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Microbiological Air Monitoring and Long-Term Evaluations of Selected Urban Areas in the City of Tirana †

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Abstract: The described experimental study, performed over the years, includes the quantitative and qualitative monitoring of the presence of microorganisms of air in outdoor and indoor environments of the Albanian Capital, Tirana, during a time when large demographic movements, accompanied by important urban interventions and infrastructural changes, have been part of our lives. A project, part of the National Program in Biotechnology (R & D—the year 2000), was the first support to obtain a database on microbiological air pollution in selected urban areas in Tirana and isolate and identify specific air microbial pollutants. The results obtained were an incentive to continue further with additional scientific evaluation monitoring research, which included the years 2011 to 2015 and then those of 2016–2020. Over the years, there has been a significant reduction in pollutant microbial loads (for selected outdoor areas of the center of Tirana, the total discovered loads decreased from values of the order 10^5–10^6, to currently about 10^2, for the same areas). A fluctuation in indoor microbial loads was observed in many cases. Additionally, a prominent presence of typical environmental fungi pollutants such as *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus terreus*, as well as bacterial pollutants, cocci, and bacilli (typical *Bacillus megatherium*) was identified during a Total Viable Count (TVC) and other microbiological tests of identification.

Keywords: Total Viable Count (TVC); outdoor pollutants; indoor pollutants; cocci; bacilli; molds; yeasts

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1. Introduction

A significant number of microorganisms are present in the air, in different layers of the Troposphere. They pass into the air from various sources. Dust, plants, animals, excrements of human bodies, waste materials, new constructions such as buildings, changes of urban infrastructure, etc., are primarily responsible for increasing the number of microorganisms in the air outside and inside.

An assessment of microbiological loads of the air is essential to ensure a clean environment for a healthy life and control the cleanliness of raw materials, food and pharmaceutical products, as polluted air can be a common source of infections. Various bacilli (*Bacillus cereus*, *Bacillus megatherium*, *Bacillus subtilis*, etc.) and various micrococci and fungi (molds and yeasts) are determined in air particles [1–3]. Air pathogens are responsible for some important human diseases, such as *Streptococcal Respiratory Infections*, *Tuberculosis*, *Pneumonia*, *Aspergillosis*, and other viral diseases [4,5]. Microbiological loads of the air were determined at both quantitative and qualitative levels, to identify the total viable count (TVC) and pathogenic loads to assess and manage their possible negative impact [2,5,6].

The experimental work was performed through a collaboration between the University of Medicine of Tirana, Faculty of Medicine, Department of Pharmacy and University of Tirana, Faculty of Natural Sciences, Department of Industrial Chemistry (group of Food

Chemistry and Industrial Microbiology). The study comes at a time when, at the national level, there is extensive scientific research focusing on evaluating chemical contaminants and less microbiological ones. Therefore, determining the total loads of microorganisms, as well as exploring the typical predominant contaminant strains in monitored environments is a modest contribution in creating a data network for the microbiological contamination of urban environments and efforts to minimize a contamination with high risk for vegetation and living beings.

2. Materials and methods

Initially, 25 outdoor and indoor environments were selected in the city center's geographical locations [7–9]. This paper describes the results taken from 5 of them, and they are coded as follows:

- OGH—University Hospital Center
- FNS—University Institution
- NM—Museum Center
- MTS—Mother Teresa Square (most famous square of the capital)
- TLP—Lake National Park of Tirana

For each area under monitoring, air samples were taken outdoors and indoors belonging to each outdoor environment mentioned above [5,10]. Passive sampling was carried out with settle plates, using the settling plate technique. The principle of this simple method is the gravitational force. Open Petri dishes with 20 mL of solid culture media, were placed at a 1-m distance from the ground level. An air volume with dust particles and microorganisms were settled onto each plate's selected solid medium. Plate Count Agar-PCA was used to determine the total number of microorganisms. Meat Peptone Agar-MPA was used for bacteria and Sabouroauds Dextrose Agar-SDA for yeasts and molds. The microbial load was evaluated as CFU/m^2 hour. The plates were incubated for total mesophilic microorganisms at 28 °C, for weak pathogens at 37 °C, for molds and yeasts at 25–28 °C [2,5,11,12].

