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BACTERIOLOGICAL POLLUTION OF THE ATMOSPHERIC AIR AT THE MUNICIPAL AND DAIRY WASTEWATER TREATMENT PLANT AREA AND IN ITS SURROUNDINGS

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Keywords: Air pollution, bioaerosol, wastewater treatment plant, *Enterobacteriaceae*, *Staphylococcus*, *Enterococcus*, actinomycetes.

Abstract: Microbiological studies were carried out of atmospheric air sampled on the area and in the surroundings of a mechanical and biological wastewater treatment plant (WTP) treating municipal sewage. The capacity of the wastewater treatment plant, which also received some wastewater from the dairy industry, was *ca* $3 \cdot 10^3$ $m^3 \cdot d^{-1}$. Counts of heterotrophic psychrophilic, psychrotrophic and mesophilic bacteria as well as some physiological groups of microorganisms which belong to *Enterobacteriaceae* family, *Staphylococcus* and *Enterococcus* genera, *Pseudomonas fluorescens* and *P. aeruginosa* species, hemolysing bacteria and actinomycetes were analyzed. Air samples were collected in summer, autumn, winter and spring seasons simultaneously by the sedimentation and impact methods at 6 sites located on the area of the WTP and at 5 sites situated in its surroundings. The background was established depending on the direction of wind, always on the windward side in relation to the location of the WTP. In addition, temperature and air humidity as well as wind speed and direction at each sampling sites were observed. Statistically significant differences were found in studied groups of microorganisms counts between air samples collected in different seasons of the year (with the exception of psychrophilic bacteria and by the two different methods (with the exception of psychrophilic bacteria) and microorganisms which belong to *Enterobacteriaceae* family). The highest mean counts of the microorganisms were usually determined in air samples collected by the sedimentation method, especially during the autumn (with the exception of actinomycetes, which are the most numerous in spring), the lowest ones in winter and/or in summer. No statistically significant differences were observed in counts of the analyzed groups of microorganisms in air sampled at particular sites (with the exception of *Enterobacteriaceae* bacteria isolated on Chromocult medium). However, higher counts of these microorganisms were typically found in the air sampled in the area of the WTP, particularly near the grit chamber, phosphorus removal tank, nitrification and denitrification chambers and secondary settling tank. According to the Polish Standards used for evaluation of atmospheric air pollution, the air sampled in the area of wastewater treatment plant and in its surroundings was classified as only slightly and sporadically strongly polluted. It was mainly in the spring and autumn seasons that the air was strongly polluted with psychrophilic and mesophilic bacteria. No increased emission of the analyzed groups of microorganisms, including faecal bacteria was determined in the air samples collected outside the WTP.

INTRODUCTION

Many municipal facilities which were originally built outside towns and cities are now, due to intensive growth of urban agglomerations, very close to areas designated for housing purposes [1]. In Poland, the number of municipal WTPs which can be a source of severe emission of chemical compounds and bioaerosols has increased five times during the past 15 years [1, 9]. Precise evaluation of the density of microorganisms in a biological aerosol is difficult due to a high number of parameters which have direct and indirect influence on counts of aerial microflora [11]. The size of emission as well as the species composition of an emitted bioaerosol depends on the source of emission. Moreover, initial density of bioaerosol is shaped by the concentration of microorganisms in sewage, growth phase of microbes, emission threshold, sewage purification technology, aeration conditions as well as meteorological and natural conditions [5–7, 11, 12]. Domestic sewage has rich microflora, which comprises numerous saprophytic microorganisms as well as potentially pathogenic or pathogenic microbes belonging to the families of *Enterobacteriaceae*, *Pseudomonadaceae* and *Micrococcaceae*. In addition, there are also large numbers of filamentous fungi, such as moulds, yeasts and yeast-like organisms [3, 11, 21]. Dark and Stretton [2] found out that the surface layer of wastewater undergoing aeration could contain highly concentrated amounts of microorganisms, the fact which facilitated their transfer to atmospheric air. The density of microorganisms in this layer of wastewater as well as in aerosols expelled to the air can be 10- to 1.000-fold higher than their concentration in the middle part of an aeration tank. Filipkowska *et al.* [7] reported that a sewage aeration system applied along with the hermetic properties of the facilities determined the size and reach of their emission. They found out that microorganisms spread over a smaller area when submerged rather than surface aeration devices were applied. Kaźmierczuk *et al.* [11] demonstrated that wastewater droplets, containing various microorganisms, could be swept from some facilities at a WTP, such as aeration chambers, biological beds and grit chambers, and could be transferred by air over long distances. Microorganisms present in a bioaerosol can cause various allergic, toxic and immunologic effects. The following are the most often responsible for causing allergies: spores of fungi and actinomycetes, flower pollens, fragmented animal tissues or dried secretions and excreta. Health risk is caused by presence of both live and dead microorganisms in air [11]. Dead microorganisms are dangerous because their cells contain various types of toxins, which cause allergies, attack the immunological system and can be cytotoxic. Such effects can be produced, for example, by bacteria of the speciesof *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* or *Enterobacter* sp. Considering the above, the aim of our study was to determine the microbiological pollution of air sampled on the area of the WTP and to find out to what extent emissions from the plant polluted its surroundings.

