A QUANTITATIVE AND QUALITATIVE ASSESSMENT OF MICROBIAL POPULATIONS ASSOCIATED WITH AIR-CONDITIONING SYSTEMS

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DECLARATION OF INDEPENDENT WORK

I, ELIZABETH LOURENS, do hereby declare that this research project submitted for the degree MAGISTER TECHNOLOGIAE: ENVIRONMENTAL HEALTH, is my own independent work that has not been submitted before to any institution by me or anyone else as part of any a qualification.

....
SIGNATURE OF STUDENT

01/12/1998
DATE

He knows your ideals and dreams because He knows you.

To my parents

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Summary

People working in indoor environments often complain of allergies, illness or discomfort. A variety of the symptoms are associated with micro-organisms occurring in these environments. At least some of these micro-organisms originate in or are distributed through the heating, ventilation and air-conditioning (HVAC) systems used in these indoor environments. A study was conducted over a 12-month period, ranging from February 1996 through January 1997, to investigate the microbial populations in selected occupational environments and related respiratory symptoms suffered by occupants of these indoor environments. Based on the type of air-supply system used in the building, four buildings were selected for sampling during the study period.

An occupational health questionnaire was compiled to cover a variety of respiratory symptoms related to microbial infections, and was completed by 101 occupants of the four sampled buildings. Certain aspects regarding influenza and hay fever symptoms, as well as a health profile for the 12-month period, were emphasised in the questionnaire.

A microbiological study of surrounding air obtained from selected sampling points was conducted to determine the predominance of specific micro-organisms in air-borne infections. The presence of bacteria, moulds and yeasts, as well as species of *Pseudomonas* were determined. Additionally, water samples were collected from one of the buildings that had a water-based air-conditioning system. Water droplets are apparently liberated into the indoor environment by the HVAC system in this building. Micro-organisms could proliferate in the water and affect occupants' health when liberated into the environment. This effect was revealed from the questionnaires.

From the questionnaires it emanated that the building occupants suffered from a variety of respiratory-related symptoms, which were more prevalent in certain buildings. As expected, a high incidence of influenza symptoms were observed in the winter months, including blocked or dry noses and gritty eyes.

The buildings showed a variation in t[...] ganisms found during the sampling period. Yeasts and moulds were present in all the sampling locations throughout the sampling period, while *Pseudomonas* species were not found in any of the buildings. Total bacterial counts were relatively high at times, especially during the summer months.

It was evident that the HVAC systems differ in their effectivity to ensure safe indoor air quality with regards to microbiological pollutants. The comparison of the microbiological data and the health profiles obtained from the questionnaires revealed that micro-organisms found in indoor environments could possibly affect the health of occupants and therefore influence productivity.

Opsomming

Klagtes van allergië, siektes en ongemak, wat met lugversorgingsisteme geassosieer kan word, word gereeld ontvang van werkers in binnenshuise omgewings. Verskeie simptome word geassosieer met mikro-organismes wat in hierdie omgewings voorkom. Dit word aanvaar dat minstens sommige van hierdie organismes deur die lugversorgingsisteme in binnenshuise omgewings versprei word.

Oor 'n periode van 12 maande, vanaf Februarie 1996 tot Januarie 1997, is 'n studie uitgevoer om die mikrobiologiese populasies in beroepsomgewings, asook respiratoriese simptome wat deur die werkers in hierdie omgewings ondervind is en wat moontlik met hierdie mikro-organismes geassosiëer kan word, te bepaal. Vier geboue is op grond van die tipe lugvoosieningsisteme wat daarin gebruik word vir die studie geselekteer.

'n Beroepsgesondheidsvraelys is saamgestel en deur 101 werkers voltooi. Die volgende aspekte is in die vraelys beklemtoon, respiratoriese simptome, insluitende verkoue en griep simptome, asook 'n gesondheidsprofiel vir die bestek van die 12 maande periode.

