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Mycological control and surveillance of biological waste and compost

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The collection and recycling of biosolids and the organic fraction of municipal solid waste (MSW) is an important factor for the success of the so-called «circle economy» as a component of modern waste management policy.
Composting is one of the major treatment processes used to transform biodegradable wastes (kitchen, garden and industrial waste and sewage sludge) into agriculturally useful products. Composting has several ecological and economical advantages: a) recycling of humigenic materials, to compensate for the important loss of humus in agricultural soils, b) reduction of treatment costs, compared to MSW incineration, c) energy gain (heat and electricity), in the case of pre-methanization, d) substitution of peat [31]. Composting at industrial scale can pose problems of occupational safety, due to the occurrence of aerosols containing allergenic / pathogenic micro-organisms and toxins.
Industrial composting has to be a controlled process, leading to optimal hygienisation and degradation of the biowaste. However, the composting procedures (systems and management) vary greatly and tend to be highly empirical. In addition, the composting waste management authorities often do not impose satisfactory plant performance criteria to the composting industry, particularly for hygienic aspects. In consequence, accidents could occur, which would lead to reject composting as a whole. It is therefore essential to shed light on the exclusion of risks associated with composting, i.e. the public health hazards due to allergenic or pathogenic micro-organisms, and in particular the mold *Aspergillus fumigatus*.

Below is a summation of some recent developments regarding the recent work on hygienic aspects, in particular the presence and dispersion of fungi (e.g. *Aspergillus fumigatus*), of biological waste and compost.
To avoid possible health risks, intervention at several levels is necessary: education of the population about what to put in the green waste container and what not, optimization of the composting process itself and the management of the sites, medical follow-up of compost

workers and the fundamental research on the detection of medically important fungi in biowaste and compost. Composting can be carried out at different scales: in the backyard, in small community composts, or in large centralized facilities. For smaller quantities of biowaste, vermicompost is an interesting alternative. Very little is known, though, about the hygienic aspects of this low-temperature process.

Composting : a microbiological process

Composting is a self-heating, aerobic solid phase biodegradative process of organic waste materials. The composting process at the microbial level involves several interrelated factors, i.e. metabolic heat generation, temperature, ventilation (oxygen input), moisture content, and available nutrients. The temperature both reflects prior microbial activity and the current rate of activity. The initial rapid increase of temperature involves a rapid transition from a mesophilic to a thermophilic microflora. The compost ecosystem then tends to limit itself due to inhibitory high temperatures, resulting from excessive heat accumulation. If a good management is continuously provided (i.e. regular aeration or frequent turning), the thermogenic stage continues until the heat production becomes lower than heat dissipation, due to the exhaustion of easily metabolizable substrates. During the terminal cooling or maturing phase, the amount of readily available nutrients becomes a limiting factor, causing a decline in microbial activity and heat output. During these temperature changes various microbial groups succeed each other, each of which being adapted to a particular environment.
A large variety of mesophilic, thermotolerant and thermophilic aerobic micro-organisms (including bacteria, actinomycetes, yeasts, molds and various other fungi) have been extensively reported in composting and other self-heating organic materials [13,11,9,7]. In function of their degradation potential and their ability to grow at elevated temperatures, they are active at different moments during the process.

Mesophilic microorganisms are partially killed or are poorly active during the initial thermogenic stage (temperatures between 40-60°C), where the number and species diversity of thermophilic/thermotolerant bacteria, actinomycetes and fungi increases. The optimal temperature for thermophilic fungi is 40-55°C, with a maximum at 60-62°C. Fungi are killed or are present transiently as spores at temperatures above 60°C.
Among the fungi, the mold *Aspergillus fumigatus* has a special significance: due to its capacity to degrade almost all components of organic waste (sugars, fatty acids, proteins, cellulose, pectin, xylane, [14]), and its thermotolerance (optimal growth at 37°C, good growth between 30 and 45°C, maximal growth at 52°C [19]), it finds ideal proliferation conditions in young compost. *Aspergillus fumigatus* conidia can survive at temperature of 55-60°C for a fairly long period. Unfortunately, *Aspergillus fumigatus* is also a known opportunistic pathogen and allergen [20].

