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EVALUATION OF MICROBIAL CONTAMINATION OF THE MIST INTENDED FOR INHALATION IN A BRINE GRADUATION TOWER

Abstract: The brine mist used for inhalation, produced by the municipal closed-circuit graduation towers, is not subject to microbiological control. There are also no studies on the microbiological safety of brine mist and its potential adverse effects on the health of people inhaling it. Therefore, a two-year study was conducted to evaluate the microbial purity of brine mist intended for inhalation, taking into account the effects of air temperature and brine concentration on microbial cell counts. Bioaerosol was tested for the presence of indicator microorganisms that are epidemiologically significant and may affect the health of inhalers (mould fungi, bacteria, *Staphylococcus* spp., actinomycetes, *Escherichia coli*). The study was conducted over two seasons from April to September in 2021 and 2022. The abundance of actinomycetes and *Staphylococcus* spp. during the holiday season has increased in this period, due to the high air temperatures. Bacteria and mould counts did not exceed the recommended limits, while *E. coli* was not found. As the air temperature increased and the brine concentration decreased, the abundance of the microorganisms analysed (especially actinomycetes and *Staphylococcus* spp.) increased. The study used Bayesian networks which showed that at high temperatures, the probability of *Staphylococcus* spp. abundance above 73 $CFU\cdot m^{-3}$ was 63 % and that of actinomycetes above 8 $CFU\cdot m^{-3}$ was 64 %.

Keywords: closed-cycle brine graduation tower, bioaerosol, indicator microorganisms, Bayesian networks

Introduction

Polish air is one of the most polluted among European countries, and Krakow is among the top Polish cities where permissible air pollution levels (carbon oxides, nitrogen oxides, sulphur oxides, particulate matter, heavy metals, dioxins and others) are notoriously exceeded [1, 2]. Despite ongoing measures to reduce low-level emissions and the introduction of regulations to limit air pollution with PM10 and PM2.5, significant exceedances of permissible pollution values are recorded in Krakow every year [3]. In 2018, an estimated 49,700 people in Poland died prematurely due to complications from smog-related diseases [4, 5]. Therefore, new methods to combat air pollution are constantly being sought. An alternative initiative is the construction of brine graduation towers in

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cities, which are intended to improve the health and well-being of the inhabitants who inhale the brine mist [1, 6].

A brine graduation tower, also known as a thorn house, is a wooden structure filled with blackthorn branches and equipped with a suction and pumping system for brine that is rich in minerals, i.e. sodium chloride, iodine, bromine, calcium, and iron compounds (Fig. 1). The wooden frame, usually made of spruce or oak, is placed on a concrete foundation. The facility serves as an open-air inhalation room [7]. The inhalation effect is as follows: the brine in the reservoir at the base of the graduation tower is pumped to the top of the structure and enters special distribution gutters, from where it trickles down the branches of the blackthorn and is concentrated by evaporation [8]. The phenomenon of brine atomisation occurs as the brine breaks down into smaller droplets. In a closed-cycle brine graduation tower, the brine flows down a gutter system and is diverted to a reservoir and then pumped to the top of the graduation tower. The air around the facility is characterised by higher humidity and lower temperature due to the brine evaporation [8-10]. The microclimate of the near-tower zone has similar characteristics and therapeutic effects to the coastal climate [11]. The process of aerosol formation is weather dependent. Brine evaporation is most intense on sunny and windy days. In contrast, during rain or high humidity, brine evaporation almost completely disappears, so that the inhalation process is not effective [9].

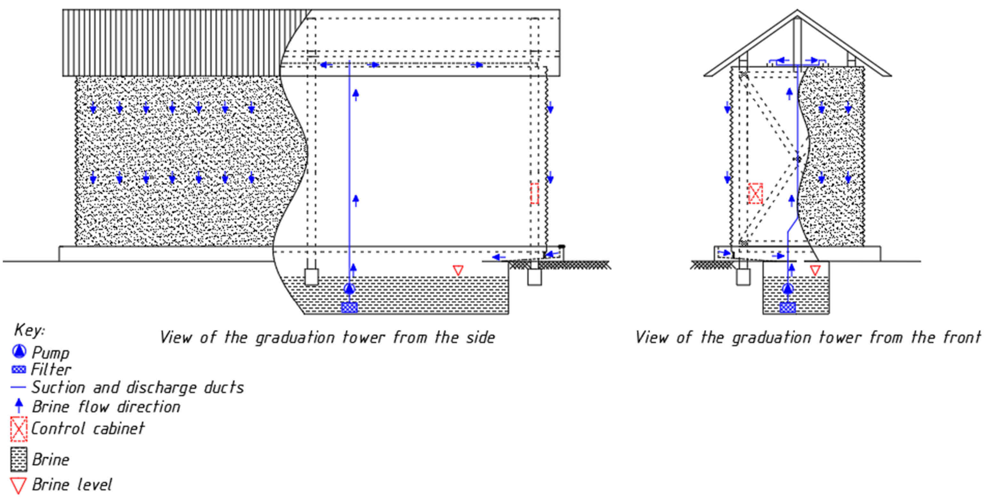

Fig. 1. Schematic of a closed-cycle graduation tower structure [7]

