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Short communication

Seasonal changes in the concentrations of airborne bacteria emitted from a large wastewater treatment plant

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ABSTRACT

Various factors influence the concentrations of bacteria and fungi found in the air surrounding wastewater treatment plants. These include the relative humidity, temperature, intensity of UV and visible radiation, and wind speed. Typically, higher emissions of bioaerosols are observed in the summer months due to the smaller amount of rainfall. The purpose of this study was to undertake a microbiological analysis of wastewater samples from a wastewater treatment plant (with samples taken before and after treatment) together with an analysis of bioaerosol samples taken in and around the plant. The study was undertaken in different seasons and samples were collected during a variety wastewater and sludge treatment operations. The most intense airborne emissions were found in the summer when, in most cases, all groups of bacteria (mesophilic bacteria, total coliforms, faecal coliforms and mannitol-positive staphylococci) were isolated from the air samples. The highest concentrations of airborne bacteria were observed at a grit chamber and a sludge storage site. The same seasonal trend was found in wastewater – bacteria numbers were significantly higher in the summer; mannitol-positive staphylococci were only present in treated wastewater in the summer.

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1. Introduction

Wastewater treatment plants emit bioaerosols containing pathogenic bacteria, fungi and viruses as well as particles of biological origin. This bacterial material comes from sewage or sludge and is capable of infecting people through inhalation, ingestion and contact with skin. Many microorganisms present in bioaerosols emitted by sewage treatment plants are resistant to antibiotics (Gregova et al., 2009). The most frequently occurring isolates are heterotrophic mesophilic bacteria belonging to the genera *Pseudomonas, Micrococcus, Escherichia, Bacillus, Streptococcus, Staphylococcus, Proteus, Enterobacter, Klebsiella, Corynebacterium, Mycobacterium, Vibrio, Acinetobacter, Actinomyces, Clostridium*. Indoors, concentration in the air may reach 10^4 cfu m^{-3} (Bauer et al., 2002), while the concentration of bacteria in the air outdoors has been estimated by other researchers to be as high as 10^7 cfu m^{-3} (Cyprowski et al., 2005). One consequence of the occurrence of gram negative bacteria (such as *Pseudomonas* sp., *Enterobacter* sp., *Klebsiella* sp., *Proteus* sp.) in bioaerosols is the presence of endotoxins. These are components of the bacterial cell wall composed of lipopolysaccharides (LPS), and they have a harmful effect on the respiratory system or pulmonary function (Douwes et al., 2003). Pathogenic organisms, such as *Clostridium perfringens, Listeria monocytogenes, Pseudomonas aeruginosa, Staphylococcus aureus, Shigella sonnei* and *Proteus vulgaris*, have also been isolated from bioaerosols (Michałkiewicz, 2008). The highest emission of microorganisms usually occurs during aeration of waste in aeration tanks and in units such as bar screens, pump stations, grit chambers and sludge storage sites.

Most bioaerosol particles emitted from sewage treatment plants have a diameter less than 4.7 μm, making them respirable. The particles can be transmitted several kilometres on the wind, creating a threat not only to workers but also to local residents (Sanchez-Monedero et al., 2008). It has been shown that relative humidity of 70–80 per cent and temperature of 12–15 °C promotes bioaerosol survival. The intensity of the UV and visible radiation, the concentration of oxygen and the presence of toxic compounds as well as the type and number of microorganisms present in the wastewater and sewage sludge also have an impact on the concentration of bioaerosols (Barabasz et al., 2003; Kulig and Ossowska-Cypryk, 2013). Wind speed, and operating practices of the treatment plants, are also important factors in the

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concentrations of microorganisms found in the air around treatment plants. One study found that the concentration of bacteria in the air around a wastewater treatment plant that employed fine-aeration techniques did not exceed 100 cfu m^{-3}, while the concentration was more than 4.5 × 10^3 cfu m^{-3} around plants using older methods of aeration (with surface aerators) (Sanchez-Monedero et al., 2008). Typically, higher emissions of bioaerosols have been observed in the summer months.

This study involved the collection and microbiological analysis of wastewater samples (before and after the treatment process) and of bioaerosol samples around a municipal wastewater treatment plant. Samples were collected in different seasons and at various stages of wastewater and sludge treatment operations. The aim was to examine seasonal patterns in the emissions of bacteria. This required a microbiological examination schedule that would allow meaningful results to be obtained for the full year. The study was also designed to identify the elements of wastewater treatment plants that contribute most to air contamination. The results can be used to set receptor points for microbiological examinations on wastewater treatment plants. These locations should be used for environmental assessment studies, due to their potential to emit pathogens as well as potentially pathogenic airborne bacteria.

