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Biological Contamination of Air in Indoor Spaces

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1. Introduction

The indoor environment is a very complex environment. Pollutants are numerous in both outdoor and indoor air. While the same pollutants (biological, chemical, or radioactive) can be identified in indoor and outdoor spaces, their concentrations can be very different. More specifically, extremely high concentrations can accumulate indoors. Many bacteria that belong to the normal microflora of the human skin are continuously emitted into the immediate surroundings of humans, and accumulate indoors.

The pollution of the indoor spaces is an important element that can affect human health. People spend around 90% or more of their time of a day indoors. Indoor spaces include the inside of houses, schools, university rooms, social buildings like shops, cars, planes, and workplaces. The outdoor and indoor environments are linked. The two elements cannot be separated. Biological agents are defined as agents or microfragments from plant or animal matter or from microorganisms.

Elements identified in the air include bacteria, either in vegetative status or spores, fungi, yeasts, microbial toxins and secondary metabolites like bacterial endotoxin, peptidoglycans or fungal $\beta(1,3)$ -glucans, volatile organic compounds, pollens, pet and insect allergens, other allergens, viruses, protozoa, etc. [1-3].

Many bacteria, fungi, viruses, and protozoa may be infectious to humans and they may cause specific infectious diseases. Infectious agents are usually received from other humans, from animals (these infections are zoonoses), or from environmental sources such as contaminated water, soil, etc.

Biological contamination in indoor air may have sources like outdoor air, human body, bacteria that are growing indoors, and pets.

2. Biological contamination of indoor air

2.1. Bacteria in the indoor environments

The most common building-associated bacteria are saprophytic bacteria of the normal human skin, mouth, and nose that are emitted into the indoor air and bacteria originating from outdoors air [1-3]. Other common bacteria of indoor environments include heterotrophic bacteria that grow in the water reservoirs or moist sites of the building, such as bathroom sinks. Specifically, legionellae and nontuberculous environmental mycobacteria develop in biofilms of water pipelines or in water reservoirs of cooling systems. Actinobacteria (especially Streptomyces), *Bacillus* species and various other bacteria grow in moist building materials together with fungi. Elements from bacterial structures released in air include bacterial cells, bacterial spores, peptidoglycans, microbial volatile organic compounds, exotoxins, and other bacteria growing metabolites [1].

Humans are an important source of indoor bacteria. The upmost layer of the normal human skin is continuously renewed, and skin scales containing bacteria are shed into the environment [1]. Bacteria in the respiratory airways are eliminated through Pflügge droplets while talking, coughing, or sneezing. The level of air contamination is dependent on the number of persons inside a room and the efficiency of the ventilation system (natural or artificial ventilation). Bacteria that can be identified in indoor air are micrococci, staphylococci, streptococci, and corynebacteria [1].

The presence of pets in indoor spaces can represent an important source of air contamination and can be linked with the level of indoor endotoxins. The presence of dogs and cats can be the main predictors of endotoxin levels in house dust [1, 4-7]. Other predictors are the presence of vermin, such as mice, and infrequent cleaning, which indicates poor hygienic conditions in the home [1]. Storage of organic household waste indoors also increases bacterial contamination in the indoor environment [1].

Bacteria actively growing or accumulating in the indoor environment may cause health effects and may require specific actions to control growth and prevent the exposures.

Bacterial growth can be found wherever standing water is present and Gram-negative bacteria can be identified in this environment. The presence of bacteria, either as viable bacteria or bacterial spores, mycotoxins, chemical markers like β -glucans and volatile organic compounds, and endotoxins, represents an indication of high humidity in the indoor environment [1].

2.2. Fungi in indoor environments

Fungi are present in both outdoor and indoor air. The levels found in the outdoor air can range from less than 100 spores/m³ up to 100 000 spores/m³. Normally, the fungi levels are lower in indoor spaces compared with outdoor spaces. If the conditions that permit the fungal growth are present, for example, in damp houses, the levels of fungi in the indoor air can reach very

high concentrations. Fungi level measurements are expressed in colony-forming units (CFUs) per cubic meter of air.

The levels of fungi and the type of fungi identified in indoor air depend on the season, construction features, age, and use of the building as well as the ventilation rate.

The most common fungal genera occurring in indoor environments are *Penicillium*, *Aspergillus*, *Cladosporium*, *Alternaria*, and yeasts [1-3]. These genera are also the most frequently occurring fungi in outdoor air.

Spores of fungi are present everywhere, and they are able to germinate wherever there is water available and the ranges between 0.80 and 0.98. Fungi need carbohydrates, proteins, and lipid to develop. They can find all these elements in house dust, construction materials like wall-paper or textiles, paint, glue, wood, paper and books, stored food, or deposit of cooking oil. Fungi can grow on inert materials like ceramic tiles [2].

2.3. Allergens in indoor environment

Allergens are a group of agents that may cause a specific IgE-mediated reaction in humans. Fungal allergens can trigger type I allergic reactions and IgE sensitization to fungal species like *Alternaria*, *Penicillium*, *Aspergillus*, and *Cladosporium* spp. All these types of fungi species can induce allergic respiratory diseases, especially acute asthma attacks. Fungal allergens can also trigger type III-IgG-mediated allergic reactions (*Penicillium* and *Aspergillus*) and even type IV allergic reactions. This is the premise of the onset of hypersensitivity pneumonitis [2].

Allergens may also be present in indoor air due to house dust mites. The mite species that produce allergens are *Dermatophagoides pteronyssinus* (major allergens—Der p I and Der p II) and *Dermatophagoides farina* (major allergen—Der f I).

2.4. Endotoxins

These are toxins integrated in the outer membrane of Gram-negative bacteria, which are composed of proteins, lipids, and lipopolysaccharides [2, 4-8]. The bacterial cell lysis determines the release of endotoxins. In the air, endotoxins are bound to coarse particles. Exposure to different levels in air has different results: if the exposure is to high levels, respiratory symptoms or nonallergic asthma can develop, while if the exposure is at low and moderate levels, it is possible to have a protective action against allergies and asthma [2, 4-8].

2.5. (1 → 3)-β-D-glucans

It is a structural element of the cell wall of fungi and some bacteria, and it can be found in house dust. (1 → 3)-β-D-glucans do not have allergic properties. However, they do have immune-modulating properties. Exposure to (1 → 3)-β-D-glucans in home environment can induce respiratory diseases [2, 9, 10] and may increase peak flow variability in asthmatic children.

2.6. Mycotoxins

These are toxins (secondary metabolites) produced by fungi that interfere with RNA synthesis and can cause DNA damage. Some mycotoxins like aflatoxins produced by *Aspergillus flavus* and *Aspergillus parasiticus* induce carcinogenic effects [2, 11]. Indoor exposure to mycotoxins produced by *Stachybotrys chartarum* can be associated with acute pulmonary hemorrhage.

2.7. Fungi and Volatile Organic Compounds (VOC) in indoor air

Fungi can produce around 200 volatile organic compounds. The identification of the VOCs in indoor air shows that there is a fungal growth present, even if the quantitative measurements are negative [11]. One of the most important substances that can be identified in indoor air is formaldehyde that can induce cancer.

2.8. Viruses

Indoor air humidity can induce a longer survival time of respiratory viruses and increased risk of respiratory infection and allergic diseases.

3. Exposure evaluation of biological contamination of indoor air

Indoor air levels of bacterial concentrations of $<1,000$ CFU/m³ can be regarded as “low,” and concentrations of $>5,000$ CFU/m³ can be regarded as “high” [1]. These numbers reflect mainly the crowdedness of the room and the efficiency of the ventilation and thus are a measure of the hygienic quality of the indoor air.

Characterizing the bacterial flora in the sample may give additional information about the sources of the bacteria present. If Gram-positive cocci and corynebacteria, the common bacteria of the human skin, are the dominating groups, this indicates human sources [1].

The presence of Gram-negative bacteria shows an active bacterial growth due to the presence of water indoors.

Gram-positive bacteria with exo- and endospores like *Streptomyces* and *Bacillus* can grow on moist building materials. Their spores are very resistant and can survive even if the air humidity is low.

The presence of fecal bacteria like the group of *Enterobacteriaceae* is considered heavy contamination of indoor air, coming probably from a sewage leakage. The presence of actinomycetes shows the growth of microbes in the building material.

