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Microbiological Contamination of Brine Feeding a Closed-Cycle Graduation Tower and Its Potential Human Exposure

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Abstract: Given the growing interest among residents of large agglomerations in inhalation treatments in urban graduation towers, as well as the lack of research on the microbiological safety of brine, an attempt was made to provide new and unique information on the potential epidemiological risks that may occur in such places. The study covered one of Krakow's brine graduation towers, opened in 2020, attracting crowds of city residents and tourists every spring/summer season. Based on a two-year microbiological study of brine, which included analysis of the presence of mesophilic and psychrophilic bacteria in it, as well as indicators of microbiological water quality, i.e., *Escherichia coli*, *Enterococcus faecalis*, *Clostridium perfringens*, *Staphylococcus* spp., and *Salmonella* spp., it was concluded that contamination increased periodically. According to standards for inhalation waters, acceptable counts of selected microorganisms were exceeded, especially during the holiday season. It was, undoubtedly, related to weather conditions conducive to outdoor inhalation treatments, and thus the large number of heath resort visitors present near the graduation tower. Despite the fact that our study provides epidemiologically disturbing results, it is extremely valuable because it constitutes a starting point for discussion of the health safety of urban graduation towers. The study is also an opportunity to take measures to improve the microbiological quality of the brine in the closed cycle of graduation towers.

Keywords: closed-cycle graduation tower; indicator microorganisms; microbiological quality of brine

Citation: Bodziacki, S.; Wolny-Koładka, K. Microbiological Contamination of Brine Feeding a Closed-Cycle Graduation Tower and Its Potential Human Exposure. *Processes* **2023**, *11*, 966. https://doi.org/10.3390/pr11030966

Academic Editor: Elisa Gamalero

Received: 2 March 2023
Revised: 19 March 2023
Accepted: 20 March 2023
Published: 22 March 2023

1. Introduction

A brine graduation tower, also known as a thorn house, is a structure built of wooden logs stuffed with blackthorn branches (Figure 1) and equipped with an integrated brine distribution system (valves, taps, pipelines, and pumps). The graduation tower serves as an open-air inhalation room [1]. The graduation tower must be built on a suitable foundation and solid wooden frames, usually made of oak or spruce. Optionally, a roof is added to protect against nesting by birds. Facilities of this type are usually large in size, such as Poland's most famous graduation tower in the health resort of Ciechocinek (16 m high, total length of 1741 m), while there are also much smaller structures, the so-called urban graduation towers with an average height of >3 m and a length of >2 m. Large structures are built in health resorts where a continuous supply of fresh brine can be ensured, e.g., Ciechocinek, Inowroclaw, Konstancin-Jeziorna, and Goldap. The key feature necessary for the proper functioning of the graduation tower is its height, which provides a long enough path for the salt droplets falling by gravity into the gutter. The aim of the entire process is to obtain a minimum 16% NaCl solution in water (max. approx. 27%). The concentration process is strongly dependent on weather conditions. A sunny and windy day favours the evaporation of brine and the formation of a therapeutic bioaerosol, while rainy and foggy days are not conductive to this microclimate [2].

