Chapter 2

PATHOGENIC MICROORGANISMS AND SITUATIONS OF RISK TO MAN

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2. Introduction

A very topical health problem is that of air pollution in enclosed environments and indoor spaces used in various ways: dwellings, offices, schools, recreation places, sports facilities, libraries, hospitals and other institutions, and vehicles (Bellante De Martiis et al., 1992).

2.1 Indoor Pollution and Effects on Human Health

A very topical health problem is that of air pollution in enclosed environments and indoor spaces used in various ways: dwellings, offices, schools, recreation places, sports facilities, libraries, hospitals and other institutions, and vehicles (Bellante De Martiis et al., 1992).

Indoor pollution becomes a public health problem both because of the duration of the exposure and because of the type and size of the population at risk. As regards exposure time, it should be considered that, in our age, people's main daily activities, both for work and for recreation, are carried out indoors: according to the World Health Organisation, European populations spend 90% of their time indoors. Two studies that were performed on the lifestyle of the inhabitants of two large cities in central Italy revealed that the average daily time spent indoors, during the winter, is as high as 94% and consists chiefly of the time spent at home (64%) (see Fig. 2.1). Some population groups (children, elderly people and sick people) can be regarded as exposed continuously to the risk of indoor pollution. As regards the type of population that is exposed, it is neither homogeneous nor selected, because it includes subjects of various age brackets and in different conditions of health (even sick people and immunodeficient people).

P. Mandrioli et al. (eds.), Cultural Heritage and Aerobiology, 31-43.

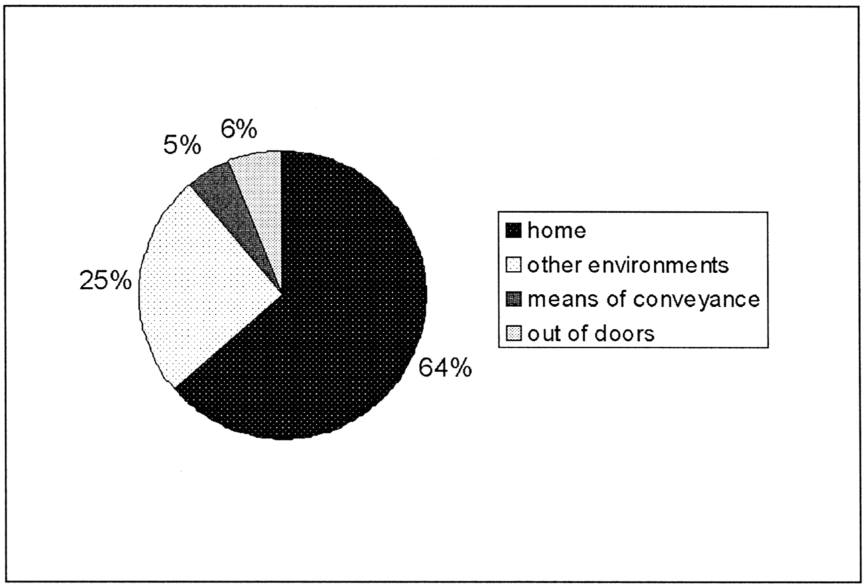

Figure 2.1. Percentage of time spent in various environments by the inhabitants of two cities of Central Italy during the winter.

