Indoor air quality inside vehicle cabins while commuting in Lisbon

Nicole D. Buitrago Russi*
*Instituto Superior Tecnico, Lisboa, Portugal. October 2019.
nbuitrago@ctn.tecnico.ulisboa.pt

Abstract:
Commuters may be exposed to a variety physicochemical and biological pollutants that could lead to adverse health effects. This study aims to evaluate the indoor air quality (IAQ) while commuting in cars, buses and trains in Lisbon to contribute in the assessment of possible effects on human health.
Particulate matter (PM) with diameter lower than 1, 2.5 and 10 µm, black carbon (BC), carbon monoxide (CO), carbon dioxide (CO_2) volatile organic compounds (VOCs) and formaldehyde (CH_2O) were measured. In cars the effect of cleaning and three ventilation modes were evaluated: fan off, fan on and air-conditioning (AC). Total airborne bacteria and fungi were estimated, and bacterial isolates identified. The inhaled dose in each vehicle was estimated.
In cars, the AC decreased the $PM_{2.5\text{-}10}$ concentration to 0.7 µg/m^3, but it increased BC concentration to 4.1 µg/m^3. The effect of cleaning was negligible. $PM_{2.5}$ concentration in buses ranged between 8 - 73 µg/m^3, similar to cars under fan off condition. BC concentration in buses was 4.5 µg/m^3 similar to cars with AC. CO_2, VOCs and CH_2O found in cars were higher under the fan off condition. In trains, the concentrations of all pollutants were low, with an exception of VOCs (2516 µg/m^3). Fungi and bacterial loads were higher in trains and buses.
In this study the ventilation mode was identified as the main factor affecting the IAQ. Additionally, it was possible to provide a general perspective of the IAQ in public transport

Keywords: indoor microbiota; Lisbon; exposure assessment; inhaled dose; vehicle cabin

1. Introduction

Along the years, cities have faced air quality detriment as a consequence of reliance on fossil fuels, degradation of green areas, dependence on private automobiles and traffic (Ozcan & Cubukcu, 2018). In countries of the EU-28, traffic emissions accounts for 46% of nitrogen oxides (NOx) and 15% of particulate matter with aerodynamic diameter $\leqq$ 2.5 µm ($PM_{2.5}$), which has lead the EU to stablish goals to improve the urban mobility and air quality (European Commission, 2017). In Lisbon Metropolitan area (LMA), cars are the main transport mode used to commute within and between different municipalities (51.6% and 50.2%), followed by buses (15.2 and 21.9%) and trains (4,7 % and 18.8%) according to the INE (Câmara Municipal de Oeiras, 2016)

It is known that commuters health is particularly affected by traffic-related air pollutants due to their proximity to the source, but additionally, due contact duration; commuters spend around 5.5% of the daily time inside vehicles cabins (Xu et al., 2016). This microenvironment is susceptible to contamination as outside pollutants can accumulate inside cabins, and interior materials can be a potential pollutant source (Barnes et al., 2018; Xu et al., 2016). Fixed monitoring stations provide a general idea of the population exposure to air pollutants, but studies have found poor correlation of personal measurements with monitoring stations (Grana et al. 2017; Rivas et al., 2017)

The short- and long-term exposure of children and adults to air pollutants has a significant influence on respiratory infections, severe asthma and reduced lung function(European Environment Agency, 2018). Apart from physicochemical pollutants, many studies fail to assess the exposure to bioaerosols. The vehicle's enclosed environment provides conditions for the transmission of a wide range of pathogens and infections. Thereby, the exposure to elevated loads of microbes is often associated with asthma, hypersensitivity pneumonitis and inflammatory reactions (Jo & Lee, 2008).

Although indoor air quality (IAQ) while commuting has been well studied, the comparison between results is complex and fail to reach a conclusion. For example, commuting with closed windows, ventilation and air recirculation, is documented to decrease the exposure to PM and BC (Hong, 2019), but other studies have proved the accumulation of CO_2 and CO when air is recirculated (Dirks et al., 2018; Grana et al., 2017). Exposure is affected by transport mode, commute location, commuting conditions and time. As such, it is important to study each case and identify the parameters affecting pollutants concentration in different microenvironments. Thus, this study aimed to evaluate the exposure to indoor air pollutants while commuting in three different transportation modes: cars, buses and trains.

2. Methods

2.1. Location

This study was carried out in the Lisbon and Loures municipalities; both located in the LMA. Lisbon has a population of 547 733 inhabitants where the center and northern neighborhoods are the most populated.
According to the 2011 census, Lisbon is the final destination of most of the commutes performed by inhabitants of Loures (45.0000 inhabitants), while only 6200 inhabitants from Lisbon have as final destination Loures.

2.2. Route selection

Three cars with different motor types (gasoline, diesel and electric) were used. The journey took place from a residential neighborhood (Moscavide) to the Instituto Superior Técnico (IST) covering a distance of 8 km (Figure 1). For each car three conditions were exanimated, without ventilation (fan off), with ventilation (fan on) and with air conditioning (AC), all cars had windows closed and recirculation mode off. Additionally, the effects of cleaning were also studied, thus, the cars were sampled before and after a typical cleaning routine performed by the owners, in total 18 commutes were sampled.

Figure 1. Routes performed in car, bus and train. Blue line for trains, orange line for cars and black line for buses.

Public transport commutes were done in Rodoviaria de Lisboa buses and Comboios de Portugal trains. All sample campaigns were taken on different days with an average return trip duration for each of 60 min. Bus commutes began at bus stop N10, Fte Campus Tecnológico e Nuclear in Bobadela, and ended at the terminal bus stop in Areeiro covering 10.5 km and 21 stops. Train commutes ran from Bobadela until Alcantara-Terra covering 17 km and 9 stops. The ventilation condition was not controlled; however, it was noticed that AC was used in all commutes.