2. Materials and methods

Samples were taken in seven series from November 2009 to September 2010 at a municipal wastewater treatment plant with a daily mean inflow of 240,000 m^3. In each series, material was collected from 12 sampling points, 3 replicates were used in each point.

2.1. Air sampling

Samples of air were taken with a Mas 100 Air Sampler (Merck) – a single stage 400-hole impactor using Petri dishes of 90 mm in diameter. Each Petri dish contained solid medium suitable to the isolated group of bacteria. The flow rate was 100 l min^{-1} and sampling time was adjusted at 1 min or 2 min. After sampling, the Petri dishes were transported immediately to the laboratory and incubated at an optimum temperature.

The following microorganisms were isolated from the air: heterotrophic mesophilic bacteria, total coliforms (*Enterobacteriaceae*, faecal coliform), *Escherichia coli* and potential pathogens (mannitol-positive staphylococci). Mesophilic bacteria were isolated on a Plate Count Agar, *Enterobacteriaceae* and *E. coli* – on MacConkey Agar, while mannitol-positive staphylococci were isolated on Chapman Agar. For *Enterobacteriaceae* both the red colonies surrounded by a turbid zone and the colourless ones surrounded by a yellowish zone were counted. Faecal coliform colonies were red and surrounded by a turbid zone. The presence of *E. coli* was confirmed on tryptophan broth and brilliant green broth.

The number of colonies counted on the solid media was corrected according to the Feller table (1959). Results of the microbiological air analysis were expressed in colony forming units per cubic metre of air (cfu m^{-3}).

During air sampling, parameters such as wind speed (m s^{-1}), air temperature (°C) and relative humidity (in percentages) were measured with the following portable equipment: Kestrel 4500 NV anemometer and Rotronic HygroPalm thermohygrometer with HygroClip2 HC2-S3 sensor. Samples were taken at different times through the day – and in three seasons (spring, summer and autumn) – without disturbing the normal operation procedure of the wastewater treatment plant. Samples were usually collected from 8 a.m. to 4 p.m. at 1,5 m above the ground and 1–3 m from the selected wastewater and activated sludge treatment processes.

Air samples were collected at these points: a) wastewater line: equalization basin, bar screens, grit chamber, primary settling tank; b) sludge line: pump station and sludge storage site. Indoor air was sampled in two locations: a building with bar screens and a pump station. A control sample of clean outdoor air was taken upwind from each sampling site at each session. Fig. 1 shows a diagram of the wastewater treatment plant.

2.2. Wastewater sampling

Samples of untreated and treated wastewater were taken from a grit chamber and a discharge canal, respectively, at the same time as the air samples were collected. Microbiological analysis of the samples was undertaken using the conventional plate count method. The same culture media and parameters of incubation were used for the wastewater analysis and the air samples analysis.

2.3. Statistical methods

The variance analysis required determination of the form of relationship between the dependent variable (number of bacteria identified during testing) and the factor (season) using a linear regression function. This specific model was subjected variance analysis. After rejecting the null hypothesis, Tukey's HSD, Least Significant Differences (NIR Fisher) and Scheffe's post hoc tests were applied, allowing several comparisons between groups.

3. Results and discussion

3.1. Wastewater analysis

Mesophilic bacteria are released into the air from warm-blooded organisms (the optimum temperature for their growth is 35–37 °C). These bacteria include pathogens from genera *Staphylococcus, Streptococcus,* and *Pseudomonas.* The main inflows to the wastewater treatment plant are primarily domestic wastewater, hospital wastewater, some industrial waste (meat, dairy) and sewage sludge. The concentration of mesophilic bacteria in the untreated effluent may be as high as 10^8 cfu cm^{-3}. In this study

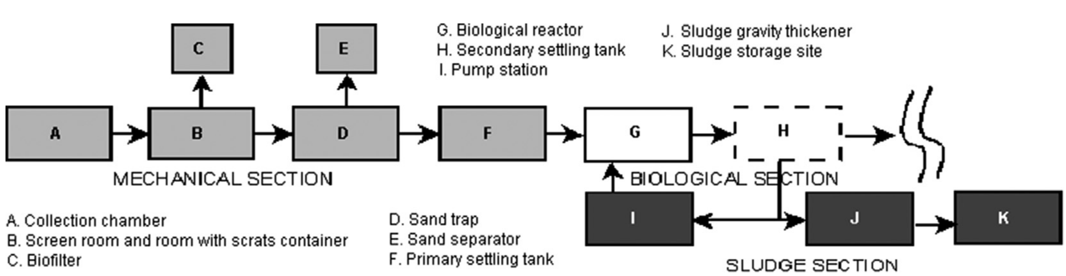

Fig. 1. Wastewater treatment plant diagram.

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