There are two technical systems of water cycle in brine graduation towers: open and closed. Open-cycle graduation towers were the first structures of this type in Poland, located at the sources of brine extraction (usually in health resorts). At present, closed-cycle graduation towers are most commonly built in towns with no brine springs. Typically, such structures do not exceed 4 m in height, 8 m in length, and 2 m in width [7]. The brine that feeds the municipal graduation towers is stored in specially designed and adapted storage tanks located at the base of the graduation towers. They have a capacity of several to tens of thousands of litres. The quality of the brine is the same as that used in brine health resorts and meets hygiene and water quality requirements [7].

Analysis of the available literature indicated that no studies were carried out on the microbial quality of brine mist intended for inhalation in a closed-cycle graduation tower. So far, the focus was on describing the graduation tower's structure, functional-technological aspects, and the health-promoting effects of brine inhalation on the human body [7, 8, 10, 12-15]. The aim of the study was to identify the potential microbiological risks associated with inhalation of brine in a municipal closed-cycle graduation tower. On this basis, it was planned to assess whether the inhalation of a brine mist in a closed-cycle graduation tower is safe from a hygienic and microbiological point of view. The effect of brine concentration and weather conditions on the microbial contamination of the air around the graduation tower was also determined.

Material and methods

Sampling site characteristics

The closed-cycle brine graduation tower (Fig. 2) under study is located in Nowa Huta (Krakow, Poland; 50°04'52.7"N 20°03'05.7"E). The base of the graduation tower is triangular in shape with a total area of 350 m^2; the 18 m long arms of the tower are made of larch wood and the whole is filled with blackthorn branches. At the base of the graduation tower there is a brine tank with a capacity of 60,000 dm^3. The brine has a high iodine content and comes from a mine in Zablocie near Pszczyna in Upper Silesia (Poland). During the operation of the graduation tower, the brine has to be replenished due to evaporation or losses caused by drops falling outside the basin. Brine shortages are replenished with mains water, and the brine concentration is checked daily and adjusted if necessary. Twice a season, the brine is replaced with fresh brine and, at the same time, the suction and pumping systems and the pool are disinfected. The graduation tower in Nowa Huta is a public facility open from April to September. According to the local regulations, an inhalation session should last about 30 minutes, but no longer than 45 minutes [16]. Penetration of the brine through the respiratory tract and skin replenishes micronutrient deficiencies in the human body and strengthens the immune system [7]. The brine used in the described graduation tower has a high concentration of sodium chloride and also contains many other minerals such as calcium, potassium, magnesium, sulphates, strontium, bicarbonates and iodides. According to the brine manufacturer, the atomised mist has an antibacterial effect, absorbs pollutants, and prevents inflammation in the body. The brine supplied has a concentration of 28 % and is diluted in the graduation tower to achieve a final concentration of 16 % - 19 % [16]. Therefore, 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 [17, 18].

Sampling

Air quality tests around the brine graduation tower were carried out for two years during the period of its operation, i.e. from April to September in 2021 and 2022. Six brine samples (1 per month) were taken each year, every 30 ±5 days to account for seasonality. Before sampling, attention was paid to weather conditions to avoid heavy rainfall that dilutes the brine, i.e. after heavy rainfall, 3 additional (rain-free) days were waited before the next sampling.

Bioaerosol samples were collected using the impact method with a MAS-100 Eco Airsampler for the food industry from MERCK (Germany) known as impactor. The air temperature at each bioaerosol sampling point was also measured using an electronic thermometer from Biowin (Poland). Samples were taken from 4 locations in 3 replicates. Points I, II, and III were located halfway up the arms of the graduation tower, 1 m from the wall of the facility (Fig. 2), while point IV was the control and was located 80 m from the graduation tower. The data on brine concentration were obtained directly from the Management of the Krakow Municipal Greenspace Authority (ZZM), which systematically monitors the brine concentration in the graduation tower using an electric refractometer.