Thermophilic bacteria are very active at 50-60°C. High temperatures (> 60°C) are often considered to reduce dramatically the functional biodiversity [13]. It is generally assumed that to obtain efficient and rapid decomposition temperatures should not be allowed to exceed 55-60°C. However, at these temperatures the thermohygienization towards potentially pathogenic and / or allergenic microorganisms is not guaranteed.
Recent results demonstrated that in the composts studied temperatures between 65-80°C were usually reached during the thermogenic phase [7, 8]. We showed in these hot composts the presence of a great variety and high numbers (10^7-10^{11} cells/g compost dry weight) of aerobic high thermophilic bacteria growing at temperatures between 60-82°C. These bacteria were

present in all types of waste (green, kitchen, sewage sludge) and industrial systems studied. The demonstrated functional bacterial diversity during the thermogenic phase seems to make it possible to compost at high temperatures (65-75°C) for a longer period of time, but not exceeding 80°C. By that, the composting process can be performed with a better destruction of potential human pathogens and allergenic molds, as well as phytopathogens and seeds.

Comments on proposals for the avoidance of health risks posed by fungi from biowaste and compost

In the newly introduced field of biological waste and composting management it should not be ignored that a variety of allergens and/or pathogens can be found in the raw material of biowaste and compost. These hazards arise from three possible sources of contamination:
a) primary pathogens of intestinal origin (bacteria, virus, intestinal parasite cysts or eggs),
b) secondary or opportunistic allergenic and / or pathogenic forms, mainly molds, developed during compost processing and stocking,
c) bacterial and fungal allergens and toxins.
The first source is linked to the fecal pollution of the raw material. This contamination is maximum for products incorporating high amounts of urban wastewater sludge or farm wastes, lower for household refuse composts and industrially processed composts of vegetable waste. The second hazard results from the development of meso- and thermophilic / thermotolerant fungi and actinomycetes which play a role in biowaste degradation and maturation.

Who knows it better than the mycologist that organic wastes occurring in the household may serve as a nutrient substratum for fungi, among which causative agents of infection, allergy or intoxication may be found. The detection of the soil of potted plants as a source of airborne fungal infections in immunosuppressed patients in the hospital by the medical mycologist and, likewise, of various materials specifically colonized by a variety of allergenic fungal species in the home environment by the allergologist in cooperation with the mycologist, have drawn attention to the ecology of fungi in our everyday life [30].
The role of *A. fumigatus* during the composting process has been a subject of interest for some time [6]. However, other fungi of medical-mycological interest have not been routinely detected in compost facilities [26]. Also, practicable rules and standards for the control of fungi from the side of medical mycology are expected by composting industries.

Already at the XI ISHAM Congress 1991 in Montreal, Beffa et al. reported on the presence of *A. fumigatus* in municipal composting facilities and the possible risks of exposure to *A. fumigatus* for compost workers and neighbors [6]. At the same congress, Staib presented a report on "Pathogenic fungi in human dwellings". He drew attention to the biological waste container as a new source of indoor fungal growth [30]. On the occasion of the XII ISHAM Congress 1994 in Adelaide, there was a report by Staib on "Health risks from decay of organic wastes and compost: Proposals for mycological control" [29]. From the viewpoint of a medical mycologist and microbiologist, 12 proposals were made on a variety of topics: education of the population; the biological waste container; waste processing and fungi of medical interest; composting and fungi, and the role of *A. fumigatus*; immunological control respecting exposure to fungi of medical interest; the need for mycological departments in reference institutions for waste and composting microbiology and their tasks e.g. elaboration of appropriate methods of mycological analysis, procedures for testing the decomposability of newly developed environmentally friendly materials by fungi of medical interest; precautions to avoid spore inhalation; surveillance of wildlife animals and pests in the environment of composting plant; and an international exchange of knowledge gained in this field and the results obtained.

