COMPARATIVE STUDIES

On

The Relationship Between Human Activities And Microbial Populations In Two Working Environments

Dissertation submitted to the Institute of Science Technology, London – United Kingdom

Through the Botany Department

Of

University of Ghana, Legon

In Partial Fulfillment of the Requirement for the Award of Higher National Diploma in Microbiological Laboratory Techniques

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Accra – Ghana

June 2000

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DEDICATION

First and foremost to God Almighty for guiding me through the test. Secondly to my wife, Mrs. Veronica Kupulii Takli and my two sons, Serofia and Selasi.

Finally to the memory of my late boss/protégée Leslie Caleb Babatunde Sawyarr. Your counselling, priceless advice, spiritual and moral encouragement has brought me this far. Unfortunately, you did not live long to reap what you have sown. May your soul rest in perfect peace.

Leslie!
He de nyuie

ACKNOWLEDGEMENT

I wish to express my sincere gratitude to my supervisor, Mrs. Mary Obodai, Head of the Mushroom Project of the Food Research Institute, for her numerous contributions towards the successful execution of this project.

I also acknowledge the support and guidance enjoyed from the head of the Microbiology Division and Acting Deputy Director, Dr. Wisdom K. Amoa-Amua and his entire team (both research and technical) at the Microbiology Laboratory of the Food Research Institute where I carried out all the experiments, especially Mr. Paul Yirenkyi for his personal interest in my work and his unflinching support.

Special thanks to the organiser and his team from the Botany Department of the University of Ghana, Legon, for the level of competence exhibited throughout the programme.

Last, but not the least, to my mother (Madam Akator Ahumah) for her instrumental contributions throughout my educational career. Finally to the Director/Management of the Food Research Institute for giving me the opportunity.

DECLARATION

I hereby declare that, except for references to other people's work which have been duly cited, this work is the result of my own research and that this dissertation has neither in whole nor in part been presented for another degree elsewhere.

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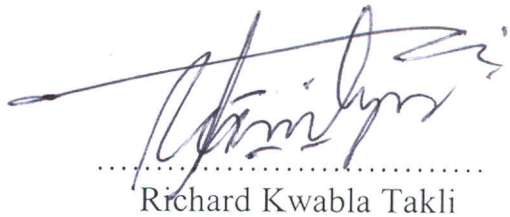
Mrs. Mary Obodai
SUPERVISOR



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ABSTRACT

The Microflora of Fruit and Vegetable Processing Plant was compared with that of Post Harvest Research Laboratory. The microbial loads in the air and on the floors were enumerated on ***Plate Count Agar (PCA), Malt Extract Agar (MEA) and Dicloran Rosebengal Chloramphenicol Agar (DRBC).***

The highest counts were recorded at Bamaf Industries during work $1.03X10^3$ Cfu/100 cm^2 and 164.7 cfu/min. whilst the least count was recorded at the post harvest research laboratory after work 5cfu/min and $3.0x10^1$ cfu/100cm^2.

Organisms isolated were mainly ***Staphylococcus, Micrococcus, Kurthia, Aspergillus, Penicillium, Neurospora*** and ***Cladosporium species,*** etc. Percentage occurrence of organism at Bamaf Industries were generally higher than the Post Harvest Laboratory, both during work and after work.

From the two sites, human activities were found to greatly affect the microbial populations in the working environments studied.

Settle plate and swab rinse methods were fully utilized in the enumeration of the entire organism isolated. However, the design of the food processing plant has greatly contributed to the high microbial populations recorded at Bamaf Industries. Low count of microbial population at the post harvest laboratory was the result of a proper architectural design of the laboratory which had an urge over Bamaf Industries.

INTRODUCTION/LITERATURE REVIEW

The estimation of the microbial population in an environment where food processing is carried out determines the quality of food being produced, the safety of the consumers and finally the level of hygiene being practiced (Collins and Lynne 1984). Likewise their application in the industrial sector has contributed in diverse ways towards the sustenance of mankind and the development of several nations in the world.

The level of air hygiene in the air is generally expressed in terms of the colony counts of bacteria/fungi of all kinds made on plate count agar/malt-extract agar plates and incubated for 48-72 hours and 5-7 days respectively at 25°c and 30°c.

Under normal conditions the air in homes, offices, schools, hospitals, factories, etc, commonly show levels of contamination in the range of 150-4000 bacteria – carrying particles per cubic metre (Slit sampler) and 10-1000 bacteria – carrying particles per square metre per minute (Cruickshank et al 1975).

The higher levels are found where there is high rate of human activities (Krzysztofik et al 1973). Provisional standards have been suggested by (Bourdillen et al 1948).

For rooms such as factories, offices and homes – not exceeding 353/m3 and in surgical theatres – not exceeding 35.3/m3. The greater proportion of bacteria found in the air are saprophytes or comensals. Even in hospital wards and other rooms only 0.0 – 0.1% are pathogenic.

As societies throughout the world are increasingly moving to greater levels of urbanization and industrial development, public concern is mounting over the state of the environment for future generations. To achieve this, there has been practically in developed nations major environmental legislation directed towards liquid, solid and hazardous wastes disposal and microbial ecology (Smith 1996).

Developed countries with good financial backgrounds now use bioreactors for the treatment of sewage, water, etc, where most wastes are reconverted into useful products using microorganisms.

On the contrary, micro-organisms present in the air, water bodies, wastes not properly disposed off, etc., are major sources of epidemics in the world, especially the less developed countries. As a result of high cost of water treatment, most people living in the rural communities do not treat their water before drinking leading to contamination by enteric and non-enteric organisms such as ***Escherichia coli*** ***Vibrio Cholorae*** ,c.

Spores of bacteria and some pathogenic organisms such as ***Staphylococcus aureus*** and ***Streptococcus pyogenes***, are the most common pathogens in the environment (Cruickshank 1975). Other causative organisms of food spoilage in the environment includes shegella, brucella, ***.Tubercle Bacilli***, ***Salmonella,*** etc. (Pike 1976, Harrington and Shanon 1976).

Food borne fungi such as the ***Aspergillus spp***.p., ***Penicillium*** species, ***Cladosporium***, ***Neurospora***, ***Fusarium*** species, etc. can also cause food spoilage in the environment by releasing their spores which in turn develop to produce aspergillic acid, a toxin leading to fungal food contamination.

The survival of micro-organisms both pathogenic and non-pathogenic, e.g. bacteria, fungi and their spores in an environment where food is being processed may lead to contamination of the finished product that may in turn result in the reduction of the micro-biological safety and quality (Hawker and Linton 1974).

Rates of infection therefore is expected to be high in the less developed countries where training for laboratory workers and safety measures are inadequate. According to (Phoon 1977) there are many complex and new occupational health hazards occurring in tropical countries which are rapidly industrializing without adequate safeguard for health and safety.

To overcome all these problems, several methods were developed for studying the environment by scientists. The emphasis is however on the swab rinse method, swab/agar slant method, use of Rhodac strips and microscopic examination of transparent cellotape pressed ~~into~~ the surface to be examined. Alternative approach is the use of non-volumetric and commercial air sampling devices such as Anderson's sampler (Jay 1992). A further environmental sampling technique that can be used conveniently following the air sampling is the sweep plate technique as described by (Cruickshank et al 1975).

Air Sampling

Various methods and devices were used for sampling air in food processing environment or rooms for the presence of micro-organisms. These include sedimentation, impingement in liquid, impacting on solid surfaces, filtration, electrostatic precipitation and thermal precipitation (Anderson 1958) and Speck 1984 described the new sampler for collection, sizing and enumeration of viable airborne particles.