2.3. Instrumentation and sampling design

In order to measure each pollutant, it was placed in the middle of the back seat of the car within a box all the equipment. Furthermore, in the following section, a brief explanation of the equipment's operation was made.
PM with diameter lower than 1, 2.5 and 10 µm and BC were measured at 10 seconds intervals from journey start to end. The Dusttrak DRX Aerosol Monitor 8533 and a MicroAeth® AE51 were used to measure PM and BC respectively. Total airborne bacteria and fungi were estimated at the beginning, middle and end of the journey via impaction of 250 L of air in Tryptic Soy Agar (bacterial population) and Malt Extract Agar (fungi population) petri dishes using the MAS-100® Microbial Air Monitoring (100 L/min). The equipment was placed in between the driver and the co-pilot.

Bacterial isolates were characterized and phenotypically typified based on Bergey's Manual of Determinative Bacteriology. The most frequent bacterial isolates were identified using RapID Systems (Remel, Thermo Scientific). Bacteria relative frequency (%) was calculated based on its detected number and total number of isolated bacteria per commute. The fungi colonies were not identified.

VOCs, were measured using the TSI IAQ-CALC™ 9565. CO and CO_2 were measured using the TSI IAQ-CALC™ 7545. These instruments have probes that allow the real-time measurement gas concentrations, at 10 seconds intervals. Furthermore, formaldehyde (a specific VOC found typically inside vehicle cabins) was measured at 1 min interval using a Formaldemeter htV.

2.4. Inhaled dose

The inhaled dose of pollutants is a representation of exposure in function of pollutant concentration and the respiratory rate. This dose is determined by multiplying the pollutant concentration, by duration time of the commute and respiratory rate (Equation 1).

$$\boldsymbol{Di} = \frac{[\boldsymbol{X}]*\boldsymbol{IR}*\boldsymbol{t}}{\boldsymbol{D}} \qquad \text{Equation 1}$$

where Di represents the average inhalation dose, µg/km; [X] is the average pollutant concentration in a specific commute, µg/m^3; IR is the respiratory rate, m^3/h; t is the commute duration and D the commute distance (km). The average respiratory rate used was 0.47m^3/h while seated or standing for people between 20 and 45 years of age according to Li et al. (2015).

2.5. Statistical analysis

Statistical tests were performed in STATISTICA software. The Mann-Whitney U test (anon-parametric test) was selected. This test is used for two independent samples (e.g. concentrations of a pollutant in two different ventilation conditions). It compares medians to suggest if two samples are from the same population or not with a significance if $p<0.05$.

3. Results and Discussion

3.1. PM

PM is composed by a variety of particle sizes; as such, it was possible to identify the fractions that contribute the most to the total PM. For all vehicle cabins, PM_1 had the highest contribution (80 to 90%), followed by PM_{4-10} (2 to 11%)(Figure 2). Frequently, this type of particle size distribution is typical in cities because of vehicular traffic, which is the main source of pollution as it emits a wide range of particles, among them the finest fraction of particulate matter (Martins & Carrilho da Graça, 2018). Moreover, it has been found that this fraction tends to penetrate and accumulate within indoor environments degrading its air quality (Li et al., 2015; Martins & Carrilho da Graça, 2018).

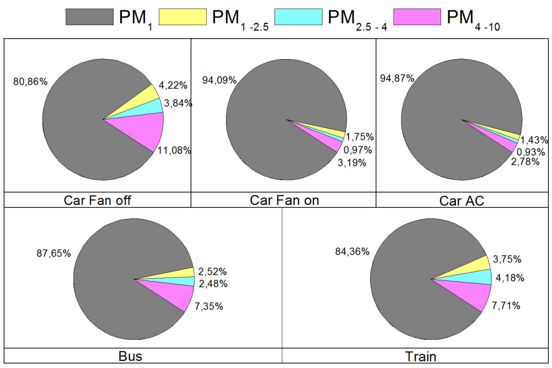

Figure 2. Contribution of PM fractions to total PM_{10} in cars (with fan off, fan-on and AC), buses and trains

In cars, no differences on the particle distribution with the cleaning of the cabin nor the type of cars was observed. In contrast it was noticed that either with Fan on or with AC the coarser fractions decreased. For public transport, the particle distribution obtained differs from Rivas et al. (2017) where the coarse fraction accounted in buses for almost 70% as a result of the constant door opening, resuspension generated by passengers and the seating materials. Here, the coarse fraction accounted for ~10%. Furthermore, the commutes performed in train had almost the same particle fraction contribution as road transportation, even though the railway was not directly affected by vehicles emissions.
The concentrations obtained for each type of car under ventilation and cleaning conditions are shown in Figure 3. In this case small differences were observed with the cleaning of the car just under fan off condition, in most of the cars, a decrease was noticed in the concentration of $PM_{2.5}$. For instance, without cleaning the average concentration was 1.3, 1.1 and 1.9 times significantly higher ($p < 0.05$) for gasoline, diesel and electric car respectively.

Regarding the coarser fraction ($PM_{2.5-10}$) only gasoline and diesel car showed a difference after cleaning with average concentrations 1.6 and 1.1 times lower respectively. However, only the gasoline car showed a significant difference between clean and not clean ($p < 0.05$). In this case, the cleaning may influence in the removal of settled particles in compartments and furniture of the cabin. However, Martins & Carrilho da Graça (2018) reported that sweeping and vacuuming may resuspend particles, which was likely the case for the electric car. The average $PM_{2.5}$ concentrations of all cars ranged between 10 and 26 µg/m³. Differences in concentrations regarding $PM_{2.5}$ were not observed to be linked to the fuel type.
Additionally, as experiments were performed in different days it is possible that differences were influenced mostly by the background concentrations of the day (Geiss et al., 2010). The most noticeable effect occurred with the ventilation mode.

