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Air Quality and Potential Health Risk Impacts of Exposure to Bacterial Aerosol in a Waste Sorting Plant Located in the Mountain Region of Southern Poland, Around Which There Are Numerous Rural Areas

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Abstract: Many studies have shown an association between working in waste sorting plants (SP) and occupational health problems, such as skin irritation or pulmonary diseases. These symptoms have been related to biological aerosol exposure. The main goal of this work was to assess the levels of concentration and the characteristics of bacterial aerosols in waste sorting plants, based on measurements taken in a plant located in the mountain region of Southern Poland, around which there are numerous rural areas. The average concentrations of culturable bacterial aerosol (CCBA) collected in the unloading hall of the waste sorting plant (UHSP) and the outdoor air of the sorting plant (OSP) were 2687 CFU/m^3 and 1138 CFU/m^3, respectively. Sampling was undertaken in the plant using an Andersen six-stage impactor (with aerodynamic cut-off diameters of 7.0, 4.7, 3.3, 2.1, 1.1, and 0.65 µm), during the spring of 2019. Size distributions were unimodal, with a peak in particle bacterial aerodynamic diameters at less than 3.3 µm, increasing the potentially adverse health effects of their inhalation. An analysis was conducted to determine the antibiotic resistance of isolated strains of bacteria. During the study, it was found that isolates belonging to the genus *Bacillus* were most frequently detected in the waste sorting plant. Isolates with the highest resistance to antibiotics belonged to the genus *Neisseria*. This test indicates that the use of personal protective equipment is necessary.

Keywords: air quality; municipal mixed-waste sorting plant; bacterial aerosol; antibiotic resistance; human health risks

1. Introduction

The employees working in sorting plants are concerned mainly about the health effects of exposure to bioaerosols and chemical particulates [1–4]. Aerosol particles can nucleate from atmospheric gases to form secondary organic aerosols (SOAs). SOAs are composed of a myriad of complex chemical compounds, exhibiting a wide range of molecular structures and phase states which influence their physico-chemical properties and reactivities [5]. There is increasing evidence showing associations between working in sorting plants and health problems such as allergies, irritation, inflammation, and pulmonary diseases [6].

Biological aerosols are always present in the air. These include viruses, viable organisms such as bacteria and fungi, and products of organisms, such as bacterial or fungal spores, plant parts, or pollen [7]. The main source of bacterial aerosols (individual organisms, conglomerates of bacteria, and bacteria attached to particulate matter) in enclosed spaces is deemed to be human and animal organisms [8–10]. Significant amounts of bacteria are also found in settled dust, from which they can be resuspended into the air as a result of secondary particulate emissions, for example, via movements in the premises. Additionally, indoor biological agents may develop in a building's heating, ventilation and air-conditioning system [11–13]. Exposure to bioaerosols occurs via inhalation, ingestion, or skin contact, and may result in considerable adverse effects on human health [14].

Emission of microorganisms at sorting plants (SPs) occurs during waste transport, unloading and processing at sorting stations. Therefore, the concentration of bioaerosols in SPs has a very wide range of values. Wet fractions of municipal wastes, in particular, may contain 10^9 bacteria or viruses per gram of wastes [15]. In Poland and in many other countries, there are still no established legal limits for occupational exposure to bioaerosols in waste treatment plants, and many studies have focused only on the total concentration of biological aerosols in this environment. However, more information is indispensable for the assessment of population exposure in sorting plants, as well as for the identification of the sources of biological aerosol emissions.

Bacterial aerosols have a significant impact on the health of waste sorting plant employees. The indoor air is characterized mainly by the presence of Gram-positive cocci and Gram-positive bacilli of the *Bacillaceae* family [11,15,16]. Since the sorting employees are in direct contact with household hygienic waste, there is a risk of exposure to microorganisms that could pose a health risk. Municipal waste mixed with plant and animal waste, as well as with hygienic waste, combined with high waste moisture levels and temperatures, especially in the spring and summer season, constitute a real microbiological hazard for people working in waste sorting plants [17].

Nowadays, the biggest problem is contact with pathogenic microorganisms that have resistance to antibiotics. At the same time, it should be noted that the waste storage method promotes the development and multiplication of microorganisms in the decay processes taking place. Currently, numerous studies show that resistance to antibiotics is not only associated with clinical strains but also with environmental strains [17]. Bacteria resistant to antibiotics are more frequently detected in bioaerosols in various environments.

