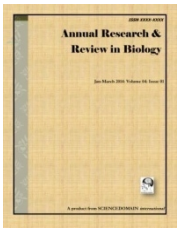

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Microbiological Monitoring of Ethnographic Ornamental Collections in the Museu do Índio, Rio de Janeiro, Brazil

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Authors' contributions

Author ACNDODS selected the objects from the ethnographic collections and investigated the historical importance of the objects to the study. Authors ACADC and OH designed the microbiological study, wrote and supervised the present paper. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: To investigate fungal contamination in ethnographic objects from the Museu do Índio (Rio de Janeiro, Brazil), from a particular indian community named Urubu-Kaapor. Results were compared to the same investigation on objects from other indian tribes, to determine possible cross-contamination between objects, if stored in the same repository in the museum.
Study Design: Selection and materials characterization of the objects from distinct ethnographic collections, followed by an investigation of the fungal contamination through the use of swab techniques and specific culture medium.
Place and Duration of Study: Museu do Índio, located in Rio de Janeiro, Brazil, between April and December 2012.
Methodology: Samples: We included 5 ethnographic objects from Urubu-Kaapor indian community, probably non-contaminated due to its chemical constitution and state of conservation and 5 ethnographic objects from Xavante, Nambikwáras and Kamayurá

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tribes, probably contaminated with fungal colonies due to its chemical constitution and visual observations.
Results: Results clearly indicated that Urubu-Kaapor objects presented, after growth in proper culture medium, small colonies of fungus collected from their surfaces. The same observations were obtained from the Xavante, Nambikwáras and Kamayurá objects. However, the main difference between the levels of contamination was observed in the type of growth observed. Differently from Urubu-Kaapor objects, fungal colonies grown on other objects presented a higher diameter, associated to a high level of spores and filamentous forms. This could be explained based on the nature of the materials.
Conclusion: Ethnographic objects from Urubu-Kaapor collections should not be put together with the remaining ones from other indian tribes, due to the distinct level of contamination. If not controlled, fungal cross-contamination can take place, with the transfer of fungal spores from one piece to another. In a near future, the whole ethnographic collections would face the same level of microbial contamination.

Keywords: Fungal contamination; ethnographic objects; colony growth; Aspergillus; Penicillium.

1. INTRODUCTION

Ornamental collections from the Museu do Índio were produced from the development of non-scientific researches, stimulated by the Brazilian Indian Protection Service. According to Couto [1] researches on ethnographic ornament collections were restricted to the gathering of artifacts from several indian groups. The objective, at that time, was to illustrate the specific characteristics of each indian group, starting the ethnographic collection of the Museu do Índio. Particularly the Urubu tribe, one of the most traditional groups, kept its original traditions, differently from the Tembé, Guajajara, Guajá, Canela, Krikat and Gavião groups. The Urubu tribe kept its traditions, including traditional rites, chants and myths, and also manufacturing of cultural materials, as presented in Figs. 1 and 2.

Ethnographic artifacts are objects derived from indian communities collected from anthropological research expeditions around the world. However, it is usual that those objects lack proper documentation associated to their occurrence or use. Consequently, it is of extreme importance that preservation of any information regarding those artifacts must be properly stored, in order to understand the sociocultural context to which it is included. The ethnographic collection from the Urubu-Kaapor tribe, organized by anthropologist Darcy Ribeiro, was formed from two scientific expeditions to the Urubu tribe, between 1949 and 1951. this includes 112 artifacts organized in seven specific categories: 38 feathered ornaments, 20 composite ornaments, 6 tissue objects, 39 weapons, 3 ritual and magic objects, 6 twisted objects and 1 non-classified piece.

