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Microbiota Diversity on the Preserved Specimens of Natural History Museum, Swayambhu, Kathmandu, Nepal

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Abstract
Microorganisms and the undesired environmental conditions in the indoor environment pose potential risk to museum collection. Simultaneously, moisture and mold problems in indoor environment are known to cause threat to preserved collections as well as to workers and visitors. With an aim to study diversity of microbiota on the preserved museum specimens, total 80 swab samples from surfaces of specimens and 30 air samples were collected from the Natural History Museum. The microbial contamination of the indoor air ranged from 2.47×10^3 - 3.61×10^3 cfu/m^3; contamination of the museum specimens ranged from 9.12×10^3 - 6.57×10^4 cfu/m^2. The predominant microorganisms isolated were *Staphylococcus aureus* followed by CoNS, *Bacillus subtilis*, *B. cereus*, *Cladosporium* spp., yeast, *Aspergillus* spp. and *Penicillium* spp. *B. subtilis* was the greatest proteolytic and keratinolytic enzyme producer which degraded feather about 74.8%. Microclimate inside the indoor environment show great fluctuations in indoor air temperature and relative humidity on different sampling days, i.e. 21.77±3.35°C and 62.26±5.93% respectively. Great fluctuations in microclimate inside museum environment and higher microbial contamination of the museum specimen indicated the inability of the museum to maintain appropriate preservative conditions. Hence, proper study of the museum environmental condition, level of the microbial contamination of the indoor environment and disinfection of museum specimens is important.

Keywords
indoor, molds, appropriate preservative, microclimate, contamination

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INTRODUCTION
Museum items include prehistoric and historic objects, artifacts, work of art, archival material, and natural history specimens possessing functional, aesthetic, cultural, symbolic, and scientific value, usually movable by nature or design [1]. Natural History Museum situated at the foothill of Swayambhu World heritage site in Kathmandu Metropolitan City was established in 1975 July 17, is an institute under the aegis of Tribhuvan University, Institute of Science and Technology. The museum houses more than 55,000 valuable Nepalese flora, fauna and paleontological

specimens. Various research and educational activities are conducted by the museum for the conservation of precious natural resources of the Nepal. The openly exhibited museum specimen (Photograph 1) needs to be preserved well by maintaining good indoor environment [2].

Photograph 1: Displayed museum specimen outside showcase

Photograph 2: Displayed museum specimen inside wooden showcase

The moisture on the surface of museum specimens can favor growth of microbes and create specific niches. A leaky roof, plumbing defects, capillary movement of water in the structures, poor ventilation or insufficient insulation, condensation on windows and subtle surfaces, and humidified air etc. are the main reasons for the creation of moisture in indoor environment (Photograph 2) [3]. The increase in moisture in indoor environment can lead to biodeterioration of building structures and museum specimens through physical, chemical processes or microbial growth. In addition, it releases various potentially harmful agents such as fungal spores, volatile organic chemicals and mycotoxins into the indoor air [4] which can cause health hazard to museum staff and visitors.

Museum specimens' exhibits are national heritage and may subject to permanent damage from biological interactions by bacteria and fungi. These microorganisms can be carried by dust particles in indoor archive repositories by means of the people and air ventilation systems [5]. The nutrients; carbon, nitrogen, phosphorus, and potassium essential for the growth of microorganisms are usually provided by most of the wood and paper present inside the buildings [6]. Trace amounts of nutrients present in house dust and water, makes the availability of nutrients to facilitate the microbial growth [7].

Colonization and biodegradation of specimens by microorganisms in closed environment depends on the species and composition of microorganism. The micro ecosystem created from the colonization of microorganisms on the museum specimens stops the normal flux of air. This conditions the surface to absorb humidity which assists additional microbial adherence and subsequent biofilm formation [8].

Different enzymes, such as cellulases, proteases, ligninases and organic acids produced by fungi and bacteria can decompose historic materials resulting its serious damage. Filamentous fungi and Actinobacteria are more efficient in degradation of complex polymers [9]. The environmental microorganisms can utilize and deteriorate different organic, natural or synthetic substrates (cellulose, polycarbonates), metals, and compounds of optical and magnetic devices [10]. A

special group of proteolytic enzymes, keratinases can catalyze the cleavage and hydrolysis of the highly stable and fibrous proteins, keratins present in feather of bird specimen. A diverse range of microorganisms, including fungi, Actinomycetes and bacteria, have been reported to produce keratinases [11]. The keratinase activity of the keratin degrading isolates can be assessed on feather medium. Keratin degradation can be measured by the release of free protein content, free amino acids and pH change in culture medium [12].

