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AGRICULTURE AND LIFE SCIENCES

Theses of doctoral (Ph.D.) dissertation

EFFECTS OF GLOBAL MEGATRENDS ON THE OCCURRENCE OF MICROSCOPIC FUNGI IN THE PANNON BIOGEOGRAPHIC REGION

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1. Introduction and aims

The complexity and change of our indoor, outdoor, artificial and natural environments, as well as the industrial and technological developments are crucial for the spread and diversity of microscopic fungi. To define the global changes resulting from human activity, we use the concept of megatrends, which are barely perceptible slow processes at first, but later elicit long-term global effects. As a result of their effects, social, political, demographic and environmental changes can be observed. The European Environment Agency (EEA) identified 11 global megatrends in 2015 (SOER report), which are the follows:

1. Diverging global population trends
2. Towards a more urban world
3. Changing disease burdens and risks of pandemics
4. Accelerating technological change
5. Continued economic growth?
6. An increasingly multipolar world
7. Intensified global competition for resources
8. Growing pressures on ecosystems
9. Increasingly severe consequences of climate change
10. Increasing environmental pollution
11. Diversifying approaches to governance.

Some of them can have a significant impact on the spread, survival, and reproduction of microscopic fungi. In Hungary, this change is particularly significant as it is located in one of the most vulnerable and geographically isolated regions of Europe, the Pannonian Biogeographical Region (PBR). The presence of these fungal species in our environment is not negligible, as their toxin-producing ability and other properties (flying spores, volatile organic compounds, infectivity) raise serious phytosanitary, animal and human health issues.

In this doctoral work, we aimed to review microfungal taxa associated with global megatrends in PBR, to fill the knowledge gaps related to the introduction of the most common fungi, and to investigate and discuss their complex effects on the local population and the environment. We have highlighted five megatrends, namely: “*Towards a more urban world*”, “*Changing disease burdens and risks of pandemics*”, “*Accelerating technological change*”, “*Continued economic growth?*” and “*Increasingly severe consequences of climate change*” (2, 3, 4, 5, and 9), which may affect the range, reproduction, and toxin production of microscopic fungi in the PBR. Approached from a mycological point of view, we have merged and redefined the above listed megatrends in order to focus on the processes that directly affect the mircoscopic fungi. The new names of the megatrends are **globalization**, **technological development**, and **climate change**. The megatrend of globalization is the accelerated transport of passengers and goods worldwide, including online shopping and the increasing burden of epidemics (which in the case of fungi has so far mainly affected flora and fauna). The "*Continued economic growth?*" and “*Changing disease burdens and the risks of pandemics*” are closely intertwined. According to studies by EHRENFELD (2003) and HULME (2009), one of the main drivers of globalization is the growth of economic revenues. Technological developments are creating new materials and modern microenvironments that support the colonization of fungi. The megatrend of urbanization (“*Towards a more urban world*”) can be combined with technological development in the sense that urbanization, the metropolitan way of life, creates habitats with new conditions for fungal species. Climate change will allow alien fungal species to colonize new natural habitats through global warming and extreme weather events.

Aims:

- Investigation of fungal contamination of imported goods and packaging materials which have tropical and subtropical origin
- Investigation of modern household appliances providing habitat for extremotolerant fungi: washing machines, bottled water dispensers, other devices
- Examination of new building materials, eg. plasterboard, MMVF, OSB
- Aerobiological examination of indoor environments made with new technology, eg. salt rooms, passive houses
- Investigation of the thermotolerance of fungal species introduced from tropical and subtropical regions, taking into account the climate forecasts for Hungary.

2. Materials and Methods

In order to achieve the objectives of the doctoral work, different environmental samples (water, air, surface and material samples) were collected for cultivations in connection with the above mentioned megatrends. Concentration data were counted from the number of colonies, and each strain was identified based on their morphological characteristics and molecular methods. Morphological identifications were performed under 300× magnification of a light microscope. For the DNA-based molecular identifications, the marker genes ITS, TEF, calmodulin, and β-tubulin were determined by conventional Sanger sequencing techniques. Isolated strains were placed in Hungarian and international culture collections. We have supplemented our collections with megatrend-related literature data published since 2001 in order to obtain a more comprehensive picture of the effects of global megatrends on the spread and occurrence of molds and yeasts in the PBR. Additional tests were also performed, which are described in the following subsections. In the following, the methodological descriptions are grouped around the studies that can be linked to each megatrend.

2.1. Research on the megatrend of globalization

In connection with the megatrend of globalization, we examined the fungal contamination of imported goods from subtropical and tropical regions, primarily fruits, spices and packaging materials. For this, material samples were collected with a sterile scalpel under the pre-disinfected epicarp tissue from fruits, and with a sterile cotton swab from spices and packaging materials.

2.2. Research on the megatrend of technological developments

In connection with the megatrend of technological development, we examined the fungal contamination of modern wet household appliances. A total of 61 washing machines operated in Budapest households were sampled. Samples were collected with sterile cotton swabs mainly from the detergent dispensers, rubber seals surfaces. A total of 31 bottled water dispensers (BWDs) were sampled in healthcare facilities. Water samples were collected with opening the tap. Swab samples were also collected from the taps and drip trays. Besides the above mentioned, other water-connected equipments (eg split air conditioner, dehumidifier) and modern building materials (eg MMVF, plasterboard) were also sampled with a sterile cotton swab. We also investigated the fungal contamination of the indoor air from special rooms built with modern technology. Air samples were collected with an Andersen-type sampler (MAS-100 Eco, Merck Millipore). Air sampling was performed in a total of 21 salt rooms and 15 passive houses.

Additional studies were performed on washing machines and BWDs in order to shed light on the relationships between fungal contamination and possible usage patterns as well as environmental parameters. A questionnaire was used to assess usage patterns and examined the conditions under which the devices were stored, operated, and cleaned. In the case of BWDs, the physical, chemical and biological water quality characteristics of the water from the device were also investigated. In the case of washing machines, tolerance tests were performed with the 5 most frequently isolated human pathogenic fungal species, during which we examined the effect of various parameters (specific to washing machines) on the growth of the selected fungi. Temperature (25 °C; 37 °C; 50 °C; 22 hours at 25 °C/2 hours at 40 °C and 22 hours at 25 °C/2 hours at 60 ° C), pH (2.09; 4.10; 7.00; 8.36 and 10.88) and halotolerance (NaCl concentrations: 0% (a_w=0.99); 3% (a_w=0.97); 6% (a_w=0.95), 9% (a_w=0.93), 12% (a_w=0.91)) were tested. We selected one filamentous fungal strain belonging to the *Fusarium oxysporum* species complex (FOSC) and four yeast strains (*Candida parapsilosis, Meyerozyma guilliermondii, Cystobasidium sloffiae* and *Cutaneotrichosporon dermatis*). During the 6-day experiments, the growth of fungal strains was measured in every 24th hours. In the case of yeasts, the average number of fungal elements was calculated from the optical density values (OD) previously measured from the broth inoculated with the fungus using a SPECTROstarNano (BMG LABTECH) spectrophotometer at a wavelength of 620 nm. In the case of the filamentous fungus, an increase in colony diameter (mm) was measured.

2.3. Research on the megatrend of climate change

In connection with the megatrend of climate change, we examined the growth of 10 fungal strains of tropical origin introduced with a commercial product at temperature values set according to climate scenarios. Two *Cladosporium* strains common in Hungary were chosen as controls (**Table 1**).