There is an increasing interest in specific proposals addressing citizens and authorities responsible for biological waste and composting management. In the editorial of the Zentralblatt für Bakteriologie, Staib made the following remark: «Unless the subject of fungi in biowaste and compost has been thoroughly examined and publicly discussed by medical-mycological societies and the ISHAM, no rules, standards or regulations should be applied in practice» [27]. Therefore, our special thanks go to the convenor of the XIII ISHAM Congress, Professor L. Polonelli, who corroborated the need of a symposium dealing with the topic of mycological control and surveillance of biological wastes and compost.
In addition to the foregoing basic considerations, some examples from everyday life shall illustrate the need for a medical mycological control.

Education of the population: Independently of the elaboration of a mycological surveillance program with the assistance of ISHAM, and in addition to information by offices of local government, education of the public should start in biology classes in schools. In 1990/91, the Mycology Unit of the Robert Koch Institute in Berlin hold lectures with practical demonstrations before members of the German Society for Teachers of Biology. A teaching program for schools dealing with molds, biological waste and composting was elaborated by these teachers and published in their professional journal [24]. Teaching programs are always welcome from that side. We hope that such education will show the young generation why mycological departments are important in institutions of medical microbiology.

Immune responses to exposure to fungi in biowaste: After a 3-4-month exposure of four HIV-positive men to *Cryptococcus neoformans* (*C. n.*), aspergilli, *Mucoraceae* and *Dematiaceae* found in pigeon droppings and waste, only one of them who exhibited a low count of 50 CD4 lymphocytes / µL (normal count 650-1250 / µL) fell sick and had a systemic course of cryptococcosis, but the others with higher CD4 counts, remained free of *C. n.* and free of infections by the other opportunistic fungi they had been exposed to [4]. This example shows that various types of specific immunodeficiency being decisive for opportunistic infections .

Surveillance for medically interesting fungi among wildlife animals living near waste and composting plant: Cases of cutaneous histoplasmosis in badgers in Germany, i.e. outside of endemic areas of histoplasmosis, have raised the question if biological wastes as feed of badgers and accidental microfocus of *Histoplasma capsulatum* (*H. c.*) may be a source of infection [16]. Research on this topic is under way.

Testing of materials for decomposability by fungi of medical interest: A new field of applied mycology will develop from the proposal No. 11, "In procedures designed to test the decomposability of newly developed environmentally friendly household materials, fungi of medical interest should be included along with other test organisms" [27].
Recently, Dill et al. reported that gardeners in a large German horticultural establishment developed inflammatory toxic reactions on their fingers. They had been exposed to compostable flower pots manufactured from recycled paste-board. A heavy growth of *Stachybotrys chartarum* (a well known mycotoxin producing fungus) developed on these pots [12]. This observation proves the recommendations made in the above mentioned proposal No. 11. It has to be noted that so far in Germany an incident of this kind has to be considered by various government institutions, e.g. the Federal Institute for Testing of Materials, the Federal Environmental Agency, the Federal Institute for Health Protection of Consumers and

Veterinary Medicine, and the Robert Koch Institute - Federal Institute for Infectious and Non-Communicable Diseases. Quite generally, the division of responsibility has resulted in problems.
Materials to be excluded from the biological waste container, e.g. materials of animal and human origin: In connection with the observations of the various opportunistic pathogens in HIV-positive persons by Arastéh et al., it was found that infections by *Cryptococcus neoformans* together with *Mycobacterium avium intracellulare* (*M.a.i.*) occurred if there was a CD4 lymphocyte count as low as 3-20 / µL [5]. Epidemiologically, this predisposition is of interest since in Berlin, Wittstatt found mycobacteriosis to be present in 7.1 % of 1067 psittacine home birds autopsied during the 1988-1996 period. In a third of these cases, *M.a.i.* was the causative agent (which was also excreted in droppings) [32]. It seems therefore logical to exclude droppings of home birds from the biological waste container, according to proposal No. 12 [27]. Compatible *C. neoformans* strains could be isolated from biological waste containing bird droppings. The perfect stage with basidiospore formation on bird filtrate agar could be reached already after 48 hours at 26°C [28]. Based on this observation, it is suggested that all types of morphogenesis of medically interesting fungi and other microorganisms may occur under the various growth conditions in biowaste.

