Aerobiologia (2009) 25:159–166
DOI 10.1007/s10453-009-9121-y

ORIGINAL PAPER

Possible cause of allergy for the librarians: books manipulation and ventilation as sources of fungus spores spreading

Ingrid Cezara Apetrei · Gilda Eleonora Drăgănescu · Ilinca Teodora Popescu · Cătălin Carp-Cărare · Eleonora Guguianu · Traian Mihăescu · Alina Ştefanache · Carmen Creţu · Xenia Patraş

Received: 8 January 2009 / Accepted: 13 May 2009 / Published online: 9 June 2009

Abstract The objective of this study was to investigate the airborne viable spore concentrations and identify the fungal species in all indoor spaces from the lending library at the Technical University "Gheorghe Asachi" Iaşi, Romania. Samples were collected using the settle plate method and swab samples from PC cooler fan grids as well as from the wall in it's vicinity and from paper/wood fragments. There were no air conditioning systems in the library rooms. The heating systems were standard with an environmental temperature of 20°C in winter, except for the storage area of old/rare books stacks II, where the temperature was below 15°C and the humidity was very high due to water infiltrations in the walls and poor maintenance. More than 296 fungal colonies from over 78 samples were identified, enumerated, and reported. Indoor airborne fungal spore deposition rates were within the range of 419–1,677 CFU/m^2, with the predominance of genera being *Aspergillus* spp., *Penicillium* spp., *Cladosporium* spp., *Alternaria* spp. and *Chaetomium* spp. Approximately ten fungal colonies could not be identified. The PC fans move particles from the low levels (floor) to the air, and are thus responsible for maintaining a constant air velocity and contribute to fungal-spore aerosolization, transport, deposition and resuspension. Book paper and wood furniture are known to be suitable substrates for cellulose degrading fungi.

Keywords Airborne · Fungi · Paper · Ventilation sources · Allergy · Librarians

I. C. Apetrei (✉) · C. Carp-Cărare · E. Guguianu · C. Creţu
Faculty of Veterinary Medicine, Department of Animal Products and Public Health, University of Agricultural Sciences and Veterinary Medicine "Ion Ionescu de la Brad", Aleea M. Sadoveanu no. 8, Iasi, Romania
e-mail: ingridapetrei@gmail.com

G. E. Drăgănescu
Library Department, University of Agricultural Sciences and Veterinary Medicine "Ion Ionescu de la Brad", Aleea M. Sadoveanu no. 3, Iasi, Romania

T. Mihăescu
Department of Pneumology, University of Medicine and Pharmacy Gr.T. Popa, University str. no. 16, Iasi, Romania

A. Ştefanache
Department of Pharmacology, University of Medicine and Pharmacy Gr.T. Popa, University str. no. 16, Iasi, Romania

X. Patraş
Pharmacology Department, University Petre Andrei, Gavril Muzicescu Nr. 6, Iasi, Romania

I. T. Popescu
Department of Allergology, Medical Center Copou, Str. Cozma Toma no. 4, Iasi, Romania

1 Introduction

Mold spores are regularly found in indoor air and no indoor space is free of them. The lack of knowledge

regarding the role of microorganisms in the development and exacerbation of diseases, found in occupants of damp indoor environments, is due largely to the lack of valid quantitative exposure assessment methods and knowledge of which specific microbial agents may primarily account for the presumed health effects. Very few biomarkers of exposure to, or doses of biologic agents have been identified, and their validity for exposure assessment in the indoor environment is often not known. The entire process of fungal-spore air dispersion, transport, deposition, and tracking—all of which determine inhalation exposure—is poorly understood, as is the significance of exposure to fungi through dermal contact and ingestion. There are several methods for measuring and characterizing fungal populations, but methods for assessing human exposure to fungal agents are poorly developed and are a high-priority research need (Committee on Damp Indoor Spaces and Health 2004). Part of the difficulty is related to the large number of fungal species that are measurable indoors and the fact that fungal allergen content and toxic potential vary among species and among morphologic forms within species. In addition, the most common methods for fungal assessment—counting cultured colonies and identifying and counting spores—have variable and uncertain relationships to allergen, toxin, and irritant content of exposures (Committee on Damp Indoor Spaces and Health 2004; Nathanson 1995). There are at least 180 fungal genera and species or bacteria capable of damaging both wood and cellulose by using them as nutritious substratum (Wood 1988). Over the past 10 years studies on the presence of bioaerosols in closed workplaces have taken on new approaches as a result of the misunderstanding or misidentification of the real causes of various respiratory dysfunctions (allergies, asthma) or skin related (contact-dermatitis) problems in humans. Airborne routes of transmission of microorganisms are extremely important and must be studied with great attention. Indoor fungi is not only a scientific issue but is gradually becoming a social one. The World Health Organization tried to define the Sick Building Syndrome (SBS) first in 1982, which proved to be extremely difficult because of insufficient scientific data available at the time, as well as obstacles of a technical and practical nature (Committee on Damp Indoor Spaces and Health 2004; Nathanson 1995). Despite the availability of effective interventions to prevent occupational hazards and to protect and promote health in the workplace, large gaps exist between and within countries with regard to the health status of workers and their exposure to occupational risks (Nathanson 1995). We attempt to demonstrate that extensive use of the personal computer in resting places and at work, at least 6 h daily, can create favorable conditions for a human respiratory affliction, by facilitating spores and mycelium fragments to become airborne, and further to be inhaled once they have reached the level of the human airways.

