doi: 10.17221/205/2014-SWR

Potential Microbiological Threat to the Vistula Waters by Its Tributaries in the Vicinity of Kraków

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Abstract

Lenart-Boroń A., Puchała M., Boroń P. (2015): Potential microbiological threat to the Vistula waters by its tributaries in the vicinity of Kraków. Soil & Water Res., 10: 130–136.

The Vistula is the longest river in Poland. It stretches from the Silesian Beskids to the Gulf of Gdańsk. Water from the Vistula is the source of drinking water for many cities and is used in industry, and therefore its quality and microbiological purity is an important issue. The quality of water in the Vistula depends, among others, on the quality of water in its basin. The aim of this study is to assess microbiological hazard to the Vistula waters from its tributaries in the vicinity of Kraków. The analyses were carried out on 10 tributaries of the Vistula: Prądnik, Dłubnia, Drwina Długa, Drwinka, Raba, Uszewka, Szreniawa, Uszwica, Kisielina, and Dunajec. The examinations were carried out in four series, from May 2012 to March 2013. The numbers of coliforms, faecal coliforms, *Enterococcus faecalis*, and sulphate reducing *Clostridium* were determined using the membrane filtration method, while the serial dilutions method was used to determine the numbers of mesophilic and psychrophilic bacteria, *Staphylococcus* and *Salmonella*. There was a large variation in the number of microorganisms in the examined watercourses, however in most cases the water could be classified as clean, whereas waters of the Vistula in the vicinity of Kraków are microbiologically contaminated. Thus, the examined watercourses do not pose a serious threat to the quality of the Vistula. Among the analyzed tributaries, the waters of the Prądnik were characterized by the worst sanitary condition, while the best microbiological quality was observed in the Dunajec.

Keywords: coliforms; faecal coliforms; microbiological pollution; water contamination

Water is one of the most important natural resources indispensable for life and almost any kind of human activity. Water quality affects health and safety of people, the plant and animal production, economic development in production and non-production sectors, as well as the condition and development potential of the natural environment. Interestingly, Poland is the country with relatively low water resources which constitute only 3% of the European total amount of water (Eurostat 2011). On the other hand, Poland's water consumption is also one of the lowest in Europe, therefore not the quantity of water, but ensuring access to water resources of appropriate quality becomes more and more challenging (Myszograj & Sadecka 2012). Groundwater, which is characterized by much higher quality, is normally used as a supply of drinking water, but surface water represents as much as 85% of Polish water resources and is used for water supply of the national economy (Myszograj & Sadecka 2012). Surface waters become contaminated as a result of domestic and economic activities, as well as industry and tourism. Pollution from urban sewage, which is the source of chemicals and nutrients, is the main threat to water reservoirs located in the vicinity of urban and semi-urban areas (Gupta & Mehra 2009). Surface water quality largely depends on the amount and type of introduced pollutants, as well as on the water's susceptibility to degradation and self-cleaning potential (Ostroumov 2006).

The Vistula is the longest river in Poland. It stretches from the Silesian Beskids to the Gulf of Gdańsk and passes through seven voivodeships (Silesian, Lesser Poland, Subcarpathian, Lubusz, Masovian, Kuyavian-

doi: 10.17221/205/2014-SWR

Pomeranian, and Pomeranian) while its catchment covers the next four voivodeships (Holy Cross, Łódź, Warmian-Masurian, and Podlaskie). Water from the Vistula is the source of drinking water for many cities and is used in industry, and therefore its quality and microbiological purity is a significant issue. On the other hand, quality of water in the Vistula depends, among others, on the quality of water in its catchment. Therefore, the aim of this study was to determine the potential microbiological hazard to the Vistula waters by its selected tributaries in the vicinity of Kraków.