Indoor air is regarded as polluted, therefore hazardous to human health, in various cases: alteration of microclimatic parameters, consequently of thermohygrometric comfort; quantitative alteration of normal constituents of air, e.g. where there is an increase in expiratory carbon dioxide and in consumption of oxygen; presence of polluting agents of various kinds. The evaluation of indoor air therefore includes both the investigation of physical, chemical and biological pollutants and the measurement of microclimatic physical parameters; other factors that determine the degree of comfort in an indoor environment are the level of lighting and noise. The evaluation of these situations of microclimatic comfort can be summarised by the indexes introduced by Fanger (1967 and 1988): PMV and PPD (based on the data relevant to the perception of the microclimatic physical factors), OLF and Decipol (indexes of sensory and perceptual pollution). The PMV index (Predicted Mean Vote) is the score that an expressly-trained group of people would assign to a certain microclimatic condition. Its evaluation is performed with reference to instrumental microclimatic values that determine a state of comfort. The scale range is from +3 (hot discomfort) to –3 (cold discomfort), with thermal comfort between +0.5 and –0.5. The PPD index (Predicted Percent of thermally Dissatisfied people) is the percentage of people who are dissatisfied, from the point of view of their comfort, with a certain microclimatic situation. In this case, too, the measurement is performed by means of instruments, and thermal comfort is defined by a PPD not exceeding 10% (Fanger, 1967). The OLF index, which defines olfactory pollution, is correlated with the emanations of bioeffluents by a person in standard conditions. The ensuing percentage of dissatisfied people (DP) depends on several parameters, including air ventilation (in inverse proportion) and human concentration (in direct proportion). The Decipol, a term that combines the words "decibel" and "pollution", is a parameter that standardises the ventilation factor and corresponds to the OLF felt with a standard ventilation of 10 litres of air per second. The maximum discomfort value (DP = 100%) is obtained with a 31.3 Decipol, equal to a 31.3 OLF with a ventilation of 10 litres of air per second. These indexes, too, can be calculated on the basis of instrumental measurements (Fanger, 1988). The temperature and humidity factors are mutually correlated for the achievement of a state of environmental comfort, and are, in their turn, affected by ventilation. The latter is important both for the state of comfort, and, if it is in relation to the change of air, for the dilution of any pollutants present in the indoor air. The temperature and humidity, moreover, are correlated with the development of bacterial and fungal species that lead to infective and allergic symptoms. During the last few years, in order to save energy, buildings have been sealed (by using highly insulating materials, eliminating drafts, locking the windows, etc.); the natural change of air has been reduced; and there has been an increase in the use of the re-circulation of indoor air in air conditioning systems. All this has resulted in a deterioration of the quality of indoor air. As regards the quality of indoor air, each individual is involved both actively (because he/she is a source of pollution) and passively (because he/she undergoes the action of various types of pollutant agents – physical, chemical and biological – coming from all sorts of widespread, not easily controlled sources: e.g. air conditioning systems, building materials, furniture, cleaning products, deodorants). A particular type of harmfulness, frequently present in workplaces, may be constituted by negative psychological factors that the user of the space associates with the physical environment, but that may actually result from other conditions (e.g. dissatisfaction with one's job, stressful situations, conflictual relationships with other people). From the point of view of their origin, sources of indoor pollution can be divided into two groups: external and internal. External sources include the ground, movable sources (e.g.. vehicular traffic) and fixed sources (e.g. industrial plants and thermal power stations). Internal sources include building materials, furniture, equipment, occupiers and human activities. Figure 2.2 shows the harmful factors in indoor environments. The percentage of pollutant substances coming from outside depends on the atmospheric pollution and on the degree of insulation of the building. In enclosed environments, the pollutants coming from outside are added to the internal ones and produce mixtures formed by a considerable variety of physical, chemical and biological pollutants that may work in synergism and cause hard-to-evaluate health damages. As regards