2 Materials and methods

2.1 Sampling and isolation methods

In the period September 15, 2007 to February 23, 2008, we collected 78 samples (settle plate method, swab sample and bulk) to analyse the viable cultivable fungal spores from all indoor spaces of the University Library. Thus, 28 swab samples were taken and immersed in 2 ml Tween 80 saline solution of 0.05% concentration to ensure a better release in solution of the spores and hyphal fragments. The samples were taken to the laboratory in the shortest time possible and were homogenized. We then placed 0.5 ml into two sterile Petri dishes and added 15–20 ml of potato dextrose agar (PDA; Merck KgaA, Germany) and malt extract agar (MEA; Merck KgaA, Germany), melted with chloramphenicol (maintained at 45°C). The swabs were also streaked immediately onto another two Petri dishes which contained PDA and MEA solid agar.

At the same time 34 Petri dishes, 90-mm in diameter and half containing PDA and half MEA solid agar, were opened and exposed to air about one meter above the ground in different parts of the rooms (near the keyboard when a PC was in the room). After 15 min, the Petri dishes were closed, collected and transferred to the laboratory and incubated at 25–27°C for 5 days. The colonies were then counted and the results were expressed as colony forming units (CFU) per square meter. Fungal isolates were identified by conventional methods including the gross morphology of the fungal colony, tape mount and slide culture for microscopic characterization of reproductive structure and accessory

structures (Ismail et al. 1999). We also collected 16 fragments of paper books and wood furniture (Fig. 1) which were immersed into 5 ml Tween 80 saline solution (0.05%) in plastic closed recipients. We placed 1 ml from the immersion solution onto the PDA solid medium and 1 ml onto the MEA and in succession we deposed all the fragments collected onto the surface of the Petri dishes PDA medium. Each colony of fungi after 5–7 days of incubation at 27±1°C was individually inoculated to MEA and PDA media for identification at species/genus level (Fig. 1). Petri plates were first examined under a stereomicroscope. Then we used a light microscope to determine the colonial features and morphological structures of the fungi. The determination of the morphological structures of fungi was carried out by mounting the colony fragments with a drop of Lacto-Cotton Blue on clean microscope slides, which is a method proposed by Sime et al. (2002). Airborne viable fungal spores and fragments were expressed as colony forming units (CFU/m^2) settled in a 90 mm Petri plate for 15 min of exposure and expressed by using the following formula (Kołwzan et al. 2006):

$$x = a \times 5 \times 10^4/\pi r^2 \times t$$

where x is the number of fungal spores in air, a is the number of colonies upon the minimum two petri dishes, πr^2 is the surface area of the petri dish (cm^2), and t is exposure time (15 min).

2.2 Identification

Many different culture media were used for identification of fungi. Fungal genera and species were identified based on micro and macro-morphology, reverse and surface coloration of colonies developed on CZ (Agar Czapeck–Dox; Merck KgaA, Germany), MEA, PDA, CGA (Chloramphenicol Glucose Agar; Scharlau Chemie S.A) and Agar Sabouraud Cloramfenicol (Merck KgaA, Germany) media. Fungi were identified to genus and species level following the indications of De Hoog et al. (2005), Samson and Vargas (2007) and Frisvad and Samson (2004).

