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Microbiological Tests of Air Quality in Car Cabins – Preliminary Tests

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ABSTRACT

The aim of this study is to determine the concentration of fungi and bacteria in the air inside the car cabin and, on this basis, to determine the air quality in the passenger car cabin. The aim of the work is also to demonstrate the impact of hygienic maintenance of filtering devices on the quality of indoor air. The subjects of the research are car cabins, as an example of small, enclosed spaces in which people may remain. The tests were carried out in the summer of 2020 in three passenger cars. Based on the conducted research, it was found that the operation time of regularly serviced air conditioning has a positive effect on the air quality in small, confined spaces. The vehicle with the longest-used filter was characterised by the highest concentration of fungi (8369 CFU/m^3) and bacteria (16563 CFU/m^3) in the environment inside the car cabin, which means that periodic replacement of the filters in the car's ventilation system is very important. In Poland, it is recommended that such a filter be replaced after a year or after driving 10,000–15,000 kilometres. In analysing the state of air quality in the examined confined spaces, it can be concluded that by ensuring regular replacement of cabin filters and air conditioning servicing, we have a very large impact on indoor air quality.

Keywords: air conditioning, indoor air quality, bacteria, indoor air, fungi, filters, car cabins.

INTRODUCTION

Indoor air quality is a topic that has become popular with the public in recent years. Society's awareness of the pollutants in the air that we inhale is increasing every year (Adamkiewicz and Matyasik, 2019). It is estimated that 30% of buildings in Poland are affected by the Sick Building Syndrome (Gładyszewska-Fiedoruk, 2019).

Car cabins are places where people spend many hours. The air in car cabins is taken from the outside, can be recirculated and can be filtered in ventilation systems and air conditioning systems (Łuniewski, 2021).

Many studies that have been carried out in numerous places around the world indicate correlations between exposure to air pollution and negative health effects (Park et al., 2020; Bukłaha et al., 2022; Sowiak et al., 2018). Air quality has a significant impact on human health and life expectancy (Zhu et al., 2021). Frequently staying in small indoor spaces is associated with the appearance of various diseases (Gładysz, et al., 2010; Gołofit-Szymczak et al., 2019A, B; Udaya Prakash et al., 2014). The World Health Organization (WHO, 2010) has released a list of symptoms that may be caused by staying in a building with poor air quality: (1) Irritation of the mucous membranes (difficulty breathing, irritation or dryness of the eyes, throat, nose); (2) Symptoms appearing on the skin (redness, dryness, peeling of the epidermis on the hands, face and ears); (3) Typically allergic symptoms; (4) Migraines, irritability, mood swings, dizziness and headaches, unnatural fatigue (Gładyszewska-Fiedoruk, 2019).

Air fungal pollution is responsible for 30% of all diseases caused by poor air quality. The growth of fungi requires trace amounts of organic substances, which are contained in commonly available building and finishing materials. Insufficient air exchange or high air humidity will provide favourable conditions for fungal growth (Li and Yao, 2023; Wang et al., 2013). Fungi produce metabolites that can cause dermatitis and problems with the human nervous system (Abbasi et al., 2020; Pertegal et al., 2023).

Buildings are also a place where bacteria occur, the main source of which are people. Bacteria constitute 19–26% of the microflora in closed rooms (Guz et al., 2023). A significant number of bacteria is not a threat at low concentrations (Gołofit-Szymczak and Stobnicka-Kupiec, 2018). Bacteria can be found on poorly protected building materials that are damp and in warm water.