No work so far has been carried out for determination of microbial contamination in museum of Nepal. To preserve the national biological heritage, investigation of microbial diversity and concentration in museum specimens and indoor air is urgently needed. It has been assumed that this study will definitely give qualitative and quantitative knowledge of indoor environment quality of museum in terms of microbial concentration and diversity.

METHODOLOGY

The museum indoor environment

Microbiological studies were performed at the Natural History Museum, Swayambhu, Kathmandu, Nepal. The physiochemical parameters of the indoor environment (i.e. temperature and relative humidity) at different sampling days were recorded using a digital thermo hygrometer.

Isolation of microorganism from museum specimens

Preserved animal specimens including hides, bones, feathers, shell and fossils from the showcases and outside the showcases were selected for sampling. Samples were collected by swabbing area of 4 cm^2 [8, 10] with sterile cotton swabs dipped in normal saline. For characterization of total aerobic bacteria, the collected samples were homogenized and suitable dilutions of each sample from the different materials were inoculated in Nutrient Agar (NA) and incubated at 37°C for 24 hours. For fungal isolation, suitable dilutions of each sample from the different materials were inoculated onto Sabouraud Dextrose Agar (SDA) and incubated at 28°C for 5 days prior to colony counting.

Microbiological sampling of air

Microbiological sampling was carried out by the sedimentation method as described by Omeliansky [8, 10]. Petri dishes containing NA for the isolation of bacteria and SDA with chloramphenicol for fungi, were exposed at approximately 1 m above the floor for 10, 20 and 30 minutes. Five different points inside museum were sampled. SDA plates were incubated at 28°C for 7 days and NA at 37°C for 24 hours. The cfu/m^3 was estimated according to Omelianskyi's formula: $N = 5a \times 10^4/(bt)$. Where, N = microbial cfu/m^3 of indoor air, a = number of colonies per Petri dish, b = dish surface, in square centimetres, and t = exposure time, in minutes.

Relative microbial distribution in air and surface samples was conducted according to Borrego et al. 2012 [10] where:

$$\text{Relative distribution (\%)} = \frac{\text{Number of colonies of the genus or species}}{\text{Total number of colonies of all genera or species}} \times 100$$

Identification of isolated organisms

Identification of fungal colonies was done by observation of cultural and morphological characteristics [13]. For bacterial identification, Gram staining and biochemical tests were performed as described in Bergey's Manual of Systematic Bacteriology [14].

Screening and determination of enzymatic activity

Skim Milk agar medium was used for the primary screening of keratinolytic bacteria. Total 50 bacterial isolates of genera *Staphylococcus, Micrococcus* and *Bacillus* already maintained in NA were inoculated in milk agar plates. The plates were incubated at 37°C for 24 hours and examined for clear zone formation on the milk agar plate. Isolates showing highest proteolytic activity on milk agar plates were incubated on modified liquid media with feather at 37°C for 10 days at 175 rpm. A control was kept with the same amount of medium and feather without organisms. The change in pH of the medium was measured at an interval of 2 days. After 10 days, the remaining feather was washed, dried and total dry weight of feather remains was measured. Degradation percentage (%) was calculated from weight loss from the feather substrate between control (uninoculated) and experimental samples [12].

Statistical analysis

Statistical analysis was conducted using the Statistical Package for the Social Sciences (SPSS) program. Chi-square test was employed to evaluate differences between total microbial prevalence in museum specimens displayed inside and outside wooden showcases. Results with $p \leq 0.05$ were considered statistically significant.

RESULTS

From the 80 swab samples collected, total 1889 microorganisms were isolated, out of which 1468 (77.7%) were bacteria and 421 (22.3%) were fungi. The quantitative analysis of microbial concentration on the preserved museum specimens displayed outside showcases showed predominance of bacterial isolates that of fungi in all type of specimens. Among the museum specimens displayed outside showcases, highest concentration of microorganisms was detected on hides (6.57×10^4) cfu/m^2 followed by bones (4.35×10^4) cfu/m^2, shell and fossils (3.20×10^4) cfu/m^2 and feathers (3.10×10^4) cfu/m^2. Among the museum specimens displayed inside the showcases, highest concentration of microorganisms was detected on bones (2.20×10^4) cfu/m^2 followed by hides (2.13×10^4) cfu/m^2, feathers (1.00×10^4) cfu/m^2 and the least on shells and fossils (9.12×10^3) cfu/m^2.

Distribution and prevalence of microorganisms on museum specimen

Among the 80 swab samples taken from different museum specimens, 75 (93.7%) samples were detected positive for bacterial contamination. The predominant bacteria on the museum specimens was Gram positive cocci followed by Gram positive sporulating bacilli, Gram positive non sporulating bacilli and Gram-negative bacilli. Gram negative bacilli was absent in all the specimens inside the showcases (Photograph 2) as shown in Figure 1.