3 Results

The main fungal species and genera identified by micro- and macro-morphology examination and which sampling method we used for each type of surfaces in all spaces analysed are detailed in Table 1. Approximately 296 fungal colonies from the initial Petri dishes were isolated in pure culture, and identified at genus/species level, thus allowing determination of the occurrence of fungal taxons for all studied spaces. The fungal deposition ranges were between 419 and 1,677 CFU/m^2 with *Aspergillus* spp., *Penicillium* spp., *Cladosporium* spp., and *Alternaria* spp. genera identified as predominant in all the examined locations (Table 2; Fig. 2). Ten fungal colonies could not be identified. *Aspergillus*, *Penicillium*, *Cladosporium* and *Alternaria* genera were consistently found in the dust collected on the ventilation grid of the PC's cooler fan, followed by *Fusarium*, *Chaetomium* and yeasts (Fig. 1). *Chaetomium* genera was identified on the old book paper surface through the swab sampling method; we then succeeded in isolating it from the three dermal lesions that had bothered a person for more than 2 months in one case (neck skin) and for more than 6 months (hands skin) in the others (Fig. 3). The lowest airborne concentration of fungi was observed in the lecture hall (near the keyboard) and the highest concentration in the basement in the storage area of valuable old books, from which wood fragments of the existing furniture and from the wall surfaces *Stachybotrys chartarum* was isolated (Table 2; Fig. 1).

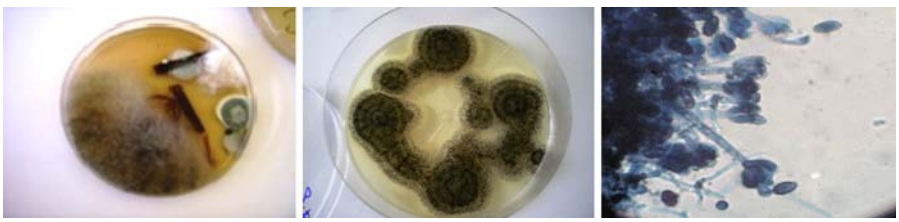

Fig. 1 Wood fragments of furniture on surface of solid PDA medium (*left*), *Aspergillus niger* isolated in pure culture on CZ medium (*middle*) and *Stachybotrys chartarum* × 40 (*right*)

Table 1 Main genera and fungal species identified in all spaces and in all types of samples analyzed

Location in the library of the Technical University "Gheorghe Asachi" IASI	Swab sampling-surface		Direct exposure of petri plates-15 min		Bulk samples
Lecture room, office	Ventilation grid of the PC Prevalent in 6 samples	Wall close to the PC cooler-fan/on the book paper surface	Near the keyboard Prevalent in 6 samples	Another location in the examined space	
Main fungal genera and species identified	*Cladosporium herbarum* *Alternaria alternata* *Chaetomium murorum* *Penicillium* spp./5 *Rhizopus microsporus* *Trichoderma viride* *Chaetomium* spp. *Aspergillus flavus* *Aspergillus fumigatus* *Aspergillus niger* *Curvularia* spp. *Alternaria* spp. *Mucor* spp. *Penicillium citrinum* *Fusarium* spp.		*Cladosporium herbarum* *Alternaria alternata* *Chaetomium* spp. *Penicillium* spp./3 *Aspergillus fumigatus* *Alternaria* spp.		

Storage area of old/rare book stacks I	Four swab samples on the book paper surface	Prevalent in 6 samples	Six samples of paper fragments from old books'
Main fungal genera and species identified	*Chaetomium* spp.	*Cladosporium herbarum*	*Cladosporium herbarum*
	Cladosporium herbarum	*Penicillium* spp./4 *Alternaria* spp.	*Penicillium* spp.
	Penicillium spp./2 *Alternaria* spp.	*Yeasts*	*Yeasts*
	Yeasts	*Mucor* spp.	
	Mucor spp.	*Rhizopus* spp.	
	Rhizopus spp.	*Rhodotorula* spp.	
	Rhodotorula spp.	*Alternaria* spp.	
	Aspergillus flavus	*Mucor* spp.	
	Aspergillus versicolor	*Fusarium* spp.	
	Aspergillus niger	*Aspergillus versicolor*	
	Fusarium spp.	*Trichoderma viride*	
	Mucor spp.	*Pseudallescheria boydii*	
	Insects	*Absidia corymbifera*	

