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Microbiological Impact Assessment of Air Environment of the Hides and Skin Processing Unit of NILEST Tannery, Zaria

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Authors' contributions

This work was carried out in collaboration between the authors. Author JOO designed the study, wrote the protocol, analyzed the study and supervised the experimental. Author RA managed the literature searches, managed the experimental process and wrote the first draft of the manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

Introduction: Microbial analysis of air is one of the most vital investigations of determining the microbial air pollution. The information on the microbial concentration of bacteria and fungi is necessary both to estimate the health hazard and to create standard for air quality control.
Aim: This study was carried out to investigate the microbiological quality of the air environment in the hides and skin processing unit of NILEST Tannery, Zaria.
Methodology: The analysis was carried out using the settle-plate method. The plates were exposed during passive and active sessions for 30 to 60 minutes.
Results: The bacteria isolated and identified include *Proteus mirablis, Pseudomonas aeruginosa, Salmonella enterica, Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Klebsiella pneumonia* whereas the fungi isolated include *Sclerotium sp., Fusarium sp., Aspergillus niger,*

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Aspergillus fumigatus, Corticium sp., and *Aspergillus ficheri*. The mean total bacteria load recorded during passive and active sessions were 180 cfu/m^3 and 254 cfu/m^3 respectively. Those of fungi were 115 cfu/m^3 and 284 cfu/m^3 respectively.
Conclusions: The isolation of these microorganisms from the study area is indicative of workers being exposed to potential bio-hazards and therefore there is the need for adequate measures to reduce the risk of exposure to pathogenic strains.

Keywords: Biohazard; air quality; opportunistic infections; epidemiology.

1. INTRODUCTION

Workers in the leather manufacturing industry have been associated with various hazards caused by microbiological, toxicological, and carcinogenic agents. The specific hazard associated with exposure in the leather industry depends upon the extent to which the worker is exposed to the agent(s) which is dependent upon the working area within the industry. The leather processing begins with the removal of the epidermis of the hide and only the dermis is transformed into leather [1]. During this process, infection is a constant hazard since the hide serves as a medium for numerous microorganisms. Tetanus, Anthrax, Leptospirosis, Epizootic aphtha and Brucellosis are possible diseases that workers of this occupation could contact due to infected hides. Skin disorders such as eczema and contact (allergic) dermatitis have also been diagnosed among leather tanners exposed to preservatives applied to hides [2]. Irritation of the mucus membranes of the throat and nose and perforations of the nasal septum may occur after inhaling chronic acid fumes liberated during tanning process.

One potential source of contamination that is often overlooked is air. Air is a potential source of contamination by pathogenic and spoilage organisms in meat, leather, food and other processing units. These biological contaminations are mostly particles which are microscopic with a diameter of 0.5 to 50 micrometres and are suspended in the air as an aerosol, (a fine solid or liquid in air or another gas). This contaminants include bacteria, fungi, viruses and even pollen which may be present as solid (dust) or as liquid (condensation and water) they may come in contact with food products, containers, equipment, raw materials and other surfaces during processing. The potentially pathogenic organisms usually found in the environment of an industry are *Escherichia coli*, *Shigella* species, *Salmonella* species, *Staphylococcus* species, and other colonies of fungi such as *Aspergillus niger* and *Pernillium glaucum* [3]. Yeasts of three genera (*Rhodotorula, Clasdosporium* and *Torulopsis*) have also been found. The above mentioned organisms have been isolated from the air environment according to research [2]. This supports the theory that bio-aerosols transport microorganisms and contributes to the further contamination of hides and skin. During slaughtering process of the animals of which the hides or skin is obtained, they may be contaminated and unknowingly be brought to the factory (Tannery) which can as well contaminate the air and surfaces in the factory.

The outer covering of animals is a carrier of particulate matters, aerosols, and microbes. Hides and skin processing is therefore a potential high risk procedure which requires high level of expertise and proper precautions to avoid the risk of infections and diseases. This study therefore, is aimed at investigating the microbial quality of the air environment of the hides and skin processing unit by morphological and biochemical characterization of isolates as well as determining the microbial load of the work environment.