Table 1 The fungal strains tested under climate scenarios

Fungal taxa	Type of product	Origin of product
Aspergillus flavus	chili pepper	India
Aspergillus niger	cable drum	India
Aspergillus tubingensis	pomegranate	Israel
Aspergillus tubingensis	pomegranate	Israel
Aspergillus tubingensis	pomegranate	Israel
Fusarium musae	bio banana	Dominican Republic
Fusarium ananatum	pineapple	Costa Rica
Fusarium bubalinum	dragon fruit	Vietnam
Penicillium adametzioides	pomegranate	Israel
Talaromyces albobiverticillius	pomegranate	Israel
Cladosporium cladosporioides	common ragweed	Hungary
Cladosporium xylophilum	common ragweed	Hungary

For the experiment, we selected the RCP 4.5 and RCP 8.5 emission scenarios adapted to Hungary by PONGRÁCZ et al. (2016), of which RCP 4.5 represents a moderately and RCP 8.5 a strongly pessimistic scenario. Based on these, a one-week heat wave was modeled in Binder KT53, 9020-0311 (Din12880) type thermostats (**Table 2**). The colony diameters (mm) of the fungal strains were measured every 24 hours.

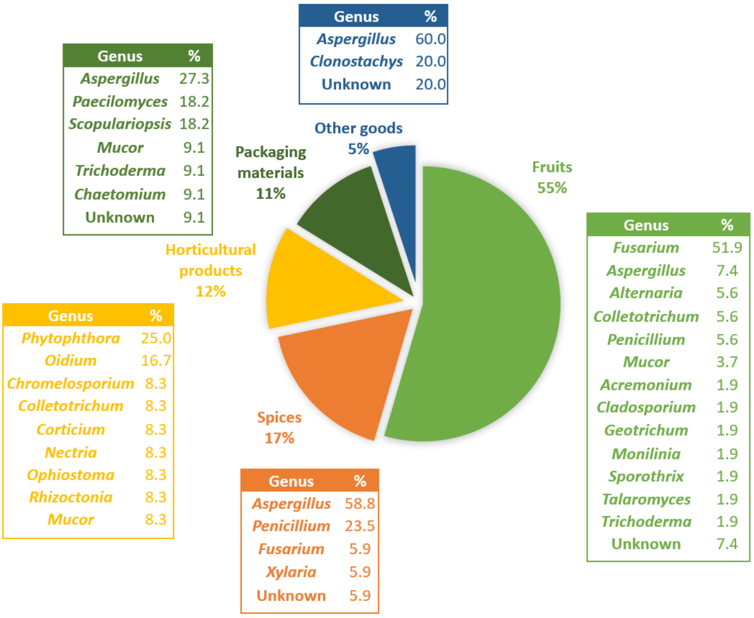
Table 2 Temperature values set for the experiment

Time intervals (h)		0-6	6-12	12-18	18-24
Temperature values (°C)		minimum	average	maximum	average
Treatments	Control	20	21.5	30	21.5
	RCP 4.5	22	27	35	27
	RCP 8.5	24	30	40	30

3. Results and discussion

3.1. Results concerning the megatrend of globalization

A total of 106 samples were collected from goods imported into the PBR, mainly of tropical origin, of which 99 samples contained one or more fungal species. A total of 54 fungal strains were isolated, which were supplemented with 45 additional occurrence data from literature. Of these, 47 fungal isolates come from tropical fruits, 7 from other imported fruits, 17 from spices, 12 from horticultural products, 11 from wood and paper packaging materials and 5 from other products (primarily coffee and tea) (**Figure 1**).



Genus	%
Aspergillus	60.0
Clonostachys	20.0
Unknown	20.0

Genus	%
Aspergillus	27.3
Paecilomyces	18.2
Scopulariopsis	18.2
Mucor	9.1
Trichoderma	9.1
Chaetomium	9.1
Unknown	9.1

Genus	%
Fusarium	51.9
Aspergillus	7.4
Alternaria	5.6
Colletotrichum	5.6
Penicillium	5.6
Mucor	3.7
Acremonium	1.9
Cladosporium	1.9
Geotrichum	1.9
Monilinia	1.9
Sporothrix	1.9
Talaromyces	1.9
Trichoderma	1.9
Unknown	7.4

Genus	%
Phytophthora	25.0
Oidium	16.7
Chromelosporium	8.3
Colletotrichum	8.3
Corticium	8.3
Nectria	8.3
Ophiostoma	8.3
Rhizoctonia	8.3
Mucor	8.3

Genus	%
Aspergillus	58.8
Penicillium	23.5
Fusarium	5.9
Xylaria	5.9
Unknown	5.9

Figure 1 Fungi isolated from imported goods, mainly of tropical origin grouped at the level of genera and commodities

Most of the identified strains belong to the genera *Aspergillus*, *Fusarium* and *Penicillium*, and species of *Chaetomium*, *Mucor*, *Paecilomyces*, *Scopulariopsis* and *Trichoderma* have also been detected. 62.5% of the examined tropical fruits imported to Hungary were infected with some kind of microscopic fungi. The majority of the identified strains from tropical fruits belonged to the storage molds which cause deterioration (*Fusarium-, Aspergillus-, Penicillium-* and *Mucor*-species), and to a lesser extent (24%) other microscopic fungal strains (*Alternaria, Colletotrichum, Acremonium, Cladosporium, Geotrichum, Monilinia, Sporothrix, Talaromyces* and *Trichoderma* spp.) were also identified. The sampled spices were mainly contaminated with *Aspergillus-* and *Penicillium*-species. *Aspergillus-, Chaetomium-, Paecilomyces-, Scopulariopsis-* and *Trichoderma*-species were isolated from packaging materials.

3.2. Results of fungal colonization associated with the technological megatrend

3.2.1. Fungal contamination of washing machines

A total of 71 fungal strains were isolated from the 61 washing machines tested; 32 yeast and 39 filamentous fungi were detected. By microscopic analysis, 8 genera were isolated based on their morphological properties (*Acremonium, Aspergillus, Fusarium, Geotrichum, Mucor, Rhizopus, Scolecobasidium,* and *Trichoderma*). Based on the sequenced DNA samples, 22 different filamentous fungi and 8 different yeast species were identified. The most frequently isolated taxa identified by molecular methods were *Acremonium sclerotigenum, Aspergillus insuetus, A. jensenii, A. niger, Candida orthopsilosis, C. parapsilosis, Cladosporium halotolerans, Cutaneotrichosporon dermatis, Cystobasidium slooffiae, Exophiala* sp., *Fusarium oxysporum, F. solani, F. fujikuroi, F. proliferatum, Meyerozyma guilliermondii, Penicillium chrysogenum, P. terrigenum, P. viridicatum, Rhodotorula mucilaginosa* and *Trichoderma orientale* (**Figure 2**).

