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ORIGINAL ARTICLE

Analysis of faecal bacteria isolated from air and seawater samples following an emergency sewage discharge into the Gulf of Gdansk in 2018 — preliminary study

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ABSTRACT

Background: *Knowing the numbers of bacteria in coastal atmospheric air as well as in coastal waters significantly contributes to a better understanding of the processes affecting the health of people who stay temporarily or permanently in areas where the synergistic effect of the atmospheric conditions and the aquatic environment on a human body is particularly strong.*

Materials and methods: *Seawater and air samples were collected from 22 May to 22 July 2018 in the seaside towns of Hel, Puck, Gdynia, Sopot, Gdansk-Brzezno, all located along the Gulf of Gdansk. The number of psychrophilic, mesophilic as well as coliform bacteria and* Escherichia coli *was determined in both the water and the ambient air samples. In total, 232 seawater and coastal air samples were collected for the study purposes.*

Results: *The study showed a deterioration of coastal waters and atmospheric air in the Gulf of Gdansk which may have resulted from an increase of potentially pathogenic mesophilic bacteria following the emergency discharge of raw sewage from the Gdansk-Wschod wastewater plant.*

Conclusions: *An increase in the number of coliform bacteria and* Escherichia coli *in the seawater and in the air across the Gulf of Gdansk is related to the emergency sewage discharge.*

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Key words: coastal air, seawater, bacteria, the Gulf of Gdansk

INTRODUCTION

The Baltic coast is one of the most popular tourist destinations in Poland. It is of key importance that coastal waters near popular seaside resorts are free from physical and microbiological contamination [1, 2]. Unfortunately, it is often the case that both treated and raw sewage from seaside towns is discharged into the sea [3, 4]. Moreover, the outflow of rainwater and floods also contributes to the biological as well as chemical contamination of the coastal seawaters [5–7]. An example of this type of event was a serious malfunction at the Mishref Pumping Station, which resulted in sewage discharge and an increase in the number of coliform bacteria, *Escherichia coli* and faecal streptococci in the waters of the Gulf of Kuwait [8]. Potentially pathogenic bacteria, mould fungi and enteroviruses are a significant factor influencing bathing water quality. In the light of the most recent studies, contamination of the seawaters with potentially pathogenic coliform bacteria (*Citrobacter*, *Klebsiella*, *Proteus*, *Enterobacter*, *Escherichia coli*) or faecal streptococci (Enterococci) poses a serious health hazard for humans. It has been indicated that these microorganisms may cause gastrointestinal disturbances, airways disorders and skin allergies [9–13]. Since microorganisms which are present in the seawater may be easily transferred into the atmospheric air, it is important to monitor the sanitary and epidemiological conditions of the seawater and coastal air [6, 14]. Thus, the supervision over the disposal of treated sewage into the Gulf of Gdansk (among others from the two largest sewage treatment plants in Poland) has become a necessity.

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As an example, the emergency discharge of raw sewage into the Motlawa river flowing into the Martwa Wisla river between 15 and 18 May 2018 caused a rapid deterioration of sanitary conditions in Gdansk. Therefore, the aim of the study was to determine the level of faecal bacteria in the waters surrounding the Gulf of Gdansk and the air over the Gulf following the emergency discharge of raw sewage from the Gdansk-Wschod Sewage Treatment Plant.

MATERIALS AND METHODS

MATERIAL SAMPLING

Samples of the seawater and the air from the seaside towns lying along the Gulf of Gdansk: Hel, Puck, Gdynia, Sopot and Gdansk-Brzezno were collected between 22 May and 22 July 2018. The sea surface microlayer of ≤ 100 µm was sampled using the glass plate method [15, 16]. In total, 116 samples of seawater were collected from the waters surrounding the Gulf of Gdansk.

Air samples were collected 50 cm above the water surface and at a 1 m distance from the shoreline in the direction of the Gulf of Gdansk. Air samples were collected for 10 min with an Air Sampler SAS Super 100 (Turin, Italy) which utilizes the impaction method. In total, 116 air samples were collected for the study purposes.