Control of Micro-organisms in the Air

Many factors have been shown to contribute to the concentration of airborne micro-organisms in the environment. These include the number of persons present and their physical and physiological activities (Krzysztofik et al 1976, Collins et al 1977). There is the need therefore to control the number of micro-organisms present in the environment to reduce the risk of air-borne cross infection.

Various physical processes and chemical agents have been employed for reducing or eliminating the viable microbial population in the environment. Air filtration and mechanical ventilation systems have proved effective in keeping the concentration of airborne bacteria below specified limits in food processing plants, laboratories, operating theatres, etc. (Clark et al 1985, Lidwell 1981, Charnley 1980).

In hospital wards, laboratories and other places such as aseptic filling rooms in the pharmaceuticals industries, ultra violet irradiation and chemical vapour such as formaldehyde and potassium permanganate have been used for air disinfection to reduce aerial contamination and to protect the workers and quality of products from contaminants.

However, very effective elaborate precautions must be applied to safeguard the health and safety of the workers when these agents are used. (Weymens 1966, Jack 1952/1954, Hawkins and Mallison 1978).

Air disinfection is also very necessary in animal houses such as sheep pens brooder units, pig styles and laboratory animals units. The effect of regular cleaning and disinfection resulted in the reduction of the number of micrococci, staphylococci, streptococci and coliforms in a sheep pen.

Common diseases of the respiratory tract as well as pueperal disorders and diseases of the lambs were also reduced (Bocklish et al 1989).

OBJECTIVES.

In this study the resident, airborne microflora of a fruit and vegetables processing plant and that of a post harvest research laboratory were investigated.

The primary aim was to:

estimate the microbial population in these two study areas and

establish the relationship between human activities and the microbial population in the two environments.

MATERIALS AND METHODS

Equipment

1. ***Autoclave:*** for sterilizing cultures media and contaminated materials
2. ***Incubator:*** for growing cultures bacteria and fungi, and also for drying plates.
3. ***Water bath:*** for holding molten media at specific temperatures prior to use and for short term incubation.
4. ***Microscope:*** for observation of specimen and cultures.
5. ***Mixer:*** for mixing and shaking cultures (VF2 Janke & Kunkel)
6. ***Stomacher (Seward):*** for emulsifying suspensions.
7. ***Balance (Sartorious):*** for weighing materials and media
8. ***pH meter:*** for determining the pH of samples and media

9. ***Refrigerator:*** for storage of cultures and media.

10. ***Inoculating loop (3mm diameter) and wires:*** for transferring culture.

11. ***Spreaders:*** 'L' shape and 'A' shape metallic spreader for spreading cultures on sterile media.

12. ***Racks:*** for holding test tubes

13. ***Bursen Burner:*** for flaming loops, wires, mouth of media, bottles, etc.

14. ***Colony Counter:*** for counting bacteria/fungal colonies on media.

15. ***Hand Magnifier:*** for examining colonies on plates

16. ***Bench Rack:*** to keep loops and wires together

17. ***Discard Bins:*** to receive cultures and specimen

Glassware

1. ***Petri dishes:*** borosilicates glass, forculture plates

2. ***Measuring cylinders:*** for measuring fluids & liquids.

3. ***Test tubes:*** for tubing culture media

4. ***Storage bottles:*** for storing media

5. ***Graduated pipettes:*** used for transferring fluids

6. ***Pipetting aid:*** an alternative to mouth pipetting

7. ***Micro-slides and cover glasses:*** for preparing smears and wet films of cultures.

8. ***Durham's tubes:*** for detecting gas formation in cultures

9. ***Discard Jars:*** for used slides and disposables

10. ***Pipette Jars:*** for receiving graduated pipettes

Chemicals

'Analar' grade chemicals from BDH Yele, England

Media

Malt extract agar (MERK 5.398 UK) (MEA)
Malt extract - 30g
Peptone from suy meal - 3g
Distilled water - 1000 ml.
Agar - 15g
pH 5.6 (0.1 @ 25°c

Plate Count Agar (PCA) (MERCK 546.3)
Peptone from casein - 5g
Yeast - 2.5g
Glucose - 1g
Agar - 14g
Distilled water - 1000 ml.
pH 7.0 (0.1 @ 25°c

Nutrient Agar (NA) (Difco)
Beef extract - 3g
Peptone - 5g
Agar - 15g
Distilled water - 1000 ml
pH 6.8 (0.2 @ 25°c

Dicloran Rose Bengal Chloraphenical Agar (DRBC) (OXOID)
Petone - 5.0g
Glucose - 10.0g
Potassium dihydrogen phosphate - 1.0g
Magnesium sulphate - 0.5 g
Dichloran - 0.002g

Rose Bengal	- 0.025g
Agar	- 15.0g
pH 5.6 ± 0.2	

METHODS

Methods of Sterilization

Read Heat Flaming – Innoculating ~~crops~~, wires and pins were sterilized by flaming to red heat.

Dry Heat – Glass, petri dishes, pipettes on canisters, flasks, etc. were sterilized in a electric ovens set at 160°c

Autoclaving – Media in flasks, MacCarthy tubes, test tubes, etc. were sterilized by autoclaving at a preassure of 15 psi and temperature of 121°c for 20 minutes. All material sterilized were packed loosely in the autoclave after filling THE CHAMBER WITH SUFFICIENT WATER. The lids were tightened and heaters switched on with the pressure value opened. Trapped air was allowed to escape from the chamber and temperature maintained at 100°c for about 5 minutes before the pressure valve was sealed. Temperature rose to 121°c and pressure 15 psi and timed for 15-20 minutes. Pressure valve was released gently and pressure within the Autoclave dropped to atmospheric pressure. The content were allowed to cool for safe handling.

Filtration - The glucose, maltose and other culture media that would have been damaged by heat were filter sterilized. A membrane about (4.5mm) was mounted on a supporting stainless steel which was sealed together between the upper and the lower funnels. Filtration was achieved by applying a negative pressure to the exit side.

Disinfection - 70% ethyl acohol was used for disinfecting working surfaces and bench tops by swabbing before and after work.

Fumigation - Potassium permanganate and formaldehyde were reacted to sterilize inoculation rooms and laboratories where experiments were carried out.

Preparation of Media - All media used were pre-prepared by adhering to the manufacturers instructions. Appropriate weights and volumes were measured, melted either on hot plate or in autoclaves or in water baths depending upon the type of media. pH of the final media after sterilization compensated to conform to manufacturer's specifications.

Preparation of Agar Plates - The molten medium was maintained at 45°c in a thermostat controlled water bath prior to pouring. About 10-15 ml was poured into each petri dish and allowed to set. The plates were packed and dried in an oven at 50°c for 30 minutes before use.

Incubation - The plates containing Plate Count Agar (PCA) were incubated in inverted forms after being exposed to o air in the study areas for 20 minutes at 30°c for 48-72 hours. Fungal Plates were also incubated at 25°c for (7) seven days.

SAFETY/PRECAUTION TAKEN

The working environment (Laboratory) was fumegated on weekly basis using potassium permaganate and formaldehyde. Bench tops were also cleaned on daily basis before and after work with 70% ethyl alcohol.

Clean laboratory coats, hand gloves where necessary were worn during the entire experiment. Hands were washed after handling culture. After sterilization autoclaves were allowed to cool to room temperature before opening.

Used pipettes and slides were discarded into a discard jar filled with clorox diluted with water over night before washing.

Thermal protective gloves were worn to prevent the hands from burns and scalds. The necks of media bottles and culture tubes were flamed before and after opening. Inoculation loops and wires were flamed red hot before and after use. Contaminated plates and cultures were sterilized at 121°c at 15 psl for 30 minutes before discarded. Hand to mouth operation in the laboratory was avoided.

