most impacted environments are surface waters: rivers and lakes being very popular tourist destinations during the summer season (Krajewska and Fac-Beneda 2016).

Rivers located in the northeastern part of Pomeranian Voivodeship are not under permanent control by the Coastal Landscape Park's employees. Their catchment is not always within the Park's area and the Park's Board has no authority over the tributaries, therefore the protection and management are very difficult. Streams and ditches supplying bigger rivers usually pass-through agricultural areas and carry organic pollution from the farms and fertilizers from the fields (Wojciechowska et al. 2019). They flow through the CLP area, carrying pollutants straight into the Baltic Sea (Zaborska et al. 2019). They also receive treated effluent from local wastewater treatment plants. During the summer, when population density dramatically increases, thousands more cubic meters of wastewater flow into the wastewater treatment plants than in other periods of the year, which raises the question of whether during the holiday season the efficiency of wastewater treatment plants located in tourist areas is sufficient.

There is need to apply a comprehensive protection program to the unique NPK area that will perfectly correspond to the priorities of the Regional Operational Program of the Pomeranian Voivodeship for 2014–2020 (ROP WP 2014–2020) funded by the European Union (EU) under the European Regional Development Fund (ERDF) and the European Social Fund (ESF).

In 2015, preparations were initiated to assess the physicochemical and microbiological risks occurring in this area. Essential physicochemical and sanitary analyses (number of *E. coli* bacteria) were performed at selected measurement points.

On the basis of preliminary observations, 40 measurement points were selected, and the scope of physicochemical and microbiological studies was extended. The CLP area was divided into two regions – catchments of the Piaśnica, Karwianka and Czarna Wda rivers which directly flow to the Baltic Sea (Part I) and rivers and smaller watercourses flowing to the Bay of Puck (Part II). The conducted research confirms the urgent need for better protection of this area to conserve both its ecosystem and value for tourism. The watercourses pass near the swimming areas and beaches, that are very popular in the summer season and may affect their quality. Moreover, due to the popularity of local tourism after the Covid-19 pandemic, these areas may still be used intensively in the future because of their unique tourist qualities.

This paper discusses the analysis of the results of the research conducted in 2016 in the Part I area, the research of the second area (Part II) is discussed in Baczkowska et al., 2022.

Materials and Methods

Study area

In this study, three rivers: the Piaśnica River, Karwianka River and Czarna Wda River, which pass through the Coastal Landscape Park (CLP), were selected in consultation with the CLP management board to conduct physicochemical and microbiological analysis, based on the representative character of these rivers.

The catchments of these rivers represent diverse physiographic conditions, e.g., isolated wetlands and coastal lowlands. The variety of relief conditions cause the occurrence of different types of soils, which entails different types of land use in the catchments. The Piaśnica River catchment is an area of 300 km^2, mainly covered by forests. Agricultural use does not exceed 50% of the region. On the contrary, the smaller catchments of the Karwianka River (60 km^2) and the Czarna Wda River (90 km^2) are covered in 65% by agricultural land dominated by farmlands. Anthropogenically transformed areas constitute a small part of the above-mentioned areas, however, the introduced changes are responsible for the diversified hydrographic network in the catchments. In the river valleys and coastal plains occupied by wetlands, the natural river system has been included in the extensive and complex system of ditches and drainage channels. A network of polders was established in the coastal lowlands, which today extends over the estuaries of the Piaśnica, Karwianka and Czarna Wda rivers (Krajewska and Fac-Beneda 2016). Each of the rivers receives treated effluent from local wastewater treatment plants: WWTP1 in Żarnowiec, WWTP2 "Krokowa" in Parszyce, WWTP3 in Kłanino and WWTP4 in Jastrzębia Góra.

WWTP in Żarnowiec collects domestic and industrial wastewater from the sanitary wastewater system as well as the wastewater delivered by tanker trucks. Its designed capacity equals 3,180 m^3/day. The treatment technology consists of mechanical treatment (screens and sand separator), biological treatment (activated sludge – JHB system) followed by secondary settling tanks with excess sludge recirculation. PIX (inorganic coagulant based on trivalent iron Fe^{3+}) and PAX (inorganic coagulant, whose main ingredient is aluminum) dosing systems are occasionally used. Treated wastewater is discharged via a drainage ditch to the Piaśnica River after additional stages of purification: constructed wetlands (gravel-plant filter) and retention ponds.

WWTP "Krokowa" in Parszyce collects domestic and municipal wastewater. Its capacity equals 655 m^3/day and it receives a pollutant load corresponding to 3,021 PE (population equivalent). The actual volume of wastewater coming to the WWTP in the summer season is nearly twice as big as in the winter season (567 m^3/day versus 293 m^3/day in winter). Excessive wastewater is transferred to the wastewater treatment plant in Kłanino. Krokowa WWTP technology includes mechanical (screens and sand separator) and biological treatment (two sequencing batch reactors with activated sludge). The PIX dosing system is occasionally used for additional phosphorus removal. Excessive sludge is transported, stabilized and dewatered in Żarnowiec WWTP. Treated wastewater is discharged to the Karwianka River via a melioration ditch.

WWTP in Kłanino serves approximately 3,000 inhabitants, its capacity equals 491 m^3/day, and it receives overflow wastewater from WWTP Krokowa. The treatment process consists of mechanical (screens and sand separator) and biological treatment (low-loaded activated sludge system with secondary settling tanks with excess sludge recirculation – two SBR reactors operating cyclically). The PIX agent is occasionally used. Treated wastewater is discharged via a ditch to the Czarna Wda River.

WWTP in Jastrzębia Góra serves a population of 3,500 which in summer increases to even 30 000 people due to the tourism. Its designed capacity equals 7,305 m^3/d, and a pollutant load corresponds to 62,000 PE. Treatment technology includes mechanical step and advanced biological step based on activated sludge with chemical precipitation to increase phosphorus removal (five-stage modified Bardenpho process) and UV disinfection applied before discharge to the Czarna Wda. However, WWTP has to deal with changing inflowing wastewater volume, which is highly dependent on the season and number of tourists in the region. In the summer, the wastewater volume can increase from 1.5- to 7 fold of winter inflow (peak in July and August). In 2015 the average pollutant load to the WWTP corresponded to 12,540 PE, while the average daily inflow rate equaled 1,678 m^3/day, and ranged from 529 m^3/day to 5,592 m^3/day (Luczkiewicz et al. 2019; Infoeko 2004). This variability can lead to hydraulic overload and other technological problems caused by tourism. Another important issue is the management of wastewater originating from manholes/septic tanks. In case the share of manholes/septic tank wastewater is significant in the total WWTP inflow, serious operational and technological problems may arise. However, as the treatment plant has a contaminant identification system, it can refuse to accept wastewater from a tanker truck. An increase of pollutant inflow in summer season also does not pose a threat to the technological process, because the treatment plant is equipped with a reservoir which makes it possible to establish a constant flow of wastewater throughout the year. All the WWTPs discharging treated effluent to the rivers located within the CLP were compared in Table 1.

Sampling

Sampling was carried out in July 2016 in the area of the Coastal Landscape Park (CLP), in its buffer zone and in the adjacent areas (see Figure 1). The analysis was carried out as a part of a periodic environmental monitoring program conducted in cooperation with the CLP. The selection of 14 sampling points was based on the characteristic location: on the Piaśnica River two points were located upstream from Żarnowieckie Lake (P5 and P4) and three downstream from the lake (P2 corresponds to the point of Żarnowiec wastewater treatment plant (WWTP 1) effluent inflow). On the Karwianka River, sampling was carried out at five points: downstream from WWTP in Parszyce (WWTP 2, point K3) and after branching off the river into two arms, just before its mouth: the point at the west weir (K2W) is located on the arm of the river (similar width and depth as the river) – after the weir it reconnects with the main watercourse. During the sampling, the west weir was open, and the east weir was closed. Four sampling points were located on the Czarna

Wda River: two upstream from the wastewater treatment plant in Jastrzębia Góra (WWTP 3, points CW3 and CW4) and two downstream from the WWTP effluent discharge point (CW1 and CW2). For each river, water samples were taken at its mouth, at a similar distance from the shoreline of the Baltic Sea. The points are denoted after the corresponding river name abbreviation and numbers increase with the distance from the river mouth. For the detailed description of sampling points see Figure 1.

The surface water samples were collected manually at characteristic points, along the river course, using a bucket with an adjustable length handle. Water was sampled along the main river current, at 0.2 m below the water surface. The bucket was triple rinsed with the sample before its collection. Samples were collected into sterile 125 mL polypropylene containers, transported in the dark, cold storage (portable refrigerator) and analyzed immediately after returning to the laboratory.

Laboratory analyses

The scope of the research included measurements of in situ parameters (temperature, conductivity, pH, dissolved oxygen), ions, total organic carbon and chemical oxygen demand concentrations. The sanitary state of watercourses was assessed based on fecal coliform abundance. Total cell number (TCN), prokaryotic biomass (BP), average cell volume (ACV) and cell morphotype diversity were determined using microscopic analysis (Figure 3).

Table 1. WWTPs located in the region of the Costal Landscape Park and their main characteristics

WWTP	Location of WWTP and region of operation	Treatment technology	Designed capacity [m^3/day]	Treated wastewater receiver	Additional stages of purification
Żarnowiec	Żarnowiec City, municipality of Krokowa	JHB system	3,180	Piaśnica River	constructed wetlands (gravel-plant filter), retention ponds
Krokowa	Parszyce, municipality of Krokowa	SBR system	655	Karwianka River	–
Kłanino	Kłanino, municipality of Krokowa	SBR system	491	Czarna Wda River	–
Jastrzębia Góra	Jastrzębia Góra City, municipality of Władysławowo	five-stage modified Bardenpho process	7,305	Czarna Wda River	UV disinfection

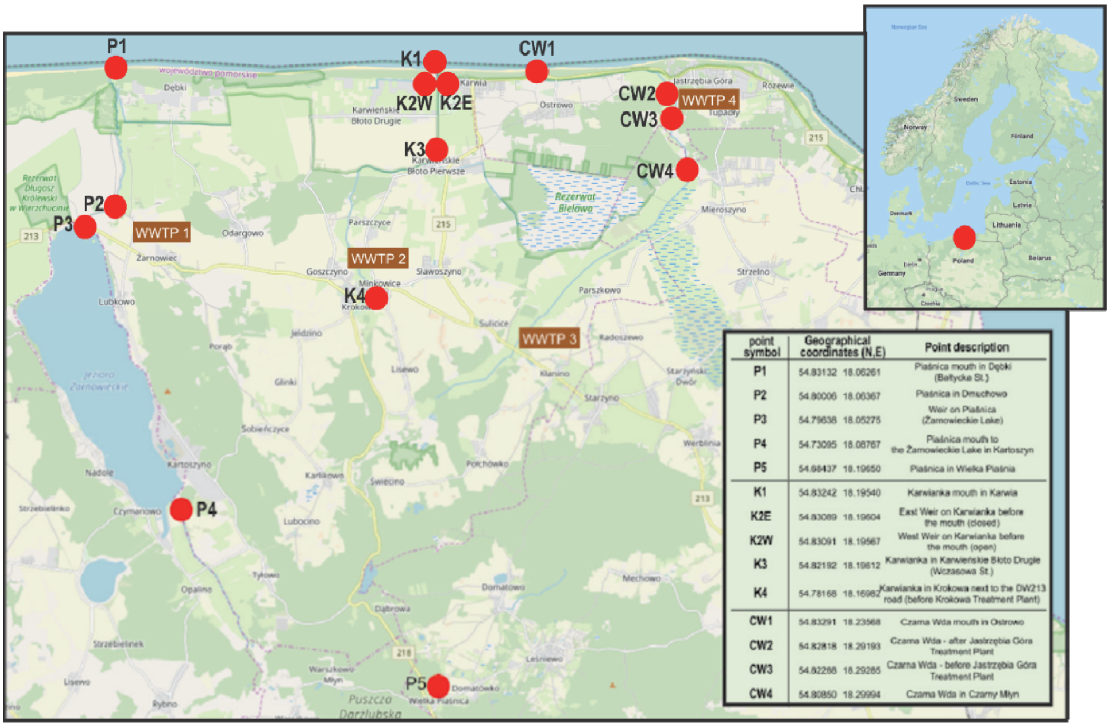

Fig. 1. Map of study the area with sampling points and WWTPs (WWTP1 – Żarnowiec, WWTP2 – Krokowa, WWTP3 – Kłanino, WWTP4 – Jastrzębia Góra) discharging treated effluent into the rivers under investigation (source: ©OpenStreetMap)