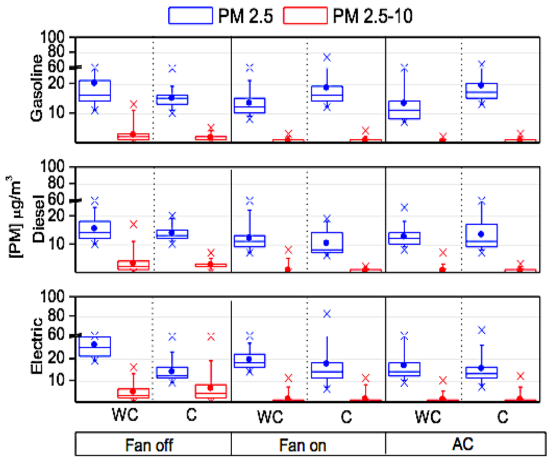

Figure 3. $PM_{2.5}$ and $PM_{2.5-10}$ concentrations boxplots for each car under fan off, fan on and AC conditions. Boxes represent 25th and 75th percentile, central circle mean, central line median, higher and lower "X" the 99% and 1% percentiles. Extending vertical lines from the box represent the variability outside the upper and lower quartile. WC: without cleaning C: clean

As observed in Figure 4, the concentrations of both fractions were significantly higher under the fan off condition ($p < 0.05$). With fan on and AC the concentration of $PM_{2.5-10}$ and $PM_{2.5}$ decreased, but in less magnitude for this latter.
Some studies have assessed the behavior of PM in cars, among them Xu & Zhu (2009) studied and quantified the behavior of UFP within cars with: fan off- recirculation (RC) off, fan on-recirculation off and fan on-recirculation on. These results showed that with fan off – RC off, the main factors affecting the pollutant entrance are the penetration factor of particles, leakage rate and deposition coefficient. In contrast, under fan on- RC off, condition, the mechanical airflow rate and the filtration efficiency are the main factors affecting particle concentration.
Hence, it is possible that with fan off some particles enter the cabin especially the largest ones, as penetration is higher for larger particles under this condition (Geiss et al., 2010;Xu & Zhu, 2009). However, when ventilation is used, coarse particles were filtered and had higher deposition. Geiss et al. (2010) observed that PM_{10} concentrations were as high as the outdoor air in cars with closed windows and with entrance of fresh air. Moreover, Xu & Zhu (2009) proved that under the fan off condition, the

dilution/diffusion of pollutants takes longer than when ventilation is used.

Furthermore, Geiss et al. (2010) found that the filtration efficiency for particles <10µm was low. Similarly, Leavey et al. (2017) found that despite the variation of ventilation, $PM_{2.5}$ concentrations remain unchanged. The low efficiency for fine particle removal may been influenced by the fact that there was entrance of fresh air during the whole experiment. In fact, Jung et al. (2017) documented that recirculation of air reduce significantly fine particle concentrations due to air passing multiple times through the filters.

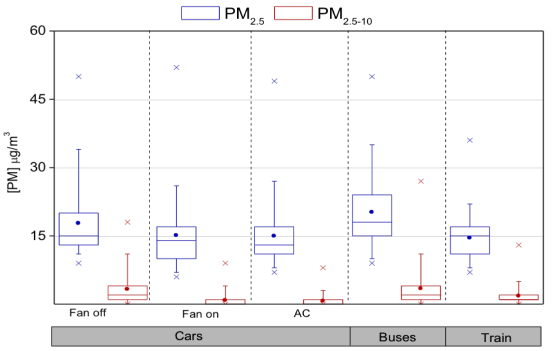

Figure 4. $PM_{2.5}$ and $PM_{2.5\text{-}10}$ boxplot concentrations per type of vehicle. Boxes represent 25th and 75th percentile, central circle mean, central line median, higher and lower "X" the 99% and 1% percentiles, the extending vertical lines from the box represent the variability outside the upper and lower quartile.

Comparing the three transportation modes it was possible to observe that the average concentration of fine and coarse particles were significantly higher ($p < 0.05$) in bus commutes (20.2 ± 8.6 µg/m³ for $PM_{2.5}$ and 3.4 ± 4.9 for $PM_{2.5\text{-}10}$) followed by cars with fan off (17.5 ± 7.7 for $PM_{2.5}$ and 3.3 ± 3.8 for $PM_{2.5\text{-}10}$). In cars with closed windows, RC-on and AC, Chaney et al. (2017) found an average $PM_{2.5}$ concentration of 5.2 µg/m³ in Salt Lake City (U.S.A), almost 3 times lower than the result obtained in the present study (14.9 ± 7.6 µg/m³). This shows that air RC and ventilation are important factor to reduce the concentration of fine particles. Moreover, Rivas et al. (2017) registered a concentration of $PM_{2.5\text{-}10}$ of 0.9 µg/m³ in cars with ventilation set at 50% velocity and closed windows, similarly to the results of this study (0.8 ± 1.6 µg/m³).

Chaney et al. (2017) determined that buses had the highest concentration of fine particles among all the transports modes studied (cars, light rails), with an average $PM_{2.5}$ concentration of 13.0 µg/m³. In contrast the average $PM_{2.5\text{-}10}$ concentration was lower compared to those registered by Rivas et al. (2017) (24.0 µg/m³), which may be influenced by the seating materials and the dust in the cabin. Furthermore, the high concentrations of particulate matter in buses are associated with the air exchange rate produced by constant door opening, resuspension by passenger movements and the proximity to vehicular emissions (Ramos et al., 2016; Rivas et al., 2017).

Ramos et al. (2016) studied human exposure to different pollutants including $PM_{2.5}$ of different transport modes (buses, cars without ventilation, cycling and metro) in Lisbon. For cars and buses their concentrations were almost a threefold higher (55 ± 13 for cars and 52 ± 27 µg/m³ for buses). This was also observed for coarser particles. These differences may be due to the experiment location. Sections of the routes in this study took place in suburban areas with less traffic compared to the Telheiras- Restauradores route, which is busier.