MATERIALS AND METHODS

Study area and sampling collection

Microbiological pollution of air sampled on mechanical and biological WTP area and its surroundings (of a 3000 $m^3 \cdot d^{-1}$ capacity) purifying municipal sewage (including up to 15% of wastewater from the dairy industry) was examined. There were six sampling sites in the WTP area (Fig. 1) near the grit chamber, phosphorus removal tank, nitrification/denitrification chambers, secondary settling tank, gravitational sludge thickener and mechanical sludge dehydration site. Five other sampling sites were situated near the wastewater treatment plant fence and at a distance of 0–50 m, 50–100 m, 100–150 m and 150–200 m from the fence of the plant. The control site, i.e. the background, was always situated on a windward side, outside the plant. When selecting the location of air sampling sites, places where emission of aerosols and bacterial dust was potentially the highest were preferred. Another factor taken into consideration was the direction of winds blowing on the days when samples were collected, both at the wastewater treatment plant and in its surroundings.

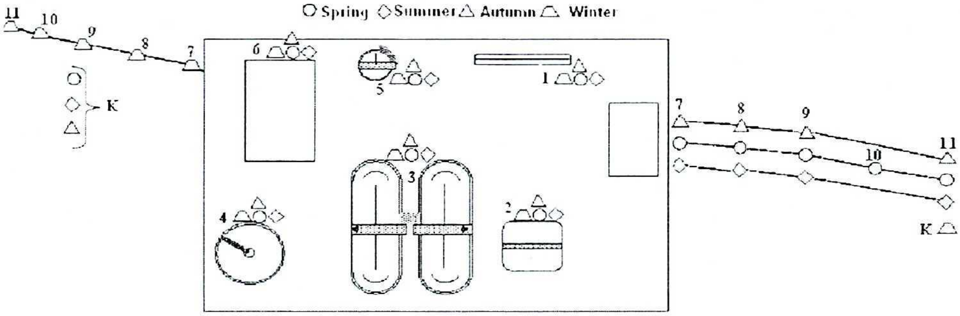

Fig. 1. Location sketch of Wastewater Treatment Plant; K, 1, 2,..., 11 – air sampling sites

K – Control site
1 – Grit chamber
2 – Phosphorus removal tank
3 – Nitrification/denitrification chambers
4 – Secondary settling tank
5 – Gravitational sludge thickener
6 – Mechanical sludge dehydration site
7 – Fence
8 – 50 m
9 – 100 m
10 – 150 m
11 – 200 m

Microbiological analyses

Collection of atmospheric air samples was carried out simultaneously by the sedimentation and impact methods. Air samples were collected in the summer and autumn 2005, and in the winter and spring 2006. In total, 24 air samples were collected from each sampling site. The microbiological studies involved determination of:

1. Total counts (CFU·m^{-3}) of psychrophilic bacteria on broth agar after 72 h and 7 days of incubation at 22°C;
2. Total counts (CFU·m^{-3}) of psychrotrophic bacteria on broth agar after 72 h and 7 days of incubation at 26°C;
3. Total counts (CFU·m^{-3}) of mesophilic bacteria on broth agar after 24 h incubation at 37°C;
4. Number (CFU·m^{-3}) of mannitol-positive and mannitol-negative *Staphylococcus* sp. bacteria on Chapman's medium after 48 hours of incubation at 37°C;
5. Number (CFU·m^{-3}) of haemolysing bacteria on agar-bullion medium with 7% sheep blood added after 48 hours of incubation at 37°C;
6. Number (CFU·m^{-3}) of *Pseudomonas fluorescens* on King B medium after 48 hours of incubation at 26°C;
7. Number (CFU·m^{-3}) of *Pseudomonas aeruginosa* on cetrimide-agar medium after 48 hours of incubation at 37°C;
8. Number (CFU·m^{-3}) of faecal enterococci on Slanetz and Bartley agar medium after 72 h incubation at 37°C;

9. Number ($CFU \cdot m^{-3}$) of *Enterobacteriaceae* bacteria on Chromocult Coliform Agar and Endo medium after 24 hours of incubation at 37°C;
10. Number ($CFU \cdot m^{-3}$) of actinomycetes on Pochon' medium after 7 days of incubation at 26°C.

The occurrence of *Pseudomonas fluorescens* and *P. aeruginosa* was verified under the light of a Wood UV lamp (wavelength 365 nm); colonies which produced fluorescein were counted. Additionally colonies which produced blue-green pyocyanin on cetrimid-agar medium were counted too.

Typical *Enterobacteriaceae* colonies grown on Endo and Chromocult media, and *Staphylococcus* sp. on Chapman's medium were inoculated onto the agar-bullion medium with 7% sheep blood added in order to multiply the bacteria and detection of haemolysins.

Additionally, bacteria of the *Enterobacteriaceae* family were analyzed for the lack of production cytochrome oxidase (using 1% tetramethylo-p-phenyldiuoamine solution). They were finally identified with API 20E tests (bioMerieux).

In addition to testing for haemolysins, *Staphylococcus* sp. were analyzed for the production of catalase (using 3% hydrogen peroxide solution) and were also checked for their capability of producing coagulase enzyme using lyophilized rabbit plasma with EDTA. Final identification was carried out with API STAPH tests (bioMerieux).

All the determinations were preceded by determination of motility of bacteria and their response to staining by the complex Gram's Method.

Parallel to the determination of bacteria presence in air samples, quantitative and qualitative yeasts and moulds contamination of air was analyzed. Results of this part of studies will be publicized in another paper.

Meteorological observation

Parallel to the microbiological assays, observations of some meteorological parameters were conducted. The direction and speed of winds, air temperatures and relative humidity at a given sampling site were observed. During the study, the speed of winds ($m\ s^{-1}$) in particular seasons of the year varied from in summer 0–3.7; in autumn 0–1.5; in winter 0–2.9; in spring 0–2.1. Temperature (°C) varied: in summer from 23 to 28.7; in autumn from 10.8 to 20; in winter from -1 to 4.4; in spring from 19 to 28. The relative air humidity in the summer was 40–61%, in autumn – 48–89%, in winter – 54–75% and in spring – 30–50%.

Statistical evaluation

In order to obtain information concerning potential differences between studied micro-organisms numbers (dependent variables) in the atmospheric air for various methods, time and place of samples collecting (independent variable), a single and double factor analysis of variance (ANOVA) was conducted, verifying the hypothesis of the equality of means (H_0: $x_1 = x_2 = \ldots = x_5$) at the level of significance $\alpha = 0.05$, assuming that the variance for the numerousness of the bacteria groups under study are uniform [20].

RESULTS AND DISCUSSION

Wastewater treatment plants are a source of biological aerosols and contribute to atmospheric air pollution. Wastewater treatment technologies (for example aeration and mixing) generate tiny droplets, which escape to the air along with the microorganisms they contain. Average counts of psychrotrophic and mesophilic bacteria in sewage collected from the nitrification/denitrification chamber of the studied plant were $1.6 \cdot 10^7$ and $9.8 \cdot 10^6$ cfu in 1 cm^3, respectively. Moreover, numbers of intestinal bacteria belonging to the *Enterobacteriaceae* family and *Enterococcus* genus (up to $2.1 \cdot 10^8$ and $1.6 \cdot 10^7$ MPN per 100 cm^3, respectively) were determined to be high. From the *Enterobacteriaceae* family, species *Escherichia coli, Klebsiella oxytoca, Enterobacter sakazaki, Proteus penneri, Pantoea* spp.2 were predominatingly isolated from sewage in the examined plant [14].