Lugmonsters is op geselekteerde bemonsteringspunts geneem om die hoeveelhede van spesifieke mikro-organismes vas te stel. Ontledings is gedoen om die teenwoordigheid en van bakterië, giste en swamme, sowel as die genus *Pseudomonas* te bepaal. Watermonsters is addisioneel by een van die geboue versamel. Hierdie gebou maak gebruik van 'n water-gebaseerde verkoelingsisteem, wat tot gevolg het dat waterdruppels in die binnenshuise omgewing van die gebou vrygestel word. Na aanleiding van die resultate wat uit die vraelyste verkry is, blyk dit dat verskeie respiratories- verwante simptome deur die werkers ondervind is. Die voorkoms van hierdie simptome was hoër in sommige geboue as in ander. Soos verwag het verkouesimptome baie gedurende die wintermaande voorgekom. Simptome soos toe-neuse en branderige oë is gereeld deur die werkers aangemeld.

Mikrobiologiese ontleding het getoon .... amme voortdurend in al vier die geboue gedurende die studietydperk voorgek .... *nonas* spesies is in geen van die geboue gevind nie. Die totale tellings was hoog, veral gedurende die somer. Sommige geboue het hoër tellings van mikro-organismes as ander getoon.

Ontleding van die data vanaf lugmonsters dui duidelik aan dat sommige tipes lugvoorsieningsisteme meer doeltreffend lug van goeie mikrobiologiese kwaliteit verseker. Vergelyking van mikrobiologiese data met die gesondheidsprofiel van werkers het aan die lig gebring dat mikro-organismes wel die gesondheid en gevolglik produktiwiteit van werkers kan beïnvloed.

LIST OF ABBREVIATIONS

ACGIH – American Conference of Governmental Industrial Hygienists

ASHRAE – American Society of Heating, Refrigerating and Air-conditioning Engineers

AVG – Average

cfu – colony forming unit

cfu/m^3 - colony forming units per cubic meter of air

HP – Hypersensitivity Pneumonia

HVAC – Heating, ventilation and air-conditioning

NIOSH – National Institute for Occupational Safety and Health

SABS – South African Bureau of Standards

SMACNA – The Sheet Metal and Air-conditioning Contractors' National Association

TWA – Time weighed average

WHO – World Health Organization

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CHAPTER 1

INTRODUCTION

1.1 Introductory remarks

According to the 0400-1990 SABS Appliance Code of the National Building Regulations, Part O (1990), a minimum air supply of 7.5 liter/second (l/s) is required per person where smoking is allowed whereas a minimum of 5.0 l/s is required per person in non-smoking areas or where the air is filtered. Nevertheless, no regulations exist in South Africa with regard to indoor microbiological pollutants. According to the Compensation for Occupational Injuries and Diseases Act (130/1993), disease occurring due to any work involving the handling of or exposure to fungal spores or any other allergenic proteinaceious material or substances emanating from the workplace, should be reported and a claim should be submitted

This study concentrated on micro-organisms associated with the occupational environment. Some micro-organisms in the occupational environment may be harmless to the occupants, while others may be infectious or allergens, or they may even have toxic or irritant components. Willeke and Baron (1993) remind us that to be infectious, an organism must be viable, but to cause allergic or toxic effects, viability is not required. Dead cells may also affect human health.

In this study we investigated the prevalence of pathogenic micro-organisms found in the water and surrounding air associated with air-conditioning systems in indoor occupational environments. In general, the aim of air sampling as performed in this study, is to identify the source of bioaerosol components so that effective corrective action may be taken. It may also be useful for documenting the contribution to the bioaerosol of previously identified sources.

In the Bloemfontein area, where the study was conducted, only a few buildings are still using air-conditioning systems in which the air and water come into direct contact with each other. Most

buildings, especially large office buildings, use closed-circuit systems, where no direct contact occurs between the water and air. This means that the water usually moves in a closed-pipe system, and air is blown over the pipe or coil system. Only one of the buildings included in the study had a system in which water and air come into direct contact with each other. The three closed systems differed in their use of recycled air. These differences ranged from 20% to 100% fresh air. There was also a significant difference in the ages or period of use of the buildings.