Crusts of human skin lesions: A HIV-positive male person who had a CD4 lymphocyte count of 30 / µL, was diagnosed with histoplasmosis as first opportunistic infection. He had disposed of the crusts of the skin lesions loaded with *H. c.* by putting them into the biological waste container [16]. He had done so as a daily routine over weeks. Thus, attention is drawn on the possible presence of microfoci of *H.c.* outside endemic areas, i.e. sources of infection for persons handling wastes and operating compost processing installations as well as for wildlife animals living in the vicinity of waste handling and composting plant.

Follow-up of compost and waste workers: evolution of immune response to compost dust and *Aspergillus fumigatus*

Positive epidemiological evidence is the best argument to conclude from potential hazard to actual risk. However, precise scientific data are not available at the moment. Emphasis must be placed on possible biases, which may lead to some underestimation of long-term effects. In fact, many compost workers consider their job only as temporally acceptable, while they look for a less hard, better paid and better regard work. Such an unsteadiness makes long-term surveys difficult, many people vanishing before the end of the study. In addition, as soon as some possibly compost-related clinical symptoms are observed, doctors in charge of occupational medicine for the plant immediately request that the employee changes his work for an unexposed environment.

In a preliminary study, the occupational risk factors for workers in municipal composting facilities had been evaluated [17].
As part of a 3 year multidisciplinary pilot follow-up study that began in 1992 in a large composting facility in the Geneva area, we have studied the evolution of the immune response towards a panel of *Aspergillus fumigatus* antigens in a group of ten exposed compost workers (CW) recruited on a voluntary basis. This facility was visited twice per year and each CW was submitted to: i) lung function tests, ii) laboratory investigations, iii) non-invasive microbiological samplings.
Lung functions: Spirographic measures (Vitalograph^{R}) consistently showed a transient weak to moderate decrease of the lung functions after work periods of over 3-4 hours on compost piles. However, no significant deterioration has appeared during the 3 years of the survey.

Immune response: *A. fumigatus* immunity was assessed with a purified precipitate (F27) from the culture filtrate, and various mycelial and purified extracts for both Ig-specific ELISA tests and lymphocyte transformation tests (LTT). The cellular and specific IgG, IgM, IgA and IgE responses toward *A. fumigatus* antigens were compared to the responses observed in 10 *A. fumigatus* allergic patients (AP) and in 10 normal non-exposed subjects non-allergic to *A. fumigatus* (CP). LTT responses were significantly increased in CW and AP subjects in comparison to the responses observed in CP. No statistical significant differences could be observed between CW and AP. Specific IgG and IgA responses were increased in CW compared to CP. Again, no statistical significant differences could be observed between CW and AP. With IgM specific Ab, no differentiation between the three groups could be made (no statistical difference). IgE were present in all AP and could be detected in low amount in most of the CW, with a seasonal fluctuation, though all CW were asymptomatic. No IgE could be found in CP. In newly engaged CW, an hyperimmunity state towards *A. fumigatus* equivalent to that of long-term exposed CW appeared after 9-18 months. The appearance of this immunity was essentially related to the frequency of the exposure *A. fumigatus* isolates: Culture swabs (nose and/or ear external ducts) were positive for *A. fumigatus* in > 90% of CW after direct work on compost piles over 2-3 hours; positive isolates were found in 45% after 15 hours, in 15-20% after 24 hours and were all negative after 48 hours.

Elimination or reduction of pathogenic fungi during composting, in function of the type of system and management

The main objective of the compost research at the laboratory of microbiology of the University of Neuchâtel is to optimize the thermogenic phase of the composting process, in order to maximize the hygienisation (elimination of allergenic and pathogenic micro-organisms). By this, also an efficient degradation of the organic matter and a good maturation of the compost is ensured, and the recolonisation with pathogenic / allergenic microorganisms and the phytotoxicity hazards in the maturation phase are avoided. The research is supported by the Swiss National Foundation (Priority Program Biotechnology, module 5B, biosafety research), and several compost industries.
It should enable to give guidelines to collectivities, industries and privates for the establishment of composting places, for the choice of the composting system , and for its management, in order to warrant a good composting process with a high hygienic security.
The composting systems studied belong to the following types of installations:
- open-air triangular windrows (elongated heaps),
- boxes with automatic aeration and/or turning, roofed or in a closed hall,
- closed bioreactors.