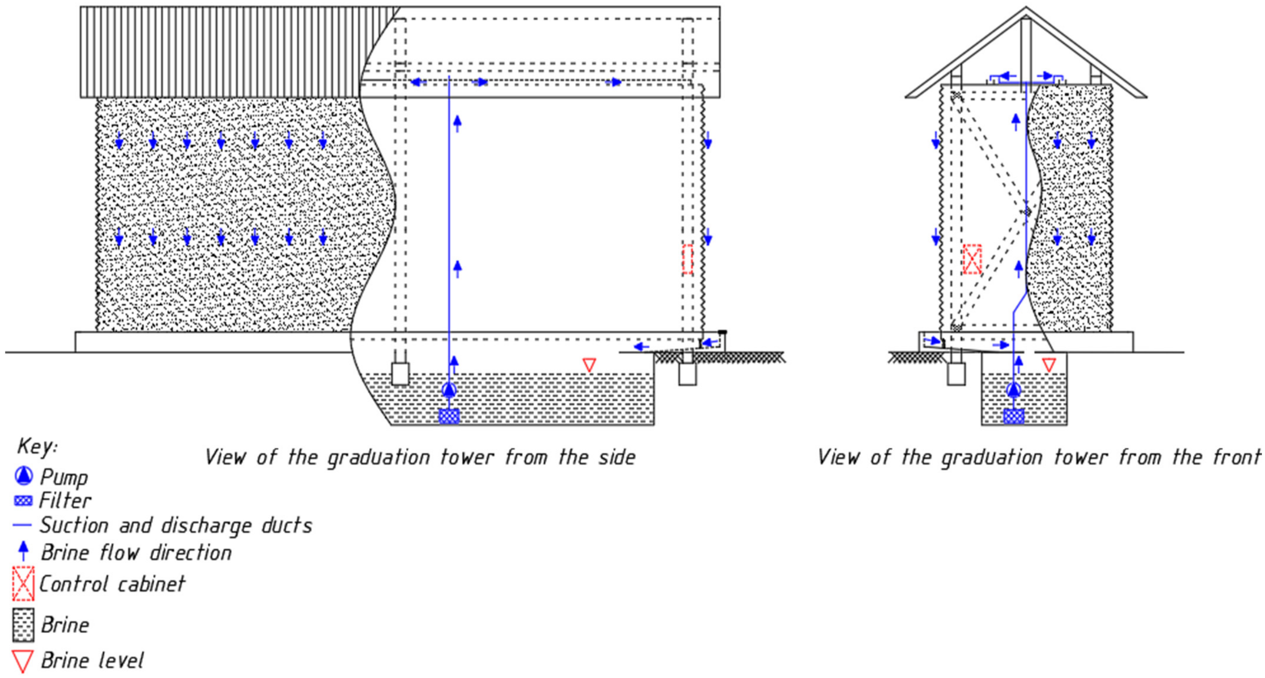

Figure 1. Schematic of a closed-cycle graduation tower structure. Source: Own materials.

Graduation towers can be divided according to the water cycle used: open or closed. The first-mentioned cycle is used in graduation towers located at the brine extraction sources in health resorts (new brine is supplied on an ongoing basis). Closed-cycle graduation towers, on the other hand, are found only in towns with no brine intakes. Closed-cycle graduation towers are small in size; usually, their height does not exceed 4 m, length 8 m, and width 2 m. They are surrounded by benches for people using inhalation treatments in the immediate vicinity of the tower. Urban graduation towers are fed with suitably prepared brine (the same as in brine spas), meeting both sanitary and water quality requirements. Brine is stored in specially designed and adapted reservoirs with a capacity of several to tens of thousands of litres, located in graduation towers [3].

The principle of operation of the open-cycle graduation tower is that the brine is first pumped from the source to the top of the graduation tower, where it enters special distribution channels, from where it flows down the slightly sloping wooden walls stuffed with blackthorn. The solution flows down by gravity, hits the bundles of brushwood, breaks into smaller droplets, and creates a bioaerosol. The brine that has flowed down is redirected through a system of gutters to the puddle or returns to the cycle and to the top of the graduation tower [4,5]. The operation mechanism of a closed-cycle graduation tower is, in principle, the same as that of an open-cycle graduation tower, except that the brine is drawn not from the springs but from a reservoir at the base of the tower. Falling droplets are collected by gutters at the base of the structure, from where they are directed back to the reservoir and again to the top of the tower by a system of pumps. Brine deficiencies caused by evaporation or droplets falling outside the gutters are replenished with water from the water supply system. Brine concentration is also controlled and, if necessary, modified accordingly [6–9].

The first graduation tower in Krakow (Figure 2) is located in Nowa Huta (city district) in the area of the Nowa Huta Lagoon. In July 2020, the graduation tower was officially opened for public use [7]. The structure is founded on a triangular base with an area of 350 m^2 and has a brine tank with a capacity of 60,000 L. Graduation tower is built with three 18 m arms made of larch wood and stuffed with blackthorn. The brine that feeds the graduation tower is supplied from Zablocie near Pszczyna in Upper Silesia, from a mine that specializes in this type of mixture and has the highest iodine content. The Nowa Huta closed-cycle brine graduation tower is a public facility, open from April to September [6].

The recommended inhalation session in the graduation tower should last about 30 min and not more than 45 min [9]. Brine has an antimicrobial effect, absorbs pollutants, and prevents inflammation in the body. The air around the brine graduation tower is characterized by a high bacteriological quality and low dustiness, thus leisurely strolls, sitting on benches, or lying down in the area around the graduation tower have a therapeutic effect, as the minerals and trace elements contained in the brine are inhaled [10]. The study by Burkowska-But et al. [11] showed that the number of bacteria in the area around the graduation tower in Ciechocinek is four times lower than in the town centre. Those therapeutic properties are recommended for people with cardiovascular and respiratory problems, such as sinusitis, asthma, rhinitis, as well as those struggling with immune system problems and iodine deficiency, dealing with hypertension or thyroid disorders [12]. Brine is also used in the treatment of people with dermatological problems, as it has keratolytic and keratoplastic properties. For example, in the case of psoriasis, it is used in balneotherapeutic treatments as an alternative or adjunctive method of drug treatment [13].