The presence of two groups of bacteria, that is, *Legionella* and actinomycetes, has a special importance in indoor air.

Legionella spp. are Gram-negative environmental bacteria that have found a remarkable ecological niche in man-made water systems, especially in those serving air conditioning systems for heat transfer [1].

4. Health effects induced by indoor exposure to biological contaminants

Indoor air contamination can generate a multitude of effects on human health. The effects are especially on the respiratory system. Allergic reactions can be induced by the exposure to different biological agents that are identified in indoor air. Also, there is evidence to suggest that exposure to biological pollutants in indoor air can be associated with symptoms with neurological basis like headaches, fatigue, and forgetfulness.

Some of the effects on the respiratory system related to the biological contamination of indoor air are due to the inflammation of the airways. This can appear in both allergic (with the implication of IgE and IgG antibodies) and nonallergic respiratory diseases. The separation between those two entities is important in the case of asthma and rhinitis.

4.1. Rhinitis

Rhinitis is induced by the inflammation of the mucosa of the upper respiratory tract. Symptoms of rhinitis are nasal congestion, rhinorrhea, sneezing, and conjunctivitis. Rhinitis can be of allergic or nonallergic cause [1, 11].

4.2. Asthma

Allergic asthma involves IgE-mediated sensitivity and eosinophils in presence of an atopic underlying reaction of the respiratory airways [1, 12-18]. Among adults, there is also an increasing prevalence of asthma. People with moderate to severe asthma will react adversely toward biological and other contaminants in the indoor environment due to a nonspecific bronchial hyperresponsiveness. That is an exaggerated bronchoconstrictor response to numerous numbers of stimuli. The severity of asthma and the need of medication in house dust mite allergic patients is closely related to the amount of endotoxin in house dust in a dose-dependent manner [1].

Pollen allergens interact with other sources of fine particles in polluted air, which concentrate the allergens and trigger asthma attacks. As for the interaction between biological and nonbiological agents, airborne particles such as diesel exhaust particles have an adjuvant activity for IgE antibody and cytokine production [1]. The intranasal instillation of ragweed allergens with diesel exhaust particles in allergic patients significantly increased allergen-specific IgE production and enhanced Th2-type cytokine mRNA expression compared to allergic patients instilled with ragweed alone [1].

Both allergens and viruses have been known to worsen asthma symptoms. A study in the UK demonstrated that allergen exposure and viral infections act synergistically to trigger exacerbations in adults [1].

4.3. Chronic bronchitis

Chronic bronchitis is defined as cough with phlegm (productive cough) for at least 3 months per year for 2 years or more [1, 17-19]. Chronic bronchitis may be present with or without

airways obstruction. The underlying mechanism is an increase in mucus secreting glands and alterations in the characteristics of the mucus itself [1]. Airway inflammation is present, and an increased number of neutrophils can be observed [1].

Subjects with chronic bronchitis may also have symptoms such as dyspnea, chest tightness, and wheezing, and there is thus a considerable overlap in symptoms between allergic asthma, nonallergic asthma, and bronchitis [1]. Biological contamination through the presence of endotoxin and β -glucan can be incriminated in the pathogenesis of bronchitis [1].

4.4. Chronic airflow obstruction

Several studies have shown associations between chronic airflow obstructions as measured by spirometry (particularly FEV1) and organic dust exposures in industrial and agricultural occupational populations [1, 19]. Endotoxin exposure may play an important role.

4.5. Hypersensitivity Pneumonitis (HP) or Extrinsic Allergic Alveolitis (EAA) [1, 2, 20]

The disease, also known as “farmers lung” or “pigeon breeder’s lung,” is one of the most severe diseases induced by fungi and thermophilic actinomycetes (spore-forming bacteria) exposure. The clinical aspect of HP is represented by severe pulmonary disease with pneumonia-like fever, cough, tightness of chest, shortness of breath, inflammation of pulmonary parenchyma, and development of pulmonary granulomas. HP is very similar to organic dust toxic syndrome (ODTS).

4.6. Organic Dust Toxic Syndrome (ODTS)

The disease is similar to influenza and is characterized by symptoms like acute fever, dry cough, dyspnea, chest tightness, headache, shivering, malaise, fatigue, and muscle and joint pains. ODTS is a nonallergic neutrophil-mediated airway inflammation [1].

4.7. Atopic allergic dermatitis

This disease can be induced by exposure to the biological indoor air pollutants.

4.8. Sick building syndrome

In the 1980s, the concept of “sick building syndrome” (SBS) was established by the WHO [1, 3, 21, 22]. The label sick building syndrome (SBS) can be applied when the inhabitants or occupants of different indoor spaces develop acute health problems or discomfort inside the rooms. The exposed people have the following symptoms: eye, nose, and throat irritation and dryness; itching and dryness of skin and eyes; dry cough; nausea; vomiting; headache; difficulty in concentration; fatigue; etc. The symptoms disappear after cessation of the exposure.

5. Aim and purpose of the study

The aim of the study was to assess indoor air contamination from the point of view of bacteriological and fungal contamination of different indoor spaces like houses, schools, offices, archives, and spaces with commercial destinations [23-25].

6. Materials and methodology of investigation

The methodology of the study implies the investigation of the levels of indoor air contamination with fungi and bacteria. The destination of the investigated indoor spaces was very diverse. The levels of bacteriological contamination were assessed in indoor spaces designated as living spaces, social use spaces like shops, and work and education spaces like schools, offices, and archives.

Quantitative and qualitative assessments were made to evaluate the levels of air contamination. Passive and active methods were used to sample the air from the investigated sites. The results were compared to the enforced recommendations in Romania, at this moment.

6.1. Investigated places

6.1.1. Houses in Bucharest City

There were 10 investigated apartments, with different numbers of room situated at different floors and with different geographical orientations and different characteristics.

Type of investigated apartments:

- a. One apartment with one room, one inhabitant, nonsmoker
- b. One apartment with one room, two inhabitants, one smoking inhabitant
- c. Apartment with two separates rooms, two nonsmoking inhabitants
- d. Apartment with two separates rooms, three smoking inhabitants
- e. Apartment with two rooms, with five nonsmoking inhabitants
- f. Apartment with two separates rooms, four inhabitants, one smoking person
- g. Apartment with three separates rooms, three nonsmoking inhabitants
- h. Apartment with three separates rooms, five inhabitants, one smoking person
- i. Apartment with three rooms, five nonsmoking inhabitants
- j. Apartment with three rooms, with four inhabitants, one smoking person

For an accurate assessment of the biological contamination of indoor air, the measurements were made three times in a year, in the winter, in the summer, and in the autumn, 3 days consecutively, in the kitchen and in the living room of each investigated apartment.

6.1.2. Schools

There were two investigated schools, one in Bucharest and one in Giurgiu City.

In the school in Bucharest, the investigations were made

- in an empty classroom, before 8:00 a.m.
- during the school day (at 9.30 a.m.), in a classroom with 23 children
- in a classroom with 36 children and 1 teacher
- in a classroom with 23 children and 1 teacher
- in a classroom (at 11:00 a.m.) with 24 children and 1 teacher
- in the school library (at 10:00 a.m.) with 3 people inside
- in study room number 1, next to the library with 15 children inside
- in study room number 2, next to the library, room that is connected to the hall in front of the school library
- in the hall of the school
- in the medical ward, with 8 people inside the room.

In the school in Giurgiu, investigations were made

- in a classroom at 8:00 a.m.
- in a classroom at 11:00 a.m.
- in a classroom at 1:00 p.m.
- in the school hall.

6.1.3. Offices

Fungi were investigated in eight offices.

6.1.4. Archives

Fungi were investigated in 10 archive rooms, where the possibility of contamination was presumed.

6.1.5. Shops

The levels of bacteria in air were investigated (a) in a supermarket in Bucharest City with ground floor and 4 floors and (b) in a shop with only a ground floor.

The investigations were made in the morning when the shop opens and in the evening when the shops were nearly to be closed.

6.2. Sampling methods

For the bacteriological and fungal evaluation, the sampling methods used were a passive method and an active one [26, 27].