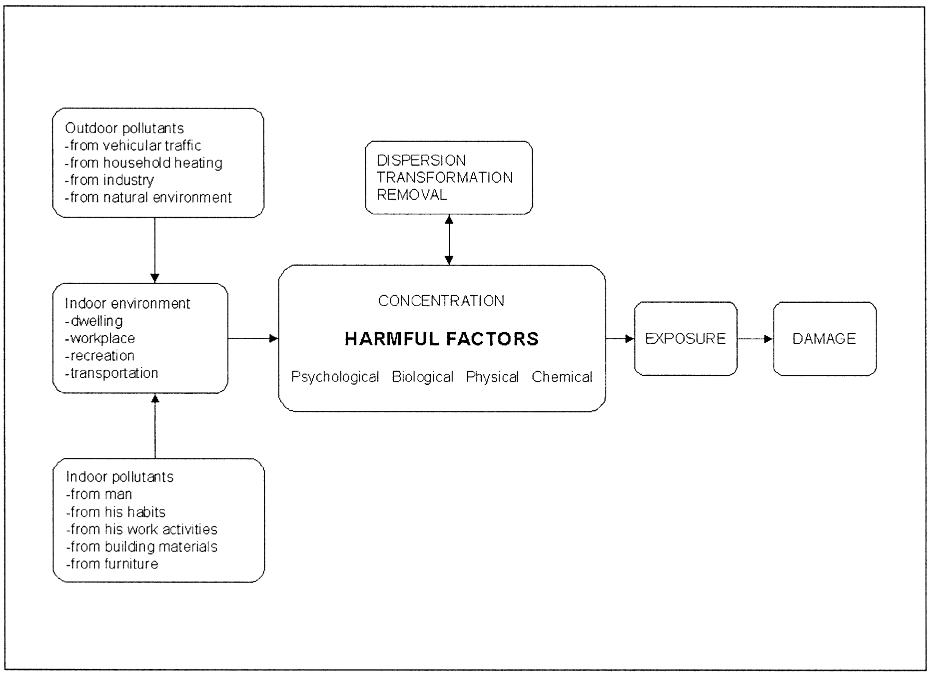

Figure 2.2. Harmful factors in indoor environments.

the effects of pollutants on health, considering their great variability and the difficulty of defining a specific action for many of them, it is possible to remark that the damage due to indoor pollution for the occupants of a building may show itself with a sensory feeling of discomfort or with a group of immediate, short-, medium- or long-term symptoms (see Table 2.1). The effects of pollution on human health include some diseases for which it is possible to identify a specific aetiological factor, and non-specific diseases for which it is not possible to demonstrate a causal relationship with a certain pollutant, also because the damages to human health are increased by the sum and synergism of the various pollutants. Among the short- and medium-term effects, two syndromes stand out: building related illness (BRI) and sick building syndrome (SBS). BRI denotes diseases determined by clearly identifiable causal factors that affect a very limited number of occupants of a certain building and may be correlated with the presence of a specific pollutant. Bioaerosol plays an important role in the aetiology of BRI forms, by means of three mechanisms: infective, allergic and toxic. SBS is a syndrome with a non-specific aetiology that affects at least 20% of the occupants of a building that is therefore called "sick".

This disease includes symptoms with different characteristics: general symptoms (drowsiness, headaches, fatigue, difficulty in concentrating); and symptoms related to the upper respiratory tract (nose congestion, parched throat), to the eyes (smarting, dryness of mucous membrane), and to the skin (dryness, erythema). The symptoms have a typical evolution: they appear at the beginning of the week, increase during the week and disappear when the individual is absent from the "sick" building.

Table 2.1. Indoor pollution and effects on health.

EFFECTS ON HEALTH	CAUSAL AND CONCAUSAL AGENTS			
	psychological	biological	physical	chemical
IMMEDIATE				
headache			•	•
irritation of eye/upper respiratory tract			•	•
nausea/discomfort			o	o
allergy		•		•
mental confusion/irritation	o			•
SHORT-TERM				
airborne infections		•	o	
B.R.I.:				
- legionnaires' disease/Pontiac's fever		•	o	
MEDIUM-TERM				
B.R.I. :				
- humidifier fever		•	o	o
- allergic alveolitis		•	o	o
asthma		•		•
S.B.S.	•	•	•	•
LONG-TERM				
chronic bronchopneumopathy		•	o	•
mutagenic and carcinogenic activity			•	•

Legend: • causes o concauses ; Source: Bellante et al., 1992 (modified)

They are more frequent in females, in people with a higher education, in people who are more senior in service and in people who are not fulfilled by their work (economic dissatisfaction, thankless tasks). A great number of investigations of sick buildings have led to the identification of several factors that come into play in the determination of SBS. They include organisational factors, adjustment to the job, microclimatic factors, ventilation factors or incorrect management of ventilation, materials such as paint or carpeting, cleaning products, furniture, artificial lighting and noise.