Ion (anions and cations) concentrations were obtained by DIONEX ICS-3000 chromatographs (DIONEX, USA). For anion analysis Dionex IonPac AS22 analytical column was used (eluents: 4.5 mM Na_2CO_3 and 1.5 mM $NaHCO_3$, flow rate: 0.3 mL min^{-1}). For cation analysis Dionex IonPac CS16 analytical column was used (eluent: 38 mM methanesulfonic acid, flow rate: 0.36 mL min^{-1}). Conductometric detection for both cation and anion analyses was applied. Total organic carbon (TOC) was determined on a Total Organic Carbon Analyser TOC-VCSH/CSN (Shimadzu, Japan) using catalytic oxidation method with oxygen at 680°C, with non-dispersive infrared spectroscopy (NDIR) as a detection method. Physicochemical measurements of temperature (T), pH, dissolved oxygen (DO) and electrical conductivity (EC) were carried out in situ using portable multifunctional measuring device ELMETRON CX-561 (Elmetron, Poland) with a set of electrodes: EPP-1 for pH measurement, ECF-1 for conductivity measurement, COG-1 for dissolved oxygen concentration measurement and temperature sensor with Pt-1000s resistor. Technical details are specified in Table 1. Chemical oxygen demand (COD) was determined according to the Standard Methods (APHA 2005), using UV-VIS spectrophotometer (Merck, Germany).

Microbiological analysis

Microbiology analyses included the cultivation of fecal indicator bacteria (fecal coliforms, including *E. coli*) and

Fig. 2. Pictures of the rivers tested in the study at the sampling points. The first row represents pictures of Piaśnica River, second row – Czarna Wda River and the third row – Karwianka River

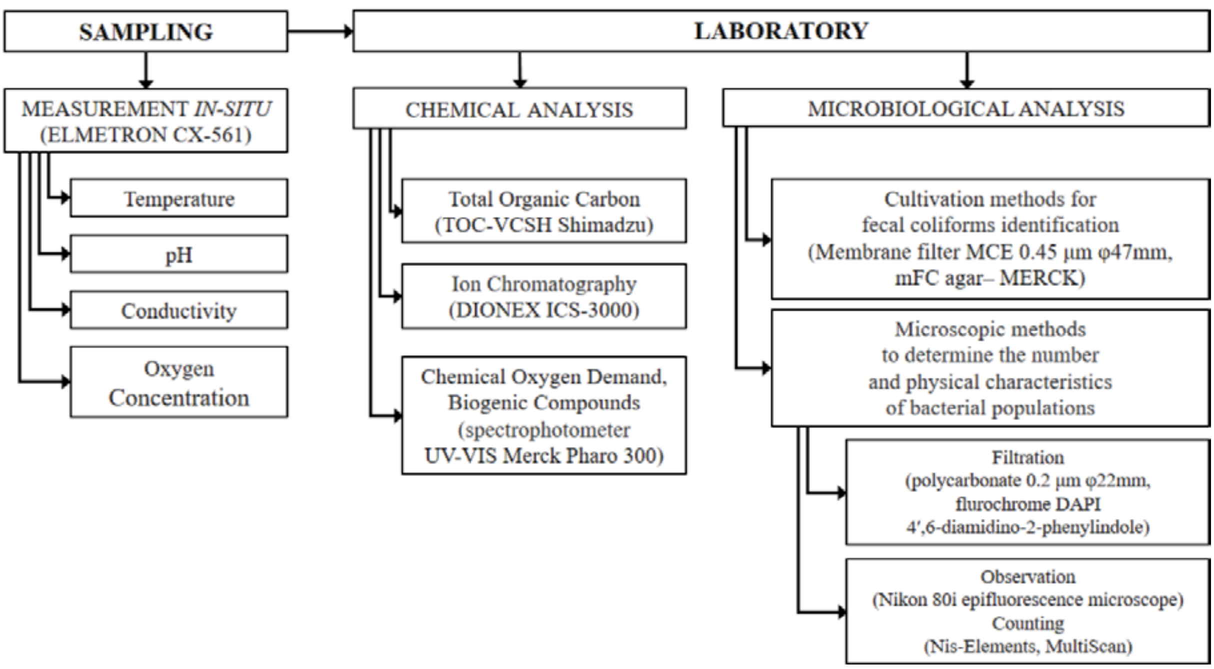

Fig. 3. Schematic diagram showing *in situ* measurements and laboratory analyses

microscopic observations to determine the abundance and physical characteristics of the prokaryotic community present in the water samples.

Detection and enumeration of fecal coliforms were carried out according to ISO 9308-1:2014, using membrane filtration. 1 and 10 ml samples were filtered on cellulose membrane filters (47 mm diameter, 0.45 µm pore diameter, Whatman, Germany) and incubation on mFC agar (Merck, Germany) at 44°C for 24 h. Blue colonies were counted as fecal coliforms.

Microbiological parameters such as total prokaryotic cell number (TCN), average prokaryotic cell volume (ACV), prokaryotic biomass (PB) and prokaryotic cell morphotype diversity were determined using the direct epifluorescent filter technique (DEFT). Water samples (50 ml) were fixed with buffered formalin (Merck, Germany) to a final concentration of 2%. In the laboratory, sub-samples of 1.5 mL were stained with DAPI – 4,6-diamidino-2-phenyl-indole (Thermo Fisher Scientific, US) to a final concentration of 1 µg/mL and filtered through a black Nuclepore polycarbonate membrane filter (0.2 µm pore diameter, Whatman, Germany) (Porter and Feig 1980). Filters were mounted on a microscopic slide with non-fluorescent oil (Citifluor AF2: Agar Scientific, US) (Fry 1980) and stored at -20°C until analysis.

Microscopic observations were carried out using a Nikon 80i epifluorescence microscope under 1,000-fold total useful microscope magnification. An HBO103 W/2 high-pressure mercury lamp (Osram GmbH, US), 330–380 nm excitation filter, 400 nm dichroic mirror and 420 nm barrier filter were used. For each sample, 20 fields of microscope view, with a maximum of 60 thousand objects, were digitized using Nikon DS-5Mc-U2 high-resolution color digital camera and NIS-Elements BR 3.0 software. Abundance (TCN), size (ACV) and geometric parameters (morphological types) of stained prokaryotic cells were determined with an automatic image analysis system (MultiScan, v.14.02) and modification of Świątecki (Świątecki 1997). The biomass was calculated by converting DAPI-stained cell volume to carbon units using the biomass conversion factor 170 fg C µm3 (Norland 1993). Prokaryotic diversity based on the presence of individual morphotypes (cocci, rods and cylindrical curved) was evaluated (Nübel et al. 1999).

The principal component analysis (PCA) for this study was performed using Rstudio v.1.3.9., using the factoextra package and prcomp function. Figures were edited in CorelDRAW X7.

Results

Chemical analysis

Measurements in situ: temperature, EC, DO and pH

During the survey, the temperature of the water did not exceed 20°C (Table 2). In each river, the water temperature was higher closer to its mouth. The largest temperature variability was noted in the Piaśnica River – both the lowest (13.8°C, P5) and the highest temperature in the dataset (19.6°C, downstream from Żarnowieckie Lake and at the mouth of the river into the Baltic Sea; points P1 and P2) were recorded for this river. Water temperature in the Karwianka River at point K4 (upstream from the WWTP discharge point) was 14.9°C, while downstream the WWTP's effluent inflow increased to almost 18.0°C. A similar dependence was noted on the Czarna Wda River: lower water temperatures (15.9°C and 16.5°C) were recorded upstream from the treated wastewater discharge (points CW4 and CW3). At points closer to the mouth, located behind WWTP, the temperature increased to above 17.3°C (CW2 and CW1).

Electrical conductivity ranged from 310 µS/cm (P2 and P3) to 949 µS/cm (K2E). On average, conductivity was the lowest in the Piaśnica River (average±SD: 343±42 µS/cm) and the highest in the Karwianka River (630±201 µS/cm). At points K2E, K2W and K1 on the Karwianka River, EC exceeded 600 µS/cm, while at points further from the mouth, slightly lower values were recorded: 498 µS/cm for sample K3 from Karwieńskie Błota Drugie, and 424 µS/cm for K4 in Krokowa (see Figure 1). In the Czarna Wda River, the conductivity at points located upstream from the treated wastewater discharge (CW4, CW3) was lower (below 440 µS/cm) than behind WWTP – the conductivity in CW2 and CW1 increased to over 500 µS/cm (Table 2).

Table 2. Validation parameters and technical specifications used in the applied analytical procedures.

Determined compounds/parameters	Unit	MR	LOD	LOQ	Measurement method/technique
Electrical conductivity	µS/cm	–	–	–	Electrochemical method: CX-561 conductometer (Elmetron), conductivity sensor ECF-1
pH	–	–	–	–	Electrochemical method: microcomputer pH-meter (Elmetron), electrode type EPP-1
TOC	mg/L	0.150–10.0	0.030	0.100	Total Organic Carbon Analyser, TOC-VCSH/CSN (Shimazu), method of catalytic combustion (oxidation) with NDIR detector
Anions	mg/L	0.030–250	0.060	0.180	Ion Chromatography with conductivity detector (DIONEX ICS-3000)
Cations	mg/L	0.030–250	0.010	0.030	

MR – measurement range; LOD – limit of detection; LOQ – limit of quantification
The limit of detection (LOD) and the limit of quantification (LOQ) were calculated based on the standard deviation of the response (s) and the slope of the calibration curve (b), according to the formulas: LOD = 3.3(s/b), LOQ = 10(s/b).

Among all the samples, dissolved oxygen concentration varied from 5.9 mg/L (CW1) to 9.6 mg/L (P3). The lowest average DO concentration was noted for the Karwianka River (6.6±0.47 mg/L) and the highest for the Piaśnica River (8.5±0.82 mg/L). No visible trend was observed regarding DO concentration and sampling point localization upstream or downstream from WWTPs, however, in all the three rivers the oxygen concentration decreased at the estuary.

In all the samples, pH was slightly alkaline (7.6–8.5). It followed the same pattern as dissolved oxygen concentration: on average it was the highest for the Piaśnica River (8.06±0.32) and the lowest for the Karwianka River (7.66±0.09). The lowest pH was noted at stations K2W, K3 and K4 and the highest on P3.

Total organic carbon (TOC) and Chemical Oxygen Demand (COD)

Among all the samples, total organic carbon concentrations ranged from 2.0 mg/L (P3) to 6.81 mg/L (K3), see Table 3. On average, the lowest TOC was noted in the Piaśnica River (2.3±0.34 mg/L) and highest in the Karwianka River (5.29±0.95 mg/L). In all rivers, the increase in TOC (concentrations above mean value) was noted at sampling points located after the discharge from WWTPs. On the Karwianka River, the west weir (point K2W) was open during the sampling and TOC was equal to 5.54 mg/L, while on the east weir, closed during the sampling, TOC was lower and equaled 4.57 mg/L. Chemical oxygen demand values exhibited a similar pattern between rivers as TOC: the lowest COD was noted for the Piaśnica River (average below 12 mg/L) and the highest was noted in the Karwianka River (26.8±6.55 mg/L). The highest values were detected at points close to the estuary (K1, K2E, K2W, 30±1 mg/L), however, the sampling point located closer to the WWTP effluent inflow (K3) showed concentrations lower by almost half (17 mg/L).