In some composting facilities, very wet and nutrient rich materials, such as kitchen waste or sewage sludge, are treated by methanization before composting.

Undoubtedly, the biggest potential biohazard associated with composting is *A. fumigatus,* an opportunistic pathogen and allergen [2,10,15,22]. It was detected in all composting types investigated. Up to 10^6-10^7 *A. fumigatus* CFU (colony forming unit) were measured in gDW (gram dry weight) compost, and up to 10^6 *A. fumigatus* CFU per m^3 of air were measured at sites where compost was processed.

Research at our laboratory has shown that the presence and abundance of *Aspergillus fumigatus* in the composts and in the air can be taken as a bio-indicator for the presence and dispersal of other potentially pathogenic microorganisms and particles.

Temperatures up to 80°C have been observed in the center of composting heaps. These elevated temperatures are generally considered sufficient for the elimination of pathogenic microorganisms. Extensive measurements carried out at our laboratory showed, however, that important temperature gradients can exist in the compost mass, and that temperatures in the outer or lower zones can be up to 30-40°C below the core temperature. *A. fumigatus* proliferates or persists in these cooler parts. The destruction of *A. fumigatus* spores, which are very heat tolerant, requires quite high temperatures (> 65°C) [3]. Also, recolonisation of the compost by *A. fumigatus* after the thermogenic phase is frequently observed, although concentrations do not reach the initial values. The extent of the recolonisation depends on the compost maturity, which itself depends on the organic matter content of the starting material [25].

An intensive management of the compost is necessary for good hygienisation. The following parameters have to be chosen carefully to assure a correct composting process: composition of the initial substrate (C:N ratio around 30 with a good structure), adjustment of humidity, frequent mixing to redistribute microorganisms and substrate and to bring material to the hot center of the heap, adjustment of aeration (frequency, duration), duration of the process long enough to avoid recolonisation during maturation, or direct use of fresh compost without storage.

But, it is not only the composting process itself that is important, but also how the material is treated before and after it: development of *A. fumigatus* happens already at home, when biodegradable waste is stocked for an extended period of time. For this, waste collection has to happen at short intervals (not longer than 1 week between collections). Once the material gets to the composting site, it should be processed immediately, and not let sit on big heaps for days. This will also help to control odors. Special care should also be given to the finished compost. If ever possible, treatment should be continued, although at more infrequent intervals, until the compost is used, mostly for agricultural purposes. If for shortage of space this cannot be done, the compost should be stored unsifted in not too big heaps, to allow a minimal natural ventilation.

<u>Composting in open-air triangular windrows</u>: When comparing the results from different sites that compost in open-air triangular windrows, it seems that there are two main factors that influence the proliferation of *A. fumigatus*: the turning frequency, and the degree of humidity of the compost. These two parameters are in fact coupled: effective watering of the compost is only possible during turning.
Spore counts in compost were effected at an intensive managed facility, where the windrows were turned daily with a specialized machine, and compared to those of an extensively managed facility where turnings were performed only every third week with a front end loader. Although the core temperatures measured in the windrows during sampling were about the same (70°C to 77°C for the fresh composts and 63°C to 70°C for the composts of several weeks of age), the much lower mold counts at the intensive managed facility indicated that there, through the frequent turnings, the entire compost had been submitted to those high temperatures. At the extensively managed facility, where the turnings were infrequent, only the center had been heated up, while the surface stayed much cooler, furthering the proliferation of *A. fumigatus* [21].
These experiments showed that is in fact not the core temperature of a compost heap that is decisive for the elimination of molds and other pathogenic or allergenic microorganisms, but it has to be ascertained that by frequent turnings 65°C are reached in the whole compost material.