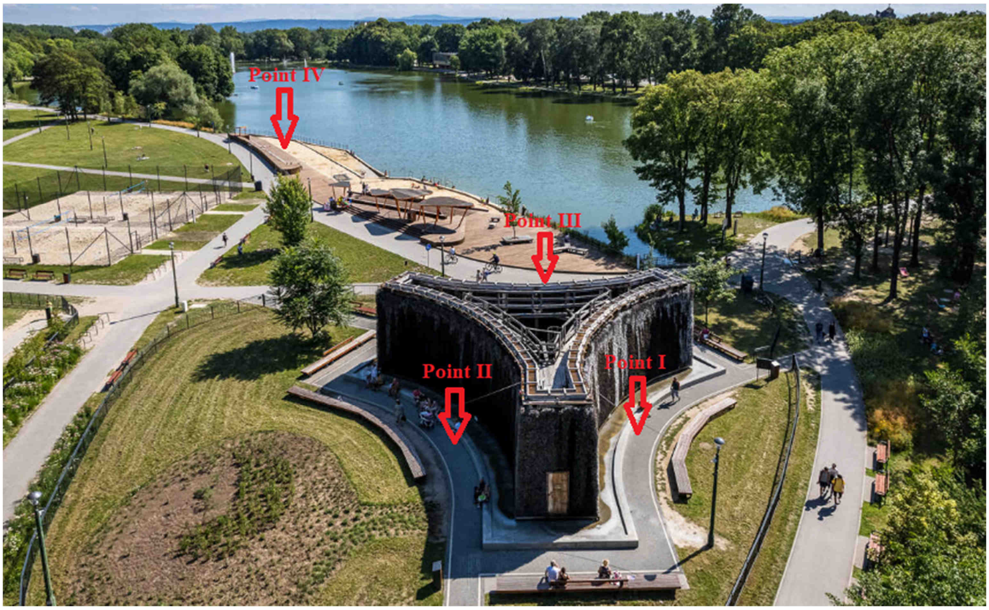

Fig. 2. Bioaerosol sampling points [19]

Microbioal analysis

The evaluation of the microbial contamination of the mist intended for inhalation in the brine graduation tower was performed according in line with the recommendations developed by the Polish Air Quality Standards [20] and other publications [21-23]. Growth media were used to determine the abundance of bacteria (Trypticasein Soy Lab Agar, BTL, Poland), moulds (Malt Extract Agar, BTL, Poland), actinomycetes (Actinomycete Isolation Lab Agar, BIOCORP, Poland). The presence of *Staphylococcus* spp. (Chapman agar, BTL, Poland) and *Escherichia coli* (TBX agar, BTL, Poland) in the brine bioaerosol was also analysed, as these are bacteria associated with humans and indicate anthropogenic causes of microbial contamination of the brine. Our previous studies [7] showed that these microorganisms are present in the brine circulating in the graduation tower. It is therefore reasonable to assess their survival in the brine mist, as this information is crucial to determine the epidemiological safety for inhalation users. After sampling, cultures were incubated under the following conditions: bacteria and *Staphylococcus* spp. 37 °C, 24 h, *E. coli* 44 °C, 24 h, fungi 28 °C, 3-5 days, actinomycetes 28 °C, 5-7 days.

After incubation, characteristic colonies of the identified microorganisms were counted and the results expressed as colony forming units per cubic metre of air (CFU · m^{-3}), taking into account the correction recommended by the impactor manufacturer. The microbial counts obtained were compared with the criteria in the Polish standards [20] and in the publications by Grzyb and Lenart-Boroń [21] and Wolny-Koładka et al. [22]. In this way, the air was qualified in terms of the degree of its microbiological contamination.

Statistical analysis

A two-factor analysis of variance was performed using Statistica v. 13.5 (StatSoft) to test the significance of the effects of temperature and brine concentration on the abundance of the microorganisms studied.

Bayes belief networks were also used for the statistical analysis of the results obtained, since the occurrence of certain types of microorganisms and their growth are strongly dependent on environmental factors. Bayes belief networks are one of the probabilistic methods based on conditional probabilities. A Bayes' network is a classifier based on maximum posterior probability [24, 25].