6.2.1. Passive sampling method

The total number of mesophilic aerobic bacteria, yeast, and molds in the air of selected rooms was determined using the Koch sedimentation method. Bacteria from air were harvested using the gravitational method on the Petri plates with specific growth media. The measurements of levels of bacteria in air were made in several points on each location. The results were expressed as CFU/m³. The equation used for calculating the results was as follows:

$$\text{CFU/m}^3 = n \times 10,000 / s \cdot t / 5$$

where n is the number of colonies on the Petri plate, s is the surface of the Petri plate, and t is the time of Petri plate exposure. Petri dishes with growth media were exposed for 10 min.

Results obtained by the Koch sedimentation method are less accurate than those from impaction methods with the use of an air sampler. The sedimentation method does not require expensive instrumentation and is cheap and simple. The sedimentation method does not permit exact quantitative determination. Still, this method permit an assessment of the types of microorganisms present in the air and can give an approximation of bacterial and fungal concentration in the air of investigated indoor spaces.

6.2.2. Active sampling method

This method used an impactor sampler M.A.S.Q.-90, with 2 l/min airflow and a total air volume equal to 200 l.

The following growth media were used [28]:

- tryptic soy agar (TSA) to determine the total number of bacteria
- blood agar to determine the presence of *Staphylococcus* and *Streptococcus*
- Czapek Dox Agar (Oxoid) for fungi identification

To obtain the total number of bacteria in the air of investigated rooms, the Petri dishes with growth media were incubated at 37°C for 24 h and then left for another 24 h at room temperature. To obtain the number of fungi colonies, the Petri dishes were exposed for 10 days at 25°C.

The identification of bacteria types was made by macroscopic estimation (description of colony) and by microscopic estimation.

Diagnosis of filamentous fungi was based on estimation of morphological features of growth on Czapek medium as well as on microscopic observation.

6.3. Indicators used to assessed the air contamination of indoor air

The following were assessed and counted on a Petri dish:

- a. The total number of bacteria, expressed in CFU/m³ (colony-forming units/cubic meter, CFU/m³)
- b. The presence of *Streptococcus* alpha and beta hemolytic
- c. The presence of *Staphylococcus aureus* and non-*aureus* with and without hemolytic proprieties
- d. The presence of coliform bacteria as an indicator of enteric contamination of air
- e. The total number of fungi expressed in CFU/m³ as quantitative assessment of contamination
- f. The types of fungi as a qualitative assessment of indoor air contamination with fungi

6.4. References values

To have a good assessment of the results that were obtained, it was useful to have as references standardized values for the biological contamination of indoor air.

At present, there are no standards or guidelines for microbiological quality of indoor air. Each country has individual regulations and recommendations.

The Commission of the European Communities (CEC) defines the following level of bacteriological air contamination: 0 undetectable, 1-499 CFU/m³ low, 500-999 CFU/m³ medium, >1, 000 CFU/m³ high, and 2, 000 CFU/m³ very high. A value of 10, 000 CFU/m³ of nondescript airborne microbes could therefore be considered a hazardous level for indoor environment [12].

In 2001, the American Industrial Hygiene Association (AIHA) published a proposition of guidelines that stipulate 1, 000 of viable CFU/m³ in air as an upper limit for concentration in indoor environment and for the amount of fungal spores in different indoor environments. It is proposed that the indoor air levels of microorganisms should be less than 500 CFU/m³ for residential buildings and less than 250 CFU/m³ for commercial buildings [29, 30].

According to current Swedish requirements, the number of 500 colony-forming units (CFU) of bacteria and 300 CFU of fungal spores in 1 m³ can be accepted in an indoor environment [31].

The regulation in Brazil stipulates that the upper limit for total amount of airborne microorganisms (especially fungi) should be 750 CFU/m³ [32, 33].

In Hong Kong, the indoor air levels of microorganism should be less than 1, 000 CFU/m³ of bacteria. The levels are considered excellent if they are less than 500 CFU/m³ [34, 35].

In Taiwan, the indoor air levels of bacteria should not exceeded 500 CFU/m³ and 1, 000 CFU/m³ for fungi [36, 37].

In India, there are no guidelines for the levels of bacteriological contamination of air [38].

In Singapore, the regulation for indoor air quality imposes an upper limit for the concentration of bacteria of 500 CFU/m³ [39].

The American Conference of Governmental Industrial Hygienists (ACGIH) stipulates that the counts for total bacteria should not exceed 500 CFU/m³[40].

WHO (1988) has set a limitation of fungi counts at 500 CFU/m³ [41].

The Korean Indoor Air Quality Control in Public Use Facilities Act prescribes a total airborne bacterial concentration below 800 CFU/m³ for hospitals, nurseries, public nurseries, and hospitals for the elderly and post partum care centers [42, 43].

In Romania, the reference value for indoor air contamination in a house is equal to 2,500 CFU/m³ and 550 CFU/m³ for fungi. In schools, the level of bacteriological contamination of air should not exceed 1,500 CFU/m³ for bacteria at 37°. The presence of coliform bacteria and hemolytic bacteria are not allowed in air inside education units.

7. Results

7.1. Houses in Bucharest City

The highest value for air contamination with germs at 37°C was equal to 21,466 CFU/m³ in one of the apartments with one room (apartment B).

In the kitchens, the highest value of contamination identified was equal to 12,701 CFU/m³, the minimum value was equal to 201 CFU/m³, and the highest average value was equal 3,200 CFU/m³ (Fig. 1).

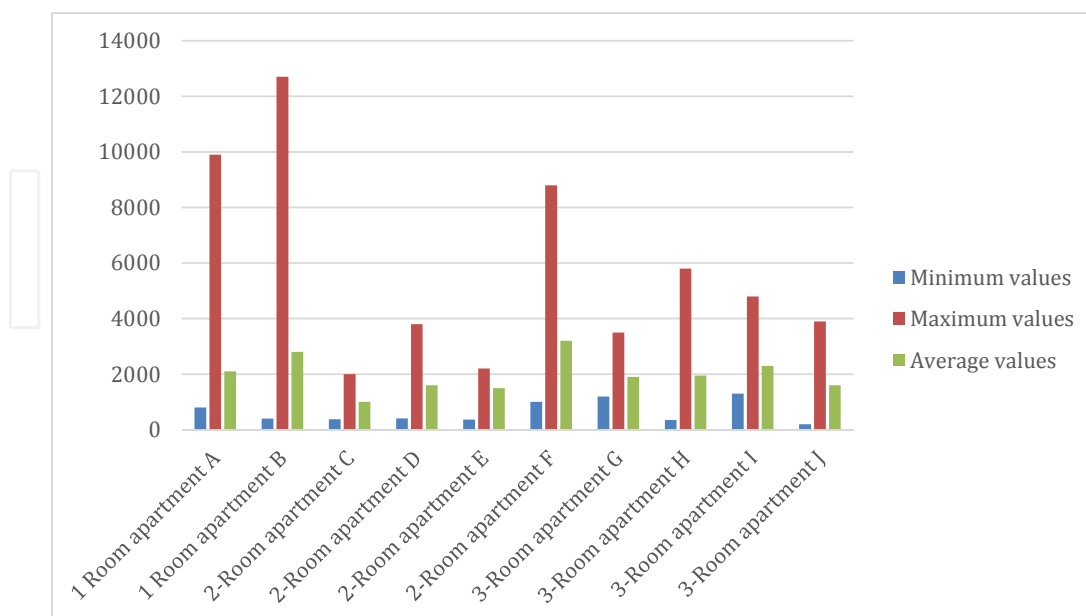


Figure 1. Bacteriological contamination of kitchen air (CFU/m³ at 37°C).

In the living rooms, the highest identified value of contamination was equal to 21, 466 CFU/m³, the minimum value was equal to 210 CFU/m³, and the highest average value was equal 4, 731 CFU/m³ (Fig. 2).

