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Letter to the Editor

Microbiological Contamination of Air within the City of Toruń

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Abstract

Research was conducted for the present paper on microbiological contamination of the air within the City of Toruń. It follows from the studies conducted that microorganisms occurred in greater numbers in the air within the area of the Old Town than in the Rubinkowo residential district. The number of microorganisms in the air was subject to seasonal changes. The highest numbers of heterotrophic mesophylic bacteria (CFU 37°C) were found in the summer season and the lowest in the winter season. The majority of isolated bacteria belonged to Gram positive forms - bacilli or cocci. A significant proportion of the isolated bacteria displayed antibiotic resistance to commonly used antibiotics.

Keywords: microflora of the air, antibiotic resistance

Introduction

The air is an environment with which land organisms have contact throughout their lives. The extent of its contamination, chemical, physical and also biological, significantly influences the health and well-being of organisms inhabiting it [1, 2]. Plant and animal organisms as well as humans are subject to the negative effects of factors found in the air. Among the biological contamination found in the air, viruses, bacteria, fungi and pollen are distinguished. Viruses, bacteria and fungi derive above all from the natural environment as typical microflora of the water or soil [2, 3, 4, 5]. Another significant source of microorganisms is land organisms themselves, acting as carriers of saprophytic and pathogenic microflora causing illness in individuals of a given species [6,7]. The next significant source that emits microorganisms into the air is domestic waste and waste from the health service and agriculture, decomposing by means of microbiological mineralization and often dumped or processed on rubbish dumps and sewage treatment plants.

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From the point of view of its properties and different physico-chemical structure, air is not a typical environment for the life of microorganisms. Air is very often defined as a "transport environment" in which microorganisms can occur and be transported, often over considerable distances. During this time they do not breed and very often their vital functions are considerably restricted or completely halted [6,8,9]. This follows from the fact that air is a very unfavourable environment for the life and development of microorganisms. The main causes are the physico-chemical conditions to which microorganisms are exposed, like large amplitudes of temperature, changes in humidity, UV radiation, the presence of ozone and the presence of contamination of industrial origin, e.g. ozone of industrial origin, sulphur oxide and nitrogen oxide [10]. The next factor limiting the presence of microorganisms in air is the way in which they are transported. Microorganisms are transported in the form of so-called microbiological aerosols formed as a result of the action of wind and evaporation of surface layers of water bodies.

For the above reasons, it is important to monitor the sanitary state of the air in places of increased risk of bio-

logical contamination (hospitals and particularly isolation wards), in storage premises where food items are stored, in places where people spend time every day, like dwelling places, public utility buildings and atmospheric air in the streets of towns with heavy traffic.

Materials and Methods

Research Site

Studies on the microflora of the air (bacteria and mould fungi) were conducted within Toruń. The research sites were situated within Toruń's residential area Rubinkowo, where 4 research points were located (map 1), and within the Old Town, where 8 research points were located (map 2).

Taking Samples

Samples were taken during the year at one-month intervals, from February 2001 to January 2002.

Samples of air were taken in accordance with the Polish Norm (PN89/Z-04008/08), at a height of 1.3 m above the ground. The collision method was used to collect the samples with a microbiological air sampler MAS-100 (Merck). The speed of air flow through the sampler was about 11 m/s, which allowed particles to be trapped with a dimension of >1 μm, which are significant in the transport of microorganisms. At every research site, air samples were taken in three replications.

Petry dishes with the collected material were taken to the laboratory and placed in a thermostat in order to incubate individual groups of the tested microorganisms. After incubation the colonies of microorganisms that had grown on the plates were counted and the results were calculated for a volume of 1 m3 of air, according to the instructions provided by Merck for the Mas-100 apparatus.