The study of biological agents in the air of waste sorting plants is not obligatory in Poland. Hence, these studies are rarely carried out, and the problem of microbiological hazards is often understated. The harmfulness of biological factors in Polish regulations is set out in the regulation dated 22 April 2005 on harmful biological factors for health in the work environment and health protection of employees exposed to these factors [18]. On the other hand, the employer is obliged to assess the occupational risk. Improper working conditions and high exposure of employees in municipal waste sorting plants, combined with biological factors, result in an increase in sickness among employees, which is associated with employee absences as well as ill-health.

The subjects of this study are the following: (a) the concentration levels of culturable airborne bacteria during spring, (b) the size distributions, with a particular focus on the respirable fraction, and (c) determination of the dominant group of bacteria in a sorting plant located in the mountain region of Poland, surrounded by rural areas. This paper aims to catalogue the aerosols in waste sorting plants in rural areas, thus increasing the awareness of their potential health risks.

2. Materials and Methods

2.1. Sampling Sites

The research was carried out in the unloading hall of a sorting plant for mixed municipal waste, located in the mountain region of Poland, in the Silesian foothills, around which there are numerous rural areas. The research was conducted during March 2019, in the sorting plant as well as outside the building (Figure 1). Within a radius of 2 km from the sorting plant, there were about 8,000 local inhabitants. Every measurement was conducted between 10:00 a.m. and 12:00 a.m., from the outdoor air (to determine the indoor/outdoor ratio) and from the indoor air in the unloading hall. The samples of bacterial aerosols were collected at a height of about 1.5 m, to simulate aspiration from the human breathing zone. During the measurements, environmental parameters were also recorded (Table 1). The spring season was selected for this study because recent research into biological air pollution conducted in Poland indicates that the highest bacterial concentration is consistently found in spring [19,20].

This sorting plant operates within the mechanical part of the mechanical-biological treatment (MBT) plant for municipal solid waste (MSW). During the study, only municipal mixed waste and garbage trucks bringing this type of waste were regularly brought into the hall.

The sorting plant, which has a capacity of 70,000 Mg/year (20 Mg/h), works in a two-shift system, and is equipped with technology adapted to segregating municipal waste, collected selectively. The volume of the waste reception hall in which the tests were undertaken was ~18,000 m^3. The type of ventilation used in the waste reception hall was exhaust ventilation (general lift with a mirror), with a total capacity of ~12,000 m^3/ h.

Supplying municipal waste into the bag disrupter and the process of tearing the bags, generate increased dust, and thus a greater threat to the health of the worker. Our previous studies indicated that the microbial concentration in the air was significantly positively correlated with the atmospheric temperature [19]. Furthermore, high relative humidity is typically favourable for bacterial growth because the bacteria can absorb this water from their living substrates, for metabolic purposes. In addition, high relative humidity may result in the clumping of cells, which possibly increases the chances of cell survival [21]. Table 1 presents a short description of the analysed sorting plant.

Table 1. Environmental parameters of the sorting plant.

Environmental parameters	Measurement Point Outside the Hall	Measurement Point in Municipal Waste Unloading Hall
Temperature, °C	10.2	12–14.2
Relative Humidity (RH), %	28	47–53
Air Pressure, hPa	961	961
Wind Velocity, m/s	3.6	-
Wind Direction	Variable, with south-west dominant	-

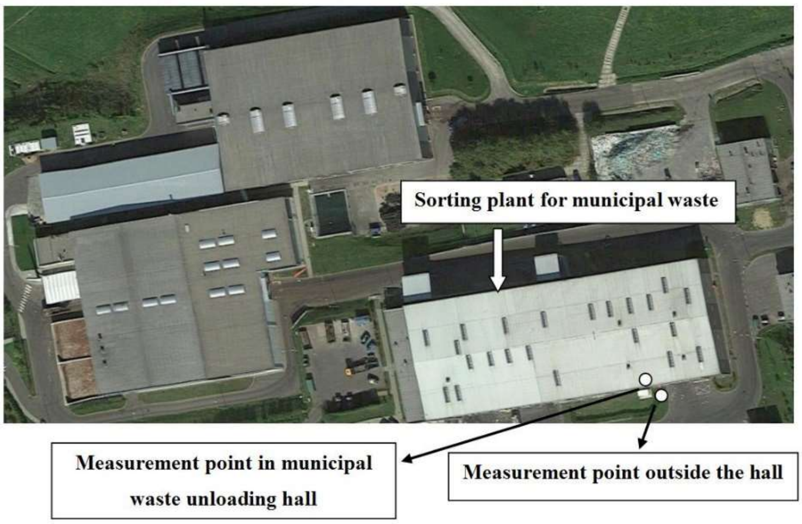

Figure 1. Location of the measurement points in the municipal mixed-waste unloading hall in the mechanical–biological treatment (MBT) plant and outside the hall, in Poland (map data: Google 2019).