Although trains also have a high air exchange rate due the doors opening in each station, separation from vehicle emissions contributed to a lower $PM_{2.5}$ concentration. However, as trains pass through inner points of the city, background and traffic levels are likely to influence the $PM_{2.5}$ concentration inside the train. This effect was also seen by Nasir & Colbeck (2009) where PM_1 and $PM_{2.5}$ concentrations were higher during peak hours than off peak. Moreover, PM_{10} increased in each stop.

Even though in all cases average concentrations were not above the limit values set by the WHO, ASHRAE nor the Portuguese legislation ($PM_{2.5}$: 25 µg/m³ and PM_{10} 50 µg/m³), during some commutes there were peaks that reached maximum concentrations above the limits for short periods of time.

3.2. BC

Under the fan off condition, the electric car had lower BC concentrations compared to non-electric cars (e.g. 1.7 compared to 3.6 and 4.7 µg/m³ within the diesel and gasoline vehicle) (Figure 5). In addition, it was observed under the same condition, that the average concentrations of BC significantly decreased after being cleaned ($p < 0.005$). Despite the significant statistical differences, due to the low number of measurements it is not possible to establish whether the type of car or the cleaning were responsible for this result. Other variables were likely to have affected the experiment, as such, background concentration and traffic intensity of the day are likely to have influenced the results as BC is a specific tracer of traffic.

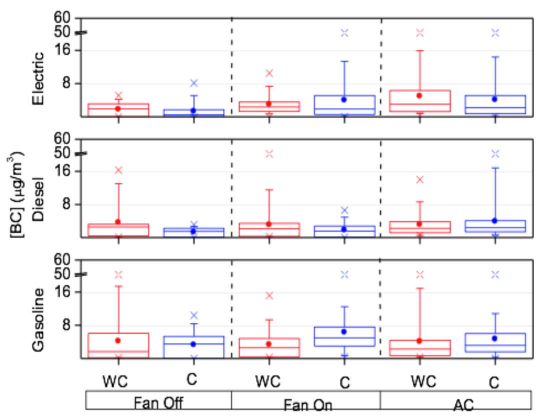

Figure 5 Boxplot of BC concentrations for each car under fan off, fan on and AC conditions. Boxes represent 25th and 75th percentile, central circle mean, central line median, higher and lower "X" the 99% and 1% percentiles. the extending vertical

lines from the box represent the variability outside the upper and lower quartile. WC: without cleaning C: clean

When ventilation was used either with fan on or AC, in all cars BC average concentration significantly increased ($p< 0.05$)(Figure 6). The average concentration increased by 2.7± 3.8, 3.5 ± 4.5 and 4.1 ± 5.1 µg/m^3 for fan off, fan on and AC respectively. Moreover, a particular aspect noticed in cars was, that while $PM_{2.5}$ did not change much with ventilation, BC concentrations tended to increase. This behavior may be caused by the particle size of BC. Kocbach et al. (2006) described the size of vehicular emission of BC to have a size of 0.024 ± 0.006 µm (within the range of UFP). Which may explain why the same behavior was not observe for both pollutants. Thereby, when ventilation is used the higher exchange rate, continuous entrance of fresh air, low filtration efficiency and low deposition of the finest fractions contributed to an increase of BC concentrations.

Average BC concentrations obtained in this study are higher than those obtained by Merritt et al.'s (2019) in Sweden, where the average concentration measured in cars was 2.0 µg/m^3 and ranged between 0.03–19.0 µg/m^3. Train commutes showed significantly lower BC concentrations (1.13 ± 0.58 µg/m^3) compared to other transports ($p < 0.005$) (Figure 6). As the railway is separate from the roads and there were no direct vehicular emissions, this was expected. Furthermore, concentrations obtained were very similar to those presented by Onat et al. (2017) and (4.9 µg/m^3).
In contrast with buses, the average concentration obtained was almost three times higher than trains (4.5 ± 3.5 µg/m^3), but were lower than those found in previous studies (11.2 ± 7.0; 5.5 and 5.4 µ/m^3) (Moreno et al., 2019; Rivas et al., 2017).

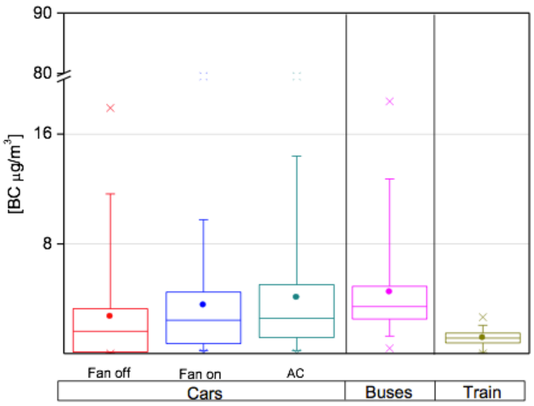

Figure 6. Boxplot of BC concentrations for each type of vehicle. Boxes represent 25th and 75th percentile, central circle mean, central line median, higher and lower "X" the 99% and 1% percentiles. the extending vertical lines from the box represent the variability outside the upper and lower quartile.

3.3. Bacterial and Fungal loads

As seen in Figure 7, in all cases the bacterial loads were higher when the ventilation was off. In some cases, higher than outdoor loads. In contrast, fungal loads were always lower inside the car. In this case, since the air exchange was lower under the fan off condition, the main sources of these bacterial and fungal loads may be linked to the human microbiota and dust settled in the furniture of the car. Furthermore, it was also observed that cleaning decreased both bacterial and fungal loads in all cars. This would mean that cleaning may contribute in reducing the dust inside the vehicle and consequently the presence of fungi spores or vegetative bacteria as Sattar et al. (2016) mentioned.
In situations where moderate ventilation and AC was used, fungal and bacterial loads decreased. This effect may be linked to dilution due to the higher air exchange. Additionally, this result agrees with the literature, where ventilation has been shown to reduce up to 80% the microbe loads (Li et al., 2013). This is mainly caused due to air filtration rather than the microclimate conditions generated by the ventilation as Gołofit-Szymczak et al. (2019) established.

