

DIURNAL VARIATION IN THE SELECTED INDICATORS OF WATER CONTAMINATION IN THE BIAŁKA RIVER AFFECTED BY A SEWAGE TREATMENT PLANT DISCHARGE

Anna Lenart-Boron^{1,*}, Justyna Prajsnar², Kinga Krzesiwo³, Anna Wolanin⁴, Łukasz Jelonkiewicz⁴, Ewelina Jelonkiewicz⁴, Mirosław Żelazny⁴

¹ Department of Microbiology, University of Agriculture in Cracow; Mickiewicza Ave. 24/28, 30-059 Cracow, Poland

² Faculty of Biotechnology and Horticulture, University of Agriculture in Cracow, 29 listopada Ave. 54, 31-425 Cracow, Poland

³ Department of Entrepreneurship and Spatial Management, Institute of Geography, Pedagogical University of Cracow, Podchorążych str. 2, 30-084 Cracow, Poland

⁴ Department of Hydrology, Institute of Geography and Spatial Management, Jagiellonian University in Cracow; Gronostajowa 7, 30-387 Cracow, Poland

ABSTRACT

This study was aimed to assess the diurnal variability in the number of microbial indicators of water quality and the content of the selected main ions at 3 sites located on the Białka river, Podhale. The examined sites included the stream being a receiver of sewage from the treatment plant, before the treatment plant and few kilometers downstream of the sewage discharge - at the water intake for artificial snowing of the largest ski station in the region. Twelve series of samples were collected over 36 hours period. Temperature, pH, electrical conductivity and dissolved oxygen were measured onsite, microbiological analyses included the numbers of mesophilic bacteria, fecal *Escherichia coli* and *Enterococcus faecalis*, chemical analyses determined the concentration of main ions. *E. faecalis* was not detected in this study, while the concentration of mesophilic bacteria and *E. coli* varied largely between sites and hours of sampling. The smallest content of microorganisms was observed before the treatment plant and significantly increased at the sewage discharge. It was found that the cycle of the sewage treatment plant operation affects microbial contamination of water both in the stream which receives effluent from the treatment plant and in the downstream part of the river.

KEYWORDS:

Białka river, *Escherichia coli*, mesophilic bacteria, nutrients, sewage treatment plant

INTRODUCTION

From 1992 the European Union has been implementing the "Natura 2000" system, which is aimed for the protection of the European biological

diversity, preserving natural habitats, plants and animal species [1]. One of the sites covered by this program includes the valley of the Białka river, starting from the Leśnicki Stream's mouth to the Białka until the Białka river's mouth to the Czorszyńskie Lake. Moreover, waters of the Białka river are considered as ones of the most pure in Poland. It is among the class I (the best) rivers in terms of biological, hydromorphological and water indexes quality [2]. Many animal species, characteristic of mountain streams with small degree of conversion and high class of water purity have been found to dwell in this river [3].

However, it has appeared recently that the natural characteristics of the Białka river have been strongly reduced by human activity. There are several reasons for such situation including, among others, anthropogenic transformations of the riverbed, both conducted under the supervision of hydrotechnical companies and by local tenants as a prevention measure against flooding [4]. Another aspect is the exploitation of the riverbed material, in the form of pebbles and wood rubble – the first one being used for building e.g. house foundations and hardening roads, while the latter one being used as firewood [4]. Another two aspects of anthropogenic disturbance of the Białka river are directly related to the issue analyzed and discussed in this paper. The first one is related to increasing water uptake from the Białka river – for drinking water as well as for artificial snowing of several ski stations operating in the neighborhood of the Białka valley, which results in the total consumption of more than 1,350 m³/h of water [5]. The last and the most severe problem is related to the fact that the river acts a sewage receiver from households of the municipality – Bukowina Tatrzańska, in which only 49.8% of the population uses sewerage system [6]. Studies conducted previously in the catchment of the Białka river [7], indicate that the sewage treatment plant, operating in



the Bukowina Tatrzańska municipality is insufficient, which results in large loads of pollutants being discharged into the river. Moreover, there are numerous discharge sites of untreated sewage, which during winter increase the amount of sewage inflow and may degrade the quality of water in this region [8].

There are studies, reporting that some microbial genera and species are able to survive the process of freezing, required for the production of artificial snow [9, 10], thus causing the threat of microbial contaminants being transferred to snow cannons and then to artificial snow deposited over numerous slopes in the vicinity of the Białka river catchment.