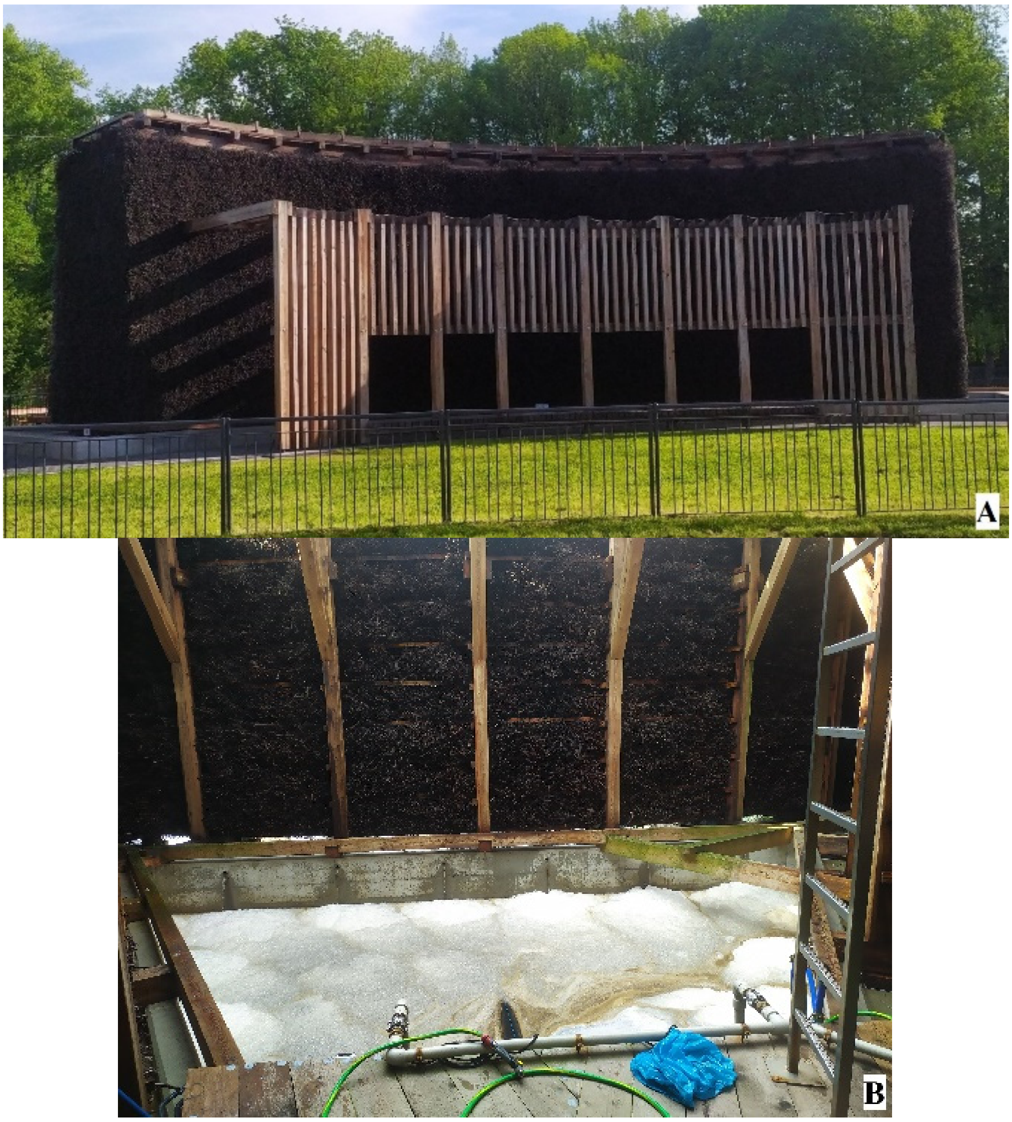

Figure 2. Sampling site ((**A**). graduation tower, (**B**). brine reservoir).

Analysis of the available literature revealed that there have been no studies on the microbiological quality of brine used to feed urban graduation towers to date. The only research papers on graduation towers describe and analyse their structure, functional-technological aspects, and pro-health effects on the human body [3–5,14–18]. However, to the best of our knowledge, there are no studies on microbiological contamination of brine in the context of epidemiological risk. It is, therefore, extremely encouraging to conduct research in this very area, and thus provide new and unique results and information in the field of environmental microbiology.

