Neurospora sitophila in indoor environment of buildings

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Abstract. Neurospora sitophila is a microscopic filamentous fungus of the Ascomycete taxon that rapidly expands in the environment. Although the fungus has been recorded primarily as a known laboratory contaminant, this contribution presents evidence of its frequent occurrence in indoor environment of offices at the university, especially in places where a coffee maker was part of the equipment. A total of 50 smears were made from the environment of the University of VŠB - TU Ostrava from various parts of the fixtures. It was found that spores and fragments of N. sitophila mycelia are an active part of the building air using the BIOLOG identification system. Although no direct evidence has yet been obtained that N. sitophila is the causative agent of human disease or infection, the presence of this fungus in the environment should be regarded as a risk factor for human health. Exposure to fungal spores and fragments of mycelia in the indoor environment of buildings can cause hypersensitivity known to be an influencing factor in allergic reactions and asthma.

1. Introduction

The European Commission through the Scientific Committee on Health and Environmental Risks emphasises the indoor air quality by understanding and controlling common pollutants indoors to help reduce the risk of indoor health risks and worsened quality of life [1]. Besides various factors affecting the indoor air quality, microorganisms in bioaerosols, their spores and metabolites as aeroallergens can significantly affect human health in building environments. Bioaerosol consists of particles in the air that may contain various microorganisms, e.g. viruses, bacteria or microscopic filamentous fungi that enter the air by spreading from the sites of their occurrence. Many species of microscopic filamentous fungi, e.g. Aspergillus, Penicillium, Cladosporium etc. produce large amounts of conidia and various types of spores that are released and then spread through the air. Besides spores, filaments and their fractions can also be released from fungal growths; the health effects (e.g. allergenic potential) of mycelial fragments and spores are the same. The cultivation of microorganisms becomes important, which is also direct evidence of their presence in bioaerosols, especially in indoor environment of public buildings, residential areas, medical facilities and various types of the working environments. Humans in this environment may experience various types of allergies that can lead to chronic respiratory failures (bronchitis, allergic alveolitis, "farmer's lung", pulmonary mycotoxicosis and others) [2].

Neurospora sitophila is a microscopic filamentous fungus of the Ascomycota division, which spreads rapidly in the environment through vegetative spores of orange colour formed on the lateral branches of conidiogenous hyphae. This organism grows rapidly and propagates easily on defined growth
media. Due to its high growth rate (3-5 mm/h) and the ease with which arthroconidia become airborne, *N. sitophila* is often referred to as a known laboratory contaminant. As a part of bioaerosols, it can occur and survive also in very high altitudes in the stratosphere (19 km) [3]. In our paper, *N. sitophila* is shown also as a frequent contaminant in offices, especially where coffee makers are located, as it can spread well through coffee grounds and coffee espresso residues. *Neurospora sitophila* syn. *Monilia sitophila* (Ascomycota, Sordariales, Sordariaceae) is an asexual state (anamorph), also referred to as *Chrysonilia sitophila*, the sexual state (teleomorph) of this microscopic fungus [4].

2. Material and Methods
The smears were collected from various places in the offices and laboratories in the building of the VŠB – Technical University Ostrava (Table 1) using sterile swabs (1601 Amies, Dispolab, Czech Republic). The swabs were then plated on Sabouraud dextrose agar in Petri dishes (M063, HiMedia Laboratories, India). All Petri dishes were incubated at room temperature and checked regularly for 14 days. The developed colonies of microscopic fungi were inoculated into fresh Petri dishes with Sabouraud agar until clear colonies were obtained.

After the selection of pure colonies, the isolated fungi were maintained on a 2% Malt Extract Glucose Agar (M1874, HiMedia Laboratories, India) to confirm their identification with the BIOLOG system (Biolog™, USA). BIOLOG MicroStation is an advanced testing system that serves to identify and phenotypically characterize microorganisms based on highly accurate and patented biochemical tests [5]. A unique biochemical profile can be obtained for individual strains by using 96-well FF-microplates; the phenotypic profiles of the tested fungi accurately reflect their catabolic potential, and therefore it was used to identify these fungi using phenotypic profiling.

3. Results and Discussion
A total of 50 smears were made from indoor environment of the office and laboratory rooms at the VŠB – Technical University Ostrava, especially from furniture and plants. Positive samples in which *N. sitophila* species have been identified are listed in Table 1.

| Sampling place          | Number of positive samples /total amount |
|-------------------------|-----------------------------------------|
| laboratory environment (air) | 5/10                                     |
| office environment (air)   | 4/10                                     |
| plant surface              | 2/6                                      |
| dust on furniture          | 2/6                                      |
| coffee maker               | 4/4                                      |
| desk                      | 0/4                                      |
| carpet                    | 0/4                                      |
| window frame               | 0/4                                      |
| PC keyboard                | 0/2                                      |

Identification of *N. sitophila* was performed on 7-day cultures based on a biochemical profile using a Biolog MicroStation system using substrates in FF-microplates; the results are summarized in Table 2.

| Carbohydrates | Sugar derivatives | Organic acids | Amino acids |
|---------------|-------------------|---------------|-------------|
|               |                   |               |             |

Table 1. Obtained isolates from the university building environment (positive samples / total amount of samples).