A Bayesian network is a mathematical and graphical model that represents a probabilistic relationship between a set of previously discretised random variables. A Bayesian network is an acyclic directed graph in which each node of the network represents a random variable along with the probabilistic relationships between each of them. The quantitative part of the network represents the probability distribution for these variables [25-27]. The nodes of the network are identified as variables, while the arrow indicates the causal relationship between variables. Associated with each node of the network is a conditional probability distribution of a given component (characteristic, variable) being in a certain state (group or class of values), conditioned by the state of the elements (factors) represented by the elements associated with it. The GeNie 2.0 software developed by the University of Pittsburgh (USA) was used to model Bayesian networks [28, 29]. The Bayes formula was used to calculate probabilities [27, 30, 31]:

$$P(A/I) = \frac{P(A) \cdot P(I/A)}{P(I)} \tag{1}$$

where *P*(*A*/*I*) is probability of event *A* after information I is available; *P*(*A*) is probability of event *A* before information *I* is available; *P*(*I*/*A*) is probability of information *I* occurring if A is true; *P*(*I*) is total probability of event *I*.

We hypothesise that the number and composition of microorganisms may be statistically dependent on temperature only under certain conditions. The modelling of these relationships is possible after discretisation of the measured data. Discretisation of the data involves the creation of intervals (classes) for each variable. The most common notations for grouping are: below, mean, and over. The results of the discretisation are shown in Table 2. These classes can be generated automatically in GeNie 2.0 (3 available methods) or manually. The Jenks natural breaks method [32, 33] was used to group the data.

Results and discussion

A two-year study of the microbial purity of the brine mist taken from the surroundings of the Nowa Huta graduation tower provided epidemiologically worrying information. Comparing the results of our own study (Table 1) with the limit values contained in the Polish standards [20] and in the publications by Grzyb and Lenart-Boroń [21] and Wolny-Koładka et al. [22] it can be stated that in the case of actinomycetes the requirements regarding the number of microorganisms tested were not exceeded. Among the microorganisms studied, no *E. coli* were found in any of the samples (including the control sample taken at point IV away from the graduation tower). On this basis, it was considered that although these bacteria were detected in our previous study [7] in the brine circulating in the closed cycle of the graduation tower, they do not enter the mist used for inhalation. This is extremely valuable information, as the survival of *E. coli* in the brine mist would call into question the reasonableness and health safety of the construction of municipal graduation towers. As shown in the studies by Jureczko et al. [34] and Hwang et al. [35], even a 6 % brine concentration inhibits the growth of *E. coli*, while since the brine concentration in the described closed-cycle graduation tower is 16 % - 19 % [7], the transition of bacteria from the aqueous environment (brine) to the gas phase (air) seems to be crucial. This is because air is an environment that is not conducive to microbial life, in which they cannot grow and divide. It is only a place for their periodic residence and a centre for their movement [36].

The data in Table 1 clearly show some trends in the changes of microbial abundance both in the mist and in the control samples which were the air around the lagoon. The distribution of microbial abundance in both years of the study was as follows: the first months of operation of the graduation tower (April and May) were characterised by significantly lower microbial abundance compared to the summer months. The summer holiday period (June, July, August, and September) is a time when tourists and residents of Krakow visit the Nowa Huta graduation tower in large numbers due to the favourable weather. Seasonality and the impact of anthropopressure are very important in terms of microbiological contamination of brine water. Bacteria and fungi did not pose a health risk to inhalation users as their numbers did not exceed acceptable standards. Their abundance increased as the holiday season approached and decreased at the end of the summer season. In most cases, the abundance of indicator microorganisms was higher in the brine mist than in the air sampled at the control point. The abundance of *Staphylococcus* spp. was particularly high during the holiday season (June-August), when it exceeded 150 $CFU \cdot m^{-3}$. *Staphylococcus* spp. are microorganisms associated with humans, who are their main source, as well as pets and wild animals living in the lagoon area, hence the high abundance of these bacteria found in the mist during the holiday season, when visitors to the graduation tower are most numerous [37]. From an epidemiological point of view, the presence of the indicator microorganisms analysed in the brine is unfavourable, since these microorganisms can cause a series of very serious infections, both gastrointestinal and generalised. Direct contact and inhalation of contaminated mist can lead to pneumonia (*Staphylococcus* spp., actinomycetes), endocarditis (*Staphylococcus* spp.), osteomyelitis (*Staphylococcus* spp., actinomycetes), gastrointestinal poisoning (*Staphylococcus* spp.), skin infections (*Staphylococcus* spp., actinomycetes), nervous system diseases (actinomycetes), fungal pneumonia and bronchitis (fungi), allergies (fungi) [38-43].

