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## *Escherichia coli* as a potential indicator of Biebrza River enrichment sources

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### Abstract

The number of *Escherichia coli* cells in the waters of the Biebrza River was analyzed. The results were compared with the values of select chemical water quality indicators and with the Biebrza River catchment development. Organic contamination was detected along the entire run of the River and was caused by the presence of substances washed off the adjacent marshy areas and the influx of household and agricultural wastewaters. The highest number of bacteria was found in the Middle and Lower Basins of the Biebrza River. The increase in the number of *E. coli* in the spring to over 24 MPN·cm<sup>-3</sup> is a result of the numerous presence of water birds in the area of the National Park. Of consequence are also meltwaters that introduce contamination from peat lands into the watercourse. The increased number of *E. coli* cells in the summer is related to runoff from the areas along the River with numerous farms and dairy cattle pastures. The number of bacteria in the river is most probably influenced by changes in water levels and types of accumulated sediment. The *Escherichia coli* count allows determining the source of the contamination of surface waters, which is of particular significance for planned water management on protected areas. The number of *E. coli* below 7 MPN·cm<sup>-3</sup> in the waters of the Biebrza River is at the natural background level, whereas counts of over 24 MPN·cm<sup>-3</sup> are related to the influx of household wastewaters.

**Key words:** *Escherichia coli*, indicator, water quality, sources of contamination

### INTRODUCTION

Protected areas, in which water is a major factor shaping habitats, should be embraced by long-term monitoring. The range of protective actions taken should be based, amongst others, on the determination of the qualitative status of a habitat. Analysis of results obtained should allow precise determination of the sources of pollution, and therefore the potential sources being a threat to an ecosystem.

The Biebrza River valley is embraced by legal protection as a national park and by the Natura 2000 Network. Because of the specificity of the terrain it is subject to the inflow of pollution of natural origin, as well as that related to farming on the adjacent areas

[BIELAK 2009; MIODUSZEWSKI *et al.* 2004]. The river valley is the refuge of many valuable species of animals, but is also used by the locals as a cattle grazing area. Many farms are located in the close neighbourhood of the river. There are also numerous transiently used recreation buildings that can be a source of illegal discharges of wastewaters. The variety of causes of the contamination of the Biebrza River prompts searching for solutions allowing the precise pinpointing of the individual sources [BIELAK 2009; FRAK 2010; MIODUSZEWSKI *et al.* 2004].

Even though the Biebrza River Valley is considered a pure and reference environment the presence of allochthonous contaminants is still recorded. Studies indicate that the waters of the Biebrza periodically



discharge outlets, special management areas). Samples of water from the main current of the River were collected into sterile containers in accordance with PN-EN ISO 5667-1:2008 and PN-EN ISO 5667-3:2005 during 4 measurement periods, that is in April, June, August and September of 2010.

The occurrence and number of *Escherichia coli* cells in the collected water samples was determined using the MPN (most probable number) method in accordance with PN-ISO 7251:2006 at temperature 37°C, and the obtained results were confirmed on Endo medium at 44°C (results are given as MPN·cm<sup>-3</sup>; accordance with methodology: MPN in 100 cm<sup>3</sup>). The levels of select chemical parameters, in accordance with the recommendations in ISO 8245 EPA 415.1 (DOC), PN-ISO10304-1:2009E (NO<sub>3</sub><sup>-</sup>), PN-ISO 14911:2002 (NH<sub>4</sub><sup>+</sup>), were also determined. The concentrations of nitrates (mg NO<sub>3</sub><sup>-</sup>·dm<sup>-3</sup>) and ammonia (mg NH<sub>4</sub><sup>+</sup>·dm<sup>-3</sup>) were assayed by liquid chromatography (Dionex ICS – 1000). The concentration of dissolved organic carbon (DOC, mg C·dm<sup>-3</sup>) was determined using the high temperature method in continuous flow analyzer (SKALAR Formacs HT/TN). Analysis of the results also included field observations and information catchment development of the studied area.