Table 1 continued

Lecture room	Prevalent in 6 samples	Prevalent in 4 samples	Prevalent in 6 samples	Four samples of wood fragments from the existing furniture
Main fungal genera and species identified follow up	*Cladosporium herbarum* *Penicillium* spp./4 *Aspergillus flavus* *Yeasts* *Cladosporium* spp. *Alternaria* spp.	*Cladosporium herbarum* *Penicillium* spp. *Aspergillus flavus* *Yeasts* *Cladosporium* spp. *Alternaria* spp.	*Cladosporium herbarum* *Penicillium* spp./2 *Aspergillus flavus*	*Cladosporium* spp. *Penicillium* spp. *Aspergillus versicolor* *Fusarium* spp.

Storage area of old/rare book stacks II	Prevalent in 6 swab samples on the book paper surface	Prevalent in 6 samples	Four samples of wood fragments from the existing furniture and 2 samples of old books' fragments
Main fungal genera and species identified	*Cladosporium herbarum* *Cladosporium cladosporoides* *Fusarium* spp. *Penicillium* spp./2 *Aspergillus flavus* *Rhodotorula* spp.	*Fusarium* spp. *Rhodotorula* spp. *Candida* spp. *Penicillium* spp./3 *Cladosporium herbarum*	*Stachybotrys chartarum* *Penicillium* spp./2 *Fusarium* spp. *Rhizomucor* spp. *Cladosporium. cladosporoides* *Alternaria* spp.

Editorial office, 3 personal computers	Prevalent in 6 samples	Prevalent in 6 samples
Main fungal genera and species identified	*Cladosporium herbarum* *Alternaria alternata* *Penicillium* spp./3 *Rhizopus stolonifer* *Trichoderma viride* *Chaetomium* spp. *Aspergillus flavus* *Aspergillus fumigatus* *Aspergillus niger* *Alternaria* spp. *Mucor* spp. *Penicillium commune* *Fusarium* spp. *Absidia corymbifera* *Yeasts* *Stachybotrys chartarum*	*Cladosporium herbarum* *Penicillium* spp. *Aspergillus candidus* *Pseudallescheria boydii*

Table 2 Fungal deposition rates and dominant fungus taxons identified for all indoor spaces examined

Location in the library of the Technical University "Gheorghe Asachi" IASI	Number of Petri plates exposed 15 min for each space analyzed	CFU counted after 5–7 days of incubation	CFU fungal deposition rates/m^2	Dominant fungus identified
Lecture room, office room and near the keyboard	2	10	524	*Aspergillus*
	2	12	629	*Cladosporium*
	2	8	419	*Cladosporium*
Storage area of old/rare book stacks I	2	32	1,677	*Penicillium*
	2	27	1,415	*Aspergillus*
	2	25	1,310	*Alternaria*
Lecture hall, near the keyboard of all computers in the examined space	2	15	786	*Cladosporium*
	4	17	891	*Penicillium*
	2	9	471	*Aspergillus*
	2	11	576	*Aspergillus*
Storage area of old/rare book stacks II	2	22	1,153	*Aspergillus* *Penicillium*
	2	23	1,205	*Aspergillus* *Alternaria*
	2	31	1,625	*Aspergillus*
Editorial staff office, near the keyboards of 3 personal computers	2	17	891	*Aspergillus*
	2	13	681	*Cladosporium*
	2	24	1,258	*Penicillium*