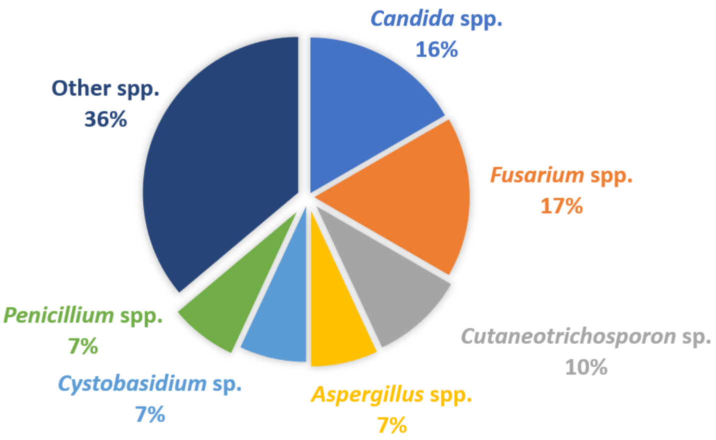


Figure 2 Fungal strains isolated from washing machines at the level of genera

The number of fungal species found in washing machines has been influenced by a number of environmental factors and usage patterns. The presence of the genus *Penicillium* showed a significant negative correlation with the insulation of buildings: the incidence of the genus *Penicillium* was six times higher in partially insulated houses compared to fully insulated ones (OR=6, CI: 0.9832–49.7003, p=0.0613) and eight times higher in uninsulated houses (OR=8, CI: 1.5301–62.0262, p=0.0216). The number of fungal species was higher in washing machines placed in kitchens (mean: 3.33) than in bathrooms (mean: 1.74) or in other rooms (mean: 2.00) (OR=1.9, CI: 0.9273–3.5203, p=0.0544), however, only 6% (n=4) of the users placed the washing machine in the kitchen. The genus *Penicillium* was significantly less common in washing machines from which visible contamination was regularly removed by mechanical or chemical methods (OR=0.14, CI: 0.0226–0.7134, p=0.0214). Significantly more *Cladosporium* strains were isolated from front-loading washing machines than from top-loading washing machines (OR=3.9, CI: 1.0031–17.2016, p=0.0551). The explanation for this is presumably that the presence of fungal species in washing machines is highly dependent on the ability of the fungus to tolerate the dry surfaces. The often remaining stagnant water in the door seals of front-loading devices, provide the preferred conditions for these fungi. 30% of users keep their washing machine in unheated rooms, where the number of species was significantly higher inside the devices (mean: 2.10) (OR=2.39, CI: 1.3700–3.9296, p=0.0011) than in those stored in a heated place (average: 1.72). This is probably due to the fact that the internal surfaces of the devices dry out more slowly at lower temperatures.

None of the strains tested were able to grow at 50 °C. The two-hour daily treatments at 40 and 60 °C inhibited their growth (**Figure 3**). The growth of the tested strains was inhibited by the highly acidic (pH: 2.09) and highly alkaline pH (pH: 10.88), but the slightly alkaline treatment (pH: 8.36) did not inhibit their growth (**Figure 4**). The strains tested were able to grow at the salt concentration values we set, however, the higher concentrations delayed and reduced their growth (**Figure 5**).

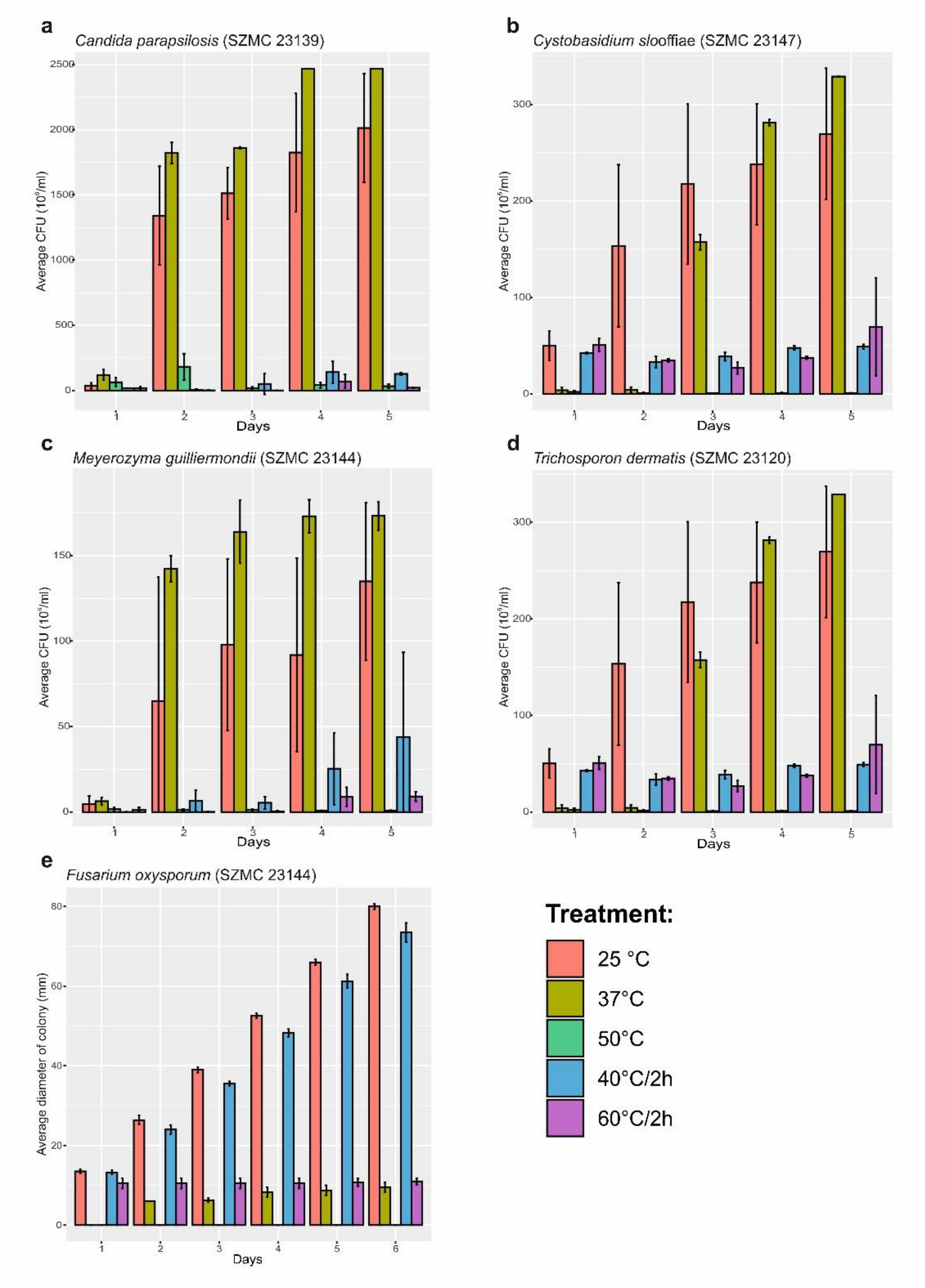


Figure 3 Results of the temperature tolerance tests for four yeasts and one filamentous fungal strains (x-axis: days, y-axis: average number of fungal elements * 10^6/ml (average colony diameter (mm) for e figure))

