CHAPTER ONE

INTRODUCTION AND OBJECTIVES

1.1. Introduction

The airborne microbial contamination can cause health problems and can also compromise the normal activities in the work environment, such as hospitals, dental offices, pharmaceutical and cosmetics facilities and could affect the performance, morale, and productivity of staff (Cellini *et al*., 2001; Katušin *et al*., 2003; Venkateswaran *et al*., 2003; Pasquarella *et al*., 2007).

Exposure to bio-aerosols, containing airborne microorganisms and their by-products, can result in respiratory disorders and other adverse health effects such as infections, hypersensitivity pneumonitis and toxic reactions (Gorny *et al*., 2002; Fracchia *et al*., 2006). Fungi are common in indoor and outdoor environments and nearly 10 % of people worldwide have fungal allergy (Pasanen *et al*., 1996).

The findings of epidemiological research indicate that exposure to high concentrations of microbes in the air frequently leads to allergies, asthma, hay fever (Björnsson *et al.*, 1995; Newson *et al.*, 2000), pneumonia (Siersted and Gravesen, 1993), and many other health side-effects, including infections (Renn *et al.*, 2001).

Airborne bacteria enter the atmosphere from practically all kind of surfaces (e.g., soil, forest, river, desert, ocean, agricultural area, road dust) as aerosol particles. Once in the air, the bacteria are carried upwards by air currents and can remain in the atmosphere until being removed by precipitation or direct deposition onto surfaces (Burrows *et al.*, 2009). Because aerosol-associated bacteria are carried with the air mass, atmospheric

bacterial communities show strong variations in time according to the physical and chemical changes of the air masses (Fierer *et al.,* 2008; Maron *et al.,* 2005).

Air sampling is useful for monitoring airborne biological agents and can be conducted qualitatively or quantitatively (Asefa, 2009). There are many different methods to measure microbial air contamination and air samples can be collected in two ways: by active air samplers and passive air sampling (settle plates). The settle plates represent an economical and simple method, are sterile, available everywhere and give results that are reproducible (Pasquarella *et al.*, 2000).

Airborne microorganisms can arise from many sources including air-conditioning systems, raw materials and specific food production systems (APHA, 2001).

Air sampling of microorganisms is a popular method of conducting microbial examinations, as it allows a direct toxicological evaluation (Velmurgan *et al.,* 2008; Cuthbertson *et al.,* 2010).

1.2. Rationale

There is an increased tendency to consume meals at dining facilities outside the home, moreover; meals supplied in food businesses have been involved in many foodborne disease outbreaks. Therefore, microbial air contamination in food processing facilities could be a concern and an increase of microbial loads could represent a risk factor, especially for the potential contamination of foods due to undesirable spoiling and pathogenic bacteria. Therefore, this study was undertaken to assess bacterial load and type in air of cafeteria at Sudan University of Science and Technology because food poisoning has been reported in one of them.

1.3. Objectives

1.3.1. General objective

To assess bacterial load and types in cafeterias at Sudan University of Science and Technology.

1.3.2. Specific objectives

1- To isolate bacteria from cafeterias air using the settle plates method.

2- To calculate the bacterial numbers in air of cafeterias.

3- To identify the types of bacteria.

CHAPTER TWO

LITERATURE REVIEW

2.1. Background

Air is a resource that supplies us with oxygen which is essential for our bodies to live. Pure air is a mixture of gases that are invisible, colorless and odorless consisting of 78% nitrogen, 21% oxygen and other gases as well as varying amounts of water vapor. This pure air can become contaminated in various ways affecting humans, plants and animals (Murray *et al*., 1995).

Indoor air quality is defined by the air parameters that found inside buildings, businesses, schools, and homes. The source of contamination can vary they can be biological resources or chemical resources (EPA, 1995). Some environmental factors such as high temperature and humidity. Bacteria, mould, fungi, viruses, mite, cockroaches, pollen and animal particles contributes to indoor air quality and are known as biological indicators of air quality (Sexton and Dyer, 2004)

Air is not natural environment for the growth and reproduction of microorganisms because it does not contain the necessary amount of moisture and kinds of nutrient in a form utilizable by these organisms, therefore air does not possess a flora, yet organisms are found in air. This may be due to the fact that air is an effective medium for distributing microbes because of its continuous movement and mixing on many scales (Gregory, 1973).

The presence of few numbers of airborne microorganisms in indoor environments is a normal condition, but an increase of their concentration could represent a disease risk

factor (Douwes *et al.,* 2003). The concentration of indoor bacteria is affected by humidity, the number and density of humans, the amount and type of activity, and air circulation. A typical aerobic concentration of bacteria may approximate 15 to 500 colony-forming units (CFU)/m^3 (Cousins and Collett, 1989).