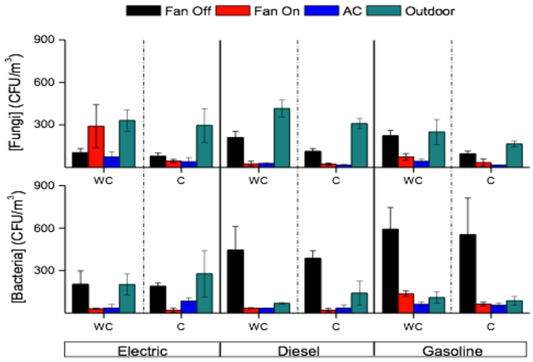

Figure 7. Fungal and bacterial loads per type of car. The bars represent the confidence interval (CI) 95%. WC: without cleaning C: clean.

Overall, cars had lower fungal and bacterial loads than public transport (Table 1). This result was expected as both buses and trains have higher occupancies.
Furthermore, Barnes et al. (2018) evaluated the microbial quality in 51 vehicles with the AC on; reporting an average bacterial and fungal loads of 350 and 13 CFU/m^3 respectively, and only three cars had bacterial loads above 1000 CFU/m^3. In buses with AC, Prakash et al. (2014) reported an average fungal load of 713 CFU/m^3 and Onat et al. (2019) reported a bacterial load of 712 ± 330 CFU/m^3. Results obtained in this study were higher than both, likely affected by occupation density, hygienic conditions of the filters or resuspension from the vehicle floor and fabric seats.
Bacterial and fungal loads in trains reported by Xu et al. (2016) were 417 and 413 CFU/m^3 respectively. These results are similar to those in this study. Taking into account that the Portuguese legislation established that the bacterial loads should be less than the sum of outside bacterial load with 350 CFU/m^3, in scenarios like cars with fan off and trains, the limits were surpassed. Fungal loads remained lower indoors than outdoors, in compliance with the Portuguese legislation.

Table 1 Fungal and Bacterial loads per type of vehicle

Vehicle	Condition	Fungi CFU/m³		Bacteria CFU/m³	
		Mean	SE	Mean	SE
Cars	Fan off	137.0	14.4	395.0	46.6
	Fan on	82.0	25.4	51.0	11.7
	AC	35.8	6.1	51.0	5.8
Buses	AC	560.0	98.1	460.0	58.8
Train	AC	203.5	13.8	728.0	173.2

SE: Standard error of the mean.

3.4. Characterization of bacterial isolates

Cultured based methods were performed to quantify and identify bacterial isolates. Gram-positive cocci were the highest prevalence group (60 and 85%), similarly to the findings of Gołofit-Szymczak et al. (2019) were Gram-positive cocci range 40 to 54% prevalence. Furthermore, Gram-negative bacteria were the lowest group found in the samples. This result is expected since cell wall of this type of bacteria has a lower resistance to temperature and humidity variations, which reduce the viability in the air (Matković et al., 2007). Moreover, Gram-positive cocci are considered human-borne, thus, it was higher dominance of this type of bacteria in all samples were expected (Greene et al.,1962)

Taking into consideration of Gram-positive cocci genera, *Micrococcus, Kocuria* and *Staphylococcus* were identified in all transports (Figure 8). Considering all bacterial isolates, *Micrococcus* genus accounted for 45.6 % frequency followed by *Staphylococcus* and *Kocuria* (16.8 and 5.4 % respectively). This is similar to the studies performed by Leung et al. (2014) where most of the bacteria found in public transports belong to these genera. These genera are commonly found in indoor environments as most of them inhabit the human skin and mucous membranes and are rarely associated with infection (with exception of some species of Staphylococcus genera) (Aydogdu et al., 2010; Folayan et al., 2018).

The specie *Micrococcus luteus* was found to be the most common in all transports, with relative frequencies ranging between 28 and 75%. In recent investigations, *Micrococcus luteus* has been reported in cases of skin infections and pneumonia (Folayan et al., 2018).

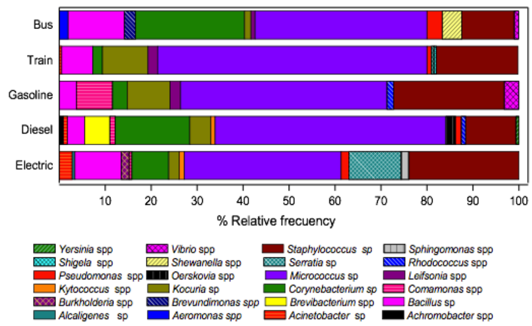

Figure 8. Distribution of bacterial isolates according to the genera

Staphylococcus genus has many pathogenic species often found in the skin. In this study, the species found were *S. aureus, S. epidermidis, S. hominis ss hominis* in cars *S. conhii ss conhii, S. sprophyticus* in trains and *S. xylosus* in buses. Onat et al. (2019) also found high levels of *Staphylococcus* sp. in buses and light trains mostly due to overcrowding and the ability of this species to remain viable in surfaces, dry air and harsh environmental conditions. Several studies have found that large amounts of *S. aureus* identified are MRSA (methicillin resistant *Staphyloccoccus aureus*) or vancomycin resistant. Conceição et al., (2013) evaluated the hand touched surfaces of buses in Lisbon and found in 72 buses contamination with MRSA. These types of MRSA were related to those found in hospitals. In this study, it was not possible to determine whether the species identified were MRSA or antibiotic resistant.