Sites of Investigation

The fruit and vegetable processing plant (Bamaf Industries)

Bamaf Industries is a non-governmental organization established in Ghana in 1998 by a private entrepreneur to process non traditional export crops such as mushrooms, okro, pepper, ginger, pineapple, tomatoes, etc for both local and export market.

It is located at Sakaman near Dansoman, a suburb of Accra in the Greater Accra Region of Ghana. Even though the factory is young and small in size, its currently processing about 12.5 tons of fresh mushrooms; 20 tons of okro, 15 tons of ginger and about 50 tons of other spices and fruits into dehydrated and powdered forms for export and local consumption per annum.

The fresh fruits and vegetables are normally purchased directly from farmers and transported to the factory for processing. The produce e.g. mushrooms are normally received at the factory in fresh forms. The mushrooms are sorted out, cleaned and sliced if possible, before packed on trays and dried in hot air ovens at temperatures of about 45°c to 60°c for 10 to 14 hours depending upon the moisture content of the product under processing.

After adequately dehydrating them (10-12% moisture content) they are removed after cooling and packaged for sale. Some are milled and packaged as powdered mushrooms for thickening soups and stews.

The study areas at Bamaf Industries was made up of three main rooms – processing room, packaging and storerooms. The dryer is kept in the proecessing room. Sorting, cleaning and dehydration of the products are done in this room. Design blocks were used to create room for aeration.

The second sampling site – packaging room. This opens into the store with a wooden partition separating them. In a similar manner the room above was ventilated with floors and walls very smooth (not slippery). The packaging room houses the milling machines, working benches covered with formica, sealing machines, etc. which are used for packaging the products. There are two main doors provided. The main door opens into the main street (sales point/store) and interconnected with common entrance from the store into the packaging room. One other door (emergency door) has also been provided from the packaging room to the outside. The entire floor of these two rooms are lined with rubber carpets to pave way for effective disinfection of the rooms.

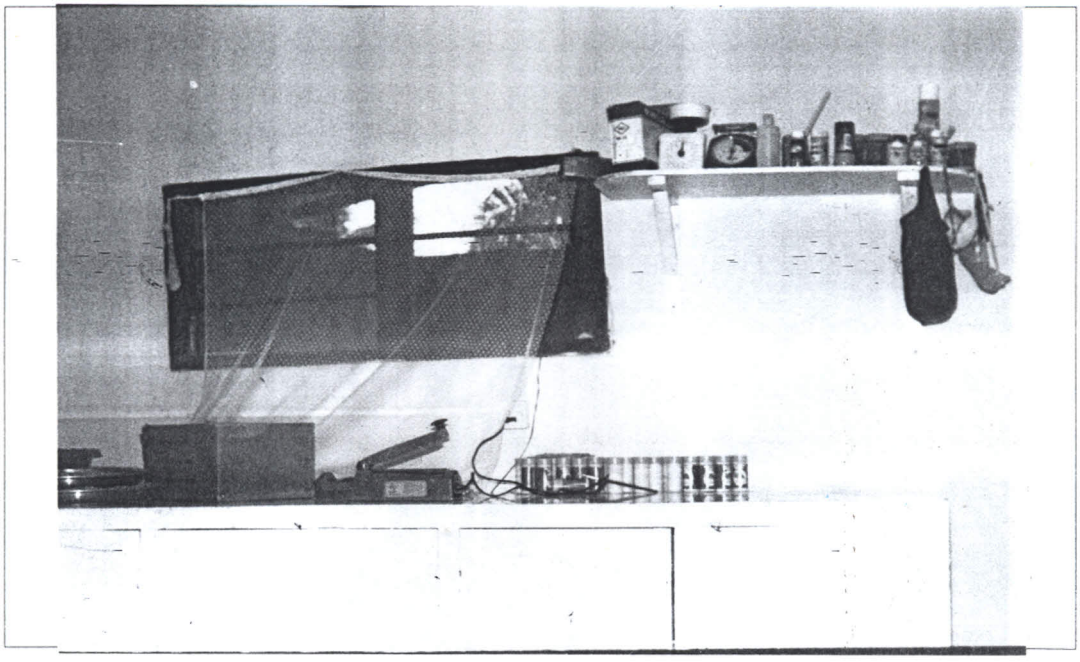

Plate 1: The processing plant at Bamaf Industries

THE POST HARVEST RESEARCH LABORATORY

The above laboratory was set up by the Food Research Institute of the Council for Scientific and Industrial Research (CSIR) under the National Agricultural Research Programme (NARP) to conduct research into the post harvest losses of Ghanaian farmers, especially those dealing with fruits, vegetables, roots and tuber crops throughout the country.

The Laboratory is located within the premises of the Food Research Institute Pilot Plant, Okponglo, Accra. This Laboratory was basically designed as a laboratory and has few advantages over the processing plant. The floors and the walls are very smooth (terrazzo floor), however, not slippery. It is furnished with formica top working benches and air-conditioned. All windows are made from glass. Sinks, fume chambers, chemical shelves and book shelves are also provided. All electrical wiring passed through pipes and water, air under pressure, etc. are also provided through pipes.

The room has only one door, windows are always kept closed to prevent entry of dust and floors mopped regularly and fumigation carried out on weekly basis.

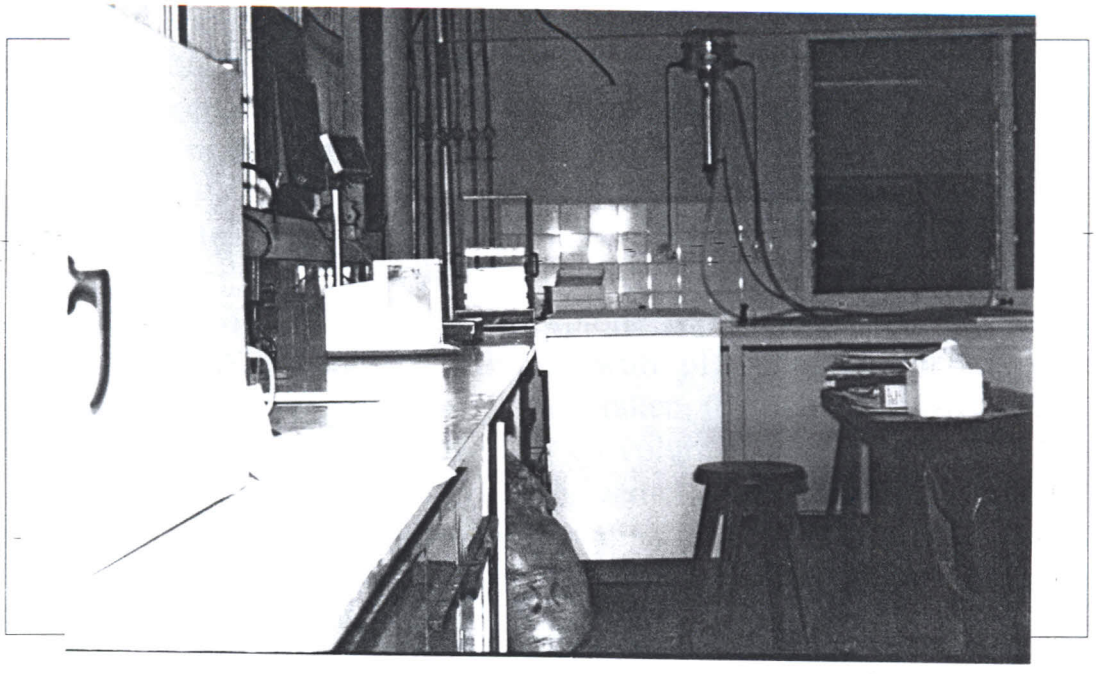

Plate 2: Post Harvest Research Laboratory

METHODS

Air Sampling

Agar plates (3 PCA plates, 3 MEA plates and 3 DRBC plates each were exposed in the rooms under consideration/sampling for 20 minutes (20 minutes was used because from the test trials several plates containing different media was exposed at different times ranging from 5 to 30 minutes; only 15 and 20 minutes gave very good results.

