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JAGIELLONIAN CURSE, ENVIRONMENTAL REQUIREMENTS IN BUILDINGS AND TOXICITY OF MOULD FUNGI

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1. Introduction

In Poland we have our own "curse of the Jagiellonian dynasty". Some authors quote the connections between fatal accidents and a large number of deaths and curses.

As early as 20 years ago, *Aspergillus flavus*, a toxic fungus was identified in the Wawel Castle vault. The mycotoxin (aflatoxin) produced by this fungus has been recognised as a potential culprit responsible for the misfortunes accompanying the acts of disturbing the peace of ancient kings. Thus the myth of a "curse" could be discarded.

Mycotoxins are also produced by other fungi. The observations indicate that the environment in which the fungi develop and its characteristics (including, among other elements, the base on which the fungi grow, and the microclimate) is one of the factors influencing the production of mycotoxins.

Has it been the curses, or perhaps was it the mould fungi, that were the reason of unfortunate events which happened alongside the exploration of the tombs? Can the risk similar threats be faced during the renovation of old historic buildings?

The author of this paper has explored the influence of the environment on the toxic effect of mould fungi growing on building materials. The results of the research are presented below.

2. Royal vaults and their curses

In the 1980s, in Cracow, restoration and maintenance work was carried out on the grounds of the royal castle of Wawel, an ancient seat of Polish kings. After the graves had been opened in the royal vault of King Casimir the Jagiellonian, the members of the restoration and maintenance team began to rapidly die off, one after another [13]. Some maintain that it was mould fungi that were the source of toxic effect on people entering graves.

A similar story, highlighted by a Latin inscription *Violator operis infelix esto* (Be damned, you who shall destroy my work) placed on the wall of former Royal Chapel in the Vilnius Cathedral, and a series of deaths of those who explored the royal graves, strengthened the belief in the "Jagiellonian curse" [14].

During archaeological work carried out at the Wawel Castle, the crypt of King Casimir the Jagiellonian was examined. The crypt had not been opened since the day of the king's funeral in 1492 [7].

In 1973, that is, nearly 500 years after the burial ceremony, microbiological tests were carried out in order to identify the microbiological organisms present in the crypt (in the air, on the walls and ceiling, in the samples of wood and mortar from and around the coffin, and the coffin dust). As a result of the research the following species of mould fungi were identified there: *Aspergillus niger, Chaetomium globosum, Penicillium, Trichoderma* [11]. Bibliographical source [13] maintains that the *Aspergillus flavus* was found in the Wawel Castle crypt.

The characteristic feature of the mould fungi identified in the Wawel Castle crypt is their strong biochemical activity in the process of wood and silicone and lime mortar degradation, they adapt very well to the environment in the old buildings.

It was the mould fungi that were blamed for the misfortunes accompanying the disturbance of peace of kings and rulers. As it turned out, the killer was lurking from the walls of crypts, and thus, the myth of a "curse" had been discarded.

Therefore, if it is not a curse that kills, but toxic mould fungi, could such a danger be come across in old, fungus-infested buildings?

3. The mould fungi

3.1. Environment conditions determining the development of mould fungi

Mould fungi is a large family of heterotrophic organisms (25 000 species), rather undemanding as far as nutrition needs are concerned [1÷5, 9, 10, 15].

The mould fungi which grow in buildings, use the components of paint and wallpaper and micropollutants which land, with dust, on the surfaces of partitions and walls as their nutritional base. The partition walls are also easily contaminated, because there will always be fungal spores in the atmosphere. Parameters of the indoor microclimate are nearly optimal for the development of moulds (the temperature of air indoors is t_i=20 to 25^{o}C, and there is access to natural light and oxygen). Mould fungi, nonetheless, may only develop if the humidity in their environment is high. They can take in water both from the atmosphere and from the base on which they grow.