Gram-positive bacilli were the second most dominant group present for most of the samples. This result is similar to the findings of Aydogdu et al. (2010); after Gram-positive cocci, Gram-positive bacilli were the most frequent group in childcare centers. At the same time, Gram-positive bacilli were more dominant in the outdoor air than indoors, which indicates that most of these bacteria came from outdoor environments through ventilation. Within this group, the *Corynebacterium, Bacillus, Oerskovia, Leifsonia* and *Rhodococcus* genera were identified. *Leifsonia* and *Oerskovia* are mainly found in soil, water reservoirs, and other environmental sources (Folayan et al., 2018;Oikonomou et al., 2018). This could explain the low frequency of this specie over all samples (0.98%). *Rhodococcus* was found in two cars and in low frequency (0.97 and 2.8%). However, the specie *R. equi* has been found to be an opportunistic pathogen, involved in cases of pneumonia (de la Paz et al., 2010)

On the other hand, *Corynebacterium* and *Bacillus* genera were found in all vehicles. The *Corynebacterium* genera was more frequent over all samples (6.8 and 11.4% for *Bacillus* and *Corynebacterium* respectively) due to the fact that this genera inhabits the human skin (Leung et al., 2014). Kim et al. (2010) examined the size distribution of the most frequent specie in hospitals and found that *Corynebacterium* along with other bacteria (*Micrococcus* sp. and *Staphylococcus* sp.) were distributed in sizes between 0.65 to 4.7 µm also known as the respirable fraction. In contrast with *Bacillus* that was in the size of >7 µm. This fact could explain the low frequency of this genera in all transports, where most of them could be retain in the filter of the cabin along with coarser particles. In this study, *B. subtilis* specie was found in only one car and in low abundance (1.6%). Among the species of *Corynebacterium,* just two from the species isolated were reported to be opportunistic pathogens *C. jekeium* (found in two cars and trains) and C. *pseudodiphtheriticum* (found in one car). Yang et al. (2018) reported cases of *C. jekeium* and *C. pseudodiphtheriticum* causing pneumonia in immunocompromised patients.

As discussed before, Gram-negative bacilli were found in most of the samples in low frequency, but it was a very diverse group. Around 14 different genera were found. The results obtained indicated that the most frequent genera were *Comamonas, Pseudomonas* and *Serratia.* Similarly, Li et al. (2013) reported *Pseudomonas* sp. as a dominant genus in AC filter dust. In this study, the specie

P. aeruginosa was identified in just one car (2.0 % relative frequency). For other isolates in this genus, it was not possible to determine the species. Furthermore, this genus is the most renowned in respiratory infections, especially *P. aeruginosa,* which is common in patients with cystic fibrosis lung disease (Knibbs et al., 2014). Likewise, Knibbs et al. (2014) studied the transmission and persistent of this microorganism in the air and found that when a person coughs the aerosols can travel up to 4 m distance and after 45 min this bacterium was still viable.
According to the results obtained, there is a high diversity of bacterial species in all vehicles. Despite the identification of microorganisms in these sources, it is difficult to predict if people will eventually be affected by respiratory infections. As seen in most of the cases, these bacteria may act as an opportunist. These findings show the potential of these microenvironments for the transmission of diseases. Thereby, it is known that simple actions like coughing could transmit a variety of pathogens that remain in distance and time (Knibbs et al., 2014).

3.5. CO_2 and CO

CO_2 concentrations were higher with the fan off. This result was expected as CO_2 concentrations exhaled by people range between 38,000 and 56,000 ppm and accumulate under low air exchange rates (Hudda & Fruin, 2018; Jung et al., 2017). Lower CO_2 concentrations were observed with the cleaning of the car under the fan off condition. However, the effect could be attributed to the traffic intensity of the day rather than the cleaning, since Leung et al. (2014) determined that CO_2 is a function of commuter density and it is influenced by traffic conditions and vehicle speed. Hudda & Fruin (2018) reported that CO_2 tends to accumulate less under high speed conditions. Furthermore, the differences between cars is attributed to the characteristics of the car. Since all cars had different cabin volumes, the air exchange may also vary.
Moreover, it was observed that continuous entrance of fresh air and ventilation (fan on or AC) reduce the CO_2 concentration significantly ($p < 0.05$). As discussed before, the air exchange increases with the ventilation and thus CO_2 is diluted. In contrast with CO, it was observed that the ventilation contributed to register higher spikes and concentration of this pollutant that could be due to the increase of the air exchange rate and the proximity to the front vehicle emissions and to the congestion events. Dirks et al. (2018) found that CO levels decrease when driving with open windows and fresh air entrance due higher exchange rates of air as well as avoiding the use the ventilation when the vehicle is close to the vehicle in front (e.g. in intersection, light stop) to prevent spikes. However, the opposite was found by Leavey et al. (2017) where driving with windows closed and AC reduced the diffusion of CO. Overall, CO_2 and CO concentrations in cars were close to those registered in buses and trains, even with their lower occupancies (Table 2).

This result is similar to the findings of Odekanle et al. (2017), where cars had higher concentration of CO compared to buses and rapid transit buses and Grana et al. (2017) who reported similar maximum concentrations while commuting in two areas in Rome (7.1 ppm – 5.6 ppm). The reasons for this result could be the low vehicle cabin volume, which decreases the dilution of gases; the proximity of the cars to tailpipe emissions from cars in front and lower exchange air rates compared to buses. Regarding trains, CO concentrations were significantly lower ($p < 0.05$) due to their location farther away from the vehicular emissions.

Recent studies have proved a decline in the decision-making performance when people are exposed to CO_2 concentrations up to 1000 ppm (Satish et al., 2012). This study proved that CO_2 may accumulate up to 4000 ppm in certain conditions and is much higher than the average ambient CO_2 concentrations. The effect that which commuters may suffer are not clear in this study, thus more studies should be done to determine the effects on mental performance during short commutes (30-60 min).
CO concentrations registered in the commutes did not exceed the limit value set by the WHO for a 1 h average (25 ppm). In comparison with other studies, the concentration registered here are negligible to the concentrations obtained in a study performed by Odekanle et al. (2017) in Lagos city (Nigeria). In that study the average concentrations registered were 40 times higher (32.3 ppm in cars and 23.7 ppm in buses) than the measured in this study.

