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Characteristics and Assessing Biological Risks of Airborne Bacteria in Waste Sorting Plant

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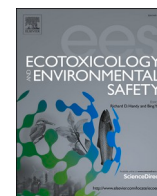
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Characteristics and assessing biological risks of airborne bacteria in waste sorting plant

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ABSTRACT

Examining the concentration and types of airborne bacteria in waste paper and cardboard sorting plants (WPCSP) is an urgent matter to inform policy makers about the health impacts on exposed workers. Herein, we collected 20 samples at 9 points of a WPCSP every 6 winter days, and found that the most abundant airborne bacteria were positively and negatively correlated to relative humidity and temperature, respectively. The most abundant airborne bacteria (in units of CFU m⁻³) were: *Staphylococcus* sp. (72.4) > *Micrococcus* sp. (52.2) > *Bacillus* sp. (30.3) > *Enterococcus* sp. (24.0) > *Serratia marcescens* (20.1) > *E. coli* (19.1) > *Pseudomonas* sp. (16.0) > *Nocardia* sp. (1.9). The lifetime average daily dose (LADD) for the inhalation and dermal routes for the intake of airborne bacteria ranged from $3.7 \times 10^{-3} \leq \text{LADD}_{\text{Inhalation}} \leq 2.07 \times 10^1 \text{ CFU (kg d)}^{-1}$ and $4.75 \times 10^{-6} \leq \text{LADD}_{\text{Dermal}} \leq 1.64 \times 10^{-5} \text{ CFU (kg d)}^{-1}$, respectively. Based on a sensitivity analysis (SA), the concentration of airborne bacteria (C) and the exposure duration (ED) had the most effect on the $\text{LADD}_{\text{Inhalation}}$ and $\text{LADD}_{\text{Dermal}}$ for all sampling locations. Although the Hazard Quotient of airborne bacteria was $\text{HQ} < 1$, an acceptable level, the indoor/outdoor ratio ($1.5 \leq \text{I/O} \leq 6.6$) of airborne bacteria typically exceeded the threshold value ($\text{I/O} > 2$), indicating worker's exposure to an infected environment. Therefore, in the absence of sufficient natural ventilation the indoor ambient conditions of the WPCSP studied should be controlled by supplying mechanical ventilation.

1. Introduction

One of the main sources of airborne bacteria emissions into the ambient air or indoor air is municipal and industrial solid waste (M&ISW) (Wikuats et al., 2020b). The main components of M&ISW products contain glass, paper, metals, cardboard, etc., which are recycled for reusing (Baghani et al., 2016, 2017; Farzadkia et al., 2021). As a consequence of the recycling of these main components in waste sorting plants, bacteria can be emitted to air in the form of bioaerosol (Solans et al., 2007). Previous studies found that the concentrations of

culturable bacteria emissions from municipal landfill sites in southern Taiwan ($> 10^3 \text{ CFU m}^{-3}$) were higher in winter than in other seasons (Huang et al., 2002). In addition, the main source of bacteria in a waste sorting plant was related to household waste sorting activities (Dequois et al., 2017).

Airborne bacteria (bioaerosols) can be generated in a waste sorting plant by mechanical agitation by front-end loaders and farm tractors hauling wastes into other sites, during the pick-up and manual separation of solid wastes, by the movement of wheels and tires of the cars and trucks, and during draining solid waste by trucks (Baghani et al.,

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2020b). Direct or indirect contact with the created bioaerosols from the solid waste can spread human diseases (Baghani et al., 2020b). Exposure to such bioaerosols may induce intestinal and infectious diseases of exposed persons. Genitourinary tract infection, respiratory system infection, pneumonia, allergies, acute toxic effects, diarrhea, acute toxic allergies, sore throat, and even cancers have been documented among waste sorting workers, landfill workers, compost and garbage handlers, and M&ISW employees (Borrego and Molina Veloso, 2018; Hossain et al., 2013; Tang and Stratton, 2010). There is also a fair concern for bioaerosol exposure not only to the plant workers but also to nearby residents to the plants, who can experience health hazards (skin and respiratory issues) (Baghani et al., 2020b; Degois et al., 2017).

The number and type of airborne bacteria is a useful metric to assess the adverse effects owing to human exposure to these emissions (Wikuats et al., 2020b). Such human health risk assessment serves to evaluate the health hazards associated to airborne bacteria exposure (Li et al., 2012). E.g., the total non-carcinogenic risk from exposure to airborne bacteria from wastewater treatment plants (WWTP) has been reported for children and adults in China (Wang et al., 2018). However, to the best of our knowledge, no standard values of exposure to different airborne bacteria in waste sorting plants have been specified by pertinent organizations and authorities (Baghani et al., 2020b). Previous epidemiological works have recommended a threshold value for airborne bacteria in these workplaces during 8 h should not exceed 5000 CFU m⁻³ (Li et al., 2012; Wang et al., 2018).

Nevertheless, the severity of exposure to bioaerosols varies with its pathway (dermal contact, ingestion, or inhalation), weather conditions, the use of personal protective equipment (PPE), ventilation equipment, type and capacity of the factory, and performed activities (Li et al., 2013; Sigsgaard et al., 1990). Furthermore, additional factors in human exposure are played by the nature and processing volume of the plant, the types of bacteria released, and seasonality (Degois et al., 2017; Solans et al., 2007; Wikuats et al., 2020b). Thus, the recent global raise in the number of waste paper and cardboard sorting plants (WPCSP) for economic benefits can result in detriment to the health of staff exposed to airborne bacteria during the classification of solid waste (Baghani et al., 2020b; Degois et al., 2017; Lavoie et al., 2006; Madsen et al., 2016; Park et al., 2013b).

In this study, we report the characteristics and effects of airborne bacteria discharged from a WPCSP in Tehran and evaluate their transport from indoor to outdoor. While other studies were focused on airborne fungi and bacteria discharged from composting facilities and landfills (Bru-Adan et al., 2009; Gamero et al., 2018; Liu et al., 2021), this is the first work evaluating the airborne bacteria emitted from a WPCSP, which provides a health risk assessment (HRA) of the exposed workers. Thus, the work expands the previous scarce information available regarding plant workers' risk of exposure in a WPCSP. The present study examines the concentration and type of airborne bacteria species, and their heatmap and Venn diagrams visual relationships, the effect of atmospheric conditions and particulate matter, and provides a health risk assessment for workers. The results of this work have broad implications for other regions owing to the pervasiveness of waste sorting plant and airborne bacteria. Hence, the results below can be used by WPCSP around the world to evaluate previously unknown health problems suffered by workers and take action for controlling airborne bacteria contamination.

2. Materials and methods

2.1. Descriptions of study area

This research was conducted in a WPCSP located at 35°32'42"N, 51°23'35"E in the north of Iran (Fig. S1) (Baghani et al., 2020a; Norouzzian Baghani et al., 2020). The study area of the processing units contained a conveyor belt, two hand-picking or manual separation routes (labeled I and II), the tipping floor, and a baling machine or

automatic pressing system (Fig. 1). A total of 6 indoor processing units and 2 outdoor (positioned to the north and south of the plant) sampling locations were selected. About 3000 kg of solid waste per day are delivered to the WPCSP, with > 95% made of paper and cardboard and < 5% comprised of organic waste, plastic, glass, aluminum, textiles, metals, leather, and wood. The processed paper and cardboard were collected by scavengers, institutions, and official organizations from Tehran recycling centers, supermarkets, industrial factories, and residential, commercial, and landfill sites. After the arrival to this plant by trucks or private vehicles of the transported paper and cardboard, 102 workers processed the material. Eighty-eight of them worked in the processing units (10 in the baling machine, 12 in the conveyor belt, 28 in the hand-picking route I, 28 in the hand-picking route II, 4 in storage, and 6 in the tipping floor), and 14 in the main headquarters. The typical weight of each package (bale) generated after pressing the waste paper and cardboard in the baling machine of this plant was 1000–1700 kg. The plant occupied an area of 16,000 m² provided of 2 fans for mechanical ventilation. However, both fans were inactive during the sampling periods. Furthermore, most workers did not utilize personal protective equipment (PPE), especially N95 respirator masks, safety goggles or gloves.

2.2. Sampling methods

All sampling was completed by duplicate in 9 sites of the WPCSP following the United States Environmental Protection Agency (EPA) guidelines (EPA, 2006) for airborne bacteria. The sampling sites were chosen to include all major units, dissimilar workers conditions, and to register the effect of background ambient air from the north and south of the plant (Norouzzian Baghani et al., 2020). A total of 180 bacterial samples (twenty samples for each of the 9 sites) were taken with a 6 day frequency between 22 December 2019 and 21 January 2020.