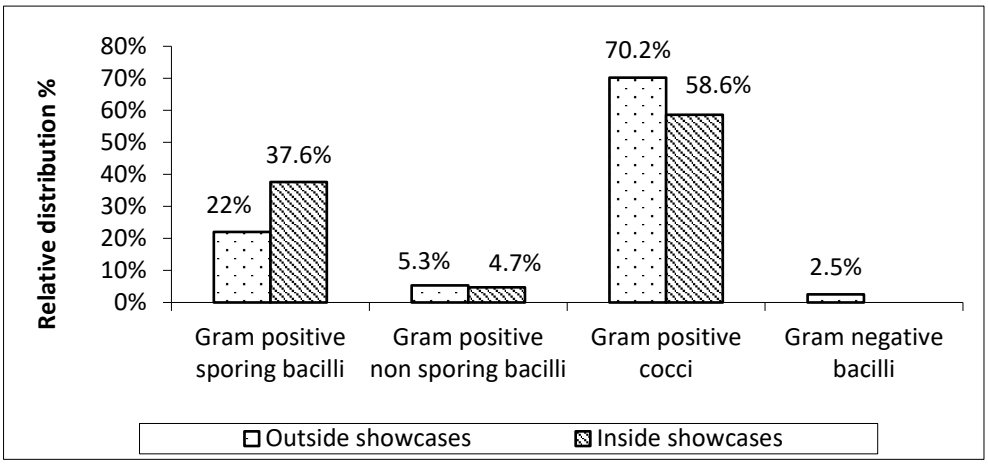

Figure 1: Relative distribution of bacteria on museum specimens

Among the 40 swab samples collected from the museum specimens displayed outside the showcases for detection of fungal contamination, 31 (77.5%) samples were found positive. *Cladosporium* spp. 118 (34.4%) was the prevailing fungi followed by yeasts 76 (22.3%), *Aspergillus* spp. 52 (15.2%) and *Penicillium* spp. 37 (10.8%). The least detected fungi were *Alternaria* spp. 6 (1.6%), *Pecilomyces* spp. 5 (1.5%), *Sporothrix* spp. 5 (1.5%) and *Curvalaria* spp. 3 (0.8%) (Figure 2).

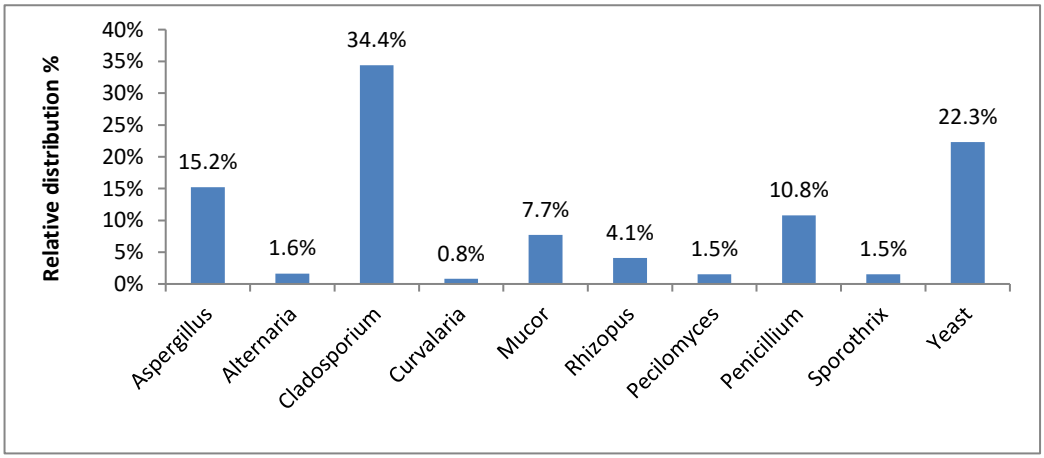

Figure 2: Relative distribution of fungal genera detected in museum specimens (outside showcases

Similarly, out of 40 samples collected from 27 (67.5%) were found positive for fungal contamination. *Cladosporium* spp. 43 (54.7%) was the predominating fungal genera followed by yeasts 21 (26.5%), *Aspergillus* spp. 7 (8.8%), *Penicillium* spp. 3 (3.7%), *Rhizopus* spp. 3 (3.7%) and *Mucor* spp. 2 (2.7%) (Figure 3).