Many authors implied an aeration chamber as the biggest source of biological aerosols [1, 4]. In their study on a WTP similar in its parameters to the one we investigated, Kaźmierczuk *et al.* [11] most often determined presence of bacteria of the *Enterobacteriaceae* family and haemolysing bacteria in the air collected near aeration chambers, which could suggest that in this part of a WTP, irrespective of the direction of a current wind, there were always some indicator microorganisms, most probably derived from the sewage. In air samples collected near the edge of an aeration chamber, these authors isolated bacteria of the genera *Klebsiella* and *Proteus*. They are potentially pathogenic bacteria, which are responsible for diseases of respiratory track and/or other internal organs in humans with weak immunological system [16]. Likewise, air samples collected near the aeration chambers of the examined WTP contained increased levels of mesophilic bacteria as well as intestinal bacteria belonging to the *Enterobacteraceae* family, reaching $5 \cdot 10^3$ and $6 \cdot 10^2$ cfu·m^{-3}, respectively (Fig. 2). Kaźmierczuk *et al.* [11] concluded that two zones could be distinguished on the WTP area from microbiological menacing. One was the aforementioned area near the reactors with surface aerating turbines, where the counts of bacteria, yeasts and yeast-like fungi were higher. The other zone in the WTP area was the area near the facilities used for mechanical sewage purification. Although high numbers of microorganisms in air sampled in vicinity of aerating chambers have been affirmed in literature, it does not find the confirmation in studied WTP. This could be connected with using horizontal roller to aerating sewages, placed under the platform, as well as high concrete screens around aerating chambers, which limited emission of bioaeroslol from these elements of technological process. The air samples collected near the grit chamber in the examined WTP were characterized by higher numbers of mesophilic microorganisms (up to $3.5 \cdot 10^3$ cfu m^{-3}) and bacteria of the *Enterobacteriaceae* family (up to $3 \cdot 10^2$ cfu·m^{-3}) as well as greater species diversity among the latter bacteria [14]. Moreover, at this sampling site higher numbers of α- and β-haemolysing bacteria were determined too ($2 \cdot 10^2$ and $5.5 \cdot 10^2$ cfu·m^{-3}, respectively). This could be connected with lack of casing of mechanical sewage treatment facilities. Similar results were obtained by Fracchia *et al.* [8], who analyzed air at two WTP in Piedmont province, northern Italy. Their analyzes proved that mesophilic and thermophilic bacteria appeared in higher counts (up to $4 \cdot 10^4$ cfu·m^{-3}) in air near a grit chamber and a retarding reservoir. This may have been due to the fact that sewage flowing fast through the grit chamber splashed on its concrete walls and became dispersed. The flow of influent sewage generates a stream of air, which can easily carry dispersed sewage particles. Furthermore, the grit chamber is fitted with a pump which sucks the mineral suspension solids from the bottom of the chambers and expels it with great force to a trough along the grit chamber. This may have had an additional effect on the results of the microbiological assays [11]. Higher counts of mesophilic bacteria and *Enterobacteriaceae* cells were also determined in air sampled near the phosphorus

