ABSTRACT

Early detection of respiratory diseases in humans and animal segment, determining the prevalence of unknown risk factors or neglected ones until recently and the establishment of a community health professional or a target group were based on determining the existence of unexplored associations till now. Individual testing is first time showing the influence of fungal pollution in working and living spaces, adding for the investigations results a predictive value for respiratory allergic and non-allergic diseases at humans and animals. Although allergic sensitization is not a disease itself, it' s causes deserve to be analyzed and decoded because of its association with asthma and other diseases, many of them quite serious. Unique environmental microclimate conditions from the indoor spaces makes it possible for fungal development, their predominance in the inner atmosphere can be attributed to their capability to grow in almost any conditions. Among fungal micro-habitate from a living space are: waste storage containers, food storage areas (including freezer), vinyl wallpaper, decorative plants, books (anything containing cellulose), windows and close to them , bathroom, air-conditioning and office equipments, etc.. Observations valid for a microclimate can not be automatically extrapolated to the other microclimate, due to existence of large variations among the factors that contributes to the stimulation or maintenance of indoor fungal development. Fungal diversity in work or living spaces has been much less studied than that outside due to the existence of technical impediments. In part, we talk about the difficulties in choosing the location to study and free access to them especially when it comes to private homes or offices of the commercial companies. Health diagnosing and accounting issues that may arise consecutive indoor fungal exposure may be of great use in assessing the cause-effect relationship. Non-allergic effects of fungi on humans and animals are very difficult to prove by direct evidence that's why any clue has been detected was excessively explored. Central units of computers are equipped with a ventilation system for cooling components. Ventilation, even at low levels, contributes to the removal of moisture from a space. Inside equipment fitted with ventilation systems can be a "database" that includes the most relevant information about "resident fungi", in a given area at a time. Because fungal adhesion mechanisms (matrix

glycoprotein) in computer components (motherboard, cooler-fan, etc.) particle crossing through the ventilation system due to electrical loading of metal or celluloid surfaces is being facilitated their static sedimentation as accession followed by overheating computers, leading to lower relative humidity especially in the "personal risk area" of users with negative health impact.

Electrostatic charging is a physical process by which the solids (eg carcasses and parts of electrical appliances) appear charge, due to mechanical actions such as air friction, vibration, proximity of metal wires crossed by electrical currents, etc. Typical problems caused by static charging are the attraction, adhesion and storage of dust so by default are the fungal spores and fragments. All this will be followed by constant air dispersion of fungal particulate and so pollution is kept at levels that are at least warring for human and animal health. We intend to demonstrate the existence of "personal areas of risk" for personal computer users and that the central computer unit can be considered a "database" that can accumulates and shows the diversity of fungal species and genera existing at a time in each area considered in the experimental conditions imposed. Major goal of research was to find a direct connection between the degree of impairment of health and fungal pollution in the studied spaces, between the viable airborne fungal elements and inhaled ones, per unit of time by assessing the quantity, quality and distribution of fungal species identified. Also we will recommend values for "safe" limit's concerning fungal pollution in work and living spaces. We wish that through this research of the clinically healthy individuals, young and with immunological competence and by a comprehensive multiphase screening, to "fill a gap" for understanding of the beginning and evolution of allergic and non-allergic respiratory diseases. The thesis consists of two distinct parts. "The current state of knowledge" first part is drawn over 3 chapters comprising 52 pages. In the **first chapter** are briefly presented a number of concepts on the fungal biology, morphology and physiology with a short classification, being reviewed the main features that are relevant for the aerobiological research conducted. **Chapter II** presented a number of elements of both clinical significance and factors that may influence the presence of certain fungal species and genera in work or living spaces, focusing on ecological factors on inter-conditionality between microclimate and fungal dynamics. Are presented mechanisms underlying air dispersion and germination of fungal spores and fragments in indoor environments. In the second part of this chapter are shown a series of statistics, epidemiology of allergic and respiratory disorders and distribution of some fungal species in both hospital and in working and living spaces and are also presented difficulties faced by clinicians in diagnosing fungal infections. Also still in this chapter are presented in the light of scientific latest concepts and assumptions in aeromicology, studies designed to facilitate understanding of the occurrence