Table 2 CO_2 and CO concentration per type of vehicle

Vehicle	Condition	CO_2 ppm				CO ppm			
		Mean	SD	Min	Max	Mean	SD	Min	Max
Cars	Fan off	2136.8	960.7	376.0	4336.0	0.8	0.4	0.0	2.3
	Fan on	633.9	99.4	367.0	1159.0	0.6	0.5	0.0	5.2
	AC	662.7	144.0	367.0	1099.0	0.4	0.5	0.0	5.2
Bus	AC	753.8	222.0	388.0	1357.0	0.6	0.2	0.1	1.5
Train	AC	747.1	156.4	372.0	1531.0	0.3	0.2	0.1	0.9

3.6. VOCs and CH_2O

Several types of VOCs have been found in cars such as benzene, toluene, alkanes, etc. (Moreno et al., 2019; Xu et al., 2016). In the present study, only Total Volatile Organic Compounds (TVOCs) and the carbonyl compound formaldehyde (CH_2O) were studied. Cars with fan off showed significantly higher concentrations ($p < 0.05$) of VOCs and CH_2O compared to the other modes of ventilations. Similar to result reported by Xu et al. (2016) where the change of ventilation mode from fan on to fan off, caused a 50% increase on the average concentration of aromatic hydrocarbons.
Furthermore, higher variations were found in each type of car, this major difference between cars could be driven by the off-gassing of the cabin trim materials of each. However, there are many other factors that can influence the concentration of VOCs inside the cabin, such as the temperature, humidity, vehicle age and type of fuel. Duh (2015) reported average concentrations of VOCs of 0.25 ppm (~4000 µg/m3) when the temperature was around

35ºC and decreased to 0 ppm when fresh entrance of air was allowed.
Regarding the cleaning condition, when the electric and diesel cars were cleaned, higher average concentrations were registered (3370 ± 2387 and 4450 ± 890 μg/m^3, respectively) compared to their uncleaned counterparts (697.8 ± 241.4 and 3595 ± 513 μg/m^3). Here the type of product used to clean the surfaces of these cars may have influenced VOCs concentration. Both cars were cleaned with alcohol 70% v/v, in contrast with the gasoline car, which was cleaned with window cleaner.

Taking into consideration the different types of vehicles, cars under the fan off condition had the highest concentration of VOCs and CH_2O followed by trains (Table 3). Xu et al., (2016) reported that cabins with lower volumes accumulate higher concentrations of VOCs. Furthermore, the air exchange in public transports is much higher than cars, which allows VOCs inside the cabin to dilute. In comparison with other studies, Faber & Brodzik (2017) reported concentration of VOCs and CH_2O inside cars of 612.2 ± 188.9 and 16.4 ± 4.9 μg/m^3 respectively, this result is similar to the ones obtained for AC cars. Compared to the results obtained by Ramos et al. (2016) for cars without ventilation, the levels registered here for the same condition were almost 3 times higher (471.0 ± 29.0 μg/m^3). Similar differences were registered with buses where concentrations were almost 3 times lower (287.0± 69.0 μg/m^3).

Lau & Chan (2003) measured the concentration of four specific VOCs: benzene, toluene, ethylbenzene and xylene (BTEX) and recorded higher concentrations of BTEX in buses than railway transports. This is due the proximity of buses to vehicular emissions and these VOCs being released in vehicular tailpipe emissions. This result disagrees with the results of this study; as seen in Table 3 the average concentration in trains were almost 3 times higher than buses. Lau & Chan (2003) explained that the concentrations on trains are strongly influence by the surrounding air, similar to the findings of Gong et al. (2017) who studied VOCs concentration in underground and aboveground trains. Thereby, the aboveground train had higher concentration of VOCs (143.9 ± 3.0 μg/m3) than the underground due the influence of vehicular emissions. However, the concentrations in that study are almost 17 times lower than the result obtained here, which may indicate that VOCs could be released from other sources such as the internal materials, interference with other types of substances or internal sources i.e. during the measurements, bad odors within the carriage were noticed.

Table 3. Concentration of VOCs and CH_2O per type of vehicle

Vehicle	Condition	VOCs (μg/m^3)				CH_2O(μg/m^3)			
		Mean	SD	Min	Max	Mean	SD	Min	Max
Cars	Fan off	3235.0	1872.0	100.0	8100.0	124.0	260.0	0.0	2708.0
	Fan on	1251.0	1797.0	0.0	23403.0	15.0	14.0	0.0	88.0
	AC	733.0	739.0	0.0	3083.0	10.0	6.0	0.0	54.0
Bus	AC	881.6	394.1	269.0	4636.1	8.5	5.0	0.0	19.0
Train	AC	2516.1	5529.6	472.1	67965.1	24.7	44.1	1.0	272.0