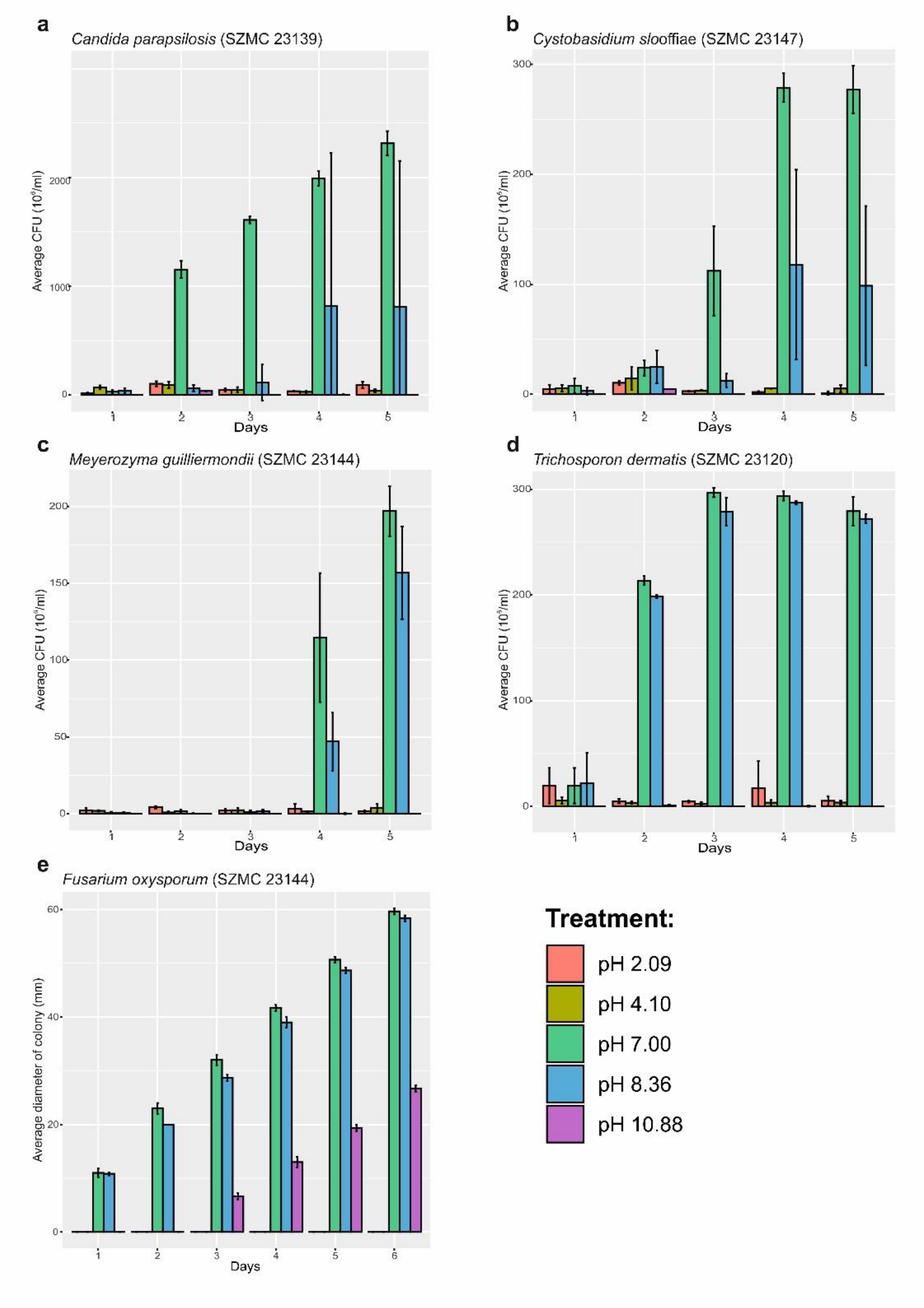


Figure 4 Results of the pH tolerance tests for four yeasts and one filamentous fungal strains (x-axis: days, y-axis: average number of fungal elements * 10^6/ml (average colony diameter (mm) for e figure))

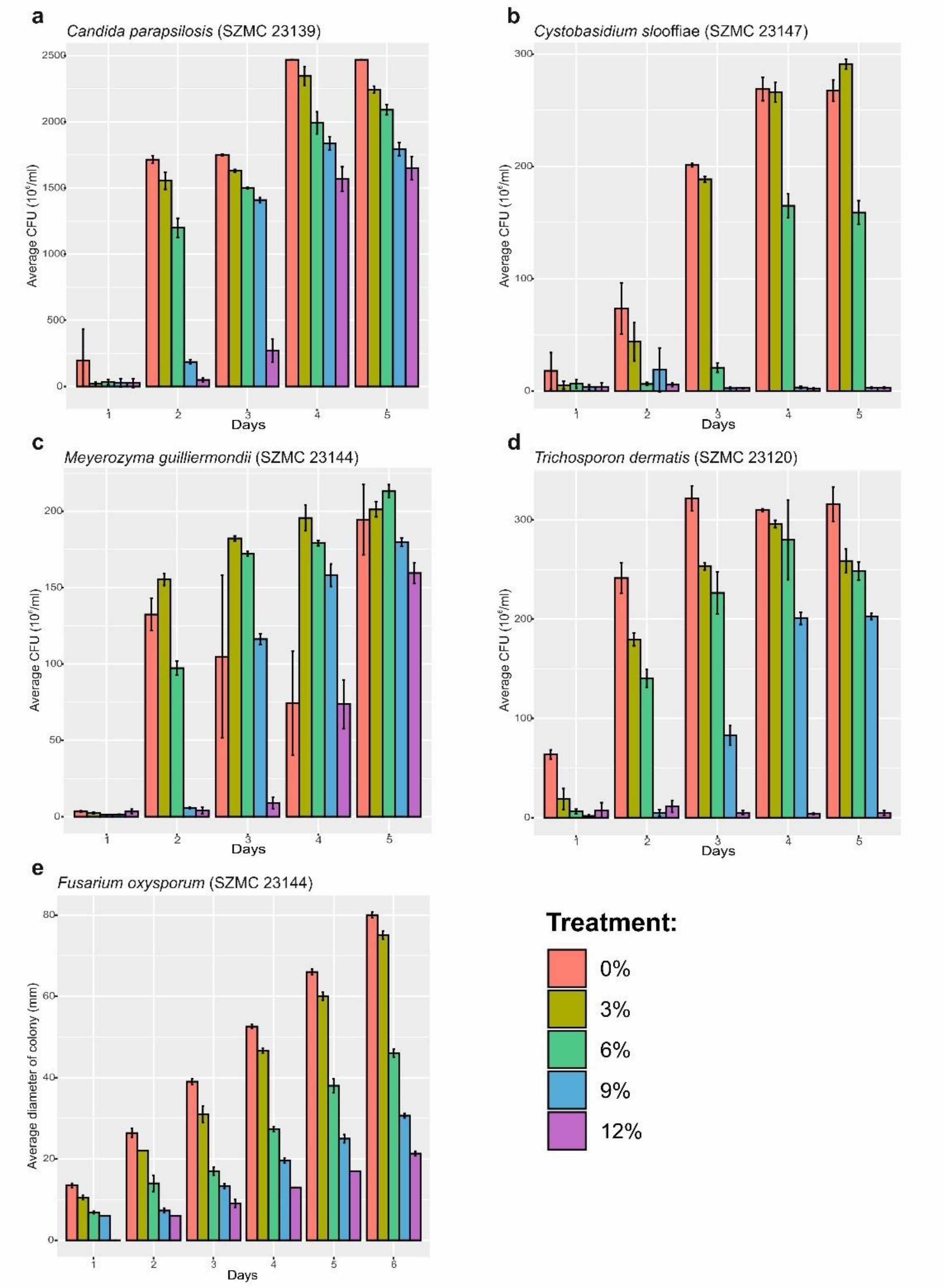


Figure 5 Results of the halotolerance tests for four yeasts and one filamentous fungal strains (x-axis: days, y-axis: average number of fungal elements * 10^6 /ml (average colony diameter (mm) for e figure))

3.2.2. Fungal contamination of bottled water dispensers

56.76% of the water sampled from BWDs was highly contaminated with fungi. A high concentration of filamentous fungi was detected in 51.35% and a high yeast concentration of was detected in 29.73% of the examined water samples from the devices. A total of 36 and 38 fungal taxa were identified from the water and swab samples, the most common genera were *Acremonium* spp., *Cladosporium* spp., *Cystobasidium* spp., *Penicillium* spp., *Rhodotorula* spp. and *Simplicillium* spp. (**Figure 6**).