Two QuickTake® 30 air sample pumps were equipped with a single-stage impactor (BioStage, SKC, USA) for sampling at a flow rate $Q = 28.30 \text{ L min}^{-1}$ during a time $t = 10 \text{ min}$ (Dashti et al., 2021). The workers respiratory zone was sampled with the impactors positioned 1.5 m above the ground level (Chegini et al., 2020; Dehghani et al., 2018a). The air sampler calibration was performed at each sampling location according to the BioStage impactor directions in Cat. nos. 225-9611 and 225-9610. A portable instrument (Preservation Equipment Ltd, UK) was utilized for recording simultaneously the percent relative humidity (RH%) and temperature of the sampling sites. The concentration of airborne bacteria in colony forming units per metric cube (CFU m⁻³) (Faridi et al., 2015; Naddafi et al., 2019b) was computed based on the number of colonies counted on the plates (N) as described by Eq. (1) (Mosalaei et al., 2021; Wang et al., 2018):

$$\text{Concentration}(\text{CFU m}^{-3}) = \frac{10^3 \times N}{Q \times t} \quad (1)$$

where Q is the flow rate of the sampling pump (L min^{-1}) and the sampling time is indicated by t (min).

2.3. Characterization and quantification of bioaerosols

Tryptic soy agar (TSA) culture media (Merck Co, Germany) with cycloheximide was used to identify and differentiate bacterial bioaerosols (Chegini et al., 2020; Naddafi et al., 2019a). Bergey's Manual and biochemical tests were used for the identification of the airborne bacterial species (Brown and Smith, 2014; Faridi et al., 2015; Naddafi et al., 2019a). For the taxonomy characterization, we follow the binomial nomenclature of bacteria that includes a genus and a species. Due to the presence of a mixture of bacteria on the culture media, this characterization required first to isolate and purify each type of bacteria on differential and enriched culture plates. The colonies grown on Tryptic Soy Agar (TSA) culture media were subjected to differential tests. The main method of bacteria identification involved the following



Fig. 1. Map of various processing units in the WPCSP (Baghani et al., 2020b).

biochemical assays typically used to differentiate members of the genera given in parentheses: 1) catalase test (*Staphylococcus* and *Micrococcus* spp. vs *Streptococcus* and *Enterococcus* spp.); 2) mannitol salt agar (MSA) (*Staphylococcus aureus* vs *Staphylococcus epidermidis*); 3) taxos A, a bacitracin sensitivity testing (*Staphylococcus* vs *Micrococcus* spp. and group A *Streptococcus* vs various types of streptococci); 4) blood agar plates (*Staphylococcus aureus*, *Streptococcus pyogenes* and *Streptococcus agalactiae* vs *Streptococcus pneumoniae* and *Streptococcus* vs *Staphylococcus epidermidis*); 5) DNase test agar (*Staphylococcus aureus* vs *Staphylococci*, *Serratia* vs *Enterobacter* sp., and *Moraxella catarrhalis* vs *Neisseria*); 6) oxidase test (*Pseudomonadaceae* vs *Enterobacteriaceae*); and 7) macConkey agar (for *Enterobacteriaceae*). The results of using these tests in our samples were compared with Bergey's Manual.

Specifically to this work, catalase and oxidase tests and the bacitracin susceptibility test (disks) were performed for identifying gram-positive cocci. Then, catalase positive and oxidase negative samples were transferred to mannitol salt agar (MSA) and DNase test agar. In addition, resistance or susceptibility to novobiocin disk for these bacteria was investigated in Mueller Hinton culture media. The SXT disc susceptibility test and the CAMP test were performed for catalase-negative colonies with beta-hemolysis. In addition, for catalase-negative colonies with alpha-hemolysis, bile esculin test and salt tolerance test were performed. For example, if the results of catalase test, oxidase test and bacitracin test are positive, positive and sensitive, respectively, it indicates the presence of *Micrococcus* sp. However, catalase test, oxidase test and bacitracin test are positive, negative and resistant, respectively,

it demonstrates the presence of *Staphylococcus* sp. Moreover, if the results of catalase test, bile esculin test and salt tolerance test are negative, positive and positive, respectively, it shows the presence of *Enterococcus* sp.

2.4. Quality control

2.4.1. Quality control of culture media

Quality control of growth media is a very important factor in the study of bioaerosols (Basu et al., 2005; Chegini et al., 2020; Therkorn et al., 2017). The batch of culture media was thoroughly investigated for contamination before its utilization in the laboratory. We also checked for contamination of the entire batch of the prepared media by maintaining plates at room temperature for at least 3 days. Two plates from the test batch were saved and incubated at 37 °C for 24 h to examine any bacterial growth. In the case of observing growth on the last plates, the previous process was repeated to save again two culture media from the same batch. In practical terms, if contamination of the plates is confirmed a second time or surpasses a 10% contamination threshold (Basu et al., 2005; Chegini et al., 2020; Therkorn et al., 2017), the produced media batch was discarded. Overall, following the previous protocol ensured that bacterial growth was neither observed on the two plates incubated at 37 °C for 24 h nor on the plates saved for at least 3 days at room temperature.

2.4.2. Quality control of samples

The quality control of samples included the analysis of field blanks and shipping blanks (or transport blanks). The precision of the measurements is determined by duplicate sampling (EPA, 2000). The potential for contamination resulting from the handling of the culture media was evaluated by analyzing field blanks (EPA, 2000; Therkorn et al., 2017). The blank value for bacteria was less than ten percent of the post-sampling values for all samplers. In addition, the sterility of the plates was checked by returning one unexposed shipping blank of each TSA medium. The shipping blanks consisted of unused plates in petri dishes that remained closed at the sampling site and were transferred to the laboratory with the collected air samples (EPA, 2000). The sterilized plates in the shipping blanks were confirmed neither to produce bacterial colonies nor to become contaminated during transport. For reproducibility (precision) purposes, all sampling and analysis were respectively conducted and evaluated in duplicate samples. One set of duplicate samples was gathered at a particular indoor site and another set of duplicate samples was gathered in a surrounding site (outdoor) (EPA, 2000). The reported concentrations for each sampling location correspond to the average of duplicate samples.

2.5. Statistical analysis

Analysis was performed by the statistical program R (version 3.0.1 (2013-05-16)). Analysis of variance was used to assess the differences in the concentrations of airborne bacteria at different sampling sites, and to compare them with the background (such as south and north sites in the plant), storage and office locations. The Fligner-Killeen test was applied to assess for the homogeneity of the variance and identifying the type of analysis (parametric and non-parametric tests). If the p-value obtained from the Fligner-Killeen test exceeded 0.05, the ANOVA test and Tukey test were performed for further analysis. Instead, if the p-value was less than 0.05, the Kruskal-Wallis test and the Kruskal-mac post hoc analysis were applied. The relationship between airborne bacteria concentrations and particulate matter (PM) concentrations (i.e., PM₁, PM_{2.5}, PM₁₀, and PM_{Total}) and meteorological conditions (i.e., temperature, relative humidity) were quantified using Spearman's ρ correlation coefficient.

A Venn diagram was used to graphically represent the collection of bacteria and the logical relationships between them at different processes of the WPCSP. The behavior and concentrations of different bacterial species at different sampling sites was evaluated using heat-map charts that indicated maximum (red), intermediate (black) and minimum (green) density of bacteria. In addition, indoor/outdoor ratios of bacteria (I/O Bacteria) were used to quantify the nature of pollution exchange among indoor and outdoor environments and the potential impact of ventilation and air distribution. Figures were drawn using GraphPad Prism 7 and R Statistical Software version 3.0.1.

2.6. Health Risk Assessment (HRA)

After determining the concentration of airborne bacteria in the various stages of the WPCSP, the lifetime average daily dose (LADD) in CFU (kg d)⁻¹ units was computed as described in the U.S. EPA procedure (EPA, 2011a). Since a very small number of mesophilic bacteria such as *E. coli* and *Staphylococcus aureus* discharged from WPCSP enhance the risk of cancer, the majority of mesophiles identified in this work were considered non-carcinogenic airborne bacteria (Li et al., 2013; Morgado-Gamero et al., 2019; Yan et al., 2019). In addition, the workers could be exposed to airborne bacteria mainly through dermal contact (skin) and inhalation, with the possible input from ingestion being negligible for the non-carcinogenic risk (Cangialosi et al., 2008; Li et al., 2013; Yan et al., 2019). Moreover, exposure as described by the LADD can be calculated for dermal contact and inhalation pathways (EPA, 2011c; Li et al., 2013; Yan et al., 2019). The LADD for inhalation and dermal contact were calculated from Eqs. (2) and (3), respectively:

$$\text{LADD}_{\text{inhalation}} = (C \times \text{IR} \times \text{ED} \times \text{EF}) / (\text{AT} \times \text{BW}) \quad (2)$$

$$\text{LADD}_{\text{dermal}} = (C \times \text{ESA} \times \text{SAF} \times \text{DAF} \times \text{ED} \times \text{EF}) / (\text{AT} \times \text{BW}) \quad (3)$$

where C represents the mean bacterial concentration at exposure for each sampling site (CFU m⁻³), IR illustrates the inhalation rate (m³ Day⁻¹), ED describes the exposure duration (year), EF expresses exposure frequency (days year⁻¹), AT indicates mean lifetime (year), and BW describes the body weight (kg). In Eq. (3), ESA shows the exposure skin area (m²), and SAF expresses the skin adherence factor (kg (m³ d)⁻¹). In addition, in Eq. (3), DAF represents the dermal absorption factor (unitless). The probabilistic computation was performed by Monte Carlo simulations (Oracle Crystal Ball, Version 11.1.2.4).