Białka Tatrzańska is the resort, located in the Bukowina Tatrzańska municipality, by the Białka river. This locality is specialized in ski tourism, whose role is dominant in the structure of their overall tourism function. It is one of the largest and the best ski stations in Poland [11]. In the winter season 2014/2015 the ski base in this territory comprised 23 ski lifts, including 8 cableways with a total transportation capacity of 28,400 people/hour and 27 ski runs with a total length of 17.2 km. As many as 88.9% of them allows skiing after dark, and 81.5% is equipped with snow cannons. The ski runs are located in the eastern, north-eastern and south-eastern slopes of Kotelnica (918 m a.s.l.), Jankulakowski Wierch (934 m a.s.l.) and Horników Wierch (926 m a.s.l.). The largest ski resorts in Białka Tatrzańska are: Kotelnica Białczańska, Bania and Kaniówka [11]. In this area tourist traffic in winter significantly predominates the one in summer [11]. In recent years there was a significant increase in the number of tourists visiting Białka Tatrzańska in the period of winter holidays, starting from mid-January and ending in late March. People skiing or snowboarding usually use long-term stays in Białka Tatrzańska or in the Bukowina Tatrzańska municipality, which causes shortages in accommodation places in this area [11]. For instance, in the winter season 2014/2015 Kotelnica Białczańska ski resort (the largest ski resort in Białka Tatrzańska) was visited by between 320 and 340 thousand tourists [12]. Such situation resulted in a deterioration of the efficiency of the sewage treatment plant operating in the Bukowina Tatrzańska municipality and therefore a significant decrease in water quality from the section of Bukowina Tatrzańska until the river mouth to the Czorszyńskie Lake [7].

Water quality monitoring programs vary widely with different numbers of samples being collected for the analysis and possible decisions [13] as sampling design is rarely based on empirical and/or anticipated variation. According to Whitman & Nevers [13] these limitations in monitoring protocols cause that the efforts should be made to provide accurate and reliable results. Understanding

the processes affecting the water quality requires sound scientific data on the variability of the concentration, sources and faith of fecal contaminants [14].

Having this in mind, a study was undertaken in order to assess a diurnal variability in the abundance of microbial indicators of water quality and the content of the selected main ions in three sites located on the Białka river – by the mouth of a stream being a direct receiver of sewage from the treatment plant in Bukowina Tatrzańska, before the treatment plant and in the neighboring village - Białka Tatrzańska at the water intake for artificial snowing of the slopes of the largest ski station in the region.

The selection of the sampling sites was a direct effect of a 2-year study conducted throughout the Białka river [7], which indicated that even though large proportion of the municipality is not sewered, resulting in numerous households discharging their effluent directly into the river, still the insufficiently operating treatment plant is the most important factor which affects dramatically decreased quality of the Białka waters.

The studies will allow for more detailed assessment of the operation and efficiency of the sewage treatment plant and its direct effect on the quality of water in the river, which has been widely considered as pure, as well as on the quality of water being deposited over the mountain slopes in the form of artificial snow.

METHODS

Sampling strategy. Three measuring points were selected for the study, two of which were located on the river Białka. The first one is situated before the municipal sewage treatment plant (1), the second one – by the sewage discharge from the treatment plant (2) – both located in Bukowina Tatrzańska. The third measuring point was situated at the site of water intake (3) for artificial snowing of the slopes of the biggest ski station in the region, located in Białka Tatrzańska – the village neighboring Bukowina (Fig. 1.). The study was conducted over a weekend in mid-February, which is the middle of the “high ski season” in Poland, when the considered area is visited by the greatest number of tourists throughout the year [8]. Water samples were collected during 36 hours at 2 to 4-hour interval. The samples were collected into 1,000 ml and 500 ml autoclaved polypropylene bottles. During sampling, measurements of temperature (T), pH, electrical conductivity (EC_{25°C}) and dissolved oxygen (DO) were conducted by a digital multimeter Multi 3430 (WTW) with a combined glass electrode type SenTix 940 (WTW), a conductometric sensor TetraCon 925 (WTW) with a constant k=0.475 and optical dissolved oxygen sensors FDO 925 (WTW).

Each sensor had a built-in temperature sensor.

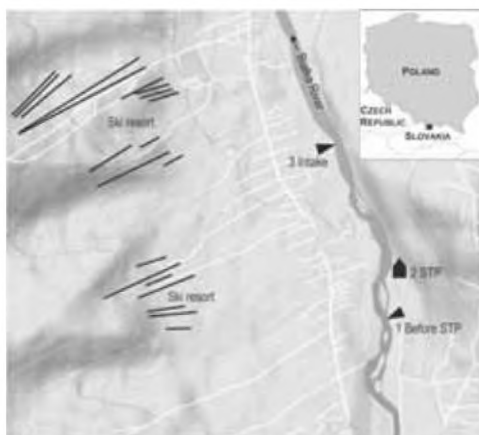


FIGURE 1
Study area

Laboratory analyses. *Escherichia coli* (Blue-green colonies on TBX agar, incubated at 44°C, 48 h) and *Enterococcus faecalis* (small dark red to light brown colonies on Slanetz-Bartley agar, 37°C, 72 h) were enumerated using the membrane filtration method, while the number of mesophilic bacteria (trypticase soy agar, 37°C, 48 h) was determined using the serial dilutions method. After incubation, grown colonies were counted and expressed as colony forming units per 100 ml of water for the membrane filtration method and per 1 ml for the serial dilutions method (CFU/100 ml and CFU/ml, respectively).