Ions (including biogenic compounds)

Tested forms of biogenic compounds included ammonia, nitrate, nitrite and phosphorus (in the form of $N\text{-}NH_4^+$, $N\text{-}NO_2^-$, $N\text{-}NO_3^-$ and $P\text{-}PO_4^{3-}$), see Table 4. Ammonia concentrations in all locations on the Czarna Wda River were equal to 0.13 mg/L, varied between 0.19–0.96 mg/L in the Piaśnica River, and was the highest in the Karwianka River (1.24–3.70 mg/L). In the Czarna Wda River ammonia values were steady along the watercourse, in the Karwianka River the highest value was noted at point K3 located after the WWTP effluent inflow, whereas in the Piaśnica River the highest value was detected at the estuary (0.96 mg/L, point P1) and it was over 5 times higher than the concentration noted at the previous sampling station, located at the treated wastewater inflow (P2, 0.16 mg/L). Nitrate concentrations presented a different trend than ammonia: the lowest concentrations were noted in the Piaśnica River (0.03–0.39 mg/L) and the highest in the Czarna Wda River (0.34–1.32 mg/L), while in the Czarna Wda River almost 3-fold increase of nitrate was noted after the WWTP effluent inflow (points CW2 and CW3). Nitrite was higher in the Karwianka River (0.34 mg/L) than in the Piaśnica River (0.04 mg/L). Phosphate values were similar for the Piaśnica and Czarna Wda rivers (2.00±2.69 mg/L and 2.02±3.49 mg/L, respectively), but higher in the Karwianka River (4.47±6.81 mg/L).

The analysis of remaining cations and anions in the water showed trace amounts of such elements as lithium, potassium, fluorine and bromine (below 0.1 mg/L, 0.71–3.84 mg/L, 0.18–0.49 mg/L and 0.01–0.78 mg/L, respectively), Table 4. Sodium, calcium and magnesium were the cations of the

Table 3. Results of *in situ* measurements: average temperature (T), electrical conductivity (EC), pH and dissolved oxygen (DO), as well as total organic carbon (TOC) and chemical oxygen demand (COD). Grey field indicates points below the WWTP discharge. In case of Czarna Wda River different shading has been applied to indicate that CW4 and CW3 points are located below WWTP3 discharge, while CW2 and CW1 are located directly below WWTP4 discharge. Blue, green and yellow fields indicates rivers' classification (explained in discussion).

Sampling point	Date of sampling	JCW types	T [°C]	EC [μS/cm]	pH [–]	DO [mg/L]	TOC [mg/L]	COD [mg/L]
P1	06.07.2016	22	19.6	354	7.90	8.10	2.64	11.0
P2	06.07.2016	23	19.6	310	8.30	8.80	2.00	14.0
P3	06.07.2016	23	19.2	310	8.50	9.60	2.69	<10
P4	06.07.2016	17	14.4	410	7.80	8.70	2.18	<10
P5	06.07.2016	17	13.8	329	7.80	7.40	2.01	11.0
K1	07.07.2016	22	17.6	652	7.70	6.60	5.06	29.0
K2E	07.07.2016	23	18.5	949	7.80	7.40	4.57	31.0
K2W	07.07.2016	23	17.7	626	7.60	6.50	5.54	30.0
K3	07.07.2016	23	17.9	498	7.60	6.60	6.81	17.0
K4	07.07.2016	23	14.9	424	7.60	6.10	4.47	<10
CW1	07.07.2016	22	18.4	524	7.80	5.90	3.49	18.0
CW2	07.07.2016	22	17.3	517	7.80	7.80	3.42	23.0
CW3	07.07.2016	22	16.5	439	7.90	7.10	2.02	14.0
CW4	07.07.2016	22	15.9	437	7.90	7.00	2.23	10.0

highest concentration in all the samples. Sodium ranged from 2.42 mg/L (P4) to 48.80 mg/L (K2E), calcium from 16.1 (P1) to 49.1 mg/L (K2E) and magnesium from 4.73 (P5) to 14.1 mg/L (K2E). Among anions, sulfate and chloride were the most abundant in all the samples (20.3 – 72.8 mg/L and 11.7–137 mg/L, respectively). Among all the samples, K2E frequently represented the highest concentrations of ions in all the samples (Na^+, Mg^{2+}, Ca2$^+$, Cl^-, Br^- and SO_4^{2-}) and K3 presented the highest concentrations of K^+.

When analyzing the percentage cationic and anionic composition of the samples, it can be noted that for the Czarna Wda River, samples before WWTP discharge (CW1 and CW2) were similar to each other but differed from the ones after the inflow of the treated effluent (CW3 and CW4). However, a similar pattern was observed neither in the case of the Piaśnica River, nor for the Karwianka River (Table 4). Interestingly, K2E and K2W, being the neighbor points in the east and west weir on the Karwianka River differed from each other, mainly by sodium and chloride ions concentration (almost three times higher in K2E). Also, the influence of the treated wastewater was not observed for the Piaśnica River, where samples P2 and P3 were similar to each other.

The abundance of fecal coliforms, determined based on cultivation method, is defined in CFU/100 mL unit, where CFU stands for Colony Forming Units. In contrast, total prokaryotic cell number (TCN) determined with the use of microscopic analysis is reported in cells/100 mL. Morphotypes (cocci, rods and curved) were divided into five volume (size) classes: very small (below 0.1 μm^3), small (0.1–0.2 μm^3), medium (0.2–0.5 μm^3), large (0.5–1.0 μm^3) and very large (above 1.0 μm^3).

The Piaśnica River

The highest abundance of fecal coliforms was noted behind the treated wastewater inflow – 3.2×10^3 CFU/100mL was recorded at point P2 and 2.9×10^3 CFU/100 mL at point P1 (Figure 4a). In other locations, indicator bacteria were less abundant. At the furthest point from the river mouth, P5, only 0.28×10^3 CFU/100 mL were noted. The lowest abundance of fecal coliforms was noted at point P4 before the lake: 0.20×10^3 CFU/100 mL. Downstream from Żarnowiec Lake, at point P3, indicator bacteria abundance was equal to 0.25×10^3 CFU/100 mL (Figure 4a).

The total number of prokaryotic organisms (TCN) at P5 was equal to 3.0×10^8 cells/100 mL which was the highest value noted in the Piaśnica River (Figure 4a). A similar value (2.9×10^8 cells/100 mL) was observed at P1. At the stations further from the river estuary, the total number of prokaryotic organisms was lower. At point P3, TCN was equal to 1.8×10^8 cells/100 mL and at points P2 and P4 1.0×10^8 cells/100 mL were recorded.

At point P1, where an increased TCN value was noted, the average volume of prokaryotic cells (ACV) was equal to 0.27 μm^3 and their biomass was the highest among all the water samples from the Piaśnica River (103 μg C/mL, Figure 4b). However, at point P2, where TCN was three times smaller, the biomass of prokaryotic cells (PB) equaled 57 μg C/mL but composed mainly of bigger cells (ACV equal to 0.59 μm^3). In this sample, ACV was influenced mainly by the presence of medium, large and very large cocci of 0.2–1.0 μm^3 volume (30% of the total prokaryotic community) and of >1.0 μm^3 volume (more than 18% of the community), see Figure 4e.

In the P1 sample, the most abundant were small (<0.1 μm^3) rods, however, the largest share of the biomass was corresponding to medium, large and very large cocci. The lowest PB value among the points was noted at point P3 (28 μg C/mL), where ACV was only 0.05 μm^3 (Figure 4b). Such low values resulted from the presence of very small rods and cocci which constituted over 80% of the prokaryotic community in that sample (Figure 4e).

At P4, small and medium (0.1–0.5 μm^3) rods were observed (>16%), and their share in the biomass was significant (25% of PB value) (Figure 4f). However, the TCN value at this point was the lowest among the samples and very small rods (<0.1 μm^3) were the most abundant (Figure 4e). Therefore, this resulted in the low AVC (0.12 μm^3) and the lowest prokaryotic cell biomass among all the samples from the Piaśnica River (25 μg C/mL) (Figure 4b).

At P5, very small rods and cocci were the most numerous, however, a very large share in the biomass was constituted by medium and large (0.2–0.5 μm^3 and 0.5–1.0 μm^3) cells. With TCN of 3.0×10^8 cells/100mL, the average PB value in this point was equal to 74 μg C/mL, while the average cell volume was 0.14 μm^3 (Figure 4b).

The Karwianka River

Compared with the Piaśnica River, the total number of prokaryotic organisms (TCN) in the Karwianka River was similar, but the number of fecal coliforms was several times higher (Figure 5a). Also, in the Karwianka River, the amount of indicator bacteria was the highest among the analyzed watercourses.

Along the river, TCN was rather stable. The smallest TCN was noted in point K4 (1.6×10^8 cells/100 mL) and the highest at K3, K2E and K2W points (around 3.0×10^8 cells/100 mL), which was similar to the number of microorganisms that were observed at points P1 and P5 on the Piaśnica River (Figure 5a). However, in the Karwianka River the number of fecal coliforms was one or two orders of magnitude higher than in the Piaśnica River: at the eastern and western weirs (K2E and K2W), more than 1.0×10^4 CFU/100 mL was recorded, and in Karwieńskie Błoto Drugie (K3) more than 2.5×10^4 CFU/100 mL. On the other hand, at the river mouth (K1), the total number of prokaryotic cells was 2.3×10^8 cells/100 mL, while fecal coliform abundance was equal to 1.3×10^4 CFU/100 mL (Figure 5a).

At points K1, K2E, K2W and K4, the most abundant morphotypes were very small rods (<0.1 μm^3). However, their high abundance had no dominant influence on PB value in these points (Figure 5e and 5f). At point K4, the largest rods (0.5–1.0 μm3 and larger, Figure 5f) had the largest share (36%) in biomass. At points K2E and K2W, medium and very large size cocci had a significant impact on PB value (27% and 28%, respectively).

At point K2E, the mean cell volume was equal to 0.25 μm^3. In this sample, the highest biomass value was recorded (97 μg C/mL, Figure 5b). For the water sample collected at the west weir (K2W), almost identical AVC was noted (0.24 μm^3), but it corresponded to slightly lower biomass (89 μg C/mL, Figure 5b), which results mainly from the difference in TCN between those two points (difference equal to 0.1×10^8 cells/100 mL, Figure 5a).

Table 4. Concentrations of cations and anions determined by ion chromatograph

Sampling point	JCW	N-NH_4^-	N-NO_2^-	N-NO_3^-	P-PO_4^{3-}	Li^+	Na^+	K^+	Mg^{2+}	Ca^{2+}	F^-	Cl^-	Br^-	SO_4^{2-}
	types	[mg/L]	[mg/L]	[mg/L]	[mg/L]	[mg/L]	[mg/L]	[mg/L]	[mg/L]	[mg/L]	[mg/L]	[mg/L]	[mg/L]	[mg/L]
P1	22	0.96	<LOD	0.05	0.19	0.10	10.3	1.09	7.38	16.1	0.24	33.7	<LOD	37.5
P2	23	0.16	<LOD	0.03	0.12	0.10	5.42	0.84	6.11	19.9	0.20	24.0	<LOD	35.8
P3	23	0.19	<LOD	0.04	0.11	0.10	6.37	0.83	6.64	22.9	0.27	24.3	<LOD	40.1
P4	17	0.22	0.04	0.39	0.25	0.10	2.42	0.71	7.43	36.5	0.33	11.7	<LOD	33.8
P5	17	<LOD	<LOD	0.06	0.31	<LOD	3.50	<LOD	4.73	28.5	0.30	15.5	<LOD	20.3
K1	22	1.24	<LOD	0.11	0.37	<LOD	18.8	2.54	9.41	43.2	0.29	59.3	0.35	49.8
K2E	23	2.18	<LOD	0.22	0.55	0.10	42.8	2.64	14.1	49.1	0.38	137.0	0.78	72.8
K2W	23	1.25	<LOD	0.10	0.35	0.10	14.5	2.28	8.57	42.8	0.24	51.4	0.27	48.9
K3	23	3.70	0.34	0.36	0.62	<LOD	6.10	3.84	6.20	35.9	0.27	22.0	<LOD	50.4
K4	23	<LOD	<LOD	0.60	0.39	<LOD	2.82	1.33	6.44	37.0	0.49	16.7	<LOD	35.8
CW1	22	0.13	<LOD	1.32	0.25	0.10	10.1	2.73	7.01	39.5	0.18	32.6	<LOD	50.3
CW2	22	0.13	<LOD	0.99	0.16	0.10	10.2	3.20	8.33	38.4	0.37	30.5	<LOD	51.2
CW3	22	0.13	<LOD	0.34	0.31	<LOD	3.53	0.93	7.75	39.1	0.29	16.8	<LOD	41.4
CW4	22	0.13	<LOD	0.37	0.24	0.10	4.24	0.94	8.26	36.4	0.46	17.8	<LOD	41.4

At point K2W, the largest share in the entire prokaryotic community was represented by very small rods (over 25%, Figure 5e). However, the prokaryotic biomass at this point was dependent not only on this form but also on the presence of medium and large cocci (Figure 5d and 5f). Their share in the total PB was similar and equaled to 13% for medium and 14% for very large cells (Figure 5f).