Because the workers could be exposed to a variety of bacteria and not just *E. coli* or *Staphylococcus aureus*, it was useful to calculate the Health Risk Assessment (HRA), as exemplified in the literature (Li et al., 2013; Morgado-Gamero et al., 2019; Yan et al., 2019) by the mean concentration of measured airborne bacteria for each sampling site. To recognize the non-cancer risk of pollutants, we computed the Hazard Quotient, HQ, a ratio for the lifetime average daily dose (LADD_{dermal} or LADD_{inhalation}) to the reference dose for chronic exposure (RfD) (CFU (kg d)⁻¹) (Jafari et al., 2021; Yan et al., 2019):

$$\text{HQ} = \text{LADD}_{\text{dermal or inhalation}} (\text{CFU (kg d)}^{-1}) / \text{RfD} (\text{CFU (kg d)}^{-1}) \quad (4)$$

In addition, the sum of total hazard quotient values computed individually for each pathway was determined as the hazard index, HI, as indicated in Eq. (5):

$$\text{HI} = \sum \text{HQ} \text{ (individually for dermal and inhalation pathways)} \quad (5)$$

The potential risk can be considerable if HQ > 1 or HI > 1, while a HQ ≤ 1 or HI ≤ 1 means that non-cancer health effects of airborne bacteria are unlikely to raise (an acceptable hazard level) (Jafari et al., 2021; Li et al., 2013). The average concentration of airborne bacteria in the various operational units was applied to compute the LADD. Risk variables employed for Monte Carlo simulations for calculating HQ, LADD, and a sensitivity analysis (SA) for airborne bacteria in the various units of WPCSP according to the average (± SD) are portrayed in Table S1. In order to determine which of those parameters in Eqs. (2) and (3) had the most effect on the LADD, a SA was performed by Monte Carlo simulations. The workers were 21–61 years old and had a 1–6 years of work experience. Therefore, for computing the lifetime average daily dose, the average work experience or the ED (year) and BW (kg) of workers were employed (Table S1). Besides, the HRA was performed for workers in the WPCSP site using the daily mean working duration of 8 h (except Fridays). Taking into account thirty holidays and the eight-hour workdays, the exposure frequency (EF) for workers was computed as follows (Baghani et al., 2020b; Durmusoglu et al., 2010):

$$\text{EF (74 days)} = [52 \text{ weeks year}^{-1} \times 6 \text{ days} \times (8 \text{ h day} / 24 \text{ h}) - 30 \text{ days of vacation}] \quad (6)$$

The HRA of microbial agents such as airborne bacteria in the air cannot be easily determined, due to the fact that the specific RfD of the respective airborne microorganisms has not yet been recommended by the scientific and medical communities. Furthermore, the allowable level of airborne bacteria in the workplace in Iran has not yet been determined because local and regional agencies have not implemented such monitoring programs yet. Nevertheless, some epidemiological works have recommended that the threshold value for airborne bacteria in the workplace for eight-hour work days should not exceed a threshold value of 5000 CFU m⁻³ for the RfD of HRA (Wang et al., 2018; Yan et al., 2019). The work below addresses the recent call to provide new experimental data (Aghaei and Yunesian, 2021) aimed to interpret and generalize the implementation of a scientific RfD value for airborne bacteria.

3. Results and discussion

3.1. Mean concentration of total bacteria in different sampling locations of WPCSP

The mean (\pm SD) concentration (CFU m^{-3}) of total bacteria in all sampling sites compared with the average concentration of total bacteria in the background locations to the north and south of the plant is shown in Fig. 2. The mean concentration for the North and South background locations in Fig. 2 were 58.5 (\pm 26.0) and 77.8 (\pm 26.6) CFU m^{-3} , respectively, which are only higher than the mean for the office (20.8 \pm 7.5 CFU m^{-3}). Fig. 2 shows that the average concentration of total bacteria in all other sampling sites (except for the office) was higher than the mean concentration of total bacteria in the North and South background points. Accordingly, the minima bacteria concentration could be bracketed between 20.8 (\pm 7.5) and 77.8 (\pm 26.6) CFU m^{-3} in the office and background locations. These minima are similar to the airborne bacteria determined in Copenhagen's ambient air of residential and reference (noncontaminated) areas that were in the range from 11 to 50 CFU m^{-3} (Madsen et al., 2016).

The maximum bacteria concentration in Fig. 2 was observed in the conveyor belt and hand-picking route I, ranging between 385.6 (\pm 89.6) and 354.6 (\pm 103.6) CFU m^{-3} . The main reason for the higher airborne bacteria concentration in these sites may be related to the early step of the waste manipulation at a relative high relative humidity for the setting, as reflected by the flow of the process and the 42.66 (\pm 1.14)% and 41.64 (\pm 5.19)% relative humidity of the conveyor belt and the hand-picking route I, respectively (Table S2). During this winter study, the ventilation system was inactive due to the cold weather, and the sack coverage the hand-picking route I for safeguarding workers from the cold weather. Moreover, hauling the waste on the conveyor belt by front-end loaders might exceed the discharge of airborne bacteria into the air (Millner et al., 1994; Park et al., 2013b; Schlosser et al., 2009). Because most workers in all units of the WPCSP did not wear personal protective equipment (PPE) (especially N95 respirator masks, safety goggles and gloves), they were exposed to the amounts of airborne bacteria depicted in Fig. 2. These values range from 117.2 (\pm 60.2) to 385.6 (\pm 89.6) CFU m^{-3} , which are in line with past works that confirmed the sorting of the solid waste was the major variable for discharging of bioaerosols into the ambient air (Baghani et al., 2020b; Nielsen et al., 1997). The main difference with the studies listed above is that the input materials in the present work included mostly paper and cardboard (> 95%) and other wastes (< 5%) (Baghani et al., 2020b). Hence, we interpret that the main factor for bioaerosol emission into ambient air is the type of the solid waste originating from activities such as collecting, composting, and recycling waste. Other studies showed

higher concentrations of 1088.8 (\pm 825.2) CFU m^{-3} for the surroundings of a waste recycling in Brazil (Wikuats et al., 2020a) and from 1395 to 5280 CFU m^{-3} for a household recycled container sorting plant (Solans et al., 2007).

The reasons for the higher concentrations detected by Wikuats et al. (2020a, 2020b) and Solans et al. (2007) than in our study may be described by differences in atmospheric conditions and difference in the amount of organic waste (Patil and Kakde, 2017; Solans et al., 2007; Viegas et al., 2014a; Wikuats et al., 2020a), waste combination/waste kind (Park et al., 2013b; Solans et al., 2007), ventilation systems (Wikuats et al., 2020a), the kind of waste-handling activities in different countries (Baghani et al., 2020b; Park et al., 2013b), and that sampling was performed during various seasons (Baghani et al., 2020b; Madsen et al., 2016). The main reason for the low concentration of airborne bacteria in this work is the lower content of organic waste (< 5%) reaching the WPCSP. For comparison, the levels of airborne bacteria and fungi in municipal landfill sites in southern Taiwan during a 3-yr study were all far above 10³ CFU m^{-3} due to high level of organic waste (Huang et al., 2002). Thus, we interpret that the concentration of airborne bacteria increases with the content of organic waste (Park et al., 2013a; Wei et al., 2017).