Microbiological Research

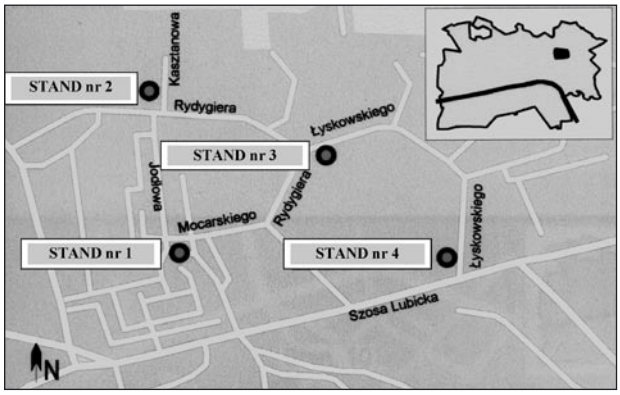
Microbiological studies of the air involved the determination of: 1) the number of heterotrophic mesophylic bacteria, 2) the number of bacteria from the *Enterobacteriaceae* family and 3) the number of mould fungi.

1) The number of mesophylic bacteria was determined according to the Polish Norm (PN-89 Z-04111/02). Plate Count Agar (by Difco) was used as the medium, as proposed by the norm PN-ISO 4833. Incubation of the bacteria which had grown on the plate was conducted at 37°C for 48 h, taking readings of CFU every 24 h.
   Morphological identification of strains was carried out among the mesophylic bacteria in the dyed preparations using the Gram method.
2) Bacteria from the *Enterobacteriaceae* family were isolated using MacConkey medium. The culture was performed in accordance with the Polish Norm (PN-64 A-04023) at 37°C for 48 h, after which the colony of bacteria that had grown up was counted.
3) The number of mould fungi was determined using Martin medium. The culture was performed at 26°C for 5 days.

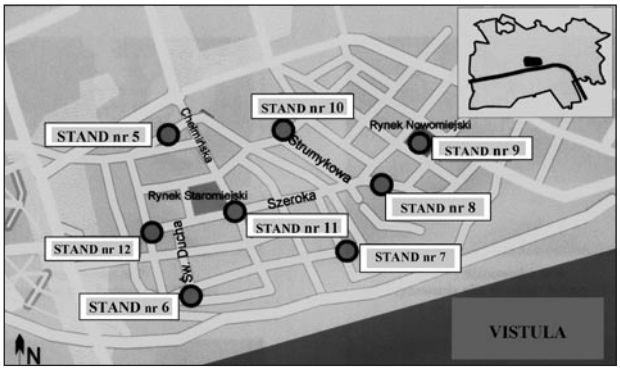
At the same time as the samples of air were taken for microbiological research, certain physico-chemical parameters of the air were noted at the sites being studied, such as temperature, air humidity, sunlight radiation, precipitation, direction and speed of the wind, all of which affect the degree of air contamination by microbiological aerosols [10, 11]. The results of the measurements of the above-mentioned parameters were obtained from the air monitoring stations within the town of Toruń run by the Provincial Inspectorate for Environmental Protection and Water Management.

Research on the Antibiotic Resistance of Bacteria

The research was conducted according to the recommendations of the State Hygiene Institution [12]. 100 strains of bacteria isolated from the studied air samples



Map 1. The location of stands in the Rubinkowo area.



Map 2. The location of stands in the Old Town area.

Table 1. Physico-chemical properties of the air during the research period.

Month	Temperature [°C]	Humidity [%]	Wind direction	Wind speed [m/s]	Sunlight radiation [lux]	Precipitation
January	4.5	88	193	2.3	64.5	0
February	7.9	82.6	154	2.3	232.2	0
March	7.2	46.8	154	2.8	913.2	0
April	19.9	14.9	206	3.5	444.9	0
May	20.4	15	31	2.6	1488.7	0
June	15.5	53	270	4.7	835.8	0
July	26.1	42.6	120	1.3	594	0
August	21.9	73.5	242	3	745	0
September	17.6	53	110	0.9	236.7	0
October	14.1	89	217	1.4	189.9	0
November	5.9	46.9	281	1.6	346.2	0
December	2	83	183	4.7	77.5	0

during the studied seasons, i.e. from spring, summer, autumn and winter, were used for the research.