There have been no polish standards or guide lines for microbiological quality of indoor air. Furthermore, there isn't any European Union directive addressing this; therefore, it is assumed to be based on particular European countries' requirements and scientific propositions (Gorny, 2004). According to current Swedish requirements the number of 500 colony-forming units (cfu) of bacteria and 300 cfu of fungal spores in 1 m^3 can be accepted in an indoor environment (Abele *et al*., 2002)

According to Berk (1979) exposure of 20 cfu/m^3 to over 700 cfu/m^3 has no harmful effect. In Hong Kong good microbiological class air should include less than 1000 cfu/m^3 of bacteria. If it includes less than 500 cfu/m^3 air is classified as excellent. In Singapore requirements for indoor air quality strictly describe concentration of bacteria on the maximum level of 500 cfu/m^3 (Obbard and Fang, 2003).

2.2. Common types of bacteria in air:

The most commonly associated with airborne bacteria is *Mycobacterium tuberculosis* which is carried by droplet nuclei (CDC, 2003). The organisms such as *Staphylococcus aureus, Micrococcus* spp, alpha-hemolytic *Streptococci* and Gram-negative rods, *Streptococcus pyogenes, Neisseria meningitidis, Corynebacterium diphtheria* and *Mycobacterium tuberculosis* are known to be transmitted predominantly by airborne droplets from infected people and they may cause nosocomial infection (Jaffal *et al*.,

1997). *Staphylococcus* is the most frequently occurring bacteria, followed by *Micrococcus,* which together contributed 58-78% of total bacteria concentration (Pastuszka *et al*., 2005). Gram-positive cocci (i.e., *Staphylococcus aureus,* also important pathogens are resistant to inactivation by drying and can persist in the environment and on environmental surfaces for extended periods (Bennett *et al.*,1998). These organisms can be shed from heavily colonized persons and discharged into the air. Airborne dispersal of *S. aureus* is directly associated with the concentration of the bacterium in the anterior nares (White, 1961). Approximately 10% of healthy carriers will disseminate *S. aureus* into the air and some persons become more effective disseminators of *S. aureus* than others (Sherertz *et al*., 1996).

Other Gram-positive bacteria linked to airborne transmission include *Bacillus* spp. which is capable of sporulation as environmental conditions become less favorable to support their growth. Gram-negative bacteria rarely are associated with episodes of airborne transmission because they require moist environments for persistence and growth. The main exception is *Acinetobacter* spp which can withstand the inactivating effects of drying (McDonald *et al.*, 1998).

The poor condition of an air condition system can harbor psychrophilic bacteria like *Legionella pneumophilia* which is responsible for the legionnaires disease (WHO, 2002).

2.3. Mode of Transmission of Airborne Diseases:

A variety of airborne infections in susceptible hosts can result from exposures to clinically significant microorganisms released into the air when environmental reservoirs (i.e., soil, water, dust and decaying organic matter) are disturbed. Once these materials

are brought indoors can transmit by number of vehicles (e.g., people, air currents, water, construction materials and equipment). Respiratory infections can be acquired from exposure to pathogens contained either in droplets or droplet nuclei. Exposure to microorganisms in droplets (e.g., through aerosolized oral and nasal secretions from infected patients constitutes a form of direct contact transmission (Schaal, 1991).

The spread of airborne infectious diseases via droplet nuclei is a form of indirect transmission. The microorganisms in droplet nuclei persist in favorable conditions in dry or cool atmosphere with little or no direct exposure to sunlight or other sources of radiation (Osterholm, 2000).

2.4. Common techniques use for microbial air detection:

2.4.1. Active air sampling:

The microbial air contamination can be measured by counting the number of cfu per cubic meter (cfu/m^3) of air. For this purpose active air samplers are used, which collect a known volume of air, blown on to a nutrient medium by different techniques (Cage *et al*., 1996).

2.4.2. Passive air sampling: (settle plates)

Passive air sampling is performed using settle plates. Petri dishes containing a solid nutrient medium are left open to air for a given period of time. Microbes carried by inert particles fall onto the surface of the nutrient, with an average deposition rate of 0.46 cm/s being reported. After incubation at 35 ˚C they grow colonies in a number proportional to the level of microbial contamination of the air.

The main criticism of settle plates is that the measured microbial fallout is not at all or is only weakly correlated with the counts determined by other quantitative methods and with a defined volume of the surrounding atmosphere (Radmore and Luck, 1984). Therefore, gravity or depositional sampling is considered a non-quantitative collection method, affected by the size and shape of particles and by the motion of the surrounding atmosphere. The volume of air from which the particles originate is unknown (Buttner *et al*., 1997).

The use of settle plates is the length of the time required to collect samples: from 15 minute to 1 hour or more. According to the United States Pharmacopeial, the settle plate method is still widely used as a simple and inexpensive way to qualitatively assess the environments over prolonged exposure times. Settle plates are not to be used for quantitative estimations of the microbial contamination levels of critical environments (Humphreys, 1992).

2.5. Previous studies:

In study conducted in the University of Chieti (Italy), to evaluate the airborne microflora in research laboratories. A quantitative evaluation of the index microbial air contamination was performed using the settle plate method. The microbial air contamination was evaluated during 6 months in three university buildings (A, B and C). Nutrient agar plates were exposed, monthly, for 1 hour at the morning and the afternoon to evaluate the colony forming units per plate per hour. With the quantitative analysis, the most frequent bacterial and fungal colonies isolated were also characterized. The most common microorganisms detected in the indoor air of the examined buildings were

Gram-positive bacteria, belonged to the genera *Staphylococcus*, *Bacillus* (Giulio *et al*., 2010).