## RESULTS AND DISCUSSION

The number of *Escherichia coli* bacteria in the collected water samples was determined (Fig. 2). In April their highest number of 13 MPN·cm<sup>-3</sup> was found at point 1 (the value at all the other points was below 7 MPN·cm<sup>-3</sup>). This value suggests the inflow of fecal pollutants. Upstream of point 1 the Biebrza River is joined by the tributaries Sidra and Kropiwna, which collect, amongst others, wastewaters from local treatment plants in Różanystok and Dąbrowa Biało-

stocka. The inflow of wastewaters from the purification plant in Lipsk and the Kamienna River downstream of p. 1 does not indicate any significant effect on the quality of the Biebrza River waters. At the remaining measurement points the number of *E. coli* ranged from 0.05 (pts. 8, 9, 10) to 7 (p. 6) MPN·cm<sup>-3</sup> (accordance with the methodology, from 5 to 700 MPN in 100 cm<sup>3</sup> water from the river). Such low bacteriological contamination can result from, amongst others, thermal conditions [BUCCI *et al.* 2013]. Low water temperature does not favour the propagation of mesophilic bacteria (to which *E. coli* belongs) but allows their long-term persistence in the environment [FLINT 1987]. Moreover, April is the time of strong inundations in the Biebrza valley – the river waters strongly mingle throughout several months in the year with meltwaters that flow into the valley from the area of the catchment [CHORMAŃSKI *et al.* 2000]. Massive volumes of water result in the dilution of inflowing pollution. The greatest volumes of inundation waters are recorded every year in the lower basin of the Biebrza River and this results in low concentrations of most of the recorded parameters (pkt. 8, 9, 10) [KARDEL *et al.* 2007]. In June, in the final stages of recession of the inundation, the number of *E. coli* increased: from 0.13 MPN·cm<sup>-3</sup> at p. 11 to 24 at p. 1. A higher number of bacteria was found in the Upper and Middle Basins (13–24 MPN·cm<sup>-3</sup> at pts. 1, 3, 6). This was probably related to the receding of the catchment basin along this section of the river (and thus smaller water volume) and regular inflow of sanitary pollutants. The increase in the number of bacteria may also be the result of picking up the pieces of bottom sediments into the water deep. The result obtained for p. 1 continues to confirm the inflow of increased amounts of faecal contaminants (24 MPN·cm<sup>-3</sup>). The disappearance of the catchment basin as well as increased temperatures enabled the grazing of animals (both

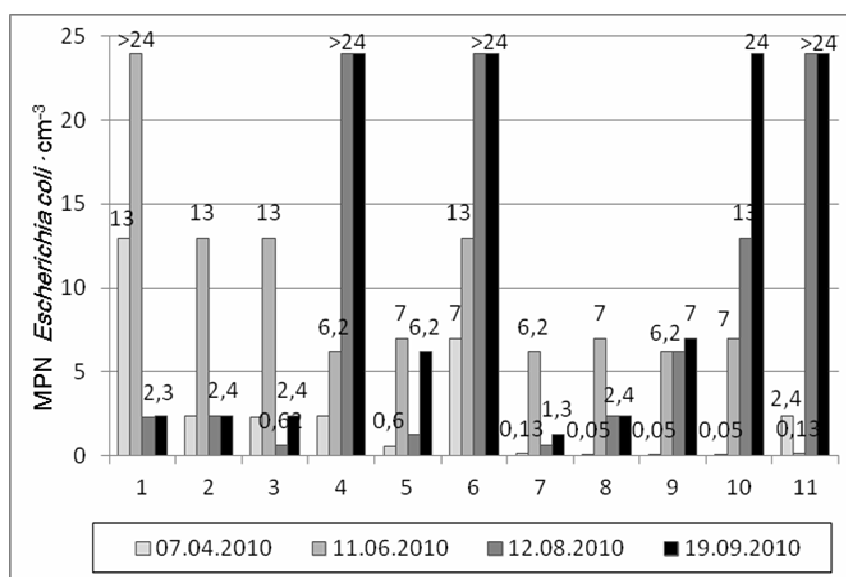


Fig. 2. Number of *Escherichia coli* in the measurement points; explanations as in Fig. 1; source: own study