At point K2E, over 50% of the biomass was composed of a medium, large and very large cocci (0.2–1.0 μm^3). Among rods, the smallest size class was most abundant, while very large rods were least abundant, however, their influence on the PB at this point was fairly equal and constituted approximately 8% for each of the size classes (Figure 5f).

At point K3, AVC was 0.23 μm^3, and PB was 93 μg C/mL. Similarly to K2W, over 50% of the biomass was composed of the biomass of medium, large and very large cocci (Figure 5f), where almost 25% PB was due to the presence of medium size cocci only. Rods of different size classes contributed to the next 25% of the biomass.

At point K4, located before the wastewater treatment plant outlet, a low PB value (59 μg C/mL) was noted, together with the highest ACV value among all the samples from the Karwianka River (0.3 μm^3, Figure 5b). Despite high ACV, PB remained low due to the lower TCN noted at this point. In TCN, the highest share was represented by very small rods. However, the highest share in the prokaryotic biomass was due to the presence of very large rods – over 20% (differently to other points, Figure 5e).

From point K4 to the river mouth, the share of the largest rods in the PB structure dropped (Figure 5e). In the prokaryotic community, the morphology of the cells changed – the percentage of the largest cocci increased. At point K1, despite a large number of prokaryotic cells, the lowest PB value was noted (40 μg C/mL) due to the smallest ACV (0.07 μm^3, Figure 5b). At this point, biomass consisted mainly of the smallest rods (37% of PB, Figure 4e) and in terms of abundance this group was the most numerous (Figure 5e), which finally resulted in a low ACV value (Figure 5b).

The Czarna Wda River

Total prokaryotic cell number in the Czarna Wda River was of the same order of magnitude as in the Piaśnica River and the Karwianka River and varied from 1.64×10^8 cells/100 mL (CW2) to 3.04×10^8 cells/100 mL (CW1). Fecal coliforms varied from 0.9×10^3 CFU/100 mL (CW3, located before WWTP effluent inflow) up to 2.9×10^3 CFU/100 mL in CW2 located after the WWTP (Figure 6a).

Prokaryotic biomass pattern along the watercourse partially followed the pattern presented by average cell volume (Figure 6b). At point CW4, PB was equal to 87 μg C/mL corresponding to ACV equaled 0.33 μm^3. While at point CW3, the average cell volume was slightly lower (0.3 μm^3), PB

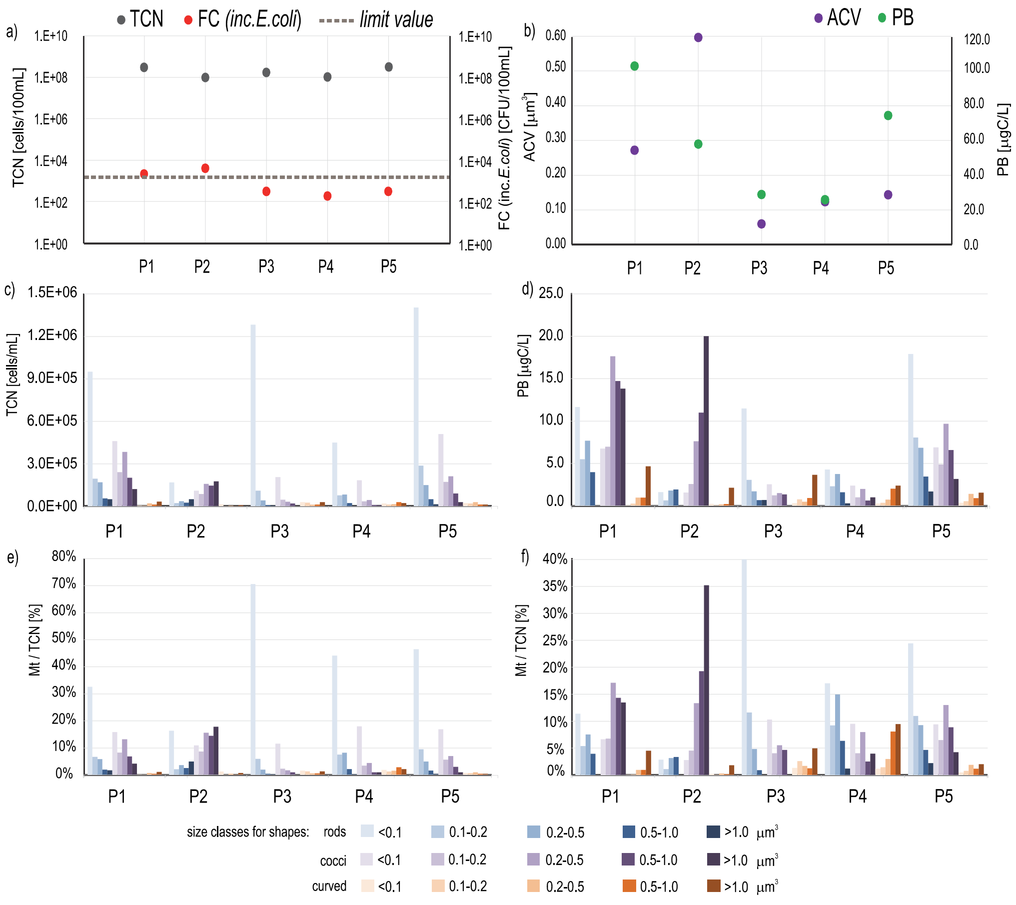

Fig. 4. Results of microbiological analysis for Piaśnica River: a) total prokaryote cell number (TCN) and abundance of fecal coliforms, b) average cell volume (ACV) and prokaryote biomass (PB), c) abundance of morfhotypes (Mt) in the total cell number (TCN), d) morphotypes' biomass in the total prokaryotic biomass, e) percentage share of morphotypes in TCN, f) percentage share of morphotypes' biomass in the total prokaryotic biomass

increased to 110 µg C/mL due to an increase in TCN (from 2.06 to 2.97×10^8 cells/100 mL, Figures 6a and 6b). Point C2, located behind WWTP inflow, was characterized by the smallest ACV, PB and TCN among the samples from the Czarna Wda River (Figure 6b). At the river mouth (CW1), the biomass increased to the level observed at the beginning of the river (due to the highest TCN value), while the ACV remained smaller than downstream from the wastewater discharge (0.18 µm^3).

Similarly, as in other rivers, sampling points closer to the Czarna Wda River mouth were dominated by very small rods, in terms of their abundance (points CW3, CW2 and CW1, respectively: 32%, 42% and 28% of TCN, Figure 6e). In terms of morphology, points CW3 and CW4 located behind WWTP discharge were similar to each other, as well as CW1 with CW2. In CW3 and CW4 the dominant cell morphotypes were very small rods and medium-size cocci, while in CW1 and CW2 smallest rods are followed by the smallest size class cocci (Figure 6c). Larger cocci and rods almost did not occur. In CW3 and CW4 medium size cocci constituted 21–24% of the biomass, while in CW1 and CW2 they did not exceed 20% of the total biomass (Figure 6f). Also, a larger contribution of the largest cocci in PB could be noted in CW3 and CW4 (very large cocci constituted 15–20% of PB, Figure 6f). At points before WWTP on the Czarna Wda River, cocci constituted approximately 60% of biomass and rods constituted approx. 25% of biomass (Figure 6f). At sampling points after WWTP discharge, the contribution to PB changed to approx. 50% for cocci and 40% for rods.

Principal component analysis

According to principal component analysis (PCA), almost 60% of the dataset variability was explained by two principal components. Most of the variability, 43.5%, is explained by the Dim1 component: the variability of samples was mainly shaped by chemical parameters such as ions, conductivity and organic compounds: TOC and COD (Figure 7a, b). Among the microbial parameters, the presence of Fecal Coliforms (FC) seemed to play the greatest role, while the variables obtained from microscopic analysis (Total Cell Number (TCN), Average Cell Volume (AVC) and Prokaryotic Biomass (PB)) were of less importance. The Dim2 component explained about 15.6% of the total variance and was mainly defined by temperature and, to a smaller extent, by pH and DO values.

Among the biogenic compounds, phosphorus played the greatest role in shaping the variability. Most microbiological parameters correlated relatively closely and positively with N-NH_4^+ and TOC; these values were the highest for the Karwianka River, which was reflected on the PCA plot. The water sample collected at point K2E had the highest conductivity, concentrations of most of the ions and COD. The Czarna Wda River was characterized mainly by such

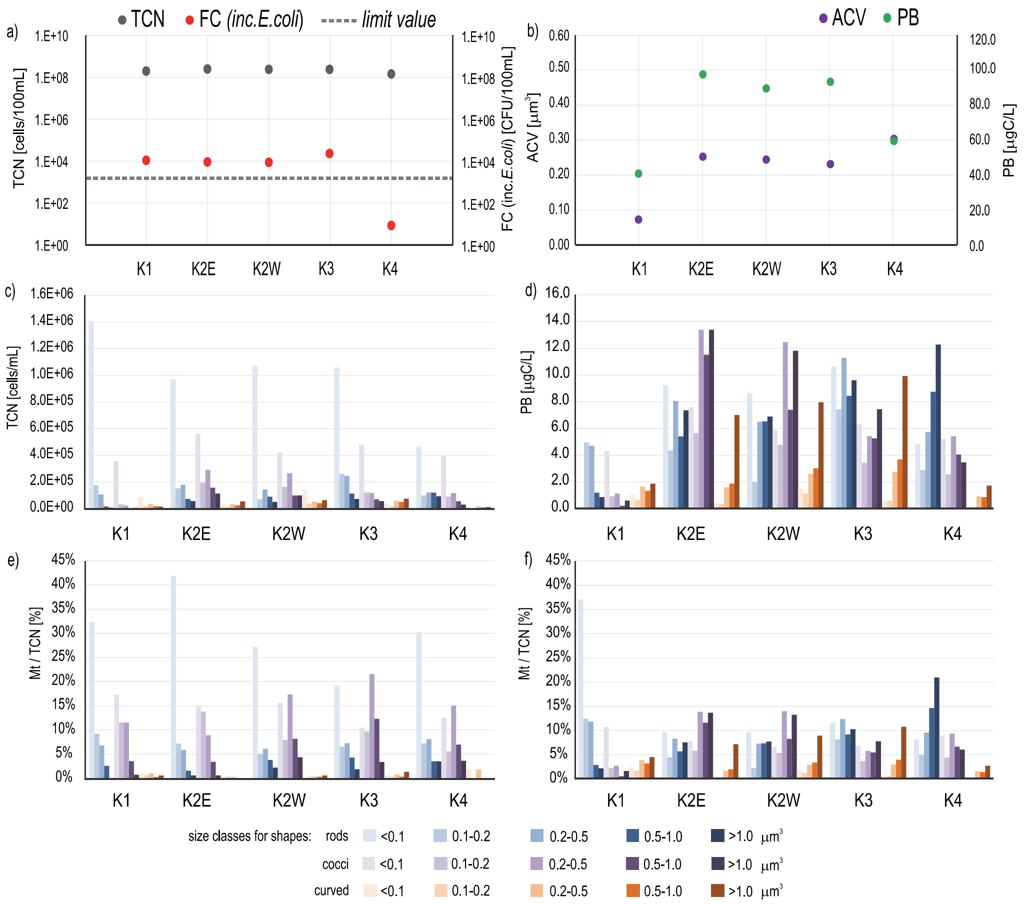

Fig. 5. Results of microbiological analysis for Karwianka River: a) total prokaryote cell number (TCN) and abundance of fecal coliforms, b) average cell volume (ACV) and prokaryote biomass (PB), c) abundance of morphotypes (Mt) in the total cell number (TCN), d) morphotypes' biomass in the total prokaryotic biomass, e) percentage share of morphotypesin TCN, f) percentage share of morphotypes' biomass in the total prokaryotic biomass

parameters as AVC, PB and $N\text{-}NO_3^-$. Dissolved oxygen showed a similar pattern as pH. Oxygen concentration was the highest in water samples collected from the Piaśnica River, especially at points located downstream of Zarnowieckie Lake (Figure 7b; PII). The points located upstream (PI) differed from those below the WWTP discharge (PII) regarding the majority of the tested parameters .