CFU colony forming units

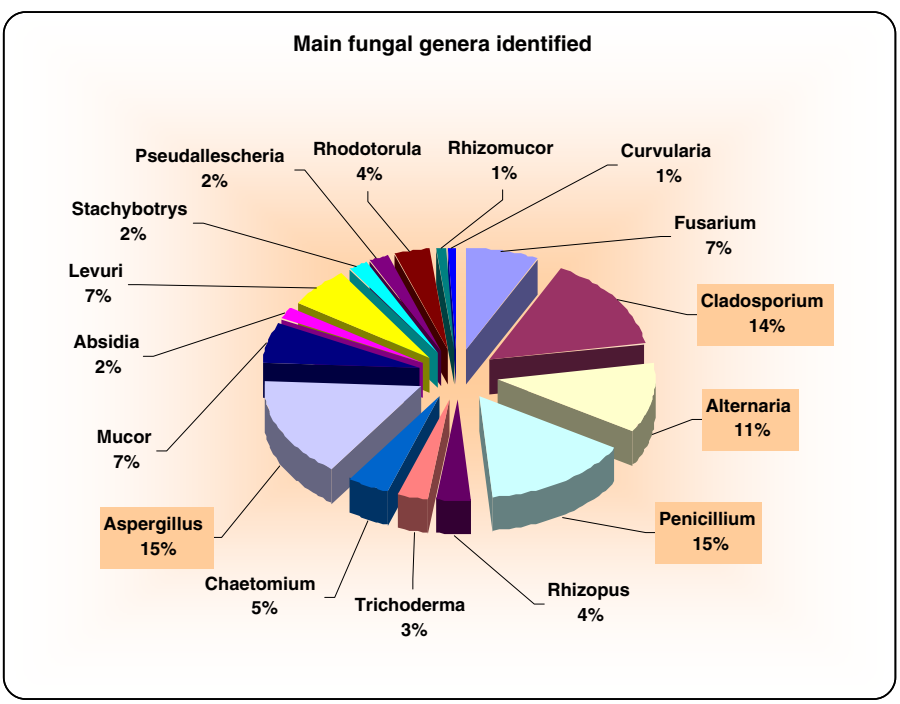

Fig. 2 Main fungal genera identified in all indoor spaces of the library

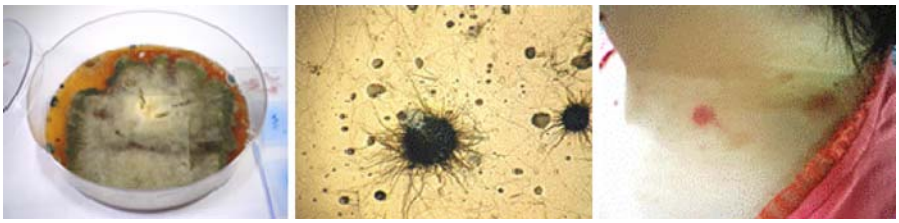

Fig. 3 *Chaetomium* sp. isolated in pure culture on MEA (*left*), *Chaetomium* spp. on cello-tape × 20 (*middle*), and dermal lesions (*right*) caused by *Chaietomium* sp.

4 Discussion

Turbulence of air in closed spaces plays an essential role in the movement and sedimentation of viable fungal structures. The PC fan cooler is a means to move particles from the low levels (floor) to the air. The study design and execution had some restrictions that limited interpretation of quantitative aspects but offer a good image about the fungal diversity in indoor spaces. There are no previous studies on airborne fungi in working and living indoor spaces in Romania and this is a preliminary one. We intend to pursue study on clinical and immunological affects on librarian's health. Everything may be exposed to molds, but fungal density in air and time of exposure near the personal computer—cooler-fan or near the key-board—can be considered as a possible cause of negative impact on human health. Over the last decade we have witnessed a huge growth both in number and density of PC's in offices and homes. This can be a possible explanation for the process of fungal-spore air dispersion, transport, deposition, and resuspension that may be a bridge between human exposure to fungi through dermal contact, inhalation or ingestion and number of hours spent daily using personal computers. On the other hand, in a lending library, book paper and wood furniture are sources for growth and dispersal of fungal contaminants in indoor spaces.

Aspergillus and *Penicillium* spores are the most widespread air allergens in the world. According to qualitative and quantitative reports, the former is the dominant species in tropical regions while the latter is dominant all over the world (Şen and Asan 2001). Anderssen et al. (2003) noted that *Alternaria* is known to be allergic and is one of the most common fungi worldwide, and they suggested that *Alternaria* allergens can contribute to severe asthma. Myszkowska et al. (2002) noted that 4–7% of the European population show sensitivity to *Alternaria* and *Cladosporium* spores. *Alternaria* and *Cladosporium* were also the predominant genera in our study. Therefore, it is necessary to reveal the concentration percentages of these fungi in order to take some health precautions for allergen sensitive people. Vesper et al. (1999) demonstrated that isolation of *S. chartarum*, even in a small percentage, in indoor air samples or on different surfaces is a harmful toxin producer fungus that has been previously associated with a number of human and veterinary health problems. Without data on what is acceptable in buildings, it is difficult to identify unacceptable indoor levels of airborne micro-organisms. Many fungi are cellulose degraders in the natural environment, meaning that paper books or wood furniture are perfect substrates for fungal development in the lending library. It has been experimentally proven that fungi can eat up iron and other elements in the composition of ink, while the effects of chemical degradation are even harder to eliminate, especially due to its permanent character. Species belonging to the genera *Aspergillus* and *Penicillium* can also digest additives, adhesives (glue) or textile fabrics used in book-binding.