of allergies in relation to fungal environmental biology and activities conducted in work and living spaces. Are presented in **chapter three** fungal pathogenic mechanisms known to date and a number of particularities for allergic respiratory disease that develops both in humans and animals. **Part 2** of "own research" is written over 6 chapters covering 173 pages. In **Chapter IV**, entitled "The aim and research objectives" are presented in detail the main directions. Assessment of quantity, quality and fungal genera and species distribution in work and living spaces, proving the existence of "personal risk zone " for personal computer users and that the central computer unit can be considered a "**database**" which accumulates and shows the diversity of fungal species and genera present at one point in space are just some of the proposed objectives. Using for the first time in Romania an intranasal air sampler, evaluation of two new types of questionnaires and assessing the health status of participants in this study, humans and animals, using serology and skin tests were the means by which we have proposed to complete the objectives set out above. Evaluation of a new fluorochrome for direct examination of clinical specimens, obtaining fungal culture extracts for assessing skin reactivity in allergy tests in humans and animals, observing the hemolytic and acute toxicity effect of these extracts on the earthworm - *Lumbricus Terestris* and demonstrate the existence of a new fungal pathogenicity factor are part of the mycological corollary diagnostic methods and techniques that have served to achieve all these objectives. In **Chapter V** it was intended to achieve four main objectives, assessment of quantity, quality and distribution of fungal genera and species identified. Were monitored all sources of ventilation and identified the fungal genera and species being presented the sampling techniques used for each urban area and for all examined spaces. We isolated and identified major fungal genera and species of work and living spaces, by taking samples at different distances from the source of ventilation units of computing power but also from other equipment with similar corporate. They are presented here techniques used for sampling and basics criteria for fungal identification. Were taken 433 samples by applying several techniques. We sought to cover many destination buildings but the choice of participants in the study was based on a number of criteria, such as the smallest age, clinically healthy, using the personal computer minimum of 6 hours daily and having a pet.

Samples were collected from nine urban areas and spaces of Pneumology Hospital, using sampling techniques adequate to the nature of surfaces and analyzed substrates. In areas where pets were present, sampling technique was tailored for the study. By gravitational sedimentation technique for fungal identification at genus and species were collected about 179 samples and examined over 1800 slides from cultures. 189 samples were collected with swab sampler from the ventilation grid of personal computers being examined over 2000 slides from

cultures. Were collected around 32 samples by brushing and clipping 0.5 g. of hair of the animal's body surface and we examined over 350 slides from cultures . We collected 28 bulk fragments, in particular pieces of furniture and pages of books and we examined more than 100 slides from fungal cultures. Between 20. 11. 2007 - 1. 06. 2008 we collected about 153 samples from all spaces of Pneumology Hospital - Iasi, and only on this segment to identify fungal colonies at genus and species level we examined more than 800 slides from cultures. In general microclimate fungal concentration should have correspondence with the exterior one concerning the composition of genera identified but in reality it is not so. The investigations conducted show that the most numerous species and genera were found in indoor spaces at ventilation grid at the source of the personal computer but also on filters of the air conditioning and medical equipment, here being identified viable spores, cultivable, from a surprisingly wide range of fungal genera. Most fungal species isolated can harm employees causing allergic respiratory diseases or skin diseases. For most samples, identification to species level was done and when it was not possible at gender. We found that in most cases values registered fall within the range of 900 - 1700 CFU/m^3 air, most values were well above those recommended as "safety limits". We didn,t found a difference value between spaces where there are pets and others and in some cases only from the pet were identified some fungal species that were not found in the air and on the surfaces of living space, thus the presence of pets contribute to the diversification of resident fungal genera and species. Seasonal correlations can not be achieved because in the indoor spaces are acting different factors, other than in the external environment.

The results obtained in a relatively closed space can not be extrapolated to another one, each situation must be treated differently depending on the results of investigations and laboratory tests after the assessment of the individual's health. In terms of percentage prevalence fungal species belonging to the genera *Penicillium, Aspergillus, Cladosporium, Alternaria, Rhizopus* and *Chaetomium*. Of the total aspergillus species isolated more predominance had *Aspergillus fumigatus, Aspergillus flavus, Aspergillus versicolor, Aspergillus niger. Cladosporium herbarum* is the predominant specie in many of the samples examined was isolated from both surface and from the air-conditioning. Spores of these fungi are known to be highly allergenic, may cause epithelial, keratitis, onycomycosis, sinusitis or upper air tract infection. In samples taken from the Hospital a very high proportion had the genera *Aspergillus* and *Penicillium* sometimes even isolated in pure culture. On the fifth floor of the Hospital we observed the highest count of CFU/m^3 of air with values in the range from 1467 to 1572, this being one of the causes for contamination of the lower floors. In the Pneumology Hospital wards we counted a very high CFU/m^3 of air, which could be explained by uncontrolled access