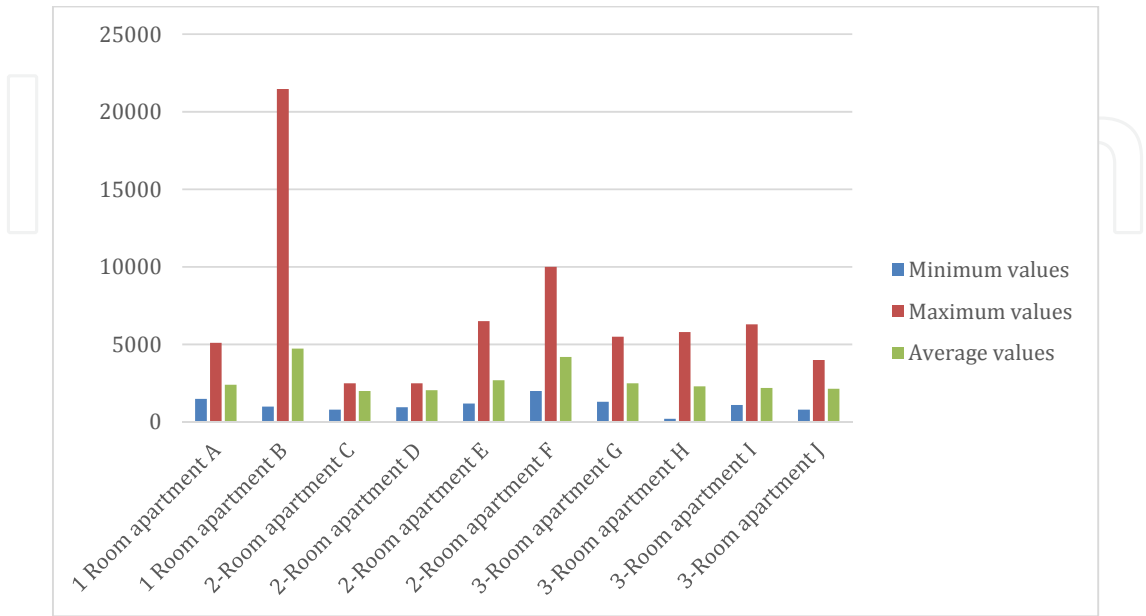


Figure 2. Levels of bacteriological air contamination in living rooms (CFU/m³ at 37°C).

Taking into account the moment of the day, the highest bacteriological contamination (average values) was found in the living room, in the evening, and was equal to 6, 665 CFU/m³ in the one room apartment—apartment B (Fig. 3).

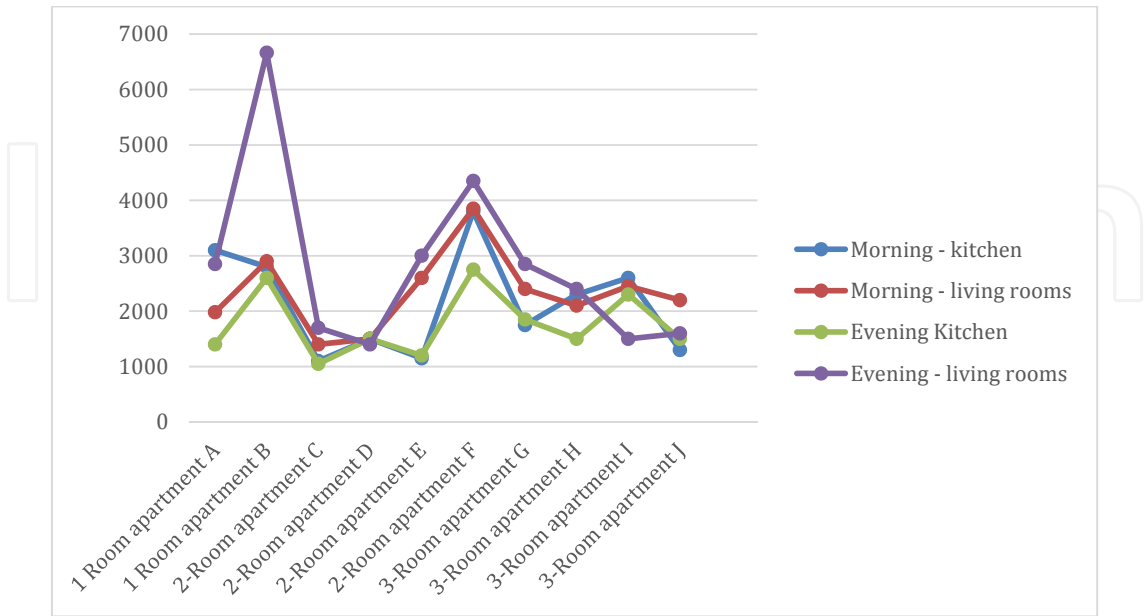


Figure 3. Average numbers of germs at 37°C in the morning and in the evening in investigated apartments.

The indoor air contamination is higher in the fall and in the winter, reaching a level equal to 7, 853 CFU/m³ in one room apartment (average values)—apartment B (Fig. 4).

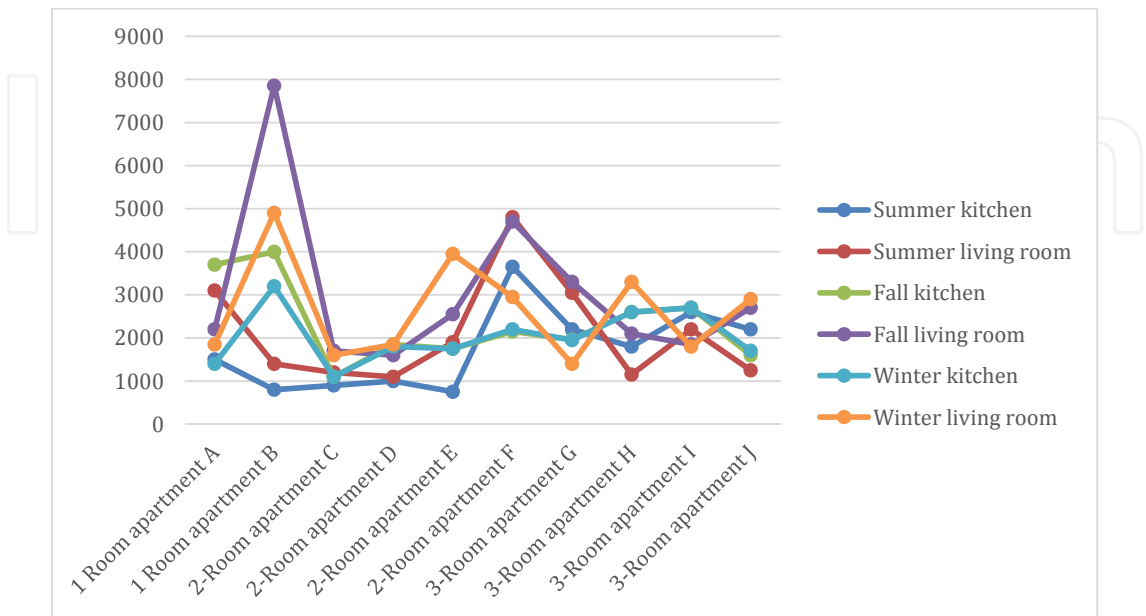


Figure 4. Average numbers of germs at 37°C according to the season in investigated apartments.

In connection to the smoking habit of the inhabitants, apartments with one and two rooms had a higher bacteriological contamination of air (Fig. 5).

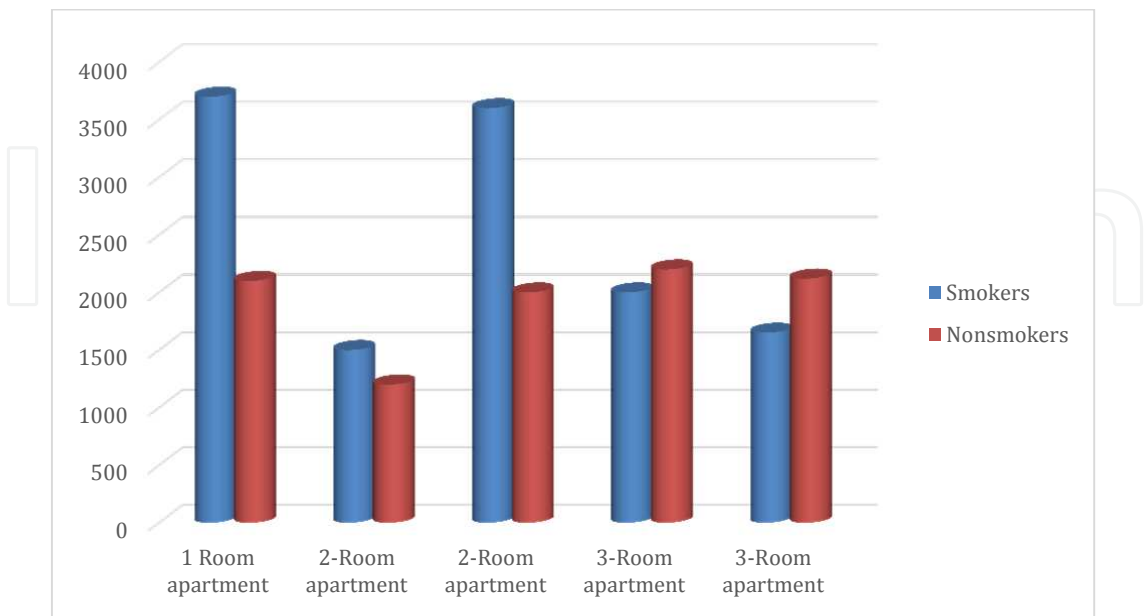


Figure 5. Number of germs at 37°C in houses according to the smoking habit.

