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ASSESSMENT OF THE MICROBIOLOGICAL CONTAMINATION OF AIR IN A MUNICIPAL SOLID WASTE TREATMENT COMPANY

OCENA ZANIECZYSZCZENIA MIKROBIOLOGICZNEGO POWIETRZA NA TERENIE ZAKŁADU PRZETWARZANIA ODPADÓW KOMUNALNYCH

Abstract: The subject of the study is the analysis of the number of microorganisms forming a microbiological aerosol in a municipal solid waste treatment plant in Krakow. The storage time of mixed municipal solid waste in the plant hall is 6–48 hours and the storage time of the produced alternative fuel is within the range of 12–96 hours. 18 employees work in the three shift system in the sorting facility. The air for research was sampled using a MAS-100 impactor (Merck, Switzerland) in three locations within the plant, four times within a year to assess the effect of meteorological conditions (temperature, humidity and dustiness) on the number of selected groups of microorganisms. It was found that the number of microorganisms changes with seasons and depends on the meteorological conditions as well as the air sampling location. Since the border values of bioaerosol concentration were exceeded, further research is required to assess the changes in the number of microorganisms with potential negative impact on human health.

Keywords: microbiological aerosol, air, municipal solid waste

Waste management is a civilizational problem. The human residence and business activity results in the creation of waste [1]. Due to the possibility of penalties incurred by Poland for large scale storage of biodegradable waste, mechanical, biological and thermal treatment and processing of municipal waste in special installations has become more popular in recent years. Bis at al [1] as well as Przybulewska at al [2] claim that the direct surroundings of this type of facilities may be contaminated microbiologically, with bacteria, viruses and fungi. This is the reason why every environmentally noxious

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facility of this type should be monitored, provided with a safety zone and recultivated after it is no longer in use. The problem of the microbiological contamination of air within and in proximity of waste storage and treatment facilities is growing [3]. More attention is given to the hygiene and sanitary work conditions for persons employed in waste segregation and treatment. Sufficient insulation of staff and office facilities from the working area in order to minimise the propagation of organic dust containing potentially pathogenic microorganisms is an important issue in waste sorting facilities. Unfortunately, there is little testing provided in Poland in the area of the microbiological analysis of air inhaled by the employees of such facilities. Due to occupational safety and health regulations, the dust content levels and noise emission levels are monitored in waste treatment plants. Analyses are also provided regarding the exposure to harmful factors, including microbiological aerosol in work areas. Possible health effects are also monitored, since work in the waste treatment industry involves the risk of allergies, upper respiratory tract infections, skin and mucous membranes conditions as well as immunotoxic and infectious diseases [4–9]. The degree of microbiological contamination of air in waste management facilities varies and depends on multiple parameters, such as: number of employees, room construction and ventilation, storage conditions, operation system, air temperature, humidity and dust content level [3, 10, 11]. This is the reason why, due to sanitary reasons, observing safe work principles should be promoted among employees [10]. Employees of waste management facilities are under the risk of exposure to aerosol-forming microbiological factors, such as: viruses, prions, bacteria, fungi, biological allergens and endotoxins [12–14]. Krajewski et al [15] studied the number and species composition of microorganisms present in the breathing areas available to employees working with the collection and treatment of municipal solid waste. Air samples were collected in selected work areas, *ie* waste collection point, reloading, sorting facilities and composting plant. In all analysed locations, the number of mesophilic bacteria and filamentous fungi exceeded 10^4 cfu $\cdot$ m^{-3} of air, an increased number of actinobacteria was found. A number of microorganisms potentially dangerous to human health was found, such as: *Escherichia coli, Enterococcus faecalis* and *Enterococcus faecium* as well as *Pseudomonas* bacteria.

Despite the fact that the air is not a natural habitat for microorganisms, as it does not favour their growth and divisions, it may form a temporary environment for them and enables their long distance transport. The time for which the microorganisms can survive in these conditions depends on their resistance to drying and availability of nutrients. They can be transported for long distances from their original habitat with wind [16]. Microorganisms are most frequently found in air in the form of endospores, spores, mycelium fragments as well as vegetative forms (bacteria, viruses), while still maintaining their potential harmfulness to the health of humans, animals and plants [10].