However, 20 minutes was outstanding in almost all the plates hence 20 minutes was maintained constant throughout the air sampling. The lids were replaced after 20 minutes. The PCA plates were inverted and incubated at 30°c for 48 to 72 hours while the MEA and DRBC plates were incubated at 25°c for seven days. The temperature of sampling were taken at the time of exposure of the plates.

Swabbing/Sampling of Surfaces

Standard cotton swabs were placed in test tubes and plugged with non-absorbent cotton wool and sterilized at 121°c for 30 minutes in an autoclave.

An area of 10 cm by 10 cm from each sampling site was swabbed. Sites swabbed includes bech tops and floors at Bamaf Industries processing room, packaging room and storeroom. From the post harvest laboratory bench tops and floors at three different places were swabbed.

Swab Procedure

The swab was dipped into sterile peptone water (SPW) containing 0.1% peptone and 0.85% sodium chloride with pH adjusted to 7.2 prior to swabbing. Immediately the sample was taken, the swab was plunged into a medium of (SPW) in a test tube of 10 ml volume and the swab stick broken off and the stopper timing applied. The samples were placed in an ice chest and transported to the laboratory for examinations

Micro-biological Analysis

The samples from each study site was pooled together after shaking to dislodge micro-organisms from swab and homogenized in a stomacher for 30 seconds at normal speed. From appropriate 10 fold dilutions in sterile salt peptone water enumeration of bacteria was carried out on plate count agar.

Yeast and moulds on malt extract agar and dicloran rose bengal chloranphicol agar. Using sterile pipette 0.1 ml of the test sample was transferred to each of two sterile agar plates previously dried in an oven at 50°c for 30 minutes. The procedure was repeated for 10^{2}, 10^{3}, etc. dilutions where necessary.

For the moulds, the innoculum was spread as quickly as possible over the surface of the agar plates using metallic spreaders each to a plate. The plates were allowed to dry with their lids on for about 10 to 15 minutes at room temperature.

Incubation

The PCA plates were inverted and incubated at 30°c for 3 days. The MEA and DRBC plates were incubated at 25°c for 7 days. Incubated alongside the plates were uninoculated plates of PCA, MEA and DRBC to test for sterility.

IDENTIFICATION OF BACTERIA

Bacteria isolates were identified according to Cowan and Steel (1974) scheme used for identifying medical bacteria. After counting the colonies from each plate using the colony counter, the isolated colonies were examined with hand lens. A single colony of each strain was gram stained and examined under the microscope for their grouping into their various genera and biochemical test were carried out for their identification. About 20 colonies from a segment of a suitable plate were sub-cultured into corresponding broth and streaked unto agar plates until pure colonies were obtained. PCA colonies were sub-cultured onto nutrient broth and MEA & DRBC onto MEA broth respectively.

The pure cultures were grouped into rods and cocci shapes/forms. From each group a number of representative cultures were selected for further investigations. Moulds and yeasts were identified based on their morphological characteristics.

Pure isolates of bacteria were identified by morphological and biochemical test such as colony and cell morphology, gram reaction, oxidase test, catalase tests, gas production from glucose, acid production from glucose and Hugh and Leifson Tests.

Gram Staining

Thin smear of the organisms were made on clean slides, dried air and heat

fixed. The fixed smear was flooded with diluted crystal violet and allowed to stand for 30 to 60 seconds. The slide was washed well in low running tap water and then flooded with iodine mordant for 1 to 2 minutes.

The slide was washed in running tap water and decolourised with acetone – alcohol while holding the slide at an angle. The slide was again washed in water and counter stained with safranine for 30 seconds and finally washed in water drained and blotted dry. Examination was done under oil immersion with x1000 magnification and gram reaction noted.

Catalase Test

A 3% hydrogen peroxide was dropped on a glass slide and bacterial colony from a pure culture was picked using sterile loop and teased out on the slide. Gas bubble production observed for catalase positive organisms.

Oxidase Test

(Merck 1992 - Test strip – Bactident) - The oxidase strip was smeared with the culture under test across the impregnated tip with the edge of a cover-slip or platinum loop. A positive reaction was indicated by the appearance of dark purple colour within 10 seconds.

Gas Production From Glucose

A tube with inverted Durham tube and nutrient broth (Broth Sugar) was inoculated with colony mass from a single colony. The tube was incubated at 30°c for at least 72 hours. Following metabolism of the sugar, acidic products were produced and the acidity was detected by pH indicator. Gas produced accumulates in the Durham's tube.

Oxidation and Fermentation Test

One percent filtered sterile glucose was added to the basal medium. Two tubes of the Hugh and Leifsin O.F medium were innoculated with the bacteria isolates. The medium was tubed aseptically in narrow tubes in 8 to 10 ml amounts. Sterile parafin oil was added to one of the tubes to obtain anaerobic condition. The tubes were incubated at 30°c for 2 to 7 days.

Fermentation reaction was indicated by a yellow colour in both tubes whilst an oxidation reaction was indicated by a yellow colour in the aerobic tub only.

Presence of Spores and Motility

The presence of spores and cell motility were observed in wet preparation by using Phase Contrast Microscope with 400 and 1000 magnification. Motility was observed as microbial cells which showed a definite movement and spores as rods bearing phase bright spores.

IDENTIFICATION OF FUNGI

After enumerating the various colonies on both Malt Extract Agar (MEA) and Dichloran Rosebengal Chloranphenical Agar (DRBC), the fungal colonies were sub-cultured into MEA broth and incubated for seven days. The broth was then sub-cultured unto MEA plates and three points as described by (Sampson and Pit – 1985)

The pure strains (isolates were then mounted on slides. Using Lactophenol/Lactophenol cotton blue the cells were identified under the low power magnification (10 and 100).

Some Fungal Species Identified

Aspergillus Flavus: Colonies on MEA at 25°c attained a diameter of 305 cm within 7 days, forming a dense felt of yellow- green conidiophones. Conidial heads typically radiate, later splitting into several loose columns, yellow-green becoming dark yellow-green. Vosicles globose to sub-globose. Philiades borne directly on the vesicle or on the metulae. Conidia globose to sub-globose 3.6 cm in diameter plae gree and echinulate.

Aspergillus Niger: Colonies on MEA at 25°c thin, but sporulating densely attaining a diameter of 4-5 cm within 7 days consisting of compact white or yellow based felt within a dense layer of dark radiate tending to split into loose columns with age. Conidiophene stipes smooth – walled, hyaline, but also in brown columns. Vesicles globose to sub-globosephialides borne on metulae. Metulae hyaline to brown often septate conidia globose to sub-globose, brown ornamented with irregular wart spines and ridges.

RESULTS AND DISCUSSION

Results

In this study, two working environments – Bamaf Industries and Post Harvest Research Laboratory – were investigated.

The microflora of Bamaf Industries (a processing plant for fruit and vegetables) was compared with a Post Harvest Research Laboratory also dealing with similar products. The microbial loads were enumerated on PCA, MEA and DRBC and the various organisms isolated and identified from which relationships between human activities and the microbial populations in these working environments were established.

In all, a total of 197 different species of organism were isolated from the two study areas investigated. A sub-total of 145 organisms were isolated from Bamaf Industries and 52 other species from the Post Harvest Laboratory. Table 9 & 10.