In microbiological laboratories, mould fungi are grown on special organic media, in the conditions of stable, relatively high temperature t=25÷30^{o}C. Their water requirements is described by means of a water activity index, a_w. It describes the humidity of the medium in the conditions of hygroscopic equilibrium with atmospheric air, and is equivalent to the relative humidity of the air, φ (for example, $a_w = 0{,}6$ is equivalent to $\varphi = 60\%$). The majority of mould fungi grown in laboratories, do grow when a_w>0,6, and as to the *Aspergillus flavus* fungus, when a_w>0,9. Therefore, microbiologists use a single parameter to describe both the humidity of the air, and the humidity of the medium on which the mould fungi grow.

Unfortunately, the water activity index should not be used to identify the humidity conditions in buildings, because it does not reflect the character of phenomena which occur there. The water content in inorganic materials, under the conditions of hygroscopic balance, results from the humidity of air, but also depends on ambient temperature and on the structure of a given material, e.g. on its texture and porosity.

Another separate issue is the humidification of materials in partitions, resulting from vapour condensation on their surface, and from the capillary movement. In those cases, there will be no direct relationship between the a_w factor which describes humidity requirements of the mould fungi and the instantaneous relative humidity of air and the water content in the material.

3.2. *Aspergillus* fungi

It is assumed that the oldest generic names of mould fungi (*Aspergillus* and *Mucor*) have been given to them by the Royal Botanist of the Prince of Tuscany as early as in the 17th century. Since then, a large number of botanists have described new species and genera of mould fungi. Identifying the species of mould fungi requires extraordinary precision.

Both in the Jagiellonian Kings crypt, two species of *Aspergillus* fungi have been identified: *Aspergillus flavus* and *Aspergillus niger*. The *Aspergillus flavus* [13] species is regarded to be the major producer of aflatoxins (ccerogenous toxins), whereas *Aspergillus niger* [11] is a species commonly occurring in buildings which are damp, but not as dangerous.

a) b)

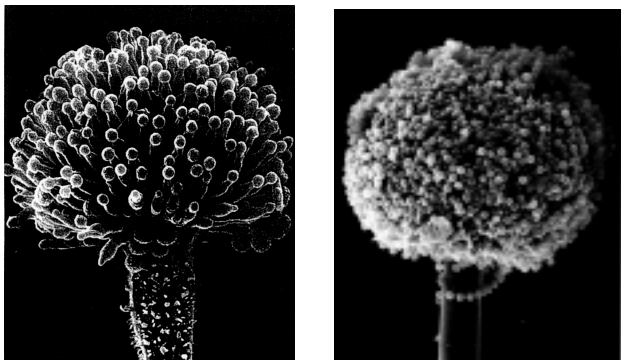

Fig 1. The structure of *Aspergillus* fungi [2];
a - *Aspergillus flavus* , b - *Aspergillus niger*

4. Historic buildings and the mould fungi

4.1. Problems of toxicity in historic buildings restoration

The problem of toxic character of mould fungi may be pertinent either to the toxic character of preparations used as fungicides, or the toxic products of the mould fungi themselves which may result in many diseases,

including allergies, athlete's foot, they may hinder normal development or organs, and even cause cancer [1].

In recent years, it has been proved that many species of mould fungi produce mycotoxins (*aflatoxins, ochratoxins*, and so on) – toxic compounds which can be produces at all times, or only under specific conditions. One of the factors mobilising fungi to produce toxins is, for example, overcooling.

It has been a strong belief that those mycotoxins are dangerous which penetrate into the human body through the digestive tract. Recent research has proved, nonetheless, that the compounds are most strongly toxic when they are inhaled. The inhaled mycotoxins are approximately 40 times more toxic than those which penetrate through the digestive tract [6, 10].

Today's chemical analysis (cytotoxic screening test, chromatography) is able to identify mycotoxins and assess the toxic compounds in the air and building materials in the restored historic buildings.