This is the reason for the assessment of the microbiological contamination of air in selected locations of a municipal solid waste treatment company in Krakow. Furthermore, the purpose of the analyses completed four times within a year was the determination of the impact of seasonal changes, including temperature, humidity and dust content to the number of the selected groups of microorganisms.

Materials and methods

Microbiological testing of air was completed using the MAS-100 impactor (Merck. Switzerland), in compliance with the guidelines of Polish Standards: PN-Z-04008-08, PN-89/Z-04111/02 and PN-89/Z-04111/03 [17–19]. The analysis consisted of collecting air samples in three locations within MIKI Recykling Sp. z o.o. in Krakow – a company producing alternative fuel from municipal waste and other types of waste. The air was sampled in two locations within the hall used for storage of waste intended for processing (point A) and alternative fuel – processing remnants (pint B) as well as in one location outside the hall (point C). The waste sorting hall has 3 entry gates (Fig. 1), gravitational and forced ventilation systems. Inside the production hall, there are 13 belt conveyors and 5 machines marked on Fig. 1 with the following numbers:

1 – two preliminary shredders with magnetic separators,
2 – drum sieve (downstream of a magnetic separator),
3 – air separator (under the belt conveyor),
4 – final shredder,
5 – sorting chamber for recycling material separation from municipal waste.

Samples were collected using microbiological growth media for: bacteria (bacteriological agar, BTL), fungi (glucose-potato agar PDA, BTL), actinobacteria (Pochon agar, BTL) and staphylococci (Chapman agar, BTL), *Escherichia coli* (Endo agar, TBX agar, BTL), *Salmonella* spp. and *Shigella* spp. (SS agar, BTL), *Enterococcus faecalis* (Slanetz Bartley growth medium, BTL), *Pseudomonas fluorescens* (King B agar, BTL). Air temperature and humidity were also measured (HT-9213 thermohygrometer, ATM,

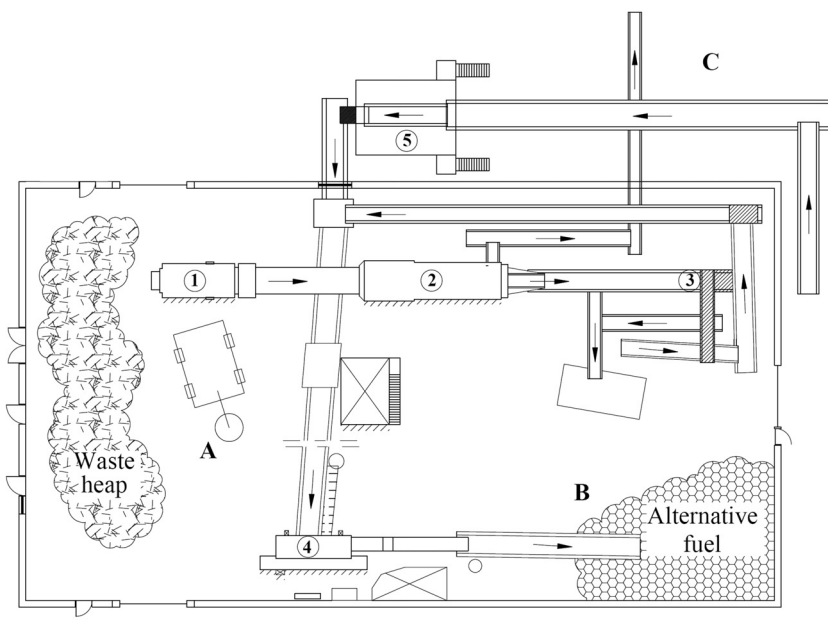

Fig. 1. Simplified diagram of the MIKI Recykling Sp. z o.o. alternative fuel production installation and microbiological analysis sampling points (A, B, C)