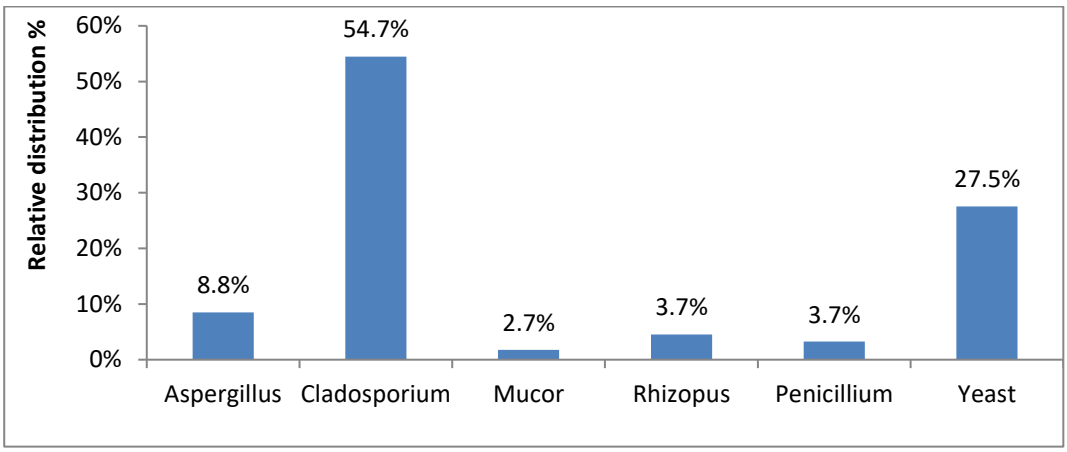

Figure 3: Relative distribution of fungal genera detected in museum specimens (showcases)

The concentration of bacteria and fungi on museum specimens by statistical analysis showed that there was significant difference between bacterial and fungal concentrations on museum specimens outside and in showcases as shown in Table 1.

Table 1: Distribution of microorganisms in surface sample

Site	Concentration of bacteria	Concentration of fungi	p-value*
Outdoor	25,050	17,875	0.018**
Showcases	23,700	3,250	

***Chi-square test; **Significant at p≤0.05**

Distribution and prevalence of microorganisms in indoor air

From 30 air samples collected, total 691 microorganisms were isolated, i.e. 438 (63.7%) bacteria and 253 (36.3%) fungi. The average concentration of microorganisms in air per cubic meter of the museum was 2.49×10^3 cfu/m^3 and 6.21×10^2 cfu/m^3 respectively for bacteria and fungi. The entrance zone had a greatest total microbial concentration (3.61×10^3 cfu/m^3) followed by geological collection room (3.29×10^3 cfu/m^3), middle of the museum hall (3.25×10^3 cfu/m^3), corner of the museum hall (2.93×10^3 cfu/m^3) and the least at botanical collection room (2.47×10^3 cfu/m^3).

Out of 15 SDA plates exposed, all plates were positive for contamination of fungi that accounts for 100 % of the total plates exposed. The predominant fungi isolated from air were yeasts 76 (30.1%), *Cladosporium* spp. 58 (22.9%) and *Aspergillus* spp. 45 (17.8%). The least isolated fungi were *Sporothrix* spp. 9 (3.5%) and *Pecilomyces* spp. 3 (1.2%) (Figure 4).

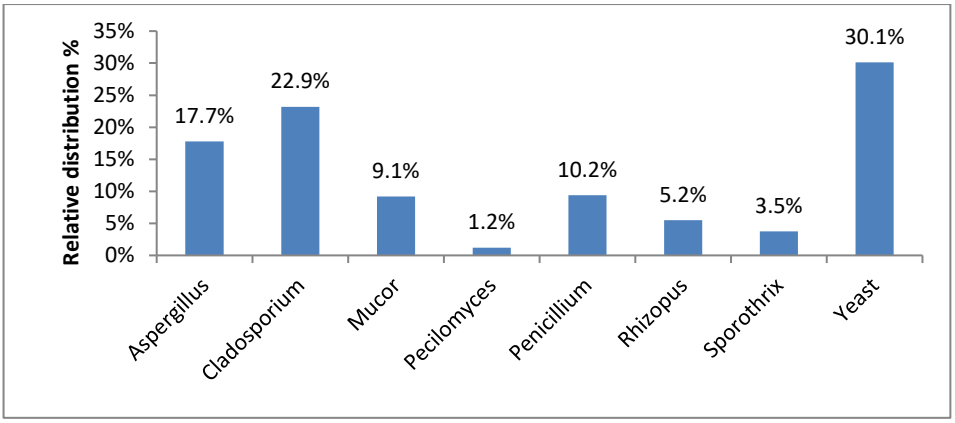

Figure 4: Relative distribution fungi in indoor air at different sites

Out of total 15 NA plates exposed for bacterial isolation, all the plates (100%) were positive for bacterial contamination. Among the isolates, Gram positive cocci (73.1%) were the most predominant bacteria followed by Gram positive sporing bacilli (22.6%), Gram positive non sporing bacilli (2.6%) and the least, Gram negative bacilli (1.5%) (Figure 5).