Out of the 197 isolated from Air Bench Tops and Floors, 102 were identified as fungi whilst 95 were made up of different species of bacteria. The fungi isolated from the two study areas belongs to six different genera - ***Altenaria, Aspergillus, Cladosporium, Neurospora, Penicillium and Rehizopus,*** whilst bacteria were from six different genera – ***Bacillus, Kurthia, Micrococcus, Pasteurella, Staphylococcus and Streptococcus.***

With exception of ***Kurthia, Pasteurella*** and ***Altenaria,*** most organisms isolated were common to both sites. ***Staphylococcus*** and ***Micrococcus*** were the most occurring organisms isolated from the two environments (Fig. 2 and 3) during working hours, whilst the least occurring organisms were ***Bacillus, Cladosporium*** and ***Staphylococcus*** from the two sites after work.

The occurrence of the organisms followed a general trend, most frequest organisms (aerobic mesophiles and food borne fungi) dominated the environments, especially when human activities were in progress (Figure 1 to 3).

The Microflora at Bamaf Industries recorded the highest number of organisms during working hours and after work, whilst Post Harvest Research Laboratory recorded the least in both cases (fig. 1, 2 & 3).

Enumerating the organisms from air on PCA, MEA and DRBC, it was revealed that the highest counts were made on PCA 164.7 cfu/min. at Bamaf Industries during work whilst the least count was recorded at Post Harvest Research Laboratory after work, 5cfu/min. (Fig 1)

Swabs from the two environments – floor and bench tops also presented a similar scenario. Highest counts were made on PCA from Bamaf Industries floors, $1.03x10^3$, whilst the least count was made on DRBC (Bench Top) from Bamaf Industries, $3.0x10^1$ cfu/100cm^2.

Discussions

The levels of contamination estimated from the two environments were in the rage of 5-164.7cfu/min from the air samples and $3.0x10^1$cfu/100cm^2 from the swabs. According to Cruickshant et al (1975), the levels of air contamination at homes, hospital, schools, factories, etc. range between 150-4000 bacteria carrying particles per cubic metre. The levels are however higher at environments where the rate of human activities are higher (Bourdillon et al 1948 and Krzystofik et al 1973)

Comparing the above levels of contamination in the two study areas, it is clear that the levels conform to the earlier works done by scientists.

The general trend observed from figure 1 to 3 also supports earlier work done by Krzysztofik et al 1973. From the graphs (figure 1 to 3) the occurrence of the organisms during work were generally higher than when no human activities were on progress from the two environments studied. This fact is further strengthened by the presence of ***Staphylococcus spp.*** occurring at almost all the sites.

Since these ***Halophilic mesophiles*** are commonly found in sweats on the body. Presence of ***Kurthia species*** from Bamaf was due to the dehydrated fruits and vegetables that are worked on in the environment (Cowan and Steel 1974).

From figure 1, PCA supported the growth of bacteria with the highest counts occurring during work. Growth of the organisms on DRBC was better compared with their appearance on MEA. Such a pattern was observed because both sites, especially Bamaf Industries was heavily loaded with spores of food borne fungi, such as ***Aspergillus spp, Cladosporium spp, Penicillium spp, Neurospora,*** etc.

According to Sampson R. A., Haekstra 1995, DRBC supports the growth of food borne fungi better than the other media.

Methodologies employed had few limitations for instance all experiments carried out could have produced more interesting results if replicates were observed under anaerobic conditions.

CONCLUSTION AND RECOMMENDATIONS

In this study the swab rinse and the settle plate methods were successfully used to examine the microbial load in the two study areas. Due to few limitations in the design of Bamaf Industries, the microflora there was found out to be more loaded than the Post Harvest Research Laboratory. Most importantly, it was established that human activities affects the microbial population in the work environment.

Organisms isolated from the two environments such as ***Staphylococcus, Kurthia, Micrococcus, Aspergillus spp, Neurospora, Penicillium,*** etc. were a confirmation of what transpired in other fruit and vegetable processing environments.

Since some of the organisms isolated from the study sites are pathogenic, it is highly recommended that steps should be taken to improve:

i. health hazards at the work place;

ii. adequate and effective means of decontamination Should be provided e.g. Biological Safety Cabinets, Ultraviolet Irradiation, etc;

iii. architectural designs should facilitate thorough disinfection such as fumigation of the processing rooms;

iv. adequate mechanical ventilation systems which provide an inward air flow with exhausts without recirculation should be provided in specified working areas;

v. a comprehensive health record of each worker should be kept and workers given periodic medical examinations; and

vi. Every workplace should have Biological Safety Officer.