Discussion

The chemical composition of the environment defines what organisms can survive. Nutrients, temperature and pH are important factors influencing whether the conditions for bacterial development and survival are favorable or not. Therefore in this study, the presence of organic substances,

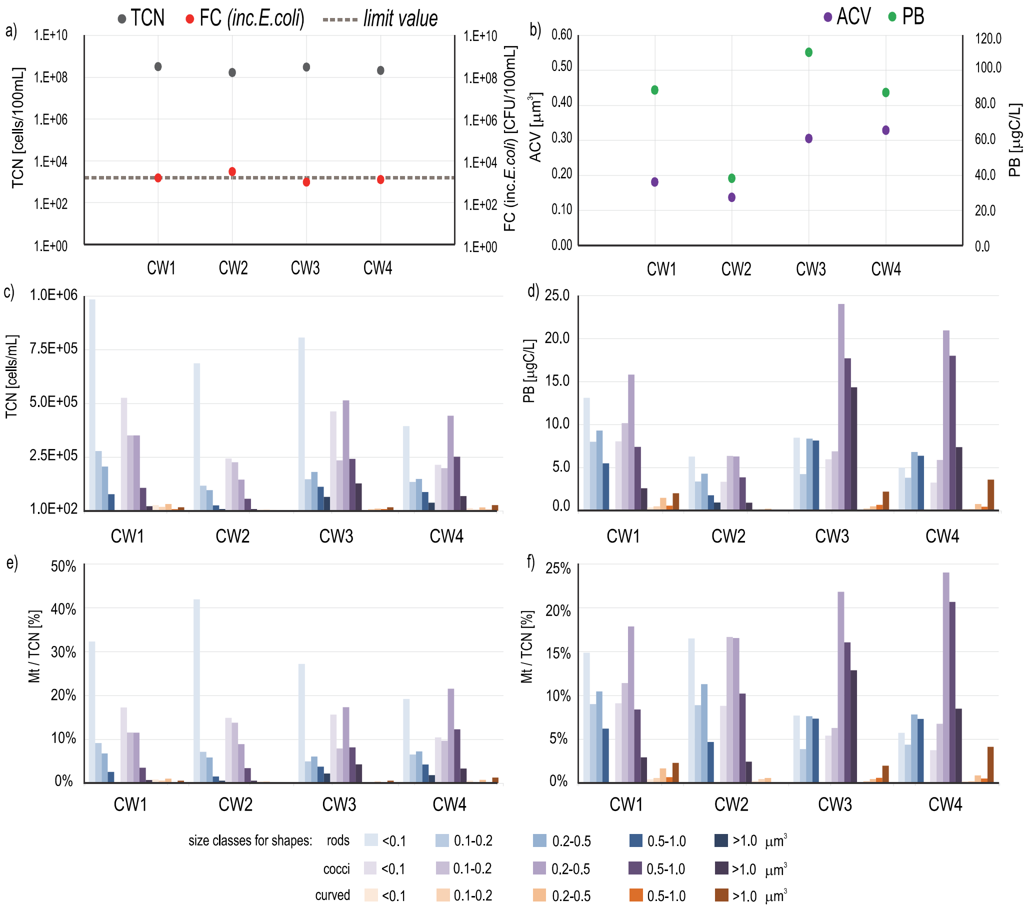

Fig. 6. Results of microbiological analysis for Czarna Wda River: a) total prokaryote cell number (TCN) and abundance of fecal coliforms, b) average cell volume (ACV) and prokaryote biomass (PB), c) abundance of morphotypes (Mt) in the total cell number (TCN), d) morphotypes' biomass in the total prokaryotic biomass, e) percentage share of morphotypes in TCN, f) percentage share of morphotypes' biomass in the total prokaryotic biomass

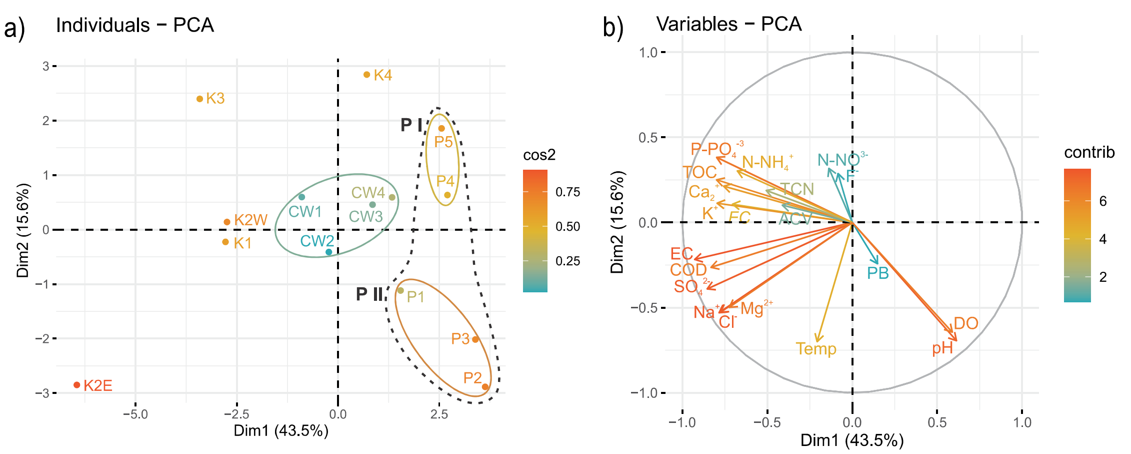

Fig. 7. Principal component analysis of the dataset: a) samples b) variables, plotted in space defined by principal components Dim1 and Dim2

nutrients and ion concentration was analyzed together with microbiological parameters: total prokaryotic cell number, prokaryotic biomass and occurrence of fecal pollution indicator bacteria (fecal coliforms, including *E. coli*) in order to assess the quality of three watercourses located in the area of Coastal Landscape Park.

During the study period, intensive legislative work was carried out in Poland on clarifying the legal standards for assessing the status of unified water bodies (in Polish: jednolite części wód – JCW). The assessment of the current ecological status or potential and the assessment of the risk of not achieving the environmental objectives were carried out. In 2016, the Council of Ministers introduced an update of the Vistula River Basin Management Plan (Council of Ministers 2016b). It characterized in general the area of the Vistula River basin and divided it into 4 water regions. The rivers discussed in this paper are in the fourth area described as "Lower Vistula River Water Region covering the catchment area of the Vistula River from Korabniki to the mouth to the sea and the basin of the Pomeranian rivers".

The plan presented a list of surface water bodies in the area of the Vistula River basin which were given codes and were classified according to types with a division into categories according to the Regulation of the Minister of Environment on the classification of ecological status, ecological potential and chemical status of surface water bodies (Council of Ministers 2011). The Classification and rating of assessments for the rivers discussed in this study are presented in Table 5. The table also indicates measurement points selected for analyses in this study.

During the sampling campaign in 2016, the Regulation of the Minister of Environment (Council of Ministers 2014) was in force. The current regulation did not link the permissible values of particular parameters with the detailed classification of water bodies. However, a few days after the end of the study, a new regulation came into force which enforced such a distinction (Council of Ministers 2016a). Thus, in this paper we referred to these classifications (see Table 6).

Chemical analyses

Water temperature among all the samples was lower than 22°C (1st water quality class). Dissolved oxygen concentration in surface waters of the 1st quality class should not be lower than 7.5 mg/L for lowland streams and 7.1 mg/L in the mouth. Measurements in the Piaśnica River showed values exceeding this standard in all the samples except point P5 which met the standards for the 2nd class. In the Karwianka River, DO in all the samples (apart from K2E) was below this value, but most the points presented DO concentrations above 6.2 mg/L (limit value for the 2nd class water quality). Only at point K4 the value was below the standards. In the Czarna Wda River, the only point with DO values which did not meet the standards was CW1. Studies on water quality of other small rivers in Poland showed even lower dissolved oxygen values (Brysiewicz et al. 2019). However, in protected areas and areas particularly sensitive to anthropogenic pollution any exceeding of the limit values should not be underestimated. Decreasing dissolved oxygen concentration at the rivers' mouth may be partly related to increasing temperature.

Electrical conductivity in all the sampling points was below 1000 µS/cm, but due to the standards only in the Piaśnica River the observed values corresponded to the 1st water quality class. The highest EC value among all the rivers was noted in the Karwianka River in the vicinity of the weir (K2E), where the river passes under the road located above the channel. This can introduce some pollution to the river. Increased EC value in this point results from increased concentrations of sodium and chloride (Table 2). Both values for this point were the highest from the whole dataset.

The source of TOC in the environment may be anthropogenic, but a significant part is also naturally formed by the metabolic processes of aquatic organisms (Kosek and Polkowska 2016). TOC in aquatic ecosystems includes all kinds of organic compounds of plant origin, as well as waterborne bacteria and microorganisms (biomass) (Kozak et al. 2017). Nevertheless, the increase in the value of TOC in surface waters may indicate environmental pollution caused by human

Table 5. Classification and rating of the analyzed rivers

Typology JCW	Code JCWP	Sampling points	Status JCW	Current ecological status or potential	Assessment of the risk of not reaching the environmental goals
17	PLRW200017477259	P5, P4	heavily modified water body	good	not threatened
23	PLRW200023477289	P3, P2	heavily modified water body	good	not threatened
22	PLRW20002247729	P1	natural	bad	not threatened
23	PLRW200023477324	K4, K3, K2W, K2E	heavily modified water body	bad	threatened
22	PLRW200022477329	K1	natural	bad	threatened
22	PLRW200022477349	CW4, CW3, CW2, CW1	natural	bad	threatened

JCW – „jednolite części wód" – unified water bodies
JCWP – „jednolite części wód powierzchniowych" – unified water bodies of surface water
17 – a sandy lowland stream
22 – a stream or estuarine stream influenced by saltwater
23 – a stream in a valley with a high proportion of peatlands

activities (Kosek and Polkowska 2016). Chemical oxygen demand (COD) is also a good proxy for the contamination of surface waters, and therefore, how much oxygen will be used for its mineralization (including inorganic pollutants). As TOC corresponds to the amount of organic carbon, while COD reflects the oxygen needed to oxidize organic and inorganic compounds, the values of these parameters may correlate but are not synonymous.

According to Polish standards being in force in 2016 (Council of Ministers 2016a) and regarding the water quality of the surface waters, all the rivers filled in the requirements for the 1st class water quality (except point K1 which met the standards for the 2nd class). The Czarna Wda River presented higher values at points CW2 and CW1 downstream the discharge of WWTP. The Karwianka River did not exceed the permissible COD value for the 1st and 2nd water quality class, but the COD values were the highest for that river. Among the rivers, a pattern similar to the one exhibited by COD, could be seen also for TOC concentration (on average it is the highest for the Karwianka River and the lowest for the Piaśnica River), however, the allowed permissible limit for the 1st quality class (≤10 mg/L) was not exceeded at any of the sampling points. In terms of TOC and COD, the Karwianka River presented concentrations two times higher than the Piaśnica River. It should be noted that TOC includes compounds that are difficult to degrade and the size of the COD/TOC ratio depends on the types of organic compounds. This is related, in particular, to the oxygen content in the molecule of the compound (Kowalski, 1989). Unfavorable nutrient ratios and high concentrations of specific substances can affect the decomposition process during self-purification of the rivers. Therefore, it is important to control the inflow of pollutants into the environment and constantly monitor the ecological condition.