In order to determine the chemical composition, water samples were collected to 500 ml polyethylene bottles. Chemical composition of water was determined in the laboratory of the Institute of Geography and Spatial Management, Jagiellonian University in Kraków. After filtration of water with a 0.45 µm PTFE syringe filter, the chemical composition of water was determined by ion chromatography using two chromatographs DIONEX ICS-2000 and an autosampler AS-40. The chromatographic system composed of anionic and cationic modules allows the simultaneous separation and determination of the following ions in water: Ca²⁺, Mg²⁺, Na⁺, K⁺, NH₄⁺, SO₄²⁻, Cl⁻, NO₃⁻, NO₂⁻, PO₄³⁻, F⁻. HCO₃⁻ was determined by titration using 855 Robotnic Titrosampler (Metrohm). Mineralization of water (total dissolved solids – TDS) was also calculated as a sum of individual ions concentrations.

Statistical analysis. Statistical analysis was performed using Statistica v. 10 (StatSoft) software, by calculating basic descriptive statistics and a one-way T-test [15] was used to verify the significance of differences in the number of microbial indicators between the sampling sites and different times of sampling. In order to verify the differences in the

investigated physical and chemical parameters between the sampling sites, the analysis of variance (ANOVA) and post-hoc Scheffe test were applied for p=0.95. On the other hand, a one-sample T test was applied to verify the significance of differences in physical and chemical characteristics of water over the study period. The confidence interval was 0.05. Spearman's correlation coefficient was used to determine the relationship between the tested parameters for each of the sampling sites.

Multivariate statistical analysis, particularly Principal Component Analysis, has been successfully applied in a number of hydrochemical studies [16, 17]. On the other hand, Thyne et al. [18] believe that this method can be applied in order to extract key information from large sets of data on water quality. Principal Component Analysis (PCA) was used to identify factors affecting the microbiological and hydrochemical parameters in the investigated points. In the case of points STP (No. 2) and Intake (No. 3) the analysis included data on mesophilic bacteria, *E. coli* and T, EC_{25°C}, DO, Na⁺, K⁺, NH₄⁺, Cl⁻, NO₃⁻ and PO₄³⁻. On the other hand, in the case of the point Before STP (No. 1) only data on mesophilic bacteria, *E. coli*, NH₄⁺ and NO₃⁻ were included due to the small amount of water samples collected during the experiment and also small variation of the obtained results. Before conducting the analysis, data were standardized and normalized. The factors for the analysis were selected based on the Kaiser criterion (eigenvalue >1 and when the value of the explained variance was greater than 10%).

RESULTS AND DISCUSSION

Table 1 presents the results of varying concentrations of microorganisms over the 36-hour period of the study. *E. faecalis* was not detected in the examined samples, which may indicate that the origin of the microbial contamination is of anthropogenic character, as there have been studies showing that the number of fecal coliforms in human feces significantly outnumbers fecal streptococci [19]. Also, ratios between fecal coliforms and fecal streptococci greater than 4 have been suggested to indicate a human source of water pollution, while ratios less than 0.7 would indicate animal source [20].

The concentration of another indicator of fecal contamination, *E. coli* varied significantly, both between the examined sites and within individual sites – between different hours of sampling. At the site located before the treatment plant the concentration of *E. coli* ranged from 17 to 164 CFU/100 ml of water (with a mean of 87 CFU/100 ml) and it increased severely at the site located by the STP, where it ranged from 71 thousand CFU/100 ml

of water in series No. 4 and 9 to even 1.5 million CFU/100 ml of water in series 1 and 8 with the mean

TABLE 1
The number of microbial indicators of fecal contamination recorded during the diurnal study.

Location	Sample No.	<i>E. coli</i> (CFU/100 ml)	<i>E. faecalis</i> (CFU/100 ml)	Mesophilic bacteria (CFU/ml)
Before STP	1	17	0	50
Before STP	2	120	0	42
Before STP	3	164	0	105
Before STP	4	47	0	70
Mean	-	87	0	67
Std. deviation	-	67	0	28
STP	1	1,450,600	0	159,800
STP	2	788,800	0	102,100
STP	3	1,156,000	0	93,950
STP	4	71,540	0	36,400
STP	5	1,142,000	0	79,450
STP	6	952,000	0	79,200
STP	7	1,224,200	0	416,000
STP	8	1,496,000	0	136,700
STP	9	71,120	0	64,000
STP	10	816,100	0	261,300
STP	11	140,080	0	5,650
STP	12	680,000	0	32,500
Mean	-	832,370	0	122,254
Std. deviation	-	509,373	0	114,633
Intake	1	18	0	95
Intake	2	14	0	58
Intake	3	23	0	50
Intake	4	51	0	97
Intake	5	816	0	7,480
Intake	6	571	0	480
Intake	7	470	0	4,510
Intake	8	512	0	765
Intake	9	115	0	210
Intake	10	1,870	0	2,340
Intake	11	2,320	0	1,175
Intake	12	860	0	340
Mean	-	637	0	1,467
Std. deviation	-	754	0	2,303