In study conducted in Nigeria about airborne bacterial flora of Ahmadu Bello Univeresity Health Clinic (Sickbay) ZARIA. Airborne bacteria were sampled in various environments of the Sickbay in 2001 using exposed plate technique. The highest mean bacterial count of the indoor air was obtained in the antenatal clinic (299 cfu/m ^{3}) and the least in the toilet environment (98 cfu/m^3). Among the isolated airborne bacteria, Gram-positive rods predominated (60%), Gram-positive cocci constituted 30% while the Gram-negative rods are the least (10%). The bacterial species isolated include *Bacillus* spp., *Esherichia* spp., *Staphylococcus* spp., *Streptococcus* spp., *Pseudomonas* spp., *Micrococcus* spp., *Proteus* spp., *Klebsiella* spp. and *Corynebacterium* spp. (Shiaka and Ado, 2011).

A study done in Poland in indoor air microbiological contamination in various rooms of University buildings in Poznań. Investigations were conducted in the period September-October 2002 and the same period in 2003. Air samples were taken twice a day: in the morning and in the afternoon. In all of the tested places a multiple growth of bacteria and significant increase of mould spores were observed in afternoons. The predominant bacteria and isolated from investigated air samples were: *Staphylococcus* spp., *Micrococcus* spp., *Serratia* spp. Among these microbes the presence of pathogenic and strongly allergenic microorganisms was detected (Stryjakowska *et al*., 2007).

In study done in Korea to assess the levels of airborne bacteria, Gram-negative bacteria, and fungi in six hospitals lobbies, to investigate the environmental and hospital

characteristics that affected the airborne microorganism levels. Nutrient plate agar was used to collect the samples. The three types of microorganisms were repeatedly collected at a fixed location in each hospital with a sampling time of less than 5 minutes. Temperature and relative humidity were simultaneously monitored. Multiple regression analysis was used to identify the major factors affecting microorganism levels. The average levels of bacteria (720 CFU/m^3), indicated that all hospital lobbies were generally contaminated, were contamination was the highest during the summer (Park *et al*., 2011).

In study done about indoor and outdoor air of child day-care centers (CDCCs) in the city of Edirne, Turkey. Air samples were collected using settle plate method from the indoor and outdoor air of CDCCs. Counts of airborne bacteria were measured as colony forming units (CFU). Samples were taken monthly over a period of 12 months between January and December 2004. A total of 3,120 bacteria colonies were counted on 192 Petri plates. Four groups of culturable bacteria were identified: Gram-positive cocci, Gram-positive bacilli, endospore-forming Gram-positive bacilli, and Gram-negative bacteria. Airborne Gram-positive bacteria were the most abundant at more than 95% of the measured population. While Gram-positive cocci were more common in indoor environments, Gram-positive bacilli were more dominant in outdoor air. Bacteria commonly isolated from CDCCs were identified at a genus level. *Staphylococcus* (39.16%), *Bacillus* (18.46%), *Corynebacterium* (16.25%) and *Micrococcus* (7.21%) were dominant among the genera identified in the study (Aydogdu *et al*., 2010).

Study was conducted in Kuwait for assessment of airborne bacteria and fungi in an indoor and outdoor environment were assessed during the spring season using conventional methods to investigate the enumeration and identification of airborne micro-organisms. This was determined through air quality sampling using the 'open plate technique' in four different locations. The public places included kitchens, classrooms, recreational areas, laboratories. 26 groups of bacteria and fungi, either of human or environmental origin were detected. Bacteria showed higher growth numbers as opposed to the slow growing fungi (Yassin and Almouqatea, 2010).

Study was done in stability of airborne microorganisms in the Louvre Museum using high-throughput molecular tools and to underline the microbial signature of indoor air in this human-occupied environment. A common bacterial diversity was underlined, corresponding to 58.4% of the sequences. The core species were belonging mostly to the *Proteobacteria* , *Actinobacteria* , *Paracoccus* spp., *Acinetobacter* spp., *Pseudomonas* spp., *Enhydrobacter* spp., *Sphingomonas* spp., *Staphylococcus* spp. and *Streptococcus* spp. (Gaüzère *et al*., 2011).

CHAPTER THREE

MATERIALS AND METHODS

3.1. Study design

3.1.1. Type of study

Cross- sectional study to assess bacterial load and types in cafeterias at Sudan University of Science and Technology

3.1.2. Study area

The study was carried out in cafeterias at Sudan University of Science and Technology, Khartoum, Sudan.

3.1.3. Study duration

The study was carried out during the period from June to August 2013.

3.2. Sample size

Twenty (n=20) air samples were collected randomly from 20 cafeterias in Sudan University of Science and Technology.

3.3. Ethical consideration

This study was approved by Sudan University of Science and Technology, Ethical Committee Board and an informed consent was obtained before collecting samples from cafeterias.