2.2. Sampling and Analysis Methods

Measurements of the bacterial aerosol concentrations were conducted using a six-stage Andersen impactor with cut-off diameters of 7.0, 4.7, 3.3, 2.1, 1.1, and 0.65 µm (Figure 2). During the measurements, the air flow was 28.3 dm^3/minute (min.) and the sampling time, calculated following Nevalainen et al. [22] was 10 min. Before and after sampling, the flow rate was measured using a rotameter. Impactors were disinfected using cotton balls immersed in 70% ethanol between each sampling. Tryptic soy agar (TSA) was used for bacteria, with cycloheximide added to inhibit fungal growth. The Petri dishes were incubated for 48 hours (h) at 36 ± 1°C. Enumeration of bacteria was conducted according to the Polish standard. Total colony counts were corrected for multiple impactions by the positive hole method and expressed as colony forming units (CFU) per cubic metre of air [23].

Quality control was practised using PN-EN 12322 [24] and ISO 11133 [25] standards, with the same operational details as in our previous studies.

Figure 2. Six-stage Andersen impactor used during measurements in the waste sorting plant.

2.3. Identification of Selected Bacteria

The bacterial isolates obtained as a result of air intake were identified using Gram-staining and the Biolog OmniLog system (Biolog, Haward, CA, USA). After a 24-h incubation on TSA plates, single colonies were passaged on Biolog Universal Growth Agar (24-h incubation at 37 °C). The initial characterization of isolates was performed via Gram-staining and cell morphology.

The selected strains were then identified using the Biolog OmniLog system. The system software was used to identify bacteria on the basis of the phenotype using a GEN III MicroPlate™. Each plate contained 71 different carbon sources and 23 chemical sensitivity assays. Next, each of the isolates was transferred to the inoculation fluid B. In the next stage, GEN III microplates were prepared for each isolate, as recommended by the manufacturer. The inoculated plates were incubated for 48 h at 37 °C, with measurements taken every 15 min. using the OmniLog incubator/reader. The identification was based on the phenotypic similarity between the data collected by the OmniLog GEN III data collection software and the control strains in the Biolog database.

2.4. Antibiotic Susceptibility Test

The Kirby–Bauer disc diffusion sensitivity protocol was used [26]. A total of 36 antibiotics were tested, belonging to the following groups: aminocoumarin (novobiocin, amikacin, gentamicin, neomycin, netilmicin, tobramycin), carbapenems (doripenem, ertapenem, imipenem), and cephalosporins (cefaclor, cefadroxil, cefepime, cefoxitin, ceftaroline, ceftazidime), and drugs against mycobacteria (rifampicin), glycopeptides (teicoplanin, vancomycin), macrolides (azithromycin, erythromycin), monobactams (aztreonam), nitrofurans (nitrofurantoin), penicillins (amoxicillin, ampicillin, piperacillin, ticarcillin), quinolones/fluoroquinolones (ciprofloxacin, nalidixic acid, norfloxacin, ofloxacin), sulfonamides (trimethoprim-sulfamethoxazole), tetracyclines (doxycycline, minocycline), and others (metronidazole, mupirocin, trimethoprim). The inoculated Petri dishes were incubated at 37 °C for 24 h. After incubation, areas of inhibited growth were measured according to a three-step scale, to assess the sensitivity of the bacteria to antibiotics.

The diameter of growth inhibition was classified as follows

- <15 mm—resistant to antibiotics (R),
- 16 to 25 mm—the average level of resistance to antibiotics (I),
- >25 mm—bacteria were sensitive to antibiotics (S).

Each test was performed in triplicate.