The growing popularity of brine graduation towers and their potential therapeutic effects contributed to the establishment of such facilities outside of health resorts, mainly in large urban agglomerations. Currently, there are two closed-cycle structures in Krakow, and the first one was built near the lagoon in Nowa Huta (city district). Brine graduation towers attract visitors with their beneficial effects on health, delivering valuable compounds through the skin and respiratory tract. A fact that raises doubts about a graduation tower located in a large urban area is the potential contamination of the brine that feeds it. There are a number of factors that potentially contribute to the contamination of the medicinal water in the reservoir, which translates into the quality of the bioaerosol produced and its health-promoting properties. Those include: prolonged circulation of the same brine in a closed system, dilution by tap water, atmospheric factors, as well as improper use of the facility by visitors, e.g., by washing hands, seeking refreshment on hot days, etc. The high popularity of graduation towers, as evidenced by the number of people visiting them, became a reason to focus on the actual microbiological condition of brine. Therefore, the aim of the study was to determine selected groups of microorganisms in the brine, which are epidemiologically significant and can negatively affect human health. Depending on the obtained results, it was planned to propose appropriate management methods to minimize the risk of microbiological contamination of the brine feeding the graduation tower.

2. Materials and Methods

2.1. Sampling

The study of the microbiological quality of the brine feeding the graduation tower in Nowa Huta, Krakow, Poland (50°04′52.7′′ N 20°03′05.7′′ E) (Figure 2) was carried out for two years in the period of its operation, i.e., from April to September 2021 and 2022. Each year, 6 brine samples were collected (1 per month) to account for seasonality. Brine in the amount of 1000 mL was taken from the supply reservoir of the graduation tower into a sterile container; in addition, one sample of water from the lagoon (control) of the same volume was also collected [19,20]. The temperatures of water taken from the lagoon and brine taken from the reservoir inside the graduation tower were also measured using an electronic thermometer (Biowin). The data related to brine concentration were obtained directly from the Management of the Krakow Municipal Greenspace Authority (ZZM), which monitors this parameter.

2.2. Microbiological Analysis

The filtration method was used to determine: *Escherichia coli* (TBX agar, 44 °C, 24 h, BTL, Poland), *Enterococcus faecalis* (Slanetz-Bartley agar, 37 °C, 48 h, BTL, Poland), and *Clostridium perfringens* (Wilson-Blair agar, 37 °C, 48 h, BTL, Poland). The serial dilution method by Koch was used to determine: mesophilic bacteria (TSA agar, 37 °C, 24 h, BTL, Poland), psychrophilic bacteria (TSA agar, 22 °C, 72 h BTL, Poland), *Staphylococcus* spp. (MSA agar, 37 °C, 24 h, BTL, Poland), and *Salmonella* spp. (SS agar, 37 °C, 24 h, BTL, Poland) [20–24]. After the incubation, the colonies were counted and the analysis results were expressed, depending on the method used, as the number of colony-forming units in 100 mL of brine—by the membrane filtration method (CFU·100 mL^{-1} for *E. coli*, *E. faecalis*, *C. perfringens*) or in 1 mL of brine—by Koch's serial dilution method (CFU·mL^{-1} for mesophilic and psychrophilic bacteria, *Staphylococcus* spp., *Salmonella* spp.).

2.3. Statistical Analysis

The Statistica v. 13.5 software (StatSoft) was used for a two-factor analysis of variance to test how significant were the effects of temperature and brine concentration on the number of tested microorganisms.