3.4. Methods

3.4.1. Air sampling technique

Air sample was performed using settle plate's methods. Petri dishes containing nutrient agar were transported to cafeteria in sealed plastic bags. The plates were labelled with

sample number, time and date of collection. The plates were placed at three chosen places in cafeteria at about 1 meter above the ground, and 1 meter from the wall. All the plates were exposed for 10 minutes. After this exposure, the plates were covered with their lids and taken to laboratory in sealed plastic bags and incubated at 37°C for 24 hours. All microorganisms concentrations were expressed as colony-forming unit, "CFU" per plate (Napoli *et al*., 2012). The settle-plate technique was determined and the number of microorganisms expressed as cfu/m^3 was estimated for the settle plate technique using Koch's sedimentation method according to Polish standard PN89/2- 04088/08, according to which,

$$Cfu/m^3 = a.x1000 / p\ x.t.x\ 0.2$$

Where:

a= the number of colonies on the Petri plate.

p= the surface measurement of the the plate used in cm^2.

t= the time of exposure of the Petri plate (Friberg *et al*., 1999).

Obtained micro-organisms were streaked and subcultured on to Nutrient agar for further identification.

3.5. Identification of the isolates

3.5.1. Morphological characters

The isolated micro-organisms were characterized morphologically and based on its colony size, shape, margin, opacity, elevation, pigment production and Gram's character.

3.5.2. Gram stain

A drop of normal saline was placed on clean and dry slide. The smear was fixed by dry heat, covered by crystal violet for 1 minute and then the stain was washed with clean water. Lugol's iodine was added for 30-60 seconds and then washed by clean water. The smear was decolorized by 70% alcohol for few seconds, and then washed by clean water. Saffaranine was added for 2 minutes, and then washed by water. Smear preparations were air-dried and examined by microscope using high-resolution objective power (×100) (Collee *et al.*, 1996; Cheesbrough, 2000).

3.5.3. Spore stain

By flooding the slide with 5% aqueous malachite green and steamed for 1 minutes, then was washed under running water. Then safranin added for 15 seconds. Rinse with water and dried. Bacterial bodies stain red, spores green.

3.6. Biochemical tests

3.6.1. Indole test

The test is based on bacteria that break down the amino acid tryptophan with release of indole. The test was done by inoculating the tested organism on peptone water and then incubated at 37˚ C for 24 hours. Indole production was detected by adding Kovac's reagent. The result was observed by formation of red ring in the surface of the tube (Collee *et al.*, 1996; Cheesbrough, 2000).

3.6.2. Citrate utilization test

This test is based on the ability of bacteria to utilize citrate as source of carbon and ammonium as source of nitrogen in the presence of bromothymol blue as an indicator.

Inoculation was done by a sterile loop into broth medium and incubated at 37˚ C for 18-24 hours. Positive result detected by changing of the indicator**'**s color from green to blue (Cheesbrough, 2000).

3.6.3. Urease test

This test is used to identify bacteria, particularly those growing naturally in an environment which produce urease enzyme to break down the urea into ammonia and carbon dioxide, which lead to change the pH to alkaline in presence of phenol red indicator. The test was done by inoculating the test organism in urea agar and then incubated at 37 ˚C for 24 hours. Positive result appeared in the changing of indicator's color from yellow to pink (Collee *et al.*, 1996; Cheesbrough, 2000).

3.6.4. Oxidase test

The test depends on production of oxidase enzyme that catalyzes the transport of electrons in the bacteria and redox dye-tetra methyl-p-phenylene-diamine in which the phenylene-diamine in the reagent is oxidized to indophenol blue with a deep purple color.

3.6.5. Catalase test

This test is used to differentiate between staphylococci (which produce catalase enzyme) from streptococci (which cannot produce catalase enzyme). One ml of hydrogen peroxide (H_2O_2) solution was placed in a test tube, and small amount of bacterial growth was added by wood stick. The formation of air bubbles indicated positive result (Collee *et al.*, 1996; Cheesbrough, 2000).

3.6.6. DNAase test

This test is used to identify *S. aureus* which produces deoxyribonuclease (DNA) enzymes. By using sterile loop test organism was inoculated under aseptic condition on DNA medium. After overnight incubation at 37 ˚C, hydrochloric acid (1N) was added to the plate. The colonies which produced DNase were surrounded by clear areas indicating DNA positive hydrolysis (Cheesbrough, 2000).

3.6.7. Coagulase test

This test is used to identify *S. aureus* which produces coagulase enzyme. This enzyme can clot the plasma by converting fibrinogen to fibrin. The test was done by placing drop of plasma on slide and then the organism under test was added and mixed gently. Positive was detected by the clumping of bacterial cells within 10 seconds (Cheesbrough, 2000).

3.6.8. Manitol fermentation

Test organism was inoculated into manitol salt agar, incubated at 37˚ C and examined after 24 hours for manitol fermentation; it was indicated by formation of yellow color around the growth (Cheesbrough, 2000).

3.6.9. Starch hydrolysis test

To determine if a bacterium produce the enzyme amylase , which breaks down starch to glucose which indicated by blue halo surrounding the growth.

3.6.10. Manitol egg-yolk polymyxin agar

Bacteria that ferment manitol result in acid production and produce yellow colony. *B. cereus* is manitol negative and result in pink colonies and lecithinase positive. Polymyxin B solution inhibits the growth of most other bacteria.