3. Results and Discussion

3.1. The Concentration of Culturable Bacterial Aerosol (CCBA)

Table 2 presents the average concentrations of bacterial aerosol collected in the unloading hall of the waste sorting plant (UHSP) and the outdoor air of the sorting plant (OSP). The average concentration in the OSP was 1138 CFU/m^3. In the case of indoor air, the average concentration of bacterial aerosol in the UHSP was 2687 CFU/m^3. The bioaerosol concentrations indoors are constant during working.

The indoor-to-outdoor ratio (I/O) indicates where the source of bioaerosol might be found. The average I/O ratio calculated for UHSP bacteria concentrations was 2.36 (Table 2). Since the I/O ratio was > 1, it can clearly be concluded that the main source of the bacterial aerosol is stored waste. This indicates that ventilation plays an important role in improving the quality of air in the unloading hall [27,28].

Ventilation design has an important impact on the concentrations of bacterial aerosols in the indoor environment. A high-quality ventilation system can help remove and/or dilute more than 80% of aerosol pollutants from the air [29]. Inadequate maintenance of ventilation systems or low efficiency can often lead to unintentional contamination in indoor air [30].

In Bydgoszcz, Poland, the level of airborne bacteria in a waste sorting plant in April was 15,200 CFU/m^3 [15]. Studies carried out during different seasons in a sorting plant in Finland showed that the concentration of culturable bacteria in the indoor air of the unloading hall ranged from 480 to 1430 CFU/m^3 [31]. Definitely higher exposure level to viable airborne bacteria in sorting plant was found in Korea (ranged from 0.13×10^5 to 23.5×10^5 CFU/m^3). The mainly possible reason for this high exposure in Korea was no facility for ventilation or the ventilation was inefficient [32]. Previously conducted studies have shown that the concentration of bioaerosols is different, depending on the prevailing season and the climatic conditions [19,33,34]. Geography and climate play an important role in determining the biological pollutant concentration in the air, because the transport of bioaerosol is primarily governed by hydrodynamic and kinetic factors, while their fate is dependent on their specific chemical composition and the meteorological factors to which they are exposed [35]. Bioaerosols making their way from stored wastes into the air are subjected to numerous factors unfavourable to their development, and many of them die in the first few seconds, mainly due to drying, high temperatures or excessive UV radiation. Only those microorganisms which are the most resistant and adapted to the highest degree to the unfavourable living conditions, retain their vitality for long in the air [15,36].

Table 2. Average concentration in CFU/m^3 (total bacterial colony-forming units per cubic metre) in the unloading hall of the waste sorting plant (UHSP) and outside the waste sorting plant (OSP).

Location	Average Concentration CFU/m^3	SD	Min.	Max.	I/O Ratio
UHSP	2687	912	2124	3121	2.36
OSP	1138	401	692	1714	-

SD—standard deviation.

3.2. The Size Distribution of the Bacterial Aerosol

Figures 3 and 4 present the analysis of the numbers and aerodynamic diameters of viable bacteria collected from the different stages of the impactor in the waste sorting plant and outside the building. It can be seen that the size distributions of the bacterial aerosols, both in the indoor and the outdoor air, were characterized by a large share of the respirable fraction (particles less than 3.3 µm). These results could indicate the existence of a serious potential exposure to particles of respirable sizes, especially for workers, which may reach the trachea, bronchi, and alveoli and contribute to adverse symptoms in the respiratory system [37,38]. Additionally, the shape of the size distributions may indicate that the particles of culturable bacteria are relatively fresh, and mostly of waste-sorting origin.

Workers primarily exposed in the reception hall for mixed municipal waste are working on a bunker conveyor (no more than 10 persons), and their task is to pull out wastes with larger dimensions that could block the automated line. The danger from the concentration of bioaerosols is greatest for them, due to the fact that mixed municipal waste is fed by means of a bag-fender loader, from where it reaches the bunker conveyor, and these processes are carried out within the workplace. Supplying municipal mixed waste to the bag disrupter and the process of tearing the bags, generate increased dust, and thus represent a greater threat to the health of the workers.

The highest peak of the particle bacterial aerodynamic diameters was in the range from 2.1 to 3.3 µm in the unloading hall (about 35%). For particle bacterial aerodynamic diameters outside the building, it was in the range from 3.3 to 4.7 µm (about 30%).