of c.a. 832 thousand CFU/100 ml. Also at the Intake the number of these indicator bacteria varied significantly depending on the hour of sampling and

it ranged from only 14 CFU/100 ml (series. 2) to as much as nearly 2500 CFU/100 ml (series 11) with a mean of 637 CFU/100 ml. The number of *E. coli* at the water intake for artificial snowing allowed to classify these waters either to class A1 or to A2. This means that they require typical physical and chemical treatment, including disinfection by chlorination [21]. This is one of the most important observations resulting from this study, as on one hand the waters of the Bialka river have been considered exceptionally clean, while on the other hand they are being constantly contaminated with undertreated sewage. Fecal pollution of surface waters is of particular concern, as the most dangerous bacterial gastrointestinal human infections, such as cholera, salmonellosis or shigellosis are primarily transmitted by water polluted with feces of infected people [22].

The number of mesophilic bacteria may be also used to determine the extent of microbiological contamination of water, as this group of microorganisms consists of most species related to sewage contamination [23]. Most of these bacteria are incapable of proliferating in water environment, therefore their presence may indicate relatively fresh contamination with sewage [23]. The number of mesophilic bacteria at the site No. 1 (before the treatment plant) ranged from 42 to 105 CFU/ml of water. According to the Regulation of the Minister of Environment on the requirements for surface water used for supplying people in drinking water [21], water at this site can be classified to A1 or A2 category. However, the prevalence of these microorganisms increased significantly by the sewage discharge from the treatment plant - No. 2 (range between 5,650 CFU/ml and 416 thousand CFU/ml), which then affected the quality of water at the intake for artificial snowing (No. 3), where the number of mesophilic bacteria ranged from 50 CFU/ml (series 3) to almost 7,500 CFU/ml (series 5).

The diurnal variation in the microbial indicators' concentration in both of the sites, i.e. STP and Intake, located downstream of the discharge from the treatment plant, are consistent with the observations of Meays et al. [14] and confirm the conclusions drawn from their study, as well as the remarks given by Whitman & Nevers [13] concerning the main limitations related to bacterial monitoring protocols. As also shown in our study, if a monitoring was designed to obtain a single sample per site per day, the results of the *E. coli* concentrations at the intake for artificial snowing could range from only 14 or 18 CFU/100 ml to even 2,320 CFU/100 ml. The situation is similar by the discharge from the STP – even though the *E. coli* concentrations are huge, they still could vary from 71 thousand CFU/100 ml to as many as 1.5 million CFU/100 ml (Table 1). The assessment of the fecal contamination based on these outermost results will

be very different and can lead to incorrect conclusions.

Given the fact that the examined three sampling sites are located within a few kilometer distance from each other, it appears that also designing a reliable network of sampling sites and selecting the most representative ones is not only a very significant matter, but it can be also very difficult task. The location of a sampling site within a catchment can affect the concentrations of *E. coli* and other microbial indicators of fecal contamination of water [14]. The concentrations and sources of *E. coli* can be also affected if a sampling site is located below a highly used area versus a more remote one [14].

Statistical analysis showed that the differences in the numbers of *E. coli* between the sampling sites, i.e. STP/before-STP and STP/Intake, are statistically significant (T-values 3.19 and 5.66, respectively). There were also statistically significant differences in the number of mesophilic bacteria between the sampling sites (T-values of 2.08 and 3.65, respectively). Moreover, there were statistically significant differences in the concentrations of *E. coli* and mesophilic bacteria over the study period in each of the measuring points.

Determination of the Spearman's correlation coefficient revealed no significant correlation between the microbial indicators (*E. coli* and mesophilic bacteria) and physico-chemical characteristics of water at the measuring point Before STP (Tab. 2). At the measuring point No. 3 (Intake) a significant positive relationship between the concentrations of *E. coli* and mesophilic bacteria, and EC_{25°C}, Na⁺, NH₄⁺, Cl⁻, PO₄³⁻ was found. Similarly, in the case of mesophilic bacteria, there was no significant correlation only with phosphate ions. At the measuring point STP there was a

significant positive correlation between *E. coli* and EC_{25°C}, Na⁺, K⁺, NH₄⁺, Cl⁻ and PO₄³⁻, and significant negative correlation with DO and NO₃⁻. On the other hand, there is a significant relationship with mesophilic bacteria only in the case of DO and K⁺. There was no significant correlation with the water temperature in any of the measuring points, although other Authors report such relationship in their papers [24, 25].