Table 1

Average abundance of microorganisms [CFU·m^{-3}] isolated in 2021 and 2022

Month	**April**		**May**		**June**		**July**		**August**		**September**	
Microorganisms	**Mist**	**Cont-rol point**	**Mist**	**Cont-rol point**	**Mist**	**Cont-rol point**	**Mist**	**Cont-rol point**	**Mist**	**Cont-rol point**	**Mist**	**Cont-rol point**
Bacteria’21	4a	5	10a	8	17a	6	6a	5	7a	4	2a	1
Bacteria’22	3a	4	5a	5	9a	8	4a	5	6a	4	2a	2
Fungi’21	34a	28	34a	23	54a	40	64a	69	61a	59	51a	26
Fungi’22	24a	19	27a	20	33a	39	62a	66	65a	67	50a	26
Actinomycetes’21	2a	2	3a	4	22a	16	7a	7	8a	7	2a	1
Actinomycetes’22	1a	1	2a	4	15a	16	7a	8	10a	10	3a	5
Staphylococcus spp.’21	3a	2	14a	14	291ab	29	196ab	13	173ab	13	58a	4
Staphylococcus spp.’22	3a	2	3a	4	53a	1	154ab	1	159ab	3	55a	3
Temperature’21 [°C]	13.5	14.0	15.8	16.8	20.1	20.8	21.2	26.2	25.0	26.2	15.4	19.6
Temperature’22 [°C]	12.7	13.2	15.5	16.5	19.4	19.4	21.2	26.2	25.0	26.2	15.4	19.6
Brine’21 [%]	19.1		18.2		7.0		16.1		19.1		18.1	
Brine’22 [%]	19.1		19.0		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

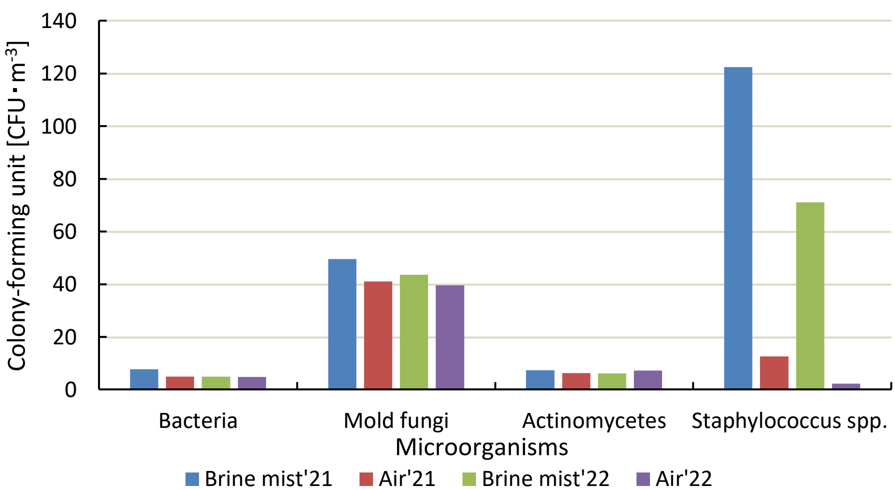

Fig. 3. Average abundance of indicator microorganisms in mist and air

Figure 3 shows the average abundance of all microorganisms analysed in the brine mist and the control point (air). In 2021 there were more microorganisms in the brine mist than in 2022. This was due to the prolonged rainfall in June that contributed to dilution of the brine water in the graduation tower, causing its concentration to drop to 7 % (Table 1), which in turn resulted in a rapid increase in microbial abundance. Individual microbial groups were observed to be present at similar levels in the mist and control (air) samples.

The exception was *Staphylococcus* spp. that were much more abundant in the mist than at the control point away from the graduation tower in both years of the study. This resulted from the fact that *Staphylococcus* spp. are human-associated microorganisms, extremely frequently isolated from humans, much more frequently than from air, water, and soil [37]. In addition, as demonstrated in our previous study [7], the brine circulating in a closed-cycle graduation tower is contaminated by those bacteria which survive in the brine for a longer period of time and can therefore enter the mist intended for inhalation.