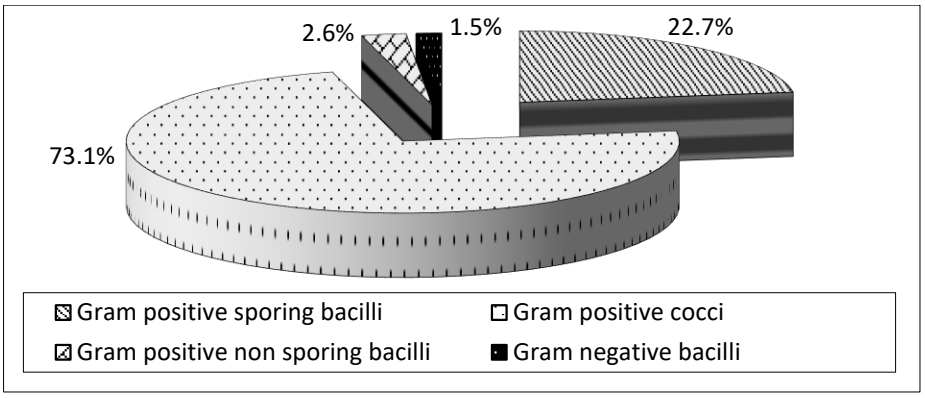

Figure 5: Occurrence of different bacteria on indoor air

Table 2: Frequency of isolated bacteria and fungi and classification on the basis of risk group

Isolated microorganism	Frequency on samples		Risk group
	Surface (%)	Air (%)	
Bacteria			
Actinomyces spp.	10	0	2
B. subtilis	56.3	100	2
B. cereus	33.3	33.3	2
B. licheniformis	6	26.7	2
B. megaterium	9.3	0	2
Bacillus spp.	7.6	0	2
CoNS	53.7	100	2
Corynebacterium spp.	26.3	20	2
S. aureus	78.7	100	2
Staphylococcus spp.	16.3	86.7	2
Micrococcus spp.	23.7	60	1
P. aeruginosa	15	13.4	2
Fungi			
A. niger	17.5	60	2
A. fumigatus	8.7	13.4	2
A. flavus	6.3	0	2
Alternaria spp.	21.3	0	2
C. cladosporides	42.5	80	2

P. notatum	‘16.3	6.7	2
Penicillium spp.	7.5	40	2
Mucor spp.	15	40	2
Rhizopus spp.	8.7	26.7	1
Sporothrix spp.	3.7	26.7	2
Pecilomyces spp.	2.5	13.4	1
Rhodotorula spp.	11.3	66.7	1
Candida albicans	18.7	46.7	2
Curvalaria spp.	1.25	0	1
A. niger	17.5	60	2
A. fumigatus	8.7	13.4	2
A. flavus	6.3	0	2

1-Risk group 1 (No hazard) 2- Risk group 2 (Potential hazard)
3-Risk group 3 (serious hazard) 4- Risk group 4 (Highly serious hazard)

The classification of potential pathogens among the isolated microorganisms was done according to classification of biological agents, National institute for public health and the environment, Ministry of health welfare and support, RIVM Letter report 205084002/2012 [15]. These belong to the genera: *Bacillius, Staphylococcus, Pseudomonas, Alternaria, Aspergillus, Cladosporium, Pecilomyces, Mucor* and *Penicillium* as shown in Table 2.

Enzymatic activity of bacterial isolates

Bacterial species of genera *Staphylococcus, Micrococcus, Bacillus* isolated from the museum specimen as well as in air sample was screened for their proteolytic and keratinolytic activity. *B. subtilis* was most efficient in keratin degradation which accounted upto 74.8% (Table 3).