Many of the isolated fungi should not have been found in the indoor spaces of the buildings studied, for example, *S. chartarum* and *Trichoderma viride* (Szczepanowska and Lovett 1992). In order to determine whether a certain indoor space meets all the microbiological safety conditions necessary for an activity to be carried out under proper conditions, whatever the activity might be, it is imperative that we make efforts to establish some limits to the indoor fungal airborne concentrations.

References

Anderssen, M., Downs, S., Mitakakis, T., Leuppi, J., & Marks, G. (2003). Natural exposure to *Alternaria* spores induces

allergic rhinitis symptoms in sensitized children. *Pediatric Allergy & Immunology, 14*, 100–105. doi:10.1034/j.1399-3038.2003.00031.x.

Committee on Damp Indoor Spaces and Health. (2004). *Damp indoor spaces and health* (pp. 29–89). Washington: National Academies Press.

De Hoog, G. S., Guarro, J., Gené, J., & Figueras, M. J. (2005). *Atlas of clinical fungi, 2nd ed. Utrecht/Reus, CD-Rom version* (p. 1126). The Netherlands: Centraalbureau voor Schimmelcultures.

Frisvad, J. C., & Samson, R. A. (2004). Polyphasic taxonomy of *Penicillium* subgenus *Penicillium*. A guide to identification of the food and air-borne terverticillate Penicillia and their mycotoxins. *Studies in Mycology, 49*, 1–173. Utrecht, The Netherlands: CBS Fungal Biodiversity Centre.

Ismail, M. A., Chebon, S. K., & Nakamya, R. (1999). Preliminary surveys of outdoor and indoor aeromycobiota in Uganda. *Mycopathologia, 148*, 41–51. doi:10.1023/A:1007195708478.

Kołwzan, B., Waldemar, A., Kazimierz, G., & Pawełczyk, A. (2006). *Introduction to environmental microbiology*. Wroclaw: Oficyna Wydawnicza Politechniki Wrocławskiej.

Myszkowska, D., Stepalska, D., Obtulowicz, K., & Porebski, G. (2002). The relationship between airborne pollen and fungal spore concentrations and seasonal pollen allergy symptoms in Cracow in 1997–1999. *Aerobiologia, 18*, 153–161. doi:10.1023/A:1020603717191.

Nathanson, T. (1995). *Indoor air quality in office buildings: A technical guide* (pp. 10–53). Ottawa: Minister of Supply and Services Canada.

Samson, R. A., & Vargas, J. (2007). Aspergillus systematics in the genomic era. *Studies in Mycology, 59*, 75–203. CBS Fungal Biodiversity Center, Institute of the Royal Netherlands Academy of Arts and Sciences, Utrecht, The Netherlands

Şen, B., & Asan, A. (2001). Airborne fungi in vegetable growing areas of Edirne, Turkey. *Aerobiologia, 17*, 69–75. doi:10.1023/A:1007604417192.

Sime, A. D., Abbott, L. L., & Abbott, S. P. (2002). A mounting medium for use in indoor air quality spore-trap analyses. *Mycologia, 94*, 1087–1088.

Szczepanowska, H., & Lovett, C. M., Jr. (1992). Fungal stains on paper: Their removal and prevention. *Journal of the American Institute for Conservation, 31*, 147–160.

Vesper, S. J., Dearborn, D. G., Yike, I., Sorenson, W. G., & Haugland, R. A. (1999). Hemolysis, toxicity, and randomly amplified polymorphic DNA-analysis of *Stachybotrys Chartarum* strains. *Applied and Environmental Microbiology, 65*, 3175–3181.

Wood Lee, M. (1988). *Prevention and treatment of mold in library collections with an emphasis on tropical climates: RAMP study for the general information programme and UNISIST* (pp. 23–24). Paris: UNESCO. PGI-88/WS/9.