The Total Viable Count (TVC) and qualitative analysis were performed every year during the same period (in April, around 12 o'clock), in order for temperature and climate not to have significant effects on results.

3. Results

The determination of Total Viable Count is presented in Table 1.

Table 1. Total Viable Count (CFU/m^2 hour) of outdoor and indoor samples in years.

Nr.	Samples	TVC-2016	TVC-2017	TVC-2018	TVC-2019	TVC-2020
1	OGH—outdoor environment	3.65×10^4	1.51×10^4	4.04×10^3	6.49×10^3	3.52×10^2
1-1	OGH—indoor environment	2.31×10^1	2.22×10^2	5.71×10^2	2.78×10^2	2.70×10^1
2	FNS—outdoor environment	7.23×10^5	8.85×10^4	4.44×10^3	5.59×10^3	4.55×10^2
2-1	FNS—indoor environment	1.22×10^3	7.41×10^4	3.02×10^3	3.26×10^2	2.20×10^1
3	NM—outdoor environment	1.41×10^2	2.91×10^2	4.34×10^4	11.43×10^2	1.42×10^2
3-1	NM—indoor environment	2.45×10^1	2.90×10^2	9.10×10^3	3.33×10^3	2.53×10^1
4	MTS—outdoor environment	1.39×10^2	2.78×10^2	6.71×10^3	6.71×10^3	5.67×10^3

Table 1. *Cont.*

Nr.	Samples	TVC-2016	TVC-2017	TVC-2018	TVC-2019	TVC-2020
4-1	MTS—indoor environment	1.25×10^2	3.44×10^2	7.39×10^3	1.56×10^3	2.30×10^1
5	TLP—outdoor environment	5.30×10^1	6.52×10^2	9.91×10^2	4.98×10^2	2.35×10^2
5-1	TLP—indoor environment	6.00×10^1	8.51×10^2	7.26×10^2	7.36×10^2	4.59×10^2

As can be seen from the table, the year 2019 marks a significant decrease in loads due to the monitored areas' infrastructural systematization. The increased loads in some outdoor and indoor environments can be associated with a larger number of people circulating in those environments (MTS and TLP outdoors). In some indoor environments, ventilation was sufficient for good microbial decontamination. In April 2020, there was a drastic decrease in the air contamination due to the local COVID-19 lockdown.

In all the cases of the selected most important microbial contaminants that were identified during the qualitative tests, decontamination over the years is evident. For *Bacillus megaterium*, a weak environmental pathogen, the collected data show a decrease in its presence from 24 to 18% (2016–2019). A similar situation (decrease from 32 to 9%, in the years 2016–2019) is also presented for *Aspergillus niger*, a highly prevalent mold that can cause specific cases of pneumonia or food spoilage. Additionally, complementary evaluations indicate a decrease in the percentage of presence of *Bacillus cereus* from 24 to 8% over the studied years, of *Aspergillus flavus*, a source of an aflatoxin production, from 11 to 3%, as well as a decrease in the presence of *micrococci, diplococci* and streptococci bacteria.

Very rarely were yeast cultures, such as *Saccharomyces* spp., *Rhodotorula* spp. (no contaminants, the first, fermented yeasts and the second, important for the biomolecules production), *Basidiomycetes* such as *Aureobasidium pullulans*, and others, observed and then identified. Over the years, only 0.2% of yeasts were detected, among them some colonies of *Candida albicans,* which is a pathogen and source of human infections. In the assessment of microbiological air pollution during 2016 and 2017, a considerable presence of *Fungi imperfecti*, vegetative cells, and spores of *Cladosporium* spp. and *Alternaria* spp. were observed in the MTS and TLP outdoor samples.

4. Conclusions

In conclusion, we would like to emphasize that study monitoring activity evidenced significantly reduced loads present in both the analyzed outdoor and indoor environments. This decrease is noticeable with the values of total loads reaching one and two logarithmic scales.

Highly reduced loads were identified in the results obtained during the years 2019 and 2020, but local COVID-19 lockdown restrictions highly impacted the data of 2020.

Positive infrastructural changes, an increase in vegetation, and hygienic control of open spaces are some of the beneficial changing factors directly impacting the environmental microbial pollution of Tirana, the capital of Albania. However, much remains to be done, mainly focusing on the negative role of construction activities in overcrowded areas.

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