3. Results and Discussion

The two-year study of the microbiological quality of brine taken from the Nowa Huta graduation tower revealed the presence of microorganisms in it, which was alarming from the epidemiological point of view. No tested *Salmonella* spp. were found in any of the samples (including the lagoon water, control). Comparing the results of our own tests (Table 1) with the standards contained in the Regulation of the Minister of Health [25] on the requirements to be met by medicinal water used for inhalation, it was concluded that the acceptable bacteria counts were exceeded. The presence of *E. coli*, *E. faecalis*, and *C. perfringens* in water used for inhalation was unacceptable (0 CFU·100 mL^{-1}) [25]. In contrast, in our tests, *E. coli* was present in all brine samples, and *E. faecalis and C. perfringens* were determined in samples collected in June 2021 and 2022 (Table 1). By analysing the microbial contamination of brine over two years (Figure 3), it was found that the number of microorganisms was the highest in June, with the highest average temperature and, most importantly, with the lowest brine concentration. June 2021 was a month in which there were heavy downpours and flooding in Nowa Huta; every few days it rained, flooding the streets and causing measurable economic losses. The graduation tower was also flooded, with a brine concentration of 7% found at that time (Table 1). As can be seen, a radical decrease in brine concentration translated into a rapid increase in the number of microorganisms. On the other hand, it was significant that none of the brine concentrations (15.1–19.1) ensured full elimination of bacteria, including pathogenic ones (*E. coli* was present in all samples). As shown by studies of other authors, salinity of 6% to 10% inhibits the growth of bacteria. Gram-positive bacteria, due to the structure of their cell wall (presence of teichoic acids, high amount of murein), have higher resistances to salinity than Gram-negative bacteria [26]. Abdulkarim et al. [27] studied the growth of *E. coli* in a saline environment. They showed that brine with a concentration of up to 0.5% had no inhibiting effect on the growth of *E. coli*. However, an increase in brine concentration observed by Abdulkarim et al. [27] to 1% and 1.5% contributed to a proportional decline in *E. coli* counts of 9.8·107 CFU·mL^{-1} and 7.8·107 CFU·mL^{-1}, respectively.

Table 1. Average number of microorganisms isolated in 2021 and 2022 (CFU·100 mL^{-1}: *E. coli*, *E. faecalis*, *C. perfringens*; CFU·1 mL^{-1}: mesophilic and psychrophilic bacteria, *Staphylococcus* spp.).

Month of Sampling	**April**		**May**		**June**		**July**		**August**		**September**	
Microorganisms'Year	**Brine**	**Water**	**Brine**	**Water**	**Brine**	**Water**	**Brine**	**Water**	**Brine**	**Water**	**Brine**	**Water**
Staphylococcus spp.'21	127 a	90	123 a	142	547 ab	371	400 a	253	450 a	357	240 ab	90
Staphylococcus spp.'22	85 a	84	123 a	135	276 a	371	541 a	390	563 a	443	260 ab	73
Mesophilic bacteria'21	358 ab	217	1250 ab	1900	3333 ab	1515	1507 ab	850	1667 ab	1433	233 ab	220
Mesophilic bacteria'22	350 ab	210	1321 ab	2085	2361 ab	1515	1633 ab	1177	1683 ab	1313	317 ab	240
Psychrophilic bacteria'21	207 a	153	163 a	1267	2533 ab	1067	1667 ab	1700	2233 ab	2433	263 ab	326
Psychrophilic bacteria'22	215 ab	108	149 a	1038	1700 ab	1067	1930 ab	1980	2000 ab	2133	230 ab	137
E. coli'21	2 a	5	11 a	2	46 a	22	7 a	63	22 a	60	5 a	5
E. coli'22	2 a	4	12 a	2	17 a	74	20 a	81	22 a	97	3 a	6
E. faecalis'21	0a	0	0 a	0	206 ab	0	0 a	0	0 a	0	0 a	0
E. faecalis'22	0 a	0	0 a	0	93 a	88	0 a	47	0 a	63	0 a	0
C. perfringens'21	0a	0	0 a	0	50 a	0	0 a	0	0 a	0	0 a	0
C. perfringens'22	0 a	0	0 a	0	3 ab	43	0 a	18	0 a	20	0 a	0
Temperature (°C)'21	9.3	13.7	14.3	14.3	17.4	23.6	19.1	22.7	21.5	24.6	12.2	14.1
Temperature (°C)'22	9.2	13	13.2	14.5	17.5	23.4	18.9	23.2	20.4	24.5	12.3	14
Brine (%)'21	19.1		18.2		7		16.1		19.1		18.1	
Brine (%)'22	19.1		19		18.1		16.3		18.1		15.1	

The different letters within a column indicate a significant difference at $p < 0.05$ according to Tukey's test.

The control in the present study was water taken from the lagoon, the microbiological parameters of which were referenced to the Regulation of the Minister of Health on the supervision of bathing areas and sites occasionally used for bathing [28], as well as previous publications describing tests conducted in this water body [20]. The lagoon in Nowa Huta has been revitalized in recent years, and the water was purified using biological methods (vegetation and pollutant-degrading microorganisms) and mechanical methods (desludging) [8]. Those measures improved the water quality, as shown in our study. Confronting the results obtained in the present study with these in the previous paper by Wolny-Koładka [20], it is clear that the number of mesophilic bacteria decreased by an average of 2267%, psychrophilic bacteria by 211%, *E. faecalis* by 52%, and *C. perfringens* by 2530% within 6 years.