Table 3 shows mean, minimum and maximum values, as well as coefficient of variation (Cv) for physical and chemical parameters at the examined sites. In the Bialka river, both before the treatment plant and at the intake, water was characterized by high oxygen content, low temperature and alkaline pH (pH~8). Water conductivity was around 270 μS/cm and TDS around 230 mg/l. The concentration of nutrients (NH₄⁺, NO₃⁻, NO₂⁻, PO₄³⁻) was low or their values were below limit of detection. On the other hand, the outflow from the sewage treatment plant was characterized by far higher values of TDS, EC_{25°C}, temperature and the concentration of ions, as well as by significantly lower content of dissolved oxygen. Very high values of NH₄⁺ and at the same time low values of NO₃⁻ and NO₂⁻ evidence very poor efficiency of biological treatment of sewage in the examined treatment plant.

The outflow from the sewage treatment plant differs significantly (ANOVA) in terms of all examined physical and chemical parameters from the values of these parameters at the points Before STP and Intake. On the other hand, the site Before STP differs significantly from the Intake only in terms of EC_{25°C}, TDS, Na⁺, NH₄⁺, Cl⁻. The outflow from the treatment plant adversely affects the chemical composition of water in the Bialka river

TABLE 2
Significant Spearman's correlation coefficients (p<0.05) between microbiological indicators and physico-chemical characteristics of water at each of the sampling sites.

Parameter	Before STP		STP		Intake	
	<i>E. coli</i>	mesophilic bacteria	<i>E. coli</i>	mesophilic bacteria	<i>E. coli</i>	mesophilic bacteria
<i>E. coli</i>	1.00	-	1.00	0.66	1.00	0.85
mesophilic bacteria	-	1.00	0.66	1.00	0.85	1.00
DO	-	-	-0.62	-0.78	-	-
T	-	-	-	-	-	-
EC _{25°C}	-	-	0.77	-	0.65	0.64
Na ⁺	-	-	0.81	-	0.91	0.77
K ⁺	-	-	0.72	0.64	-	-
NH ₄ ⁺	-	-	0.80	-	0.96	0.77
Cl ⁻	-	-	0.80	-	0.88	0.78
NO ₃ ⁻	-	-	-0.82	-	-	-
PO ₄ ³⁻	-	-	0.76	-	0.67	-

TABLE 3
Physical and chemical water parameters during the diurnal study.

Parameter	Unit	Before STP				STP				Intake			
		Mean	Min	Max	Cv [%]	Mean	Min	Max	Cv [%]	Mean	Min	Max	Cv [%]
DO	mg/L	13.9	13.6	14.3	1.7	5.0	3.0	8.0	35.1	13.5	11.4	14.2	5.3
T	°C	0.2	-0.2	1.0	179.4	6.8	3.7	9.1	26.1	0.4	-0.1	1.0	121.6
pH		8.07	8.00	8.13	0.5	7.40	7.25	7.60	1.4	8.02	7.91	8.11	0.8
EC _{25°C}	µS/cm	264.0	257.4	270.5	1.8	1,031.7	616.4	1,436.3	30.0	272.4	263.2	283.2	2.4
TDS		231.2	227.1	236.5	1.4	808.0	529.2	1,069.1	25.1	236.3	229.0	246.2	2.2
Na ⁺		3.49	3.35	3.67	2.8	68.3	18.9	116.7	53.4	3.9	3.46	4.7	10.0
K ⁺		0.89	0.72	1.74	34.4	15.3	5.1	22.5	42.2	1.1	0.72	1.9	38.0
NH ₄ ⁺	mg/L	0.02	0.01	0.03	30.7	35.8	6.7	68.2	62.6	0.14	0.01	0.51	109.1
Cl ⁻		4.86	4.51	5.11	3.7	70.3	19.0	117.5	53.9	5.2	4.70	5.9	8.2
NO ₃ ⁻		3.56	3.44	3.69	2.3	4.11	3.70	4.89	13.4	3.64	3.48	3.97	3.9
NO ₂ ⁻		below limit of detection				0.2	0.1	0.3	47.4	below limit of detection			
PO ₄ ³⁻		below limit of detection				7.1	0.7	17.7	83.9	0.007	0.003	0.015	76.7

(point Intake). Inflow of insufficiently treated sewage causes a decrease in the dissolved oxygen content and water pH, as well as an increase in the concentration of ions in the water of Białka. This is most evident in the case of NH₄⁺, whose average increase between the points Before STP and Intake was about 60%. The average increase in the concentration of Na⁺, K⁺ and Cl⁻ ions is also evident and reaches 9.9%, 7.0% and 6.7%, respectively.

The differences in physical and chemical parameters during the day were statistically significant in each of the measuring points. The greatest diurnal changes were related to ions, whose occurrence in water can be associated with anthropogenic pressure (i.e. NH₄⁺, NO₃⁻, PO₄³⁻, K⁺, Na⁺, Cl⁻ – Fig. 2). For example, at the point Before STP the maximum daily value of NH₄⁺ was 3.5 times greater than the smallest one measured throughout the day. Much larger diurnal fluctuations were observed at the point Intake. There was a 35-fold increase in the concentration of NH₄⁺ during the day. 10-fold diurnal variation in the concentration of NH₄⁺ was observed at the STP, but the variation in the concentrations of PO₄³⁻ was even more evident (25-fold difference in the concentration values throughout the day).