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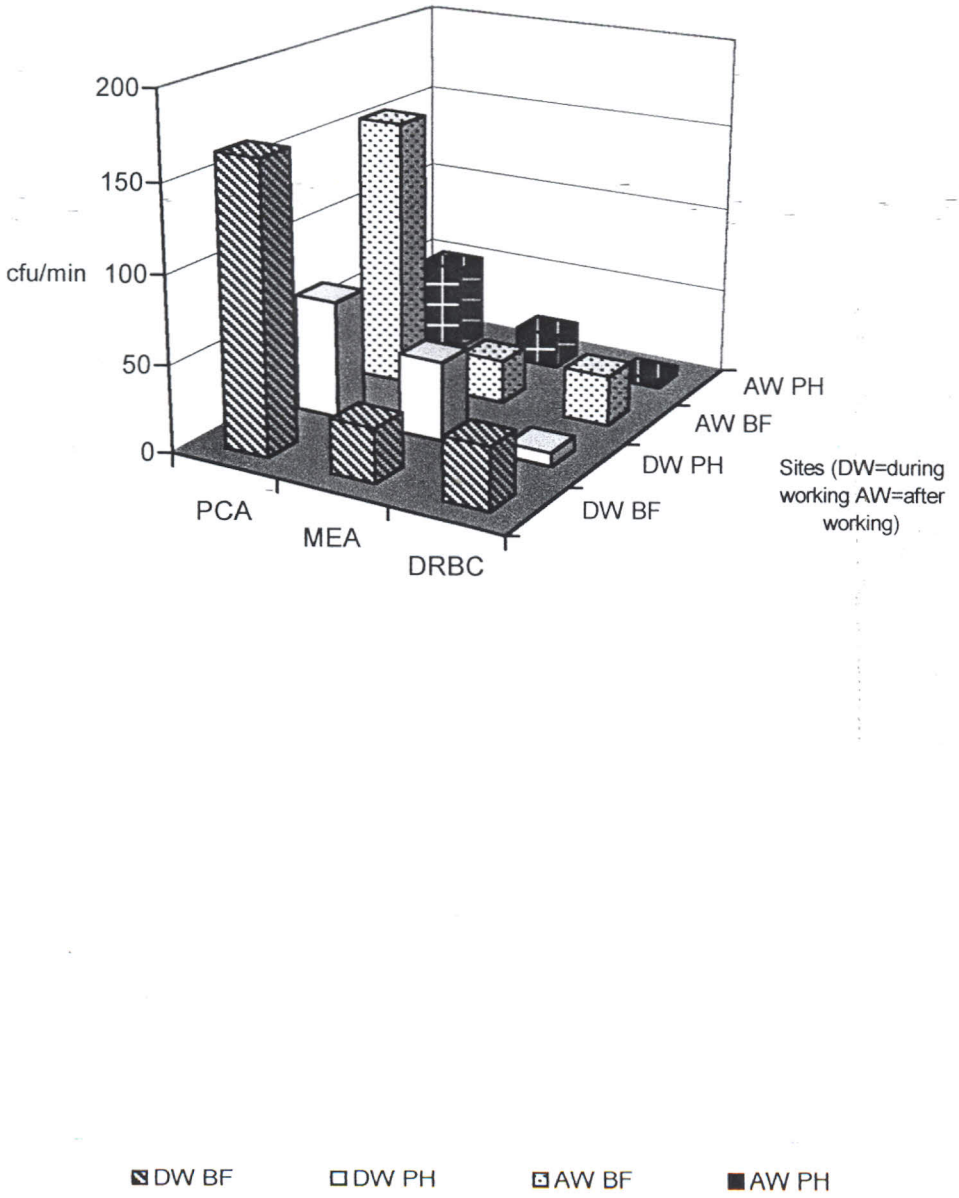
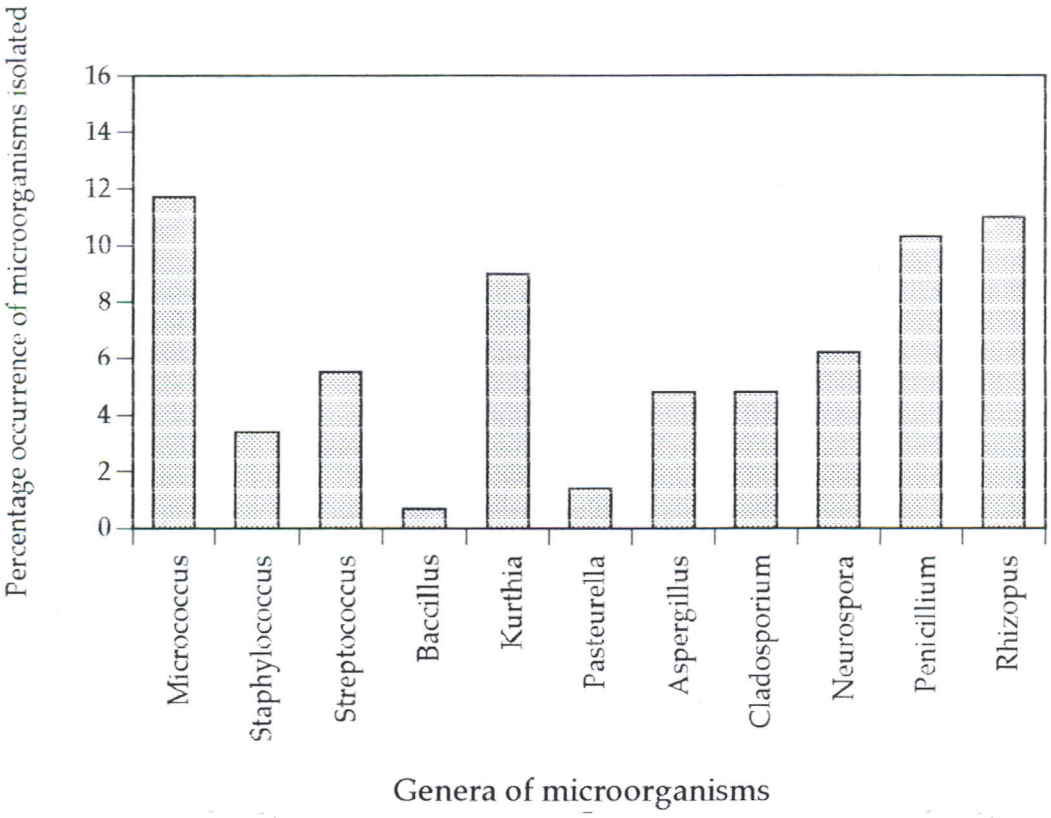
Fig. 1 Mean values of total microbial counts (air samples) in cfu/min at two different study areas on PCA, MEA and DRBC

BF - Bamaf Industries

PH - Post Harvest Lab.



(a) During work



(b) After work

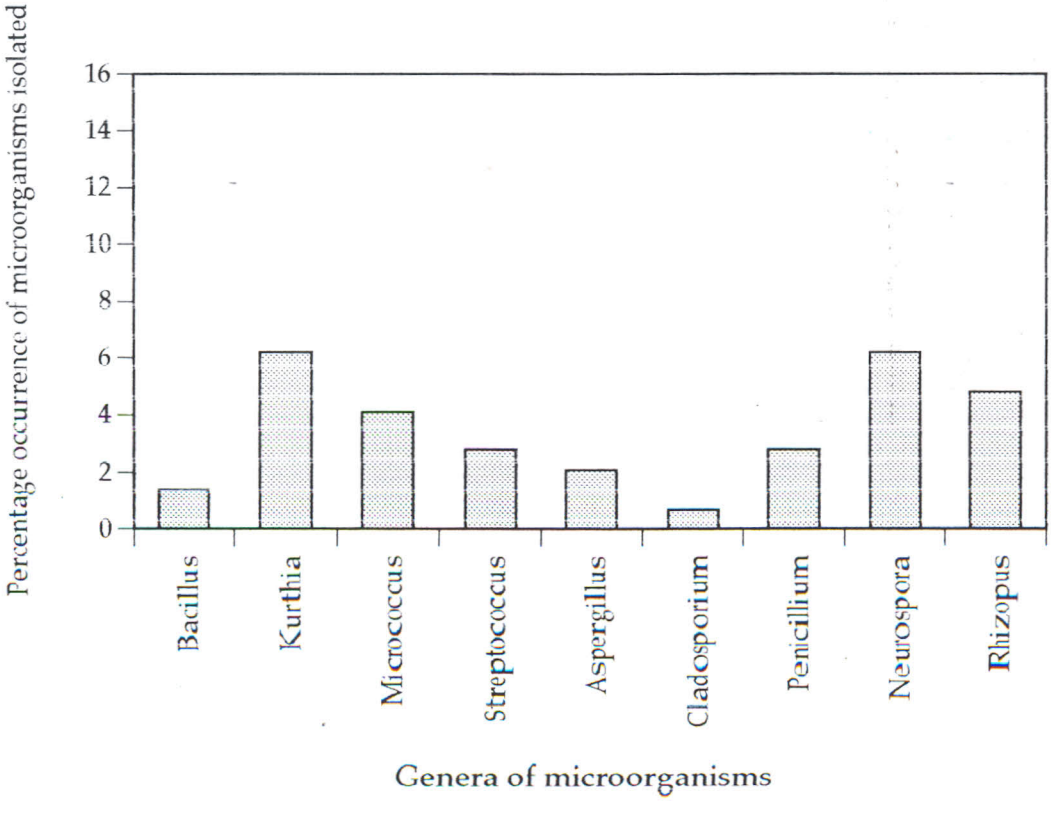


Fig. 2: Percentage occurrence of microorganisms isolated from BAMAF industries during and after work.