of foreign people - visitors, but also by the existence of medical equipment equipped with ventilation systems and operating parameters which are not respected. In video-endoscopy - intervention endoscopy CFU/m^3 amount of air is 1258, at the fibrobronchoscopy 681 CFU/m^3 air and in the perimeter of the preoperative room at 733 CFU/m^3 air. At video-endoscopy where computers and other equipment with corporate air ventilation are present the values recorded of CFU/m^3 of air are very hight. Although *Penicillium, Aspergillus, Alternaria and Cladosporium* are the dominant types in most areas studied, other genres which are not ubiquitous even in a lower concentration may be more aggressive, showing a high degree of pathogenicity and adaptation to the various substrates. Note the presence in many spaces of gender *Hormonema*/teleomorf *Sydow polyspora*, with implications for pathology etiologic agent of skin disorders and *Stachybotrys*, etiologic agent of pulmonary hemosiderosis at children. Over 60% of the identified species are known to be allergenic and with hight levels of toxicity which entitles us to say that assumptions of future installation of serious respiratory illnesses are justified. In **Chapter VI**, we propose a new method of microbiological diagnosis that is based on the determination of hemolytic ability of fungal species isolated from work and living spaces and on the results obtained from the acute toxicity test with total fungal extracts obtained in our laboratory, on the earthworm - *Lumbricus terrestris*. The main objectives were, observing the toxic and hemolytic ability of fungal species identified, obtaining extracts from fungal culture and not least testing for the first time the effect of hemolytic and toxic fungal extracts on the earthworm-Lumbricus terrestris. In this chapter we present the technique used to obtain extracts from a single species of the total fungal culture, which served for compared skin test with allergen from kits "Halcis Prick-Test". For the first time in Romania is a study of analysis and assessment of hemolytic ability of fungi, using for this purpose species and genera isolated from occupied work and living spaces as follows, *Aspergillus niger, Cladosporium herbarum, Trichoderma viride, Alternaria chlamydospora, Stachybotrys chartarum, Acremonium spp, Conidiobolus spp Chrysosporium spp, Penicillium spp, Penicillium commune, Aspergillus ochraceus, Scopulariopsis spp, Penicillium italicum, Chaetomium spp Penicillium crustosum, Aspergillus spp, Aspergillus versicolor.* For all three incubation temperatures that we tested to observe the ability of fungi to produce haemolysis on agar-sheep blood, the most effective proved to be the 27^0C. At this incubation temperature 11 fungal species tested produced complete haemolysis while at the 25^0C were nine and at 370C only eight. So temperature plays an important role in potentiality of hemolytic fungal ability. Results recorded after 14 days on control reveals that 35% of the species tested produce complete haemolysis and 23% produced incomplete haemolysis, regardless of temperature we used. Differently, depending on control

temperature, after 14 days we observed that 54% of the species tested are producing complete haemolysis, 39% incomplete haemolysis and 7% shows no haemolytic activity only at certain temperatures. It is clear that temperature may indirectly influence the haemolytic ability of fungi by restricting their development. By comparison with the bacterial hemolysis fungal hemolysis is slower and can be observed as early as three days on control. Reported to the composition of the basic medium we observed that on PDA medium, 10 species tested showed haemolysis, 9 on Merck medium and 8 on C.G.A. medium. So PDA medium showed the best base composition. In terms of speed for occurrence of fungal haemolysis can see that, *Stachybotrys chartarum* produces an medium clarification consuming erythrocytes after only three days and *Alternaria chlamydospora* after five days of development of colonies which indicates a highly level of pathogenicity of the two species. Complete fungal haemolysis is slow, can be observed at 5-14 days, for *Aspergillus versicolor, Scopulariopsis spp, Penicillium italicum, Acremonium spp* and *Aspergillus niger* species and is influenced by both incubation temperature and medium composition but is very important in terms of long term effects on the living organisms. We believe that incomplete hemolysis occurs as a consequence of the released products of metabolism from fungi in culture medium. *Stachybotrys chartarum, Aspergillus niger, Aspergillus versicolor, Rhizopus stolonifer, Cladosporium herbarum, Alternaria chlamydospora, Aspergillus ochraceus, Scopulariopsis spp, Trichoderma viride* are fungal species whose extracts were obtained in the laboratory, and were selected for inoculation in the heart node region in *Lumbricus terrestris*. In the course of the experiment we observed that the extracts of *Rhizopus stolonifer, Cladosporium herbarum, Alternaria chlamydospora, Scopulariopsis spp, Trichoderma viride*, did not affect the earthworms all duration of the experiment, while extracts of *Stachybotrys chartarum* and *Aspergillus ochraceus* showed the most rapid effect of acute toxicity on earthworms. *Lumbricus terrestris* earthworm-testing has proved a successful experimental model, earthworms may be markers for assessing the *in vivo* toxicity of total fungal extracts. **Chapter VII** is intended as the previous for the development of new mycological diagnosis technique. We obtained and evaluated a new fluorochrome for the rapid detection of fungal elements in clinical specimens. Congo red can be used as a nonspecific fluorochrome for direct examination and early detection of fungal elements in clinical specimens in native preparations or paraffin embedded ones. Is a much cheaper alternative compared to "Calcofluor white" - the fluorochrome in use worlwide. Congo red can be used on any type of clinical specimens including those already prepared by histological techniques (parafinare), is at least ten times cheaper than "Calcofluor white, is stable, can be stored up to 8 months at refrigeration temperature, can be used with a pH value between 5-7

