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Isolation of Microorgansims from the Air Conditioning System in Cars

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Abstract: Background and Objectives: Automobile air-conditioning (AC) systems are cooling equipment used in modifying air temperature inside the car. AC also can be a major source of the microbial contamination which may bring health risks to users, either by hypersensitivity or infections. This research aimed to assess the air quality inside automotive in Dammam, Eastern Province, Saudi Arabia (MACHS College). It was carried out with 34 and 3 randomly selected cars and buses, respectively (MACHS parking area). Materials and Methods: The air samples are collected starting from 9 Feb up to 27 Feb 2020 by exposing two types of Petri dishes (Blood agar and Sabouraud Dextrose agar) in front of air conditioning for 5 minutes. Dishes were incubated at 37℃ for three day and 30 °C for one day for bacteria and fungi respectively.
Results: There were deferent Bacterial species (gram positive) and many fungal species (*Aspergillus spp, and yeast*) found. There were significant relationships between the old car filter with both bacterial and fungal growth. There was a relationship between the cars' age and the presence of bacterial growth. There was no relationship observed between the cars' age and the fungal growth. There were no relationships between the cars' odometer reading and the presence of bacterial and fungal growth.

Keywords: vehicles, automotive, car, bus, air condition system, microbial load, bacteria, fungi, air quality.

عزل الأحياء الدقيقة من نظام تكييف الهواء في السيارات

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المستخلص: الفكرة والاهداف: أنظمة تكييف وتبريد الهواء هي معدات تبريد تستخدم في تعديل درجة حرارة الهواء داخل السيارة. يمكن أن يكون التيار المتردد أيضا مصدرا رئيسيا للتلوث الميكروبي الذي قد يجلب أخطار صحية للمستخدمين، إما عن طريق فرط الحساسية أو العدوى. يهدف هذا البحث إلى تقييم جودة الهواء داخل السيارات في الدمام، المنطقة الشرقية، المملكة العربية السعودية) كلية MACHS).

المواد: تم اجراء التجربة على 34 عينة تم اختيارها بشكل عشوائي في منطقة اصطفاف السيارات في الكلية.

تم اسحب العينات عن طريق تعريض اطباق تحتوي على الأوساط الغذائية لتيار الهواء الصادر من تكييف السيارة وتحضينها في الحضانة على درجات حرارة ملائمة.

إشارة النتائج لوجود بعض من البكتريا والفطريات في أنظمة التكييف وارتبط ت النتائج مع عمر السيارة - سيارة قديمة او جديدة- من حيث وجود البكتريا فقط ولم يكن لها أي تأثير في وجود الفطريات.

الكلمات المفتاحية: السيارات. أنظمة التبريد. الحافلات. الحمل الميكروبي. البكتريا. الفطريات.

Introduction:

The heat wave makes people cannot live without air conditioning system which the temperature can rise above 50 in the summer, especially in Eastern region of Saudi Arabia.

So many people cannot drive their car. Unless use air-conditioning system. The AC system established in 1933 in New York City. Furthermore, the principle of air conditioner is based on pulling

heat and humidity outside the car which the hot air in the vehicles will replace by cooler air. Indoor air may contain quantity of microorganisms, but this should be lower than the outdoor levels. The most common sources of microbial contamination of indoor air are people, dust, and the ventilation system itself which may be affected by temperature, light, nutrients availability. Human may be considered as the main source of microbial contamination of indoor air by different aspects, sneezing one of the most common way and mechanism for spreading the microbes in air (Kalwasińska A, et al.2012). Car environments considered as a close air environment which may be contaminated 10 times more than the open environment, according to the Environmental protection Agency (EPA) ARPEM Association (2017). In addition, it was found that 75% of human health impact with fungi, bacteria, viruses, mites, and chemicals in the indoor air. When a driver opens the AC system, (Kalwasińska A, et al.2012, Moazam S, Denning DW (2017). The moment that microorganisms arrived car cabin, there are potential effects on the respiratory tract with allergy, poison, and airborne infections to drivers and passengers (Kim KH et al.2018, Kalwasińska A, et al.2012). Lipson et al., 2014;) a study indicated that a few types of fungi are capable to develop more in CO2-enriched atmospheres due to internal conditions that are appropriate to fungi spores to distribute via the atmospheric air in vehicles (Latgé JP,and Chamilos G.2019, Kalwasińska A, et al.2012, Kim KH et al.2018). Over many years, little awareness paid about inner air quality of vehicles or professional drivers (truck drivers, bus drivers, taxi drivers, etc.) while people spend more time inside the transportation with constant occupation shifts of 8 hours a day, 5 days a week (Wang, et al. 2013). It was also found that all collected samples from cars driving behind poultry trucks for 17 miles were an increase in the number of the aerobic bacteria isolated from the air and suggest that poultry transportation in open crates could be a reason of an exposure to the harmful microorganisms and may separate these pathogens into the general environment (Wang et.al, 2013, Wan-Kuen Jo & Ji-Hyun Lee.2008). Overall, there is a lack of studies that is related to automotive transport about indoor air contamination, so the objective of this study is to assess the microbial load in the air conditioning system which may cause microbial contamination to the car drivers and passengers?