SAMPLE ANALYSIS

Microbiological analysis of seawater

The membrane filtration method [17] was used to examine the water samples. Seawater samples of 100 mL were passed through sterile filters (0.45 pore diameter) and placed on selective agar mediums.

The number of coliform bacteria of the Enterobacteriaceae family, including *Citrobacter*, *Klebsiella*, *Proteus*, *Enterobacter* and *Escherichia coli*, was estimated on Merck Chromocult coliform agar (Germany) after 24 h incubation at 37°C. The colony-forming unit (CFU/m^3) was used to determine the number of bacteria in seawater samples of 100 mL.

The overall number of psychrophilic and mesophilic bacteria was determined after the incubation of 1 mL seawater samples on tryptic soy agar from Merck (Germany). The results were read after 72 h and 48 h incubation at 22°C and 37°C. The colony-forming unit (CFU/m^3) was used to determine the number of bacteria in seawater samples of 1 mL.

Gram-stained preparations were made. Aminopeptidase, catalase tests were performed and the glucose fermentation activity of the examined bacteria under anaerobic conditions was measured. In addition, *Staphylococcus aureus* was differentiated from *Staphyloccocus epidermis* (*albus*) saprophytic isolates using the rabbit plasma test. *Pseudomonas aeruginosa* bacteria were determined on Merck Cetrimide Agar (Germany) after 24 h incubation at 37°C.

Microbiological analysis of bioaerosols

The number of psychrophilic and mesophilic bacteria, coliform bacteria and *Escherichia coli* in the air sampled at a distance of 1 m from the shoreline in the direction of the Gulf of Gdansk was determined as outlined in detail by Michalska et al. [18].

The colony-forming unit (CFU/m^3) was used to determine the number of bacteria in the air samples. Feller's measurement table, attached to the air sampler manual, was used for the enumeration of microorganisms in the air samples [18, 19].

Meteorological conditions

During air sampling, between 22 May and 22 July 2018, air temperature and humidity, as well as the speed and the direction of wind, were recorded with a GMH 3330 thermo-hygrometer (Greisinger, Germany). The air temperature ranged between 15°C and 27°C. The relative humidity ranged from 39% to 70%, and the wind speed was from 0 km/h to 32 km/h. Air samples were not collected during a rainfall event.

STATISTICAL ANALYSIS

The minimum and maximum mean, as well as the standard deviation of the values measured over the study period, were calculated for the overall numbers of psychrophilic, mesophilic, coliform bacteria and *Escherichia coli*. Statistical analysis was performed with Statistica 12.0 (StatSoft Inc.).

RESULTS

DETECTION AND ENUMERATION OF BACTERIA IN THE COASTAL WATERS IN SEASIDE TOWNS IN THE GULF OF GDANSK

In 2018, the mean number of psychrophilic bacteria isolated from the waters in the seaside town of Hel was 334.000 ± ± 311.500 CFU/1 mL. In another seaside town, Puck, the mean number of psychrophilic bacteria was 5.433 ± 862 CFU/ /1 mL. In Gdynia, the mean number of psychrophilic bacteria was 15.084 ± 1.326 CFU/1 mL, whereas in another seaside city, Sopot, 11.228 ± 6.621 CFU/1 mL. By contrast, the mean number of psychrophilic bacteria in the coastal waters near the seaside town of Gdansk-Brzezno was higher than the number of the psychrophilic bacteria isolated from samples collected in Sopot and Gdynia, and equal to 24.114 ± 47.309 CFU/1 mL.