2. MATERIALS AND METHODS

2.1 Media Preparation

Nutrient agar and potatoes dextrose agar were used for this work. The media were prepared according to the manufacturer's instruction, sterilized at 121℃ and 15 pounds of pressure for 15 minutes and were cooled in a 50℃ water bath for 10-15 minutes before pouring into sterile petri dishes and allowed to solidify [4].

2.2 Sample Collection of Air

The sampling was done during passive session (i.e. when no processing activity was going on) and during active session. The samples were collected using settle plate method. Potatoes Dextrose Agar (PDA) plates incorporated with

chloramphenicol and Nutrient Agar (NA) plates incorporated with griseofulvin were used for the collection of samples. Plates were exposed in pairs at intervals of 30 and 60 minutes following the methods of Rahkio et al. [5]. All plates were exposed at a height of 1m and one square metre area. This procedure was repeated during active session. The nutrient agar plates were labelled accordingly and were incubated for 24 hours at 37℃ for bacteria whereas the PDA plates were incubated at room temperature (25-28℃) for 5 days for the isolation of fungi.

2.3 Colony Count

After incubation, the total numbers of colony forming units (CFU) for bacterial and fungal air-flora were converted to organism's colony forming units per cubic metre [6].

2.4 Purification of Isolates

The isolates from both nutrient and potatoes dextrose plates were sub cultured onto freshly prepared agar plates. After incubating for 24 hours, the pure isolates were preserved on agar slants and stored in a refrigerator at 4℃ until required for further analysis.

2.5 Identification and Characterization of Isolates

2.5.1 Identification of bacterial isolates

Standard methods of identification and characterization of bacteria as described in Bergy's Manual of Determinative Bacteriology were used [7]. The final identification was carried out using the bioMérieux's API® identification Kit.

2.5.2 Identification of fungi isolates

The fungal colonies were identified based on colonial appearance and microscopic examination of the spores and hyphae [8]. The physical morphology such as colour, size, and elevation of each isolates were noted. A drop of lactose phenol cotton blue was placed on a labelled slide using a sterile inoculating needle and a small portion of the growth from the PDA plate was transferred to the drop of lactose phenol. The wet mount was covered with a cover slip and observes under a microscope using 40X objective lens [9]. Observations were recorded.

2.6 Statistical Analyses

All data obtained in this study were analysed for significant differences using paired t-test. The statistical software GraphPad Prism version 6 was utilised for these analyses.

3. RESULTS

The bacteria morphologically and biochemically identified in this study include *Proteus mirablis, Klebsiella pneumonia* with percentage prevalence of 11.11% each, *Pseudomonas aeruginosa*, *Bacillus subtilis* with prevalence of 07.41% each, with *Staphylococcus aureus* being the most prevailing bacterium with 29.63% prevalence, followed by *Escherichia coli* (18.52%) and *Salmonella enterica* (14.82) whereas the fungi isolated include *Sclerotium sp.* (05.00%), *Fusarium sp.* (10.00%), *Aspergillus fumigatus* (20.00%), *Corticium sp.* (15.00%), *Aspergillus ficheri* (10.00%) and *Aspergillus niger* (40.00%) which was the most frequently isolated.

3.1 Bacterial Counts during Passive Period

The results of settle-plates analysis during passive period are presented on Table 1. The results obtained from the plates exposed for 30 minutes shows that location L_3 is slightly higher than that from L_2 (1.6×10^2cfu/m^3/30 mins and 1.3×10^2cfu/m^3/30 mins respectively) but lower than locations L_1 and L_4 having 1.8×10^2cfu/m^3/30 mins each. There was a significant difference between plates exposed for 30 minutes and 60 minutes ($P<0.05$). The plates exposed for 60 minutes had a relatively higher number of colonies compared to those exposed for 30 minutes. This variation simply describes an increased in the number of microbial deposition with time.

3.2 Bacterial Counts during Active Period

The results of this analysis showed an increase in the number of bacteria counts with increasing time of exposure. There were observable differences in bacterial counts according to time of exposure. For corresponding locations, the counts for 60 minutes seem to be higher than the 30 minutes exposure (Table 2). Statistically, there was no significant difference between time of exposure ($P>0.05$).