The determination of antibiotic resistance was performed as follows: a suspension of bacteria was prepared from washed bacteria incubated on slants of Plate Count Agar for 72 hours at 37°C. The optical density of the suspension was established taking the strain with the weakest growth as a model, thus the lowest optical density after suspending bacteria in 3 ml of sterile physiological salt solution (0.85% NaCl). Then 0.1 ml of bacterial suspension was transferred on to Petri dishes with Plate Count Agar and spread on the surface of the medium. Discs soaked in antibiotics were placed on dishes prepared in this way (BBL™ Sensi-Disc™ Becton Dickinson France S.A.). Seeded dishes were placed in the refrigerator at 4°C for 30 minutes in order to diffuse the antibiotic into the medium and then they were transferred to a thermostat and incubated at 37°C for 72 hours. The degree of antibiotic resistance of a given strain was determined on the basis of measurements made of the zones of growth inhibition. 5 different antibiotics were used for the research, which were representative of the basic classes of antibiotics widely used in medicine: Penicillin, Erythromycin, Streptomycin, Tetracycline, Vancomycin.

The classification of strains was conducted according to data from the disc manufacturer (BBLTM Becton Dickinson France S.A.).

Results

The results obtained in the present paper indicate that the number of micro-organisms belonging to individual groups underwent seasonal variations and differed in the air at different sites.

Comparing the number of heterotrophic bacteria occurring in the air of the residential area "Rubinkowo" and the Old Town (Fig. 1), it can be seen that in general more heterotrophic bacteria occurred in the air of the Old Town. Moreover, in both studied parts of the city, the greatest number of heterotrophic bacteria was noted in the summer months (May-September). The greatest number of these bacteria occurred in July (on average: Rubinkowo - 238 cells/m^3, Old Town - 372 cells/m^3), while the lowest was in March (on average: Rubinkowo - 17 cells/m^3, Old Town - 41 cells/m^3), and in December (on average: Rubinkowo - 13 cells/m^3, Old Town - 43 cells/m^3).

Comparing the average number of heterotrophic bacteria in the air at individual sites (Table 2), it was found that the lowest number of these bacteria occurred at site I (79 cells/m^3), while the highest was at site XII, located in the Old Town (189 cells/m^3).

The number of bacteria belonging to the *Enterobacteriaceae* family in the studied air was also subject to seasonal variations (Fig. 2). In the air of the residential district Rubinkowo, there were on average more of them in July (10 cells/m^3), while in the air of Toruń's Old Town the highest number of these bacteria was noted in June and August (about 6 cells/m^3). Within the Rubinkowo district, the lowest number of bacteria from the *Enterobacteriaceae* family was noted in February and March (on average less than 1 cell/m^3). However, the lowest number of bacteria was isolated from the air of the Old Town in February (on average 0.25 cells/m^3).

Analyzing the number of bacteria from the *Enterobacteriaceae* family in the air at different sites (Table 2), it can be seen that it did not undergo significant variations, the greatest number of these bacteria being isolated from the air taken from site IX.

Table 2. Number of microorganisms in 1 m^3 air at the investigated sites (average from the entire research period).

Research site	The number of heterotrophic mesophylic bacteria	The number of bacteria from the *Enterobacteriaceae* family	The number of mould fungi
1	79	2	66
2	85	3	53
3	90	3	49
4	102	4	140
5	186	4	74
6	143	2	86
7	130	2	65
8	148	4	76
9	111	6	53
10	124	2	108
11	143	2	120
12	189	4	83

The number of mould fungi in the tested air also depended on the season (Fig. 3). In the air of the Rubinkowo district on average the highest number of these fungi occurred in June (285 cells/m^3), and the lowest in August and December (29 and 39 cells/m^3, respectively). In the samples of air taken in the Old Town, the highest number of fungi was found on average in July (188 cells/m^3), in June (171 cells/m^3) and in February (184 cells/m^3), while the lowest number of these microorganisms was observed in December (21 cells/m^3).