3.2. Dominant bacteria in different sampling locations

The mean concentration of bacteria ranked by its frequency of occurrence in different processes of the WPCSP is shown in Table 1. Accordingly, the main observed airborne bacteria for all processing units were: *Staphylococcus* sp. (72.4 CFU m^{-3}) > *Micrococcus* sp. (52.2 CFU m^{-3}) > *Bacillus* sp. (30.3 CFU m^{-3}) > *Enterococcus* sp. (24.0 CFU m^{-3}) > *Serratia marcescens* (20.1 CFU m^{-3}) > *E. coli* (19.1 CFU m^{-3}) > *Pseudomonas* sp. (16.0 CFU m^{-3}) > *Nocardia* sp. (1.9 CFU m^{-3}). For comparison, the major airborne bacteria in a French waste sorting plant separating domestic waste composed of cardboard, various kinds of plastics, cartons and metals were *Staphylococcus* sp., *Streptococcus* sp., *Prevotella* sp., *Lactococcus* sp., *Lactobacillus* sp., *Pseudomonas* sp. (Degois et al., 2021); while for waste composed of journal newspapers, food packaging, cardboards, papers, and other wastes, were *Pseudomonas* sp., *Proteobacteria*, *Acinetobacter*, *Firmicutes*, *Leuconostoc* sp., *Staphylococcus* sp., and *Lactobacillus* sp. (Degois et al., 2017). For the case of household recycling of packages made of plastics materials, ferric and non-ferric metals the dominant reported bacteria were *E. coli*, *Enterobacter* sp., *Klebsiella* sp. and *Serratia* sp. (Solans et al., 2007). In general terms, our findings are consistent with those from other researchers.

According to Table 1, *Staphylococcus* sp. had the highest frequency of occurrence compared to other bacteria counterparts for all sites. For

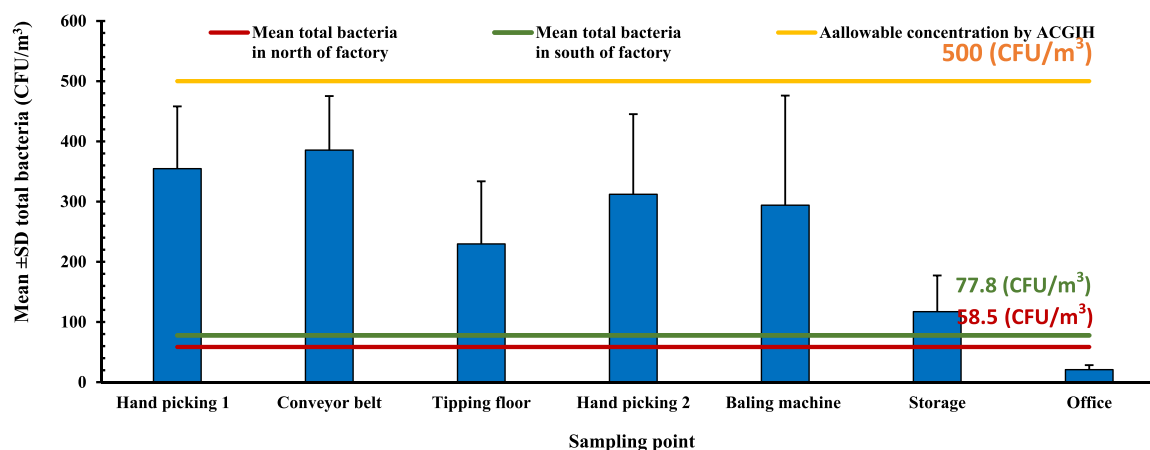


Fig. 2. The mean (\pm SD) concentrations of total bacteria in all sampling sites compared with the average concentrations of total bacteria in north and south of plant (as background locations).

Table 1
The mean concentration of bacteria ranked by its frequency of occurrence in different processes of the WPCSP.

Location	Bacteria species	Mean (UFC m ⁻³)	Standard deviation
<i>Staphylococcus</i> sp. = 80.00%			
Conveyor belt	<i>Staphylococcus</i> sp.	72.44	12.84
	<i>Enterococcus</i> sp.	22.29	9.72
	<i>Bacillus</i> sp.	12.39	23.17
	<i>Micrococcus</i> sp.	6.57	–
	<i>Serratia marcescens</i>	5.28	3.12
	<i>Pseudomonas</i> sp.	4.18	3.87
	<i>E. coli</i>	3.25	–
	<i>Nocardia</i> sp.	1.77	2.01
	<i>Staphylococcus</i> sp. = 30.77%		
Hand-picking one	<i>Staphylococcus</i> sp.	41.25	9.12
	<i>Bacillus</i> sp.	26.34	10.10
	<i>Enterococcus</i> sp.	24.03	8.57
	<i>Serratia marcescens</i>	20.11	–
	<i>Micrococcus</i> sp.	15.87	2.88
	<i>Pseudomonas</i> sp.	5.38	–
	<i>Nocardia</i> sp.	0.28	–
<i>Staphylococcus</i> sp. = 81.81%			
Hand-picking two	<i>Staphylococcus</i> sp.	57.37	12.91
	<i>Bacillus</i> sp.	22.56	9.62
	<i>Micrococcus</i> sp.	22.00	9.18
	<i>E. coli</i>	19.10	–
	<i>Enterococcus</i> sp.	16.96	5.85
	<i>Pseudomonas</i> sp.	10.10	7.61
	<i>Serratia marcescens</i>	7.66	5.08
	<i>Nocardia</i> sp.	1.85	1.38
	<i>Staphylococcus</i> sp. = 80.00%		
Storage	<i>Staphylococcus</i> sp.	57.62	20.42
	<i>Enterococcus</i> sp.	20.25	11.53
	<i>Pseudomonas</i>	15.98	–
	<i>Serratia marcescens</i>	15.46	11.82
	<i>Bacillus</i> sp.	12.21	4.98
	<i>Micrococcus</i> sp.	12.12	8.08
	<i>E. coli</i>	7.14	–
	<i>Nocardia</i> sp.	1.91	–
	<i>Staphylococcus</i> sp. = 20.00%		
Tipping floor	<i>Staphylococcus</i> sp.	67.73	–
	<i>Enterococcus</i> sp.	20.13	–
	<i>Bacillus</i> sp.	12.14	–
<i>Staphylococcus</i> sp. = 25.00%			
North of factory	<i>Staphylococcus</i> sp.	45.16	–
	<i>Enterococcus</i> sp.	25.81	–
	<i>Bacillus</i> sp.	25.81	–
	<i>Micrococcus</i> sp.	3.23	–
<i>Staphylococcus</i> sp. = 20.00%			
South of factory	<i>Staphylococcus</i> sp.	47.06	–
	<i>Micrococcus</i> sp.	20.59	–
	<i>Serratia marcescens</i>	17.65	–
	<i>Pseudomonas</i> sp.	8.82	–
	<i>Bacillus</i> sp.	5.88	–
<i>Staphylococcus</i> sp. = 0.00%			
Baling machine	<i>Staphylococcus</i> sp.	63.73	–

Table 1 (continued)

Location	Bacteria species	Mean (UFC m ⁻³)	Standard deviation
	<i>Micrococcus</i> sp.	23.69	–
	<i>Serratia marcescens</i>	6.71	–
	<i>Pseudomonas</i> sp.	5.03	–
	<i>Nocardia</i> sp.	0.84	–
<i>Staphylococcus</i> sp. = 0.00%			
Office	<i>Micrococcus</i> sp.	52.17	–
	<i>Bacillus</i> sp.	30.43	–
	<i>Staphylococcus</i> sp.	17.39	–

– The dominance bacteria in those location have been more than one species.

example, the highest frequency of *Staphylococcus* sp. was 81.81% (57.37 ± 12.91) for the hand-picking route II. However, in some locations such as the baling machine and office, more than one dominant species of bacteria are identified, which is not uncommon in the literature (Marchand et al., 1995). For example, *E. coli* and *Nocardia* sp. were abundant in all locations of the WPCSP. The difference between the bacterial species identified and those from related studies can be ascribed to the procedures applied to assess biodiversity, the management of clinical solid waste, the type of input material for sorting and recycling, and also to geographical and climatic changes in bioaerosols biodiversity (Baghani et al., 2020b; Degois et al., 2017, 2021; Hossain et al., 2013; Smets et al., 2016).

Considering that 1) *Serratia marcescens* can cause endemic and epidemic nosocomial infections such as respiratory tract, urinary tract, wounds, and bloodstream (Bremer and Darouiche, 2005; García et al., 1996; Hossain et al., 2013), 2) *Staphylococcus* sp., *Enterococcus* sp., *Serratia marcescens*, and *E. coli* can be etiological agents for nosocomial infections (Bremer and Darouiche, 2005; Hossain et al., 2013; Tang and Stratton, 2010), and 3) *E. coli* and *Serratia marcescens* can cause genitourinary tract infection, intestinal diseases and respiratory system infections (Hossain et al., 2013; Kaźmierczuk and Bojanowicz-Bablok, 2014), we conclude that the workers in WPCSP of Tehran are exposed to respiratory tract diseases and nosocomial infections. The origin of infectious bacteria such as *Staphylococcus* sp., *Enterococcus* sp., *Serratia marcescens*, and *E. coli* in the polluted air sampled at the WPCSP strongly suggest that the waste paper and cardboard was contaminated by infectious agents of some health centers. Therefore, a preventive action plan should be implemented, including some level of government regulation and inspection for monitoring the recovering, recycling and reusing of waste paper and cardboard to enhance the health protection in such factories.