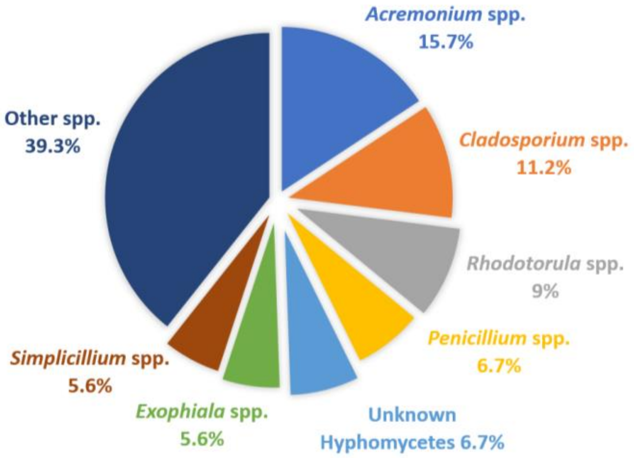


Figure 6 Fungal strains isolated from bottled water dispensers at the level of genera

The main factors affecting the total fungal count were pH, time since last maintenance or disinfection, and water temperature. The number of filamentous fungi in the water samples was significantly correlated with the number of yeasts (p=0.0002, R=0.5), as well as with the filamentous fungi of the swab samples collected from the taps of BWDs (p=0.0063, R=0.53). The number of filamentous fungi in the water samples was also significantly correlated with the expiration date of bottles, last maintenance, water pH, and other parameters. The logarithmic number of yeasts in the water samples was significantly correlated with nitrite concentration (p<0.0001, R=0.7), number of filamentous fungi (p=0.0001, R=0.5) and pH (p=0.0252, R=0.33). Detectable nitrite had a positive, solar radiation during storage had a negative effect on filamentous fungi. The number of yeasts was affected negatively by additional disinfection by staff in addition to mandatory maintenance.

3.2.3. Results of other studies related to the technological development megatrend

In addition to washing machines and BWDs, we also examined the fungal contamination of other household water-connected appliances. Of these, it is worth highlighting dehumidifiers and split air conditioners, as contamination is generated by a relatively similar mechanism. Moisture condensed from the intake air meets the dust from the air and its spore content. This leads to mold parts that come in contact with moisture. 42.9% of the isolates from the studied split air conditioners (n=7) were *Alternaria* spp., The rest were *Aspergillus* sect. *Flavi*, *Cladosporium* sp., *Scolecobasidium* sp. and *Trichoderma* sp. In the case of dehumidifiers, 27.8% of the isolated fungi (n=18) were *Penicillium* spp., 11.1% were *Cladosporium* spp. and 11.1% were *Samsoniella* spp. Furthermore *Alternaria-, Aspergillus-, Byssochlamys-, Chaetomium-, Nothophoma-, Pithomyces-,* and *Rhodotorula*-species were also isolated. Of these, *Aspergillus, Byssochlamys, Chaetomium* and *Penicillium* spp. might cause indoor wall mold. Dehumidifiers are often used in rooms where mold is present due to high humidity. In contrast, split air conditioners are mainly colonised by fungi with outdoor origin.

We have investigated the fungal contamination of indoor air from special rooms built with modern technology. In total, 29 salt rooms were examined and 18 fungal genera were detected from the indoor samples. The highest concentrations of *Cladosporium* spp., *Penicillium* spp., *Paraengyodontium album* and *Aspergillus* spp. were found in the observed salt rooms. The majority of the salt rooms were contaminated with fungi from the outdoors, but our results indicated a smaller proportion of indoor pollution. In these cases the contamination was caused by *Cladosporium* spp., *Paraengyodontium album, Penicillium italicum, Penicillium* spp. In addition to the salt rooms, we also examined the indoor air of 15 passive houses built with modern technology. There were 14 fungal genera present in the indoor air samples; the highest concentrations of *Cladosporium* spp., *Aspergillus* sect. *Nigri* and *Penicillium* spp. were found. Fungi detected from the indoor air of passive houses are also predominantly of outdoor origin, but in 4 cases of fungal contamination of indoor origin were detected. In these cases, the contaminants were *Aspergillus* sect. *Nigri*, *A. sydowii*, *Cladosporium* and *Penicillium* spp.

In connection with the megatrend of technological development, we examined new types of building materials, where the contamination was caused by *Penicillum*-species (16.4%), *Aspergillus*-species (9.8%) and other fungi (eg *Acremonium, Chaetomium, Cladosporium, Scolecobasidium* sp.). It is important to highlight, that their material is quite different, therefore the results cannot be generalized. For example, in the higher abundance MMVF samples, *Penicillium*-species were predominantly detected (75%, n=8). In contrast, no dominant genera were found among fungi isolated from plasterboard (n=8), but it can be concluded that *Aspergillus*-, *Dicyma*-, *Stachybotrys*- and *Chaetomium*-species (oral report by D. MAGYAR) are relatively common. Our results are in line with previous studies, the fungal taxa we isolated have also been detected on plasterboard in other countries (GRAVESEN et al. 1999, MENSAH-ATTIPOE 2016, BRAMBILLA and SANGIORGIO 2020). Fungal species were also detected from plastic wallpapers (n=4), ceramic tiles (n=5), linoleum (n=3) and silicone sealants (n=3) in a variety of compositions.

3.3. Results on the climate change megatrend

All the fungal strains tested were able to grow on the moderately pessimistic RCP 4.5 treatment. All *Aspergillus* sect. *Nigri* and *Flavi* strains showed significantly higher growth compared to the control from the first day of the experiment. On the contrary, the growth of *Fusarium, Penicillium, Talaromyces* and the two *Cladosporium* strains selected as controls was significantly lower than that of the control group after this treatment. Among the latter, the growth of *Penicillium, Talaromyces* and control strains was the weakest. Similar to the RCP 4.5 model, we also observed a significantly higher growth of all *Aspergillus* sect. *Nigri* strains from day 1 onwards. Among them, for *A. tubingensis* strains isolated from pomegranate imported from Israel, this increase was significantly higher than that observed in the RCP 4.5 treatment. Although the *A. niger* strain isolated from Indian cable drum also showed significantly better results ont he pessimistic RCP 8.5 treatment than the control, its growth did not exceed the growth of the RCP 4.5 treatment. The growth rate of *A. flavus* (isolated from chilli pepper imported also from India) was approximately the same as the control group. The *Fusarium, Penicillium, Talaromyces* and the two control *Cladosporium* strains showed no growth at all during the whole period of this treatment (**Figure 7**).