Comparing the average number of mould fungi in the air at individual sites (Table 2), it was found that, in the Rubinkowo district, the highest number occurred at site IV (on average 140 cells/m^3), while the lowest was at site III (49 cells/m^3). On the other hand, within the Old Town, the greatest number of these microorganisms was noted at site XI (120 cells/m^3), and the lowest at site IX - only 53 cells/m^3.

The results of the research on the morphology of the bacteria isolated from the air are presented in Figs. 4 and 5. They indicate the predominance of Gram-positive forms over Gram-negative forms. The number of Gram-nega-

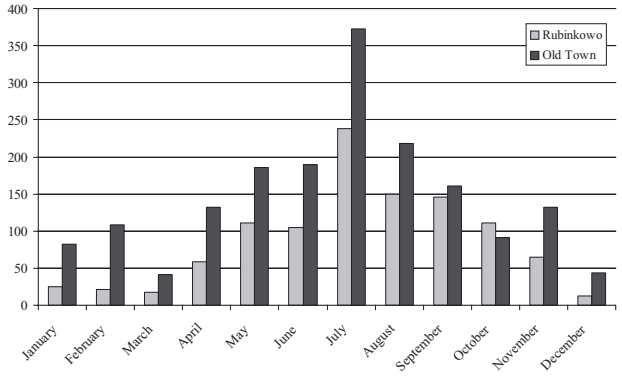


Fig. 1. Number of heterotrophic mesophylic bacteria within Toruń (cells / 1 m^3).

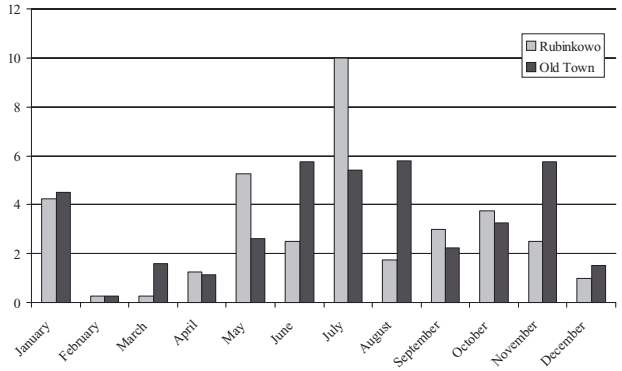


Fig. 2. Number of bacteria from the *Enterobacteriaceae* family within Toruń (cells / 1 m^3).

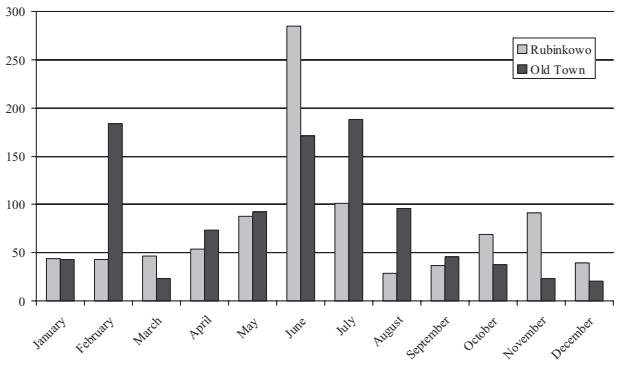


Fig. 3. Number of mould fungi within Toruń (cells / 1 m^3).

tive bacteria on the whole remained at a level not exceeding 10% in the tested samples. The samples taken in April in the Rubinkowo district and in August in the Old Town were exceptions, where the proportion of Gram-negative bacteria was 16 and 14%, respectively. In the case of Gram-positive bacteria, in the period from September to February, a greater proportion of spherical forms was observed in the air of both the Rubinkowo district and the Old Town. Comparing the results of morphological research on bacteria isolated from the air of the Rubinkowo district and the Old Town, no significant differences were found; only the proportion of pleomorphic forms was greater in the air of the Rubinkowo district.