China). Wind speed was determined using the information retrieved from the Internet weather archive [20], while the atmospheric pressure and suspended dust content (PM10 and PM2.5) were determined using the data provided by the Environment Protection Inspectorate in Krakow (PIOS) [21]. Samples were collected in three instances, four times a year (April, July, September and December of 2014) in order to account for the seasonal changes. Sample collection lasted for 1 minute, the amount of airflow through the impactor was 100 litres, the device was placed 1.5 m over ground level. Immediately after collection, the dishes were transported into the laboratory and placed in thermostats with temperatures appropriate for the growth of specific microorganisms (bacteria 37 ^{o}C for 24–48 h; fungi 28 ^{o}C for 3–5 days and actinobacteria 28 ^{o}C for 5–7 days). After the incubation period, the grown colonies were counted and the collected isolates were identified using diagnostic keys [22–25]. The results were presented as cfu (colony forming units) in m^3 of collected air (cfu · m^{-3}).

A statistical analysis was made in order to calculate the average number of microorganisms in the tested air. A variance analysis (ANOVA) was performed in order to verify the significance of temporal and spatial differences in bioaerosol concentrations. The statistical analysis was performed using the Statistica v. 10 software (StatSoft).

Results and discussion

The results of the analysis of the amount of microbiological aerosol within the municipal waste processing and treatment company from April to December 2014 are presented in Tables 1–5 below and in Fig. 2. Explanations: A, B – waste storage and processing hall, C – open area, outside the hall; * mean air contamination, ** heavy air contamination according to Polish Standards [17, 18].

Table 1

Mean number of bacteria [cfu · m^{-3}] in selected locations

Sampling point	Spring 18.04.14	Summer 21.07.14	Autumn 9.09.14	Winter 15.12.14
A	290	1980*	2890*	980
B	342	2210*	2920*	1050*
C	120	280	303	350

Table 2

Mean number of fungi [cfu · m^{-3}] in selected locations

Sampling point	Spring 18.04.14	Summer 21.07.14	Autumn 9.09.14	Winter 15.12.14
A	283	1013	2590	3520*
B	733	1316	2970	2350
C	996	823	943	720

Table 3

Mean number of actinobacteria [cfu · m^{-3}] in selected locations

Sampling point	Spring 18.04.14	Summer 21.07.14	Autumn 9.09.14	Winter 15.12.14
A	26*	593**	493**	150**
B	23*	390**	593**	110**
C	16*	43*	76*	30*

Table 4

Mean number of staphylococci [cfu · m^{-3}] in selected locations

Sampling point	Spring 18.04.14	Summer 21.07.14	Autumn 9.09.14	Winter 15.12.14
A	66	516	2890	2120
B	46	390	2760	550
C	10	86	150	290

Table 5

Mean number of *E. faecalis* [cfu · m^{-3}] in selected locations

Sampling point	Spring 18.04.14	Summer 21.07.14	Autumn 9.09.14	Winter 15.12.14
A	50	126	83	200
B	43	146	113	110
C	0	0	0	0

The number of bacteria exceeded the values recommended by the Polish Standard [17] in points A and B in July and September and in point B in December. Fungi were abundant in the analysed air and in December, their number in point A exceeded the values recommended by the Polish Standard [18]. The number of actinobacteria deserves special attention. The concentration of actinobacteria in analysed air exceeded the limit values in all measurement points in every season of the year. Based on the determined number of actinobacteria, the air was classified as averagely contaminated (spring – points A, B and C; summer, autumn and winter – point C) or heavily contaminated (summer, autumn and winter – points A and B) [17]. The presence of staphylococci in analysed air is concerning – the results from autumn show 2890 cfu · m^{-3} in point A. Furthermore, the presence of faecal bacteria *E. faecalis* in the analysed air was found in all seasons in points A and B. The samples did not show the presence of *E. coli* and *P. fluorescens* bacteria or *Salmonella* and *Shigella* bacteria. Furthermore, the highest concentration of microorganisms was found in the waste storage and processing hall (points A and B). The air samples from point C (located outdoors, outside the hall) show significantly smaller bioaerosol concentrations. Only in

the case of fungi, during the spring collection, their number was significantly smaller in point A, compared to point C.