MATERIAL AND METHODS

Water samples were collected to sterile plastic containers from 10 tributaries of the Vistula (Figure 1). The samples were collected in three replicates from each site:

– Prądnik (Białucha) – left tributary of the Vistula, 34 km long, flowing into the Vistula in Krakow-Dąbie. Its spring is located in the village Sułoszowa, Olkuska Highland;
– Dłubnia – left tributary of the Vistula, 49 km long, flowing into the Vistula in Krakow-Mogiła, having its springs in the village Jangrot;
– Drwina Długa – 5.5 km long, with its spring in the Kraków-Rybitwy district, flowing into the Vistula through the Serafa, already outside Krakow;
– Drwinka – stream having its spring in Piaski Nowe Krakow district, 5.9 km long, flowing into the Drwina Długa
– Raba – 132 km long river, with the spring in the vicinity of the village Sieniawa and flowing into the Vistula nearby Uście Solne;

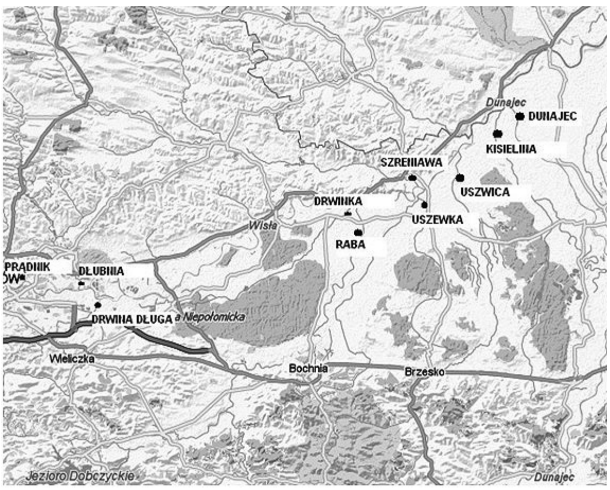

Figure 1. Location of sampling sites

– Uszewka – one of the main tributaries of the Uszwica;
– Szreniawa – left tributary of the Vistula, having its spring nearby Wolbrom and flowing into the Vistula in the village Sokołowice, total length 80 km;
– Uszwica – 61 km long river, having its spring in the Island Beskids, flowing into the Vistula in 151 km of its course in Wola Przemykowska
– Kisielina – right tributary of the Vistula, 35 km long, having its spring on the slopes of Dąbrowa nearby the village Łysa Góra;
– Dunajec – river formed through the merger of the rivers Czarny Dunajec and Biały Dunajec in Nowy Targ, flowing into the Vistula nearby Opatowiec and Ujście Jezuickie.

The samples were collected four times throughout a year – on May 21st 2012, September 11th 2012, December 19th 2012, and March 5th 2013. The membrane filtration method was used for enumeration of coliforms (purple red colonies with metallic sheen on Endo agar, incubation at 37°C, 48 h), faecal coliforms (purple red colonies with metallic sheen on Endo agar, incubation at 44°C, 48 h), *Enterococcus faecalis* (small dark red to light brown colonies on Slanetz-Bartley agar, 37°C, 72 h) and sulphate reducing *Clostridium* (black colonies on Wilson-Blair agar, 35°C, 36 h, anaerobic conditions). The serial dilutions method was used to determine the number of mesophilic bacteria (trypticase soy agar, 37°C, 48 h), psychrophilic bacteria (trypticase soy agar, 22°C, 72 h), *Staphylococcus* spp. (Chapman agar, 37°C, 48 h), and *Salmonella* spp. (SS agar, 37°C, 48 h). After incubation, visible colonies were counted and expressed as colony forming units per 100 ml in the case of membrane filtration method and per 1 ml in the case of serial dilutions method (CFU/100 ml and CFU/ml). The potential *Staphylococcus* spp. colonies were verified by Gram staining and microscopic observations.

Statistical analysis was performed using STATISTICA vers. 10 (StatSoft) software, by calculating basic descriptive statistics and a one-way ANOVA to verify the significance of differences in the number of microbial indicators between the sampling sites and study periods.

RESULTS AND DISCUSSION

Good quality water is the core not only of natural ecosystems, but it is also crucial to generate and sustain economic growth and prosperity, through farming, commercial fishing, manufacturing, trans-

doi: 10.17221/205/2014-SWR

port, and tourism. However, the quality of water deteriorates due to pollution coming from agriculture, urban developments, the use of pesticides or fertilizers, mining, forestry, and many other human activities (http://ec.europa.eu/environment/pubs/pdf/factsheets/water-framework-directive.pdf). However, microbiological quality of water is mostly affected by contamination with human or animal faeces that can originate from municipal sewage, animal husbandry, wildlife. As some of those sources are natural or semi-natural and can hardly be avoided, other result from human activity and increase along with population density.