3.7. Inhaled dose per transport mode

The inhaled dose depends on travel time, pollutant concentration and the inhalation rate. As the routes were not the same, the dose was normalized per km travelled. Trains had the longer duration trip (1.21 ± 0.03 h return trip) compared to buses and cars (0.98± 0.04 and 0.93 ± 0.17 h), however, the difference in time is negligible compared to the distance travelled (35.0, 21.4 and 15.3 km for train, buses and cars). Trains had the lowest dose for almost all pollutants (Table 4) mainly due to the low pollutant concentrations found in this vehicle. On the other hand, commutes in car without ventilation were the scenario with the highest inhaled dose for almost all pollutants despite having the lowest travel duration. The ventilation reduced the intake of toxic gases such as CO_2, VOCs and CH_2O, but the entrance of fresh air all time and the higher air exchange promoted higher BC levels that were hardly filtered. The inhaled dose in buses were similar to ventilated cars, with exception of the dose for coarser particles ($PM_{2.5\text{-}10}$).
In comparison, with the inhaled dose of BC estimated by Li et al. (2015) for different transports (subway, taxi, bus, walking and cycling), the highest dose was obtained in buses among the motorized transports (0.43 μg/km). This dose was 4 times higher than the dose estimated in this study (0.10 μg/km) despite the fact that the duration and distance were lower in Li et al. (2015) study (0.44 h and 3.5 km). The difference is influenced by the high level of BC detected in those buses (7.3 ± 1.8 μg/m^3). On the other hand, the doses estimated here were very similar to those obtained by Onat et al.(2017). In their study the estimated dose for buses for BC and $PM_{2.5}$ were 0.2 ± 0.2 and 0.7 ± 0.4 μg/km respectively; for cars with AC 0.03 ± 0.03 and 0.61 ± 0.31; for trains 0.07 ± 0.03 and 0.03 ± 0.21 for both pollutants respectively.

In Lisbon, another study estimated the inhaled dose for various pollutants in different types of vehicles (Ramos et al., 2016), in car driven without ventilation and windows closed, same as the fan off condition of this study. The $PM_{2.5}$ inhaled dose was almost 3 to 4 times higher for buses and cars (2.4 and 4.4 μg/km in buses and 1.8 and 1.9 for cars at 8 and 11 h respectively) than the doses estimated here (0.43 and 0.49 μg/km), despite their shorter distance (7 km). Similarly to particles, the inhaled dose for CO, was higher for both vehicles (40.2 - 45.3 μg/km for bus and 28.8 – 31.3 μg/km for cars) compared to this study (18.3 and 26.6 μg/km for buses and cars).
These differences are due to the fact that the concentration registered of both pollutants inside these two vehicles was much higher, possibly due to the location of the study. Their study was performed in Lisbon center on a route that passed through main squares and busy areas. Instead, in this study the routes included more suburban areas and with moving traffic. On the other hand, the CO_2 dose was lower for cars but not for buses (69.7- 97.4 for cars and 142.4-168.9 mg/km for buses). Lower concentrations were expected in those cars, since

occupation was lower (one vs two people in this study). Furthermore, it is possible that buses had higher occupations than the buses sampled here.
Regarding the VOCs inhaled dose, this study found that the dose was much higher (90 µg/km vs 14 -18 µg/km) in cars. This result could be influenced by the cleaning of the cars as discussed in the previous section. Instead the dose for buses were similar to the estimates of Ramos et al. (2016) (16.1 µg/km vs 18.9 – 24.4 µg/km). Overall, differences between vehicles may be caused by traffic differences along the days as well as congestion of each route.

Table 4 Inhaled dose estimate per vehicle

Pollutant	Unit	Train	Bus	Cars		
				Fan off	Fan on	AC
BC	µg/km	0.02	0.10	0.07	0.10	0.12
$PM_{2.5}$	µg/km	0.23	0.43	0.49	0.43	0.43
$PM_{2.5-10}$	µg/km	0.03	0.07	0.10	0.02	0.02
VOC	µg/km	43.5	16.1	90.0	38.1	26.8
CH_2O	µg/km	0.22	0.19	5.1	0.6	0.4
CO	µg/km	10.9	18.3	26.6	19.1	14.0
CO_2	mg/km	21.6	28.7	108.3	32.2	34.0

4. Conclusion

The IAQ while commuting was assessed in three different types of vehicles: cars, buses and trains. Regarding aerosols, it is not possible to determine whether cleaning had a positive or negative effect on the concentrations of these pollutants as sampling campaigns were done in different days and the cleaning was not exactly the same for all cars. In contrast, the type of ventilation had a clear influenced on these pollutants. In the case of PM, it allowed coarser particles to be filtered, but filters showed a low efficiency removing the finest particles. Additionally, the entrance of fresh air may had a great influence on this efficiency.

The airborne microbiota was highly affected by the ventilation mode and the occupancy of the vehicles. Most of the isolated species were human associated bacteria and some of the most abundant species have been linked to respiratory tract infections. However, this does not indicate that the commuter will be developed respiratory infections. The lack of representativeness of the isolated bacteria did not allow to observe whether the ventilation or the AC had an effect on the bacteria diversity.
Among the evaluated transports, train had the lowest exposure concentrations to aerosols and therefore the lowest inhaled doses. In contrast with road transportation, buses and cars had similar exposure concentrations and inhaled doses. Furthermore, the concentrations of aerosols found in this study were below the legislation limits, which was highly influenced by the location where the study was performed.

In relation to gaseous pollutant the cleaning and type of vehicle did not show any effect on the concentration of CO and CO_2. The ventilation reduced the concentration of both pollutants, but higher spikes of CO concentrations were registered. Ventilation may contribute to the diffusion of vehicle exhaust gas into the cabin when the vehicle is on standstill, idling or very close to the vehicle in front.
In contrast, the cleaning affected the concentration of VOCs and CH_2O, but this effect was observed only in two cars, mainly due to the difference in the cleaning process and products. The concentration of these two pollutants varied in all vehicles and variables such as the materials of the trim may have influenced, especially for trains, where the concentration of VOCs and CH_2O were found to be very high. Overall, the concentrations of pollutants found here, were below the limit values established by the WHO, ASHRAE and the Portuguese legislation, with exception of VOCs, however, the lack of a normative (especially for vehicles), made the comparison with the limit values a difficult task.

The IAQ was affected in different ways by the ventilation mode depending on the pollutant. For instance, under the fan off condition, the concentration of BC was lower but the concentration of gases such as CO_2 tended to accumulate rapidly. Thus, instead of choosing between one ventilation mode or another, autonomous sensors in should be introduced in order to avoid the exposure to a high concentration of any pollutant.

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