The seasonal changes in the antibiotic resistance of the tested strains are presented on Figure 6. In the case of penicillin, the sensitivity to it varied from 29% of strains in the spring period to 50% in the case of bacteria isolated in the winter months. Resistance to this antibiotic was higher among strains isolated in spring and autumn than in summer and winter.

The highest percentage of bacteria resistant to erythromycin was observed in the summer period (63% of strains were resistant), while the lowest was in the autumn period (14% of strains). In spring and winter close to 30% of strains were found to be resistant. The percentage of sensitive strains varied from 26% in the summer to 73% in the autumn.

The highest number of strains resistant to streptomycin (58%) was found in the spring. In the summer and winter, fewer than 15% of strains were resistant to this antibiotic. The percentage of sensitive strains was 30% in spring, while in the remaining seasons it varied from 62% in autumn to 79% in winter.

The number of strains resistant to tetracycline varied from 9% in winter to 29% in spring. In the summer, 10% of resistant strains were found, while in autumn it was 17%. The percentage of sensitive strains varied from 54% in spring to 77% in autumn.

The number of strains resistant to vancomycin varied from 4% of the tested samples in winter to 23% in summer. In spring and autumn resistance to the effects of vancomycin was 10 and 7% of the tested strains, respectively. The percentage of strains sensitive to this antibiotic varied from 67% in autumn to 87% in winter.

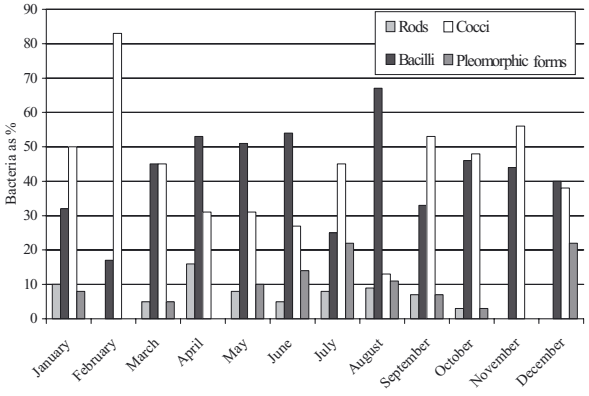


Fig. 4. Occurrence of individual morphological forms among bacteria isolated from air in the Rubinkowo district.

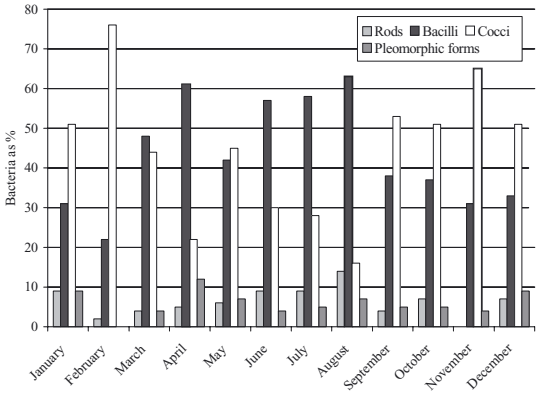


Fig. 5. Occurrence of individual morphological forms among bacteria isolated from air in the Old Town.