Figure 1 (Appendix A) shows the location of the four buildings identified for the study in the city. Two of the buildings, A and B, are government office buildings, while building D is the office building of the local municipality. Building C consists of offices and a large store for medical supplies. These buildings are installed with air-supply and conditioning systems to regulate temperatures and supply air to the indoor environments. Each person working in these buildings spends an average of 43.4 hours per week in his/her work environment. Complaints of illness, allergies or specifically discomfort were reported almost daily by occupants of affected work environments.

The Microbiological monitoring apparatus (SAS Super 90) was used in the study for collecting data on the status of hygiene, or as support for investigations into specific problems in various environments, where biological contamination was suspected. The apparatus operates on the following principle, air is aspirated at a fixed speed for a predetermined time ($\pm$ 4 min.) through a cover with a series of small holes. The resulting laminar airflow is directed onto an agar surface, placed in the apparatus. When the pre-set sampling cycle is completed, the plates are removed aseptically, covered and incubated. The organisms are then visible to the naked eye and can be counted to assess the level of contamination. The objective of this study was to determine the presence and quantity of microbial populations associated with the water in air-conditioning systems and the surrounding air in an occupational environment.

1.2 Review of literature

Biological air contaminants in indoor environments

Willeke and Baron (1993) stated that bioaerosols found in indoor and outdoor environments consist of particles with different biological origins, such as pollen, fungal spores or fragments of fungal mycelium, bacterial cells, viruses, protozoa, algae, excreta or fragments of insects, skin scales or hair of mammals, or their components, and residues of organisms. Many of these bioaerosol particles, such as fungal spores and pollen, are designed by nature for transmission to other areas and to stay viable during transmission. These hardy bioaerosols are resistant to environmental stresses like dryness, cold, ultraviolet light and toxic gases, as well as to sampling stresses (Willeke and Baron, 1993). These organisms can probably stay viable while airborne, because they adapt to the dry conditions of their environment and are protected by their original substrate. Unprotected, most vegetative cells or bacteria will be damaged as a result of becoming airborne. Microbial cells in an aerosol may be viable or non-viable. Only viable aerosol cells will be able to reproduce and colonise on a growth medium. According to Willeke and Baron (1993) and Burge (1990), it is typically possible to culture less than 1% of the microbes present in a natural soil or water sample. This may also be true for airborne microbes. Bioaerosols that are not whole cells, such as endotoxins, mycotoxins or various allergens, may also be present. Therefore, depending on the detection method, the results are commonly expressed as colony forming units (cfu) (Vincent, 1995).

Bacteria and fungal spores may be expressed as the number of bacterial entities of a given type, per unit volume of air. Viable particles may be expressed in terms of their ability to reproduce the number of colony forming units per volume of air (Vincent, 1995). Although most bioaerosols are harmless elements of normal environments, some bioaerosols may be infectious agents or allergens, or they may carry toxic components or irritants. Dead cells as well as cell residues may affect human health (Burge, 1990).

Biological contaminants of indoor environments and dust may result in allergic or pathogenic reactions, (SMACNA, 1993; Burge, 1990). Sources of these pollutants predominantly include pollens from outdoors, viruses and bacteria from humans, hair and skin flakes, and dust. According to Willeke and Baron (1993), pollen is usually produced in large amounts to ensure its successful transmission in nature. As airborne pollen types are resistant to environmental stresses such as desiccation, temperature and light, they tend to resist sampling stress. Pollen varies in size [approximately 10-100 micrometers (μm)] and shape, and many kinds of pollen contain important allergens that can cause hay fever. Godish (1989) reported that moulds are also a significant cause of common allergies and asthma in susceptible hosts. Because air currents typically disperse these mould spores, air contamination is common and the probability of inhalation is high.