wild and bred) on adjacent peat lands, which causes an increased amount of faeces in close proximity to the River [CHORMAŃSKI *et al.* 2000; FRAK 2010; MIODUSZEWSKI *et al.* 2004]. The lower basin of the Biebrza River continues during this time to be characterized by elevated level of waters that drain the peat areas and cause the leaching of contaminants into the waters of the River. In addition, in the late spring vast numbers of water birds come to this area to breed. This results in the local appearance of increased amounts of excreta, which results in elevated number of *E. coli* in the water (ca. 7 MPN  $\text{cm}^{-3}$  – pts. 8–10).

An analysis of the chemical parameters measured from April to June reveals similar tendencies among the obtained values. Increased amounts of ammonia ions were recorded at p. 1 (0.58  $\text{mg} \cdot \text{dm}^{-3}$ ), and downstream to p. 7 a gradual decrease in their concentration (0.34  $\text{mg} \cdot \text{dm}^{-3}$ ) was determined. On the other hand, the concentration of nitrates along this section slightly increases, reflecting biooxidation processes [FRAK *et al.* 2012; GÓRNIK *et al.* 1996]. In the Lower Biebrza River Basin higher concentrations of ammonia ions and reduced concentration of nitrates (Fig. 3), were recorded. The persistence of enhanced concentrations of ammonia and nitrates in the spring points to continued limited vegetation and the slow

growth of nitrifying bacteria [BELSER 1979; BRION, BILLEN 2000; FRAK *et al.* 2012]. At pts. 6 and 7 considerably elevated amounts of nitrates (over 3  $\text{mg} \cdot \text{dm}^{-3}$ ) were recorded, which may suggest an additional influx of nitrates from outside the national park area [BRION, BILLEN 2000]. Above these points the Biebrza is joined by waters of the Woźnawiejski Canal with the Rivers Elk, Jerzgnia, Berka and Krzczówka.

In the summer, the number of *E. coli* cells stabilizes. The absence of meltwaters causes that contaminants are introduced in the Biebrza River only by inflows from the area of the basin, including watercourses draining pasture areas and peatlands (wetlands). Of note is contamination by *E. coli* of the waters of the Middle and Lower Basins. The highest number of the bacteria ( $>24$  MPN  $\cdot \text{cm}^{-3}$ ) was observed at pts. 4, 6, 10 and 11. This indicates the constant inflow of fecal contaminants from various sources. Between pts. 3 and 4 there are no larger tributaries so the high level of most of the indices at p. 4 can be explained by the inflow of contaminants from the breeding of the Polish pony (Polish primitive horse – horse breeding farm near Jagłowo, upper of the p. 4) or from illegal household wastewater discharges [MIODUSZEWSKI *et al.* 2004]. Downstream of p. 4 there are extensive green crop areas, located on peat lands, on which dairy cattle grazes. Upstream of p. 5 waters are introduced by Augustów Canal and the Brzozówka River. According to FRAK *et al.* [2012], bacteriological studies of both watercourses indicate strong proteolytic and ammonifying processes and therefore the presence of nitrogen-containing compounds, including proteins. Therefore, the quality of the Biebrza River waters is significantly worsened not only by chemical compounds in leachates from marshy grounds and surface runoff, but also by pollution of anthropogenic origin [BIELAK 2009; KARDEL *et al.* 2007; MIODUSZEWSKI *et al.* 2004]. In addition, p. 6 is located below the inflow of wastewaters from the purification plant in Wrocen, which also causes high *E. coli* counts throughout the whole study period (Fig. 2). The unsatisfactory sanitary state at p. 6 is confirmed e.g. by regular closures of the urban swimming pool located downstream by the regional Sanitary-Epidemiological Station.