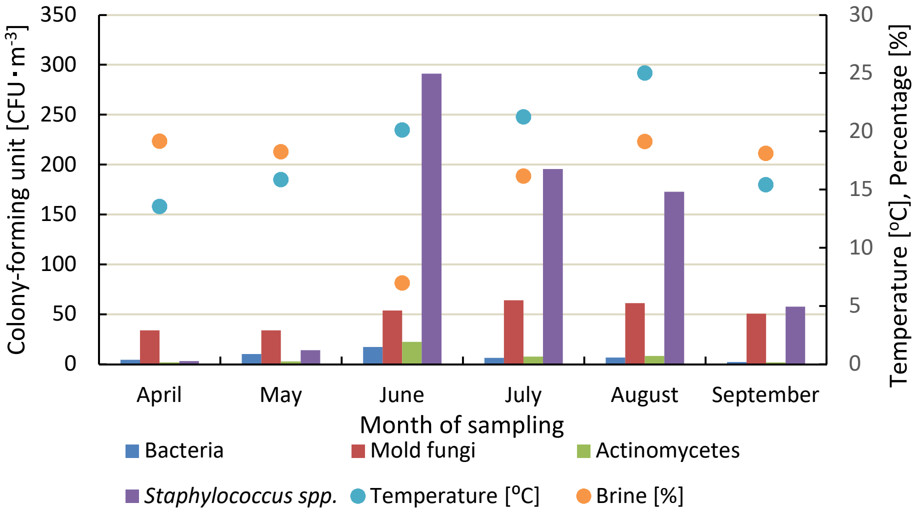

Fig. 4. Average monthly abundance of microorganisms at points I, II, and III isolated from the brine mist in 2021

When analysing the microbiological contamination of the inhalation mist over two years (Figs. 4 and 5), it should be noted that the microbial abundance was highest during the holiday season. The highest number of microorganisms was found in June 2021, when the brine concentration was the lowest (about 7 %). In June 2021, heavy rainfall occurred every few days in Nowa Huta, resulting in frequent flooding - the graduation tower was also flooded. As can be seen, a radical decrease in brine concentration translated into a rapid increase in the number of microorganisms. Interestingly, brine concentrations above 15 % did not ensure complete elimination of microorganisms. Contaminated brine water has a direct effect on the quality of the resulting aerosol in the closed-cycle brine graduation tower. During the holiday season, the higher air temperature increases the temperature of the brine circulating in the graduation tower, which results in a higher survival rate of microorganisms in the brine mist [7]. In our study, the average brine temperature during the summer months was 19 °C - 25 °C and these are very favourable temperatures for the growth of the analysed microorganisms [44-46]. As reported by Missiakas and Schneewind [45], *Staphylococcus* spp. can grow at temperatures between 15 °C and 45 °C at brine concentrations up to 15 %. In our study, *Staphylococcus* spp. had the highest abundance of the microorganisms studied (Figs. 4 and 5). They were most abundant in June and July when the salinity was around 15 % - 18 % (Table 2). Salinities up to 5 % are favourable for the growth of actinomycetes, while higher salinities limit their abundance [47, 48]. High humidity, water availability, and warm days are beneficial for fungal growth. As shown in our study, in both studied seasons, the abundance of fungi in the mist increased with air temperature (Figs. 4 and 5). The microclimate created around the brine graduation tower is characterised by high humidity, and the high air temperature promotes the proliferation of moulds. However, according to Kogej et al. [46], salinity of 10 % limits the occurrence of moulds, so their abundance in our study did not exceed the recommended limits.

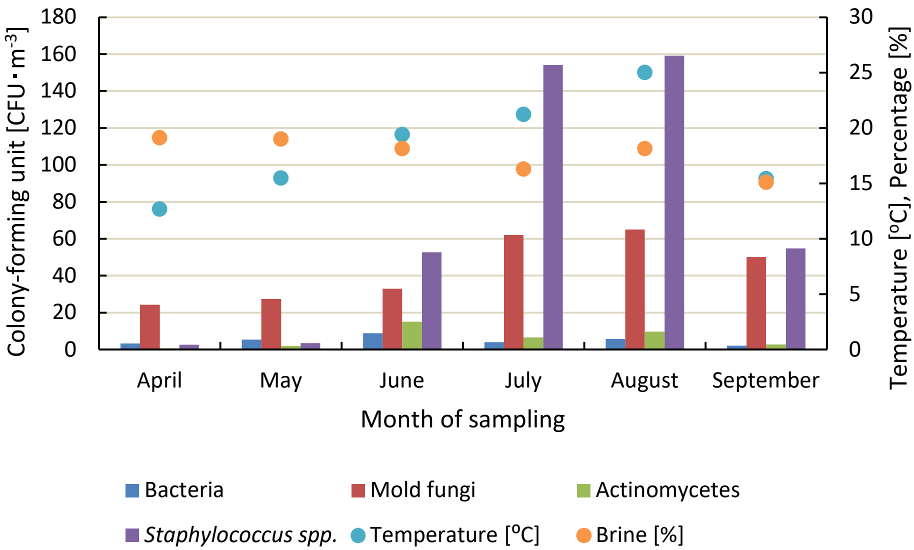

Fig. 5. Average monthly abundance of microorganisms at points I, II, and III isolated from the brine mist in 2022