As a vector for contamination, water, or its availability in any environment, is important as it acts as a reservoir and breeding-ground for biological contaminants. Moulds and bacteria easily contaminate and colonise moist or wet areas. An indoor moisture level of 30-50% relative humidity is recommended to maintain good health and comfort. A wide range of diseases (infectious and non-infectious) may be caused by biological contaminants. Pathogenic bacteria or viruses may also occur in air, although their presence can be difficult to verify. Indoor air quality can be measured by the concentration of normal human skin bacteria in air samples (Willeke and Baron, 1993). According to Walter (1969), airborne transmission of disease occurs in three basic ways, namely: 1) dust particles attached to bacteria, 2) droplets transporting bacteria, and 3) droplet nuclei harbouring bacteria. Indoor air particles can come from outdoor or indoor sources (Walter, 1969). Airborne particulate is categorised by the SMACNA (1993) according to size, and it ranges from 0.01-100 μm. Respirable particulate (10 μm and smaller) can penetrate into the lungs, whereas particles greater than 10 μm is classified as non-respirable (SMACNA, 1993; Schroder and Schoeman, 1989; Olishifski, 1981). According to Willeke and Baron (1993), bioaerosol particles cover a wide size range, with viruses the smallest potentially living particles (0.02-0.3 μm in length). Bacteria and

fungal spores range in size from about 0.5-100 µm, and pollen, algae and protozoa particles are only tens to hundreds of micrometers in diameter. When microbial cells or spores form part of a particle combined with other elements like a dust or pollen particle, their migration and deposition depend on the size of the whole unit.

According to MacFarlane *et al.* (1993), warm temperatures, contaminants and particulate matter in the water, as well as stagnant or slow flowing water, all favour the multiplication of organisms in water-based air-conditioning systems to alarmingly high numbers. Benenson (1990) stated that hot water systems, air-conditioning cooling towers and evaporative condensers, as well as taps, showers and ponds have been implicated in outbreaks of Legionnaire's disease. A common site for the causative organism of this disease is water associated with buildings, like water from air-conditioners (Nester *et al.*, 1983). *Legionella pneumophila* has been isolated from water distribution systems and air-conditioning cooling towers linked to outbreaks of pneumonia (Schaechter *et al.*, 1989). Kurtz (1985) reported that *L. pneumophila* can only be contracted from environmental sources and is not spread from person to person. *L. pneumophila* is relatively resistant to chlorine and physical parameters like heat. It has an optimum growth temperature of 30-40°C and fresh water is essential for the existence of these organisms (Parkes, 1994; Kurtz, 1985). However, Vincent (1995) stated that after effective chlorinating of the cooling tower water, the frequency of legionellosis declined. According to Tortora *et al.* (1993) and Wilson (1991), risk factors that may increase the probability of *Legionella* infection are cigarette smoke, underlying lung disease, advanced age, alcohol consumption, recent surgery or chronic illness. These factors may also increase the influence of other air-borne micro-organisms on the health of humans.

According to Brooks and Davis (1992) and Beachler *et al.* (1991), the quality of indoor air in buildings depends on a complex interaction between the sources of indoor pollutants. Factors like temperature and humidity in the building, and the removal of pollutants from outside air, may influence indoor air quality. Ventilation and air-conditioning systems may thus provide surface areas for the growth of micro-organisms.

Microbial populations associated with airborne infections

■ *Pseudomonas. spp. as infections organisms*

Members of the genus *Pseudomonas* are Gram-negative, obligate aerobic bacilli present in soil, fresh water and marine environments, with optimal growth occurring at approximately 37°C (Holt, 1984). Baron *et al.* (1994) stated that for any organism to cause respiratory-related diseases in humans, the organism must first gain a foothold within the respiratory tract in order to multiply to sufficient numbers to produce symptoms. *Pseudomonas aeruginosa* is pathogenic, causing serious lung infections and bacterial meningitis when inhaled by humans (McKane and Kandel, 1996; Wittenberg, 1987; Tietd, 1979). According to Evans and Brachman (1991), respiratory infection in the form of pneumonia caused by *P. aeruginosa* is the disease most frequently reported by the WHO. The presence of normal flora and the overall state of the host affect the ability of these organisms to cause disease (Baron *et al.*, 1994).