3.3. The relative frequency and mean concentration of various bacteria species in different locations of the WPCSP

Fig. S2 shows the relative frequency (%) of various bacteria species in different processes of the WPCSP. Accordingly, the results show that the relative frequency of *Pseudomonas* sp. in hand-picking routes I and II were lower than for other species, while for *Staphylococcus* sp. and *Bacillus* sp. were higher for the tipping floor and conveyor belt than for other species. The highest frequency of occurrence of *Pseudomonas* sp. and *Serratia marcescens* (Fig. 2) occurred in the office and background spots south and north of the plant, while their relative frequency in processing units was low. *Pseudomonas* sp. is an opportunistic airborne microorganism and gram-negative bacteria (Schlosser, 2019); and it can be emitted from fresh and stored plant materials in some locations such as hand-picking routes I and II, tipping floor, storage, and conveyor belt. Then, *Pseudomonas* sp. can be transferred from those locations into other sites such as the office and background locations.

The average (± SD) concentration of various species of bacteria in different sampling points is shown in Fig. 3. The largest mean

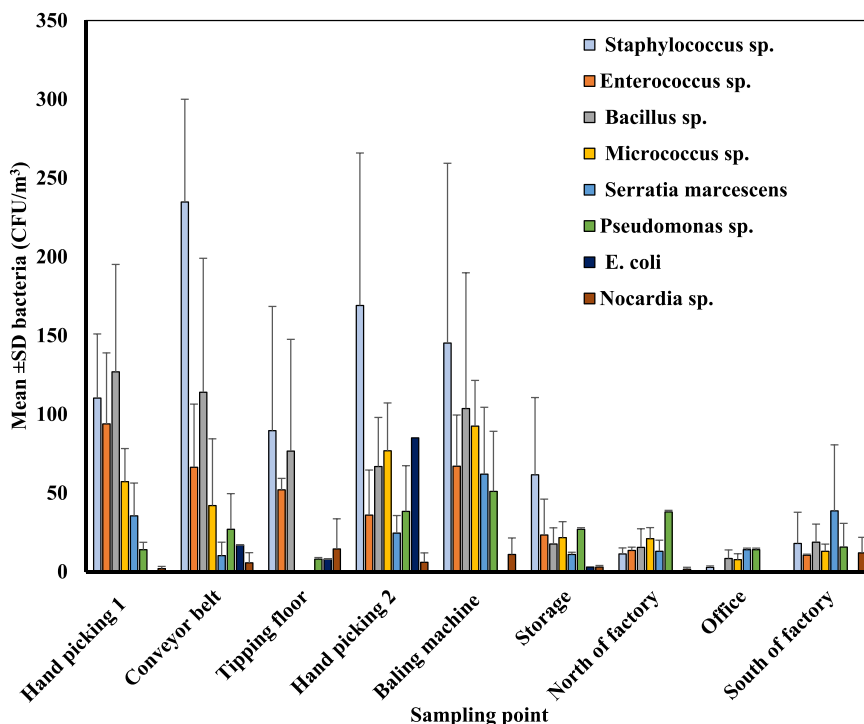


Fig. 3. The average (\pm SD) concentrations of various species of bacteria in different sampling points.

concentration in Fig. 3 were observed for *Staphylococcus* sp. in all processing units (compared to the background locations south and north of plant, storage and office). In addition, Fig. 3 shows that *Serratia marcescens* was distributed to the background locations (south and north of plant) and the office sector, where it was higher than for all processing units of the WPCSP, because its bacterial strains are resistant to various environmental stresses and produce extended-spectrum beta-lactamases (ESBL) (Bremer and Darouiche, 2005; García et al., 1996; Hossain et al.,

2013; Tang and Stratton, 2010). Past work showed that *E. coli* can be discharged into the air of a landfill in Poland with a mean concentrations from 3.3 to 62.0 CFU m⁻³ and transferred at a distance of 150–200 m from the landfill facility with concentrations of 4–12 CFU m⁻³. The reasons for the previous opposing trend to our finding can be explained by differences in the type of activities (landfilling vs recycling or paper and cardboard sorting plant), the type of input material (mixed solid waste vs paper and cardboard (90%)), metrological and geographical

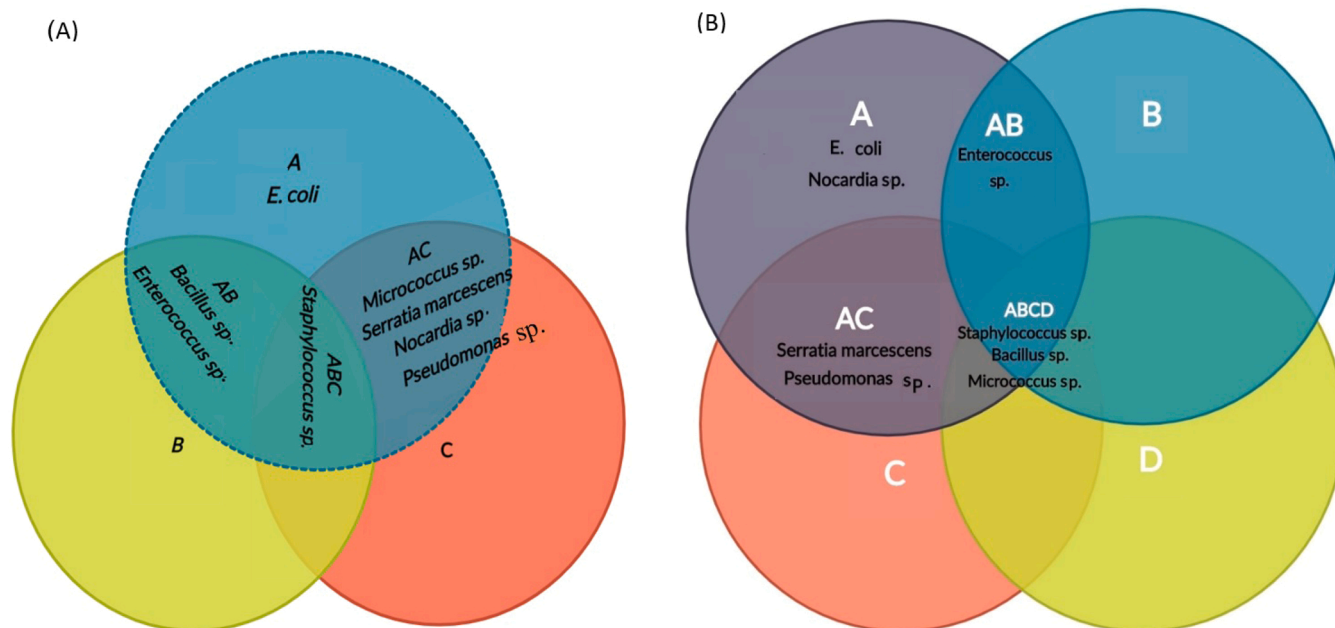


Fig. 4. A Venn diagram of different species of bacteria separately at different processes (A: conveyor belt and hand-picking route one and two; B: tipping floor; C: baling machine: Fig. 4a) and different species of bacteria in A: whole processes of WPCSP; B: north of plant; C: south of plant; D: office (Fig. 4b). Note: The points are shown in combination in Fig. 4b, such as AB, referring to a group of bacteria that are common between stages A (whole processes of WPCSP) and B (north of plant); or about ABCD, referring to a group of bacteria that are common between stages A (whole processes of WPCSP), B (north of plant), C (south of plant), and D (office).

conditions (Poland vs Iran), and the season of sampling (autumn vs winter) (Kazmierczuk and Bojanowicz-Bablok, 2014).

3.4. A Venn diagram and heatmap diagram of different species of bacteria separately at different sampling locations

A Venn diagram of different species of bacteria separated at different stages of the WPCSP (including conveyor belt, hand-picking routes I and II, tipping floor and baling machine), the background locations (north and south of plant), and the office is provided in Fig. 4a. The union and intersection of bacteria species separated at different stages of the WPCSP, show that *Staphylococcus* sp. is identified in Fig. 4 for all processing units, which is consistent with the findings of others (Islam et al., 2019). However, *E. coli* was only measured in the conveyor belt and hand-picking routes I and II, which is a similar trend to that observed elsewhere for a Tie-Stall dairy barn (Islam et al., 2019). Possibly, *E. coli* is observed only in conveyor belt and hand-picking routes I and II due to their higher relative humidity (41.64 ± 5.19 – 46.78 ± 1.33) than for background locations (south and north of factory) with low relative humidity (33.48 ± 1.07 – 33.86 ± 1.43). Low temperature and high relative humidity conditions favor the growing of *E. coli* in those processes as compared to the background locations (Wathes et al., 1986). The existent increased relative humidity during sampling can be ascribed to the inactive fans, which also allows for the growth and

accumulation of other bacteria (Viegas et al., 2014b) such as *Staphylococcus* sp., *Bacillus* sp. and *Micrococcus* sp. Indeed, the absence of mechanical ventilation and improper air exchange during the separation of paper in the waste sorting plant can increase the concentration of bio-aerosols (Viegas et al., 2014b).