Based on our results, we assume that the introduced *Penicillium, Talaromyces* and the native *Cladosporium* strains used as controls will be reduced in the future climate predicted by the models in the PBR. The spread of *Fusarium* strains from tropical fruits in the PBR is unlikely to be inhibited by the future climate. The growth of *Aspergillus* strains was clearly favourably affected by the predicted temperatures based on climate models. The introduced strains could establish stable populations in the PBR, their genes may be mixed with the genes of the native strains, which may result in the appearance of strains with new traits (STENLID 2002, STEENWYK et al. 2020). These traits may also include temperature tolerance and mycotoxin production.

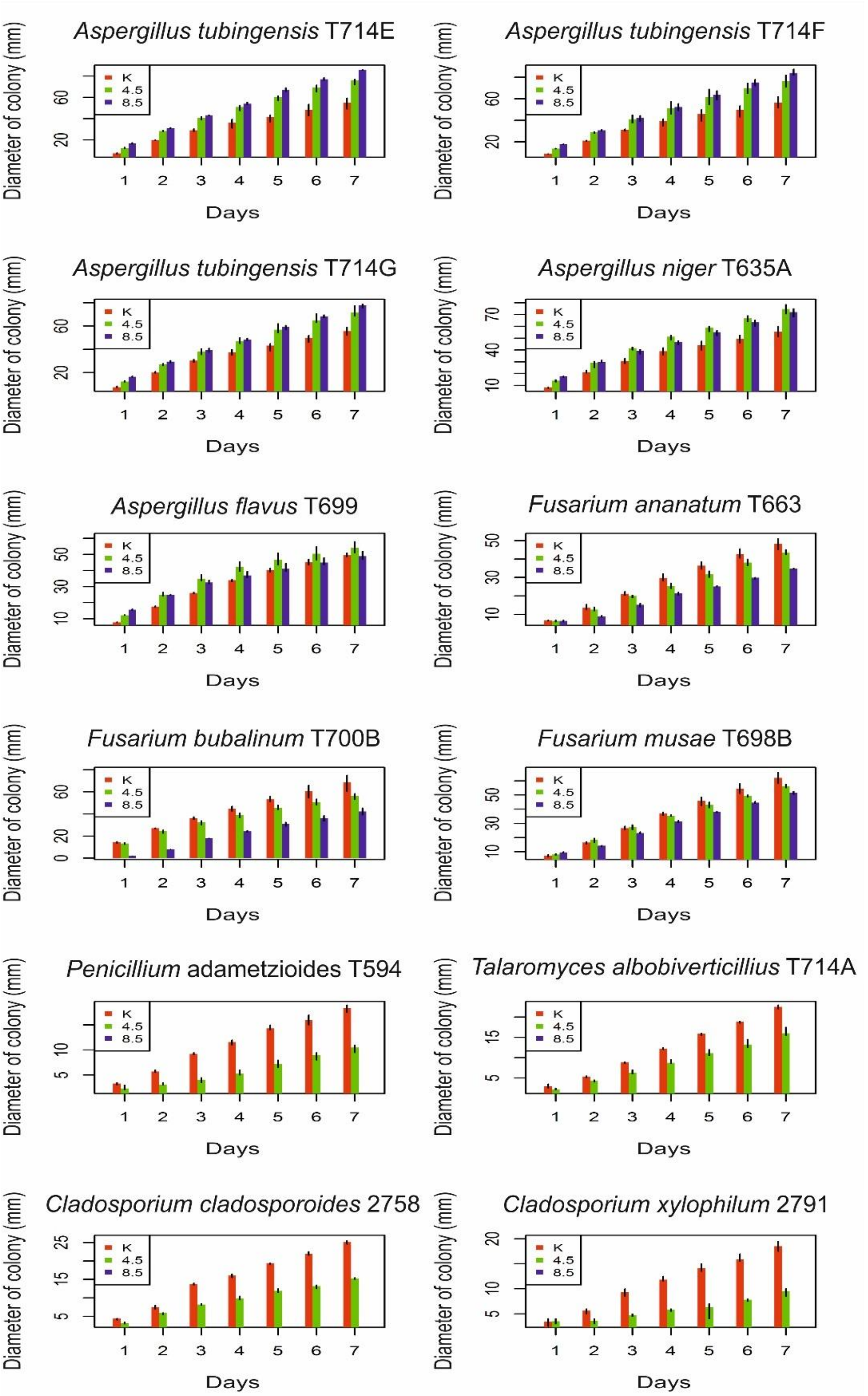
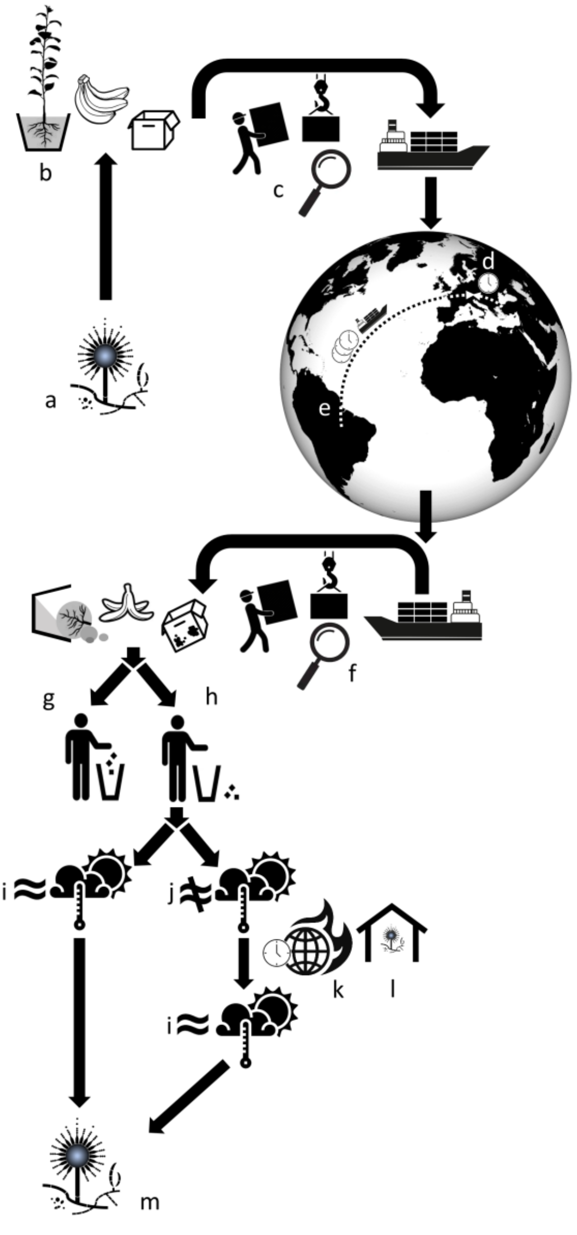


Figure 7 Growth of the tested fungal strains during the experiment (red: control, green: RCP 4.5 treatment, blue: RCP 8.5 treatment)

4. Conclusion and proposals

The unwanted, sometimes invasive spread of animal and plant species, microorganisms, including microscopic fungi, has become a common problem worldwide. Biogeographical barriers to the spread of species have been removed, and fungi can be easily transported from one place to another by cargo and travellers. Evidently, air currents, migratory birds, etc. also play an important role in the long-distance dispersal of spores (MAGYAR et al. 2016). Climate change and modern technology are creating new opportunities for these species to survive, establish new populations, become invasive or even cause epidemics in introduced locations. Global megatrends can influence the spread of various toxin-producing, pathogenic and allergenic molds and species (**Figure 8**). The emergence of these species in PBR places additional burdens on authorities in terms of preparation, planning, organisation and prevention (LÁSZLÓ 2013). Emerging fungal diseases require the development of a practical guide to facilitate adaptation to the negative impacts of global megatrends, for which our recommendations may be useful. The groups concerned are consumers, tourists, farmers, researchers, industry stakeholders and policy makers. Increased emphasis on plant protection and food safety is needed to ensure that global megatrends do not lead to public health problems in the PBR.