and is not affected by light. This fluorochrome is easily prepared with a cost effective extremely low and formalin and glutaraldehyde, included in the composition are known as fungicide those significantly reducing the risks of examiner exposure during the work. Are presented six case studies in humans and animals that illustrate and demonstrate that by fluorochrome examination we can not be disturbed by bacterial contamination being created a clear demarcation and a clear picture of what fungal presence in clinical specimens represents. In **Chapter VIII**, entitled "Increasing predictability for human respiratory diseases with fungal etiology by evaluating the two types of questionnaires" were evaluated and interpreted results for the 30 subjects that answered at the questions posed in the two types of questionnaires. All survey participants were informed about the risks posed by this experiment being made available to all data necessary for a decision well aware. Both questionnaires and "informed consent" were consulted and signed by all participants in this study. Subjects were selected for this final assessment in the following criteria, aged 18-55 years, and both sexes, without respiratory disease four weeks before the laboratory assessments or during their deployment; personal computer users at least 6 hours daily, have been favorite subjects holding pet owners of dogs or/and cats, healthy, no serious medical problems or obvious, both smokers and non-smoking and previously diagnosed as being allergic subjects only to, dust mites, pollen or food. Pregnancy status of women was the reason for their exclusion from the study. Questionnaires - for apreciate the health status and the technica onel, were completed by 30 of the participants in this study, fifteen of them holding pets. Also the 30 human participants have gone through all stages of the study and answered it,s requests. Questionnaire "I" contains 49 questions and the technical questionnaire "II" includes 36 questions and only thirteen of that have been applied for their statistical value, being relevant for interpretation of the results. Expected responses for both surveys were four types, "Yes", "No", "Do not know" and "Comments". For recording physical microclimate factors we used a portable precision instrument Kestrel 3500, and of all available parameters, given considering that research has been conducted over the past 3 years we considered for the interpretation of the results only temperature, wind speed and relative humidity in the spaces studied during the time determination. Positive responses from the two surveys are expressed as a percentage and the values obtained represent the score awarded to each of the participants interviewed. Sum values from the two questionnaires for each of the participants, will be the final amount that will be correlated both with the results of laboratory investigations and the results obtained by revealing the fungal genera and species isolated and with concentration values obtained from CFU/m^3 of air in all spaces. The value of each question is given by expressing the percentage of positive responses given by the individuals interviewed. Reported in both questionnaires of the

total of 62 questions used for the 30 subjects share of positive responses was over 40%. Was registered the unit cooling fan capacity of personal computers and any other equipment, equipped with ventilation systems to create turbulence, by measuring velocities of the air from a distance of 10 and 20 cm from them. Average of values at 10/20 cm distance of the personal computer cooling unit is about 2/1,4 m/s for desktop units and 1.5/0.8 m/s for the notebooks.