Measurements of spore concentrations in the air 2 m behind a turning machine clearly showed that lower numbers of *A. fumigatus* in the compost, due to an intensive management of the windrows (more frequent turnings and water addition) resulted in a lesser spore load of the air.
Although concentrations in the direct vicinity of turning of shredding machines were quite high (up to 10^7 CFU per m^3), exposition of the persons working on the place was often much less, because by operating the machines, they were normally standing at the side, in front or above them. In the open air, a rapid dilution of spores was observed: already in 10 m distance from the turning machine, *A. fumigatus* concentrations were 100-1000 times reduced, and in 500 m distance from the site, even downwind, not more *A. fumigatus* spores were measured (0- 20 CFU/m^3) than in places far away from composting installations.

<u>Composting in boxes, roofed or in a closed hall</u>: When composting is carried out in boxes, a new parameter for the control of the process has to be taken into consideration: aeration. Air is blown into the compost to assure sufficient supply of oxygen to the aerobic microflora. At the same time, moisture is transported out of the compost by the air stream, and heat is withdrawn through evaporative cooling.
We examined in our studies four different installations that use box composting. One problem encountered by this type of process is an excessive depth of the composting material, boxes were usually 2-4 m high filled with compost. Turning of these big masses of compost was slow, and turning frequencies were accordingly low. This led to an inhomogenous temperature distribution, vertical as well as horizontal, and consequently to an inconsistent composting process, and an insufficient thermohygienization. At one site that had no aeration system, almost no degradation of organic matter was observed during the passage in the boxes. In those heaps, *A. fumigatus* concentration was low, most probably due to the low oxygen content in the compost. But as soon as the material was stored in big heaps outside the boxes, strong heating occurred, and high concentrations of *A. fumigatus* (up to 10^5 CFU/gDW) were measured on the surface of the heaps, as in extensively managed open air windrows.

A. fumigatus spore concentrations in installations where the boxes were only roofed were similar to open air windrow sites. Emission of spores during turning depended a lot on the construction of the turning machine: more gentle mixing evoked less spore dispersion. Also, transport of spores by the wind seemed to be hindered by the box walls and the roof.
Composting inside closed buildings led to elevated concentrations of spores inside the rotting hall. Spore counts were permanently > 1000 / m^3. Even if the turning was supposed to be fully automated, the frequent presence of maintenance staff in the hall was necessary (high corrosion, clogging of the aeration system, sampling for process control). Outside the rotting hall, spore concentrations were minimal.

<u>Composting in closed bioreactors</u>: Composting in a bioreactor allows in principal the highest degree of control over the process. All input and output parameters can be easily monitored, analyzed and in function adjusted. On the other hand, visual control of the compost and sampling are often not possible. As in box composting, problems of homogeneity of the material were observed, and drying out of the compost through suboptimal aeration was frequent. Thermohygienization in bioreactors was often insufficient, in spite of quite high core temperatures due to a good thermal insulation of the reactors.
Composting in bioreactors generated no spore emission at all inside the building where the reactor were located, except for loading and emptying of the reactor.

Combined methanization and composting: When looking at *A. fumigatus* concentrations in combined methanization and composting installations, one has to look at the two processes separately. Methanization can be carried out at two different temperature ranges: Under mesophilic (≈ 40°C) or thermophilic (55-65°C) conditions. For hygienic reasons, the latter is often preferred.
The combination of elevated temperatures and acidic conditions led to a drastic reduction of molds. The sludge that resulted from the methanization process was either pressed, and subjected as is to a short composting process under aerobic conditions, or was mixed and co-composted with shredded wood. The conditions for fungal growth were enhanced through the input of air.
As composting is mostly carried out in boxes or bioreactors, if good mixing of the material is not guaranteed, a strong recolonisation of the compost can occur.

Biofilters: Five of the installations examined were equipped with biofilters. Composting was either carried out in boxes in a closed hall or in bioreactors. Spore counts carried out after the filter yielded results of a few up to several 100 CFU / m^3 of air. Taking into consideration that *A. fumigatus* concentration measured inside the rotting hall were usually a few 1000 CFU or much higher during turning of the composts, it seemed that the biofilters were reducing the amount of spores dispersed into the environment, although they were not completely holding them back.
Biofilters are usually laid out for the elimination of bad odors, and not for the retention of microorganisms.