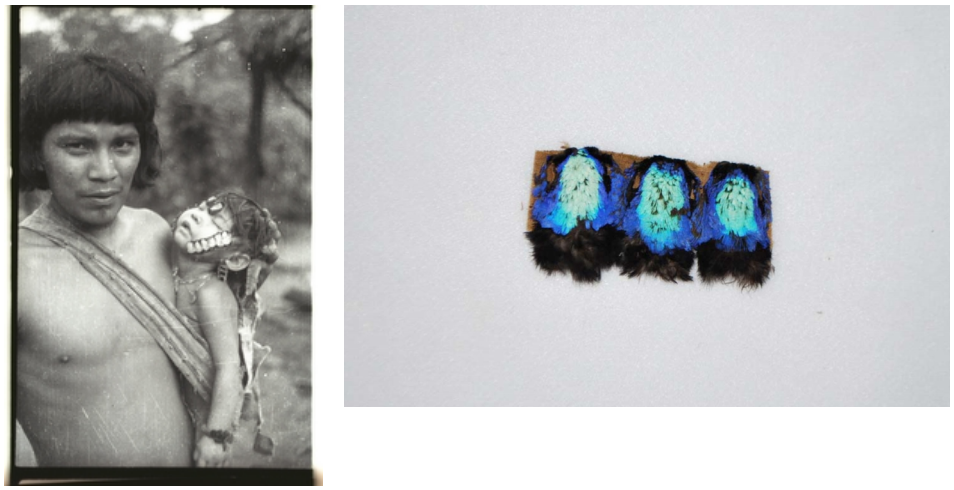

Fig. 1. Urubu-Kaapor indian adorned with a feathered frontlet. Registration SPI 4960, Photo by Heinz Foerthmann (left). Feathered frontlet. Urubu-Kaapor tribe. Darcy Ribeiro Collection. Registration 2656, Photo by A. C. Carvalho (right). Museu do Índio/FUNAI Collection, Brazil

During the 90's the Service of Musicology of the Museu do Índio starts to implement conservation policies for the ethnographic collection. At that time, the priority adopted included the adaptation of specific spaces for the storage of 13,000 objects. Those spaces were adapted for the storage of distinct materials, considering the geographic location of the museum, as well as humidity and temperature control. At the end of this step, the objects were segregated to be confined in the same space, according to their cultural and structural characteristics. This considered the type of material used in the manufacture of the piece, possible biodeteriorating agents, and environmental and chemical deteriorators.

In an excellent review Griset [2] provides information for preventive conservation of ethnographic collections, based on the knowledge of their perishable nature. The author stress the idea that, even under the best circumstances, the nature of the materials used in most objects easily leads to early deterioration.

Ethnographic objects are usually constituted of easily degradable materials, due to the organic nature of their supports, such as: vegetable fibers, shells, seeds, wood, feathers, leather, natural pigments and bones, among others. Froner et al. [3] states that most ethnographic objects are built from association between one of more elements, such as feathers and wood, vegetable fibers and teeth, resins and wood, in a wide range of possible combinations, according to techniques of each particular ethnicity, the use of certain objects and also according to the imagination and particular artistic possibilities of each individual culture. The nature of these materials was subject to damage and deterioration from chemical, physical and biological processes, and their frequent association in a single object tends to accelerate degradation.

Fig. 2. Urubu-Kaapor indian adorned with ornamental comb. Registration SPI 15206, Photo by Heinz Foerthmann (left). Ornamental comb. Urubu-Kaapor tribe. Darcy Ribeiro Collection. Registration 5452, Photo by A. C. Carvalho (right). Museu do Índio/FUNAI Collection, Brazil

Biodeterioration tends to be the main cause of damage on ethnographic artifacts, due to their organic nature, amenable to biological attack by fungal and bacterial communities. However, the lack of information on the specific nature and occurrence of fungal contamination on ethnographic objects, led to the need to search for additional information on other types of natural and organic materials. Descriptions presented below include materials from sources, different from ethnographic ones, however, subjected to the same type of microbiological decomposition and/or occurrence.

In an interesting study for the detection of moulds by volatile organic compounds and its application to heritage conservation, Joblin et al. [4] propose a methodology based on the measurements of volative organic compounds from biogenic nature to detect early stages of growth of fungi. Authors concluded that the technique was possible, not corroborating the idea that humidity could mask a difference in sensor behavior. Authors tested their protocol against the usually found genera *Aspergillus, Penicillium, Fusarium, Trichoderma* and *Ulocladium*.

Blanchette [5] observed that wooden cultural properties are degraded by microorganisms when moisture, oxygen and other environmental factors are favorable for microbial growth. The author described the structural and chemical features of wood, the degradation of wood by fungi and bacteria, associating the conclusions to the decomposition of cultural properties, both in terrestrial, aquatic and waterlogged archaeological woods. Information on the unique features associated with different types of decayed wood are also important to plan appropriate conservation procedures, and to select or develop specific consolidation procedures or other treatments for each decay situation.