The SBS risk can be reduced by means of an adequate ventilation or by a correct management of the air conditioning systems (use of appropriate filters for the elimination of suspended particulate matter, cleaning of ducts, etc.). As regards the influence of ventilation on the quality of air and, consequently, on the state of comfort of the inhabitants, the ASHRAE (American Society of Heating, Refrigerating and Air-conditioning Engineers) has defined a minimum rate of ventilation, equal to 7.5 litres per second per person in general indoor environments, and to 10 litres per second per person in office environments.

The evaluation of risk in enclosed environments is particularly important, because indoor pollution reduces the state of comfort and may be the cause of disorders and diseases. For an estimate of the risk, it is necessary to possess some data: identification of harmful pollutants, description of human exposure and analysis of the dose-response relationship. In any case, it is necessary to consider the fact that the specific dose-response relationships are not known for all pollutants and that it is difficult to evaluate the exposure to an individual pollutant, because each pollutant in an indoor environment is a part of a mixture of compounds. Moreover, there are other factors, such as the psychological ones, that are not easy to measure.

At present, for an estimate of indoor risk, data obtained by investigations into groups of exposed workers and by experimental studies on animals and *in vitro* are used. As regards the exposure of workers in industrial environments, there exists a list of limit levels for short- or long-term exposure, and of maximum acceptable concentrations, which is updated every year by the American Conference of Governmental Industrial Hygienists (ACGIH). These levels cannot be applied to the general population, because it is more composite in its age and health conditions.

The duration and mode of exposure to pollutant agents are also different between the two groups that are considered. These workers are usually exposed to a single pollutant in high concentrations and for limited periods of time (8 hours a day, totalling 40 hours a week), whereas the general population is exposed to mixtures of pollutants in low concentrations and for unlimited periods of time. Moreover, when estimating the risk for the general population, as well as for workers, it is necessary to allow both for indoor and for outdoor exposure, because a person, during a day, spends his/her time partly indoors and partly out of doors. The extrapolation of the results obtained by studies on animals also gives rise to some problems.

There are anatomical differences between the respiratory systems of animals and those of human beings, therefore there are also differences in the absorption of pollutants.

In experimental conditions, an animal is usually exposed to high doses of an individual pollutant, whereas in normal conditions the population is exposed to mixtures of pollutants in low concentrations. In order to achieve an estimate that is consistent with indoor reality, and to develop guidelines, it is necessary to use data obtained by studies performed on the general population. Some nations and institutions – also because there does not exist a comprehensive scientific literature on this subject – have proposed limit values for some indoor pollutants such as formaldehyde (1 mg/m^3), radon (200 Bq/m^3) and carbon dioxide (1,800 mg/m^3).

2.2 Biological Pollution and Risk Situations

Indoor air contains a great number and variety of biological agents that can alter the health condition of the inhabitants. They may work as infecting agents, as sensitising or aeroallergenic antigens, and as toxic agents. Table 2.2 shows the main sources and vehicles of biological pollutants.

Table 2.2. Main sources and vehicles of biological pollutants

	man and animals	building materials	hydraulic systems	humidifiers conditioners	cooling towers	domestic dust	outdoor air	furniture plants
actinomycetes thermophile bb. sporogenic bb.	•	•	•	•••	••			•
bacteria endotoxins viruses	••	•	•	••	••			•
mycetes mycotoxins		••	•	•••	•	•	••	•
pollen						••	••••	•
protozoa	(•)		•	•••	•			•
acarina	(•)	••				••••		••
dandruff excreta skin flakes saliva proteins	••					•••		•

Legend: • correlation ; Source: Bellante et al., 1992 (modified)