The chemical indices analyzed in the area of the lower section of the Biebrza (pts. 8–11) do not point to any contamination with household wastewaters. However, the detected high count of *E. coli* indicates the inflow of faecal contaminants upstream of p. 8 pollution is introduced by the Wastewater Purification Plant in Goniadz (via Czarna Struga) and Rudzki canal, downstream of the Wissa and Kosodka Rivers [BRION, BILLEN 2000; ODONKOR *et al.* 2013; RENTER *et al.* 2003]. In all the tributaries very high numbers of proteolytic and ammonifying bacteria were recorded, which confirms the additional introduction of biodegradable organic contaminants into the waters of the Biebrza River [FRAK *et al.* 2012]. Moreover, marshy areas in the Lower Basin of the River are utilized in

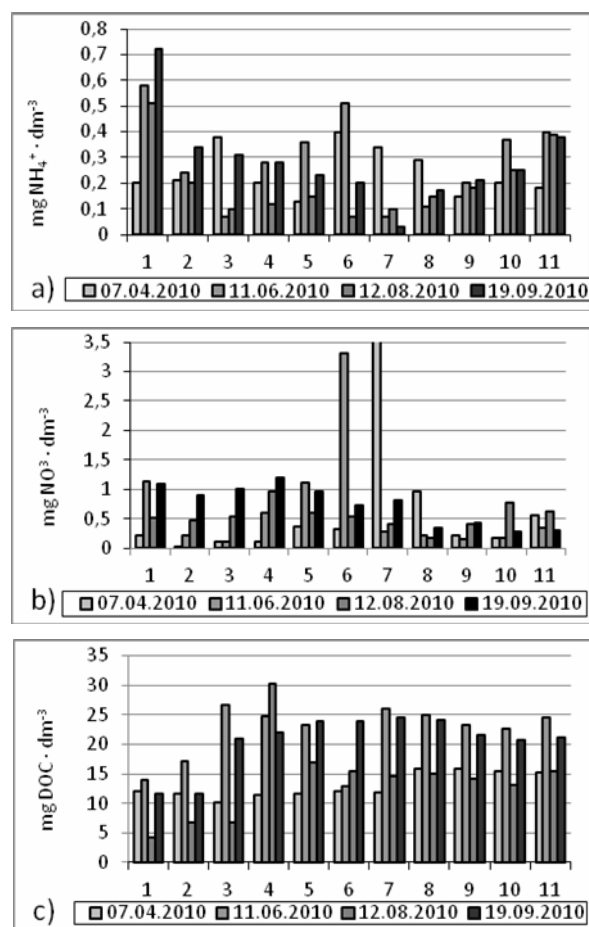


Fig. 3. Concentration of (a) ammonia, (b) nitrates and (c) dissolved organic carbon in the measurement points; explanations as in Fig. 1; source: own study

low-water areas for cattle grazing. Consequently, surface runoff may introduce not only increased amounts of organic carbon, but also faecal contaminants from animal breeding [KARDEL *et al.* 2007; RENTER *et al.* 2003]. The considerable bacteriological contamination in the lower basin of the River is also brought about by the close proximity of farm buildings and the runoff of farm wastes (pts. 10, 11). Not without significance is also the inadequate state of the water and sewerage infrastructure and the illegal discharge of wastewaters [MIODUSZEWSKI *et al.* 2004].

This can be confirmed by the recorded concentrations of ammonia (up to  $0.38 \text{ mg} \cdot \text{dm}^{-3}$  at p. 11) that are higher in the summer (that is outside the period when ammonia is intensely washed out from marshy areas and in spite of intense oxidation processes in the periods of low water-table) [BELSER 1979]. The low concentrations of nitrates in the summer time are closely related to the intense vegetation of aquatic plants, abundantly growing in the Biebrza River [PIECHOWIAK, KRASKA 2008].