Our results and the literature show that the impacts of global megatrends on fungi are complex, making it difficult to predict future trends. Complexity itself can be considered also a risk factor. The links between globalization and climate change are not yet well understood, making it difficult to predict their outcomes (RAMSFIELD et al. 2016). One of the main drivers of globalization is technological progress, which is highly dependent on income growth (EHRENFELD 2003, HULME 2009). As a result of economic growth, accelerating world trade can introduce large amounts of alien fungal spores into the PBR. As a consequence of rising temperatures, newly introduced tropical/subtropical fungi can easily spread in temperate regions (GILARDI et al. 2018).



a: native fungi in the exporting country (e.g.: tropics),
b: exported goods (e.g.: potted plants, banana, packaging material),
c: loading cargo and phytosanitary inspections,
d-e: logistics (**G**) **d:** short distance transport, **e:** long distance transport,
f: unloading imported cargo in the PBR and phytosanitary inspections,
g: residuals of imported goods (soil, banana peel, waste) and proper waste management,
h: improper waste management,
i: the climate of the exporting country is similar to the PBR's current climate,
j: the climate of the exporting country is not similar to the PBR's current climate,
k: by time, due to climate change, the climate of PBR becomes similar to the climate of the exporting country (**C**).
l: the introduced fungi survive in reservoirs: microhabitats, such as: greenhouses, household appliances that are products of modern technology (**T**).
m: Fungi waiting in reservoirs until adequate conditions to establish their outdoor populations

Figure 8 The complex effects of global megatrends (G-globalization, T-technological development and C-climate change) on the spread of microscopic fungi in the Pannonian Biogeographical Region (PBR) (MAGYAR et al. 2021).

Based on the results of the dissertation, the impacts of global megatrends on microscopic fungi and recommendations for risk reduction were formulated:

Globalization

1. ***Increase the share of local products***
2. ***Centralised repackaging.*** Repackaging of smaller products at the point of entry after entry into the European Union.
3. ***Extension of monitoring.*** Inclusion of organic products of tropical origin in the phytosanitary risk assessment monitoring programme.
4. ***Public information on the hygienic handling of tropical fruit.*** It is important to wash hands and avoid reaching into the eyes after touching or peeling tropical fruits.
5. ***Information on the correct disposal of tropical fruit waste.*** Tropical fruit waste should not be disposed in the natural environment, but placed in the designated waste containers. Transfer of knowledge in public education.
6. ***Raising consumer awareness.*** Reduction of food waste.

Technological development

1. ***Educating architects.*** It is important to further educate architects, to expand knowledge of health aspects and to incorporate it into construction training.
2. ***Educating design engineers.*** Health education should be introduced into the training of design engineers. Design should aim to ensure that wet surfaces, which are prone to contamination, are accessible, cleanable and dryable. Surfaces should be free from dripping and should not form aqueous areosols.
3. ***Information to the public.*** Ensure drying out of wet cells, e.g. ventilation, heating, wiping surfaces dry, drainage; also professional cleaning, limescale and biofilm removal, disinfection, boiling. Water replacement.

Climate change

1. ***Achieving mitigation objectives.*** Aim to meet the 1.5°C limit set by the Paris Agreement (UNFCCC 2015).
2. ***Following the "One health" concept.*** Be prepared for the expected rise of mycotoxin-producing fungi and the increased risk they pose for plant protection and food safety.
3. ****Following NÉS-2 climate policy.*** Education and learning of adaptation strategies in each sectors.

*It is of utmost importance to know and comply with the mitigation guidelines, adaptation strategies and climate education contained in the Parliamentary Decision 23/2018 (X. 31.) on the Second National Climate Change Strategy (NÉS-2), which defines the climate policy of our country. The Food Chain Security Strategy (FCSS), adopted by the government in 2013 and described in the resolution, highlights the threats of global environmental and climate change to agriculture: "A changing climate could lead to the spread of new food pathogens, new weeds and mycotoxin-producing molds. Pests from other continents pose a particular threat to the growing and natural environment, and the risks have recently increased significantly as a result of global trade and climate change." Efforts should be made to implement the actions set out in the FCSS; the agricultural sectors should be prepared, for the expected emergence of mycotoxin-producing fungi and the emergence and management of increased plant protection and food safety risks.

5. New scientific results

THESIS I. (based on the results presented in chapter 3.1.):

We have demonstrated by culture and molecular taxonomic studies that a significant amount of microscopic fungi arrives with goods (primarily fruits) imported to Hungary from tropical and subtropical regions. The majority of the strains identified by us and in the literature belonged to the group of spoilage pathogens (*Fusarium* sp. (51.9%), *Aspergillus* sp. (7.4%), *Penicillium* sp. (5.6%) and *Mucor* sp. (3.7%)); but also to a lesser extent (24%) other microscopic fungal strains (*Alternaria, Colletotrichum, Acremonium, Cladosporium, Geotrichum, Monilinia, Sporothrix, Talaromyces* and *Trichoderma* spp.) were detected. The sampled spices were contaminated with *Aspergillus*- and *Penicillium*-species, while strains of *Aspergillus, Chaetomium, Paecilomyces, Scopulariopsis,* and *Trichoderma* were isolated from the surface of the packaging materials.

THESIS II. (based on the results presented in chapter 3.2.1.):

Examining the effects of the technological development megatrend, we were the first in Hungary to measure the fungal contamination of washing machines and its possible causes. Of the 61 washing machines tested, 53 were contaminated with microscopic fungi, and 32% of the machines were heavily contaminated. We proved that the most common species in washing machines in Hungary are *Candida parapsilosis* (12.5%), *Fusarium oxysporum* (8.3%), *Cystobasidium slooffiae* (7%), *Cutaneotrichosporon dermatis* (7%), *Fusarium solani* (4, 2%), *Meyerozyma guilliermondii* (4.2%) and *Rhodotorula mucilaginosa* (4.2%) and *Candida orthopsilosis* (3%).

THESIS III. (based on the results presented in chapter 3.2.1.):

Using sampling and a questionnaire based survey, we have demonstrated for the first time that microscopic fungal contamination of washing machines is significantly influenced by certain usage patterns. The number of species was significantly higher in appliances that were stored in unheated rooms and where deposits were observed in detergent dispensers. Significantly more *Cladosporium* sp. were found in front-loading washing machines, as stagnant water often remains in the door seals of these appliances, which provides preferred conditions for these fungi. Tolerance tests have shown that certain fungal strains isolated from washing machines can be reduced by high temperature treatment, acidic detergents and drying out of wet surfaces (detergent dispensers, rubber seals).