Figures 6-9 show the distribution of cause and effect relationships between the analysed variables assigned to the respective classes as a result of the discretisation (Table 2) and the probability distribution for a specific state of the analysed variables by air temperature and brine concentration. An algorithm based on Bayesian networks confirmed the existence of a relationship between temperature and the occurrence of microorganisms, as well as between brine concentration and the number of fungi and *Staphylococcus* spp. However, it did not confirm a cause and effect relationship between bacterial abundance and temperature or brine concentration. The analysis showed that at brine concentrations below 18 % there was a high probability of the greater number of *Staphylococcus* spp. (5 CFU · m^{-3} and above). A low brine concentration did not significantly differentiate the fungal abundance (Fig. 6). However, at brine concentrations above 19 %, the probability of fewer *Staphylococcus* spp. increased (Fig. 7).

The Bayes model did not show a cause and effect relationship between brine concentration and *Staphylococcus* spp. and air temperature. Regarding the temperature effect on microorganisms, high air temperature (above 21 °C) favoured the proliferation of fungi, actinomycetes, and *Staphylococcus* spp. as well as their occurrence in significant numbers with a probability of 86 %, 64 %, and 64 %, respectively (Fig. 9). According to Figure 9, air temperatures below 16 °C did not favour the proliferation of the above microorganisms and their lower number can be expected.

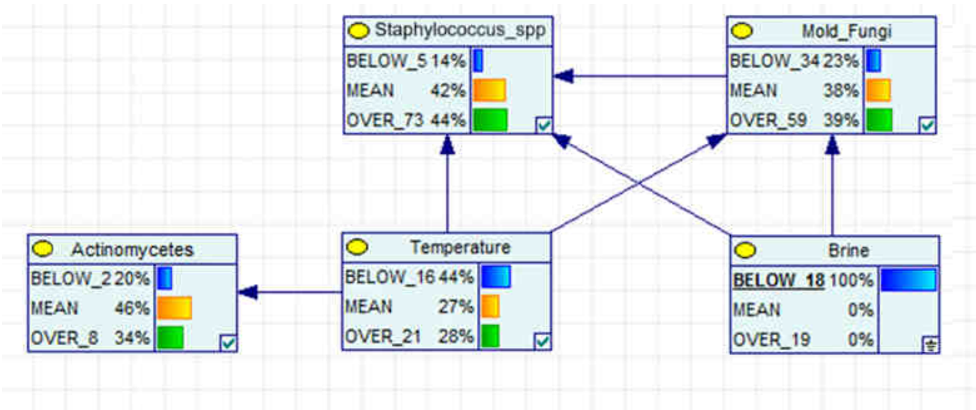

Fig. 6. Bayes belief network with probability distribution for the brine variable set at lowest values (Below_18)

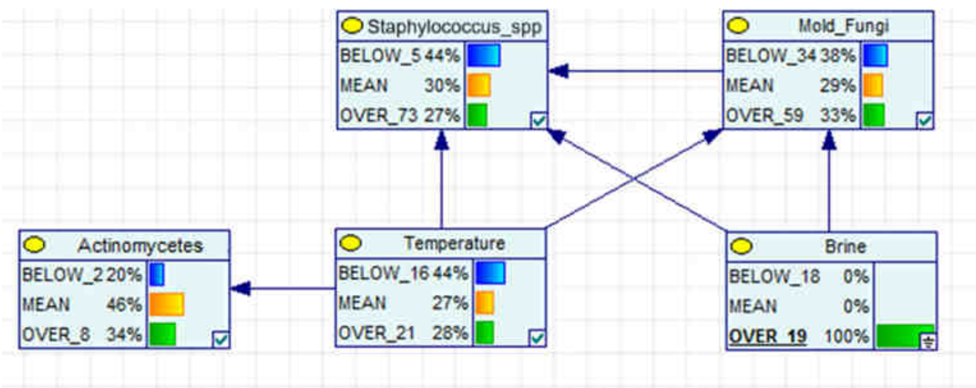

Fig. 7. Bayes belief network with probability distribution for the brine variable set at highest values (OVER_19)