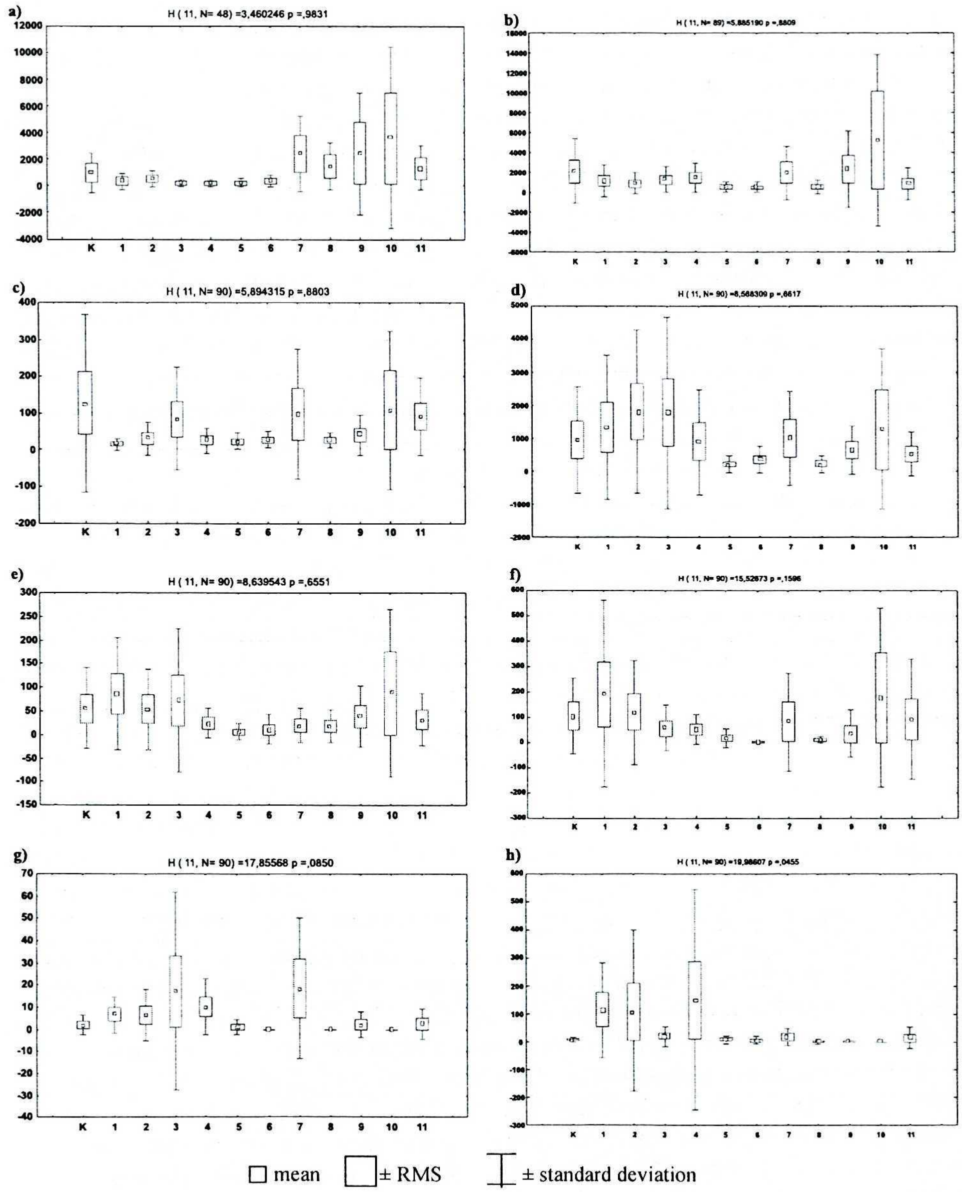

Fig. 2. Average numbers (± standard deviation and ± random mean square-RMS) [cfu·m^{-3}] of a) psychrophilic, b) psychrotrophic, c) actinomycetes, d) mesophilic e) α-haemolysing, f) β-haemolysing bacteria and g) *Enterobacteriaceae* bacteria isolated on Endo medium and h) *Enterobacteriaceae* bacteria isolated on Chromocult medium from air samples collected at different site during whole time of study; independent variable (assembling): site of samples collection; ANOVA test of Kruskal-Wallis' ranges

K – Control site
1 – Grit chamber
2 – Phosphorus removal tank
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removal tank and secondary sedimentation tank, where the respective counts were $4.5 \cdot 10^3$ and $4 \cdot 10^2$ cfu·m^{-3} as well as $2.5 \cdot 10^3$ and $5.5 \cdot 10^2$ cfu·m^{-3}. Nonetheless, statistical analyses of these results did not prove the existence of significant differences between counts of most of the studied microorganisms (with the exception of *Enterobacteriaceae* bacteria isolated on Chromocult medium) in air sampled at different sites.