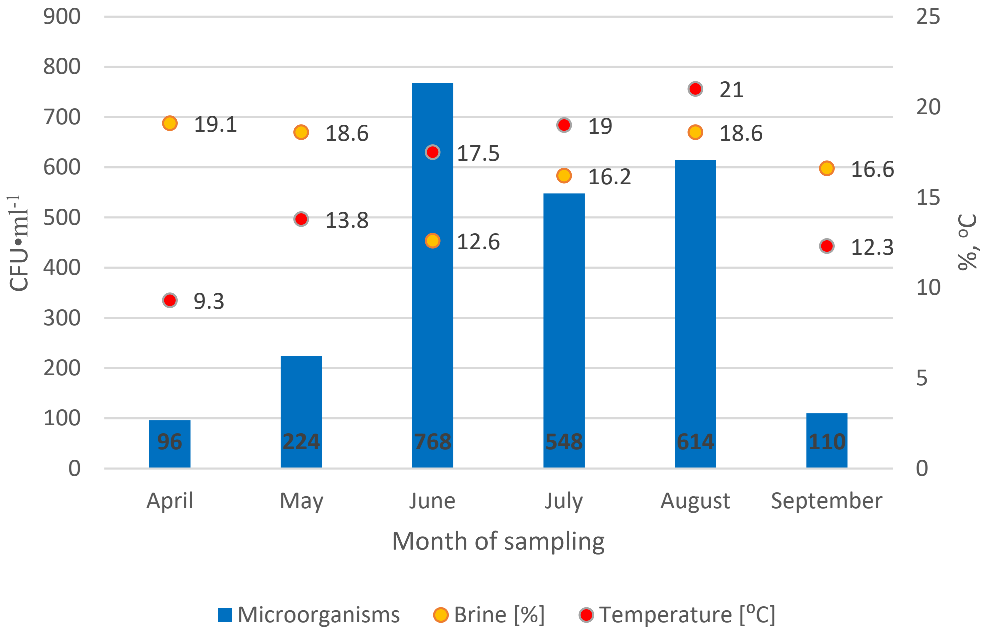

Figure 3. Average monthly number of all microorganisms and measurements of brine concentration and temperature over two years.

As observed, June is the month in which the people of Krakow begin to visit the graduation tower in Nowa Huta in great numbers. This is due to the favourable weather (spring/summer season) and the fact that the school year ends and the summer break begins. Seasonality and the impact of anthropopressure are very important in the context of the microbiological contamination of waters. The study by Topić et al. [29] showed that of the 258 official bathing areas in Primorje-Gorski Kotar County (Croatia) and the Kantrida and Ploče beaches with the highest traffic had, at the same time, the highest microbiological contamination index. That contamination was due to the higher number of people bathing (overcrowded beaches) compared to other bathing areas. In our study, it was the holiday months (June, July, August) that saw a significant increase in the number of microorganisms tested. This is also closely related to the fact that the air temperature in Poland was favourable for the development of microorganisms both at night and during the day, which directly affected the temperature of the brine circulating in the closed system of the graduation tower. In the present study, the average brine temperature in the summer months was: 17.5 °C, 19 °C, and 21 °C. Needless to say, these temperatures favoured the growth of the analysed microorganisms [30]. Therefore, based on our study, we can distinguish several reasons for changes in the number of microorganisms inhabiting the brine. These were an increase in tourist traffic and the number of visitors to the graduation tower (anthropogenic factor); an increase in air temperature, and thus the temperature of brine conducive to microbial growth (atmospheric factor); and violent factors that were beyond our control and difficult to predict (storms, heavy rainfall, which caused a sharp decrease in the concentration of brine, and thus the loss of its antibacterial properties).

When analysing the data in Table 1, one can distinguish clear trends in changes in the number of microorganisms in both the brine and control samples. The distribution of microbial abundance in both years of the study was as follows: the initial months of the graduation's tower operation (April and May), as well as the month when the tower's shutdown began (September), were characterized by significantly lower number of bacteria relative to the holiday months. In addition, these were also months in which pathogenic bacteria, i.e., *E. faecalis* and *C. perfringens*, were not found in the brine. As for *E. coli*, even if present, they were found in trace amounts compared to the holiday months. The other bacteria were also most numerous from June to August, which was related (apart from the reasons stated above) to the fact that in this period the most nutrients for microorganisms were available in water [31].