The inflow of sewage from an ineffectively operating sewage treatment plant together with numerous unregistered discharge points of untreated sewage in the Bukowina Tatrzńska municipality result in the deterioration of water quality in the Białka river. However, the mountainous nature of the river, characterized by turbulent water flow, low temperature of water and very good oxygenation causes that in terms of physical and chemical parameters it can be classified as the highest, first

class of water quality [26].

The Principal Component Analysis (PCA, Fig. 3.) allowed to determine the main factors affecting the concentration of microbial indicators of fecal contamination, as well as hydrochemical characteristics of water at the examined sampling points in the diurnal cycle. At the site No. 1 (Before STP), PCA allowed to distinguish one factor that explains 72.7% of variance. Based on the factor coordinates, a strong correlation between *E. coli*, mesophilic bacteria and NH₄⁺ and NO₃⁻ ions can be observed. This factor can be explained by the anthropogenic influence, as the higher nutrient concentrations, whose origin is mainly related to human activity, the higher the prevalence of bacteria.

By the discharge from the sewage treatment plant (site No. 2 – STP), PCA also allowed to recognize one factor that explains 74.5% of variance. It is characterized by a strong positive relationship between the number of bacterial indicators and T, EC_{25°C}, Na⁺, K⁺, NH₄⁺, Cl⁻ and PO₄³⁻ and negative relationship with pH, DO and NO₃⁻. This factor may evidence the discharge of fresh, untreated sewage from the treatment plant, where the nitrification process did not occur. This is particularly apparent in the negative relationship between NO₃⁻ and NH₄⁺, i.e. the occurrence of large concentrations of ammonium ions with very low concentrations of nitrate ions [27]. Usually, untreated sewage contains nitrogen in the form of NH₄⁺, while the amount of nitrates and nitrites is small.

Principal Component Analysis carried out for the sampling site No. 3 (Intake) showed that there are two factors affecting the quality of water throughout the day. Factor 1 explains 56.2% of variance and represents positive relationship between the content

of bacteria in water and the values of $EC_{25^{\circ}C}$, TDS, Na^+ , NH_4^+ , Cl^- , NO_3^- , PO_4^{3-} . In contrast, factor 2 explains 21.4% of variance and represents negative relationship showing that the higher the values of $EC_{25^{\circ}C}$ and NO_3^- the lower the values of T, K^+ and PO_4^{3-} . In the case of factor 1 we can consider the anthropogenic effect and deterioration of water quality through the sewage discharge to the Bialka

river. This is evidenced by the increase in the number of bacteria and NH_4^+ , NO_3^- , PO_4^{3-} ions, which are of anthropogenic origin. Factor 2 is related to the diurnal activity of people, resulting in the production of smaller amount of sewage during night. Therefore, the efficiency of sewage treatment is greater and the process of nitrification is more effective which results in higher NO_3^- values in