In connection to the type of apartment, bacteriological contamination is higher in the apartments with two linked rooms. Bacteriological contamination is also presented in the three-room apartment (Figs. 6 and 7).



Figure 6. Number of germs at 37°C and the type of apartment (apartments with two rooms).

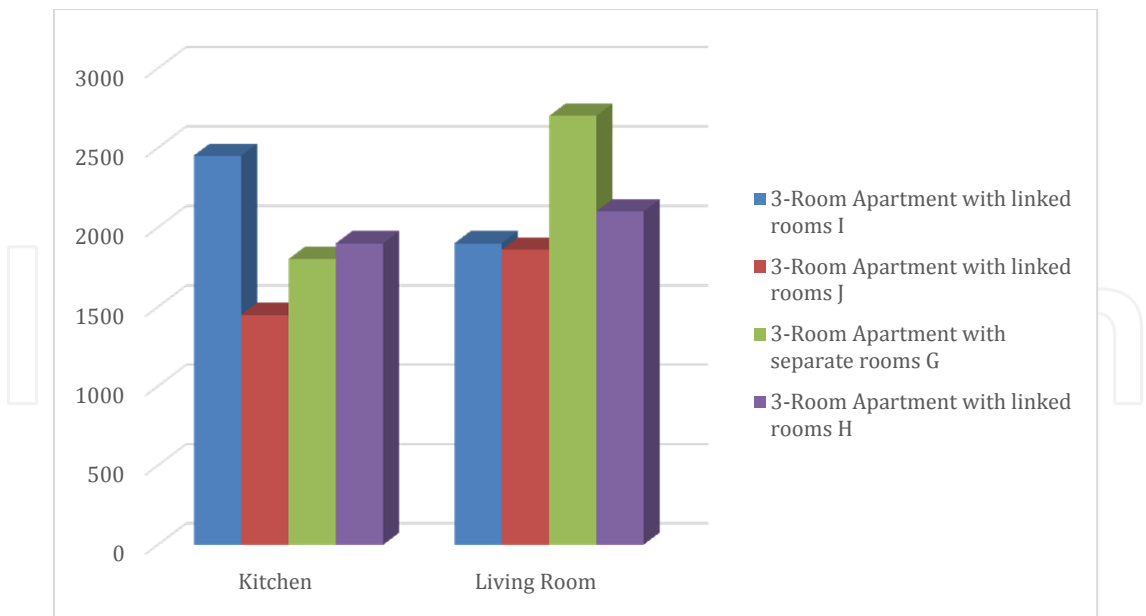


Figure 7. Number of germs at 37°C and the type of apartment (apartments with three rooms).

Contamination of air with staphylococci reached the highest value equal to 7, 988 CFU/m³ in the kitchen and 10, 157 CFU/m³ in the living room (Figs. 8 and 9).

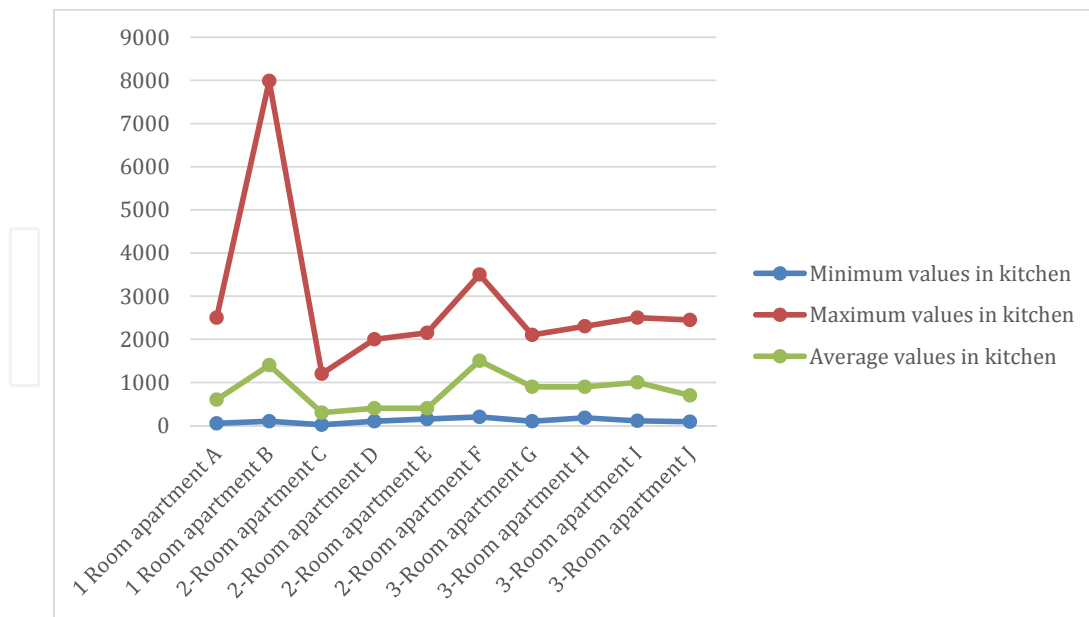


Figure 8. Contamination with staphylococci in kitchen air (CFU/m³).

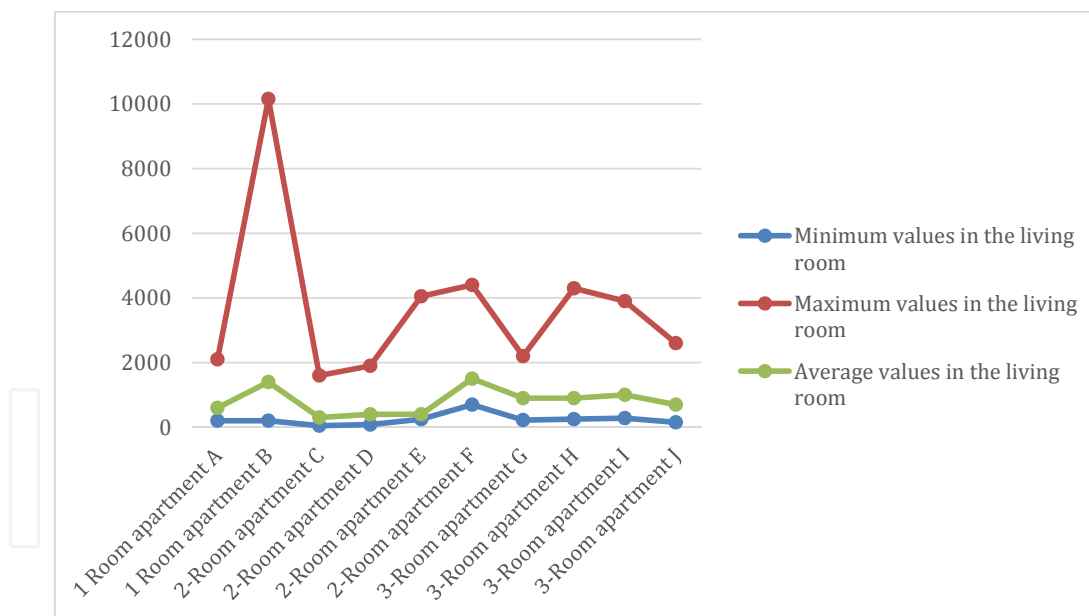


Figure 9. Contamination with staphylococci in living rooms air (CFU/m).

The ratio between staphylococci/germs at 37°C is around 40% in the kitchen and over 50% in the living rooms (Fig. 10).