CHAPTER FOUR

RESULTS

Twenty (20) air samples were collected from cafeterias in Sudan University of Science and Technology in the period from June to August, 2013. All samples were yielded bacterial growth on nutrient agar.

Table (1) showed the bacterial load in all air samples at those cafeterias. 43% of the isolates were Gram-positive cocci, while 57% were Gram-positive bacilli. In table (2) showed the characters and biochemical properties of Gram-positive cocci.

Table (3) displayed the biochemical tests of Gram-positive rods. The most abundant bacteria corresponded to species of *Bacillus cereus* 16 (34%), followed by *Staphylococcus aureus* 13 (28%), *Bacillus alvei* 11 (23%) and *Micrococcus* spp. 7 (15%)**.**

Table (4) displayed the number and percentage of bacterial species isolated from different cafeteria.

Table 1. Bacterial load in all cafeterias at Sudan University of Science and Technology

Cafeterias No.	load in all cafeterias	
No	**Mean of cfu/plate**	**Mean of cfu/m^3**
1	106.3	5905
2	145	8055
3	138	7666
4	90.6	5033
5	103	5722
6	120	6666
7	98	5444
8	86.6	4811
9	63.3	3516
10	81.3	4516
11	99.3	5516
12	80.3	4461
13	104.3	5794
14	99.3	5516
15	91.6	5088
16	79.6	4422
17	91	5055
18	108	6000
19	98	5444
20	92	5111
Total	**1975.5**	**109741**

Table 2. Characters and biochemical properties of Gram-positive cocci

Test	***Staphylococcus aureus***	***Micrococcus* spp.**
Growth in air	+	+
Catalase	+	+
Oxidase	-	+
Coagulase	+	-
DNAse	+	-
Manitol Salt Agar	Fermentation (yellow colony)	No fermentation

Table 2. Biochemical tests of Gram- positive bacilli

Organism	Gram stain	Oxidase	Urease	Citrate	Indole	Starch	Glucose	Egg-yolk	Spore
B.cereus	+	-	-	+	-	+	+	Growth	XV
B.cereus	+	+	-	+	-	+	+	Growth	XV
B.cereus	+	+	-	-	-	+	+	Growth	XV
B.alvei	+	+	-	-	+	+	+	No growth	XV
B.cereus	+	+	-	-	-	+	+	Growth	XV
B.alvei	+	+	-	-	+	+	+	No growth	XV
B.alvei	+	+	-	+	+	+	+	No growth	XV
B.alvei	+	+	+	-	+	+	+	No growth	XV
B.alvei	+	+	-	-	+	+	+	No growth	XV
B.alvei	+	+	+	-	+	+	+	No growth	XV
B.cereus	+	+	-	-	+	+	+	Growth	XV
B.alvei	+	+	-	-	+	+	+	No growth	XV

X= central spore **V**= oval

Table 4. Number and percentage of bacterial species isolated from different cafeteria

Organism	No	Percentage
B. cereus	16	34
S. aureus	13	28
B. alvei	11	23
Micrococcus spp.	7	15
Total	**47**	**100**

CHAPTER FIVE

DISCUSSION

The main objective of this study was to assess bacterial load and types in cafeterias at Sudan University of Science and Technology.

The study was carried out in the period from June to August, 2013. Sample size included was twenty (20) cafeterias. The results obtained showed that; among the twenty (20) cafeterias examined, (100%) showed significant growth on nutrient agar.

The present study demonstrates that the results of the isolated airborne bacteria counts expressed in colony forming unit (cfu/m^3) which ranged from 8,055 cfu/m^3 and 3,516 cfu/m^3 with the highest mean counts in cafeteria No.2 and lowest in cafeteria No.9.

Cafeteria No.2 and No.3 were yielded high bacterial counts (8,055 cfu/m^3 and 7,666 cfu/m^3 respectively). This may be due to increased number of students and activtities including coughing, sneezing, and talking, irregular and infrequent cleaning at the periods of investigation.

In comparison to many other studies, Giulio *et al*., (2010) found that the most common microorganisms detected in the indoor air of the University buildings were Gram-positive bacteria, belonged to the genera *Staphylococcus* and *Bacillus.* The results of the present study confined to their results.

Shiaka and Ado, (2011) found that the bacterial species isolated include *Bacillus* spp., *Esherichia* spp., S*taphylococcus* spp., S*treptococcus* spp., *Pseudomonas* spp., *Micrococcus* spp., *Proteus* spp., *Klebsiella* spp. and *Corynebacterium* spp..

In our study Gram-positive rods had the highest incidence accounting for 57%, followed by Gram positive cocci (43%). Most of these isolated bacteria are spore formers and were represented in almost all the environments investigated (e.g *Staphylococcus* spp., *Micrococcus* spp., *Bacillus* spp.) and were represented in almost all the environments studied.