Table 3: Assessment of enzyme activity of bacterial isolates

Isolate	Bacteria	Zone of skimmed milk hydrolysis (mm)	Initial pH	Final pH	Weight loss (%)
NHM3	*B. subtilis*	20	6.9	8.2	61.2
NHM8	*B. subtilis*	23	6.9	8.5	74.8
NHM11	*B. cereus*	19	6.9	8.4	72.6
NHM13	*B. cereus*	18	6.9	8.2	59.2
NHM24	*S. aureus*	17	6.9	8.4	71.8
NHM30	*S. aureus*	14	6.9	8.1	46.6
NHM38	*B. licheniformis*	16	6.9	8.3	68.4
Control			6.9	7.0	0.986

The analysis of the microbial contamination of the preserved museum specimens showed a great variability in concentrations of microorganisms on the basis of type and location of the museum specimen (Table 4). The highest concentration of microorganism was recovered from hides (6.57×10^4) cfu/m^2 followed by bones (4.35×10^4) cfu/m^2, shell and fossils (3.20×10^4) cfu/m^2 and feathers (3.10×10^4) cfu/m^2. Similarly, the analysis of microbial concentration on museum specimens displayed inside the wooden showcases showed highest concentration of microorganisms on bones (2.20×10^4) cfu/m^2 followed by hides (2.13×10^4) cfu/m^2, feathers (1.00×10^4) cfu/m^2 and the least on shell and fossils (9.12×10^3) cfu/m^2.

Table 4: Quantitative analysis of microbial contamination of museum specimens

Museum specimens	Number of microorganisms (cfu/m^2)			Percentage	
	Bacteria	Fungi	Total	Bacteria	Fungi
Hides (Outside showcases)	$9.07\times10^4 \pm 5.82\times10^4$	$4.07\times10^4 \pm 6.9\times10^4$	$6.57\times10^4 \pm 6.75\times10^4$	69.1%	30.9%
Feathers (Outside showcases)	$5.07\times10^4 \pm 3.22\times10^4$	$1.12\times10^4 \pm 1.07\times10^4$	$3.10\times10^4 \pm 3.10\times10^4$	81.8%	18.2%
Bones (Outside showcases)	$6.92\times10^4 \pm 3.40\times10^4$	$1.80\times10^4 \pm 2.72\times10^4$	$4.35\times10^4 \pm 4.0\times10^4$	79.6%	20.4%
Shell and fossils (Outside showcases)	$4.75\times10^4 \pm 4.27\times10^4$	$1.65\times10^4 \pm 2.00\times10^4$	$3.20\times10^4 \pm 3.60\times10^4$	74.2%	26.8%
Hides (Inside showcases)	$3.75\times10^4 \pm 3.42\times10^4$	$5.25\times10^3 \pm 7.00\times10^3$	$2.13\times10^4 \pm 2.92\times10^4$	87.7%	12.3%
Feathers (Inside showcases)	$1.52\times10^4 \pm 7.01\times10^3$	$4.75\times10^3 \pm 5.94\times10^4$	$1.00\times10^4 \pm 8.3\times10^3$	76.2%	23.8%
Bones (Inside showcases)	$3.80\times10^4 \pm 3.12\times10^4$	$6.0\times10^3 \pm 6.77\times10^4$	$2.20\times10^4 \pm 2.72\times10^4$	86.4%	13.6%
Shell and fossils (Inside showcases)	$1.47\times10^4 \pm 1.54\times10^4$	$3.5\times10^3 \pm 7.07\times10^3$	$9.12\times10^3 \pm 1.30\times10^4$	80.8%	19.2%

The analysis of microbial contamination in indoor air of museum (Table 5) showed greater prevalence of microorganisms at the entrance zone (3.61×10^3 cfu/m^3) than the inner areas of the museum hall which might be due entry of microorganisms during circulation of outdoor/indoor air through the doors. The close proximity of museum building to the street is more likely to favor the inflow of large amount of dust with microorgansms. Furthermore, museum personnel or the visitors contribute to the ease of transport of undesired polluting compounds [16]. The lowest microbial concentration was at Botanical collection room (2.47×10^3 cfu/m^3). This might be due to less visit frequency of workers and visitors as well as the windows of the room remain closed most of the time. The microbial prevalence in air was determined using the Omeliansky's formula [8, 10]. In compare to international studies the present study showed low contaminations as it carried out in single museum only whereas other studies involved different archives and documentary heritages.

Table 5: Quantitative analysis of microbial contamination of indoor air

Sampling site	Number of microorganisms (cfu/m^3)			Percentage	
	Bacteria	Fungi	Total	Bacteria	Fungi
Entrance zone	$2.95\times10^3 \pm 3.59\times10^2$	$6.55\times10^2 \pm 1.09\times10^2$	$3.61\times10^3 \pm 1.27\times10^3$	81.8%	18.2%
Middle of hall	$2.77\times10^3 \pm 5.52\times10^1$	$5.37\times10^2 \pm 1.42\times10^1$	$3.25\times10^3 \pm 1.23\times10^3$	83.5%	16.5%
Corner of Hall	$2.45\times10^3 \pm 7.1\times10^1$	$4.73\times10^2 \pm 1.77\times10^2$	$2.93\times10^3 \pm 1.08\times10^3$	83.8%	16.2%
Paleontological room	$2.39\times10^3 \pm 3.12\times10^2$	$8.98\times10^2 \pm 3.74\times10^2$	$3.29\times10^3 \pm 7.76\times10^2$	72.7%	27.3%
Botanical room	$1.93\times10^3 \pm 1.56\times10^2$	$5.46\times10^2 \pm 1.51\times10^2$	$2.47\times10^3 \pm 7.76\times10^2$	77.9%	22.2%