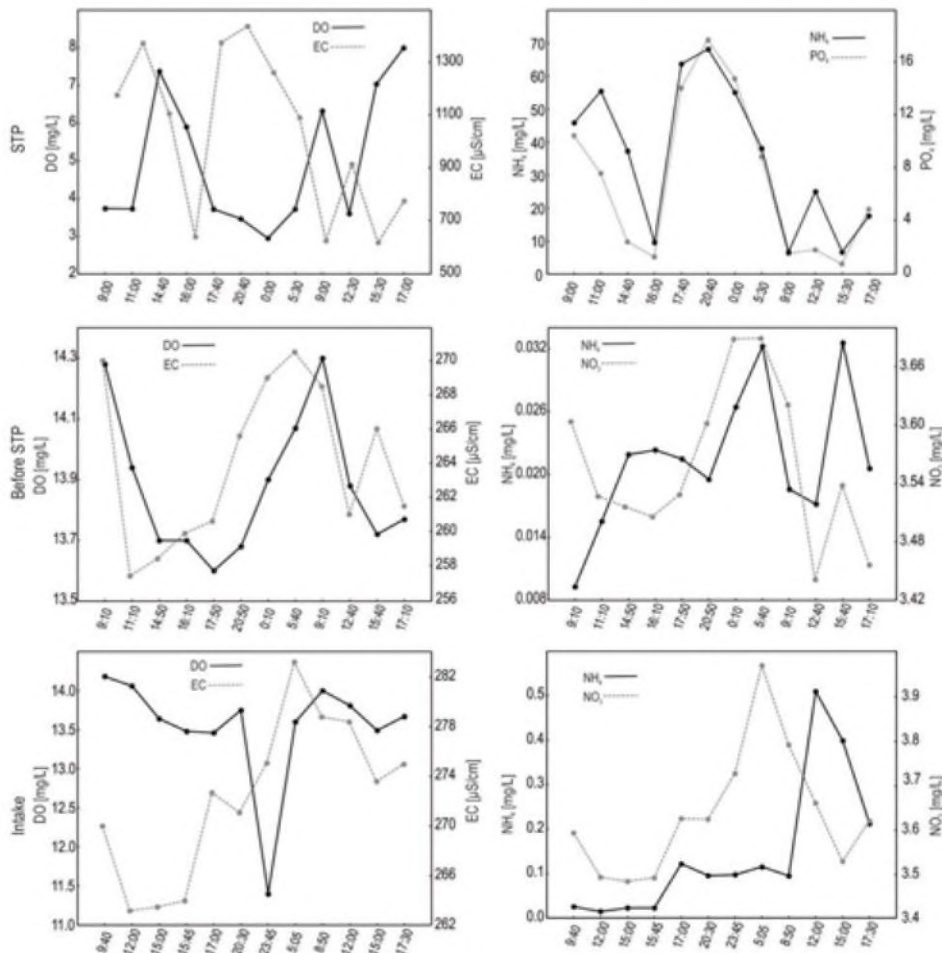


FIGURE 2
Changes in the selected physico-chemical parameters at the measuring points throughout the study period.

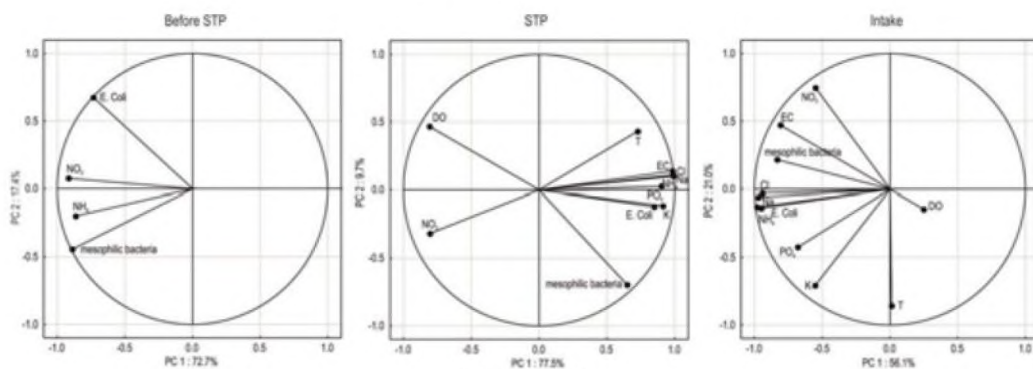


FIGURE 3
Principal Component Analysis of microbiological and physicochemical parameters determined for the sampling sites.

sewage discharged to the river. At the same time, low temperature of water at night causes that snow does not melt, thus not causing dilution of water and therefore the water conductivity being higher.

CONCLUSIONS

The number of microbial indicators of fecal contamination depends on the location of the sampling sites and their location towards the main source of water contamination, i.e. the sewage treatment plant. A variation in a diurnal cycle of the concentration of the tested microbial parameters of water quality, as well as the physico-chemical characteristics of water was also evident. The cycle of the sewage treatment plant operation affects the number of microbial contaminants of water both in the stream which receives the effluent from the treatment plant and in the part of the river downstream of the treatment plant.

The conducted analyses revealed that in terms of chemical parameters water in the Białka river can be assessed as good quality, while the detected microbiological contamination indicates that without very thorough treatment, those waters cannot be used neither for consumption or for the technological use. It is important to carefully select sampling sites, as their location within catchments may affect the concentrations of microbial indicators of fecal contamination.

ACKNOWLEDGEMENTS

This study was funded by statutory measures of the University of Agriculture in Kraków, within a grant No. BM 4149/2014 and a grant No. DS 3102/KM/2014, as well as by the Jagiellonian University grant No. K/KDU/000153 - Hydrochemical and hydrological monitoring of the Białka subcatchments in the neighborhood of Kotelnica (2013-2015).