Table 2

Variable discretisation criteria

Original name of the variable	**Unit**	**Variable name in the model**	**Values and limits of variables**
Bacteria	[CFU·m^{-3}]	Bacteria	Below (< 4), Mean (4-9), Over (> 9)
Fungi	[CFU·m^{-3}]	Mold fungi	Below (< 34), Mean (34-59), Over (> 59)
Actinomycetes	[CFU·m^{-3}]	Actinomycetes	Below (< 2), Mean (2-8), Over (> 8)
Staphylococcus spp.	[CFU·m^{-3}]	*Staphylococcus* spp.	Below (< 5), Mean (5-73), Over (> 73)
Air temperature	[°C]	Temperature	Below (< 16), Mean (16-21), Over (> 21)
Brine concentration	[%]	Brine	Below (< 18), Mean (18-19), Over (> 19)

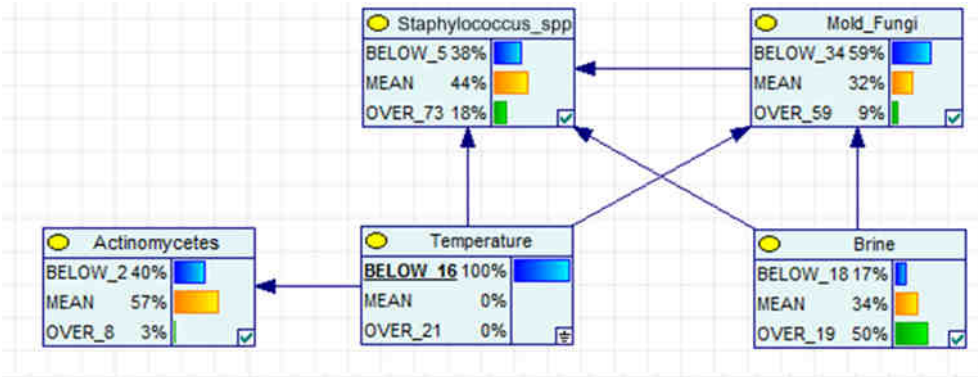

Fig. 8. Bayes belief network with probability distribution for the temperature variable set at lowest values (BELOW_16)

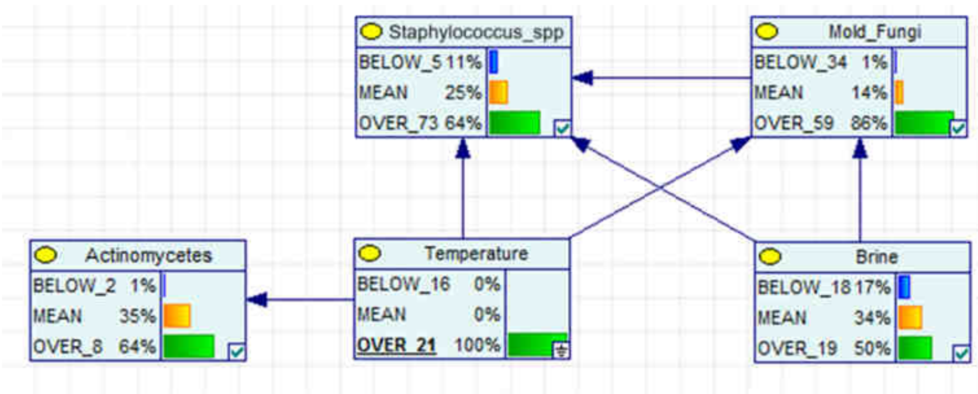

Fig. 9. Bayes belief network with probability distribution for the temperature variable set at highest values (OVER_21)

Conclusion

Our analyses of the microbial safety of the brine mist are crucial in the context of monitoring potential microbiological hazards affecting the health of the residents of Krakow, as well as tourists who use the graduation tower in large numbers. Based on the conducted research, it can be concluded that:

- The brine mist produced by a closed-cycle brine graduation tower is not free of microorganisms. Nevertheless, these concentrations do not pose a direct threat to the health of people using saline inhalations.
- An average brine concentration of 18 % did not ensure that the inhalation mist was completely antimicrobial.
- High temperatures and low brine concentrations favoured the proliferation of microorganisms, especially *Staphylococcus* spp.
- Factors that contributed to the microbiological contamination of the brine mist were: the large number of inhalation users (especially during the holiday season), non-

compliance with the rules and regulations for using the graduation tower (washing of hands and face in the basin into which the brine flows), the presence of domestic animals (mainly dogs entering the basin) and wild animals, mainly birds, which have unrestricted access to the graduation tower structure and can contaminate it with faeces.

- It is necessary to continue to monitor the microbiological quality of both the brine and the mist, and to take measures to encourage responsible and law-abiding behaviour by visitors to the graduation tower.

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