The amount of microbial contaminants entering rivers through municipal sewage that is properly managed and treated with sewerage systems can be easily estimated. On the other hand, the impact of small, sometimes illegal, sewage discharges in rural areas is difficult to be quantified. Also, animal faeces, utilized as natural fertilizers in rural areas, are a very rich source of bacterial contaminants. Those two factors affect primarily small watercourses that subsequently supply larger rivers, thus decreasing their water quality.

All of the examined watercourses, as well as the Upper Vistula, are subject to anthropological pressure, mostly due to agriculture, domestic activities or industry (Chełmicki & Siwek 2001; Miernik & Wałęga 2008). All these activities may contribute to the permanent or temporary increase in the water contamination measured by the number of microbiological indicators. Among those contaminants, faecal bacteria pose the greatest potential risk. The number of coliforms and faecal coliforms is considered to be the most important water quality indicator (Guentzel 1996). The quality of examined waters varied greatly in terms of the number of those bacteria, both when comparing individual samples and the dates of collection. For example, the smallest number of coliforms and faecal coliforms in the Prądnik, i.e. 340 CFU/100 ml and 100 CFU/100 ml, respectively, was recorded in December. On the other hand, the greatest number of coliforms in the Prądnik, i.e. 4000 CFU/100 ml, was recorded in September, while the greatest number of faecal coliforms (1220 CFU/100 ml) – in May (Table 1). The mean number of coliforms ranged from 13 CFU/100 ml in the Raba to 1620 CFU/100 ml in the Prądnik, while the mean number of faecal coliforms ranged from 1 (Dunajec) to 650 CFU/100 ml (Prądnik, Figure 2). Analysis of variance showed that both the differences in the numbers of coliforms and faecal coliforms between the study sites were statistically significant ($P < 0.05$; $F = 3.22$ and 3.80, respectively). The group of coliforms consists of a large *Enterobacteriaceae* family, including *E. coli*, as well as *Klebsiella*, *Enterobacter* or *Citrobacter* (Guentzel 1996). This is particularly important because the presence of coliforms and faecal coliforms may indicate the presence of enteric pathogens in aquatic systems (Rompré *et al.* 2002). On the other hand, the use of coliform group as an indicator of pathogen presence has been a subject of debate for many years, as some authors reported waterborne disease outbreaks in water meeting the coliform regulations (Gofti *et al*. 1999). Nevertheless, in our study, the presence of *Salmonella* spp. was accompanied by the detection of coliforms and/or faecal coliforms (Table 1). This result indicates clearly the faecal contamination of the examined water samples.

According to Levantesi *et al*. (2012), *Salmonella* spp. is frequently detected in environmental samples and it has been repeatedly reported in various types of natural waters such as rivers, lakes, etc. In their summary of environmental analyses carried out in several countries, the authors indicated that the incidence of those bacteria can be extremely variable with detection rates ranging from 3 to 100%, while the highest frequencies were reported in watersheds highly impacted by human activities. In the present study, the presence of *Salmonella* spp. was detected in 62.5% of the conducted water examinations. Only in the Dunajec River those bacteria were not detected in all sampling periods. The mean numbers of *Salmonella* spp. ranged from 0 (Drwinka and Dunajec) to the maximum of 120 CFU/ml in the Kisielina (Figure 2). Statistical analysis indicated that the differences in the number of these bacteria among study sites were significant with $P < 0.05$ ($F = 2.93$). All serovars of *Salmonella* are considered to be potentially pathogenic to human (Chiu *et al*. 2004) and can be the cause of typhoid (*Salmonella* Typhi), paratyphoid (*Salmonella* Paratyphi) fever, infectious food poisoning (so-called salmonelloses), sepsis, enteric fever syndrome, as well as urinary tract, respiratory system and skin infections or organ abscesses (Levison 2008).

Faecal streptococci are another indicator of sanitary contamination of water. Similarly to coliforms, they are constantly present in human and animal faeces, but with one difference – they are much more abundant in animal than in human faeces. Also, similarly to coliforms, *E. faecalis* do not proliferate in water environment, therefore they indicate fresh faecal contamination of water (Biziuk & Michalska 2012).