Comparative analysis of the presence of bacteria in brine and lagoon water (control) showed, for both study years, that the numbers of *staphylococci* and mesophilic and psychrophilic bacteria were similar (Figure 4). *Staphylococci* were less abundant than mesophilic and psychrophilic bacteria. This was due to the fact that they were human-related microorganisms, whose sources were mainly humans, as well as wild and companion animals [32]. *Staphylococci* were also isolated from air and water, but in lower numbers [29,33]. Mesophilic (allochthonous) bacteria inhabited water bodies and, mainly, included those that were transferred there as a result of human activity. Thus, this meant that they were perceived as a pollutant, and their high abundance was not desirable [34,35]. Psychrophilic (autochthonous) bacteria included those that naturally inhabited water bodies and their numbers proved that the ecosystem was rich in nutrients, from which they can draw from [36]

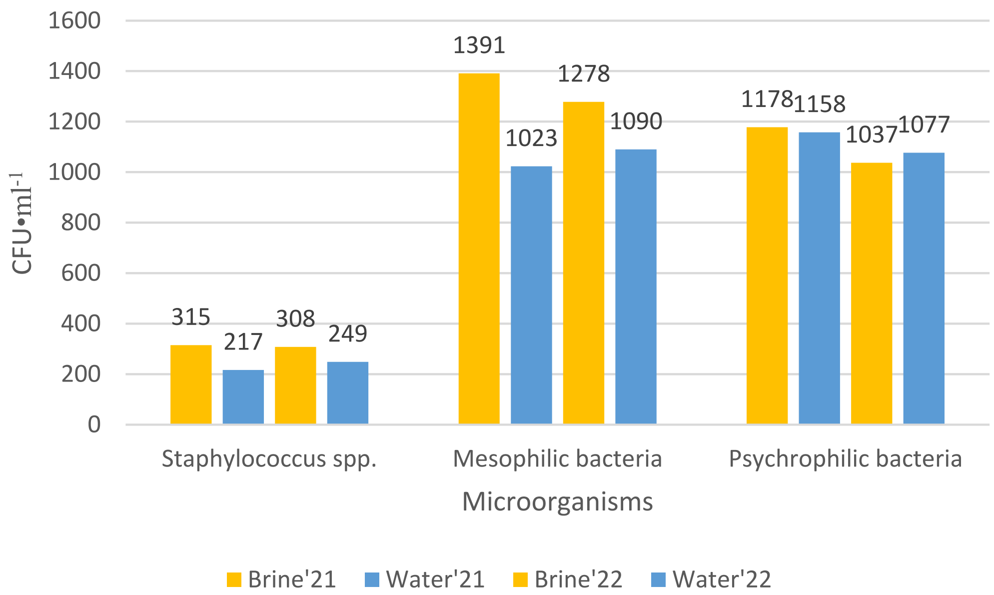

Figure 4. Average numbers of bacteria in brine and lagoon water.

Bacteria considered indicators of microbiological quality of water were found in both brine and lagoon water (Figure 5). In the first year of the graduation tower's operation, there were more of them in brine, which was probably related to the flooding that occurred in Nowa Huta in June 2021 and significantly affected the brine concentration and increased the abundance of all microorganisms. The presence of *E. coli*, *E. faecalis*, and *C. perfringes*, even in trace amounts, in brine was of great concern from an epidemiological point of view because these microorganisms cause a number of very serious infections, both gastrointestinal and generalized [37–39]. Direct ingestion (e.g., through contact with contaminated brine) or inhalation can lead to, among other things, gastroenteritis (*E. coli*, *C. perfringes*), necrotic enteritis (*C. perfringes*), food poisoning (*C. perfringes*), urinary tract infection (*E. coli* and *E. faecalis*), diarrhoea (*E. coli*, *C. perfringes*), hemorrhagic colitis (*E. coli*), kidney damage (*C. perfringes*), endocarditis (*E. faecalis*), and peritonitis (*E. faecalis*) [37,40–43]. It is worth mentioning that most strains of *E. coli* are commensal bacteria found in the human intestine (physiological microbiota), but there are also pathogenic strains that pose a growing threat to humans. These include enterohemorrhagic *E. coli* strains that cause haemorrhagic colitis [37–40].