The most common biological contaminants on filters used in air purification systems, including car air conditioning systems, are fungal spores (Staszowska, 2023). Filters may also contain bacteria, pollen and mites, which are bothersome for allergy sufferers. Particularly accumulating bacteria and mould can contribute to the increase in air pollution and, as a consequence, serious illnesses of users, so this problem should not be ignored (Cox et al., 2022; Felgueiras et al., 2022). In closed rooms, the most common fungi are *Cladosporium*, *Penicillium*, *Alternaria* and *Aspergillus*, the spores of which, when released into the air, cause serious respiratory diseases, and the released toxins may be neurotoxic, carcinogenic and cytotoxic. People who come into contact with increased quantities of fungal spores may experience allergic symptoms and reduced immunity (Shinohara et al., 2023). The most common bacterial microorganisms include *Legionella pneumophila*, *Bacillus*, *Micrococcus*, *Staphylococcus*, *Pseudomonas*, *Flavobacterium*, *Acinetobacter* and *Alcaligenes*, which may have allergenic properties. High concentrations of bacteria can cause a wide range of infections and, in certain cases, meningitis. *Legionella*, which can be very dangerous to human life, is the most common type of bacteria found in air conditioning systems. In vehicles, *Legionella* bacteria occur on air filters and in windshield washer fluids (Żak et al., 2019). There may be mites in the car cabin, so it is worth vacuuming the vehicle thoroughly, cleaning the carpets and getting rid of unnecessary items that are a habitat for allergens. Pollen accumulates in the ventilation system and on filters, so for people struggling with allergies, annual ventilation servicing and filter replacement are necessary.

The guidelines of the Polish National Institute of Public Health – National Institute of Hygiene regarding classification of the degree of air pollution are contained in PN-Z-04111/02:1989 and PN-Z-04111/03:1989. Depending on the number of bacteria in 1 m^3 (PN-Z-04111/02:1989), the air is divided into unpolluted (the total number of bacteria is less than 1000), moderately polluted (the total number of bacteria is greater than 1000 and less than 3000) and heavily polluted (the total number of bacteria is greater than 3000). In the case of assessing atmospheric air pollution with microscopic fungi, the standard PN-Z-04111/03:1989, in which the degree of atmospheric air pollution is divided into three groups depending on the total number of fungi in 1 m^3 of atmospheric air, can be referred to. In the case of the total number of fungi in 1 m^3 of atmospheric air ranging from 3000 to 5000, the air is considered to be averagely clean, especially in late spring and early autumn. For the range from 5000 to 10,000 of the total number of fungi in 1 m^3 of atmospheric air, fungal contamination may have a negative impact on the human natural environment, while for the range above 10,000 of the total number of fungi in 1 m^3 of atmospheric air, fungal contamination threatens the human natural environment.

Each vehicle should be equipped with a filter responsible for the cleanliness of the air supplied to the car's cabin. The cabin filter, also called the dust filter, is located in the ventilation ducts of the car. The purpose of using the filter is to protect the driver and passengers from dust, mould, soot and unpleasant odours. In Poland, it is recommended that such a filter be replaced after a year or after driving 10,000–15,000 kilometres.

The literature contains results of research on air in car cabins in terms of thermal comfort (Chunling et al., 2017; Gładyszewska-Fiedoruk and Teleszewski, 2020; Gładyszewska-Fiedoruk and Teleszewski, 2023) and air quality (Grzybowski, 2011; Gołofit-Szymczak and Stobnicka-Kupiec, 2018; Vonberg et al., 2010; Buitrago et al., 2021, Li et al., 2013; Luksamijarulkul et al., 2004). The main parameters of thermal comfort are temperature and humidity, while in the case of air quality these are both physicochemical parameters and microbiological pollutants. Due to the small volume of passenger car cabins, people staying in the car may be particularly exposed to the effects of microbiological contamination.

The air quality in the cabin of a passenger car may depend on many factors. The results of research (Grzybowski, 2011) on the air in 40 cars did not show any relationship between the level of bioaerosol concentration and the temperature, humidity, and the brand and production year of the car. The results of research carried out on 35 randomly selected passenger cars (Gołofit-Szymczak and Stobnicka-Kupiec, 2018) indicate that the microbiological quality of air in passenger car cabins depends on the number of kilometres travelled. In the case of high values of mould concentration outside the car, the operation of a properly functioning air conditioning system in the car reduces the mould concentration inside the car cabin compared to the mould concentration outside (Vonberg et al., 2010). According to research results (Buitrago et al., 2021, Li et al., 2013), ventilation can reduce the number of microorganisms by about 80% compared to the initial state. Filters used in cars may be a source of *Aspergillus* mould (Gołofit-Szymczak et al., 2023), so timely replacement of filters is important. The level of microorganisms in the car cabin can be reduced via air conditioning disinfection, as well as by disinfecting the surfaces of the interior walls of the car cabin (Bukłaha et al., 2022; Gołofit-Szymczak et al., 2019a, 2019b; Luksamijarulkul et al., 2004; Aquino et al., 2023).