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Table 1. Incidence of the selected microbial groups in surface waters of the examined watercourses (CFU/cm^3: mesophilic bacteria, psychrophilic bacteria, *Salmonella* spp., *Staphylococcus* spp.; CFU/cm^3: coliforms, faecal coliforms, sulphate reducing clostridia) and standard deviation (SD) of the results

Sampling period	Sampling site	Mesophilic bacteria	Psychrophilic bacteria	*Salmonella*	Coliforms	Faecal coliforms	*Staphylococcus*	Sulphate reducing clostridia
May 2012	Prądnik	1790	20 100	74	952	1220	2	20
September 2012		765	600	101	4000	320	56	41
December 2012		685	380	62	340	100	0	16
March 2013		1100	1580	98	1200	980	0	29
SD		501.8	9641.5	18.9	1625.4	529.3	27.7	11.1
May 2012	Dłubnia	826	18 000	0	1080	972	0	0
September 2012		160	100	60	700	180	81	42
December 2012		138	85	48	670	90	0	21
March 2013		530	1420	54	550	340	59	23
SD		328.6	8760.4	27.4	229.3	398.0	41.4	17.2
May 2012	Drwina Długa	440	1800	2	388	368	2	24
September 2012		145	0	246	655	80	17	36
December 2012		110	320	0	214	55	1	2
March 2013		225	1150	124	385	245	72	24
SD		148.0	816.4	117.3	182.2	147.2	33.5	14.2
May 2012	Drwinka	130	916	0	208	56	8	3
September 2012		75	100	1	0	10	0	164
December 2012		65	0	0	115	5	3	34
March 2013		110	815	0	0	35	12	121
SD		30.3	474.4	0.5	100.7	23.6	5.3	74.8
May 2012	Raba	165	1040	0	22	0	3	2
September 2012		30	0	10	0	30	11	24
December 2012		18	830	0	0	25	1	18
March 2013		75	735	8	30	45	2	13
SD		66.7	451.3	5.3	15.4	18.7	4.6	9.3
May 2012	Uszewka	806	22 100	0	294	188	0	30
September 2012		65	0	40	1200	40	10	14
December 2012		45	1250	22	190	30	0	7
March 2013		95	830	0	1250	125	30	10
SD		369.4	10 708.9	19.3	569.5	74.8	14.1	10.2
May 2012	Szreniawa	1080	2050	5	532	34	0	30
September 2012		790	1200	60	1000	80	72	83
December 2012		560	760	0	415	15	0	21
March 2013		890	1840	46	890	15	66	62
SD		215.3	592.3	29.8	280.0	30.7	39.9	28.7
May 2012	Uszwica	1250	7120	53	76	3	0	90
September 2012		165	400	30	295	180	18	51
December 2012		180	1150	21	55	20	0	35
March 2013		970	534	28	185	65	11	43
SD		552.5	3229.7	13.9	110.6	79.7	8.8	24.4
May 2012	Kisielina	524	828	204	70	3	0	27
September 2012		0	100	0	130	40	8	27
December 2012		310	200	98	45	25	0	20
March 2013		280	380	180	95	55	0	18
SD		215.1	322.2	92.3	36.3	22.2	4.0	4.7
May 2012	Dunajec	1450	34 100	0	206	4	0	5
September 2012		10	0	0	0	0	0	20
December 2012		615	0	0	20	0	0	13
March 2013		1020	0	0	110	0	0	5
SD		612.8	17 028.5	0.0	94.4	2.0	0.0	7.2

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In the present analysis, faecal streptococci were not detected in neither of the examined water samples. This suggests that the detected contamination of water samples was more likely a result of human faeces discharge than of animal faeces contamination.

The estimation of bacteriological water quality based on classical sanitary indicators may not reflect its safety for the health of bathing people. Numerous human diseases related to water use can be associated with the presence of opportunistic pathogens, belonging to the *Clostridium*, *Staphylococcus* and other genera being able to cause infections by contact with skin, mucous membrane, eyes, ears or through urogenital passage (Niewolak & Opieka 2000). Therefore, although they are not frequently studied, anaerobic sulphate reducing clostridia can also be considered as indicators of faecal contamination of water and may indicate the presence of potentially opportunistic bacterial species. They are less frequent in faeces than the previously discussed groups, but they produce spores allowing them to survive unfavourable conditions, therefore their presence may indicate remote in time faecal contamination (Tyagi *et al*. 2006). Medema *et al*. (1997) reported that sulphate-reducing clostridia were able to survive for several months in surface water, where a ten times higher decay rate was detected for *E. coli* and faecal enterococci. Sulphate-reducing clostridia were detected in all examined water samples, except for one negative measurement in the case of the Dłubnia in May (Table 1), with mean numbers ranging from 10 in the Dunajec to 80 CFU/100 ml in the Drwinka (Figure 2). The differences in the numbers of sulphate-reducing bacteria between the study sites were also statistically significant ($P < 0.05$, $F = 2.55$).