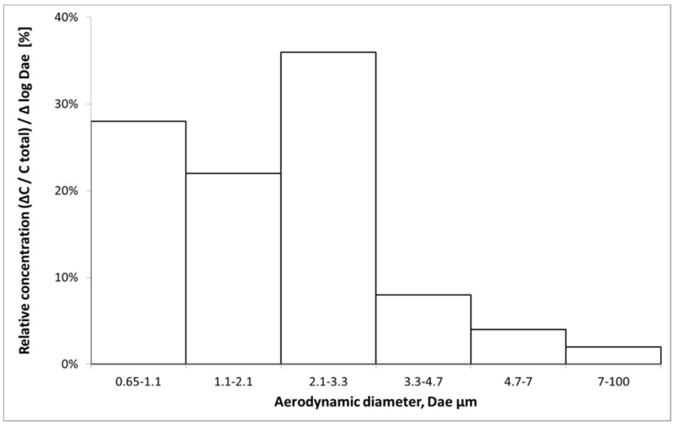

Figure 3. The size distribution of the bacterial aerosol in the indoor air of the unloading hall.

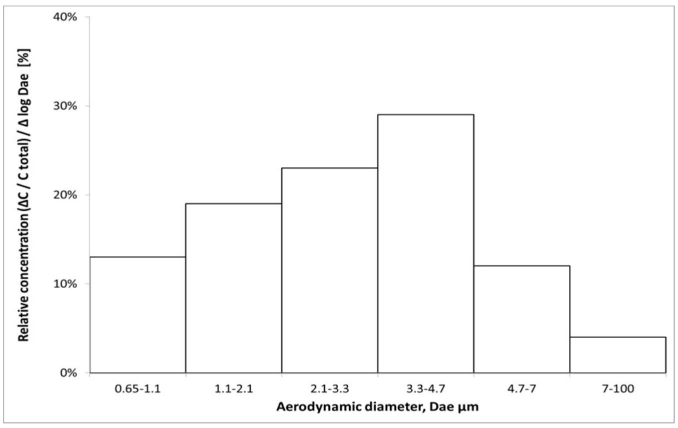

Figure 4. The size distribution of the bacterial aerosol in the outdoor air. * Dae—aerodynamic diameter, ΔC—concentration of bacterial aerosol at a particular stage of the six-stage Andersen impactor, C total—total concentration of bacterial aerosol, Δ log Dae—logarithm of differences in cut-off diameters for a particular stage of the six-stage Andersen impactor.

3.3. Identification of Bacterial Aerosol

More than 84.7% of airborne isolates were Gram-positive bacteria, while Gram-negative bacteria constituted 14.6% of isolates. The most numerous group of Gram-positive bacteria, was the *Bacillus*-type microorganisms (76.1% of all isolates). Single isolates of the genera *Micrococcus* and *Staphylococcus* were also identified. Gram-negative isolates were dominated by rod-shaped isolates (8.7% of all isolates). The representatives of the largest groups were taken for further analysis.

As a result of our research, it was found that in the sorting hall, the dominant species were *Bacillus humi, Bacillus mojavensis*, and *Neisseria shayeganii*. *Bacillus humi* and *Bacillus mojavensi* were detected in various composting plants and indoor environments. Among the dominant species, *Neisseria shayeganii* was also isolated and identified. This species is mainly associated with the presence of biofilm on human enamel, which indicates its direct origin from hygienic waste [39–42].

3.4. Antibiotic Susceptibility Test

The results of the antibiotic susceptibility test are shown in Figure 5. All strains were resistant to aztreonam and metronidazole. Aztreonam is active against Gram-negative facultative anaerobic bacteria and has no significant activity against Gram-positive or strictly anaerobic bacteria [43]. Metronidazole is an antibiotic directed against protozoa and the majority of anaerobic bacteria (Gram-positive and Gram-negative) [44], which confirms that they are not substances that affect Gram-positive aerobic *Bacillus* species (*Bacillus humi* and *Bacillus mojavensis*).