Biogenic compounds are the elements and mineral salts necessary for the development of living organisms. Increased inflow of nutrients stimulates biological productivity of microorganisms (mainly algae), which in consequence contributes to the faster oxygen consumption in rivers and lakes (Justić et al. 1995; Dodds and Smith 2016). The sources of overfertilization in rivers could be wastewater, as well as fertilizers used in agriculture and washed out from fields during the rain (Wojciechowska et al. 2019). The limit value of phosphates was exceeded in each of the studied rivers. With respect to this parameter no river could be classified even for the 2nd water quality class. Moreover, exceeded values of nitrates were observed on the Czarna Wda River. The requirements were exceeded at point CW1 and CW2 located downstream the WWTP discharge. The values nearly 3–4 times higher than upstream the discharge of WWTP (0.34 mg/L at CW3 vs 0.99 mg/L at CW2 and 1.32 mg/L at CW1). Among all the samples from the Piaśnica River, only P1 was in the 2nd class in terms of ammonia nitrogen. In the case of ammonia nitrogen concentrations, almost all the samples from the Karwianka River did not meet the standards even for the 2nd water quality class (all the samples exceeded allowed 0.738 $N\text{-}NH_4^+$ mg/L limit for lowland streams and 1.00 mg $N\text{-}NH_4^+$/L limit for the river mouth). Exceeded ammonium nitrogen along almost the entire length of the Karwianka River could indicate an inflow of untreated wastewater. This is particularly worrying because an increase in the concentration of biogenic contaminants in surface waters leads to an imbalance in biological processes and a predominance of certain forms of living organisms, e.g., algae. Eutrophication leads to excessive algal blooms causing oxygen depletion and formation of dead zones (Conley et al. 2009). However, accessibility of biogenic compounds (e.g. from the wastewater) creates a favorable niche for the development of bacteria and other microorganisms (Huo et al. 2017). As a result, the excessive inflow of pollutants drastically reduces water quality.

Regarding the remaining chemical parameters listed in the Polish standards (sulfates, chlorides, calcium, magnesium and fluoride, being a substance of special concern), all the analyzed samples did not meet the requirements for the 2nd class of water quality, because of too high concentration of sulfates and chlorides. The values of pH were in the range for the 1st water quality class (7.0–7.9; 7.4–8.2) in the Karwianka and Czarna

Table 6. Limit values for each water quality class for different types of streams and rivers.

Type:	17 (a sandy lowland stream)		22 (a stream or estuarine stream influenced by saltwater)		23 (a stream in a valley with a high proportion of peatlands)	
Class:	I	II	I	II	I	II
T	≤22.0	≤24.0	≤22.0	≤24.0	≤22.0	≤24.0
pH	7.0–7.9	7.0–7.9	7.4–8.2	7.2–8.4	7.2–8.3	7.0–8.3
EC	≤549	≤620	≤440	≤2814	≤454	≤576
COD	≤25	≤30	≤25	≤30	≤68	≤79
TOC	≤10.0	≤11.8	≤10.0	≤14.8	≤18.8	≤21.4
DO	≥7.5	≥6.8	≥7.1	≥6.5	≥7.3	≥6.2
SO_4^{2-}	≤42.0	≤57.0	≤45.9	≤114.7	≤35.2	≤64.8
Cl^-	≤26.0	≤33.7	≤37.0	≤499.0	≤10.8	≤29.4
Ca^{2+}	≤81.0	≤81.7	≤59.4	≤64.2	≤64.3	≤71.7
Mg^{2+}	≤18.4	≤22.0	≤7.3	≤40.4	≤5.8	≤10.1
$N\text{-}NH_4$	≤0.25	≤0.738	≤0.34	≤1.00	≤0.34	≤0.68
$N\text{-}NO_2$	≤0.01	≤0.03	≤0.01	≤0.03	≤0.01	≤0.03
$N\text{-}NO_3$	≤2.2	≤3.4	≤0.5	≤0.9	≤1.3	≤2.5
$P\text{-}PO_4$	≤0.065	≤0.101	≤0.065	≤0.101	≤0.065	≤0.101

Wda Rrivers. However, according to the regulation, surface waters cannot be classified as the 1st and the 2nd water quality class if at least one parameter exceeds the limit values.

The obtained results confirmed the authors' previous observations, which indicated significant improprieties in the physicochemical quality of water in the Karwianka River. In 2015, it was found that as a result of significant exceedances of TOC concentration (10–40 mg/L), PO_4^{3-} ions (2–10 mg/L) and NH_4^+ ions (5–30 mg/L), water at points K1, K2E, K2W and K3 should be classified far below the requirements for the 2nd class of water quality. Therefore, the study was extended in 2016, and showed that the rivers are still under strong anthropogenic influence and monitoring should be continued in order to achieve the set environmental objectives.

Microbiological analysis

Direct detection of all pathogenic microorganisms in the environment is practically impossible. The World Health Organization (WHO), The United States Environmental Protection Agency (US EPA) and The European Commission (EC) have developed recommendations for monitoring in recreational areas (Rees and Bartram 2002). In Poland, no sanitary requirements are set for surface waters. The quality assessment can only be done in terms of suitability for recreational use. It is currently regulated by the Regulation of the Minister of Health from 11 February 2019 on the quality of swimming areas (Council of Ministers 2019). In 2016, when the sampling was conducted, another act was in force (Council of Ministers 2015), however the permissible values of *E. coli* did not change (Table 7). As there are no quality standards, referring to the abundance of fecal coliforms, we assumed that 80% of the fecal coliforms detected on mFC agar were *E. coli* bacteria (Hachich et al. 2012).

Using simple cultivation methods, we can determine which prokaryotic microorganisms are present in the studied ecosystem. Selection of the appropriate medium and incubation temperature provides the cultivation of specific types of microorganisms and enables their enumeration. Some groups of bacteria are so characteristic that their detection in the water directly indicates the presence of domestic wastewater or other fecal contamination in the ecosystem (De Brauwere et al. 2014). *Escherichia coli* is used as one of the sanitary indicators. Together with the concentration of organic and biogenic compounds, it may indicate the pollution coming from, e.g., wastewater and, consequently, inform about a threat to the environment and the infectious risk for people in the swimming areas. The amount of *Escherichia coli* colony forming units (CFU) exceeding the limit value (1000 CFU/100 mL) (Council of Ministers 2019) indicates insufficient water quality, which is especially important in the popular tourist and recreational areas of the Baltic Sea coast.

As an additional water quality examination method, microscopic observations were applied in this study. The data obtained by applied method do not clearly indicate which bacteria are present in the water, but the analysis of changes in the composition of the prokaryotic community may be the key to understanding the interaction of prokaryotic microorganisms with the environment (Straza et al. 2009; Young 2006). Prokaryotic organisms are single-cell organisms that reproduce rapidly when the conditions allow. They use organic substances and oxygen present in water to develop and divide. However, when more substances essential for bacteria are released into the environment, they not only reproduce more intensively, but also reach larger sizes. This relationship was also observed when analyzing the results for water samples taken from the rivers of the Coastal Landscape Park. It is believed that microbial cell morphology can be a sensitive marker of changes in aquatic and terrestrial ecosystems (Straza et al. 2009; Young 2006). Analysis of various parameters of prokaryotic cells can provide information about the state of the environment. Analysis of water samples from the Piaśnica River showed a clear disturbance in the prokaryotic community at point P3 (much smaller ACV and PB compared to the other samples). From point P2 to the mouth of the river higher abundance of cocci was observed. In this localization treated effluent from the wastewater treatment plant in Żarnowiec flowed into the Piaśnica River and it could affect the prokaryotic community number and its biodiversity (Drury et al. 2013; Johnston and Roberts 2009). Chemical analyses did not show any contamination, but the highest COD value was observed at this point. This study showed that the estimated *E. coli* abundance in the Piaśnica River exceeded the allowable level for bathing sites only at points behind the outflow from WWTP in Żarnowiec. At point P4, the volume and biomass of the microorganisms were also very small, but this point is located just before the entrance to Żarnowieckie Lake, where the public beach is located. At this sampling site, a phenomenon of reversed river flow can occur, due to favorable wind direction. It can be a stress factor for the prokaryotic community and can also contribute to carrying the pollution from the swimming area back to the river. In addition, on Żarnowieckie Lake a pumped-storage hydroelectric power plant is located, which determines the constant variability of water level in this reservoir (Kutyła 2015). Behind the lake, at point P3, the number of prokaryotic cells was larger than at the sampling site before the lake. However, the microorganisms were twice smaller, which could indicate less favorable conditions for growth and division in the lake.

When comparing the microbiological results from the Karwianka and Piaśnica rivers, a general relation could be noted that with the similar TCN values in both rivers, but larger ACV in the Karwianka River, the prokaryotic biomass is also larger in the Karwianka River. In samples where cells were small, but the prokaryotic community was numerous, it could be concluded that the microorganisms found so many nutrients in

Table 7. Limit value for *E. coli* bacteria number according to Polish quality standards for bathing waters

parameter	limit value (CFU/100mL)	reference test methods
Enterococcus	≤400	PN-EN ISO 7899-1, PN-EN ISO 7899-2
Escherichia coli	≤1000	PN-EN ISO 9308-3, PN-EN ISO 9308-1

the aquatic environment that they decided to divide. Single-cell organisms have to coordinate the division with growth, therefore the number and cell size are directly correlated with the source and availability of nutrients (Chien et al. 2012). Similar to the remaining two rivers, the Karwianka River is also a receiver of treated wastewater. Additionally, a lot of drainage works were carried out in the area (Wiskulski 2015), which implies there are many unidentified tributaries from fields to the main river course. Besides, there are a lot of holiday cottages in this area that have no connection to the wastewater system and refer only to the quality of their own septic tanks, if any installed. This area is still being largely influenced by unregulated wastewater management and high popularity as a tourist resort. What is more, compared to the Piaśnica and Czarna Wda rivers, the Karwianka River is a very shallow and narrow river. All these factors may influence the increased fecal coliforms values in the river. Therefore, it can be concluded that the Karwianka River remains under the greatest anthropopressure, which is confirmed by the above results.

Similar to the Karwianka River, also the Czarna Wda and Piaśnica rivers receive treated wastewater from local WWTPs, which may create favorable conditions for microorganisms to grow and develop. In water samples taken from the Czarna Wda River, as it was noted in the Karwianka River, the estimated value of *E. coli* bacteria also exceeded the permitted amount at points located behind the waste water treatment plant (CW2 and CW1). It should be noted that at CW2, a factor inhibiting the growth and development of the prokaryotic community occurred. At the sampling stations behind the treatment plant the allowed amount of *E. coli* bacteria was exceeded, but simultaneously, the values of TCN and ACV decreased. This may have been caused by a UV disinfection system applied at the treatment plant in Jastrzębia Góra to disinfect treated wastewater, as the disinfection processes have an impact on the microbiological quality of the disinfected water (Becerra-Castro et al. 2016). The discharge of treated sewage from the treatment plant equipped with an additional wastewater treatment stage may cause a disturbance in the prokaryotic community. However, in the estuary, the number of microorganisms increased again and the prokaryotic community was dominated by very small cells. Increased number of fecal coliforms leads to the conclusion that the Czarna Wda River, similarly to the Karwianka River, remains under increased anthropopressure.