Table 6 below presents selected weather parameters, *ie* wind speed, atmospheric pressure, humidity, suspended dust levels and air temperature during sample collection.

Table 6

Meteorological conditions during sample collection

Parameter \ Sampling time	Spring 18.04.14	Summer 21.07.14	Autumn 9.09.14	Winter 15.12.14
Wind speed [km/h]	4.75	8.25	3	8
Atmospheric pressure [hPa]	987	987	993	994
Humidity [%]	61	58	77	82
Suspended dust PM2.5 [$\mu g \cdot m^{-3}$]	31*	13	18	116*
Suspended dust PM10 [$\mu g \cdot m^{-3}$]	47	23	28	166*
Temperature [^oC] in point A	17.5	25.5	22.4	2.3
Temperature [^oC] in point B	17.3	25.7	23.7	2.5
Temperature [^oC] in point C	18.1	26.7	21.8	2.1

* Value exceeds the limit specified in the Regulation of the Minister of Environment of 24 August 2012 on the levels of selected substances in air (Dz.U. of 2012, item 1031).

Air humidity and temperature are important factors for the seasonal changes in microorganisms concentrations. It is to be noted that air humidity was high during sample collections (58–82 %). Average temperature for the three collection points differed depending on the season from 2.3 ^oC to 26 ^oC. The obtained results validate the proposition of a correlation between the season of the year and the presence of microbiological aerosol. The concentration of the bioaerosol was at its lowest point in spring and grew to its maximum value in autumn. During the winter, the number of most microorganisms, apart from the fungi and *E. faecalis*, declined again (Fig. 2).

Suspended dust levels were compared to the limit values specified in the Regulation of the Minister of Environment of 24 August 2012 on the levels of selected substances

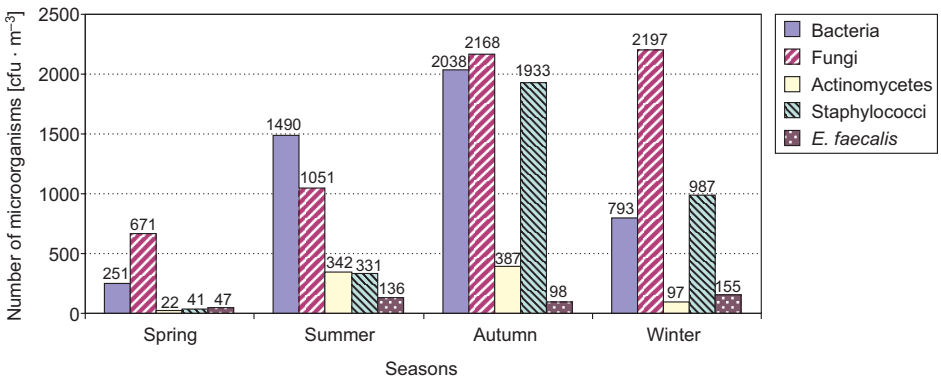

Fig. 2. Seasonal changes in bioaerosol concentrations – mean values from all analysed locations

in air [26]. It was found that the limits for PM10 (50 μg · m^{-3}), and PM2.5 (25 μg · m^{-3}) were exceeded in winter and for PM2.5 in spring. It was noted that the number of microorganisms as well as the concentration of suspended dust in the air were very high in winter. Fraczek and Grzyb [27] suspect a correlation between the presence of suspended dusts and microbiological contamination of air.