Helms et al. [6] observed that anaerobic bacteria were isolated from a 1700-year-old wooden spear shaft, excavated from an archaeological site that dates from the iron age. The bacteria were cultivated in glucose- and xylose-supplemented media at 14 ^{o}C and 20 ^{o}C. The authors could produce a gene library with 21 clones, all identified families were commonly found in soil or bog environments and many are able to utilize cellulose as their carbon or energy source. With a different approach, 20 paper samples from different centuries were studied through non-destructive techniques, where fungi developed in the areas where fluorescent response was present [7]. The authors observed a close correlation between the thermo-hygrometric measurements and the place of storage, as well as a correlation between the environment and fungal species found, mostly cellulolytic fungi. Authors observed that predominated in stained samples and maps, followed by *Chaetomium globosum* colonies. *Penicillium chrysogenum* and *Penicillium canescens* were also found, as well as a considerable number of *Trichoderma* spp. colonies. It is important to mention that xerophylic fungi were found among the species isolated.

A study of the contamination of a 1854 book by fungi, indicated that for the first time authors proved that the causative fungal species of the fungal fox spots are no longer viable; and they show that each page was contaminated by contact with contaminated materials during the papermaking or book making process and could not have been the result of airborne contamination. These observations were based on observations over a period of 14 days. Some isolated fungal colonies formed outside the marked spots and at the edges of the were observed, and were different than those observed under SEM in the outlined spots. There was no fungal growth in the outlined spots, thus the fungi that grew were from experimental or recent paper airborne contamination. This conclusion could be reached due to the presence of fungal structures in the fungal fox spots in 114 spots in the 1854 book, that were no longer viable [8].

Ethnographic objects from the Estonian National Museum (barrels, vats, butter molds and milking barrels), all made of hardwood and softwood, such as pine, linden, spruce, alder and birch, were housed at 5-28 ^{o}C and 25-55% relative humidity, measured with data loggers [8]. From 258 ethnographic objects, 11% presented signs of fungal deterioration and almost half of it presented signs of insect and rodent deterioration [9].

Thus, the objective of this work is to study fungal contamination in ten selected ethnographic objects from the Darcy Ribeiro Urubu-Kaapor collection of the Museu do Índio, located in Rio de Janeiro City, Brazil. This monitoring will bring information to support the transfer of those objects for an area subjected to microbiological cross-contamination.

2. MATERIALS AND METHODS

2.1 Selected Ethnographic Objects

Ten selected ethnographic objects from the Museu do Índio Collections, were monitored to detect fungal contamination. Five objects were apparently contaminated by fungal growth, however, not confirmed due to the organic nature of the materials on the piece; and, five additional objects from the Museu do Índio Collection where no signs of fungal contamination could be macroscopically detected. A complete description of the objects can be found in Tables 1 and 2.

2.2 Culture Medium and Sample Collection

The culture medium used for fungal growth the culture medium used was Potato Dextrose Agar [10]. Culture medium was dissolved in distilled water, autoclaved at 121°C during 20 minutes. Afterwards it was aseptically distributed in Petri dishes. Using sterilized swabs, samples were collected from each ethnographic piece presented in Tables 1 and 2. Additionally, samples were collected in order to check the natural microbial flora in the environment where objects were stored, Petri dishes containing culture medium, were opened and left stand for natural deposition of particulate matter, for two hours. Chloramphenicol at 0.05 g/L was added to the culture medium in order to prevent bacterial growth. Samples collected were incubated in microbiological chamber (Nova Ética, Model D-411) at 25°C for 1 week. Objects were monitored in triplicate.

3. RESULTS AND DISCUSSION

Figs. 3 and 4 indicate the level of contamination observed in the 5 objects from the non-contaminated collection (Urubu-Kaapor Tribe) and the contaminated collection (Several tribes), respectively.

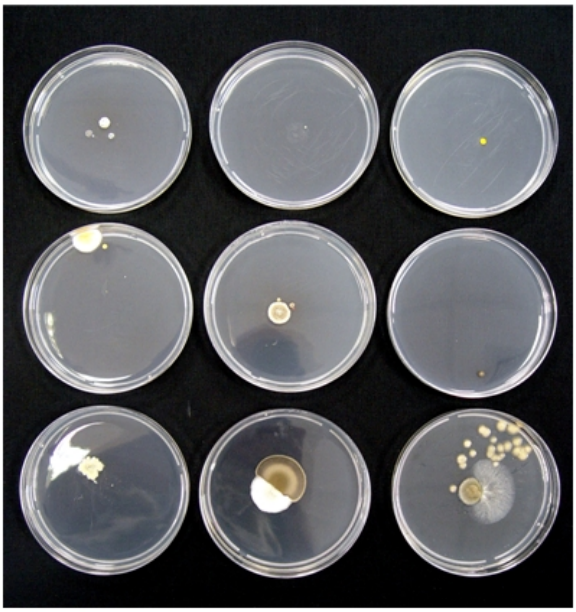

Fig. 3. Fungal colonies grown from 5 etnographic objects from the Darcy Ribeiro collection, Urubu-Kaapor tribe, Museu do Índio, Brazil.