Biopollutants are present in the form of nuclei of droplets, as droplets and as particulate matter; they contain viruses, bacteria, *Actinomycetes*, fungal spores, fragments and excrements of *Arthropoda*, animal and human residues (dandruff, skin flakes, hairs, etc.), and pollen. For the evaluation of the biological pollution of indoor air, it is very important to consider the indoor/outdoor ratio (I/O) relevant to the concentration of biopollutants. If the pollution is due to bacteria and viruses, this ratio usually exceeds 1, because most of these microorganisms are emitted by human beings when speaking, coughing or sneezing, and through the elimination of tiny skin flakes with microorganisms sticking to them. As regards fungal spores, the ratio is lower than 1, because most of them come from the outdoor environment; in poor hygienic conditions, however, or if the walls are damp or there is stagnant water, the indoor concentration of fungal spores may increase. As regards other biopollutants relevant to indoor pollution, mites live only indoors and are the biological agents that at present are most involved in the appearance of allergies. Sources of pollen, on the contrary, are chiefly or exclusively out of doors. Allergic symptoms may appear with gradual severity, ranging from a mere inflammation of the eyes and upper respiratory tract (rhinitis, sinusitis) to much more severe syndromes such as allergic alveolitis and bronchial asthma. In indoor environments, allergic symptoms are triggered by the presence of mites, skin by-products from household animals, and fungal hyphae and spores, whereas the risk connected with the presence of pollen is limited, because the concentration of pollen is lower than out of doors. Mites present in household dust are ubiquitous and include a great number of species. The genera *Dermatophagoides* and *Euroglyphus* are important for their allergenic action. These mites develop in conditions of high relative humidity (60% to 80%), feed on skin by-products and are therefore present in great quantities in blankets, mattresses, upholstered chairs, sofas, rugs and wall-to-wall carpeting. Two types of allergens are produced by mites: one is relevant to their excrements and eggs (group I) and one to their bodies (group II). As regards the evaluation of the allergic risk due to *Dermatophagoides* mites, studies performed on populations of students have revealed that the risk threshold for the development of sensitisation is equal to 2 mg/g of group I allergen dust (equivalent to 100 mites/g of dust), while an acute asthma attack in sensitised individuals is caused by a concentration of group I allergens of 10 mg/g of dust, i.e. 500 mites/g. The fungal species present in nature are numerous and ubiquitous. Their development is promoted by conditions of high relative humidity, and air temperature between 18°C and 32°C; for this reason, they are very abundant in damp buildings and in air conditioning systems. The fungi that most often cause allergic symptoms belong to the genera *Alternaria*, *Cladosporium*, *Aspergillus*, *Candida*, *Penicillium*, *Mucor*, *Fusarium* and *Rhizopus*. *Alternaria* is present chiefly out of doors, while *Aspergillus*, *Penicillium*, *Mucor* and *Rhizopus* prevail in damp indoor environments. Fungal spores cause allergic skin and respiratory symptoms that may appear continuously or with a seasonal rhythm. With respect to the toxic effects caused by biological agents, indoor air may contain fungi that produce mycotoxins (*Fusarium*, *Aspergillus*, *Trichoderma*). Some mycotoxins, in low concentrations, cause gastro-intestinal disorders, damages to the haemopoietic and genital system, and non-specific symptoms (asthenia, nausea) similar to those connected with SBS. As regards the infective risk, it is expedient to point out, once more, that indoor air may be a vehicle of contagion for diseases of the respiratory tract, and, more generally, a vehicle of airborne diseases.