Own research shows that the highest concentration of fungi can be observed in December. This tendency looks very different in the studies by [28–31] they claim that the highest concentrations of fungi in atmospheric air can be found in late summer and early autumn, while in winter, their number declines. The study found high concentration of fungi in air in autumn and the coming of winter did not reduce their number. It is to be noted, however, that air temperatures in December were above 0 ^{o}C, no recorded frosts. Fraczek and Grzyb [27] analysed the propagation of the fungi aerosol in Krakow and found a significant increase in winter. Similar results were presented by Lin and Li [32], who found a correlation between the concentration of fungi spores in the air and meteorological conditions. In summer, the number of fungi decreased with higher temperatures and in winter increased with higher temperatures.

Aspergillus, *Penicillium*, *Mucor*, *Cladosporium*, *Rhizopus* and *Alternaria* were dominant among the fungi found in the samples. Apart from the previously mentioned pathogenic bacteria, *Bacillus* spp. and *Streptobacillus* spp. spores as well as *Micrococcus* spp., *Diplococcus* spp., *Sarcina* spp. and *Streptococcus* spp. Among the actinobacteria the presence of *Streptomyces* was found. The identified microorganisms form a typical microflora of air, only the presence of toxin-forming fungi, *ie Aspergillus*, *Penicillium* and *Cladosporium*, may be harmful to the health of the employees, since they produce mycotoxins and allergic reactions [33].

Based on the statistical analysis of the results, it was found that the differences of bioaerosol concentrations between the air sampling locations are statistically significant (Table 7). The differences in the number of found microorganisms during the year are statistically significant in the case of fungi, actinobacteria and staphylococci (Table 7).

Table 7

Variance analysis results for the temporal and spatial differences in bioaerosol concentrations

Microorganism	F factor value (collection point)	F factor value (season of the year)
Bacteria	20*	1.8
Fungi	4.4*	8.7*
Actinobacteria	6.8*	9.9*
Staphylococci	5.4*	9.8*
E. faecalis	28*	2.3

* Values are significant with $p < 0.05$.

Conclusions

1. The number of microorganisms found in the microbiological aerosol depends on the season of the year, meteorological conditions and air sampling location.

2. The most contaminated air (in terms of number of microorganisms) was found in the two points inside the waste storage and processing hall.

3. At some points in time and in some locations, the air may negatively impact the human health, because the limit values for the concentrations of bacteria, fungi and actinobacteria were exceeded.

4. The presence of pathogenic bacteria, *ie E. faecalis*, *Staphylococcus* spp., and toxin-forming fungi was found in collected air samples.

5. It is necessary to continue the research of air in order to assess the changes of microbiological aerosol amounts with potential detrimental effects to human health.

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OCENA ZANIECZYSZCZENIA MIKROBIOLOGICZNEGO POWIETRZA NA TERENIE ZAKŁADU PRZETWARZANIA ODPADÓW KOMUNALNYCH

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Abstrakt: Celem pracy była analiza liczebności drobnoustrojów stanowiących biaerozol mikrobiologiczny na terenie zakładu zajmującego się przetwarzaniem odpadów komunalnych w Krakowie. Czas magazynowania zmieszanych odpadów komunalnych w hali zakładu wynosi 6–48 godzin, natomiast czas magazynowania wytworzonego z nich paliwa alternatywnego zawiera się w przedziale 12–96 godzin. W sortowni pracuje 18 osób na 3 zmianach. Powietrze do badań pobierano z użyciem impaktora MAS-100 (Merck, Szwajcaria) w trzech punktach zlokalizowanych na terenie przedsiębiorstwa, cztery razy w roku, aby ocenić wpływ warunków meteorologicznych, tj. temperatura, wilgotność oraz zapylenie na liczebność wybranych grup drobnoustrojów. Stwierdzono, że liczebność badanych mikroorganizmów podlega zmianom sezonowym i jest uzależniona od warunków meteorologicznych oraz punktu poboru powietrza. Ponieważ wartości graniczne dotyczące stężenia bioaerozolu zostały przekroczone, konieczne jest prowadzenie dalszych badań, których celem będzie ocena zmian liczebności drobnoustrojów mogących negatywnie oddziaływać na zdrowie ludzi.

Słowa kluczowe: aerozol mikrobiologiczny, powietrze, odpady komunalne