Table 1. Objects from the Urubu-kaapor Tribe - Visually non-contaminated collection

Image	Description
	**5440 – Feathered clamp (1952) -** Feathered clamp supported by a necklace of black Awai seeds. Pendant include orange papo-de-tucano feathers (*Ramphastos tucanos*), black and white mutum feathers (*Crax fasciolata*) and white seeds of lágrima-de-Nossa-Senhora (*Coix lacryme-jobi*). Photo by A. C. Carvalho, Museu do Índio/FUNAI Collection, Brazil.
	**5411 – Seeded waistband (1952) -** Seeded waistband manufactured with horizontal twine containing non-identifiable black seeds and lágrima-de-Nossa-Senhora seeds (*Coix lacryme-jobi*). Photo by A. C. Carvalho, Museu do Índio/FUNAI Collection, Brazil.
	**2607 – Seeded waistband (1950) -** Seeded waistband manufactured with non-identifiable black seeds and pendants with small cabaça spires (*Lagenaria vulgaris*, Ser. *Cucurbita lagenaria*). At the end of the pendants white lágrima-de-Nossa-Senhora seeds (*Coix lacryme-jobi*) and caraguatá twine (*Bromelia penguin*). Photo by A. C. Carvalho, Museu do Índio/FUNAI Collection, Brazil.
	**0599 – Seeded waistband (1950) -** Seeded waistband manufactured with black and white lágrima-de-Nossa-Senhora seeds (*Coix lacryme-jobi*) and yellow and black plumed rosettes. Photo by A. C. Carvalho, Museu do Índio/FUNAI Collection, Brazil.
	**0510 – Feathered clamp (1950) -** Feathered clamp supported by red colored feathers from Araracanga (*Ara macao*) and yellow Japu tail feathers (*Psarocolius decumanus*). Black and white seeds at the border of the clamp. Photo by A. C. Carvalho, Museu do Índio/FUNAI Collection, Brazil.

Table 2. Objects from several tribes - Visually contaminated collection

Image	Description
	**5714 – Auricular pin from Xavante tribe (1954) -** Auricular pin constituting a piece with 25 connected wood objects with cotton threads painted with urucum (*Bixa orellana*). Photo by M. Ferreira, Museu do Índio/FUNAI Collection, Brazil.
	**873022 – Seeded necklace from Nambikwára tribe (1987) -** Seeded necklace manufactured with Santa Fé coconut peel and twined with non-identifiable seeds containing different pendants with feathers from yellow and blue araras (*Ara macao*) and mutum (*Mitu mitu*). Photo by M. Ferreira, Museu do Índio/FUNAI Collection, Brazil.
	**83.2.13 – Wide coconut strap (1983) -** Strap manufactured with small cylindrical disks containing tucum coconut beads (*Bactris setosa*) in a vegetable fiber cord. Photo by M. Ferreira, Museu do Índio/FUNAI Collection, Brazil.
	**96.1.58 – Coconut beads necklace from Kamayurá tribe (1996) -** Coconut beads necklace manufactured with a non-identifiable coconut species, filled with cotton threads. Photo by M. Ferreira, Museu do Índio/FUNAI Collection, Brazil.
	**76.2.11 / 76.2.11a – Braided bracelet (1976) -** Braided bracelet manufactured with a cylindrical wood piece, twisted to small rhombus patterns due to the way fibers were crossed. The objects include fruit seeds in the basis of the artifact and cotton threads. Particularly in this piece the species were not identifiable. Photo by P. Monteiro, Museu do Índio/FUNAI Collection, Brazil.