No formulas for the concentration of fungi and bacteria in the air as a function of the time of use of the ventilation system in the cabin of a passenger car have been found in the literature. The aim of the study is to determine the microbiological quality of the air in the cabins of three cars during normal use over a period of 30 minutes in Poland. The research includes determining the function of changes in the concentration of fungal and bacterial colonies in 1 m^3 of air in the car cabin depending on the time from the moment the air conditioning is turned on and carrying out measurements after 5, 10 and 30 minutes of air conditioning use. Cars with different filter replacement dates were included in the tests.

METHODS

Three passenger cars were selected for the tests: sedan car No. 1 (BMW 5 Series 520D 1.2 l, production year 2015) with automatic climate control air conditioning system, crossover car No. 2 (Ford Ka+ 1.2 l, production year 2016) with manual air conditioning system, hatchback car No. 3 (Volkswagen Polo 1.2 l, production year 2012) with climatronic air conditioning system.

In a manual air conditioning system, the airflow rate, airflow direction and temperature are set independently, by manually adjusting the rotational speed of the supply fan impeller, setting the angle of the air supply louvre and the temperature controller. In the climatronic system, the specific, desired temperature in the car is maintained automatically by the control unit by mixing cold air from the evaporator with warm air from the heater. Replacement of cabin filters and disinfection of the car's ventilation cooling systems were carried out on the following dates: August 2020, July 2018 and February 2019 for car numbers 1, 2 and 3, respectively (Łuniewski, 2021). The measurements were performed after 2 months, 26 months and 18 months from the last filter replacement and disinfection of the ventilation system, respectively, for car Nos. 1, 2 and 3. All cars were equipped with anti-pollen cabin filters.

The MERCK MAS-100 Eco microbial air sampler was used to test the air quality in the cars. The tests were carried out in the autumn of 2020 using the collision method (Pu, et al., 2020; Wolny-Koładka et al., 2019). The air volume intake range is from 10 to 1000 dm^3. The nominal air flow in the device is 100 dm^3 min^{-1} (Merck, 2010). Air samples were taken in the place of the passenger seat using an additional pad. There was one person in the car during the tests. In order to obtain the most accurate test results (and with a smaller statistical error), use of three different air sample volumes: 20 dm^3, 100 dm^3 and 500 dm^3 was decided. The grown colonies were counted and the results obtained were averaged. A single sample collection was carried out in a period of several seconds. Due to the availability of only one air sampler, subsequent samples were taken directly one after the other. The experiment consisted of four stages:

1) Sampling with the car engine turned off and the air conditioning turned off (the so-called initial state). In Tables 1-3 marked as Measurement 1 – "0",
2) Sampling after 5 minutes with the air conditioning on. In Tables 1-3, marked as Measurement 2 – "5";
3) Sampling after 10 minutes with the air conditioning on. In Tables 1-3, marked as Measurement 3 – "10";
4) Sampling after 30 minutes of driving with the air conditioning on. In Tables 1-3, marked as Measurement 4 – "30".

For each stage of the research, a Petri dish with a medium on which fungal spores and fragments of thallus hyphae and bacterial cell settle were used. In total, seventy-two air samples were used throughout the study. The sanitary condition of the tested air in the car cabin was determined by calculating the total number of mould spores and the total number of bacteria. Three repetitions of each stage were performed for each volume of air intake. Two diagnostic substrates were used: (culture media by BTL Ltd.): (1) glucose-peptone agar with rose bengal (according to Martin medium – cat. No. P–0116) for the determination of the number of fungi, and (2) nutrient agar for bacterial counting (cat. No. P–0018). Cultures were carried out at 22 °C for 3 days for bacteria and 10 days for fungi. After the incubation period, the grown colonies were counted – their concentration was given in CFU (colony-forming units) per 1 m^3 of the tested air.

The number of colony-forming units of bacteria and fungi was determined in accordance with the method presented in the documentation of the MAS-100 Eco measuring device. The obtained CFU values were compared with the permissible values for atmospheric air pollutants according to the selected guidelines (PN-Z-04111/02:1989; PN-Z-04111/03:1989).

RESULTS AND DISCUSSION

The probable statistical total number (Pr) of colonies for fungi (Table 1) and bacteria (Table 2) in cars was determined based on the positive hole conversion table for the MAS-100 air monitoring system (MAS-100 Operating Manual).