No statistically significant correlation between counts of the analyzed groups of microorganisms and meteorological parameters were observed. But when comparing the results obtained from air samples collected in particular seasons of the year during the whole study period, the highest numbers of most of the analyzed groups of microorganisms were found in autumn (with the exception of psychrophilic bacteria and actinomycetes, which are the most numerous in spring), and the lowest ones – in winter (Fig. 3). This is confirmed by the statistical analyses of the results, which proved significant differences in counts of most of the analyzed microorganisms (with the exception of psychrophilic bacteria) in air samples collected in different seasons. The highest mean counts of these bacteria tended to be determined in autumn which could be connected with less insolation in autumn season during the time of study. Miquel [17], who studied microflora in atmospheric air in Paris, concluded that quantitative variations in numbers of bacteria depended on several factors, including season of the year, meteorological conditions, atmospheric precipitation, sunlight, research site and elevation. Duration of time periods when the general number of bacteria varied was also largely dependent on the overground conditions, intensity of the growth of microorganisms in soil and water as well as on different types of refuse, or plant and animal remains [15]. Thus, high numbers of psychrophilic bacteria and actinomycetes determined in air samples collected outside the examined WTP in spring could be explained by the fact that they were typical autochthonous organisms, most probably originating from a nearby forest. Kalisz *et al.* [10] claimed that actinomycetes were permanently present in atmospheric air, both in urbanized areas and near municipal facilities. Kołwzan and Traczewska [13] found out that actinomycetes occurred in lower counts in the air sampled on WTP area than in the background air. From the above it has been concluded that actinomycetes cannot be taken into consideration as indicator microorganisms when attempting to establish the range of impact of urban infrastructure facilities on the sanitary condition of atmospheric air.

While analyzing the results of microbiological studies of air samples collected on the WTP area and surroundings, we discovered that higher mean counts of the analyzed groups of microorganisms occurred in air sampled by the sedimentation method. Nevertheless these differences were statistically significant for numbers of psychrophilic bacteria and actinomycetes only. Turzeckij and Olenowa [15], who reviewed 179 research projects involving atmospheric air pollution, carried out with an aid of the sedimentation method and the impact methods using a Krotov's device, agreed that the sedimentation method generates 3- to 6-fold higher results per 1 m^3 than the impact method with a Krotov's device. This may have been attributed, for example, to the fact that some of microorganisms could be swept by a strong air current, produced by the air sampler, which means that some of them cannot settle on a medium placed in the apparatus. Furthermore, some microorganisms bounce away from the medium while air is being sucked in, which can also lower the results of analyses [11].

When considering the results of the evaluation and the current PN-89 Z-04111/02 [18] and proposed prPN-Z-04111-2 [19] Polish Standards of degree of air pollution, the air sampled both in the area of WTP and in its surroundings was classified as slightly and