These values, surprisingly hight, create and maintain a continuous movement of air masses especially in the "personal risk zone" of personal computer user. Electrostatic charging is a physical process by which the solids (eg carcasses and parts of electrical appliances) appear charge, due to mechanical actions such as air friction, vibration, proximity of metal wires crossed by electrical currents, etc. Typical problems caused by static charging are the attraction, adhesion and storage of dust so default and fungal spores and fragments. These are followed by constant air dispersion of fungal particulate so pollution is kept at threat levels for human and animal health. All these allegations are supported by the fact that at the ventilation systems of the computers were identified most fungal species and genera of an extremely wide range and most of them should not be recovered from indoor spaces, from the air of a healthy working and living microclimate. Some of the findings presented in this chapter are; in 30% of buildings constructed of prefabricated reinforced concrete slabs and concrete blocks were found condensation and damp, brick remains the recommended construction material, the tightness of the windows prevent a good natural ventilation of housing and increase the humidity so that the mold is always especially around windows. During **Chapter IX** we are trying to make a direct connection between the degree of fungal pollution in work and living spaces and the harm that can be produce to human and animal health. Experimental procedures were conducted under strict medical supervision and approved by the Ethics Committee of the Pneumology Hospital, in the Departments of Microbiology - Immunology of the Faculty of Veterinary Medicine Iassy, in the Allergy Center Affiliate at Central Medical offices from Copou-Iassy, in the Clinic and Laboratory of Pneumology Hospital-Iassy and involving laboratory Stan's Lab. Have been met national and European rules on standards of clinical practice - "Good Clinical Practice. The methodology was developed so that the procedures used to minimize possible risks and potential disadvantages in terms of participation in this study. Subjects were evaluated by; assessing lung function-spirometry, use of impact intranasal air samples, the sum of values after application of the two questionnaires, unispecie fungal skin test allergens produced in-house compared with those from test kit "Halcis Prick-Test" and assessing the immune status by two different methods, a test chromatographic, with qualitative and semi-quantitative expression, for rapid detection of total IgE with results obtained in five minutes and making immunograme

(IgE, IgA, IgM, IgG) by chemiluminescence method with an automatic analyzer. The results presented are based on the interpretation of the eight types of tests as follows;

1. Identifying key fungal genera and species in areas studied by sampling from the cooling fan system of the personal computer and its surroundings.
2. Data on fungal composition and charge obtained by sedimentation method.
3. The main fungal genera and species identified by culture, by use of RIN (intranasal sampler) and assessment of CFU inhaled in one hours reported for 60% of respiratory flow at rest (DVR) for 1.62 m3 for women and 1,94 m3 for men. during 6 hours of the experiment.
4. FEV1/FVC: where FEV1 (maximum expiratory volume in one second) is expressed as a percentage of FVC (forced vital capacity), provides clinical information on the degree of airflow limitation. FEV1 values are expressed as percentage of FVC. FEV1/FVC ratio is between 70% and 80% in normal adults, values below 70% indicates airflow limitation. Is influenced by age, sex, height, ethnicity. At the subjects examined were not recorded deviations from normal values.
5. IgE rapid test results - beyond permissible levels of 80 IU/ml serum or plasma.
6. Imunograme analysis performed by chemiluminescence and highlighting values - IgE, IgG, IgA, IgM - deviated from acceptable limits.
7. Fungal skin test with allergens produced in-house compared with those of test kit "Halcis Prick-Test".
8. Correlation between positive responses given by those asked for all questions with the results of laboratory investigations.

At the skin test with extract of *Aspergillus niger* 15 test subjects reacted by the appearance of an endurance well about 3-5 mm with erythema and 6 of them had an endurance> 5 mm with persistent erythema. In testing of *Cladosporium herbarum* extract obtained in our laboratory, 16 individuals have showed a 3-5 mm well with erythema and 3 of them an induration> 5 mm with persistent erythema the results being fully consistent with those obtained by using the extracts from the kit "Halcis Prick-Test". The extract of *Alternaria clamidospora* on 13 subjects found an induration of 3-5 mm with erythema and 3 of them showed an induration> 5 mm with persistent erythema while the application of *Alternaria alternata* extract kits "Halcis Prick-Test "reactivity was less - 9 individuals had an induration of 3-5 mm with erythema. The extracts of *Aspergillus versicolor* and *Stachybotrys chartarum* produced a well of 3-5 mm with erythema at tested subjects. There were four cases where testing was stopped because the individuals tested were overreactions first manifested by localized iritations and then generalized, feeling of suffocation, headache, dizziness and dry

mouth with tightness in the chest. Air CFU/m^3 values obtained by passive sedimentation method, in work and living spaces, where the individuals had not above the normal values of immunoglobulins were within the range from 681 to 1415 while the values of air CFU/m^3 in "personal risk zone" of origin spaces while individuals with immunoglobulin values exceeded the normal range, were within the range from 629 to 1939. By comparison, the number of viable fungal particles inhaled (CFU), after applying the correction factor was within the range from 168 to 277, for individuals with normal levels of immunoglobulins and the range from 184 to 487 subjects who presented values above the normal range of immunoglobulins. Extracts of *Aspergillus niger, Cladosporium herbarum, Penicillium spp.* and *Alternaria alternata*, are in leading positions in the degree of correlation of skin reactivity to fungal pollution in studied spaces, followed closely by *Scopulariopsis spp. Rhizopus sp.p* and *Stachybotrys chartarum.*