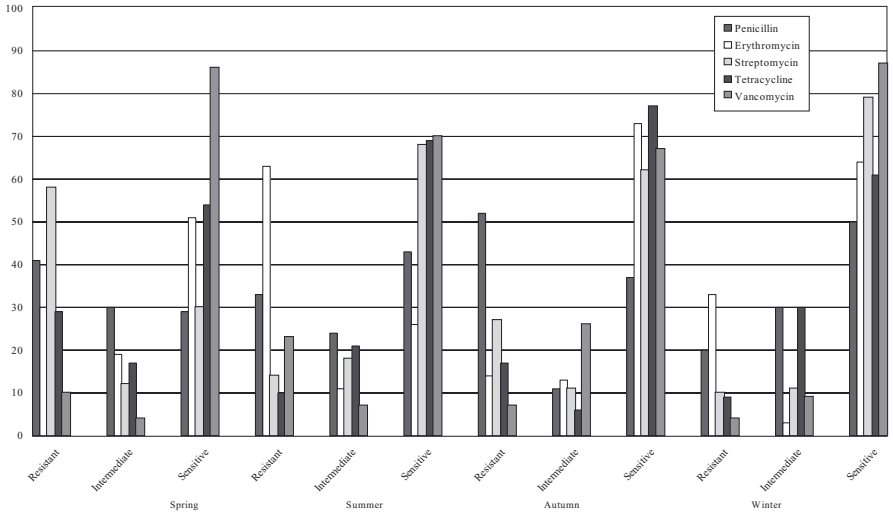


Fig. 6. Antibiotic resistance of bacteria isolated from air in Toruń.

Discussion

Analyzing the results of the microbiological research on the air, it should be remembered that the results obtained are temporary values, occurring at the moment the measurement was taken in the closest vicinity of the research site. In connection with the physico-chemical properties of the air, the degree of contamination of the air at a given point can change diametrically within a few minutes [8]. Hence, the results presented in this paper are approximate, enabling us to determine if there are many or few microorganisms at a given moment.

In recent times, microbiological analyses of the air have most often been conducted in the close vicinity of sites for collecting domestic waste and sewage treatment plants, or plants connected with the food industry [13]. Research carried out in recent years has shown that some of the emitted contamination can be transported over considerable distances. An example might be the results of research in 2000 on the area of the Municipal Rubbish Dump "Katarzynka" in Toruń, where at a distance of about 3 kilometres from the site where communal waste was collected air contamination was several times higher than in the direct vicinity of the rubbish heaps [14]. Therefore it is also important to research the emission of biological contamination in areas where people are present and are exposed to the effects of biological factors contained in the atmosphere on the one hand, and on the other are one of the sources of microorganisms in the air [15,16]. The presence of many people in the vicinity of the research point was also one of the causes of differences in the degree of air contamination in the "Rubinkowo" residential district and in the Old Town. The greater contamination in the Old Town was also connected with traffic, which raised dust containing microorganisms [10]. Not without significance for the degree of contamination of the air is the presence of buildings and trees covered in dust-containing microorganisms [17], which, when blown off by the wind, leads to an increase in the contamination of the analyzed air. In Rubinkowo, points in the west of the district were located in places with lighter traffic and far from high-rise buildings, which could have been the cause of the lower values of the tested microbiological parameters [8].

The lack of any data concerning air pollution in Toruń in earlier years (as such research was not carried out) made it impossible to compare changes in the microbiological contamination of the air in Toruń happening over the years. If such research had been carried out earlier, it would have enabled us to determine the changes in the microbiological contamination of the air happening in Toruń over time and to determine the direction of these changes in the closest vicinity to humans.

Equally, conducting future culture studies on the antibiotic resistance of bacteria in the air could signal the process of bacteria found in biological aerosols of the air breathed by people and animals, becoming resistant to antibiotics commonly used in medicine.

The results obtained in the research on the number of mesophylic bacteria and mould fungi were similar to results of research obtained in the 60s by Krzysztofik [10] in Warsaw. The Warsaw team studied the total number of bacteria using the sedimentation method and revealed higher air pollution than that found in Toruń. The highest result was obtained in July 2001 in Strumykowa street in Toruń and was 4461 cells/m^3. It was probably caused by the higher number of pedestrians, who were an additional source of these microorganisms.

The relatively high number of mould fungi testifies to the fact that these organisms are well adapted to spreading in the air [18]. These organisms can live practically anywhere, but have particularly favourable conditions in homes, where, having entered from outside, they develop, affecting the health and well-being of the residents [10, 18, 19, 20]. Apart from this they develop on the facades of houses, on the surface of the soil, and they grow over trees and bushes [21, 5]. The above factors could have affected the higher number of mould fungi in the Old Town.