The assessment of the fungal contamination showed that in many homes, the level of fungal contamination was very high in all investigated rooms. The levels of fungi were between 635 and 10,859 CFU/m³. The highest values measured were 2,802 CFU/m³ in the living room, 2,

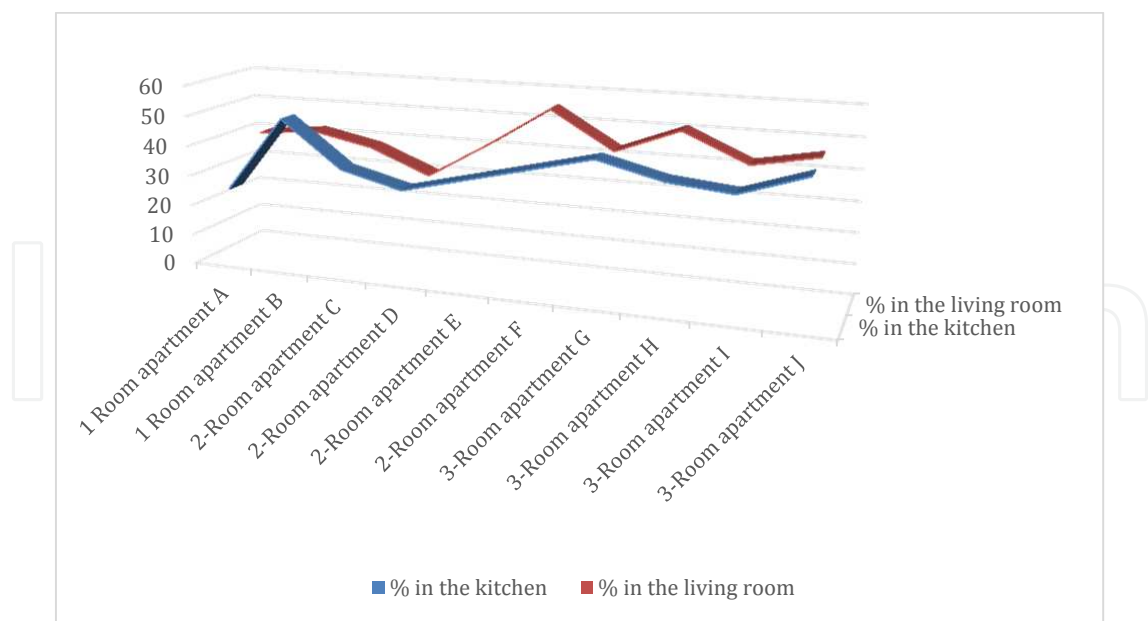


Figure 10. Ratio between staphylococci/germs at 37° in investigated houses.

302 CFU/m³ in the kitchen, 7, 675 CFU/m³ in the bathroom, and 10, 859 CFU/m³ in the bedroom (highest overall). The species of fungi identified were *Penicillium*, *Aspergillus niger*, *Rhizopus*, *Mucor*, *Cladosporium herbarum*, and *Fusarium*.

7.2. Schools

In a school in Bucharest, the following results were obtained: in an empty classroom, before 8:00 a.m., the value identified was 25 CFU/m³; during the school day (at 9.30:00 a.m.) in a classroom with 23 children, the value identified was 67 CFU/m³; in a classroom with 36 children and 1 teacher, the value identified was 229 CFU/m³; in a classroom with 23 children and 1 teacher, the value identified was 140 CFU/m³; in a classroom (at 11:00 a.m.) with 24 children and 1 teacher, the value identified was 1, 465 CFU/m³ with 38 CFU/m³ *Streptococcus* alpha-hemolytic present; in the school library (at 10:00 a.m.) with 3 people inside, the biological contamination of air was equal to 45 CFU/m³; in study room number 1, next to the library with 15 children inside, the level of air contamination was equal to 89 CFU/m³; in study room number 2, next to the library, the room connected to the hall in front of the school library, the level of biological air contamination was equal to 427 CFU/m³; the level of air contamination in the school hall was equal to 180 CFU/m³; in the medical ward, with 8 people inside the room, the level of biological air contamination was equal to 60 CFU/m³, 1 CFU/m³ *Streptococcus* alpha hemolytic, and 1 CFU/m³ *Streptococcus* beta hemolytic present (Fig.11).

In a school in Giurgiu City, the following results were obtained: at 8:00 a.m., a number of 1, 259 CFU/m³ was identified; at 11:00 a.m., a number of 1, 732 CFU/m³ was identified; at 1:00 p.m., a level of biological air contamination equal to 472 CFU/m³ was identified. In the school hall, the level of air contamination was equal to 2, 202 CFU/m³, the level of contamination of the air with *S. non-aureus* was equal to 1, 259 CFU/m³ at 8:00 a.m. and 11:00 a.m., and 472 CFU/m³ at 1:00 p.m.; the level of contamination with *Streptococcus* alpha-hemolytic was equal to 157

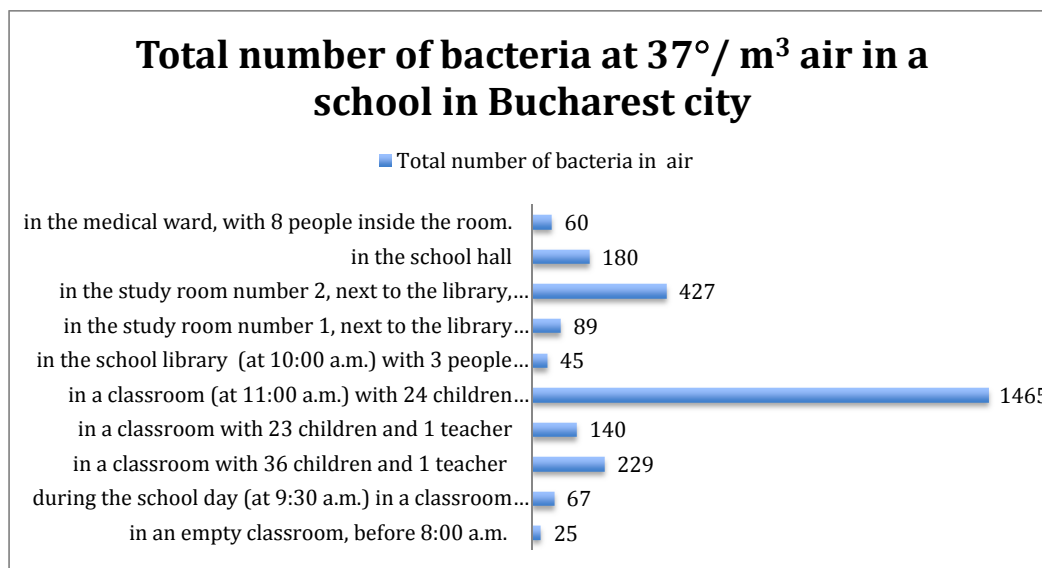


Figure 11. Level of bacteriological contamination of air in a school in Bucharest City.

CFU/m³ at 11:00 a.m. In a classroom and in the school hall, at 1:00 p.m., coliform bacteria were identified as an indicator of enteric contamination of air. The level of contamination with coliform bacteria was equal to 315 CFU/m³. The detected levels of coliform bacteria can be dangerous for children health (Fig.12).

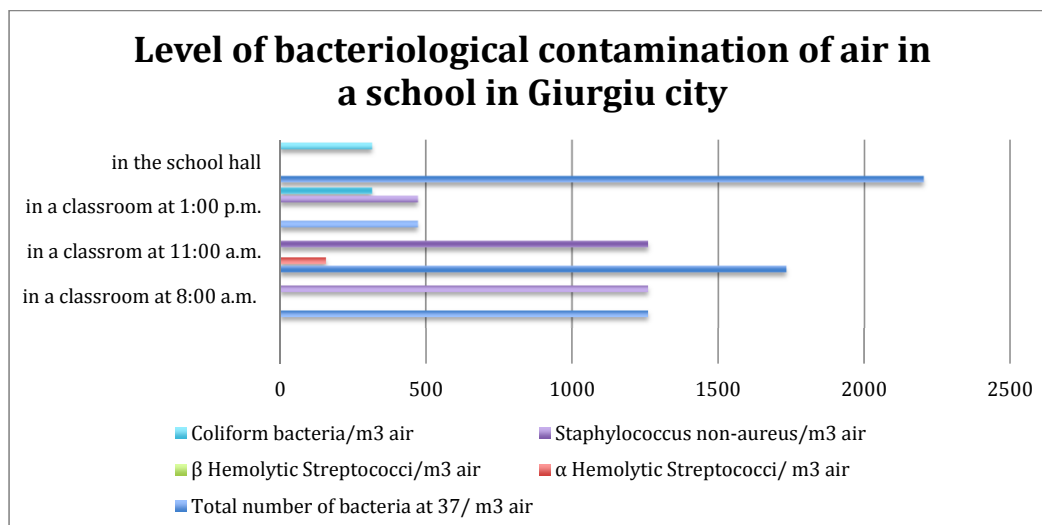


Figure 12. Levels of bacteriological air contamination in a school in Giurgiu City.