3.3 Fungal Counts during Passive Period

The results obtained from the plates exposed for 30 minutes showed that location L_1 had the highest count followed by L_4 (2.0×10cfu/m^3/30 mins and 1.5×10^2cfu/m^3/30 mins respectively)

whereas L_3 had 1.2 x 10^2cfu/m^3/30 mins and lowest count of 100cfu/m^3/30 mins was recorded for location L_2. The plates exposed for 60 minutes followed a similar pattern with those for 30 minutes (Table 3). There was no significant difference in the count according to duration of exposure (P>0.05).

3.4 Fungal Counts during Active Period

The results of this analysis showed a relative increase in the number of fungal counts according to duration of exposure (Table 4). However statistically, there was no significant difference between time of exposure (P>0.05).

3.5 Mean Bacterial Load during Passive and Active Sessions

The analysis showed that there was a significant difference (P<0.05) in the bacterial load of the air environment between the passive and active sessions. Bacterial counts were higher during active session than during passive session (Fig. 1).

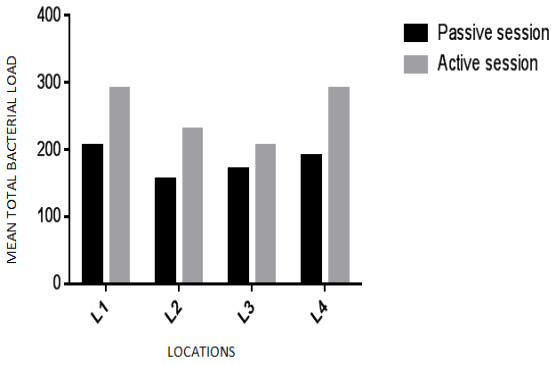

Fig. 1. Mean total bacterial count

3.6 Mean Fungal Load during Passive and Active Sessions

The analysis showed that there was a significant difference (P<0.05) in the fungal load of the air environment between the passive and active sessions. Fungal counts were higher during active session than during passive session as shown in Fig. 2.

Table 1. Bacterial viable count during passive period

Location	cfu/m^3/30 mins	cfu/m^3/60 mins	Mean total cfu/m^3
L_1	$1.8x10^2$	$2.3x10^2$	$2.05x10^2$
L_2	$1.3x10^2$	$1.8x10^2$	$1.55x10^2$
L_3	$1.6x10^2$	$1.8x10^2$	$1.7x10^2$
L_4	$1.8x10^2$	$2.0x10^2$	$1.9x10^2$

Table 2. Bacterial viable count during active work

Location	cfu/m^3/30 mins	cfu/m3/60 mins	Mean total cfu/m^3
L_1	$2.8x10^2$	$3.0x10^2$	$2.9x10^2$
L_2	$1.8x10^2$	$2.8x10^2$	$2.3x10^2$
L_3	$2.0x10^2$	$2.1x10^2$	$2.05x10^2$
L_4	$2.4x10^2$	$3.4x10^2$	$2.9x10^2$

Table 3. Fungal viable count during passive period

Location	cfu/m^3/30 mins	cfu/m^3/60 mins	Mean total cfu/m^3
L_1	2.0x10	6.0x10	4.0x10
L_2	1.0x10	1.0x10	1.0x10
L_3	$1.2x10^2$	$2.1x10^2$	$1.65x10^2$
L_4	$1.5x10^2$	$3.4x10^2$	$2.45x10^2$

Table 4. Fungal viable count during active work

Location	cfu/m^3/30 mins	cfu/m^3/60 mins	Mean total cfu/m^3
L_1	8.0x10	$2.2x10^2$	$1.14x10^2$
L_2	$1.8x10^2$	$2.8x10^2$	$2.30x10^2$
L_3	$1.6x10^2$	$6.4x10^2$	$4.15x10^2$
L_4	$1.6x10^2$	$6.0x10^2$	$3.80x10^2$