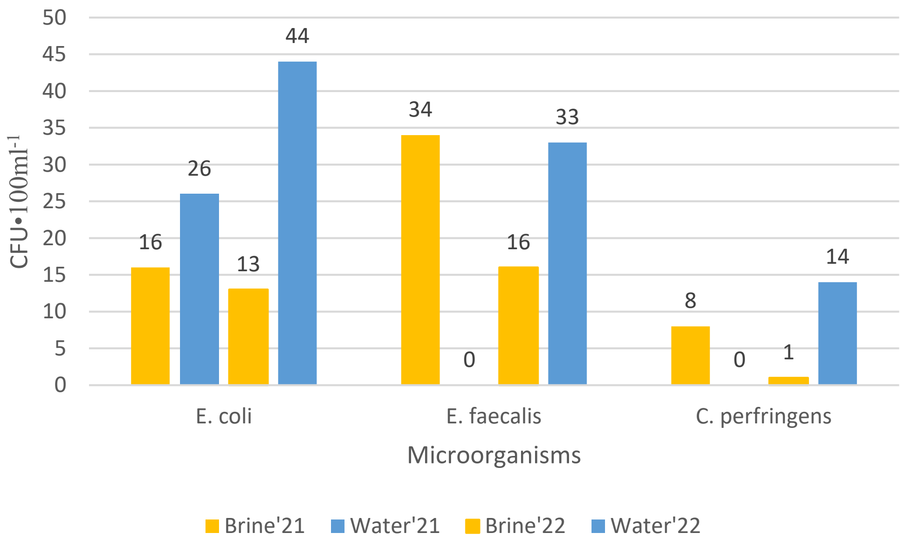

Figure 5. Average numbers of pathogenic bacteria in brine and lagoon water.

The graduation tower in Nowa Huta was opened in 2020; it was a new initiative. While it can be seen by the crowds of people visiting it, it is very necessary and useful. Given the social advantages of launching graduation towers in an urban area, efforts should be made to eliminate the epidemiological threats identified in this study, so that people using beneficial inhalation treatments can do so safely for their health.

4. Conclusions

The results obtained in the two-year microbiological study of the brine that feeds the closed-cycle graduation tower were extremely interesting and needed, as they provided new and unique knowledge on a topic that has not been addressed before and concern the health and safety of the population. As shown in the study, brine that circulated in a closed system and fed the graduation tower was exposed to microbiological contamination. It was evident that even maintaining the brine concentration at the optimal level, i.e., 17–19%, did not ensure its constant bactericidal properties. There were additional factors that had a fundamental impact on the microbiological safety of brine, and these were: the large number of people visiting the graduation tower and using inhalation treatments, the presence of wild animals living around the lagoon, where the graduation tower is located, and those accompanying people, such as dogs, as well as unpredictable weather conditions (heavy rain) and seasonality (temperature changes due to changing seasons). Every effort should be made to improve those elements of the operation closed-cycle graduation tower that are under our control, which is why we have formulated recommendations in this regard. Educating people using the inhalation facility on responsible behaviour in the graduation tower area (not soaking hands and feet in the brine flowing from the blackthorn, watching over small children and dogs so that they do not enter the basin into which the brine flows). On the other hand, the administrator of the graduation tower should measure the brine concentration on a daily basis, as before, and ensure the cleanliness of the system in which the brine circulates. Consideration should also be given to more frequent replacement of the entire brine circulating in the closed system and disinfecting the installation each time, but this must be economically justified. It is also reasonable to introduce permanent microbiological monitoring of the brine circulating in the graduation tower. Monitoring can be performed as described in the manuscript or in an automated manner. An automated sampling and analysis system could also be introduced—but such an action would require some modifications in the construction of graduation towers. Some modifications could also be made to the brine distribution system in the graduation tower. By placing additional brine filtering points (bacteriological filters), we could purify the brine during its circulation in the closed circuit of the graduation tower.

Author Contributions: Conceptualization, K.W.-K.; methodology, K.W.-K. and S.B.; validation, K.W.-K.; formal analysis, K.W.-K. and S.B.; investigation, K.W.-K. and S.B.; resources, K.W.-K. and S.B.; data curation, K.W.-K. and S.B.; writing—original draft preparation, K.W.-K. and S.B.; writing—review and editing, K.W.-K. and S.B.; visualization, K.W.-K. and S.B.; supervision, K.W.-K.; project administration, K.W.-K.; funding acquisition, K.W.-K. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the statutory measures of the University of Agriculture in Krakow and granted for the Department of Microbiology and Biomonitoring (A113, A142).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: We would like to thank Mateusz Malinowski, an employee of Department of Bioprocess Engineering, Power Engineering and Automation, and the Faculty of Production and Power Engineering, University of Agriculture in Krakow, Poland. Mateusz Malinowski supported us at the stage of writing the manuscript and gave advice related to the construction of the technical diagram of the brine graduation tower and the graphic design of the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

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