It can be quantitatively observed that fungal populations were almost in the same quantity for selected collections. However, it can also be clearly observed that the type of fungus present

in one type of collection is distinct from the fungus present in the remaining objects of the other collections. It can be seen that the growth rate in the contaminated collections (Figure 4) seems to be higher in comparison to the non-contaminated collection, thus, increasing the probability of cross-contamination if both collections are put together in the same room. This seems to be an important conclusion in relation to microbiological contamination; irrespective of the number of fungal species found, its dissemination is facilitated, considering the morphology of the colonies found. This morphology than becomes a critical parameter, more critical than the amount of species isolated. The type of growth observed for the fungus isolated from the contaminated collections, probably spore-forming species, makes their dissemination easily obtained. The monitoring of the air indicated that the fungal colonies found in the environment seem to be the same as found in the contaminated objects.

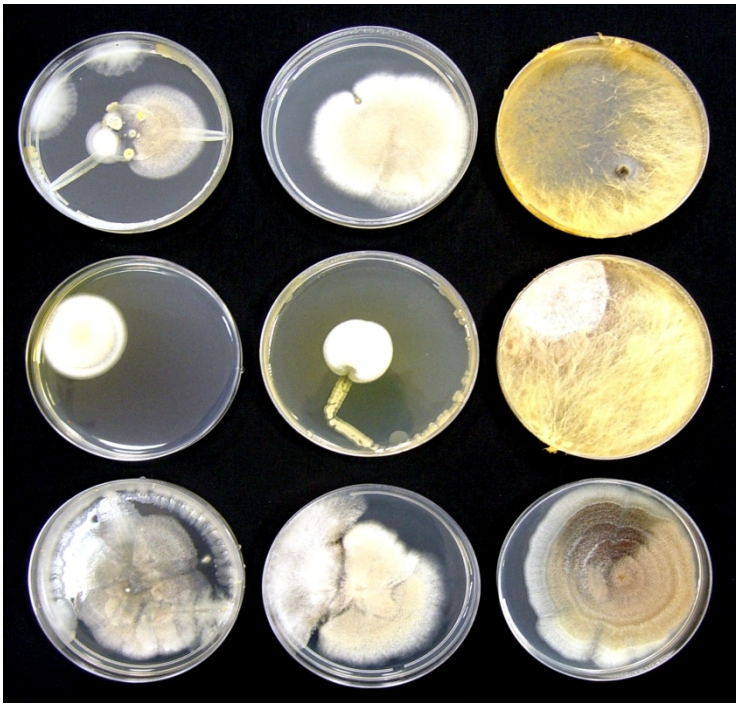

Fig. 4. Fungal colonies grown from 5 etnographic objects from several ethnographic collections. Several tribes, Museu do Índio, Brazil

This fact was not observed in the non-contaminated Urubu-Kaapor collection. Thus, this particular collection cannot be exposed in the same room as the remaining collections, because cross-contamination will probably take place.

From the total of 15 Petri dishes used for the monitoring of Urubu-Kaapor collection we decided to present the more representative dishes, just to illustrate the comments performed. Authors observed that from a total of 13 colonies grown, just 6 types of fungus were observed, based on macroscopic and microscopic observations. Considering the remaining collections we also selected a few dishes to represent the comments previously provided: we observed that from a total of 18 colonies grown, a higher number of diverse fungal types was observed, 10 in total. Beyond the fact that a higher number of typical

colonies was observed in the collections other than Urubu-Kaapor, the growth observed is clearly more representative in terms of production of biomass. The growth rate, here represented as an increase in the diameter of the colonies, proved that Urubu-Kaapor collection cannot be housed in the same space as the remaining collection. A higher biomass production is probably associated to a higher number of spores, thus increasing the deleterious effects of the biodeteriorating agents. Fungal species could not be confirmed due to the lack of molecular biology techniques available. Petri dishes located in the outer spaces of the museum indicated the presence of a much higher number of colonies and types of fungus, in comparison with the indoor evaluation of the objects. This indicated that the possible source of primary contamination is the air itself, alerting for the need to implement a strict control of air humidity and temperature. Probably some bacterial strains could also be detected. However, this was not performed in the present work, due to their reduced biodeteriorating potential in comparison to fungal biodeterioration on the objects studied.

We cannot confirm which fungal species predominate in both cases, but, for microscopic examinations probably *Aspergillus* and *Penicillium* genera occured, due to their morphological and color typical characteristics. These would be confirmed through molecular biology techniques, particularly DNA and genotypic complimentary evaluations. It is important to emphasize that the low number of fungal species isolated in both collections indicate good storage conditions for the ethnographic collections, if compared to the number of species found in external environments, where hundreds of cell colonies are found per cubic meter monitored.