Table 2. Phenotypic identification profile of *N. sitophila* showing positive and negative results in biochemical tests of substrate utilization.
|   |   |   |
|---|---|---|
| (A8) D-Arabinose | A6 Adonitol | B8 D-Galacturonic Acid |
| (A9) L-Arabinose | A7 Amygdalin | B10 D-Gluconic Acid |
| A11 Arbutin | A10 D-Arabitol | C7 2-Keto-D-Gluconic Acid |
| A12 D-Cellobiose | C1 Glucose-1-Phosphate | F3 Fumaric Acid |
| B1 α-Cyclodextrin | D1 D-Mannitol | F7 α-Keto-glutaric Acid |
| B5 D-Fructose | D8 β-Methyl-Glucoside | F9 L-Lactic Acid |
| B7 D-Galactose | E2 Salicin | F10 D-Malic Acid |
| B9 Gentiobiase | E4 D-Sorbitol | F11 L-Malic Acid |
| B12 α-D-Glucose | C5 Glycogen | F12 Quinic Acid |
| C7 2-Keto-D-Gluconic Acid | C8 α-D-Lactose | G4 Succinic Acid |
| C9 Maltose | C12 Maltotriose |   |
| D2 D-Mannose | D10 D-Psicose |   |
| D12 L-Rhamnose | E1 D-Ribose |   |
| E1 D-Ribose | E6 Stachyose |   |
| E7 Sucrose | E9 D-Trehalose |   |
| E12 D-Xylose |   |   |

Notes: white indicates positive reaction (substrate utilization), gray indicates negative reaction

The distribution of *Neurospora* species in nature varies. Most *Neurospora* species have been identified in tropical or subtropical areas where they can be easily seen and grow on the surface of recently burned vegetation. *N. discreta* is reported to be the most common species isolated in western North America [7], with *N. crassa*, *N. sitophila* and *N. tetrasperma* being observed more frequently in Europe [8].

*Figure 1.* Colonies of *Neurospora sitophila* on various growth media (from left to right): 2% Malt Extract Glucose Agar, Tryptone Soya Agar and Sabouraud Dextrose Agar. The colonies of *Neurospora* are very clear due to the accumulation of orange carotenoid neurosporaxanthin in conidia and vegetative mycelia (biosynthesis of neurosporaxanthin has been shown to be induced by light [6]).
Although *N. sitophila* has a cosmopolitan distribution [9], it is not isolated very often. It is best known for its occurrence in laboratories where it is a very dangerous contaminant due to its rapid spread and possible contamination of other samples. The production of mycotoxins has not yet been identified and so this species is not yet reported as a risk to human health. However, this is not a clear statement because some authors [10,11] have confirmed asthma provocation, especially in immunocompromised persons as an occupational disease in the professional food service industry (coffee dispenser service operator) and logging industry. This means that these people have long been exposed to the presence of this species in the environment, so the disease (asthma) may have developed. A rare occurrence of *Chrysonilia sitophila* has also been reported by Holý et al. [12] together with other types of microscopic filamentous fungi (species of the genus *Trichoderma, Aspergillus, Penicillium, Paecilomyces, Eurotium*) in the indoor air of the Transplant Unit of the University Hospital Olomouc.

The occurrence of *N. sitophila* in all samples taken from coffee makers located in several offices was very interesting finding. If there was no daily maintenance of coffee makers (removal of coffee grounds), orange spores of *N. sitophila* were observed within a few days in all parts of the machine (Fig. 2) in which coffee leaching residues were maintained. It is evident that the pressed ground coffee is one of the suitable growth substrates for this fungus, even when the coffee preparation undergoes a heat treatment in the range of 85 - 96 °C (depending on the type of coffee maker). Morenoancillo et al. [13] and Heinemann & Nolard [14] noticed the *N. sitophila* as a thermotolerant fungus. Eisenbrand & Schreier [15] stated that spores and conidia of *N. sitophila* are thermotolerant (5 min, 75 °C). Based on these findings, this microorganism can be considered as a significant contaminant not only in laboratories, but also in offices due to the presence of coffee makers, as they are now a common part of the office equipment. This is in accordance with the findings of other researchers [11, 16] in which the growth of *N. sitophila* has been demonstrated on coffee beans. There is a possible correlation between the occurrence of *N. sitophila* and the increasing incidence of sick building syndrome (SBS), which causes health problems among residents of office buildings. Although the causes of these conditions cannot often be directly identified, it is known that the key factors contributing to the development of SBS include the increased presence of biological agents in the air due to poor room ventilation systems. These biological contaminants include not only viruses and bacteria, but also spores and fragments of hyphae of microscopic fungi that can originate from different sources. Because *N. sitophila* is referred to as a fungus that can provoke allergic reactions (its extract is used in allergenic tests) (https://www.drugbank.ca/drugs/DB10969), preventive measures should be taken to prevent the possible occurrence of SBS in the presence of *N. sitophila* in the room. The basis of these measures is to accelerate the total turnover of fresh air in the room by exchanging it with outdoor air, providing hygienic conditions in the workplace, especially by regular and intensive cleaning to minimize conditions that promote increasing of allergens and growth of moulds. In places where it is not possible to fully provide for the proper room ventilation, the effective and hygienic use of the air