Fig. 4b shows that *E. coli* and *Nocardia* sp. are identified in all processing units of the WPCSP, but are not significant in the background locations (north and south of plant), storage, and office areas due to their higher temperature and lower relative humidity. Fig. 4b also indicates that *Enterococcus* sp. was the only bacteria detected among the processing units and the north background site, while *Serratia marcescens* and *Pseudomonas* sp. were merely detected among the processing units and the south background site, which can be linked to the environmental conditions and type of activities performed (Breum et al., 1999; Degois et al., 2021). Importantly, *Staphylococcus* sp., *Bacillus* sp. and *Micrococcus* sp. were commonly identified in all processing units and background locations (north and south of plant), storage, and office (Fig. 4b). Therefore, three of the most dangerous bacteria (*Staphylococcus* sp., *Bacillus* sp. and *Micrococcus* sp.) potentially affecting the health of workers.

Furthermore, the identity and concentration of different bacterial species for all sampling stations of the WPCSP, the background locations (north and south of plant), the storage, and office is displayed as a heatmap chart in Fig. 5. The highest concentrations of bacteria are

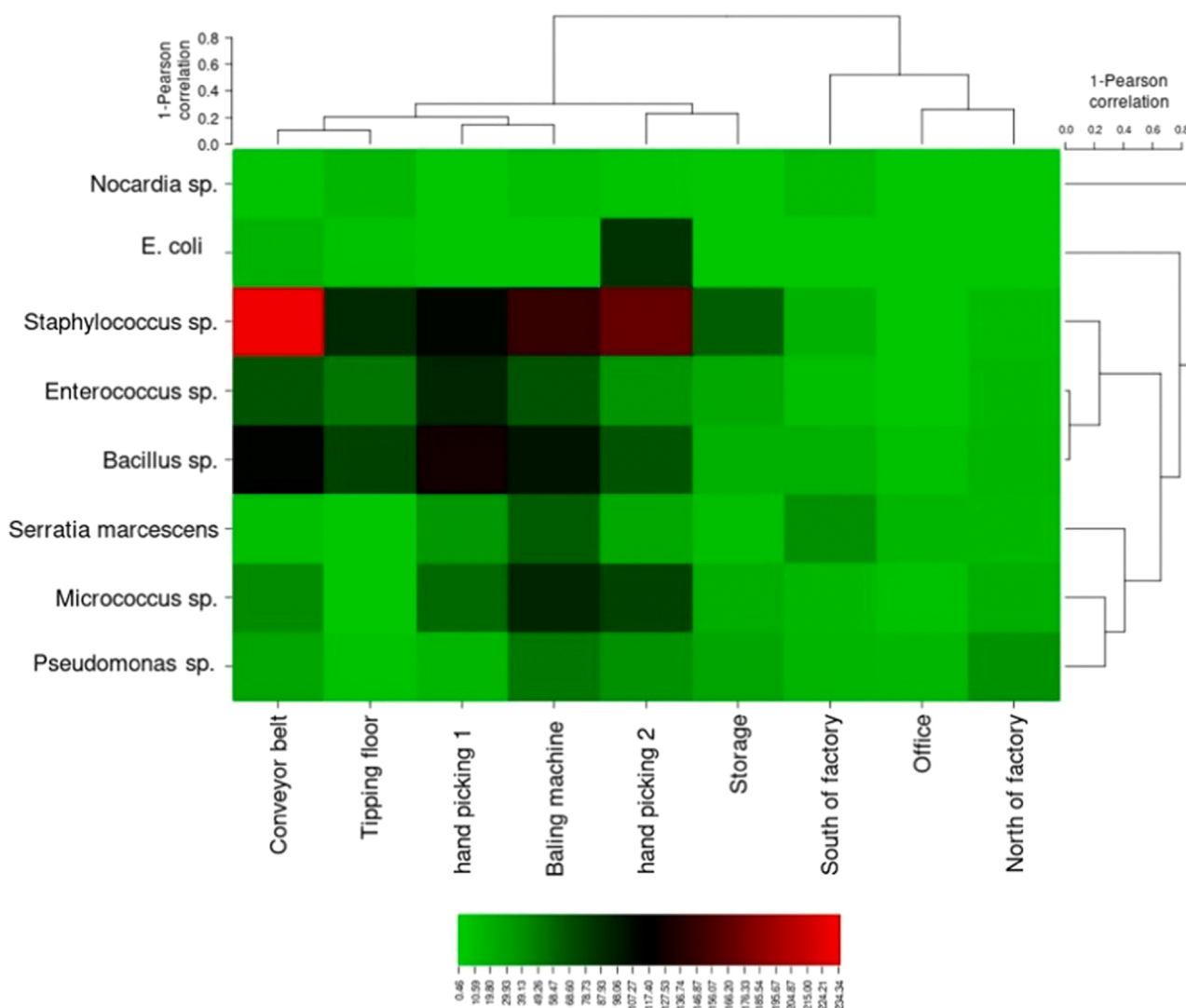


Fig. 5. A heatmap diagram for different species of bacteria at different processes of WPCSP, background points (north and south of plant) and office.

associated to the processing units in Fig. 5, especially to the hand-picking route II, conveyor belt and baling machine. The most contaminant species (red color indicates maximum concentration in Fig. 5) were *Staphylococcus* sp. > *Bacillus* sp. > *Enterococcus* sp., while the background sampling points, and the office are relatively clean (green color) as the density of bacterial in those area is low. Fig. 5 also indicates that the difference between the concentrations of bacterial in background points (north and south of plant) were statistically significant ($p < 0.05$). Moreover, Fig. 5 shows that the concentration of *Nocardia* sp. was consistently low (light green color) in all processing units of the WPCSP, background points, office, and even storage. The information in Fig. 5 reveals which site has the higher concentration of bacteria, and informs responsible parties the locations more prone to microbial contamination (possibly causing health problems) of workers. Consequently, workers can be informed about the importance of implementing and respecting PPE policies. Despite any physical discomfort from the implementation of PPE (e.g., from wearing masks), workers should understand that they serve to protect them from exposure to even low levels of potential pathogenic airborne bacteria. The high concentrations of airborne bacteria in some locations can be explained by the factory position and sampling site. For example, the wind direction and movement of front-end loaders can transfer bioaerosols from the tipping floor to the conveyor belt. Thus, the provided heatmap chart (Fig. 5) can contribute to create preventive measures for this and other WPCSP.

3.5. Consideration of airborne concentrations and proposed guidelines

Despite the health risks of exposure to bioaerosols, standard values of bacteria concentrations in the workplace have not yet been regulated (Baghani et al., 2020b; Dehghani et al., 2018b; Naddafi et al., 2019a). Nevertheless, some organization such as the American Conference of Governmental Industrial Hygienists (ACGIH), Polish standard (PN-89Z-04111/02) and Swiss OELs, have proposed total bacterial concentrations limits from 500 CFU m⁻³, 1000–3000 CFU m⁻³ and 10,000 CFU m⁻³, respectively (Božić and Ilić, 2019; Dehghani et al., 2018b; Li et al., 2013; Michalkiewicz et al., 2011; Oppliger et al., 2005). A threshold limit value (TLV) of 5000 CFU m⁻³ RfD of airborne bacteria in the workplace was suggested by the Research Center for Eco-Environmental Sciences (RCEES) of the Chinese Academy of Sciences (Li et al., 2012, 2013).

Thus, a simplistic interpretation of the data in Fig. 3 would indicate that airborne bacteria in the different sampling locations of the WPCSP were below the values listed above from ACGIH (Božić and Ilić, 2019; Jensen and Schafer, 1998), RCEES (Li et al., 2012, 2013), the Polish standard (Michalkiewicz et al., 2011), and the Swiss OELs (Oppliger et al., 2005). However, the fast surge of new bioaerosols transmitted diseases raises concerns about the validity and specificity of such limits. Furthermore, in the context of this work, there are neither standard values for airborne bacteria in the workplace in Iran nor official monitoring programs. Despite the concentration of bacteria sampled was lower than the pre-recommended standards, the fact that bacteria can create health hazards even at low concentrations cannot be disregarded, specially applied to those susceptible workers that have not been protected by PPE. Otherwise, we would be ignoring the devastating example and consequences to humanity of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Delikhoon et al., 2021; Guzman, 2021).