Table 3 presents the average value of microorganism units per cubic metre of tested air, which was determined according to Eq. 1:

$$Pr_{avg} = \frac{\frac{Pr_{20}}{0.02} + \frac{Pr_{100}}{0.1} + \frac{Pr_{500}}{0.5}}{3} \tag{1}$$

where: Pr_{20}, Pr_{100} and Pr_{500} are the probable statistical total colony counts in sample volumes of 20 dm^3, 100 dm^3 and 500 dm^3, respectively.

Due to the average number of fungal colonies (Table 3) and in accordance with the guidelines of the Polish National Institute of Public Health (PN-Z-04111/03:1989), the air in all tested cars with the air conditioning turned off is classified in the air pollution range, which may have a negative impact on the human environment. After five minutes of ventilation operation, the air in car No. 1 can be classified as unpolluted air (PN-Z-04111/03:1989, Chmiel et al., 2015), in car No. 2 as polluted air that may have a negative impact on the natural environment, while in car No. 3 it is as averagely clean atmospheric air. After ten minutes of ventilation system operation, the air in cars 1 and 3 is classified as unpolluted air, while the air in car 2 is classified as averagely clean atmospheric air. After 30 minutes of ventilation operation in all cars, the air in the cars meets the

Table 1. Probable statistical total number of colonies in cars (fungi)

Collected sample volume (dm^3)	20	100	500
Probable statistical total number of colonies	Pr_{20}	Pr_{100}	Pr_{500}
Car No. 1			
Measurement 1 - “0”	196	458	622
Measurement 2 - “5”	120	84	595
Measurement 3 - “10”	23	82	436
Measurement 4 - “30”	18	65	196
Car No. 2			
Measurement 1 - “0”	378	394	1134
Measurement 2 - “5”	281	279	67
Measurement 3 - “10”	235	219	38
Measurement 4 - “30”	110	150	34
Car No. 3			
Measurement 1 - “0”	206	642	769
Measurement 2 - “5”	148	196	449
Measurement 3 - “10”	26	35	98
Measurement 4 - “30”	8	25	60

Table 2. Probable statistical total number of colonies in cars (bacteria)

Collected sample volume (dm^3)	20	100	500
Probable statistical total number of colonies	Pr_{20}	Pr_{100}	Pr_{500}
Car No. 1			
Measurement 1 - “0”	608	1102	1209
Measurement 2 - “5”	449	289	397
Measurement 3 - “10”	226	259	295
Measurement 4 - “30”	12	6	78
Car No. 2			
Measurement 1 - “0”	783	820	1170
Measurement 2 - “5”	484	647	537
Measurement 3 - “10”	449	477	471
Measurement 4 - “30”	376	408	413
Car No. 3			
Measurement 1 - “0”	763	798	1102
Measurement 2 - “5”	706	106	1387
Measurement 3 - “10”	232	464	981
Measurement 4 - “30”	193	321	870

conditions of the PN-Z-04111/03:1989 standard and it is assumed that the air is clean.

In the case of bacteria (Table 3), the air meets the PN-Z-04111/02:1989 standard and is considered unpolluted only in the case of car No. 1 after 30 minutes of ventilation operation. In the other cases, the air is classified as heavily polluted and does not meet the requirements of the National Institute of Public Health guidelines (PN-Z-04111/02:1989).

In Portugal (Buitrago et al., 2021), the fungal load in the car cabin ranged from 165 to 416 CFU/m^3 for cars with the ventilation turned off and from 17 to 200 CFU/m^{-3} with the ventilation turned on, respectively, while the bacterial load in cars (Buitrago et al., 2021) was equal to 189 to 554 CFU/m^3 for cars with ventilation turned off and 20 to 137 CFU/m^3 with ventilation turned on. The value of fungal and bacterial loads in car cabins (Buitrago et al., 2021) was much lower compared to the results obtained, which may be due to a different climate and more frequent cleaning of the car ventilation system. In the work of (Barnes et al., 2018), fungal and bacterial loads in cars in Hong Kong also turned out to be lower compared to the obtained results, which could be caused by more frequent filter replacement.