7.3. Offices

The levels of fungal contamination were between 734 and 4, 034 CFU/m³. The fungi species identified were *Penicillium* spp., *A. niger*, *A. flavus*, *Aspergillus versicolor*, *Mucor*, and *Cladosporium*.

7.4. Archives

The levels of fungal contamination were between 655 and 1, 490 CFU/m³. By a qualitative assessment of air pollution with fungi, the following types of fungi were identified: *Penicillium* spp., *A. niger*, *A. flavus*, *A. versicolor*, *Mucor*, *Cladosporium*.

7.5. Shops

In a supermarket with ground floor and 4 floors, the levels of bacteriological contamination are shown in Table 1.

Average values		Total number of bacteria at 37°/m ³ air	Total number of staphylococci/m ³ air	Hemolytic staphylococci/m ³ air	<i>Staphylococcus aureus</i> /m ³ air	α Hemolytic streptococci/m ³ air	β Hemolytic streptococci/m ³ air
Ground floor	M	5, 140.75	3, 296	161.25	197.72	328.75	3.24
	E	13, 836.00	10, 397.25	404.5	240.75	964.25	0
First floor	M	3, 919.25	2, 015.5	137.5	170.55	213.66	0
	E	9, 258.75	7, 316	372	232.5	569.33	0
Second floor	M	6, 155.45	4, 276.33	299.75	226.25	182.5	0
	E	10, 916.75	8, 705.5	339	286.25	549.16	0
Third floor	M	6, 946	4, 914.25	280.25	141	273.5	0
	E	13, 509.25	10, 800.5	419.5	316	775.5	0
Fourth floor	M	6, 473.75	4, 292.75	301.25	189.97	228	0
	E	10, 352.25	7, 759.75	405.25	267.78	560	0

Table 1. Levels of bacteriological air contamination in a supermarket in Bucharest City (M = morning, E = evening)

The highest levels of contamination were registered in the evening and were equal to 13, 836 bacteria at 37°/m³ air at the ground floor and 13, 509 bacteria at 37°/m³ air at the third floor. In the second and fourth floors, in the evening, the level of bacteria in air were equal to 10, 917 bacteria at 37°/m³ air and 10, 352 bacteria at 37°/m³ air. In the first floor, in the evening, levels of bacterial contamination equal to 9, 259 bacteria at 37°/m³ air were registered. The levels of air contamination were the lowest in the first floor (Fig. 13).

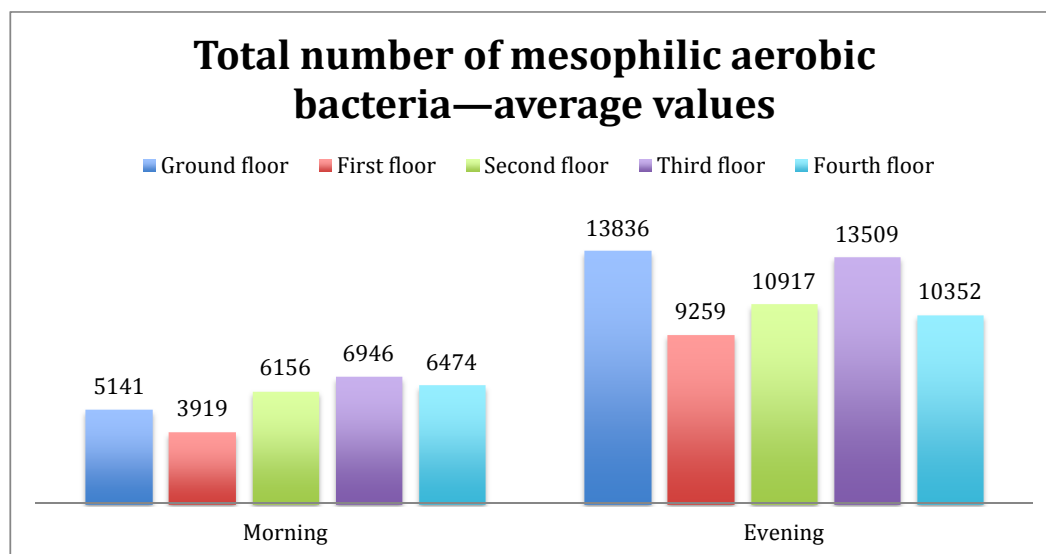


Figure 13. Levels of bacteriological contamination of air in a supermarket—average values.

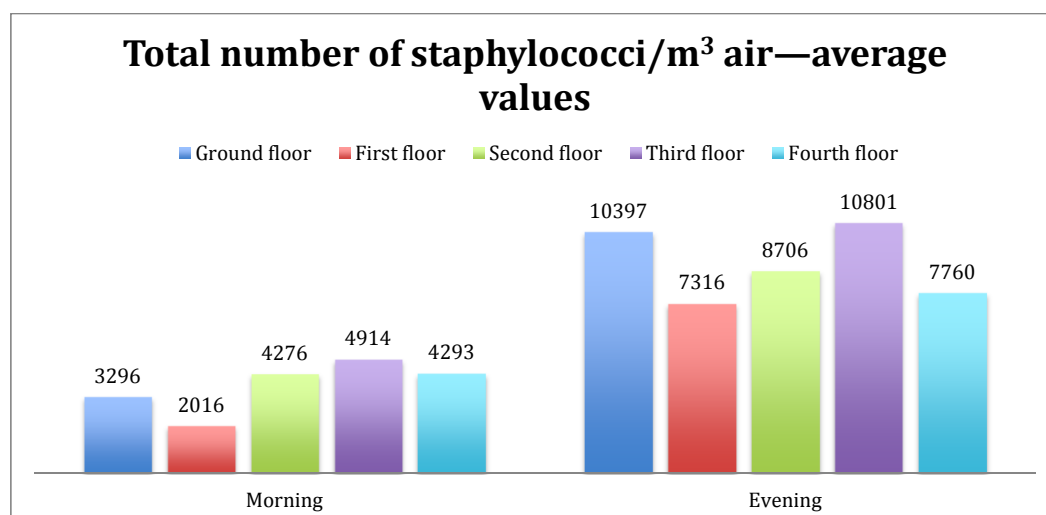


Figure 14. Total number of staphylococci/m³ air—average values.

The contamination of air, in the case of the supermarket with a ground floor and other four floors, with staphylococci was high in the evening. The highest levels were registered in the third floor with a contamination burden equal to 10, 801/m³ air, followed by the ground floor with 10, 397/m³ air, the second floor with 8, 706/m³ air, the fourth floor with 7, 760/m³ air, and the first floor with 7, 316/m³ air. It can be seen that the levels of air contamination were the lowest in the first floor (Fig.14).

Hemolytic staphylococci/m³ air and *S. aureus*/m³ air were identified in the same amount in the evening at all floors.

α Hemolytic streptococci/m³ air were identified in the highest levels in the evening at the ground floor. In the evening, at the other floors, the levels were almost equal.

β Hemolytic streptococci/m³ air were identified at the ground floor only.

In a shop with only a ground floor, the levels of bacteriological contamination are presented in Table 2.