Outdoor air contains many types of microorganisms; but they are in low concentrations and undergo self-purification mechanisms (desiccation, action of ultraviolet rays and of oxygen, washing of the air by rainwater). In enclosed spaces, the concentration of germs is considerably higher than out of doors, both because of the absence of natural purification mechanisms and because of overcrowding. Moreover, in these environments there may exist microclimatic situations (the best conditions of moisture and temperature) that promote the development of fungi, bacteria, protozoa and acari. In particular, humidifiers, evaporators, incorrectly-operating air conditioning systems, in other words any situation that allows water to stagnate, are an excellent *pabulum* for the growth of microorganisms. Microorganisms may penetrate in buildings via air conditioning systems either because they are carried by the dust and debris of building materials, or because they proliferate within the ducts (which may be an excellent breeding ground, because they offer a protected space, adequate temperature and humidity, and dirt for nourishment). In addition, microbes and spores, being small and lightweight, are carried by the flow of air that is conveyed into the building. If an air conditioning system recirculates air, polluting agents may be spread to all the rooms that are served by the same system. The microorganisms present in the air are incorporated in solid particles (dust of mineral, vegetal or animal origin, or resulting from the desiccation of saliva and secretions), or in liquid particles (Flügge's droplets), which protect them from environmental stress, allowing them to survive for a long time. The pathogenic microorganisms present in the air reach human beings chiefly by inhalation, and secondly by contact or ingestion, causing damages at several levels. The respiratory system may be regarded, in a way, as an "air sampler" where the particles are captured by sedimentation, depending on their size; their shape and chemical composition are important as well. The greater the diameter of a particle, the smaller the damage: the larger particles (diameter 3 μm to 30 μm) remain within the upper tract of the respiratory system and are subsequently eliminated with expectorated matter. The particles with a diameter of 0.5 μm to 3 μm cause the most severe respiratory damage, because they can reach the pulmonary alveoli. Particles that are even smaller are not deposited, and are eliminated with expiration.

The infective risk relevant to the inhalation of microbial aerosol is connected both with factors specific to the microorganism and with factors specific to the host. Factors specific to the microorganism are pathogenicity, virulence and bacterial load. Pathogenicity is the intrinsic ability of a microbial species to cause an infective disease in a certain animal species; virulence is the degree of pathogenicity that a certain strain of a pathogenic microbial species develops towards the animal species that hosts it (for instance, there exist some strains of the diphtherial bacillus that are more virulent to man than others). It is clear that, if the virulence is the same, the bacterial load has a considerable importance: the higher it is, the greater the likelihood that it will give rise to a state of infection and disease. This passage may take place as a result either of a massive microbial load or of repeated penetrations of a small amount of germs at short intervals. The overcrowding of enclosed spaces promotes the diffusion of infection, because the greater concentration of sources of infection in the environment results in a greater exposure to contagion. The repeated penetration of small doses of microorganisms at long intervals, on the contrary, promotes the establishment of a state of acquired immunity. As regards the host, we should consider some intrinsic factors such as age, sex, constitution and race, and some contingent factors such as lifestyle, working conditions, and state of nutrition and health. Individuals belonging to extreme age groups (early infancy and old age) are more sensitive to infections. Other subjects who are more sensitive are those who suffer from immunodepression, both congenital and acquired, and from crippling chronic diseases. Certain habits, such as smoking and excessive intake of alcohol, and conditions of malnutrition, cause the human organism to become more prone to infective diseases. For an evaluation of the biological risk of indoor environments, it is important both to measure the total bacterial load and to identify the bacterial and fungal species present in the indoor air.

The total bacterial load is an indication of a general contamination of the environment under consideration, while the identification of the species that are present gives information about the existence of specific biological risks, making it possible to identify particular sources of emission and to remove them. As a rule, in order to evaluate the quality of indoor air, a comparison is made between the data obtained by environmental monitoring and the values recommended for a hygienically acceptable environment. This criterion is valid in the case of substances for which it is possible to determine exposure limits below which no effects harmful to human health appear. It is not valid for carcinogenic substances, for which there does not exist a null-effect dose but there is only a mechanism of accumulation even in low concentrations. As regards sensitising substances, the immune response of a single individual is not always proportional to the dose that has been taken.