THESIS IV. (based on the results presented in chapter 3.2.2.):

We have been the first in Europe to demonstrate by culture and molecular taxonomic studies, that bottled water dispensers in healthcare facilities are highly contaminated with microscopic fungi. Of the 36 bottled water dispensers tested, 86.8% were contaminated with microscopic fungi. 56.8% of the water samples were highly contaminated. A high concentration of filamentous fungi was detected in 51.4% of the water samples and a high concentration of yeasts in 29.7%. The most common genera were *Acremonium* spp. (15.7%), *Cladosporium* spp. (11.2%), *Rhodotorula* spp. (9%), *Penicillium* spp. (6.7%), *Exophiala* spp. (5.6%), *Simplicillium* spp. (5.6%), *Aspergillus* spp. (4.5%) and *Cystobasidium* spp. (4.5%).

THESIS V. (based on the results presented in chapter 3.2.2.):

By sampling and questionnaire survey, we have proven for the first time that the fungal contamination of bottled water dispensers in Hungarian healthcare facilities is significantly influenced by certain environmental parameters, as well as storage, usage and maintenance habits. The number of filamentous fungi in the water samples was significantly correlated with the number of yeasts ($p=0.0002$, $R=0.5$) and with the filamentous fungi results of the swab samples collected from the taps of the device ($p=0.0063$, $R=0.53$). The number of filamentous fungi in the water samples was positively correlated with the expiration date of the bottles and the last maintenance time of the appliances, also pH, temperature, total organic carbon content of water, and negatively correlated with the heterotrophic plate count. The logarithmic number of yeasts in the water samples showed a significant correlation with nitrite concentration ($p<0.0001$, $R=0.7$), number of filamentous fungi ($p=0.0001$, $R=0.5$) and pH ($p=0.0252$, $R=0.33$).

THESIS VI. (based on the results presented in chapter 3.3.):

By modelling predicted heat waves in Hungary in 2050, we have shown for the first time that the growth of the investigated *Aspergillus tubingensis* strains of tropical origin is significantly higher under the pessimistic (RCP 8.5) climate scenario than under the more optimistic (RCP 4.5) scenario and the current climate of Hungary. The *A. niger* strain tested grew most intensively under the more optimistic climate model. The growth rate of *A. flavus* strain was not affected, whereas the growth of *Fusarium ananatum, F. bubalinum* and *F. musae* strains was reduced by the applied models. *Penicillium adametzioides* and *Talaromyces albobiverticillius*, also of tropical origin, as well as *Cladosporium cladosporioides* and *C. xylophilum* of indigenous origin, showed no growth in the pessimistic model and little growth in the more optimistic model. We have shown that the projected future climate of our country is likely to show an increase in the prevalence of some fungal strains of tropical origin and a decrease of some indigenous fungi.

6. Publications related to the topic of the thesis

Peer-reviewed, full-text scientific publication in a scientific journal:

Magyar, D., **Tischner, Z.**, Páldy, A., Kocsubé, S., Dancsházy, Z., Halász, Á., Kredics, L., (2021): Impact of global megatrends on the spread of microscopic fungi in the Pannonian Biogeographical Region. *Fungal Biology Reviews*. In Press **IF: 4.806, Q1**

Tischner, Z., Sebők, R., Kredics, L., Allaga, H., Vargha, M., Sebestyén, Á., Dobolyi, C., Kriszt, B. Magyar, D., (2021): Mycological investigation of bottled water dispensers in healthcare facilities. *Pathogens* 10(7), 871. **IF: 3.492, Q2**

Magyar, D., **Tischner, Z.**, Dancsházy, Z., Páldy, A., (2021): A globális megatrendek – világjárványok és globalizáció, technológiai fejlődés és klímaváltozás – hatása a mikroszkopikus gombák terjedésére Magyarországon. *Egészségtudomány* 65(1), 30-37.

Tischner, Z., Kredics, L., Marik, T., Vörös, K., Kriszt, B., Péter, B., Magyar, D., (2019): Environmental characteristics and taxonomy of microscopic fungi isolated from washing machines. *Fungal Biology*, 123(9), 650-659. **IF: 2.789, Q1**

Tischner, Z., Kredics, L., Marik, T., Vörös, K., Magyar, D., (2019): Hazai háztarásokban üzemelő mosógépek gombaszennyezettsége a használati szokások tükrében. *Egészségtudomány*, 1-2, 45-65.

Conference abstracts (in ISBN, ISSN or other certified publications):

Tischner, Z., Kakucs, R., Szigeti, T., Szabó, István., Kriszt, Balázs., Magyar, D., (2021): Aerobiological investigation of fungal and bacterial pollution of salt chambers in Hungarian kindergartens. *Acta Microbiologica et Immunologica Hungarica*, 68, 121-122. ISSN: 1217-8950

Tischner, Z., Kakucs, R., Szigeti, T., Szabó, István., Kriszt, Balázs., Magyar, D., (2021): Magyarországi óvodákban üzemelő terápiás sószobák biológiai légszennyezőinek vizsgálata. *Egészségtudomány*, LXV. évfolyam, 2021/3. szám. ISSN: 0013-2268

Magyar, D., **Tischner, Z.**, Dancsházy, Z., Páldy, A., (2021): Gombák által okozott pandémia lehetőségének vizsgálata a SARS-CoV-2 járvány tapasztalatai alapján. *Egészségtudomány*, LXV. évfolyam, 2021/3. szám. ISSN: 0013-2268

Tischner, Z., Sebők, R., Dobolyi, C., Kriszt, B., Magyar, D., (2019): Fungal Contamination of Bottled Water Dispensers in Health Institutions. 18th International Congress of the Hungarian Society for Microbiology, július 3-5, Budapest, *Acta Microbiologica et Immunologica Hungarica*, 66, 200-201. ISSN: 1217-8950

Tischner, Z., Kocsubé, S., Marik, T., Kredics, L., Dobolyi, C., Kriszt, B., Magyar, D., (2019): A világkereskedelem hatása a mikroszkopikus gombafajok terjedésére és hazai megjelenésére. *Egészségtudomány*, LXIII. évfolyam, 2019/3-4. szám. ISSN: 0013-2268

Sebők, R., **Tischner, Z.**, Bufa-Dőrr, Z., Khayer, B., Sebestyén, Á., Vargha, M., Kriszt, B., Magyar, D., (2019): Bacterial Contamination of Bottled Water Dispensers in Health Institutions. 18th International Congress of the Hungarian Society for Microbiology, július 3-5, Budapest, *Acta Microbiologica et Immunologica Hungarica*, 66, 185-186. ISSN: 1217-8950

Tischner, Z., Kredics, L., Marik, T., Sebők, F., Magyar, D., (2017): Hazai háztartásokban üzemelő mosógépek mikológiai felmérése; diverzitásvizsgálat és patogenitás - Occurrence of microscopical fungi in washing machines kept in Hungarian households; diversity and pathogenicity research. *Mikológiai Közlemények-Clusiana,* 56(1), 141. ISSN: 0133-9095

Tischner, Z., Vargha, M., Magyar, D., (2017): Mikroszkopikus gombák izolálása ballonos vízadagoló készülékekből nyert vizekből - Occurrence of microscopic fungi in water derived from bottled water dispensers. *Mikológiai Közlemények-Clusiana*, 56(1), 143. ISSN: 0133-9095

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