Lambda Wilksa = .54104, F(21, 215.91) = 2.4523, p = .00065

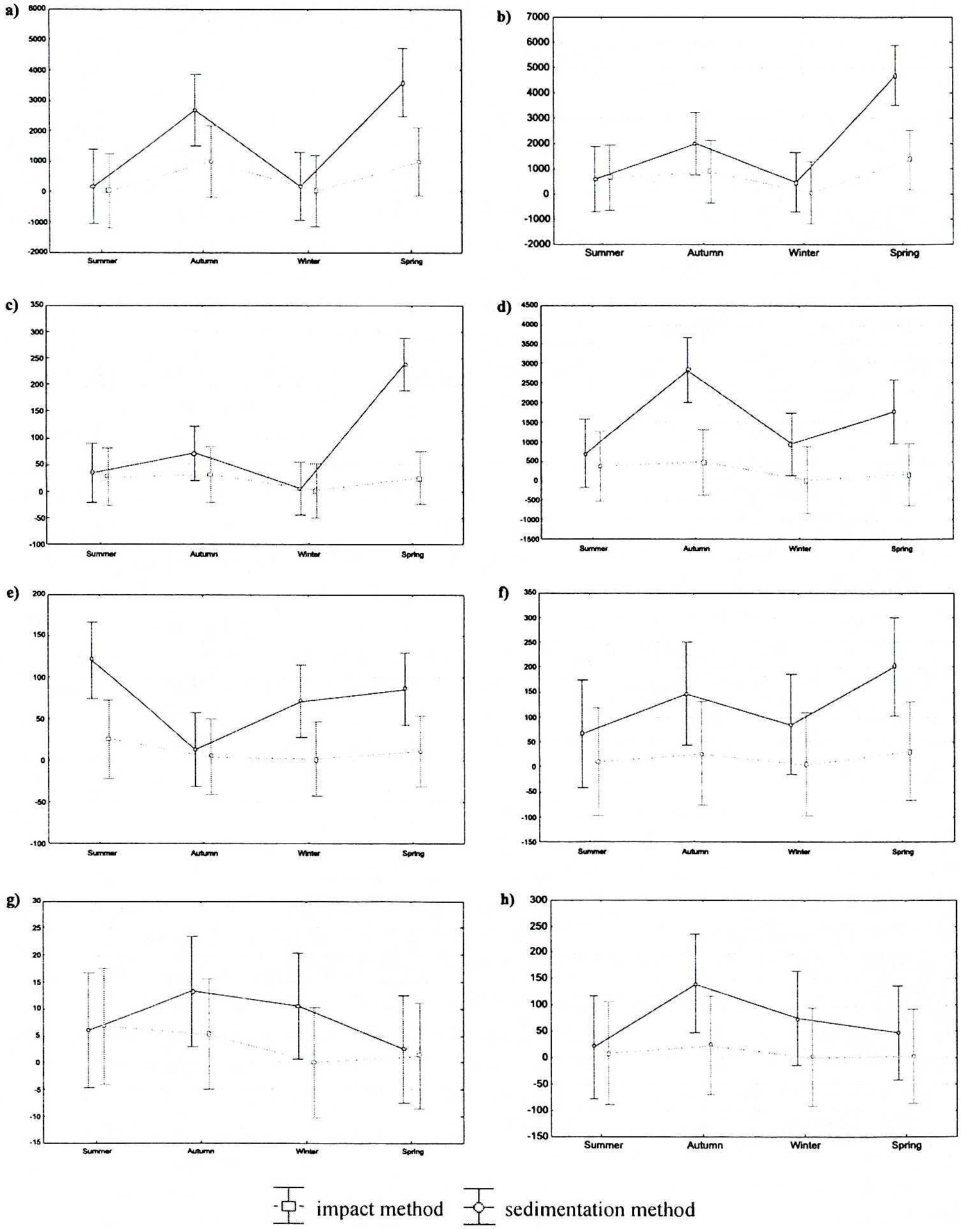

Fig. 3. Expected marginal means (some are not estimated) [cfu·m^{-3}] of a) psychrophilic, b) psychrotrophic, c) actinomycetes, d) mesophilic e) α-haemolysing, f) β-haemolysing bacteria and g) *Enterobacteriaceae* bacteria isolated on Endo medium and h) *Enterobacteriaceae* bacteria isolated on Chromocult medium from air samples collected by impact and sedimentation methods during whole time of study; independent factors: method and time of air samples collection; decomposition of effective hypotheses; vertical columns mean 0.95 confidence intervals

sporadically strongly contaminated. In spring and summer mainly there was some strong contamination of air caused by psychrophilic and mesophilic bacteria. The air at and near the WTP was not determined to have contained bacteria of the species *Pseudomonas aeruginosa*. Cells of *Psedumonas fluorescens* and bacteria of the genus *Staphylococcus* occurred only sporadically. Nonetheless, even sporadic presence of faecal bacteria, including potentially pathogenic ones, in the air sampled outside the plant suggests that it is necessary to monitor air near wastewater treatment plants, which will enable us to learn more about the impact of such objects on natural environment and human health.

CONCLUSIONS

According to the current and proposed Polish Standards, it can be said that the air sampled in the area of wastewater treatment plant and in its surroundings during whole time of study was only slightly and sporadically strongly contaminated. More severe pollution caused by psychrophilic, psychrotrophic and mesophilic bacteria occurred in spring and autumn.