Average values		Total number of bacteria at 37°/m ³ air	Total number of staphylococci/m ³ air	Hemolytic staphylococci/m ³ air	<i>Staphylococcus aureus</i> /m ³ air	α Hemolytic streptococci/m ³ air	β Hemolytic streptococci/m ³ air
Ground floor	M	2, 398.16	1, 323.2	78	47.3	86.5	0
	E	3, 107.5	2, 217.85	90.08	164.76	75.65	0

Table 2. Levels of bacteriological air contamination in a shop with only a ground floor in Bucharest City (M = morning, E = evening)

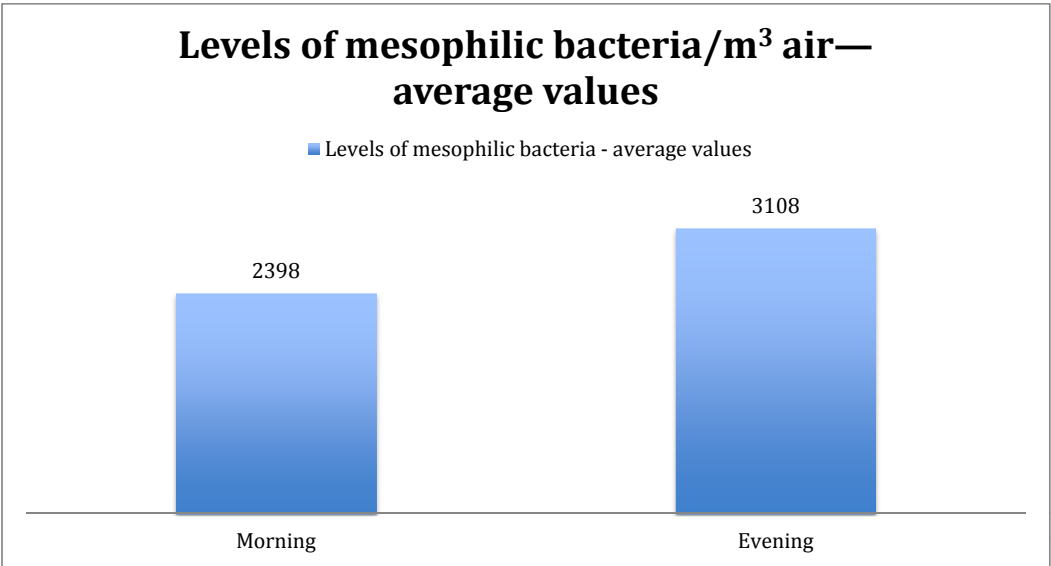


Figure 15. Levels of bacteriological contamination of air in shop with only a ground floor.

In the shop with only a ground floor, the levels of air contamination registered higher values in the evening than in the morning measurements for mesophilic bacteria, total number of staphylococci, hemolytic staphylococci, *S. aureus*, and α hemolytic streptococci (Fig.15).

8. Discussions

In the investigated houses, the highest value for air contamination with germs, at 37°C, was found in one of the apartments with one room (apartment B). Regarding the time of the day,

the highest level of bacteriological contamination was found in the evening, in the one room apartment—apartment B. Taking into account the time of the year, the indoor air contaminations were higher in the fall and winter, especially in the one room apartment—apartment B.

Considering the smoking habit of the inhabitants, apartments with one and two rooms had a higher bacteriological contamination of air where there were smokers present.

Considering the type of the apartments, in apartments with more than one room, bacteriological contamination is at the highest level in the apartment with two linked rooms.

The ratio between staphylococci/germs at 37°C is around 40% in the kitchen and over 50% in the living rooms.

In a previous study [44], the levels of bacteriological and fungal pollution of indoor air were as follows:

In summer, the maximum levels of bacteria were equal to 57, 500 CFU/m³ in the kitchen and bathroom and 32, 500 CFU/m³ in the living room with average values between 6, 090 and 13, 241 CFU/m³.

In winter, the maximum levels of bacteria were equal to 12, 600 CFU/m³ in the kitchen, 50, 400 CFU/m³ in the bathroom, and 6, 300 CFU/m³ in the living room with average values between 2, 406 and 7, 903 CFU/m³.

The highest levels of staphylococci in air were, in the summer, between 26, 125 CFU/m³ in the living room and 55, 000 CFU/m³ in the bathroom with average values between 4, 308 CFU/m³ and 7, 200 CFU/m³. In winter, the maximum values were between 10, 250 CFU/m³ in the kitchen and 55, 000 CFU/m³ in the bathroom, with the average values between 3, 219 and 6, 421 CFU/m³.

The air pollution with fungi was visualized as a layer on the Petri dish in the kitchen and bathroom, and in the living room the maximum level registered was equal to 1, 820 CFU/m³. The fungi species identified were the same like in the present study.

Another study performed in Torun University in Poland [45] had the following results:

- The levels of air contamination with bacteria were between 7 and 3, 313 CFU/m³ as the background and between 53 and 5, 673 CFU/m³ air at different sample site. The amount of bacteria found in the outdoor air ranged from 10 to 310 CFU/m³.
- The air pollution with fungi ranged between 0 and 893 CFU/m³ in the morning and 0 and 1, 373 CFU/m³ in the afternoon. The outdoor fungal background ranged between 217 and 3, 750 CFU/m³.

Another study that investigated the levels of biological contamination of indoor air in eight libraries of the Jimma University in Addis Ababa [46] had the following results:

- The levels of bacteria were between 367 and 2, 595 CFU/m³ with an average value equal to 1, 476. CFU/m³.
- The levels of fungi were between 524 and 1, 992 CFU/m³ with an average value equal to 1, 087 CFU/m³.

- The isolated bacteria were *Micrococcus* sp., *S. aureus*, *Streptococcus pyogenes*, *Bacillus* sp., and *Neisseria* sp.
- The isolated fungi were *Cladosporium* sp., *Alternaria* sp., *Penicillium* sp., and *Aspergillus* sp.

The microbiological quality of air in the investigated rooms in the Bucharest school as well as in the Giurgiu school changed significantly during the school day. The highest levels were found around 11:00 a.m. in classrooms with large numbers of persons inside. Most of the levels identified in the study were under the limit of 1,000 CFU/m³, with the exceptions of one measurement in the school in Bucharest and four measurements in the school in Giurgiu City. Also, the presence of *Streptococcus* beta hemolytic and coliform bacteria in classrooms air represents a threat to the health of children. This threat appears to be greater in the school in Giurgiu City.

The fungi levels identified in offices and archives were over the limit accepted in Romania. Isolated fungi species include *Cladosporium*, *A. flavus*, *A. niger*, and *Penicillium* sp.

In the case of the shop with only a ground floor and the supermarket, it is obvious that the size of the shop, the numbers of floors, and the numbers of departments in the shop can induce a high bacteriological contamination of air due to the high number of people visiting the shops. The air contamination in the supermarket was found to be higher at the ground floor and at the third and fourth floors. The high level of bacteriological contamination at the ground floor is probably due to the large number of visitors, while at the third and fourth floor it is probably due to the accumulation of bacteria coming from the first and second floors. In the shop with only a ground floor, the levels of air contamination in the evening were lower than in the morning. It is probable that this type of shop has better ventilation. High levels of staphylococci and the presence of *Streptococcus* in air can be dangerous for human health, and it is necessary to have a very strong disinfection action.

9. Conclusions

From this study, several conclusions can be drawn:

- a. Biological contamination (bacteriological and fungal contamination) represents a constant element that was identified in all Romanian houses.
- b. All results showed that the levels of bacteria in indoor air are higher than the allowed limit (2,500 CFU/m³ air for bacteria at 37°—limits admitted in Romania for rooms in a house). In schools, the levels of bacteriological contamination of air must not exceed 1,500 CFU/m³ for bacteria at 37°. The presence of coliform bacteria and hemolytic bacteria are not allowed in the air inside education units).
- c. Concerning the indoor air pollution with fungi, the results showed a high level of contamination exceeding the allowed limit in Romania of 550 CFU/m³ for fungi in air.
- d. Biological contamination of air in schools can represent a threat for children health. This situation is obvious especially in schools outside Bucharest.

- e. In work environment like offices or archives, the presence of fungi may represent a health risk for those who are exposed during the work process.
- f. In the shops, a high biological contamination can be identified not only due to the numerous visitors but also by the numerous vendors present inside. The employees can be exposed during the working days to an important bacteriological load.
- g. The hygienic quality of indoor air is revealed by the levels of biological air contaminants (bacteria and fungi).
- h. A poor biological quality of air in all investigated environments was identified in the present study.
- i. A constant exposure to biological contaminants (bacteria and fungi) from air can induce specific pathologies like asthma, allergic diseases, digestive diseases, and other infectious diseases. Among the isolated fungi, there were strongly allergenic and toxic species. The increased level of fungal flora in insufficiently ventilated rooms could be a reason for serious health problems of people occupying and working in those rooms.
- j. All the investigated places require measurement to ensure good ventilation and proper measures of hygiene.

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