Bacilli, Staphylococci and Micrococci predominate, occurring in almost all the environments probably because Bacilli are very resistant to many adverse conditions and their spores not repressed when exposed to air. The production of spores enables this organism to withstand unfavourable conditions such as low temperatures or heat and may improve the chances of *Bacillus* to be present in high numbers in the air (Whyte *et al.*, 2001). Micrococci and Staphylococci are known to form aggregate in nature, so they tend to give higher colony counts and also because of the possible broken up of the clusters (Lundholm, 1982). *Staphylococcus* is found in all individuals and usually expelled from the respiratory tract through the nose and mouth which may also account for their presence in the post processed product. Various researchers (Aboloma, (2008); Oyeyi and Lum-nwi, (2008) Shamsuddeen and Ameh, (2008); Wada-kura *et al.*, 2009) have reported that the presence of *Staphylococcus aureus* in food is an indication of environmental and human contamination.

5.2. Conclusion

This study concluded that there was a significant difference in air microbial loads in cafeterias of Sudan University of Science and Technology with higher count at cafeteria No 2, 3.

Bacillus spp. were determined to be most frequently detected in the air of all cafeterias followed by *Staphylococcus aureus* and *Micrococcus* spp.

Most of cafeterias were poorly ventilated which can increase level of bacteria and make health problem.

5.3. Recommendations

1. Efforts are needed to improve cafeterias environment. It is recommended to raise the awareness and educational status of workers to reduce the hazards of airborne transmission of such potentially pathogenic microorganisms.

2. Provision of clean and safe air should be regarded as not only essential to health but also a legal right.

3. Further studies are needed to reducing the contamination of air at the University.

4. Using PCR tools to diagnose resistant bacteria.

REFERENCES

1. **Abele, E., Andersson, J.V., Dawidowicz, N., Chris, T. E., Hanssen, S. O., Linden, A. L., Lindval, T. and Pasanen, A.L. (2002).** The Swedish key action, indoor air quality and Climate, California.996-1001.

2. **Aboloma, R.I., (2008).** Microbiological analysis of bread samples from bakery to sale points in Ado-Ekiti, Nigeria. *Biol. Environ. Sci. J. Tropics*, **5**(3): 77-81

3. **APHA (American Public Health Association) (2001).** Compendium of methods for the microbiological examination of foods. *American Public Health Association*, Washington DC.

4. **Asefa, D.T., Langsrud, S., Gjerde, R.O., Kure, C.F., Sidhu, M.S., Nesbakken T. and Skaar. I. (2009).** The performance of SAS-super-180 air sampler and settle plates for assessing viable fungal particles in the air of dry-cured meat production facility. *Food Control*, **20**, 997–1001.

5. **Aydogdu, H., Asan, A. and Otkun, M. T. (2010).** Indoor and outdoor airborne bacteria in child day-care centers in Edirne City (Turkey), seasonal distribution and influence of meteorological factors. *Environ Monit Assess*; **164**: 53-66.

6. **Barrow, G.I. and Feltham, R. (2003).** Cowan and Steel's Manual for the identification of medical bacteria. Third edition. University press,Campridge, UK.

7. **Bennett, J.V and Brachman, P.S. (1998)**. The inanimate environment. In: Rham FS. *Hospital Infections*, 4th ed. Philadelphia, PA: Lippincott-Raven, 299–324

8. **Berk, J.B. (1979).** Field monitoring of indoor air quality in 1979. Annual report of the energy and environment division. California.

9. **Burrows, S.M., Elbert, W., Lawrence, M.G. and Pöschl, U. (2009).** Bacteria in the global atmosphere, part 1: review and synthesis of literature data for different ecosystems. *Atmos*. *Chem*. *Phys*. **9**, 9263-9280.

10. **Björnsson, E., Norback, D., Janson, C., Widstrom, J., Palmgren, U., Strom, G. and Boman, G. (1995).** Asthmatic symptoms and indoor levels of microorganisms and house dust mites. *Clin Exp Allergy*. ; **25**: 423-431.

11. **Buttner M.P., Willeke, K. and Grinspun S.A. (1997).** Sampling and analysis of airborne microorganisms. Manual of Environmental Microbiology. Washington, DC: *American Society for Microbiology*; 629–640.

12. **Cage, B.R., Schreiber, K., Barnes, C. and Portnoy, J. (1996).** Evaluation of four bioaerosol samplers in the outdoor environment. *Ann Allergy Asthma Immunol*; **77**: 401–406.

13. **CDC (Centers for Disease Control and Prevention). (2003).** Screening for tuberculosis and tuberculosis infection in high-risk populations: recommendations of the Advisory Committee for Elimination of Tuberculosis.;**44** (11):18–34.

14. **Cellini, L., Campli, E., Candia, M. and Chiavaroli, G. (2001**). Quantitative monitoring in a dental office. *Journal of Public Health*, **115**, 301–305.

15. **Cheesbrough, M. (2000).** District Laboratory Practice in Tropical Countries. Part. The Anglo-Egyptian Bookshop. Cambridge University Press, 70-234.

16. **Collee, G.J., Marimon, P.B., Fraser, G.A. and Simmons, A. (1996)**. Mackie and McCartney Medical Microbiology.14th edition, Pearson Professional Limited. Singapore, 245-413.

17. **Cousins, D. M. and Collett, C.W. (1989).** Indoor air quality in 12 schools: A case study , *Proceeding of Ashrae Conference IAQ, The Human Equation* : *Health and Comfort*, **87**, 104-108 .