Higher counts of the analyzed groups of microorganisms were found in air sampled by the sedimentation method, although these counts were directly proportionally correlated with the numbers of bacteria isolated from air samples collected by the impact method.

Significant differences in counts of the analyzed microorganisms relative to seasons of the year were statistically confirmed for most of the groups of bacteria determined in this study. The highest mean counts of these bacteria tended to be determined in autumn which could be connected with less insolation in autumn season during time of study.

Lack or sporadic occurrence of indicator microorganisms in the analyzed air samples collected at the sites outside the plant may suggest that the wastewater plant did not produce negative influence on its surroundings. This could be connected with using horizontal roller to aerating sewages, placed under the platform, as well as high concrete screens around aerating chambers, which limited emission of bioaeroslol from these elements of technological process.

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BAKTERIOLOGICZNE ZANIECZYSZCZENIE POWIETRZA NA TERENIE I W OTOCZENIU OCZYSZCZALNI ŚCIEKÓW KOMUNALNYCH ORAZ Z PRZEMYSŁU MLECZARSKIEGO

Badania powietrza atmosferycznego przeprowadzono na terenie i w otoczeniu mechaniczno-biologicznej oczyszczalni ścieków komunalnych (z udziałem ścieków z przemysłu mleczarskiego) o przepustowości około 3000 $m^3 \cdot d^{-1}$. Badano liczebność bakterii heterotroficznych psychrofilnych, psychrotrofowych i mezofilnych oraz wybranych grup fizjologicznych drobnoustrojów: z rodziny *Enterobacteriaceae*, rodzajów *Staphylococcus* i *Enterococcus*, gatunków *Pseudomonas fluorescens* i *P. aeruginosa*, bakterii hemolizujących oraz promieniowców. Powietrze do badań pobierano w sezonach letnim, jesiennym, zimowym i wiosennym, równolegle metodą sedymentacyjną i zderzeniową na 6 stanowiskach usytuowanych na terenie oczyszczalni oraz na 5 stanowiskach usytuowanych w jej otoczeniu. Tło wyznaczano w zależności od kierunku wiatru po stronie nawietrznej w stosunku do położenia oczyszczalni. Ponadto prowadzono pomiary temperatury i wilgotności powietrza oraz prędkości wiatru na poszczególnych stanowiskach badawczych. Stwierdzono statystycznie istotne różnice w liczebności poszczególnych grup badanych drobnoustrojów w powietrzu pobieranym w różnych sezonach badawczych (z wyjątkiem bakterii psychrofilnych) oraz różnymi metodami (z wyjątkiem bakterii psychrofilnych i bakterii z rodziny *Enterobacteriaceae*). Najwyższe średnie ich liczebności stwierdzano zazwyczaj w próbach powietrza pobieranego metodą sedymentacyjną zwłaszcza jesienią (z wyjątkiem promieniowców, których maksymalne liczebności stwierdzano wiosną), najniższe zaś zimą i/lub latem. Zazwyczaj (z wyjątkiem bakterii z rodziny *Enterobacteriaceae*) nie stwierdzano istotnych statystycznie różnic w liczebności badanych grup drobnoustrojów w powietrzu pobieranym na poszczególnych stanowiskach. Jednakże wyższe ich liczebności występowały najczęściej w powietrzu pobieranym na stanowiskach usytuowanych na terenie oczyszczalni ścieków, a szczególnie w pobliżu piaskownika, komory defosfatacji, komór nitryfikacji i denitryfikacji oraz osadnika wtórnego. Uwzględniając kryteria oceny stopnia zanieczyszczenia powietrza atmosferycznego zawarte w Polskich Normach, powietrze pobierane zarówno na terenie, jak i w otoczeniu oczyszczalni zaklasyfikowano jako mało zanieczyszczone, sporadycznie, głównie w sezonach wiosennym i jesiennym występowało silne zanieczyszczenie powietrza bakteriami psychrofilnymi, psychrotrofowymi i mezofilnymi. Nie stwierdzono podwyższonej emisji badanych grup drobnoustrojów, w tym również pochodzenia kałowego, poza obszar terenu oczyszczalni.