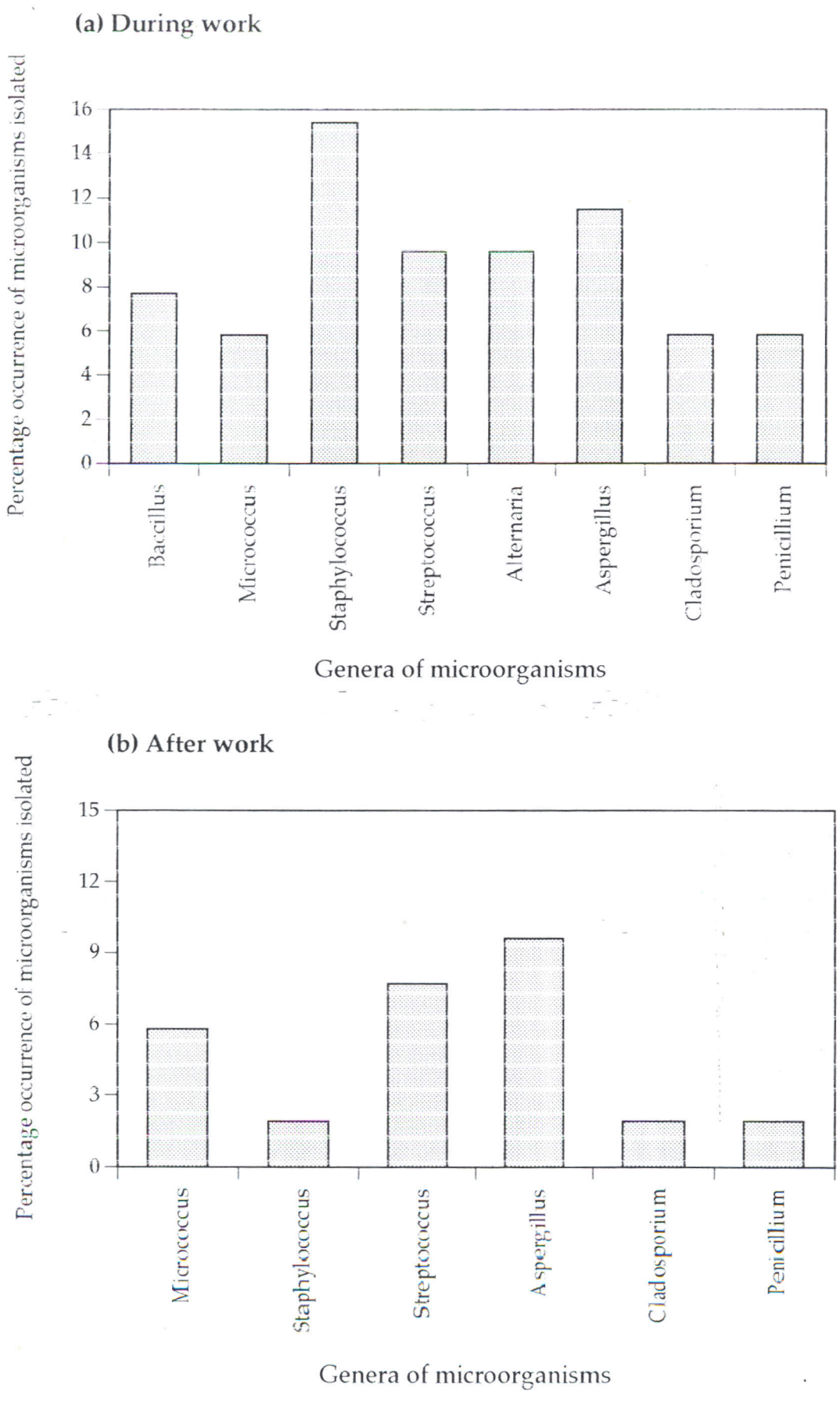


Fig. 3: Percentage occurrence of microorganisms isolated from Postharvest laboratory during and after work.

TABLE 1: TOTAL MICROBIAL COUNTS (AIR SAMPLE) CfU/MIN

SITE 1: BAMAF INDUSTRIES

DATE	DURING WORK			AFTER WORK		
	BACTERIA (PCA)	FUNGI		BACTERIA (PCA)	FUNGI	
		MEA	DRBC		MEA	DRBC
31/3/2000	223	35	36	196	27	42
	165	39	47	163	33	34
	127	27	25	190	17	24
3/4/2000	184	28	39	164	25	38
	154	33	41	152	31	22
	131	25	27	112	24	18
4/4/2000	190	31	33	113	14	22
	162	28	28	161	23	34
	146	40	40	142	30	26

TABLE II: TOTAL MICROBIAL COUNT (AIR SAMPLE) CfU/MIN.

SITE II: POST HARVEST RESEARCH LABORATORY

DATE	DURING WORK			AFTER WORK		
	BACTERIA (PCA)	FUNGI		BACTERIA (PCA)	FUNGI	
		MEA	DRBC		MEA	DRBC
10/4/2000	TNTC	108	10	52	TNTC	8
	70	23	6	36	6	4
	87	11	6	63	5	3
13/4/2000	74	70	13	48	27	7
	66	28	7	61	19	6
	81	16	5	44	24	2
14/4/2000	54	111	8	39	21	5
	68	22	9	58	28	6
	39	20	4	52	30	4

TABLE III: MEAN VALUES OF TOTAL MICROBIAL COUNTS ON PCA, MEA AND DRBC AT TWO STUDY ARES (SWABS) CfU/100CM2

SITES		PCA	MEA	DRBC
Bamaf	Bench Top Floor	4.8×10^2 1.03×10^3	9.0×10^1 1.8×10^2	3.0×10^1 3.2×10^2
Post Harvest Lab.	Bench Top Floor	3.9×10^2 2.08×10^3	3.2×10^2 4.1×10^2	4.3×10^2 3.4×10^2

TABLE IV: MEAN VALUES OF TOTAL MICROBIAL COUNTS (AIR SAMPLES) CfC/MIN.

SITES	DURING WORK			AFTER WORK		
	PCA	MEA	DRBC	PCA	MEA	DRBC
Bamaf Industries	164.7	30.7	35.1	154.8	24.9	28.9
Post harvest research laboratory	67.4	25.4	7.6	50.3	20.0	5

TABLE V: MICROORGANISMS ISOLATED IN TWO STUDY AREAS DURING WORK

SITE	ORGANISM	NO. OF ISOLATES	GENERA/SPECIES
Bamaf (Air)	Bacteria	17	Micrococus
		5	Staphylococcus
		8	Streptococcus (non-heamolitic)
		1	Bacillus
		13	Kurthia
		2	Pasteurella
	Fungi	3	Aspergillus niger
		4	A. Flavus
		7	Cladosporium herbarum
		16	Rhizopus spp.
		15	Neurospora spp.
		9	Penicillium spp.
Post Harvest Lab. (Air)	Bacteria	4	Bacillus
		5	Streptococcus
		3	Micrococcus luteus
		8	Staphylococcus
	Fungi	3	Penicrullium spp.
		5	Alternaria alternator
		2	A. flavus
		4	A. niger
		3	Caldospirium herbarum

TABLE VI: MICROORGANISMS ISOLATED IN TWO STUDY AREAS DURING WORK

SITE	ORGANISM	NO. OF ISOLATES	GENERA/SPECIES
Bamaf (Air)	Bacteria	10	Micrococus luteus
		3	Streptococus viridans
		2	Staphylococcus
		8	Kurthia
		3	Bacillus
		7	Pasteurella
	Fungi	2	Cladosporium herbarom
		8	Neurospora
		6	A niger
		3	Penicillium
		7	A. flavus
Post Harvest Lab. (Air)	Bacteria	3	Bacillus
		5	Streptococcus
		2	Micrococus
		6	Staphylococcus
	Fungi	1	Aspergillus niger
		4	A. niger
		3	A. flagus
		5	Penicillium
		2	Alternaria alternator
		1	A. tereus
		3	Cladosporium herbarum

TABLE VII: MICROORGANISMS ISOLATED IN TWO STUDY AREAS AFTER WORK

SITE	ORGANISM	NO. OF ISOLATES	GENERA/SPECIES
Bamaf (Air)	Bacteria	6	Micrococus
		9	Kurthia
		4	Streptococus
		2	Bacillus
	Fungi	9	Neurospora sp.
		7	Rhizopus sp.
		4	Penicillium sp.
		3	A. niger
		1	Cladosporium sp.
Post Harvest Lab. (Air)	Bacteria	3	Micrococus
		1	Staphylococcus
		4	Streptococcus
	Fungi	1	Penicillium
		3	A niger
		2	A flavus
		1	Cladosporium herbarum