For the infective risk, it is difficult to determine maximum levels of exposure, because, as we have already pointed out, the appearance of a pathological state is connected both with the infecting ability of the microorganisms and with the host's defence mechanisms, which are conditioned by many physiological situations (e.g. pregnancy) and pathological ones (e.g. diabetes or AIDS). For this reason, no concentration limits for biological agents have been adopted officially for indoor environments. Some institutions have proposed some limits: e.g. the OSHA (Occupational Safety and Health Administration) of the United States regards as a limit the values of 750 CFU (Colonies Forming Units) per cubic metre for the total bacterial load, 150 CFU per cubic metre for microfungi, and no allergen.

2.3 Studies on the Quality of Air in Libraries and Archives

During the last few years, several studies were carried out for the evaluation of air in libraries and archives (Gallo, 1993; Riala et al., 1993; Bellante De Martiis et al., 1996; Micali et al., 1996). Some of these researches were focused on books and documents as objects to be preserved from the risk of deterioration due to physical, chemical and/or biological agents present in them and in the air. Other studies evaluated the health risk resulting from the librarians' and users' stay in the library environment or from the use of paper materials (Tarsitani et al., 1996; Vaillant Callol, 1996).

The collection, by means of questionnaires, of data about the perception of a situation of discomfort and about any disorders correlated with a stay in the library revealed the existence of symptoms comparable to those of SBS.

Some investigations based on the detection of physical and chemical parameters found situations of objective discomfort (high temperature, excessive humidity, poor ventilation) and the presence of chemical pollutants (formaldehyde, volatile organic compounds, carbon dioxide); all this is a health risk, particularly for the staff that spends much time in these environments (Berglund et al., 1990). As regards the detection of biological agents, most investigations were focused on the search for bacteria and microfungi in the air and/or on the paper material. Studies revealed that among microfungi the genera that were most frequently identified were *Aspergillus*, *Mucor*, *Alternaria* and *Penicillium* (Kowalik, 1987), while among bacteria the most frequently identified were the genera *Bacillus*, *Staphylococcus*, *Micrococcus* and *Pseudomonas* (Gallo et al., 1976-77; Brook and Brook, 1994). Microfungi are usually of environmental origin, and their detection is important particularly from the point of view of their possible deteriorating effect on books and documents; but one should not overlook the danger to health they represent in relation to their bioallergenic nature. The discovery of bacteria, on the other hand, may point both to an environmental contamination (because of the presence of microorganisms that use the environment as a reservoir) and to contamination by man (because of the presence of microorganisms that use man as their habitat).

As regards the presence of the latter type of microorganisms, obviously much attention is given to the potential risk of contagion due to a promiscuous handling of the documents. In this regard, a study carried out by our research group in a university library confirmed that frequently-consulted books are a greater risk for the reader than rarely-consulted ones: on the surface of the latter there were almost exclusively yeasts and microfungi of environmental origin, while the former contained microorganisms of human origin. This aspect deserves to be further investigated by researchers, because epidemiologists focus their attention on the risk for librarians and archivists, neglecting the problem of the contamination of the readers. Both bacteria and fungi, as has been previously stated, may be the cause of the deterioration of paper material and of the appearance of allergies and/or infections in man. A correct management of a library is efficient from both these points of view: a good microclimate, besides being comfortable for human beings, does not allow the proliferation of potentially harmful microorganisms. Keeping the rooms clean, taking care of the books and documents, dusting them periodically, carrying out disinfection and disinfestation operations, in short all prophylactic activities are the instrument for protecting the precious content of libraries and archives, and the health of people who consult them or work in them.

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http://www.springer.com/978-1-4020-1622-6

Cultural Heritage and Aerobiology
Methods and Measurement Techniques for
Biodeterioration Monitoring
Mandrioli, P.; Caneva, G.; Sabbioni, C. (Eds.)
2003, XIV, 243 p. 56 illus., 8 illus. in color., Hardcover
ISBN: 978-1-4020-1622-6