REFERENCES

- [1] http://ec.europa.eu/environment/nature/natura2000/index_en.htm, Accessed September 20th 2015.
- [2] Pająk B. (2014). Report on the condition of the environment in the Lesser Poland voivodeship in 2013, Provincial Environmental Protection Inspectorate WIOŚ in Kraków Poland.
- [3] Heldak M. 2010 The Białka River in the Rural Landscape of Podhale. *Landscape Architecture*, 2, 39-46.
- [4] Kraż P. (2012). Anthropogenic hazards to the Białka Valley natural environment. *Geographical Papers*, 128, pp. 45-54 (in Polish).
- [5] www.supersnow.pl, Accessed July 7th 2015.
- [6] Central Statistical Office. (2013). http://stat.gov.pl/bdl/app/strona.html?p_name=indeks
- [7] Lenart-Boroń A., Wolanin A., Jelonkiewicz L., Chmielewska-Błotnicka D., Żelazny M. (2016) Spatiotemporal Variability in Microbiological Water Quality of the Białka River and Its Relation to the Selected Physicochemical Parameters of Water. *Water Air & Soil Pollution* 227(1): 10.1007/s11270-015-2725-7
- [8] Smoroń S., Twardy S. & Kowalczyk A. (2007). Surface water quality in tourist areas of the Western Carpathians. Part 1. The dynamics of chemical substances load in domestic wastewaters. *Water-Environment-Rural Areas*, 21, 155-166 (in Polish).
- [9] Parker L.V., Yushak M.L., Martel J. & Reynolds C.M. (2000). Bacterial Survival in Snow Made from Wastewater. Technical Report ERDC/CRREL TR-00-9. US Army Corps of Engineers, Cold Regions Research & Engineering Laboratory, New Hampshire, USA.
- [10] Walker V.K., Palmer G.R. & Voordouw G. (2006). Freeze-thaw tolerance and clues to the winter survival of a soil community. *Applied and Environmental Microbiology*, 72(3), 1784-1792.
- [11] Krzesiwo K. (2014). Development and functioning of ski stations in the Polish Carpathians. Kraków: Institute of Geography and Spatial Management, Jagiellonian University.
- [12] Krzesiwo K. (2015). Tourist traffic in the Kotelnica Białczańska Ski Station in the winter season 2014/2015, research report, Kinga Krzesiwo Research & Consulting, Pisarzowice, Poland.
- [13] Whitman R.L. & Nevers M.B. (2004). *Escherichia coli* sampling reliability at a frequently closed Chicago beach: monitoring and management implications. *Environmental Science and Technology*, 38, 4241-4246.
- [14] Meays C.L., Broersma K., Nordin R., Mazumder A. & Samadpour M. (2006). Diurnal variability in concentrations and sources of *Escherichia coli* in three streams. *Canadian Journal of Microbiology*, 52, 1130-1135.
- [15] <http://www.socscistatistics.com/tests/studentttest/Default.aspx>, Accessed September 18th 2015.
- [16] Wakelin S.A., Colloff M.J. & KooKana R.S. (2008). Effect of wastewater treatment plant effluent on microbial function and community structure in the sediment of a freshwater stream with variable seasonal flow. *Applied and Environmental Microbiology*, 74(9), 2659-2668.



- [17] Mishra A. (2010) Assessment of water quality using principal component analysis: A case study of the river Ganges. *Journal of Water Chemistry and Technology*, 32(4), 227-234.
- [18] Thyne G., Guler C. & Poeter E. (2004). Sequential analysis of hydrochemical data for watershed characterization. *Ground Water*, 42(5), 711-723.
- [19] Sinton L.W., Finlay R.K. & Hannah D.J. (1998). Distinguishing human from faecal contamination in water: a review. *New Zealand Journal of Marine Freshwater Research*, 32, 323-348
- [20] Cabral J.P.S. (2010) Water Microbiology. Bacterial Pathogens and Water. *International Journal of Environmental Research and Public Health*, 7(10), 3657-3703.
- [21] Regulation of the Minister of the Environment of 27th November 2002 on the requirements to be met by surface water used to supply people with drinking water. *Journal of Laws of the Republic of Poland No. 204, item 1728.*
- [22] Grabow W.O.K. (1996). Waterborne diseases: update on water quality assessment and control. *Water SA*, 22, 193-202.
- [23] Kukuła E. (2006). Assessment of microbiological quality of waters of the San river. *Proceedings of the 2nd Scientific and Technical Conferences "Blue San"*, Rzeszów University, 121 – 125.
- [24] Kolarevic S., Knezevic-Vukcevic J., Paunovic M., Vasiljevic B., Kracun M., Gacic Z. & Vukovic-Gacic B. (2012). Seasonal variations of microbiological parameters of water quality of the Velika Morava River Serbia. *Archives of Biological Sciences, Belgrade*, 64(3), 1017-1027.
- [25] North R.L., Khan N.H., Ahsan M., Prestie C., Korber D.R., Lawrence J.R. & Hudson I.J. (2014). Relationship between quality parameters and bacterial indicators in a large prairie reservoir: Lake Diefenbaker, Saskatchewan, Canada. *Canadian Journal of Microbiology*, 60(4), 243-249.
- [26] Regulation of the Minister of Environment of 22nd October 2014 on the classification status of surface waters and environmental quality standards for priority substances. *Journal of Laws of the Republic of Poland No. 2014, item. 1482.*
- [27] Bothe H., Jost G., Schloter M., Ward B.B. & Witzel K-P. (2000). Molecular analysis of ammonia oxidation and denitrification in natural environments. *FEMS Microbiology Reviews*, 24, 673-690.

Received: 07.01.2016
Accepted: 17.08.2016

CORRESPONDING AUTHOR

Anna Lenart-Boron

Department of Microbiology, University of Agriculture in Cracow Mickiewicza Ave. 24/28, 30-059 Cracow, Poland

e-mail: a.lenart-boron@ur.krakow.pl