In the studies, it was observed that the dominant species in all cultures was the fungus *Aspergillus*, the characteristic feature of which is that the conidiophore takes the shape of a vesicle, it can be club shaped, round or ellipsoid depending on the species. The conidiophore of the fungus *Aspergillus* is covered with a phialide, on the top of which chains with spores are formed. These fungi can pose a significant threat to humans due

Table 3. The average value of microorganism units per cubic metre of indoor air

Pr_{avg} fungi (CFU/m^3)			
Measurement	Car No. 1	Car No. 2	Car No. 3
Measurement 1 - “0”	5208	8369	6086
Measurement 2 - “5”	2677	5658	3419
Measurement 3 - “10”	947	4672	615
Measurement 4 - “30”	647	2356	257
Pr_{avg} bacteria (CFU/m^3)			
Measurement 1 - “0”	14613	16563	16111
Measurement 2 - “5”	8711	10581	13045
Measurement 3 - “10”	4827	9387	6067
Measurement 4 - “30”	272	7902	4867

to the high allergenicity of their spores. In addition, various species of *Aspergillus* secrete dangerous mycotoxins that can cause chronic and acute poisoning, and their high concentration can cause many diseases of the respiratory, digestive and liver systems. *Aspergillus* fungus also causes a weakening of the immune system. *Aspergillus* fungus is common in car cabins in Poland (Gołofit-Szymczak and Stobnicka-Kupiec, 2018, Gołofit-Szymczak et al., 2023) and has also been detected in car cabins in Brazil (Lima and Borrely, 2023) and Spain (Fernández-Iriarte et al., 2021) and the Republic of Korea (Ji-Hyun Lee and Wan-Kuen Jo, 2005).

Based on the average values from Table 3, it can be concluded that the worst air quality occurs before the start of forced air exchange in the car cabin and air filtration when the car is parked. At this time, the concentration of fungal colonies in vehicle No. 2 is as much as 8369 CFU/m^3 and this is the least favourable situation when travelling by car.

Figure 1 shows how effective the filtration in the vehicle is, depending on the time for whichthe filters were used. In car No. 2, where the filters were used for the longest time, the concentration of fungi is the highest. Figure 3 also shows that the concentration of microorganisms decreases with the passage of air filtration time. In the case of mould, after 30 minutes of using the ventilation system, the number of mould fungi decreased by about 88%, 72% and 96% for car Nos. 1, 2 and 3, respectively.

The longer the time elapsed since the last filter replacement service and disinfection of the car's cooling system, the worse the air quality in the car's cabin was. The trends of the obtained results are consistent with the work of (Skowron et al., 2018). It should be emphasised here that the concentration of microbiological pollutants in the air in the passenger car cabin also depends on the method of disinfection of the car cooling system (Brodzik and Faber, 2017; Bukłaha et al., 2022; Gołofit-Szymczak et al., 2019a, Aquino et al., 2023).

In the case of bacteria, the air in car No. 1 was the least polluted (Fig. 2). This is due to the cabin filter being used for the shortest time and the disinfection of the air conditioning system. The filter in car No. 1 showed the greatest effectiveness in reducing the number of bacterial colonies in the air. The concentration of bacterial colonies in the car was reduced from 14613 CFU/m^3 to 272 CFU/m^3 after 30 minutes of using the car's ventilation system. Car No. 2 has the poorest air quality because its air conditioning service and filter replacement were carried out 26 months before the start of the study. The filter in car No. 2 was probably the most contaminated due to the longest filter life, which could have contributed to the increase in the number of so-called secondary pollutants in the air (Mingxing et al. 2024). In all three cars, during the use of the ventilation system, the concentration of bacteria showed a downward trend, which proves the effectiveness of air filtration during the test. The reduction in the concentration of bacteria in car Nos. 1, 2 and 3 was 98%, 70% and 52%, respectively. The greatest reduction in the concentration of bacteria was recorded in car No. 1, in which the filter was used for the shortest time, and the smallest reduction in the concentration of bacteria occurred in car No. 2, in which the filter was used for the longest time. The above results indicate how important the work of the ventilation system in the car is, as well as the systematic replacement of car cabin filters.

Figure 1 shows the averages of fungal and bacterial colony-forming units as a function of time. Based on the interpolation of the average values of colony-forming units (Table 3) as a function of time t, a formula was developed (Eq. 2), which can be used to estimate the values of colony-forming units for fungi and bacteria in the cabin of a passenger car during a 30-minute use of the ventilation:

$$CFUm^{-3} = \frac{a+bt}{1+ct} \tag{2}$$

where: a, b and c are the coefficients of Eq. (2) shown in Table 4.