TABLE VIII: MICROORGANISMS ISOLATED IN TWO STUDY AREAS AFER WORK

SITE	ORGANISM	NO. OF ISOLATES	GENERA/SPECIES
Bamaf (Air)	Bacteria	5	Kurthia
		2	Bacillus
		3	Staphylocucus
		2	Streptococus
		1	Pasteurella
		4	Micrococus
	Fungi	5	Neurospora
		2	Rhizopus
		3	A. niger
		1	A. flavus
Post Harvest Lab. (Air)	Bacteria	3	Micrococus
		1	Staphylococcus
		4	Streptococcus
	Fungi	1	Alternaria alternator
		1	A. tereusCladosporium
		1	herbarom
		2	Penicillium
		5	A. niger

TABLE 9: FREQUENCY OF MICROORGANISM ISOLATED FROM BAMAF LABORATORY DURING AND AFTER WORK

SITES	GENERA OF ORGANISMS	FREQUENCY	PERCENTAGE OCCURRENCE
BAMAF D.W. DURING WORK	*Micrococcus*	17	11.7
	Staphylococcus	5	3.4
	Streptococcus	8	5.5
	Bacillus	1	0.7
	Kurthia	13	9
	Pasteurella	2	1.4
	Aspergillus	7	4.8
	Cladosporium	7	4.8
	Neurospora	15	10.3
	Penicillium	9	6.2
	Rhizopus	16	11
AFTER WORK A.W	*Bacillus*	2	1.4
	Kurthia	9	6.2
	Micrococcus	6	4.1
	Streptococcus	4	2.8
	Aspergillus	3	2.1
	Cladosporium	1	0.7
	Penicillium	4	2.8
	Neurospora	9	6.2
	Rhizopus	7	4.8
	TOTAL	145	100

TABLE 10: FREQUENCY OF MICROORGANISM ISOLATED FROM POSTHARVEST LABORATORY DURING AND AFTER WORK

SITES	GENERA OF ORGANISMS	FREQUENCY	PERCENTAGE OCCURRENCE
POST HARVEST LAB D.W.	*Bacillus*	4	7.7
	Micrococcus	3	5.8
	Staphylococcus	8	15.4
	Streptococcus	5	9.6
	Alternaria	5	9.6
	Aspergillus	6	11.5
	Cladosporium	3	5.8
	Penicillium	3	5.8
AFTER WORK	*Micrococcus*	3	5.8
	Staphylococcus	1	1.9
	Streptococcus	4	7.7
	Aspergillus	5	9.6
	Cladosporium	1	1.9
	Penicillium	1	1.9
	TOTAL	52	100

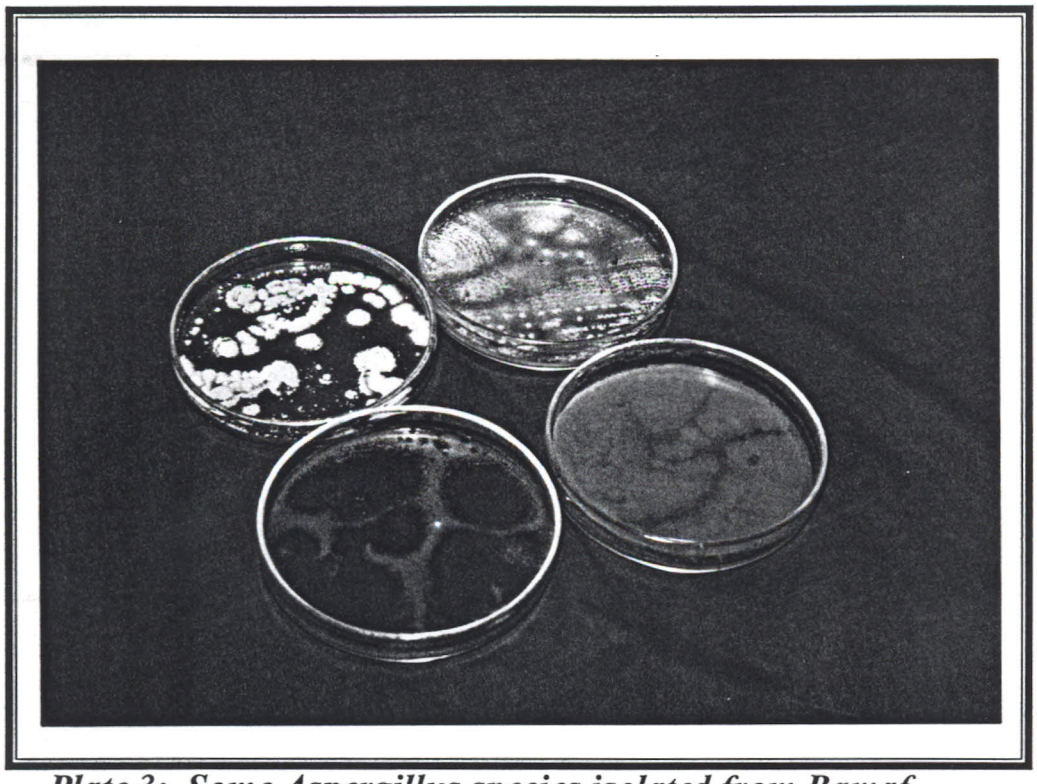


Plate 3: Some Aspergillus species isolated from Bamaf Industries

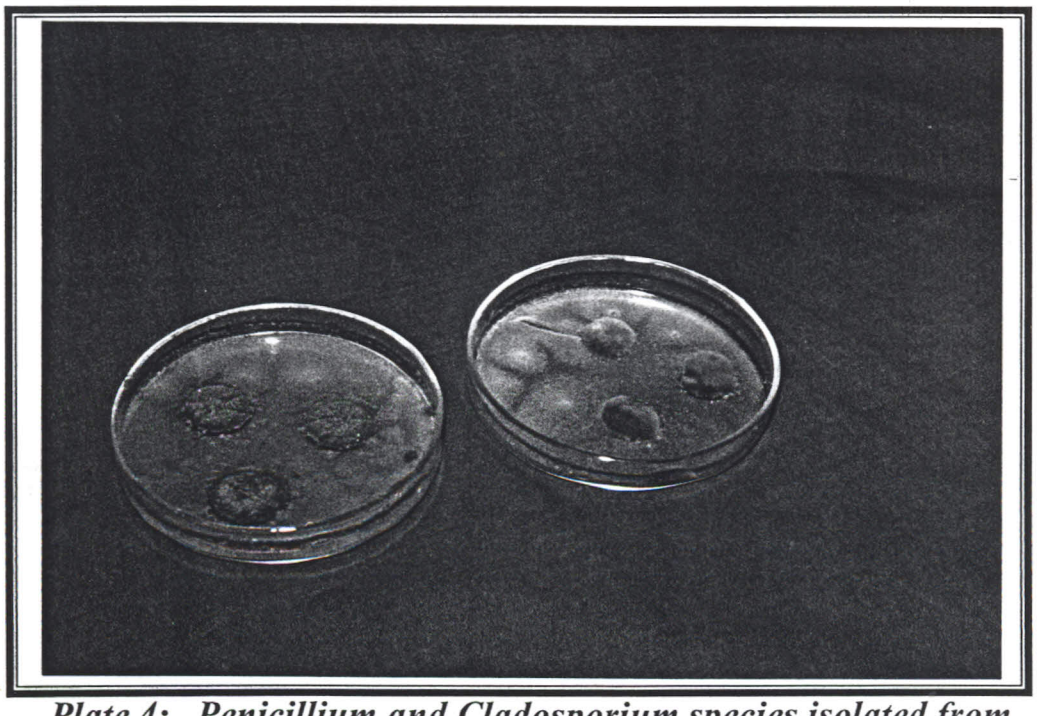


Plate 4: Penicillium and Cladosporium species isolated from the Post Harvest Research Laboratory