Some species of the genus *Staphylococcus* can cause skin, nasopharyngeal cavity, eyes or outer ear infections in bathing people (Niewolak & Opieka 2000). Those bacteria survive in water longer than classical indicators of sanitary condition and they are not related to faecal contamination of water (Niewolak & Opieka 2000). Variation in the number of *Staphylococcus* spp. was observed by Niewolak and Opieka (2000) in the Czarna Hańcza River, as they recorded numbers ranging from 0 to even 128 000 CFU/100 ml with mean numbers ranging from 760 to 29 700 CFU/100 ml. In our study, the numbers of *Staphylococcus* spp. fluctuated between 0 and the maximum of 81 CFU/ml. These bacteria were not detected in the waters of the Dunajec River and were most abundant in the Dłubnia and Szreniawa (in both rivers mean number of *Staphylococcus* spp. was 35 CFU/ml). Nevertheless, the differences in the number of these bacteria between the examined water samples were statistically not significant ($P < 0.05$; $F = 1.21$). Even though they were less frequently observed than the remaining indicators, still their presence was detected in 55% of the conducted measurements (22 out of 40). The frequency of detecting *Staphylococcus* spp. in water samples, as well as illnesses of bathing people depending on the number of those bacteria (Niewolak & Opieka 2000) suggest the necessity of monitoring these pathogens in surface water.

The enrichment of water with organic substances is reflected in the development of heterotrophic bacteria and their proportion in individual physiological groups.

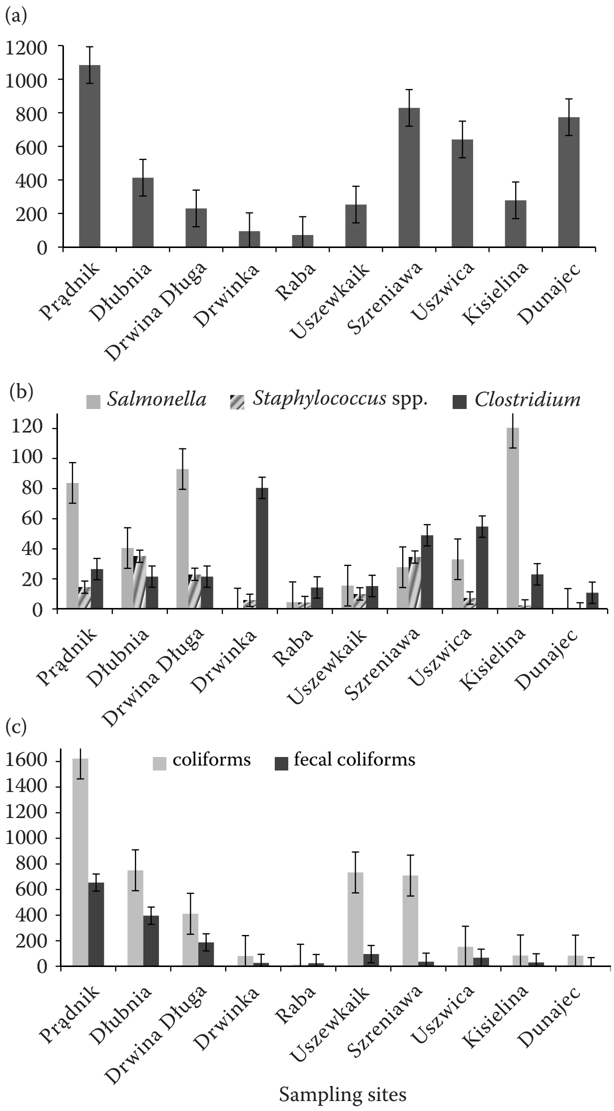

Figure 2. Mean numbers and standard errors of the analyzed microbial groups; A – mesophilic bacteria (CFU/ml); B – *Salmonella*, *Staphylococcus* spp. (CFU/ml), and sulphate reducing clostridia (CFU/100 ml); C – coliforms and faecal coliforms (CFU/100 ml)