Only in two cases - Subjects 5 and 24, we observed skin reactivity to one of the fungal species identified in the areas examined. Subjects 1, 3, 6, 7, 11, 13, 14.16, 17, 23, 25, responded by skin endurances of 3-5 mm with erythema and 5 mm with persistent erythema at least at two of the fungal extracts obtained from fungal pecies isolated from their living and working spaces. Was appreciated the degree of risk of subjects analyzed, to develop an allergic respiratory disease or non-allergic fungal disease in the coming years, taking into account the score obtained from questionnaires, the number of viable fungal particles inhaled, the total number of fungal species in which they reacted at skin tests by at least 3-5 mm endurance with erythema and elevated immunoglobulin values. The following reasoning was applied, the number of viable fungal particles inhaled, the values obtained by the subjects to the questionaires I and II and the total number of fungal species in which each of the subjects responded by skin endurances of 3-5 mm with erythema, adding 10 points for the CFU/m^3 if value was over 1000, and another 10 points when the subjects analyzed had values above the normal limits of immunoglobulins. In 24 of the 30 areas examined, the 1000 CFU/m3 air has been exceeded. Each of the subjects received a score allowed their classification into three risk groups, as noted, RG I, RG II, RG III, where RG I is treated as a major risk group to develop an allergic or non - allergic respiratory disease with fungal etiology in the coming years and RG III a minor risk, if it maintained the same coordinates on fungal pollution, habitual features and microclimate conditions existing during the present study. Engagement in these risk groups was done under the score obtained by subjects after adding all the values above, RG III between 200 - 350, RG II, 350 to 450 and RG I 450 to 600 points. Following analysis the RG I have employed 13 individuals, all showing values above the reference of Ig in RG II, 8 of which 7 subjects showing the values of IgE, IgG above reference limits and in RG III, 9 of which 3

subjects shows values above the reference IgE 80UI/ml. It was established a control group composed of six participants. Values on the number of viable fungal particles inhaled by using intranasal impaction, in the "personal risk zone" of personal computer user defined as the space of about 3m3 where he acts were much different from the values recorded by the same subjects in a room without computers or any other device equipped with a ventilation system that can create turbulence of the air masses. Six of the subjects examined were subjected to this kind of determination as follows; Subject 13, 302 CFU in the "personal risk"/control 134 CFU, Subject 14, 352 CFU in the "personal risk"/ control 92 CFU, Subject 15, 369 CFU in the "personal risk" /control 142 CFU, Subject 16, 277 CFU in the "personal risk"/control 159 CFU, Subject 17, 394 CFU in the "personal risk"/control 151 CFU, Subject 27, 487 CFU in the "personal risk"/ control 109 CFU. Clearly we prove the existence of "personal zone of risk" for personal computer user.In all 30 cases after skin testing, was found that at least one fungal species in which subjects responded by skin reactions, could be identified both at the ventilation sistem of personal computers or other equipment and from the filters of intranasal air samples and from samples obtained by passive sedimentation. All the results of research clearly demonstrates the connection between fungal pollution of space and inhalation of spores and fragments as risks to human health, individuals being susceptibility to develop an allergic or non-allergic respiratory disease with fungal etiology in the coming years. In **Chapter X** "Final conclusions and recommendations, are summarized the main results of research from which are separated a series of recommendations on the proper conduct of monitoring the degree of fungal pollution in indoor spaces. It is essential that both sources and routes of exposure could be determined correctly. The major goals of the research was focused on improving the quality of human and animal life and also being directed at developing new techniques/methods for mycological diagnosis and a natural approach to this will be that the results of this research will be presented at competent authorities as a legislative initiatives for establishing safe limits concerning fungal pollution in working and living spaces and adopting a new effective diagnostic methodts for the prevention of the respiratory segment diseases.