The concentration of dissolved carbon organic (DOC) was found to range from  $11.41 \text{ mg C} \cdot \text{dm}^{-3}$  at p. 4 in April to  $30.25 \text{ mg C} \cdot \text{dm}^{-3}$  at the same point in August. These values qualify the waters of the Biebrza as heavily contaminated. GÓRNIAK [1996], WYSOCKA-CZUBASZEK, BANASZUK [2003] and KARDEL [2007], amongst others, have indicated that geological conditions significantly affect the chemical composition of adjacent waters. The studies of these authors confirm the significant effect of marshy area on the high organic carbon content in the Biebrza. Watercourses that drain marshy areas leach organic compounds (including humus compounds) from peats, and then introduce them into the waters of the Biebrza. DOC contamination can also be caused by the inflow of local household and agricultural wastewaters [BIEŁAK 2009; GÓRNIAK 1996; KARDEL *et al.* 2007;]. The studies of FRAK *et al.* [2012] on the presence of proteolytic bacteria in the waters of the Biebrza, demonstrated their occurrence in numbers of even up to  $27\,067 \text{ units} \cdot \text{cm}^{-3}$ . Their high number confirms high pollution of organic origin. The highest number

of proteolytic bacteria was found at pts 5, 10 and 11 (identical numbering of measurement points).

These studies point to intense proteolytic and ammonification processes in the waters of the Biebrza, in spite of the low detection of nitrogen forms in chemical analyses. This indicates the absorption of ammonia ions produced from organic contaminants by aquatic vegetation (macrophytes, phytoplankton) that intensely grows in the Biebrza when it is warm [BELSER 1979; FRAK *et al.* 2012; PIECHOWIAK, KRASKA 2008]. Moreover, in conditions of high content of organic carbon compounds – nitrogen, formed in the course of their mineralization, is taken up in its entirety by microorganisms, without any release of free ammonia to the environment. When the organic substrates are mostly humus compounds, nitrogen accumulates in the bacterial cells [BELSER 1997; GÓRNIAK 1996; PIECHOWIAK, KRASKA 2008]. Thus, when management of the surrounding areas is taken into account, contamination of the Lower Basin of the Biebrza River in the summer time is related to runoffs from farm buildings and animal grazing areas.

Statistical analysis of the results showed positive correlations (Fig. 4) between the number of *E. coli* and  $\text{NH}_4^+$ ,  $\text{NO}_3^-$ , DOC. If the *E. coli* increase there is also seen grow of  $\text{NH}_4^+$ ,  $\text{NO}_3^-$ , DOC. The largest increase was observed in the case of DOC.

Studies of the Biebrza River indicate that high faecal contamination is regularly determined at similar measurement points. The studies carried out in 2007 [FRAK 2010] also indicate the constant contamination by *E. coli* in the summer time of the waters of the middle and lower basins of the River: values over  $24 \text{ NPL} \cdot \text{cm}^{-3}$  were recorded at pts. 4, 8 and 9 in June and at pts. 4, 6 and 11 in July.

To sum up, a significant increase in the number of *Escherichia coli* cells was determined in the summer time, and also in sections of its lower run. This is typical for the dynamics of the persistence of allochthonous microflora in the environment. The transient increase to over  $24 \text{ NPL} \cdot \text{cm}^{-3}$  is enhanced by the inflow of wastewaters from the area of the farms in the vicinity of the River, numerous dairy cattle pas-