This contributes to the discussion about whether enhanced treatment methods should be widely applied, including the disinfection of the treated wastewater prior to discharge to the natural water bodies. The results indicate that WWTPs based on widely applied biological methods efficiently and satisfactorily remove biogenic substances, even when operating under highly variable inflow conditions based on dramatic seasonal changes in the prokaryotic community size. However, despite WWTPs' modernization, the growing number of tourists still promotes system overload, which can lead to the deterioration of water quality of the receivers. Current wastewater treatment technologies usually do not focus on the reduction or removal of bacteria, even though the effectiveness and necessity of disinfection have long been proven (Trussell 1990) and increasingly recommended (Olańczuk-Neyman et al. 2015). The use of UV radiation (at the Jastrzębia Góra WWTP) did not seem to have a high impact on the amount of fecal coliforms in the Czarna Woda River after wastewater inflow, but a clear signal of the disturbance in the prokaryotic community was noted in terms of total prokaryotic cell number and average cell size. Therefore, we also suggest that microscopic methods could be used to detect the changes in the prokaryotic community resulting from the change in environmental conditions. Such observations can provide information about the disturbance in aquatic habitats.

Principal component analysis

In this study, three watercourses of different characteristics were analyzed: (1) the Czarna Wda River, characterized by relatively constant parameters, but with small differentiation between the group of points upstream and downstream of WWTP; (2) the Piasnica River divided into two different groups – upstream (PI) and downstream (PII) from Zarnowieckie Lake and (3) the Karwianka River, characterized by largest variability of the tested parameters along the watercourse.

The Czarna Wda River presented the lowest variability among the tested watercourses (Fig. 7a). It was mainly affected by COD, TOC and N-NO_3^-) which were higher at points below the treatment plant and clearly can be attributed to the treated wastewater discharge. Some of the ions (Na^+, K^+, Cl^- and SO_4^{2-}) also showed increased concentrations at points CW1 and CW2, however, as these points are located in the coastal area, these values might be additionally affected by the sea proximity. This has been also taken into account in the guidelines for the classification of water status, as all the tested watercourses in their lower part were classified as estuarine rivers under saltwater influence (Council of Ministers 2016c). Regarding the microbiological parameters, the most significant role was played by fecal coliforms (FC) and their increased abundance directly below the WWTP4 discharge. The microbial community parameters obtained during microscopic analysis (TCN, ACV, PB) did not present any clear pattern and were of the smallest importance on the PCA plot (Fig. 7b, blue arrows).

The inflow of treated wastewater clearly separates points located upstream (CW4 and CW3) and downstream from WWTP4 (CW1 and CW2). By the way, it is worth noting that the Czarna Wda River is exceptional among the tested watercourses, as in fact all the tested points are below the WWTP discharge (WWTP3 is located upstream from CW4) – this may result in its relative uniformity regarding the tested parameters in this watercourse. On the other hand, the impact of WWTP3 effluent was less pronounced than in the case of WWTP4, as WWTP3 is a much smaller object and is located several kilometers from the sampling point (CW4). Nevertheless, the Czarna Wda River is subjected to strong anthropogenic pressure, what has been also noted in the risk assessment (Council of Ministers 2016c). The river is at risk of not achieving the environmental objectives and its current status is assessed as bad.

In the case of the Piaśnica River, it was clearly divided by Żarnowieckie Lake into two groups. Points downstream from the lake (PI) were similar to each other from the chemical point of view and were characterized by higher temperature and pH, as well as increasing Na^+ and Cl^- concentrations, attributed to the sea proximity. The WWTP effluent discharge impact was also visible, but mostly in terms of microbiological parameters: increased ACV, PB and fecal coliform presence.

The third object, the Karwianka River, was characterized by the highest variability of the tested parameters among the watercourses examined in this study, which was reflected by the large dispersion on the PCA plot. At the same time, almost all the highest values presented in this paper were recorded on this river. Points K1 and K2W were similar to each other in terms of most of the tested parameters. They were also grouped near CW1 and CW2 points, which might reflect their estuary character. On the other hand, K4 located upstream from the WWTP is most closely related to P5 and P4 – points at the Piaśnica River also located before the WWTP discharge. Therefore, one may note that the upper right quarter of the PCA plot is represented by points closer to the river source and subjected to smaller anthropogenic (wastewater) impact. K2E point was clearly separated from all the others, as it presents completely different chemical characteristics: the highest concentration of ions and thus the highest conductivity among all the examined points. Even if K2E was similar to other points located below the WWTP discharge in terms of the microbiological parameters, they did not play a significant role. It is worth remembering that K2E was a closed, cut off branch of the canal with basically stagnant water, which clearly influenced water quality parameters. The highest FC and TOC values observed at point K3 may result from the WWTP2 discharge proximity.

Conclusions

The results obtained in this study showed that none of the inspected rivers was heavily polluted, however, some of the physicochemical or microbiological parameters were exceeded. The best water quality was noted in the case of the Piaśnica River, whose catchment is mostly covered by forests. The Karwianka and Czarna Wda rivers, having much smaller and more agricultural catchments, presented worse quality in terms of organic compounds, nutrients or indicator bacteria presence. The Karwianka River is under the greatest anthropogenic pressure, being surrounded by many recreational plots, not always connected to the collective sewage system and frequently relying on septic tanks of various quality. This river is mostly exposed to a risk of pollution, especially during the summer months, when tourist activity in the area increases. The conducted research has shown that the chemical and microbiological quality of the Karwianka River is much worse than that of the Piasnica and Czarna Wda rivers, which, due to higher water flow and lower anthropopressure, have shown better water quality, despite the fact that they are also recipients of treated wastewater.

Fecal coliforms variability did not seem to depend highly on TOC or COD concentration, which however was not extremely high in this study. Fecal coliform variability was mostly following the changes in $N\text{-}NH_4^+$ concentration and seemed to be partly related to $P\text{-}PO_4^{3-}$ concentration. An increased number of fecal coliforms is the indicator of fecal pollution, e.g., potential signal of illegal untreated wastewater discharge or inefficiently working and deregulated treatment plant.

The conducted Principal Component Analysis showed that three rivers located relatively close to one another indicated very different chemical and microbiological characteristics along their course. It may be highly influenced by the presence of wastewater infrastructure in the area and the efficiency of wastewater treatment plants. In addition, the tourist character of the region: the presence of hotels and summer cottages, large number of tourists and therefore the highly variable population on the Baltic Sea coast may play a significant role. Those rivers also have additional and various functions, e.g., kayaking or fishing. Furthermore, of note is the character of their catchment area (agricultural, built-up area with housing or small industry).

The PCA analysis also revealed that the holistic approach combining the physicochemical and microbiological analysis is necessary. Also the hydrologic characteristic of each watercourse and the type of catchment coverage should be taken into account. However, in the case of wastewater impact, it is the presence of fecal coliforms that seem to characterize best the scale of the influence. Nevertheless, this parameter has not been included among the microbial quality indicators in the latest legislation (Council of Ministers 2021).

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Conflicts of Interest

The authors declare no conflict of interest.

References

Amin, A., Ahmed, I., Salam, N., Kim, B.Y., Singh, D., Zhi, X.Y., Xiao, M. & Li, W.J. (2017). Diversity and Distribution of Thermophilic Bacteria in Hot Springs of Pakistan. *Microbial Ecology*, *74* (1), pp. 116–127. DOI: 10.1007/s00248-017-0930-1

APHA (2005). Standard methods for the examination of water and wastewater. In *21st ed. Washington DC, USA*.

Baczkowska, E., Kalinowska, A., Ronda, O., Jankowska, K., Bray, R., Płóciennik, B. & Polkowska, Ż. (2022). Microbial and chemical quality assessment of the small rivers entering the South Baltic. Part II: Case study on the watercourses in the Puck Bay catchment area. *Archives of Environmental Protection*. (under review)

Becerra-Castro, C., Macedo, G., Silva, A.M.T., Manaia, C.M. & Nunes, O.C. (2016). Proteobacteria become predominant during regrowth after water disinfection. *Science of the Total Environment*, *573*, pp. 313–323. DOI: 10.1016/j.scitotenv.2016.08.054

Borkowski, R. (2019). Wyzwania i zagrożenia dla turystyki na Półwyspie Helskim w XXI wieku. *Bezpieczeństwo. Teoria i Praktyka*, *3*, pp. 55–70. DOI: 10.34697/2451-0718-b. (in Polish)

Brysiewicz, A., Bonisławska, M., Czerniejewski, P. & Kierasiński, B. (2019). Quality analysis of waters from selected small watercourses within the river basins of Odra river and Wisła river. *Rocznik Ochrona Srodowiska*, *21*(2), pp. 1202–1216. (in Polish)

Bugajski, P. & Satora, S. (2009). Bilans ścieków dopływających i dowożonych do oczyszczalni na przykładzie wybranego obiektu. *Infrastruktura i Ekologia Terenów Wiejskich*, *5*, pp. 73–82. (in Polish)

Cai, L. & Zhang, T. (2013). Detecting human bacterial pathogens in wastewater treatment plants by a high-throughput shotgun sequencing technique. *Environmental Science and Technology*, *47*(10), pp. 5433–5441. DOI: 10.1021/es400275r

Caruso, G., La Ferla, R., Azzaro, M., Zoppini, A., Marino, G., Petochi, T., Corinaldesi, C., Leonardi, M., Zaccone, R., Fonda, S., Caroppo, C., Monticelli, L., Azzaro, F., Decembrini, F., Maimone, G., Cavallo, R., Stabili, L., Todorova, N., Karamfilov, V. & Danovaro, R. (2016). Microbial assemblages for environmental quality assessment: Knowledge, gaps and usefulness in the European marine strategy framework directive. *Critical Reviews in Microbiology*, *42*(6). DOI: 10.3109/1040841X.2015.1087380

Chien, A.C., Hill, N.S. & Levin, P.A. (2012). Cell size control in bacteria. *Current Biology*, *22*(9), R340–R349. DOI: 10.1016/j.cub.2012.02.032

Conley, D.J., Paerl, H.W., Howarth, R.W., Boesch, D.F., Seitzinger, S.P., Havens, K.E., Lancelot, C. & Likens, G.E. (2009). Ecology – Controlling eutrophication: Nitrogen and phosphorus. In *Science* (Vol. 323, Issue 5917, pp. 1014–1015). American Association for the Advancement of Science. DOI: 10.1126/science.1167755

Council of Ministers, 2011: Rozporządzenia Ministra Środowiska z dnia 9 listopada 2011 r. w sprawie klasyfikacji stanu ekologicznego, potencjału ekologicznego i stanu chemicznego jednolitych części wód powierzchniowych, (2011) (testimony of (Dz. U. poz. 1549, zał 6). (in Polish)

Council of Ministers, 2014: Rozporządzenie Ministra Środowiska z dnia 22 października 2014 r. w sprawie sposobu klasyfikacji stanu jednolitych części wód powierzchniowych oraz środowiskowych norm jakości dla substancji priorytetowych, (2014) (testimony of Dz.U.2014 poz.1482). (in Polish)

Council of Ministers, 2015: Rozporządzenie Ministra Zdrowia z dnia 3 lipca 2015 r. zmieniające rozporządzenie w sprawie prowadzenia nadzoru nad jakością wody w kąpielisku i miejscu wykorzystywanym do kąpieli, 1 (2015) (testimony of Dz.U. 2015. poz. 1510). (in Polish)

Council of Ministers, 2016a: Rozporządzenie Ministra Środowiska z dnia 21 lipca 2016 r. w sprawie sposobu klasyfikacji stanu jednolitych części wód powierzchniowych oraz środowiskowych norm jakości dla substancji priorytetowych., (2016) (testimony of Dz.U.2016 poz.1187). (in Polish)

Council of Ministers, 2016b: Rozporządzenie Rady Ministrów z dnia 18 października 2016 r. w sprawie Planu gospodarowania wodami na obszarze dorzecza Wisły, (2016) (testimony of Dz.U. 2016 poz.1991). (in Polish)

Council of Ministers, 2016c: Rozporządzenie Rady Ministrów z dnia 18 października 2016 r. w sprawie planu gospodarowania wodami na obszarze dorzecza Wisły, (2016) (testimony of Dz.U. 2016 poz. 1911). (in Polish)

Council of Ministers, 2019: Rozporządzenie Ministra Zdrowia z dnia 17 stycznia 2019 r. w sprawie nadzoru nad jakością wody w kąpielisku i miejscu okazjonalnie wykorzystywanym do kąpieli, (2019) (testimony of Dz.U. 2019 poz. 255). (in Polish)