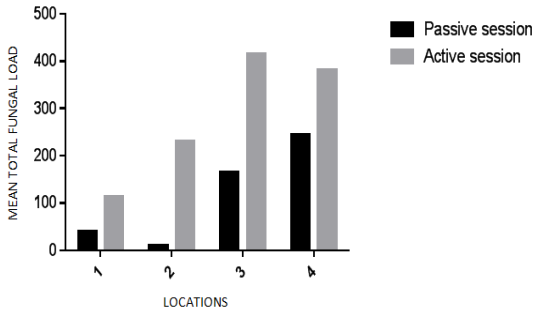

Fig. 2. Mean total fungal count

4. DISCUSSION

The study of airborne microorganisms in indoor environments is important to understand the dissemination of airborne microbes particularly the pathogenic ones [10]. It is believed that animals carry a wide variety of microorganisms within and on their outer covering, some of which may be pathogenic or opportunistic pathogens of man [11]. The daily exposure of personnel to these microbes during the processing of these animal products to finished goods is of great health concern. It is therefore, important to evaluate the quality of the air whether indoor or outdoor in the processing environments. The number and type of airborne microorganisms can be used to determine the degree of cleanliness. In this study, the isolated bacterial isolates were *Proteus mirablis, Pseudomonas aeruginosa, Salmonella enterica, Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Klebsiella pneumonia*. The most prevalent bacterium was *Staphylococcus aureus.* These airborne micro-flora obtained were similar to those obtained by Ekhaise and Ogboghodo [6]. Among the bacteria isolated in their study, *Staphylococcus aureus* was the most prevalent and has been reported to cause infections of the skin, deeper tissue and organs as well as pneumonia [12]. However, bacteria isolated in this present study are known primary pathogens.

The most frequently isolated fungus in this study was *Aspergillus niger*. The fungi recovered were majorly of soil origin. This suggest the conditions in which the animals were reared or the unsanitary condition of slaughtering process and as well as storage of the hides and skins. However, these fungi may not be primary pathogens but have been found to cause opportunistic infections in immunosuppressed individuals [13,14]. This therefore is relevant indicator of health hazard as the number and type of airborne microorganisms can be used to determine the degree of cleanliness.

The statistical significant differences ($P<0.05$) in the bacterial and fungal count between passive and active sessions is suggestive of the fact that this unit under study is not a safe place for rest. The bacterial count was directly proportional to the duration of exposure of culture plates. Therefore the longer an individual remains in the unit the higher the numbers of microbial cells and spores that will rest on the individual skin or being inhaled if protective cloths as well as nose and face masks are not used.

5. CONCLUSIONS

Air contains microbial forms maintained in suspension coming from soil, water, plants, and animals including man. Air movement favours the maintenance of microorganisms in the aerial media while their deposition is barely affected by gravity due to small sizes. The skin and hide processing unit of the NILEST tannery is laden with potentially pathogenic microorganisms as several species of bacteria and fungi have been isolated with tanning processes contributing to the concentration of bio-aerosol in the air environment. The microbial load of both bacteria and fungi is considerably high which is capable of causing infection and diseases. Frequent exposure of workers to this bio-aerosols is a potential risk therefore, workers in this environment must be well kitted to protect them from the contaminating biohazards. However, the results of this research provide adequate data for better planning, organisation, and implementation of necessary policies to cater for the reduction and/or prevention of risk factors of biohazards in the tannery.

6. RECOMMENDATIONS

It is hereby recommended from the reports of this study that:

1. Regular environmental and microbial assessment of the tannery should be carried out for the sake of prompt attention to tackle unsuspected bio-hazards.
2. Nose masks, laboratory coats, boots, and other necessary protective cloths should be worn when working or visiting the processing unit to prevent microbial infections.
3. Employees in the tannery should be enrolled in health insurance program of the

government and should have them medically checked at regular intervals.

4. However, further studies should be carried out in this study location to molecularly characterize the prevalent isolates and determine their antibiogram profile.

ETHICAL CONSIDERATION

The study proposal was presented and defended before institute's ethical panel and it was accepted by the Local Ethics Committee and approval given before commencement of research.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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