The mean number of mesophilic bacteria in the waters near Hel was 311.500 ± 350.681 CFU/1 mL. In the seaside town of Puck, the mean number of mesophilic bacteria was 3.967 ± 611 CFU/1 mL. By contrast, the mean number of mesophilic bacteria in Gdynia was 7.008 ± 4.195 CFU/1 mL, and in Sopot it was 9.146 ± 5.606 CFU/1 mL. In Gdansk--Brzezno the mean number of mesophilic bacteria was

Table 1. Minimum and maximum number of bacteria detected in the atmospheric air over the Gulf of Gdansk in 2018

	The number of psychrophilic bacteria [CFU/m^3]		The number of mesophilic bacteria [CFU/m^3]		The number of coliform bacteria [CFU/m^3]		The number of *Escherichia coli* [CFU/m^3]		The number of *Psudomonas aeruginosa* [CFU/m^3]		The number of *Staphylococcus aureus* [CFU/m^3]	
	Min	**Max**	**Min**	**Max**	**Min**	**Max**	**Min**	**Max**	**Min**	**Max**	**Min**	**Max**
Hel	890	3543	866	3150	0	551	0	8	0	95	0	16
Puck	803	1181	370	724	32	47	0	8	0	0	0	0
Gdynia	650	984	540	870	10	32	0	0	0	0	0	0
Sopot	32	3386	27	3307	0	7	0	6	0	167	0	19
Gdansk-Brzezno	55	5774	16	4856	0	149	0	95	0	236	0	0

higher in comparison to the number of mesophilic bacteria in Sopot and was equal to 15.850 ± 24.230 CFU/1 mL.

At a further stage of the study, it was attempted to detect potentially pathogenic bacteria, including coliform bacteria, *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* in the seawaters of the Gulf of Gdansk. In the studies carried out in 2018, the mean number of coliform bacteria in the seaside town of Hel was 2.805 ± ± 2.800 CFU/100 mL. The mean number of coliform bacteria in Puck was 1.157 ± 169 CFU/100 mL and in Gdynia it was 2.500 ± 381 CFU/100 mL. The mean number of coliform bacteria isolated from samples collected in Sopot was significantly higher when compared to the samples from Gdansk-Brzezno, 32.803 ± 80.058 CFU/100 mL and 5.439 ± 7.452 CFU/100 mL, respectively.

The mean number of *Escherichia coli* in the waters surrounding the town of Hel was 206 ± 396 CFU/100 mL, whereas in Puck this number was 223 ± 12 CFU/100 mL. In Gdynia, the mean number of *Escherichia coli* detected in the waters of the Gulf of Gdansk was 10 ± 7 CFU/100 mL. On the other hand, the mean number of *Escherichia coli* seen in Gdansk-Brzezno was slightly higher than in Sopot (211 ± ± 228 CFU/100 mL vs. 139 ± 179 CFU/100 mL, respectively).

DETECTION AND ENUMERATION OF BACTERIA PRESENT IN THE AIR OF THE SEASIDE TOWNS IN THE GULF OF GDANSK

The studies carried out in 2018 demonstrated that the mean number of psychrophilic bacteria detected in Hel was 1.645 ± 1.273 CFU/m^3. The number of psychrophilic bacteria in Puck was 1.021 ± 195 CFU/m^3. In Gdynia, the number of psychrophilic bacteria was 811 ± 165 CFU/m^3, whereas in Sopot it was 506 ± 1.165 CFU/m^3. The number of psychrophilic bacteria in air samples from Gdansk-Brzezno was significantly higher (2.287 ± 2.677 CFU/m^3) in comparison to the number of psychrophilic bacteria isolated from samples collected in the above mentioned coastal towns.

According to the study findings, the mean number of mesophilic bacteria in samples from the town of Hel was 1.510 ± 1.101 CFU/m^3. The number of mesophilic bacteria in the air of the Gulf of Gdansk in Puck was 588 ± 191 CFU/m^3. The number of mesophilic bacteria in Gdynia was 702 ± ± 163 CFU/m^3, while in Sopot it was 486 ± 1.141 CFU/m^3. The number of mesophilic bacteria was significantly higher in samples collected in Gdansk-Brzezno (1.832 ± 2.104 CFU/m^3), in comparison to Puck, Gdynia and Sopot.