4.2. Environment conditions and mycotoxic pollution in the historic buildings

Old buildings, covered by dust and layers of dirt, are usually damp. In such conditions, mould fungi may grow on the surfaces of walls and ceilings, and on some elements of the interior decoration. Historic buildings are often unused and, consequently, not heated and this, in turn, may be a reason of overcooling and an impulse for the production of fungal toxins. Restoration work (for example, replacing old plasterwork, painting the walls) usually increases the humidity of partitions.

There are occasions, such as restoration works, when mycological investigation may be carried out. For example, the species and types of fungi found during research in a number of historic buildings, have been listed below:

- *Aspergillus niger*, *Aspergillus flavus, Chaetomium globosum, Penicillium, Trichoderma* were identi-fied in the crypt of Jagiellonian Kings [11, 13],
- *Penicillium, Auerobasidium* and *Aspergillus, Cladosporium* occurred in the air and on the walls of historic buildings of Cracow [8], *Aspergillus, Cladosporium* and *Penicillium, Stacybotrys atra, Alternaria* were discovered in the old manuscripts storage areas, located in old buildings in the centre of Cracow [12],
- *Penicillium, Aspergillus, Alternaria, Fusarium* and *Cladosporium* are fungi identified mostly in historic buildings [15].

These fungi are potential producers of mycotoxins.

If, therefore, mould fungi grow in old buildings, the companies carrying out building restoration works are exposed to inhaling fungal spores and, consequently, may be exposed to toxic influence of mycotoxins.

5. Author's own mycological and toxicological research

5.1. Research description

The research entailed the following:

- Growing mould fungi in different environments,
- Identification of the species of fungi and assessment of the degree of mycological pollution (mycological investigation),
- Carrying out tests to state the presence of mycotoxins (toxicological research).

Between September 1999 and March 2000, in two buildings (portacabin type containers meant for human habitation), mould fungi were grown on the surface of different building materials placed on free standing internal partition walls. The following elements were assessed: plasterboard, wallpaper, cellular concrete building blocks, thermal insulation materials.

From September until the end of December, the portacabins were not heated. The heating and humidification was turned on in January. In some interiors, forced air circulation was initiated, in other, the air was still. The conditions fostering the growth of mould fungi were created: the levels of relative humidity were kept at $\varphi \geq 70\%$, and the ventilation had been limited.

The surfaces to be tested were polluted with fungal spores (sprayed by sediment, or the pollution was self-induced, straight from the air – by way of a bioaerosol). Some of the samples had been earlier covered by a thin layer of organic medium standing in place of common dirt, other samples were deprived of the medium.

The following mould fungi were used to contaminate the materials: *Stachybotrys atra* Corda, *Aureobasidium pullulans* (de Bary) Arnaud., *Ulocladium*

tuberculatum E. Simmons, *Alternaria alternata* (Fr.) Keissler, *Cladosporium sphaerospermum* Penz,

5.2. Temperature and humidity of samples in the experimental structures

During the experiment, the temperature and humidity of air indoors were controlled, as well as the temperature and humidity of the materials being investigated. The measurements were taken using non-invasive methods.

The investigation embraced three characteristic periods of time. In September and October, the temperature was kept at the levels of t_i = 25÷15°C, in November and December, it dropped to the levels of t_i=-5÷0°C, and from January to March 2000, the temperature was kept at the level of t_i = 20÷25°C).

The temperature of surfaces of the materials was close to the temperature of the air indoors.

The mass humidity of plasterboard was found to be close to the level of sorption humidity $u_M \cong 1\%$.

Concrete cellular building blocks dried out during the investigation – from the humidity level of u_M=30% to the sorption humidity at $u_M \cong 7 \div 9\%$.