18. **Cuthbertson, A. G. S., Blackburn, L. F., Northing, P., Luo, W., Cannon, R. J. C. and Walters, K. F. A. (2010).** Chemical compatibility testing of the entomopathogenic fungus Lecanicillium muscarium to control Bemisia tabaci in glasshouse environment. *Int. J. Environ. Sci. Tech.*, **7** (2), 405-409.

19. **Douwes, J., Thorne, P., Pearce, N. and Heederik, D. (2003).** Bioaerosol health effects and exposure assessment: Progress and prospects. *Annals of Occupational Hygiene*, **47**, 187–200.

20. **EPA (Environmental Protection Agency) (1995).** Indoor air pollution. A self-placed learning module. (EPA 402-K- 95-001). Washington, DC:USA.

21. **Fierer, N., Liu, Z., Rodríguez, M., Knight, R., Henn, M. and Hernandez, M.T., (2008).** Short-term temporal variability in airborne bacterial and fungal populations. *Journal of Environmental Microbiology* **74**, 200-207.

22. **Fracchia, L., Pietronave, S., Rinaldi, M. and Martinotti, M. G. (2006).** The assessment of airborne bacterial contamination in three composting plants revealed siterelated biological hazard and seasonal variations. *J. Appl. Microbiol*., 973-984.

23. **Friberg, B., Friberg, S. and Burman, L.G. (1999).** Correlation between surface and air counts of particles carrying aerobic bacteria in operating rooms with turbulent ventilation, *Journal of Hospital Infection*, 61-68.

24. **Gauzere, C., Moleta, M., Blanquart, H., Ferreira, S., Moularat, S., Godon, J. and Robine, F. (2011).** Stability of airborne microbes in the Lourve Museum overtime. *Journal of Environmental and Health*, France, **24** (1) : 29-40.

25. **Giulio, M.D, Grande, R., Campli, E.D, Bartolomeo, S. and Cellini, L. (2010).** Indoor air quality in University environments. *Environ. Monit. Assess*. ; 170:509.

26. **Gorny, R. L. (2004).** Harmful biological factors: standards, recommendations and proposals for admissible values. Basis and Methods of estimation of Work environment, *J. of Environ*. **3** (41), 17.

27. **Gorny, R. L., Reponen, T., Willeke, K., Schmechel, D., Robine, E., Boissier, M. and Grinshpun, S. A. (2002).** Fungal fragments as indoor air biocontaminants. *Appl. Environ. Microbiol*., **68** (7), 3522-3531.

28. **Gregory, P.H. (1973).** Microbiology of the Atmosphere. 2nd editon. Leonard Hill Aylesbury, 7-214.

29. **Humphreys H**., **(1992).** Microbes in the air – when to count (the role of air sampling in hospitals). *J Med Microbiol;* **37**: 81–82.

30. **Jaffal, A.A., Banat, A.A., El Mogheth, H. Nsanze, A., Benar A.S. and Ameen. (1997).** Advances in the Management of Nosocomial infections. *J. Hosp Infect* ; **19** (4) :327-38.

31. **Katušin, R.B., Mihaljevi´c., B. and Razem, D. (2003).** Microbial decontamination of cosmetic row materials and personal care products by irradiation. *Journal of Radiation Physics and Chemistry*, **66**, 309–316.

32. **Lundholm, I.M. (1982).** Comparison of Methods for Quantitative Determinations of Airborne Bacteria and Evaluation of Total Viable Counts. *Applied Environmental Microbiology* **44**: 179-183.

33. **Maron, P.A., Lejon, D., Carvalho, E., Bizet, K., Lemanceau, P., Ranjard, L. and Mougel, C. (2005**). Assessing genetic structure and diversity of airborne bacterial communities by DNA fingerprinting and 16S rDNA clone library. *Journal of Atmospheric Environment* **39**, 3687-3695.

34**.** **McDonald, L.C., Walker, M. and Carson, L. (1998).** Outbreak of *Acinetobacter* spp. bloodstream infections in a nursery associated with contaminated aerosols and air conditioners. *J. Pediatr Infect Dis* ;**17**:716–22.

35**.** **Murray, R.P., Rosenthal, S.K., Kobayashi, S.G. and Pfather, A.M. (1995**). Medical Microbiology. 4th edition, Mosby, 269-270.

36. **Napoli, C., Marcotrigiano V. and Montagna M.T. (2012**): Air sampling procedures to evaluate microbial contamination: a comparison between active and passive methods in operating theatres. *BMC Public Health*, **12**:594

37**.** **Newson, R., Strachan, D., Corden, J. and Millington, W. (2000).** Fungal and other spore counts as predictors of admission for asthma in the Trent region. *Occup Environ Med*. **57**: 786-792.

38. **Obbard, J.P. and Fang, L.S (2003)**. Airborne Concentration of Bacteria in Hospital Environment in Singapore. *Water, Air and Soil Pollution*, **144** (1), 333.

39. **Osterholm, M.T., Hedberg, C.W. and Moore, K.A. (2000)** Epidemiology of infectious diseases. Principles and practice of infectious diseases, 5th ed. Philadelphia, PA: *Churchill Livingstone* ;156–67.