Earth-worm compost and fungi

Earth-worm compost (vermicompost) is advertised as an effective, low price, "natural and ecological clean" fertilizer. Vermicomposting is used in some industrial biowaste systems, at animal enterprises, in small farms, and in private gardens. The application of earth-worm compost increases crop production of vegetables (tomatoes, cucumbers, salads), and improves the growth of flowers and indoor plants. Thus, these composts are attractive for many citizens. In the former Soviet Union for example, millions of town people have a small suburban garden-plot, and are interested in their own cheap and "clean" vegetable production. These gardeners do not only use commodity vermicompost, but try to make their own vermicomposting systems. Vermicomposting is also beneficial for farmers, because of the possibility to use the both the compost, and the earth-worms for poultry feeding. Nevertheless, very little is known about the health risk from the fungal growth in earth-worm composts.

We examined different types of earth-worm composting systems, where compost was produced from biowaste, pulp trash, mushroom farm waste, and poultry and pig manure. Some of these systems are primitive, and not controlled technologically, but are often preferred because of their cheapness. Earth-worms of the species *Eisenia fetida (Sav.)* were used in the composting process.
Temperatures in the composting environment were between 20 - 25°C. The structure of microfungal communities was described for the species composition, their frequency of occurrence (%) and abundance. To compare microfungal communities in different vermicomposts and in composts without earth-worms, the original program of the cluster analysis BIOMATRIX v.2.2 [23] was used. To determine the incidence of microfungi in the air of composting environments, open Petri dishes were exposed (at the height 1,5 m) for 15 and 30 min for sedimentation plating.

We found that microfungal communities in vermicompost differed in several respects from similar communities in compost without earth-worms, namely in their diversity, species composition, and abundance. The overall diversity of microfungal species in compost without worms was somewhat higher than in vermicompost. This difference was observed distinctly at the late stages of the composting process. The simplification was due mainly to a reduction in the number of rare microfungal species. It was very important that the abundance and frequency of occurrence of some microfungi of medical interest was, as a rule, somewhat higher in vermicompost than in compost without earth-worms. This trend was observed for representatives of genera *Aspergillus* (*A. fumigatus*, *A. niger*, *A. flavus*), *Fusarium* (*F. oxysporum*, *F. moniliforme*), and *Chrysosporium spp*. The highest level of abundance was determined in the environment of vermicompost based on poultry manure.
Simultaneously to the elimination of some dark-colored fungi - *Cl. cladosporioides, Al. alternata, Doratomyces stemonitis*, dark-colored sterile mycelium appeared. The distinctions tended to be significant after 40 days of composting, when a clear decrease in overall species diversity occurred.
In some vermicomposts a distinct difference in *Aspergillus* species distribution between different layers of compost was observed.