3.6. Correlation of bacteria concentration with environmental factors and particulate matter

Fig. S3 illustrates the correlation between the average concentration of airborne bacteria and RH% and temperature. For example, the lowest temperature and the highest RH% were registered for both hand-picking routes I (12 ± 0.35 °C and $42 \pm 5\%$) and II (12 ± 0.24 °C and $47 \pm 1.4\%$). Spearman's correlation analysis ($p \leq 0.001$) reveals that

RH% and temperature had a significant positive ($r = 0.59$, Fig. 3a) and negative ($r = 0.49$, Fig. 3b) correlation, respectively, with the concentration of airborne bacteria.

Temperature and RH% are they key factors to regulate the survival of airborne bacteria in air (Dashti et al., 2021; Dehghani et al., 2018b; Faridi et al., 2015). Bacteria growth in indoor air at RH% of 30–60% is well documented (Dehghani et al., 2018c; Qudiesat et al., 2009), and death rates (i.e., for *Serratia marcescens*, *E. coli*, *Salmonella* sp., and *Pseudomonas* sp.) for RH% of 50–70% are low (Tang, 2009). In addition, *E. coli* and *Enterococcus mundtii* can remain alive after aerosolization from several minutes to more than one hour for the temperature range from 10 to 30°C and RH% from 40% to 80% (Hoeksma et al., 2015). Meteorological conditions (high RH% and low temperature) not only favor the survival of airborne bacteria, but have also been associated to their enhanced protection from UV-induced inactivation (Dehghani et al., 2018b; Korzeniewska, 2011). Logically, the concentration of airborne bacteria in the morning (8:30–10:00 A.M.) and evening (5:30–8:30 P.M.) can be higher due to temperature drop and RH% rise (Faridi et al., 2015). If all other factors affecting airborne bacteria concentration are standardized, a temperature > 24 °C results in a drop of airborne bacterial survival for *Pseudomonas* sp., *E. coli*, *Serratia marcescens*, and *Bacillus* sp. The Supporting information provides more specific details related to this matter.

Fig. S4 shows the correlation between the mean concentration of airborne bacteria (CFU m⁻³) and particulate matter ($\mu\text{g}/\text{m}^3$). The average concentration of all bacteria was positively correlated to all types of suspended particles: PM₁₀ ($p < 0.001$, $r = 0.660$), PM_{2.5} ($p < 0.001$, $r = 0.710$), PM₁ ($p < 0.001$, $r = 0.710$) and PM_{total} ($p < 0.005$, $r = 0.70$). In other words, for increasing concentration of target particular matter (pollutants), there is a boost in the observed concentration of airborne bacteria in the WPCSP atmosphere (Islam et al., 2019; Liu et al., 2018).

For the variable temperature, Spearman's correlation analysis shows a negative correlation with the concentration of suspended particles ($p \leq 0.001$ and $r = -0.36$ for PM₁, $p \leq 0.001$ and $r = -0.36$ for PM_{2.5}, $p \leq 0.001$ and $r = -0.32$ for PM₁₀, $p \leq 0.001$, and $r = -0.34$ for PM_{total}) (Table S3), which is consistent with past work in ambient air in Erzurum (Turkey) ($r = -0.795$ and $p < 0.001$) (Turahoglu et al., 2005). This means that with decreasing temperature the concentration of suspended particles were incremented, which led to increased concentrations of bacteria. In addition, our data in Table S3 does not show a significant correlation between RH% with the concentration of PM₁, PM_{2.5}, PM₁₀, and PM_{tot}, (Al-Taai and Al-Ghabban, 2016). More details about Section 3.6 are available in the Supporting Information.

3.7. Statistical analysis of bacteria bioaerosol in different sampling locations

3.7.1. Comparison of different operational units

A box plot for the distribution of the total bacteria characterized at different processes of the WPCSP is provided in Fig. S5a, and for background locations (north and south of plant) in Fig. S5b, and for the sum of all processes in the WPCSP vs the background locations, office and storage in Fig. S5c. The analysis of Fig. S5a using the Fligner-Killeen test provided a p -value of 0.345 for bacterial bioaerosols for the different processes of the WPCSP, suggesting a non-significant difference in the variance of all sampling sites ($p > 0.05$). The ANOVA analysis reveals a p value of 0.525 for bacterial concentrations, demonstrating non-significant differences in bacterial concentrations between the different processing units ($p > 0.05$).

3.7.2. Comparison of all sampling locations (processes of WPCSP, background sites, storage, and office)

The minimum and maximum quartiles (Q1 and Q3) and the median concentration of bacteria are represented in Fig. S5c, which clearly highlights that the distribution of total bacteria in all the processes of the

WPCSP is different from those in background locations, the office and storage unit. The mean concentration of total bacteria in all the processes of the WPCSP is $323 (\pm 125) \text{ CFU m}^{-3}$, which is higher than for the storage ($117 (\pm 60) \text{ CFU m}^{-3}$), office ($21 (\pm 7) \text{ CFU m}^{-3}$), and background sites to the north ($59 (\pm 26) \text{ CFU m}^{-3}$) and south ($78 (\pm 27) \text{ CFU m}^{-3}$) of the plant. The Fligner-Killeen test showed a p value of 0.004 for bacteria bioaerosol in all sampling stations, indicating a significant difference in the variance of all sampling locations ($p < 0.05$). Given this $p < 0.05$ value, the Kruskal-Wallis test and the Kruskal-Mac post hoc analysis were applied for further analysis. Hence, the results of the Kruskal-Wallis test on bacteria concentrations in different sampling locations show a significant difference between processing units with background sites, the storage and office. In addition, the results of the Kruskal-Mac post hoc test show the largest differences correspond to those of the total processing units with the office and background locations (Table S4). There was no difference between the processing units and the storage, and the north of south background

sites, background sites and office, and background sites and storage.

3.8. Ratio of indoor bacteria to outdoor bacteria (I/O bacteria)

The I/O Bacteria is usually used to quantify the nature of pollution exchange among indoor and outdoor ambient environments, and the potential impact of ventilation and air distribution in indoor air quality (Borrego and Molina, 2019; Borrego et al., 2020; Chegini et al., 2020). I/O Bacteria values > 2 , between 1.5 and 2, and ≤ 1.5 are typically interpreted in terms of weak, normal, and well ventilated indoor air, respectively (Baghani et al., 2020b). For the conveyor belt, hand-picking route I, hand-picking route II, storage, tipping floor, and the baling machine the I/O Bacteria are 5–6.6, 4.6–6.1, 4–5.3, 1.5–2, 3–3.9, and 3.8–5, respectively (Table S5), which demonstrate the indoor air of the WPCSP was contaminated, and weak air circulation and ventilation (Baghani et al., 2020b; Borrego and Molina, 2019; Chegini et al., 2020; Harbizadeh et al., 2019; Wikuats et al., 2020a). In contrast, the I/O

Table 2

Comparison of the results of LADD ($\text{CFU (kg d}^{-1})$), HQ (dimensionless) and SA (%) for dermal and inhalation for all sampling locations and HI dermal and inhalation for processes units, in this work and similar studies (LADDs: life time average daily dose, HQ: hazard quotient, SA: sensitivity analysis, HI: total health risk (dimensionless), C: concentration of the pollutant, BW: body weight, ED: Exposure duration).