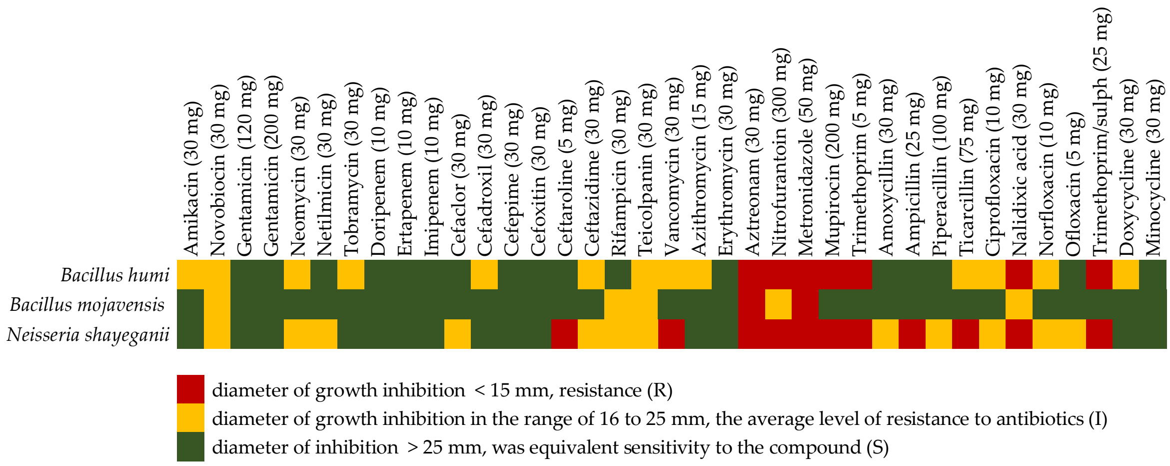

Figure 5. The pattern of sensitivity to antibiotics.

The *Neisseria shayeganii* isolated in our study showed resistance to 29.7% of the tested antibiotics. In his work, Meiden showed that the genus *Neisseria* is a group of related microorganisms that live only on the surfaces of human mucous membranes and demonstrate a large diversity in their degree of pathogenicity. However, Bennett, in his work, shows that a comparison of nucleotide sequences indicates that *N. shayeganii* is the most distant species within this group [45]. To date, there have been few Neisseria detections that did not directly involve humans. On the other hand, Neisseria can be detected in environments closely associated with people, e.g., showerhead biofilm or mattress dust [46]. Detection of these organisms as dominant in the waste sorting plant bioaerosol is very interesting. Due to the characteristics of this strain, it seems reasonable to investigate whether its presence may pose a potential health risk to employees.

The detection of *Bacillus mojavensis* in the bioaerosol is also interesting. It is a strain that does not show resistance against the tested antibiotics. Comparing our results with the results obtained by Choi, the strain isolated by us exhibits a similar pattern of sensitivity to that of the strain *B. mojavensis* KJS-3, which was isolated from food waste [47]. This species is also detected at various stages of composting of food substances [48], which allows us to assume that in the sorting plant, food waste was present in the mixed waste, indicating that the degree of segregation of waste in households is still insufficient.

Bacillus humi was first isolated from an agricultural area in the Netherlands and described in 2005 by Heyrman [49]. However, it was also detected in bioaerosols of a composting plant [41,50]. The strain was considered non-pathogenic [51], but its antibiotic resistance has not yet been studied. In our studies, the isolated *Bacillus humi* was characterized by no inhibition of growth (d = 0 mm) for aztreonam, metronidazole, mupirocin, trimethoprim, and nalidixic acid.

4. Conclusions

The average concentrations of culturable bacterial aerosol (CCBA) collected in the unloading hall of the waste sorting plant (UHSP) and in the outdoor air of the sorting plant (OSP) were 2687 CFU/m^3 and 1138 CFU/m^3, respectively. Our study clearly suggests that it is very important to improve the microbiological quality of the air in SPs by providing adequate good-quality ventilation, which can help remove and/or dilute more than 80% of aerosol pollutants from the air [29].

The size distributions of the bacterial aerosols indicate that biological particles less than 3.3 µm represented more than 80% of the total concentration of bacterial aerosol inside the unloading hall of the SP, increasing the health risk for exposed personnel. Biological particles less than 3.3 µm constituted up to 50% of the total bacterial aerosol outside the studied building, which could potentially pose a threat to the inhabitants of the surrounding rural areas.

Relatively high bacterial concentrations were measured in the unloading hall, at a level which may represent a health risk. Therefore, it is suggested that the analysed sorting plant should improve its indoor ventilation. It is also recommended that employees use masks or respirators while working in the unloading hall.

These results could help in the development of control strategies that will benefit the health and living conditions of both the workers and the people living in the vicinity of the waste sorting plant.

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