The mean number of coliform bacteria isolated from samples collected in Hel was 198 ± 261 CFU/m^3. The number of coliform bacteria in the air in the seaside town of Puck was 39 ± 8 CFU/m^3. In Gdynia, the number of coliform bacteria (20 ± 10 CFU/m^3), was higher in comparison to the number of coliform bacteria detected in Sopot. In Sopot, the number of coliform bacteria was 3 ± 2 CFU/m^3. A distinctly higher number of coliform bacteria was detected in Gdansk-Brzezno — 67 ± 68 CFU/m^3.

In 2018, the number of *Escherichia coli* strains isolated from samples collected in Hel was 2 ± 4 CFU/m^3. In Puck the number of *Escherichia coli* in the air was 3 ± 5 CFU/m^3. No *Escherichia coli* strains were detected in the air of the seaside city of Gdynia. The number of *Escherichia coli* in the air in Sopot was 1 ± 2 CFU/m^3. In 2018, a higher mean number of *Escherichia coli* was detected in Gdansk-Brzezno — 18 ± 31 CFU/m^3.

The minimum and maximum numbers of the bacteria detected in the atmospheric air across the Gulf of Gdansk within the study period are presented in Table 1.

IDENTIFICATION OF THE BACTERIA ISOLATED FROM AIR SAMPLES

Gram-positive cocci were detected in the air samples collected in the seaside towns of Hel, Puck, Gdynia, Sopot and Gdansk-Brzezno (79.96%), including *Micrococcus sp.* (33.27%), *Sarcina lutea* (46.55%) and *Staphylococcus aureus* (0.14%). Gram-positive bacteria of the *Bacillus* genus (*Bacillus sp.* 12.86%) as well as Gram-negative bacilli (7.19%), including *Pseudomonas aeruginosa* (1.35%), *Escherichia coli* (0.36%) and other bacilli of the *Enterobacteriaceae* family (5.49%) were also isolated (Fig. 1).

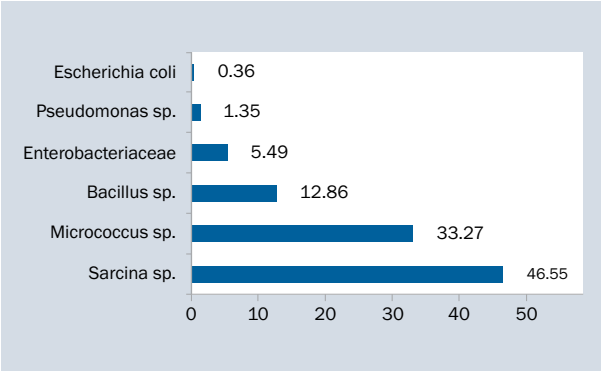

Figure 1. Percentage distribution of the bacteria isolated from the air samples collected in the Gulf of Gdansk in 2018

THE INFLUENCE OF METEOROLOGICAL PARAMETERS ON THE NUMBER OF BACTERIA IN THE AIR IN THE SEASIDE TOWNS LOCATED ALONG THE GULF OF GDANSK

At a later stage of the study, an analysis was carried out on the influence of the meteorological parameters such as relative humidity, air temperature, speed and direction of wind on the number of psychrophilic, mesophilic, coliform bacteria and *Escherichia coli* in the atmospheric air at the Gulf of Gdansk.

The maximum number of psychrophilic, mesophilic, coliform bacteria and *Escherichia coli* in the atmospheric air was recorded when the air temperature ranged between 19.5°C and 25°C, and the relative humidity was between 53% and 65%. The number of psychrophilic, mesophilic, coliform bacteria and Escherichia coli in the air was also significantly higher when the wind speed exceeded 32 km/h and the direction of the wind was north or northeast (N, NE), i.e. when the air blown towards the shoreline had been in contact with the water surface for an extended period of time.