The microclimatic conditions prevailing in a given area have a significant influence on the number of microorganisms in the air [22, 23]. Thus, in the winter months, when the weather conditions are less favourable for microorganisms than in other seasons, lower numbers of bacteria and fungi were noted than in the summer-autumn period, when microorganisms can develop with no difficulty in the environment and spread aerogenically.

The great sensitivity of pathogenic organisms to the negative effect of meteorological factors added to the lack of sources of emission could be the cause of the small number of these bacteria in the studied air. The best example to confirm this phenomenon is the small number of enterobacteria, which are a classic indicator of water contamination [24, 25]. As the research conducted by Filipkowska [26] shows, the number of bacteria from the *Enterobacteriaceae* family in the air in the vicinity of sewage treatment plants can reach considerable values [27, 28]. Research conducted using the collision method on the area of the Municipal Sewage Treatment Plant in Toruń [29, 30] did not reveal large differences in the number of enterobacteria in comparison with the results of research conducted on densely populated areas. It should be taken into account here that the results of the research, lower than those obtained by the Olsztyn group, could have been affected by the modern construction of the treatment plant in Toruń, resulting in a low emission of biological contamination into the air. However, further research would be required in order to verify this hypothesis unequivocally.

Examining the results of the morphological research on bacteria in the air in the town of Toruń, the predominance of Gram-positive forms should be emphasized. They almost always constituted over 90% of the studied microflora. From March to August a predominance of bacilli was observed, which produced spores, enabling them to bear for longer the unfavourable conditions prevailing during transport in atmospheric air [1]. In February 2001

and from September 2001 to January 2002, a predominance of spherical forms was observed. They constitute a significant component of the microflora on the surface of the bodies of humans and animals [31]. The particularly high proportion of spherical forms in February, when the number of bacteria in the air was small, may suggest that the main sources of microorganisms in the air are people and dogs passing close by the sites for taking air samples. Considering the percentage of morphological forms in the studied air samples, it should be remembered that only mesophylic bacteria were studied, as recommended by the Polish Norm [32]. Taking into account psychrophylic forms, which include the majority of autochtonic bacteria living in the natural environment, could have an effect on the percentage of individual morphological forms in the studied samples.

The results of the research on the antibiotic resistance of bacteria isolated from the air during a year revealed the presence of a relatively high number of bacteria resistant to antibiotics. The relatively high percentage of mesophylic bacteria resistant to antibiotics allows us to assume that these strains came into contact with antibiotics in the past, as a result of which they developed resistance, or that they came into contact with resistant bacteria and obtained from them genes giving them resistance to antibiotics. The large number of strains resistant to penicillin might be connected with the fact that this antibiotic has been used for many years in medicine, as a result of which many strains have become resistant to it. The observed seasonal changes in the number of bacteria resistant to the antibiotics used are probably not connected with the changing environmental conditions. Unfortunately, there is a lack of comparative data which would allow the results from this paper to be compared to results obtained in other towns and to conduct a broader discussion.

Conclusion

This work shows the results of the studies on the microbiological contamination of the atmosphere in Toruń. The results indicate that the microorganisms were more numerous in the air on the area of the Old Town than in Rubinkowo. This result was strongly influenced by the intensive movement of people in the proximity of the control points. The elevated contamination in the Old Town was also related to traffic that caused the movement of dust particles containing microorganisms. The number of microorganisms underwent seasonal changes. The maximum mesophylic heterotrophic bacteria was found in summer while the minimum in winter. Most of the isolated bacteria belonged to Gram positive forms. They constituted almost always more then 90% of studied microorganisms.

The results of the studies on the resistance to antibiotics showed that the bacteria isolated from the air during the year were in quite a significant percentage resistant to antibiotics. A high proportion of antibiotic-resistant bacteria suggest that these strains had contact with antibiotics in the past and have acquired resistance to them or the resistance genes were transferred to them from other antibiotic-resistant bacteria. The highest number of resistant strains were selected if penicillin was used. This finding may be related to a long-term application of this antibiotic in medicine.

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