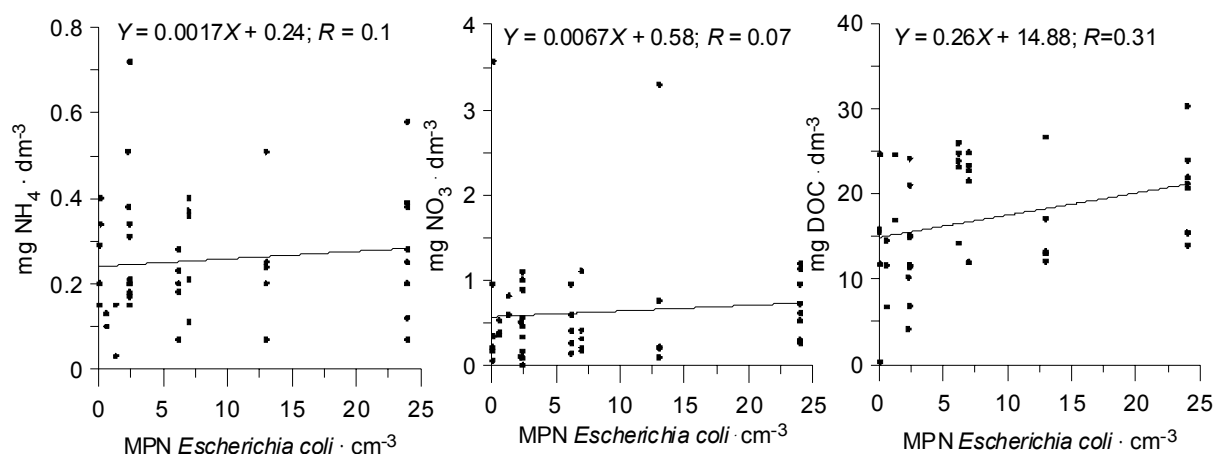


Fig. 4. Diagrams of relationship for number of *Escherichia coli* and  $\text{NH}_4^+$ ,  $\text{NO}_3^-$ , DOC; source: own study

tures (mainly in the areas of the Middle and Lower Biebrza Basins) and wastewater purification plants in Dąbrowa Białostocka, Dolistowo and Goniądz. It would thus be advised in the management of water resources to have constant monitoring of the drainage network introducing waters from areas under agricultural use into the Biebrza River. In the spring the local increase in the count of faecal microflora in the waters of the River is also related to the numerous occurrence of water birds (e.g. geese).

In analyzing the collected data it can be concluded that the contamination of the waters of the Biebrza River by *Escherichia coli* can be affected by runoffs from areas inhabited by humans (both local and transient), from dairy cattle grazing areas and areas of the numerous occurrence of wild animals. However, the unequivocal determination of the sources of contamination when comparing information about the catchment development and the chemical and bacteriological quality parameters, is not possible. Their identification in the Lower Biebrza Basin is strongly hindered. It can only be said that the number of *E. coli* over  $24 \text{ NPL} \cdot \text{cm}^{-3}$  suggests a significant influx of faecal wastewaters, with no possibility of exactly determining their source (man – household wastes, dairy cattle – wastewaters from breeding). The degree of contamination of the waters of the lower basin by *E. coli* also seems to be strongly affected by the inflow of faecal matter from wild animal habitats. *Escherichia coli* counts lower than  $7 \text{ MPN} \cdot \text{cm}^{-3}$ , on the other hand, point to sources of natural contamination, not the inflow of household wastes. It is also important to include in the analysis the results of hydrological data. They will help in determining the impact of bottom sediments on the water river quality.