	Sampling location	LADD _{Inhalation}	HQ _{Inhalation}	LADD _{Dermal}	HQ _{Dermal}	Site	Ref.
Mean	Baling machine	1.56×10^1	3.02×10^{-3}	1.25×10^{-5}	2.48×10^{-9}	In WPCSP, Tehran (Iran)	Present work
SD		1.34×10^1	2.52×10^{-3}	1.01×10^{-5}	2.05×10^{-9}		
Mean	Conveyor belt	2.07×10^1	4.13×10^{-3}	1.64×10^{-5}	3.27×10^{-9}	In WPCSP, Tehran (Iran)	Present work
SD		1.18×10^1	2.23×10^{-3}	9.01×10^{-6}	1.74×10^{-9}		
Mean	Hand picking 1	3.7×10^{-3}	3.76×10^{-3}	1.47×10^{-5}	2.91×10^{-9}	In WPCSP, Tehran (Iran)	Present work
SD		2.13×10^{-3}	2.34×10^{-3}	8.66×10^{-6}	1.72×10^{-9}		
Mean	Hand picking 2	1.67×10^1	3.25×10^{-3}	1.35×10^{-5}	2.63×10^{-9}	In WPCSP, Tehran (Iran)	Present work
SD		1.09×10^1	2.33×10^{-3}	9.19×10^{-6}	1.79×10^{-10}		
Mean	North of factory	3.08	6.27×10^{-4}	2.48×10^{-6}	5.05×10^{-10}	In WPCSP, Tehran (Iran)	Present work
SD		2.13	4.17×10^{-4}	1.74×10^{-6}	3.63×10^{-10}		
Mean	Office	1.11	2.21×10^{-4}	8.66×10^{-7}	1.76×10^{-10}	In WPCSP, Tehran (Iran)	Present work
SD		7.31×10^{-1}	1.37×10^{-4}	5.57×10^{-7}	1.13×10^{-10}		
Mean	South of factory	4.07	8.23×10^{-4}	3.34×10^{-6}	5.52×10^{-10}	In WPCSP, Tehran (Iran)	Present work
SD		2.51	5.06×10^{-4}	2.44×10^{-6}	4.13×10^{-10}		
Mean	Storage	6.05	1.26×10^{-3}	4.75×10^{-6}	9.66×10^{-10}	In WPCSP, Tehran (Iran)	Present work
SD		4.53	9.84×10^{-4}	3.69×10^{-6}	7.17×10^{-10}		
Mean	Tipping floor	1.21×10^1	2.47×10^{-3}	9.68×10^{-6}	1.94×10^{-9}	In WPCSP, Tehran (Iran)	Present work
SD		8.43	1.71×10^{-3}	6.65×10^{-6}	1.34×10^{-9}		
Mean	¹ HI _{Inhalation} in processes units	–	2.7×10^{-2}	–	–	In WPCSP, Tehran (Iran)	Present work
SD		–	1.3×10^{-2}	–	–		
Mean	² HI _{Dermal} in processes units	–	–	–	1.54×10^{-8}	In WPCSP, Tehran (Iran)	Present work
SD		–	–	–	8.63×10^{-9}		
Mean	HQ _{Inhalation} and HQ _{Dermal}	–	1.08×10^{-3} -6.37×10^{-7}	–	1.22×10^{-7} – 1.21×10^{-11}	Wastewater Treatment Plant of Xi'an, China	(Li et al., 2013)
Mean	HQ _{Inhalation} and HQ _{Dermal}	–	72.91×10^{-4} – 13.73×10^{-4}	–	72.91×10^{-4} – 13.73×10^{-4}	Wastewater treatment plant, Tianjin (China)	(Wang et al., 2018)
Mean	HQ _{Inhalation} and HQ _{Dermal}	–	5.123×10^{-5} (PCDD/Fs)	–	1.672×10^{-8} (PCDD/Fs)	Municipal solid waste incineration plant (Taranto's MSWI), Taranto, Italy	(Cangialosi et al., 2008)
Sensitivity analysis for inhalation and dermal (SA _{Inhalation} and SA _{Dermal})							
	SA _{Dermal} ED (%)	BW (%)	C (%)	SA _{Inhalation} ED (%)	BW (%)	C (%)	Ref.
Baling machine	23.2	4.9	71.7	26.8	4.2	68.6	Present work
Conveyor belt	66.8	12.5	20.1	69.5	10	20.4	Present work
Hand picking 1	58.4	13.6	27.8	56.7	11	31.9	Present work
Hand picking 2	52.2	6	41.3	40.9	7.8	50.9	Present work
North of factory	50	6.3	43.6	38.8	5.3	55.7	Present work
Office	54.5	6.4	38.6	50.2	6.6	43	Present work
South of factory	51.9	10.4	37.2	52.7	9.5	37.4	Present work
Storage	31.5	5.5	62.3	30.4	7.2	61.9	Present work
Tipping floor	40.2	5.7	53.7	42.4	5.1	51.9	Present work

* The hazard index (HI) represents the sum of hazard quotient for each pathway in operational units (processes units) that included tipping floor, baling machine, hand picking 1 and 2, and conveyor belt.

Bacteria for the office is 0.3–0.4 (Table S5), suggesting it had good air circulation and ventilation.

3.9. Health Risk Assessment (HRA)

The findings of the airborne bacteria LADD, HQ, SA, and total health risk in processing units (HI) are presented in Table 2. It is apparent that the HI of the inhalation pathway (2.7×10^{-2}) was larger than for the dermal pathway (1.54×10^{-8}) for the processing units. Therefore, inhalation dominated the intake pathway of airborne bacteria by workers, which agrees observations for wastewater treatment plants in Xi'an (HQ for inhalation vs HQ for dermal: 1.08×10^{-3} – 6.37×10^{-7} vs 1.22×10^{-7} – 1.21×10^{-11}) (Li et al., 2013) and Tianjin (HI for inhalation and HQ for dermal: 72.91×10^{-4} – 13.73×10^{-4}) (Wang et al., 2018). Adverse health effects are associated to exposure to air with HQs ≥ 1 , whereas HQs < 1 indicates an acceptable level of risk (EPA, 2011b; Nazmara et al., 2020; Wang et al., 2018). The calculated HQs of the inhalation pathway in the processing units is between 1.26×10^{-3} and 4.13×10^{-3} , whereas the HQs of the dermal pathway is even smaller (between 9.66×10^{-10} and 3.27×10^{-9}), which are acceptable levels of risk.

Because the HQs and HIs of airborne bacteria (Table 2,) in the work sites are < 1 in this work, in principle there should be no concern about the non-carcinogenic risk of airborne bacteria. The LADD sensitivity for airborne bacteria via inhalation and dermal pathways is also provided in Table 2. The LADD_{inhalation} in processing units is between 3.7×10^{-3} and 2.07×10^1 CFU (kg d)⁻¹, while LADD_{dermal} ranges from 4.75×10^{-6} to 1.64×10^{-5} CFU (kg d)⁻¹. According to Table 2, the ED $> 50.2\%$ had the most effect on the LADD in the processing units such as the conveyor belt and hand-picking route I, in the office and in south background site for both inhalation and dermal pathways. However, the concentrations of airborne bacteria ($C > 51.9\%$) had the most effect on the LADD in the baling machine, storage and the tipping floor for both pathways. In addition, the concentration of airborne bacteria ($50.9 \leq C \leq 55.7\%$) had the most effect on the LADD_{Inhalation} for the hand picking route II and the north background site. The LADD_{Dermal} was most influenced by the ED (50–52.2%) in the hand picking route II and the north background site. Finally, the third most influential factor on the LADD_{Inhalation} and LADD_{Dermal} (after ED and C) was BW% for all sampling locations.

4. Conclusions

The identified and quantified airborne bacteria produced from a WPCSP in Tehran can represent a skin and respiratory health hazard to plant workers and adjacent residents in winter. The results of this work showed that the conveyor belt ($385.6(\pm 89.6)$) and hand-picking route I ($354.6(\pm 103.6)$) generated high concentrations of airborne bacteria. The LADD_{Inhalation} in processing units ranges between 3.7×10^{-3} and 2.07×10^1 CFU (kg d)⁻¹, while the LADD_{Dermal} was between 4.75×10^{-6} and 1.64×10^{-5} CFU (kg d)⁻¹. The high frequency of airborne bacteria in the air of all operational units in the WPCSP, containing *Staphylococcus* sp., *Enterococcus* sp., *Serratia marcescens*, and *E. coli*, may represent a health hazard for the WPCSP workers that remained inside the WPCSP for a long day. The workers in the WPCSP studied can be exposed to respiratory tract diseases and nosocomial infections by *Staphylococcus* sp., *Enterococcus* sp., *Serratia marcescens*, and *E. coli* that are released by paper and cardboard sorting in the plant. Although the numerical value of HQ of airborne bacteria was less than 1 (indicating an acceptable level), the airborne I/O bacteria ratio for the processing units of the WPCSP (1.5–6.6) was typically > 2 , which indicates infectious conditions for workers exposed to indoor air. Thus, indoor air should be controlled by supplying mechanical or natural ventilation. Hence, the results of this study emphasize the necessity to control WPCSP workers' exposure to bioaerosols when airborne bacteria become aerosolized during waste sorting. These findings stimulate

improved tactics for recycling activities, composting, and landfilling that restrict the exposure of workers to discharged mycotoxins, microbial volatile organic compounds (MVOC), airborne fungi and bacteria, and fungal spores.

CRedit authorship contribution statement

Conceptualization, A.N.B., G.E., M.D., M.I.G, and R.N.; Methodology, A.N.B., M.D., M.I.G., and S.G; Writing – original draft, A.N.B. and M.D.; Writing – review & editing, A.N.B., G.E., M.D., M.I.G, and R.N.; Visualization, A.B., A.N.B., M.D., and M.I.G.; Software, A.B., A.N.B., M.J.R., and S.G.; Supervision, A.N.B. and R.N.; Project administration, A.N.B., M.I.G and R.N. Formal analysis, A.B., A.N.B., M.J.R., and S.G; Validation, A.B., A.N.B., G.E., M.D., M.I.G, M.J.R., R.N., and S.G. All authors have read and agreed to the published version of the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2022.113272.

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