Council of Ministers, 2021: Rozporządzenie Ministra Infrastruktury z dnia 25 czerwca 2021 r. w sprawie klasyfikacji stanu ekologicznego, potencjału ekologicznego i stanu chemicznego oraz sposobu klasyfikacji stanu jednolitych części wód powierzchniowych, a także środowiskowych norm, (2021) (testimony of Dz.U. 2021 poz. 1475). (in Polish)

Curr, R.H.F., Koh, A., Edwards, E., Williams, A.T. & Davies, P. (2000). Assessing anthropogenic impact on Mediterranean sand dunes from aerial digital photography. *Journal of Coastal Conservation*, *6*(1), pp. 15–22. DOI: 10.1007/BF02730463

De Brauwere, A., Ouattara, N.K., & Servais, P. (2014). Modeling fecal indicator bacteria concentrations in natural surface waters: A review. *Critical Reviews in Environmental Science and Technology*, *44*(21), pp. 2380–2453. DOI: 10.1080/10643389.2013.829978

de la Vega, C., Schückel, U., Horn, S., Kröncke, I., Asmus, R. & Asmus, H. (2018). How to include ecological network analysis results in management? A case study of three tidal basins of the Wadden Sea, south-eastern North Sea. *Ocean and Coastal Management*, *163*(May), pp. 401–416. DOI: 10.1016/j.ocecoaman.2018.07.019

Dodds, W.K. & Smith, V.H. (2016). Nitrogen, phosphorus, and eutrophication in streams. *Inland Waters*, *6*(2), pp. 155–164. DOI: 10.5268/IW-6.2.909

Drury, B., Rosi-Marshall, E. & Kelly, J.J. (2013). Wastewater treatment effluent reduces the abundance and diversity of benthic bacterial communities in urban and suburban rivers. *Applied and Environmental Microbiology*, *79*(6), pp. 1897–1905. DOI: 10.1128/AEM.03527-12

Fry, J.C. (1990). Direct Methods and Biomass Estimation. In Grigorova, R. & Norris J.R.B.T.-M. (Eds.), *Techniques in Microbial Ecology* (Vol. 22, pp. 41–85). Academic Press. DOI: 10.1016/S0580-9517(08)70239-3

García-Llorente, M., Harrison, P.A., Berry, P., Palomo, I., Gómez-=Baggethun, E., Iniesta-Arandia, I., Montes, C., García del Amo, D. & Martín-López, B. (2018). What can conservation strategies learn from the ecosystem services approach? Insights from ecosystem assessments in two Spanish protected areas. *Biodiversity and Conservation*, *27*(7), pp. 1575–1597. DOI: 10.1007/s10531-016-1152-4

Gössling, S., Hall, C.M. & Scott, D. (2018). Coastal and Ocean Tourism. *Handbook on Marine Environment Protection*, pp. 773–790. DOI: 10.1007/978-3-319-60156-4_40

Grabic, J., Duric, S., Ciric, V. & Benka, P. (2018). Water quality at special nature reserves in Vojvodina, Serbia. *Croatian Journal of Food Science and Technology*, *10*(2), pp. 179–184. DOI: 10.17508/cjfst.2018.10.2.05

Hachich, E.M.; Di Bari, M.; Christ, A.P.G.; Lamparelli, C.C.; Ramos, S.S.& Sato, M.I.Z. (2012) Comparison of thermotolerant coliforms and Escherichia coli densities in freshwater bodies. *Brazilian J. Microbiol.*, 43, pp. 675–681.

Huo, Y., Bai, Y. & Qu, J. (2017). Unravelling riverine microbial communities under wastewater treatment plant effluent discharge in large urban areas. *Applied Microbiology and Biotechnology*, *101*(17), pp. 6755–6764. DOI: 10.1007/s00253-017-8384-4

Infoeko, 2004: Available online: http://www.infoeko.pomorskie.pl/InformacjeZbiorcze/2004/Szczegoly/26. Accessed on 20 October 2020. (in Polish)

Johnston, E.L. & Roberts, D.A. (2009). Contaminants reduce the richness and evenness of marine communities: A review and meta-analysis. *Environmental Pollution*, *157*(6), pp. 1745–1752. DOI: 10.1016/j.envpol.2009.02.017
Justić, D., Rabalais, N.N., Turner, R.E. & Dortch, Q. (1995). Changes in nutrient structure of river-dominated coastal waters: Stoichiometric nutrient balance and its consequences. *Estuarine, Coastal and Shelf Science*, *40*(3), pp. 339–356. DOI: 10.1016/S0272-7714(05)80014-9
Kaczor, G. (2011). Wpływ wiosennych roztopów śniegu na dopływ wód przypadkowych do oczyszczalni ścieków bytowych. *Acta Sci. Pol., Formatio Circumiectus*, *10*(2), pp. 27–34. (in Polish)
Kosek, K., Kozak, K., Kozioł, K., Jankowska, K., Chmiel, S. & Polkowska, Z. (2018). The interaction between bacterial abundance and selected pollutants concentration levels in an arctic catchment (southwest Spitsbergen, Svalbard). *Science of the Total Environment*, *622–623*, pp. 913–923. DOI: 10.1016/j.scitotenv.2017.11.342
Kosek, K. & Polkowska, Ż. (2016). Determination of selected chemical parameters in surface water samples collected from the Revelva catchment (Hornsund fjord, Svalbard). *Monatshefte Fur Chemie*, *147*(8), pp. 1401–1405. DOI: 10.1007/s00706-016-1771-1
Kowalski, T. (1989). Analiza chemicznych i biochemicznych właściwości zanieczyszczeń występujących w ściekach. *Ochrona Środowiska*. (in Polish)
Kozak, K., Ruman, M., Kosek, K., Karasiński, G., Stachnik, Ł. & Polkowska, Z. (2017). Impact of volcanic eruptions on the occurrence of PAHs compounds in the aquatic ecosystem of the southern part of West Spitsbergen (Hornsund Fjord, Svalbard). *Water (Switzerland)*, *9*(1). DOI: 10.3390/w9010042
Krajewska, Z. & Fac-Beneda, J. (2016). Transport of biogenic substances in water-courses of coastal landscape park. *Journal of Elementology*, *21*(2), pp. 413–423. DOI: 10.5601/jelem.2015.20.1.800
Kutyła, S. (2015). Characteristics of water level fluctuations in Polish lakes – a review of the literature. *Ochrona Srodowiska i Zasobów Naturalnych*, *25*(3), pp. 27–34. DOI: 10.2478/oszn-2014-0011
la Ferla, R., Maimone, G., Azzaro, M., Conversano, F., Brunet, C., Cabral, A. S. & Paranhos, R. (2012). Vertical distribution of the prokaryotic cell size in the Mediterranean Sea. *Helgoland Marine Research*, *66*(4), pp. 635–650. DOI: 10.1007/s10152-012-0297-0
Luczkiewicz, A., Jankowska, K., Bray, R., Kulbat, E., Quant, B., Sokolowska, A. & Olańczuk-Neyman, K. (2011). Antimicrobial resistance of fecal indicators in disinfected wastewater. Water Science and Technology, 64(12), 2352. DOI: 10.2166/wst.2011.769
Luczkiewicz, A., Jankowska, K., Langas, V. & Kaiser, A. (2019). Inventory of existing treatment technologies in wastewater treatment plants Case studies in four coastal regions of the South Baltic Sea.
Łuczkiewicz, A., Jankowska, K., Fudala-Książek, S. & Olańczuk--Neyman, K. (2010). Antimicrobial resistance of fecal indicators in municipal wastewater treatment plant. Water Research, 44(17), pp. 5089–5097. DOI: 10.1016/j.watres.2010.08.007
Majdak, P. (2008). Tourist amenities of Hel and conceptions of their development. *Turystyka i Rekreacja Tom 4.* (in Polish)
Michałkiewicz, M. (2018). Ścieki i ich negatywna rola w środowisku. *Technologia Wody*, *5*(61), pp. 30–33.
Munksgaard, D.G. & Young, J.C. (1980). Flow and load variations at wastewater treatment plants. *Journal of the Water Pollution Control Federation*, *52*(8), pp. 2131–2144.
Norland S. (1993). The relationship between biomass and volume of bacteria. In Cole, J.J. (Ed.), *Handbook of methods in aquatic microbial ecology* (pp. 303–308). Lewis Publishers,.
Nübel, U., Garcia-Pichel, F., Kühl, M. & Muyzer, G. (1999). Quantifying microbial diversity: morphotypes, 16S rRNA genes, and carotenoids of oxygenic phototrophs in microbial mats. *Applied and Environmental Microbiology*, *65*(2), pp. 422–430.
Olańczuk-Neyman, K., Quant, B., Łuczkiewicz, A., Kulbat, E., Jankowska, K., Sokołowska, A., Bray, R. & Kulbat, E. (2015). *Dezynfekcja ścieków*. Seidel-Przywecki sp. z o.o. (in Polish)
Olson, D.M. & Dinerstein, E. (1998). The global 200: A representation approach to conserving the earth's most biologically valuable ecoregions. *Conservation Biology*, *12*(3), pp. 502–515. DOI: 10.1046/j.1523-1739.1998.012003502.x
Ostroumov, S.A. (2017). Water Quality and Conditioning in Natural Ecosystems: Biomachinery Theory of Self-Purification of Water. *Russian Journal of General Chemistry*, *87*(13), pp. 3199–3204. DOI: 10.1134/S107036321713014X
Porter, K.G. & Feig, Y.S. (1980). The use of DAPI for identifying and counting aquatic microflora. *Limnological Oceanography*, *25*(5), pp. 943–948.
Rees, G. & Bartram, J. (2002). Monitoring bathing waters: a practical guide to the design and implementation of assessments and monitoring programmes. CRC Press.
Statistics Poland, 2016: Available online: https://stat.gov.pl/obszary-tematyczne/ludnosc/ludnosc/ Accessed on 20 October 2020, https://stat.gov.pl/obszary-tematyczne/kultura-turystyka-sport/turystyka/ Accessed on 20 October 2020. (in Polish)
Straza, T.R.A., Cottrell, M.T., Ducklow, H.W. & Kirchman, D.L. (2009). Geographic and phylogenetic variation in bacterial biovolume as revealed by protein and nucleic acid staining. *Applied and Environmental Microbiology*, *75*(12), pp. 4028–4034. DOI: 10.1128/AEM.00183-09
Świątecki, A. (1997). Zastosowanie wskaźników bakteriologicznych w ocenie wód powierzchniowych. (Monografie). Wyższa Szkoła Pedagogiczna. (in Polish)
Trussell, R.R. (1990). Evaluation of the Health Risks Associated with Disinfection. *Critical Reviews in Environmental Control*, *20*(2), pp. 77–113. DOI: 10.1080/10643389009388392
Wiskulski, T. (2015). *Geography For Society* (Issue January 2015).
Wojciechowska, E., Pietrzak, S., Matej-Łukowicz, K., Nawrot, N., Zima, P., Kalinowska, D., Wielgat, P., Obarska-Pempkowiak, H., Gajewska, M., Dembska, G., Jasiński, P., Pazikowska-Sapota, G., Galer-Tatarowicz, K. & Dzierzbicka-Głowacka, L. (2019). Nutrient loss from three small-size watersheds in the southern Baltic Sea in relation to agricultural practices and policy. *Journal of Environmental Management*, *252*(May). DOI: 10.1016/j.jenvman.2019.109637
Young, K.D. (2006). The Selective Value of Bacterial Shape. *Microbiology and Molecular Biology Reviews*, *70*(3), pp. 660–703. DOI: 10.1128/MMBR.00001-06
Zaborska, A., Siedlewicz, G., Szymczycha, B., Dzierzbicka-Głowacka, L. & Pazdro, K. (2019). Legacy and emerging pollutants in the Gulf of Gdańsk (southern Baltic Sea) – loads and distribution revisited. *Marine Pollution Bulletin*, *139* (November 2018), pp. 238–255. DOI: 10.1016/j.marpolbul.2018.11.060