DISCUSSION

The study carried out in 2018 demonstrated a higher number of psychrophilic, mesophilic, coliform bacteria and *Escherichia coli* both in the coastal waters and in the atmospheric air of the seaside towns and cities (Hel, Puck, Gdynia, Sopot, and Gdansk-Brzezno) located at the Gulf of Gdansk, in comparison to previous studies (the studies carried out at the seaside locations between 1998 and 2005 showed a lower number of psychrophilic and mesophilic bacteria). For instance, in Gdansk-Brzezno the maximum number of mesophilic bacteria in the coastal air was 204 CFU/m^3 (period 1998–2005), whereas in 2018 the number reached 4856 CFU/m^3. In Sopot, the number of the isolated mesophilic bacteria was 550 CFU/m^3 vs. 3307 CFU/m^3 in 2018 (Table 1) [20, 21]. A study by Kruczalak et al. [22] conducted in 1998 also demonstrated a lower mean number of psychrophilic bacteria — 50 CFU/m^3, and mesophilic bacteria — 15 CFU/m^3.

A hypothesis was put forward that the results of the present study demonstrating a higher number of psychrophilic, mesophilic, coliform bacteria and *Escherichia coli* in the seawater and the atmospheric air could be the consequence of a sewage discharge into the Motlawa River. In May 2018, there was an emergency discharge of raw sewage into the Motlawa River. As a result, 2,300 m^3 of sewage per hour was flowing into the Gulf of Gdansk. Similarly, due to a malfunction at the Mishref Pumping Station, massive quantities of raw sewage was being discharged directly into the gulf for the period of 3 years (2009–2012); this-led to bacteriological contamination of a coastal section of about 20 km [8]. Other studies, however, suggest that it is heavy rainfall or floods which contribute to a higher number of faecal bacteria in coastal seawaters. As an example, heavy rain (precipitation rate of 2.5 to 7 cm) caused the contamination of the coastal seawaters between California and Mexico with faecal bacteria [5]. At a further stage of the study it was attempted to assess the influence of meteorological conditions on the presence of bacteria in the air of the seaside towns of Hel, Puck, and Gdansk-Brzezno as well as the seaside cities of Gdynia and Sopot, all lying along the Gulf of Gdansk. The maximum number of bacteria was detected at the air temperature between 19.5°C and 25°C and the relative humidity between 53% and 65%. The speed and direction of wind increased the number of psychrophilic, mesophilic, the coliform bacteria and *Escherichia coli*. The present study also demonstrated that the number of the above mentioned bacteria isolated from air samples was higher when the wind was blowing from the north and northeast (towards the land). The results of previous studies also indicated a statistically significant correlation between the speed and direction of wind and the number of psychrophilic and mesophilic bacteria in the coastal air over the Gulf of Gdansk [20, 21, 23]. It has also been confirmed that bacteria present in the seawater are capable of transferring themselves to the air [20, 21, 23]. Our studies indicated that the direction and speed of wind affect the composition of coastal aerosol, and consequently change the atmospheric conditions for people who sunbathe, swim in the sea or do water sports. This is caused by the fact that apart from the ions of sea salts and iodine, the sea aerosols contain microorganisms such as cyanobacteria, diatoms, bacteria, fungal spores and products of their metabolism [24–26]. The results of previous studies indicate that the presence of bacteria and their endotoxins in the air may contribute to the development of many diseases [10, 18, 24, 27–30].

CONCLUSIONS

In 2018, significantly higher concentrations of psychrophilic, mesophilic, coliform bacteria and *Escherichia coli* were observed in the seawater and air of the Gulf of Gdansk. It is important that the authorities and enterprises

responsible for the sanitary and epidemiological supervision of coastal waters warn people of seawater contamination as promptly as possible. At the same time, they should impose restrictions on the use of bathing areas in case of any malfunctions at the sewage treatment plants, sudden downfalls of rain or floods.

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