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Therefore, the determination of the number of heterotrophic bacteria provides a basic piece of information on water quality (Małecka & Donderski 2006). The number of mesophilic bacteria in our study ranged from 0 (Kisielina, September 2012) to 1790 CFU/ml (Prądnik, May 2012) (Table 1) with mean values ranging from 72 (Raba) to 1080 CFU/ml (Prądnik) (Figure 2). The presented differences were statistically significant ($P < 0.05$; $F = 3.67$). On the other hand, the number of psychrophilic bacteria ranged from 0 to over 34 000 CFU/ml (Dunajec, May 2012), with mean values ranging from 380 (Kisielina) to 8510 (Dunajec), however the differences were statistically not significant ($P < 0.05$, $F = 0.58$). Similarly to the studies conducted by Donderski and Wilk (2002) on the river Vistula, it is evident that psychrophilic bacteria dominate over mesophilic bacteria in most of the examined watercourses. From a health point of view, it is more dangerous if the number of mesophilic bacteria is greater, as they may contain pathogenic microorganisms, while in most cases the group of psychrophilic bacteria consists of non-pathogenic microbes (Donderski & Wilk 2002). However, according to Tyski and Krogulska (1999), Gram-negative aquatic bacteria produce lipopolysaccharides of the cell wall that may have toxic effect. Therefore, monitoring the number of both microbial groups seems to be justified.

Hydrometeorological conditions prevailing in river catchments, such as air temperature, amount of precipitation or water level, have direct effect on the quality of river water (WIOŚ 2012). This was also recorded in the present study (Table 1). The numbers of the majority of the examined microbial indicators of water quality were most abundant in spring and summer months which were abnormally or extremely warm in 2012 with the amount of precipitation significantly lower than the monthly average in a multiannual period. On the other hand, winter months of 2012 were classified as extremely cold (WIOŚ 2012). The statistical analysis showed that the seasonal differences in the numbers of mesophilic and psychrophilic bacteria, as well as of *Staphylococcus* spp. were statistically significant ($P < 0.05$, $F = 5.15$, 7.72, and 4.74, respectively).

Numerous studies conducted within the catchment of the Vistula River indicate the impact of anthropogenic pressure on the water quality, particularly in the area of the Upper Vistula (Buszewski *et al.* 2005). Various analyses indicate also that the quality of water in the Vistula River in the vicinity of Kraków is below standards (Buszewski *et al.* 2005; WIOŚ 2012), especially due to the intense industrial concentration. According to the Regulation of Minister of Environment from February 11th 2004 (Journal of Laws of the Republic of Poland 2004, repealed), in terms of the coliform and faecal coliform numbers, the majority of the examined watercourses would fall into classes of very good and good quality waters. This means that the quality of water recorded in the examined Vistula tributaries is better than in the Vistula itself (WIOŚ 2012). In general, the Prądnik was the most microbiologically contaminated river with the greatest mean numbers of mesophilic bacteria, coliforms and faecal coliforms. This is not unexpected, as among the analyzed rivers, the Prądnik passes through the most densely populated areas around Kraków. On the other hand, the Dunajec, as the mountain river passing through sparsely populated areas, turned out to be characterized by the best sanitary condition with the lowest recorded numbers of four out of the examined microbial indicators.

CONCLUSIONS

The Dunajec was characterized by the highest microbiological quality among the analyzed watercourses, while the Prądnik was found to be the most contaminated river, showing that population density is one of the most important factors affecting microbiological water quality of small watercourses.

The incidence of the examined microbial groups also appeared to be affected by varying environmental conditions resulting from the seasonal changes.

Quality of water in the Vistula River can be affected by its inflows, however the majority of the examined watercourses that are located in the catchment of the Upper Vistula would not deteriorate its quality, as their waters proved to be less microbiologically contaminated than the Vistula itself (WIOŚ 2012).

The present study has only indicated the significance of the problem, but to take measures aimed at improving the quality of water in the Vistula River through the improvement of the sanitary condition of its tributaries, a more precise research including the size of watercourses and their average water flows would be necessary.

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Received for publication September 10, 2014
Accepted after corrections November 19, 2014

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