Purpose of the study is to assess and evaluate the microbial load as species of bacteria and fungi may live inside the AC system of the car that could cause a negative influence on car drivers and passengers.

Hypothesis: the air conditioning system cause microbial contamination in car drivers and passengers.

Materials and Methods:

Study Design:

The study performed in the duration from 9 Feb up to 27 Feb 2020by a quantitative experimental method in which the samples collected in the base of randomization with the consideration of our variables. The dependent variables in this experiment were the quantity and quality of bacteria and fungi growth while the independent variables were the cars characteristics like the year of production, distance consumed by the car and the filter quality.

Population and Sample:

Samples collected as bacterial and fungal from the air conditioner in the cars by randomly selecting 34 and 3randomly selected cars and buses respectively from different model like Toyota, Hyundai, Nissan, Mazda, Havel, Chevrolet, Changa, Ford, Greely, KIA, BMW, Mercedes, GM, Daihatsu, and Fuso.

Data collection :

Samples were in 2 types of growth media: Blood Agar; Blood Agar (BA) are enriched medium used to culture those bacteria or microbes that do not grow easily, 0.5% Peptone 0.3% beef extract/yeast extract, 1.5% agar, 0.5% NaCl, Distilled water

for bacteria collection while fungi isolated by using Sabouraud Dextrose Agar; The composition (g/l) is potato infusion from 200 g potatoes; 20 g of D (+) glucose; 15 g of agar-agar and pH = 5.6. with adding chloramphenicol antibiotic to inhibit bacterial growth. The petri dishes were incubated at different temperatures (at 37 °C for three days and 30 °C for 2 days for fungi and bacteria respectively). The colonies were identified by using different culture techniques, manual procedures, and microscopic examination (Vijayakumar R et al, 2021, Aquino Simone et al 2018).

Samples collected from cars and few numbers of buses in the parking area around the buildings in the eastern region (MACHS College Parking Area). All samples processed and analyzed in Microbiology Laboratory CLS department- MACHS.

Data analysis procedure:

Data is analyzed using Jamovi statistical program and specifically by the Independent T-test and measure the p- value to know the significant of bacterial and fungal growth depending on the car age, distance consumed by the car and the quality of the filter.

Results:

To evaluate the microbiological quality of indoor environments of the car air conditions systems and quantify the microbial load in the air in different collected samples (table 1). According to the microbial load, cars models less than 2015 years were considered as an old while the equal or greater than 2015 years considered as a new. It was found that the percentages were 70.3 and 29.7% for new and old cars respectively. To explain, the data indicated there is 0.8 % of the probability that may the results were affected by the age of cars according to the (p-value is < 5%) which means that the age of car significantly affecting the bacterial count. Old cars have more positive bacterial count with 7 cars out of 11 while the new ones have 5 out 25 positives which means that the old cars have more bacterial load. In the other hand, it was indicated that there is 58.8% of the probability that may the results were not affected by the age of cars according to the (p-value is > 5%) which means that the age of cars didn't affecting of the fungal count in the car (table 2).

Table 1: The count of bacteria on the AC of old and new cars.

	Car Age		
Bacterial Growth	New	Old	Total
Positive	5	7	12
Negative	21	4	25
Total	26	11	37

(*P<0.05 is considered statistically significant).

Table 2: The count of Fungi on the AC of old and new cars.

	Car Age		
Fungal Growth	**New**	**Old**	**Total**
Positive	3	2	5
Negative	23	9	32
Total	26	11	37

(*P>0.05 is considered statistically insignificant).

To evaluate the distance consumed by the cars, it was considered that, the car that consumed 100,000 Km/hr. or greater as s long distance while if it consumed less than 100,000 km/hr. it considered as a short distance. In addition, the percentages were 37.8% and 62.2 % for long and short distance respectively. The data (table 3&4) with the probability of 29.1% for bacterial growth and 95.5% for fungal growth indicated that may the results were not affected by the distance consumed by cars according to the (p-value is > 5%).

Table3: The count of bacteria on the AC of car according to the distance consumed.

	Car odometer		
Bacterial Growth	long Distance	Short Distance	Total
Positive	6	6	12
Negative	8	17	25
Total	14	23	37

(*P>0.05 is considered statistically insignificant).

Table4: The count of Fungi on the AC of car according to the distance consumed.

	Car odometer		
Fungal Growth	long Distance	Short Distance	Total
Positive	2	3	5
Negative	12	20	32
Total	14	23	37

(*P>0.05 is considered statistically insignificant).