40**. Oyeyi T.I. and Lum-nwi, M.E. (2008).** Bacteriological quality of some street vended foods in Bayero University campuses, Kano, Nigeria. *Biol. Environ. Sci. J. Trop*., **5** (4): 239-243.

41**. Park, D.U., Ryu, S.H., Kim, S.B. and Yoon, C.S. (2011).** An assessment of dust, endotoxin, and microorganism exposure during waste collection and sorting. *J. Air Waste Manage. Assoc*., **61**, 461–468.

42. **Pasanen, A. L., Lappalainen, S. and Pasanen, P. (1996**). Volatile organic metabolites associated with some toxic fungi and their mycotoxins. *Analyst*, **121**, 1949-1953.

43**. Pasquarella, C., Pitzurra, O. and Savino, A. (2000**). The index of microbial air contamination. *The Journal of Hospital Infection*, **46**, 241–256.

44**. Pasquarella, C., Sansebastiano, G. E., Ferretti, S., Saccani, E., Fanti, M. and Moscato, U. (2007).** A mobile laminar air flow unit to reduce bacterial contamination at surgical area in a conventionally ventilated operating theatre. *The Journal of Hospital Infection*, **66**, 313–319.

45. **Pastuszka, J., S., Marchwinska, W. and Wlaz E. (2005).** Bacterial Aerosol in Silesian Hospitals: *Journal of Environmental Studies*, **14** (16), 883- 890, Bytom, Poland.

46. **Radmore K and Luck H. (1984).** Microbial contamination of dairy factory air. *SA Dairy Technol*; **16**: 119–123.

47. **Renn, P., Jankun. T.M., Belanger, K., Bracken, M.B and Leaderer, BP. (2001).** The relation between fungal propagules in indoor air and home characteristics. *Ann Allergy Asthma Immunol*. **56**: 419-424.

48. **Schaal, K.P. (1991).** Medical and microbiological problems arising from airborne infection in hospitals. *J Hosp Infect*; **18**: 451–9.

49. **Sexton, K. and Dyer, R. (2004).** Indoor air and human health. (2nd edition). USA CRC press, 456-460.

50. **Shamsuddeen, U. and Ameh, J.B. (2008).** Survey on the possible critical control points during the production of 'balangu' in Kano. Bayero, *J. Pure Appl. Sci.*, **1**(1): 76-79.

51. **Sherertz, R..J., Belani, A. and Kramer, B.S. (1996).** Impact of air filtration on nosocomial Aspergillus infections: unique risk of bone marrow transplant recipients. *Am J Med* ; **83**:709–18.

52. **Shiaka, G.P. and Ado S.A. (2011).** Airborne bacterial flora of Ahmadu Bello University Health Clinic (SICKBAY) Zaria, Nigeria. *Continental J. Biomedical Sciences* **5** (2): 7 - 13,

53. **Siersted, H.C. and Gravesen, S. (1993**). Extrinsic allergic alveolitis after exposure to the yeast Endotorula rubra. *Allergy*. **48**: 298-299.

54. **Stryjakowska, S., Piotraszewska, P., Szyszka, M., Nowicki, M. and Filipiak (2007**). Microbiological Quality of Indoor Air in University Rooms. Microbiological Quality of Indoor Air in University Rooms. *Polish J. of Environ. Stud*. **16** (4):623-632.

55. **Velmurugan, N., Chun, S. S., Han, S. S. and Lee, Y. S. (2008).** Characterization of chikusaku-eki and mokusakueki and its inhibitory effect on sapstaining fungal growth in laboratory scale. *Int. J. Environ. Sci. Tech*., **6** (1), 13-22.

56. **Venkateswaran, K., Hattori, N., La Duc, M. T. and Kern, R. (2003**). ATP as a biomarker of viable microorganisms in clean-room facilities. *Journal of Microbiological Methods*, **52**, 367–377.

57. **Wada-kura, A., Maxwell, R.G., Sadiq, H.Y., Tijjani, M.B., Abdullahi, I.O., Aliyu, M.S. and Adetunji, O.A. (2009).** Microbiological quality of some ready-to-eat foods and fomites in some cafeterias in Ahmadu Bello University, Zaria. *Biol. Environ. Sci. J. Trop.,* **6** (1): 6-9.

58. **White A**, **(1961).** Relation between quantitative nasal cultures and dissemination of staphylococci. *J Lab Clin Med*, **58**: 273–7.

59. **(WHO) World Health Organization (2002).** Guidelines for concentration and exposure-response measurements of fine and ultra-fine particulate matter for use in epidemiological studies. Geneva, Switzerland.

60. **Whyte, P., Collins, J.D., McGill, K., Monahan, C. and Mahony, H. (2001).** Distribution and prevalence of airborne microorganisms in three commercial poultry processing plants. *J. Food Prot*., **64**: 388-391.

61. **Yassin, M.F and Almouqatea, S. (2010**). Assessment of airborne bacteria and fungi in an indoor and outdoor environment. *Int. J. Environ. Sci. Technol*., **7**(3): 535-544.