Therefore, for the planned protection of the Biebrza Valley it would be of importance to determine the genetic identity of the *Escherichia coli* strains isolated from the waters. This would allow pinpointing the causes of sanitary contamination: faeces of wild animals, faeces of farm animals, human faeces. Finding a correlation between the number of *Escherichia coli* and the way the basin is used can be enabled by studies on the genetic relatedness of the strains isolated from waters of the Biebrza River. Consequently, studies aimed at determining the genotypes of the isolates, using amplification of the gene coding 16S rRNA by the triplex PCR method [LUCZKIEWICZ *et al.* 2010; ORSI *et al.* 2007; RENTER *et al.* 2003] should be planned. Sequencing of PCR products could allow determining the genetic identity of the strains isolated from the faeces of specific species of animals characteristic for the studied area. In the case of studies on the contamination of the Biebrza River an analysis of the strains characteristic for *Homo sapiens*, *Bos taurus* as well as *Alces alces*, *Castor fiber*, *Anser anser* and *Chlidonias leuapterus* (dominants by Werpachowski) is proposed.

Combining the results of regular monitoring based on hydromorphological, chemical, bacteriological with genetic indices could enable not only the defining of threats to the functioning of the ecosystem, but also the pinpointing of causes of sanitary waste contamination. Determination of the actual sources of *E. coli* contamination would also allow evaluation of the current water management in the Biebrza Valley area and ensure the implementation of proper principles of water management, which is of particular significance in the case of all legally protected watercourses.

## CONCLUSIONS

1. The number of *E. coli* cells is higher in the summer time, which is compatible with the seasonal variation in the occurrence of waste contaminants in surface waters. However, the threat posed by the bacteria is decidedly higher in the Middle and Lower Basins of the Biebrza River.

2. The high number of *E. coli* at pts. 4, 6, 10 and 11 as well, points to the constant inflow of fecal pollutants, most likely from the adjacent dairy cattle pastures, farming areas or wild animal refuges (habitats of water and mud birds).

3. The number of *E. coli* in the water of the studied river waters reflects the way the adjacent areas are used. Consequently, by determining the number of *E. coli* cells it is possible to conjecture their source to be natural or of anthropogenic origin, but it is not possible to accurately define it.

4. The analysis of the results should include the measurements of river discharge and the impact of sediments on the Biebrza river water quality.

5. In order to precisely pinpoint the causes of the sanitary contamination of the watercourse it is necessary to determine the genetic identity of the isolated strains.

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**Magdalena FRAK**

### ***Escherichia coli* jako potencjalny wskaźnik wzbogacania wód rzeki Biebrzy**

#### **STRESZCZENIE**

**Słowa kluczowe:** *Escherichia coli*, wskaźnik, jakość wody, źródła zanieczyszczeń

W wodzie rzeki Biebrza analizowano liczebność *Escherichia coli*. Wyniki zestawiono z poziomem wybranych wskaźników jakości chemicznej wody oraz danymi, dotyczącymi zagospodarowania zlewni. Stwierdzono zanieczyszczenie organiczne na całej długości biegu rzeki, wywołane obecnością substancji wymywanych z okolicznych terenów bagiennych oraz dopływem ścieków bytowych i rolniczych. Największą liczebność bakterii stwierdzono w środkowym i dolnym basenie rzeki Biebrzy. W okresie wiosennym wzrost liczebności *E. coli* do ponad 24 NPL·cm<sup>-3</sup> wynika z licznego występowania ptactwa wodnego na obszarze Parku Narodowego. Wpływ mają też wody roztopowe wnoszące do cieku zanieczyszczenia z terenów torfowych. Latem wzrost liczebności *E. coli* związany jest ze spływem z sąsiadujących z rzeką terenów, na których są gospodarstwa i pastwiska bydła mlecznego. Na liczebność bakterii w wodzie mają także prawdopodobnie wpływ zmiany poziomu wód, uruchamiające zasoby osadów dennych. Dzięki określeniu liczebności *Escherichia coli* można określić źródła zanieczyszczenia wód powierzchniowych, co jest szczególnie istotne dla planowej gospodarki wodnej na obszarach chronionych. Liczebność *E. coli* w wodach Biebrzy poniżej 7 MPN·cm<sup>-3</sup> jest naturalnym poziomem, natomiast ponad 24 MPN·cm<sup>-3</sup> wskazuje na napływ ścieków bytowych.