With regard of quality of the filter, if the filter is changed in less than 6 months it considered as a new while if the filter is changed in 6 months or greater it considered as an old. Data was indicated that, there were 51.4% and 48.6 % old and new filter respectively. Furthermore, there are <0.1% probability of bacterial growth and less than 2% of fungal growth which indicated that quality of car filters affected both the bacterial and fungal growth significantly (P<0.05), (table 5 &6). Old filter had more microbial load than new one, it found that old filter had 11 positive filters, and 5 positives out of 19 for both bacteria and fungi respectively.

Table 5: The count of bacteria on the AC of car according to the filter quality.

	Filter Quality		
Bacterial Growth	Old	New	Total
Positive	11	1	12
Negative	8	17	25
Total	19	18	37

(*P<0.05 is considered statistically significant).

Table 6: The count of fungi on the AC of car according to the filter quality.

	Filter Quality		
Fungal Growth	Old	New	Total
Positive	5	0	5
Negative	14	18	32
Total	19	18	37

(*P<0.05 is considered statistically significant)

Generally, there are significant relationship between the old car filter that changed in more than 6 months with both bacterial and fungal growth. Also, there are relationship between the cars that less than 2015 years with bacterial growth only. In, contract, there are no relationship between the distance consumed by the car if it is less or more than 100,000 km /hr with both bacterial and fungal growth.

Discussion:

In this study, 32.4 % of the samples were positive in bacterial growth while only 13.5 were positive for fungal growth, however, the pathogenic fungi identified are *Aspergillus ssp.* They are known to cause invasive aspergillosis and produce carcinogens. Most infections commonest being aspergillosis can occur in immune compromised host or as a secondary infection following inhalation of fungal spores or the toxins produce by them. Symptoms include persistent cold, watery eye, prolong muscle cramps and joint pain. The bacteria identified are *ranged from cocci, diplococcic, gram negative and positive bacteria (*table7).More organisms were reported to be attributed to the changes in weather condition with dry season having a lot of dust particle that could have contained microbial spores as they are blown into the air by the wind, these results were similar to the Study conducted by (Vijayakumar R et al, 2021, Sattar AS et al 2017), estimated the level of bacteria and fungi concentration prior and after using of the automobile air conditioning. Moreover, the qualitative analysis of bacterial and fungal aerosols found the car air cabins before the AC service used were 28 bacterial species from 10 genera and 31 fungal species belonging to 18 genera. Analysis of qualitative study represented the major prevalent bacterial species found in all the vehicles were Gram-positive cocci such as Staphylococcus and *Micrococcus/ Kocuria* genera (from 40% to 54%). Also, there were represented of endospore Gram-positive rods such as the *Bacillus* genus (between 20% to 29%). In addition, the data was similar with the studies that were conducted to measure the concentration of microbes found in the Automobile AC system of cars. (Wan-Kuen Jo & Ji-Hyun Lee 2008). For cars, the maximum bacterial concentration (2,550 CFU m-3) was 46 times higher than the in-vehicle background concentration (55 CFU m-3). Three fungi (Cladosporium, Penicillium, and Aspergillus) exhibited the highest concentrations for most sampling periods of the ACs and heater (Wan-Kuen Jo & Ji-Hyun Lee 2008).

Overall, the maximum concentration of fungi and bacteria in transportation is 1,000 CFU/m3 which is recommended by the Environmental Protection Agency (EPA) in Taiwan. The average bacterial concentration was 282 CFU/m3 while fungi were 674 CFU/m3, so the total bacterial and fungal concentration did not exceed the recommended EPA value in Taiwan. The only limitation of this study is the sample size was small (Wan-Kuen Jo & Ji-Hyun Lee 2008). The outcome of the vehicle's study appeared (100%) contamination of fungi and showed 17 genera of fungi. This study showed that non-

sporulation fungi (NSF) 81% were found in filter samples while the second elevated pollution observed via *Aspergillus spp.* (76%), then by yeasts (62%) and *Cladosporium spp.* (52%) ((Lukaszuk et al. 2017)

Table 7: The type of Microbe with the number of cars

	Type of bacteria						Type of fungi	
	Gram positive				Gram negative		Molds	Yeasts
	Cocci	Diplococcic	Micrococci	Bacilli	Cocci	Bacilli	Asp. Fumigatus	Yeats
Number of cars	4	2	1	9	0	0	3 buses	2 cars I bus

Conclusion:

This study suggested that there is strong relationship between the old car filter that changed in and less than 6 months with both bacterial and fungal growth. Also, there are relationship between the cars that produced in less than 2015 years with bacterial growth only. On the other hand, there are no relationship between the distance consumed by the car if it is less and more than 100,000 km /hr with both bacterial and fungal growth. The car filters may have Dust and liquid drops which may considered as a medium for microorganisms to spread. It is necessary to clean or change the components in air-conditioning systems (e.g., filter, heat exchanger and muffler). Filtration is a efficient method of improving air-conditioning system, and it is represent a good solution for the improvement of Indoor Air Quality (IAQ), and its considered a method to prevent the accumulation of microorganisms which contributes to the improvement of air quality.

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