DISCUSSION

The possible cause of difference in concentration of microorganisms in different museum specimens might be due to difference in the surface properties and location of the museum specimens as explained by similar studies [8, 10]. In this study, the average microbial concentration on swab samples taken from museum specimens ranged from 9.12×10^3 - 6.57×10^4 cfu/m^2. The result obtained in this study showed lower microbial contamination compared to similar studies which demonstrated the ranges of 2.5×10^3 cfu/m^2 - 2.4×10^6 cfu/m^2, 1.8×10^2 - 3.7×10^5 cfu/m^2 , 1.4×10^4 - 1.7×10^6 cfu/m^2 [8, 16, 17, 18]. Previous similar studies from other countries show higher contamination of proteolytic, amylolytic and cellulolytic microorganisms on samples taken from paper, silk, maps and paintings. The higher concentration of microorganisms on the surfaces might be possibly due to presence of organic matter on these museum collections which supported the growth of microorganisms.

The concentration of bacteria was greater than fungi in all types of museum specimens analyzed. The possible cause of predominance of bacteria in all types of museum specimen might be due to high protein content in the specimens which support the proteolytic bacteria [19]. In addition, the lower number of fungi on the surface of museum specimens might be possibly due to fact that light fungal spores do not sediment readily. Previous studies suggested that the spores less than 5 µm require winds stronger than 25 m/seg to be deposited [20]. However, bacterial cells are generally carried on dust which makes them heavy enough to be deposited on museum specimens. The resultant p-value ($p \leq 0.018$) indicated that there was significant difference in the concentration of microorganisms on museum specimens inside and outside showcases.

The concentration of microorganisms on the preserved museum specimens outside the showcases was relatively higher than specimens inside the showcases. The lowest microbial concentration was detected on the fossils inside the showcases 9.12×10^4 cfu/m^2 whereas highest concentration of microorganisms was detected on the hides displayed outside the showcases 6.5×10^4 cfu/m^2 (Table 4). The showcases act as barrier against direct contamination from the indoor air to some extent reducing the microbial load. However, museum specimens which seemed to be clean inside the showcases are contaminated too. Since, the routine cleaning practice of the showcases is not carried out from time to time; the environment inside showcase does not seem to be sterile. So, the microbes already present can thrive if effective preservatives were not used. In addition, the preservatives like naphthalene balls are inadequate for preservation. Among the bacterial isolates detected from the swab samples, predominance of Gram-positive bacteria was observed in all type of museum specimens which corroborate with reports from Borrego et al. (2012) and Guiamet et al. (2011) [8, 10]. Gram positive bacteria were prevalent in most of the environmental samples due to their ability to withstand unfavorable environmental stress [21]. All the samples collected from the preserved animal specimens showed prevalence of *S. aureus* (36.5%). *S. aureus* is a commensal microorganism in animals that colonize nose and skin. In addition, Gram positive bacteria like CoNs, *Micrococcus*, *Corynebacterium* inhabit as normal flora of the skin. *Bacillus* spp. was the predominant spore forming Gram positive bacteria detected. The ability to produce highly resistant spores enables them to withstand unfavorable environmental conditions [22].

As for the fungi, *Cladosporium* spp. was predominant followed by yeasts, *Aspergillus* and *Penicillium*. The bacteria and fungi detected in air were identical to that on the museum specimens. This relates the contamination of the museum specimens with the microflora from the air. It's worth mentioning that the *Cladosporium* spp., yeasts, *Aspergillus* spp. and *Penicillium* spp. were isolated from the air samples of these rooms, which can colonize different surfaces due to their cosmopolitan distribution [19, 23]. For the preservation of the museum specimens, sun drying of the specimens was common method which could be the possible cause of contamination of specimens from outdoor environment.