![Figure 2. *N. sitophila* on coffee grounds after preparation of coffee at various stages of development (photo by the author).](image-url)
conditioner is an important remedy, especially regular safety service, including filter replacement. Although there is no enough results, air conditioned spaces can be suitable for growing of *N. sitophila*. For example, Udaya Prakash et al. [17] showed that *N. sitophila* was the second dominant fungus (76 CFU/m$^3$ of air, 10.65 % contribution), after *Astergillus niger*, in air conditioned buses. The benefits of exposure to sensitizing agents in the workplace should be considered especially in the diagnosis of asthma in adults as it has been reported that occupational asthma may account for up to 15% of cases. The first case of occupational asthma caused by exposure to *N. sitophila* was described in a plywood factory worker in 1991 [18]. After that there were published several other cases of occupational asthma caused by *N. sitophila* [19, 20, 21, 22, 23]. Heffler et al. [11] described an example of a young man with asthma who was diagnosed with an occupational disease caused by unusual exposure to *N. sitophila*. This is the first known case describing occupational asthma caused by *N. sitophila* in a worker exposed to coffee. Also other authors confirm the occurrence of an occupational asthma caused by *N. sitophila* in coffee industry [16, 19]. These and other published information on the occurrence of *N. sitophila* on the coffee bean surface was also confirmed by our finding; *N. sitophila* was repeatedly observed and isolated from the coffee grounds remaining in the coffee maker. According to Pitt and Hocking [24] for long a common sight in bakeries and on bread, *Chrysonilia sitophila* is less commonly encountered now, but can still be a great source of trouble as persistent contaminant in laboratories. It has sometimes been reported also from pastries, hazelnuts and meat products. Kolodziejczyk and Bozek [25] found out in the group of patients monosensitized to molds that the prevalence of allergy to *N. sitophila* was 9.2 % (22 patients from 239 patients were positive by skin prick tests and increased concentration of sIgE). They stated that exposure and sensitization to fungal allergens can promote the development and worsening of allergic rhinitis and bronchial asthma. Slightly lower results were obtained by Jerath et al. [26] which tested 1500 patients of nasobronchial allergy to positive reaction to fungi. According skin prick tests, 41 patients were positive to *N. sitophila* (2.5 %). And there is a discrepancy because the pathogenicity of *Neurospora* has not been reliably proven or refuted. However, it should be suggested that it is difficult to identify individual species of *Neurospora* due to external morphology, and, as reported in [27], even some *Neurospora* species have previously been used in production and food processing in Java, Brazil or France (to flavour cheeses). In addition, the genus *Neurospora* has been reported to be particularly suitable in a variety of genetic, biochemical, developmental and subcellular genetic engineering studies [28, 29, 30]. *N. sitophila* is also being used in modern biotechnology [31], e.g. for the production of microbial phytase [32] or for the production of carotenoids [33, 34, 35]. However, doubts about its pathogenicity still remain. Although most medical mycology textbooks do not admit any significant pathogenic effects of *N. sitophila*, either by not mentioning them at all or by mentioning this species solely as a potential laboratory contaminant, there are several studies that admit the pathogenicity of this species. An example is the older study of 1962 [36], which lists *N. sitophila* as a fungus growing in the inner eye following cataract surgery, but this study also mentions other random eye infections due to other non-parasitic fungi after surgery and emphasises the importance of ensuring that operating theatres do not contain fungal spores. Also Sirikul et al. [37] and Thomas and Kaliamurthy [38] showed that *N. sitophila* can be a predisposing factor of mycotic ulcerative keratitis. However, despite no direct evidence that *N. sitophila* is the causative agent of any human disease or infection, the presence of this fungus in the environment should be considered a risk factor for human health.

4. Conclusion

Exposure to fungi in the indoor environment may trigger hypersensitivity to a variety of fungi and is known to be an influencing factor in allergic rhinitis and asthma. Although, *Neurospora sitophila*, a filamentous fungus studied in this research, is generally considered to be only a laboratory contaminant, it can be connected to occupational diseases, especially as an allergen for occupational asthma and related respiratory health problems. Moreover, it can be found on various indoor surfaces including food products such as coffee beans and coffee grounds.
Acknowledgements
This research was financially supported by the Project No. 847205-RECOVERY from The European Commission and the VEGA projects (Scientific Grant Agency) Nos. 1/0424/18 and 1/0658/19.

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