Although not exactly from the same type of ethnographic materials, Irbe et al. [11] studied the biodeterioration of external wood of Latvian Ethnographic Open-Air Museum indicating the predominating fungal groups found. Fungi from the phyla Basidiomycota, Ascomycota and Protozoa (Myxomycota) were identified. Common fungal genera were *Antrodia, Gloeophyllum, Athelia, Hyphoderma, Hyphodontia, Pharenochaete, Postia* and *Botryobasidium*, mostly filamentous fungi.

Air-conditioned areas of a museum were monitored for the presence of total microbes in air. Results were evaluated based on a Brazilian resolution that regulates accepted contamination levels in air-conditioned spaces, and on World Health Organization. Results indicated low levels of bacterial and fungal populations, smaller than 50 CFU/m^3. These results, compared to the outside area of the museum indicated a low internal to external ratio, around 0.131, a value far from the limit of 1.5 in the Brazilian legislation. Although those values clearly indicate low levels of contamination for human comfort, the presence of fungi from the genera *Cladosporium, Aspergillus* and *Penicillium* requires attention due to their possible cellulolytic activity. The spaces should be permanently controlled for their temperature and relative humidity levels, to be used as a permanent repository. The presence of cellulose-degrading microbes can jeopardize the effective occupation of the areas due to their biodegradation effects [10].

Konsa et al. [9] discussed biodeterioration in museums, for the collections of the Estonian National Museum, mainly wooden objects. Those objects include furniture, working tools, household utensils, basketry etc. Those objects were suffered from varying degrees of fungal deterioration. Authors stated that biodeterioration of objects provides information about their environmental conditions and the use patterns of these objects. As observed in the article, the risk of biodeterioration of wooden objects is small but present.

Bjordal and Nilsson [12] worked with archaeological wood samples as substrate for aerobic fungi. In fact, the authors decided to inoculate white and brow-rot fungi on sterilized 1600 year old ash degraded by bacterial cells. They observed that the white rot fungus was able to use the degraded wood, and that the attack was observed at a macroscopic level after eight months of incubation. In contrast to degradation on fresh ash, development of mycelia was significantly repressed. This result corroborates our idea that mixing contaminated and non-contaminated objects in the same space, can move to contamination.

In a study on culturable fungi and bacteria in the air and settled dust in the storerooms of five Polish libraries and archives [13]. Authors concluded that in all studied rooms, total microbial concentrations ranged from 100 to 1,000 CFU/m^3. Authors concluded that the predominating group was filamentous fungi, followed by bacteria.

From the results here presented it can also be observed that they are in close agreement with World Health Organization and Brazilian ANVISA. The main conclusion obtained is that, based on this evaluation, Urubu-Kaapor collection should not be stored in the same space with other ethnographic collections in Museu do Índio, due to its low level of contamination, probably related to its nature and chemical composition. This would avoid long-term cross-contamination.

To end, Sterflinger [14] informs that fungi play a marked role for the deterioration of cultural heritage. Due to their high enzymatic activity and ability to grow at low water activity, they inhabit and decay paintings, textiles, paper, parchment, leather, oil, casein, glue and other materials. Skills and close collaboration of mycologists and restorers are necessary in order to develop specific methods for the conservation and treatment of contaminated objects.

4. CONCLUSIONS

The level of contamination of the Urubu-Kaapor ethnographic collection was equivalent to the level of contamination of the remaining ethnographic collections from the Museu do Índio, Rio de Janeiro, Brazil. However, the type of fungal contamination present indicates the presence of different fungal types.

In the Urubu-Kaapor objects investigated the fungal types isolated did not markedly grow in proper culture medium, just developing small fungal colonies. On the other hand, the objects monitored from other ethnographic collections indicated the presence of highly filamentous and spore-forming types of fungus.

Objects from the Urubu-Kaapor collection were basically composed of bird feathers and seeds, while the remaining objects from other collections were composed of wood objects, feathers and vegetable fibers and nuts, probably much more amenable to biodeterioration.

This clearly indicates that ethnographic collections, presenting different degrees of fungal contamination should not be placed in the same storage room in the Museu do Índio, in order to prevent cross-contamination from one collection to another.

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COMPETING INTERESTS

Authors have declared that no competing interests exists

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