The bacterial concentration 438 (63.7%) in the indoor air was greater than that of fungi 253 (36.3%). The lower concentration of fungi in museum air might be due to the absence of proper ventilation system which facilitates water absorption by conidia and their sedimentation inside the building [24]. In this study, *S. aureus* was the most predominant bacteria isolated from the air samples. *Staphylococcus* spp. is known to form aggregate in nature, so they tend to give higher colony counts. *Staphylococcus* spp. are found in skin and mucous membrane of all individuals and animals, usually expelled from the respiratory tract which may account for the presence of this bacteria inside the indoor materials [25]. Similar to museum specimen, *Bacillus* spp. was the most frequently isolated Gram-positive spore former *Pseudomonas* spp. was the only Gram-negative bacterial genera isolated.

In this study, commonly detected fungi in air were *Cladosporium* spp., yeasts, *Aspergillus* spp., *Penicillium* spp. as shown by other studies [26]. Here, *Aspergillus* and *Penicillium* genera were dominant due to their cosmopolitan nature [27]. As a consequence of different climatic conditions at different sampling days, large fluctuations in relative humidity and temperature were observed. The average temperature and relative humidity recorded at different sampling days were 21.7±3.4°C and 62.3±5.9% respectively. Steady microclimatic parameters, i.e. temperature of 20±2°C and relative air humidity of 50±3%, are recommended for collection storage in the studied institution like libraries and museums [28]. Large fluctuation in temperature and relative humidity indicates the inability of the showcases as well as museum building to create favorable preservative conditions [29]. This may be due to improper ventilation and air conditioning system.

The fungi isolated can potentially cause allergic reaction in museum staffs and visitors. The toxicity of these species has been confirmed in a study performed by Tuomi et al. (2000) [30]. Similarly, bacteria like *Staphylococcus, Bacillus, Micrococcus* and CoNS can cause opportunistic infections.

It has been suggested that environment with microbial prevalence more than 7.5×10^2 cfu/m^3 should be considered as contaminated [5]. The ministry of

culture in Italy has established that the air of a good quality in Italian archives, libraries, and museums should not exceed 7.5×10^2 cfu/m^3 of bacteria or the 1.5×10^2 cfu/m^3 of fungi [31]. In all 5 different sites inside the museum, the number of microorganisms was in the range of 2.47×10^3 - 3.61×10^3 cfu/m^3 which exceeded the permissible limit of microbial concentration in the air. This indicates that the indoor air of the Natural History Museum is considered to be contaminated.

Proteolytic and keratinolyic activity of 50 different bacterial isolates from different museum specimens were performed to assess the ability to produce enzymes susceptible to biodeterioration. *Bacillus* and *Staphylococcus* were the highest protease producers. *B. subtilis* showed the highest zone of hydrolysis on skimmed milk agar (23 mm). Assessment of keratinolytic activity of seven efficient protease producers was assessed for feather biodegradation ability. The change in the pH of the media from initial neutral pH 6.9 to alkaline pH 8.5 indicated keratinolytic activity of the bacteria to utilize the substrate causing deamination and ammonia production. During keratinolytic activity, microorganisms increase the pH to at least 8 [32].

B. subtilis was the most effective feather degrading bacteria (74.8%). Similar studies showed bacteria of genera *Actinomyces, Bacillus, Staphylococcus,* and *Pseudomonas* produce hydrolytic enzymes to degrade complex organic compounds like cellulose, pectin, chitin, starch and protein [8, 10]. The potential of microorganisms to produce acids and different pigments on the substrates forms biofilms which accelerates the deterioration of the different substrates [8, 10]. This study includes simple sampling technique and basal culture media for the isolation and characterization of microorganisms from the indoor environment. Hence, for gaining more accurate results, systematic sampling is needed with diverse type of culture media to characterize fastidious microorganisms.

CONCLUSION

Natural History Museum, Swayambhu, Kathmandu is an exhibition space for museum specimens with great value was ideally considered to be free from internal sources of pollutants. But the indoor air environment of museum and museum specimens was found to be contaminated with a large number of bacteria than the fungal microflora. The air inside the museum gets polluted primarily from the outdoor environment and may transferred to indoor environment during the outdoor/indoor air exchange through crevices in walls or opening and closing activities during entering the building, or through open windows or doors and through ventilation systems. Different polluting parameters were measured with different parameters. The results allowed an estimation of the outdoor and indoor air quality, giving an insight to possible risks for museum collections exhibited in the galleries and showcases. The enzymatic activities (i.e. proteolytic and keratinolytic) demonstrated by bacterial isolates suggest the potential biodeterioration of museum specimens. *B. subtilis* showed the highest protease and keratinase activity. The microbial concentration in indoor air of Natural History Museum was higher than the permissible limit of microorganism i.e. 1.0×103 cfu/m^3. Thus, it is necessary to clean the showcases before placing specimens into the cases in order to minimize the chance of microbial growth proliferation. There should be frequent surveillance and cleanliness should be done within the museum.

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