The Pearson correlation coefficient for fungi and bacteria was $R^2 = 0.69$ and $R^2 = 0.80$, respectively. A graphical comparison of the value of Eq. 2 with experimental data is shown in Figure 3.

The greatest decrease in microbial air pollutants was observed in the first 5 minutes of operation of the car ventilation system. Operation of the air conditioning in the three tested cars, from the moment the air conditioning was turned on to 30 minutes of fan operation, resulted in an average reduction of the concentration of fungi

Table 4. Coefficients of Eq. 2

CFU/m^3	Eq. (2) coefficients		
	a	b	c
Fungal CFU/m^3 air	6593	-40.45	0.152
Bacterial CFU/m^3 air	15867	127.57	0.127

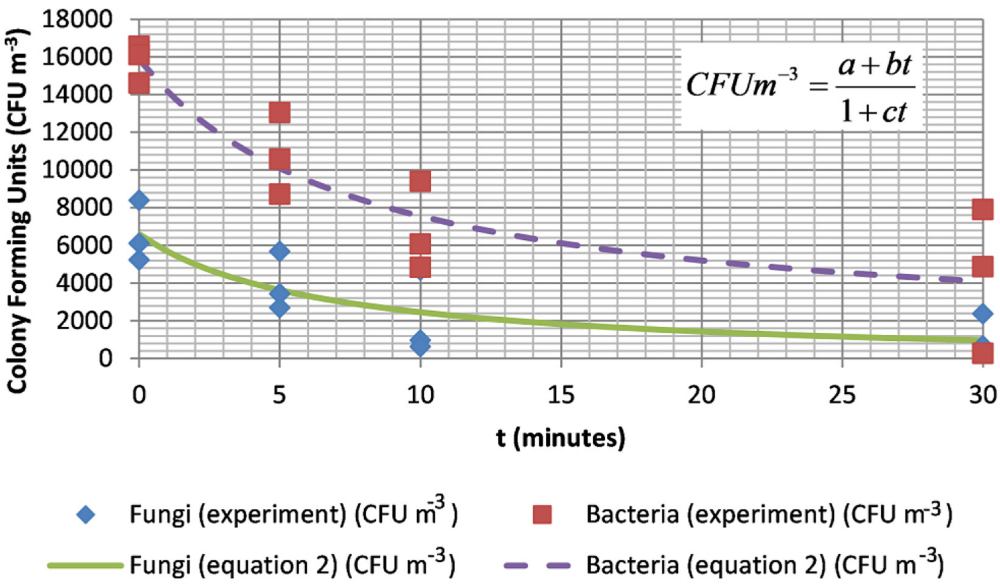

Figure 1. Graphical comparison of the average CFU/m³ values for fungi and bacteria with the interpolation formula (2).

and bacteria in the air of approximately 83% and 72%, respectively. The percentage reduction of microbes in the passenger car cabin by ventilation is consistent with other works (Buitrago et al., 2021, Li et al. 2013), where a percentage reduction of fungi and bacteria of approximately 80% was achieved.

CONCLUSIONS

In this work, the sanitary quality of air in small, enclosed spaces was assessed, using the example of car cabins. This topic is important due to the threats that may result from staying in an air-polluted environment. Based on the conducted research, the following conclusions can be drawn:

1. The longer the time of operation of the air conditioner and the fan, the better the quality of the air, the less fungi and bacteria.
2. The highest concentration of microbiological contamination in the vehicle occurs at the moment when there is no air supply from the fan and no air filtration.
3. The car with the longest filter life had the lowest air quality in terms of microbiological pollutants. Using the filter for more than two years may cause extreme microbiological contamination of the air in the passenger car cabin.
4. The course of the decrease in the load of fungi and bacteria as a function of time in the air of the car cabin from the moment the ventilation in the car is turned on can be described by the rational function, where the greatest decrease in microorganisms was recorded in the first minutes of ventilation operation.
5. The main limitation of the presented research results is the small number of cars tested.

Analysing the state of air quality in the examined car cabins, it can be concluded that regular replacement of cabin filters and air conditioning servicing have a significant impact on air quality in small indoor spaces.

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