We found that vermicompost application did not increase the abundance of problematic molds in the compost-amended soils. The formation of mycobiota in vermicompost are influenced by biotic and abiotic factors : type and concentration of organic substances, temperature, pH, microbial interactions, and earth-worm influence. It is known [1] that earth-worm activity usually leads to the neutralization of the pH of soils and substrata, in which where they are living. As previously mentioned, the vermicomposting conditions are characterized by rather high temperatures and neutral pH. This ecological situation is known to be often preferable for the growth of some opportunistic microfungi, for example representatives of the genera *Aspergillus* and *Fusarium*. Very little is known about the influence of some biotic factors (microbial-invertebrate interactions) on the microfungal community formation in vermicomposts. The significance of the interaction between fungi and the earth-worms had been demonstrated in our experiments. We found that some changes in microfungal communities composition in vermicompost could be connected to the feeding preferences of worms. In was shown experimentally that the earth-worm *E. fetida* did prefer dark-colored fungi (*Cl. cladosporioides*, *Al. alternata*) as a food. On the contrary, the substrata with addition of mycelium of *Aspergillus* species (*A. niger*) were not attractive for earth-worms. In the investigations of the earth-worms feeding capacities it was also observed that microfungal growth was drastically changed after fungal spores had passed through the earth-worm intestine. The spores of dark-colored fungi were the most sensitive to the influence of worms digestion. For *Al. alternata* and *Cl. cladosporioides,* a clear increase of spore germination time, decrease of germination level and mycelial growth was observed in earth-worms excretes. In contrast, no changes in the life cycle of *A. niger* were found after the earth-worms had been fed with *A. niger* spores. Germination and mycelial growth are strongly influenced by the microflora, in particular by the mechanism of fungistasis. The fungistasis is caused by microbial products. In the experiments with microfungi and bacteria isolated from earth-worms and vermicompost it was shown that vermicompost colonization by fungi can be partly suppressed or activated by the initial bacterial population [18]. The bacteria of the genus *Bacillus*, which were isolated as common components of the gut-microbiota of *E. fetida* and from vermicompost, inhibited spore germination, mycelial growth and gave rise to microcycle conidiation for *Cl. cladosporioides*, *T. harzianum*, and *Al. alternata*. On the other hand, the metabolites from bacteria of the genera *Pseudomonas* and *Spirillium*, which were isolated from vermicompost, activated spore germination and mycelial growth of some fungal species, including *A. niger*. In conclusion, I would like to draw attention to the high possibility of specific microfungal communities formation in vermicomposts. It should be stressed that vermicompost microfungal communities often include species from the genera *Aspergillus*, *Fusarium*, and *Chrysosporium,* which are of priority interest to human medicine. The specificity of microfungal communities formation in vermicompost may have some ecological reasons: 1) the support of special conditions for the growth of the earth-worm population during the vermicomposting process, and 2) the earthworm - microfungal interactions. The presence of problematic molds should become a point of mycological control in vermicompost during the composting process (particularly during the active composting phase), in the prepared compost, and in the vermicomposting environment.

Conclusions

It is hoped that the content of this paper will contribute to the ongoing discussion on a mycological surveillance program for biowaste treatment and composting, and complete the proposals presented previously [10, 29]. However, further multidisciplinary studies involvinging engineers, biologists/microbiologists, allergologists/epidemiologists, and competent authorities, are necessary to establish definitive recommandations on the management of biowaste and compost, and on the control of its hygienic and agronomic quality.

The aspects that should be investigated or improved in priority are the following :
1. The precise dose of allergenic/pathogenic microorganims (e.g. *A. fumigatus*) and toxins required to elicit adverse health effects in either healthy or sensitive individuals has not been determined. Long-term epidemiological studies at industrial composting facilities are indispensable to determine the effect of elevated allergen/pathogen concentration on the health of compost workers.
2. A growing number of citizens are engaged in the manipulation of smaller quantities of biowaste and compost (e.g. backyard, small communities, and vermicompost). Because these systems are managed extensively, thermohygienization of the compost is not guaranteed. Hygienic controls of these systems are necessary.
3. Composting is a very complex biological process. Much more research is needed to effectively control the process parameters (aeration cycles, turning frequency, etc, in function of the starting material), indispensable for an optimal degradation, hygienisation and maturation.
4. Collaboration of research institutions, regulatory agencies and the composting industries could lead to the establishment of « good composting practices », which would be the base for the training of compost site managers. Also, information has to be delivered to the medical community concerning the health risks encountered by compost workers or persons being in contact with biowaste.

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CONTRIBUTORS

The contributors of this symposium were: **F. Staib**, Comments on proposals for the avoidance of health risks posed by fungi from biowaste and compost; **T. Beffa**, Composting: a microbiological process; **J. Lott Fischer, P.-F. Lyon and T. Beffa**, Elimination or reduction of pathogenic fungi during composting, in function of the type of system and management; **O. E. Marfenina**, Earth-worm compost and fungi; **P. Gumowski, S. Dunoyer-Geindre, F. Georgen, R. Roch-Susuki, L. Gallaz and J. P. Latgé**, Follow-up of compost and waste workers : evolution of immune response to compost dust and *Aspergillus fumigatus*. The co-convenors were **T. Beffa** and **F. Staib**.

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