5.3. Mycological and toxicological research results

The species content of fungi isolated on the surfaces of the materials under investigation had been identified as early as 3 weeks since the beginning of the experiment, and was as follows [4]:

- from among the species used for the experiment, the occurrence of: *Alternaria alternata, Stachybotrys atra, Cladosporium sphaerospermum, Auerobasidiom pullulans, Ulocladium tuberculatum* was stated on the surfaces of samples,
- there were fungi which had not been included in the contamination mixture, nonetheless, they grew on the containers, namely: *Penicillium*, *Aspergillus*,
- *Penicillium*, *Aspergillus, Cladosporium,* were identified as dominating species.

The results of the toxicological tests (see Table 1.) confirmed the occurrence of *ochratoxin A* in the samples of the tested materials (tests to corroborate the presence of only this mycotoxin were performed [6]). Simultaneously, no correlation was noted between the amount of the toxin and the amount of spores contaminating the surfaces of the investigated materials. Nevertheless, the biggest amounts of the toxin were found on samples placed in the room where the circulation of the air was forced.

Table 1. The results of mycological and toxicological investigations

Type of material	**room 1**	**room 2**	**room 3**	**room 2**	**room 3**
	self-induced bioaerosol	**sprayed by sediment**			
	organic medium			**no medium**	
mycological pollution [jtk/g]					
wallpaper I	$6{,}3 \times 10^3$	$8{,}0 \times 10^3$	$3{,}0 \times 10^5$	$2{,}3 \times 10^4$	$8{,}2 \times 10^5$
wallpaper II	$1{,}7 \times 10^3$	$1{,}46 \times 10^6$	$3{,}7 \times 10^6$	$2{,}1 \times 10^4$	$1{,}2 \times 10^6$
mineral wool	$2{,}0 \times 10^5$	$3{,}0 \times 10^6$	$3{,}4 \times 10^6$	$4{,}6 \times 10^4$	$6{,}2 \times 10^5$
cellular concrete	$5{,}4 \times 10^3$	$1{,}7 \times 10^7$	$5{,}5 \times 10^5$	$7{,}5 \times 10^4$	$3{,}7 \times 10^6$
styrofoam	$2{,}2 \times 10^5$	$1{,}4 \times 10^7$	$3{,}7 \times 10^6$	$2{,}6 \times 10^5$	$8{,}2 \times 10^5$
amount of *ochratoxin A* mycotoxin [ppb]					
wallpaper I	0,61	0,49	0,53	0,61	2,69
wallpaper II	0,14	0,27	0,36	0,22	2,74
mineral wool	not found	not found	4,90	not found	not found
cellular concrete	not found	not found	not found	not found	not found
styrofoam	not found	not found	not found	not found	not found

Ochratoxin A is produced by different species of fungi classified as *Penicillium* and *Aspergillus*. The *Penicillium* and *Aspergillus* fungi did not constitute any part of the contaminating mixture but, as it was shown, grew naturally, on their own. Also, *Stachybotrys atra* fungus, producing *satratoxin*, a very strong toxin, was identified on the samples.

As it results from the research, mould fungi develop on the surfaces of building materials. The fungi may produce mycotoxins if the interiors are damp and temporarily not heated. In the air indoors, there is a huge number of fungal spores, microscopic in size. And the air, therefore, is also contaminated by mycotoxins.

6. Summary

- The conditions in historic and old buildings often foster the intensive development of mould fungi, such as dirty areas where dirt nurtures the fungi, as well as high humidity of air and in partitions and walls. Initiating air circulation inside the unused spaces and rooms makes it easier for the spores to spread and increases the degree of mycological pollution of the air. The humidity and overcooling in historic buildings may be an impulse mobilising mould fungi to produce mycotoxins.
- Human exposure to mycologically polluted interiors is dangerous, because the harmful toxins are inhaled in the process.
- The recently proved harmful effect of mould fungi on human health forces a new approach to the problem of toxic character of fungi during the renovation of historic buildings. There is a need of defining methods of renovation and restoration of historic buildings in view of the danger carried by mycotoxins.

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