According to Meyer (1983), air-conditioners, filters and humidifiers can be a breeding-ground for *P. aeruginosa.* Micro-organisms can spread in a variety of ways, including wet aerosols or dry particles. According to a study by Marthi *et al.* (1990), the survival of aerosolised *Pseudomonas spp.* is dependent on a number of factors, including droplet size, temperature and relative humidity.

Malangoni *et al.* (1994) emphasised the obvious fact that pneumonia caused by *P. aeruginosa* is of great concern to the medical profession. *P. aeruginosa* has been associated with a high likelihood of treatment failure and the development of resistance to antimicrobial agents. Therefore, pneumonia caused by this opportunistic pathogen needs to be identified early for treatment to be effective. Furthermore, a high mortality rate is associated with pneumonia caused by *P. aeruginosa* (Malangani *et al.,* 1994; MacFarlane *et al.*, 1993). According to Benenson (1990), increased use of antimicrobial agents and immunosuppressive therapy are as a result of an increase in the incidence of pneumonia due to endemic Gram-negative bacilli, especially species like *P. aeruginosa*. The aforementioned organism is responsible for one of the widest ranges of infections of all pathogenic

micro-organisms (McKane and Kandel, 1994). Bacterial meningitis caused by *P. aeruginosa* has also become a more common occurrence in recent years (Baron *et al.*, 1994).

■ *Fungi as airborne organisms causing infections*

Fungal spores are airborne particles that occur in great abundance in the atmosphere. Spores less than 10 μm in size can penetrate deep into the lungs (Stephen and Pietrowski, 1986). According to Baron *et al.* (1994), about 50 to 75 species of fungi are recognised as human pathogens. Walsh *et al.* (1990) found that various fungal spores have been implicated in outbreaks of building-related diseases. As stated by the ACGIH (1989), most spores produced by fungi are designed to be transported through the air. Environmental factors that influence the distribution of fungi include the availability of water and nutrients, temperature and light. Although temperature and light affect the growth of fungi, such growth is rarely limited by these factors (ACGIH, 1989). Fungi can, furthermore, utilise almost any carbon-containing material as a nutrient, but water is not always readily available. Thus, the presence of water in the indoor environment is an important cause of fungal contamination. Since fungi are disseminated primarily through the air and over long distances, there are few places on earth that are completely fungus free. Even dry environments are never sterile unless special precautions have been taken. According to Ahearn and Crow (1994), cellulose and fibreglass air filters, particularly when moist, are subjected to colonisation by *Aspergillus* and other fungi.

Fungal spores are usually the primary dissemination for the organism and are well adapted to airborne transport. The size of fungal spores is in the range of 0.5-30 μm or sometimes even larger, allowing transport over long distances by wind. In addition fungal spores are often resistant to various environmental stresses like desiccation, cold, heat and ultraviolet radiation (Willeke and Baron, 1993). Moisture levels required for fungal growth are often low and, therefore, if the relative humidity exceeds 70%, sufficient moisture may even be absorbed from the air by some organic materials. Ingold and Hudson (1993) and Godish (1989) reported that moisture in buildings is necessary for the prevalence of moulds. Therefore, the growth of moulds is more likely where water

is readily available. Most fungal aerosols can cause allergic reactions and diseases such as asthma, allergic irritation or hypersensitivity pneumonitis. A few fungi, including the genera *Cladosporium* and *Alternaria*, and spores including basidiospores and ascospores, dominate outdoor aerosols world-wide, although some geographical variation exists. In areas with great seasonal variation, the levels of fungal spores are highest in summer and autumn and lowest in winter (Ingold and Hudson, 1993).

Studies of moulds in indoor air indicate strong seasonal variation in levels (Godish, 1989). Studies also show that in office and residential environments, the outdoor air is an important source of fungal spores. The detection of airborne spores resulting from growth occurring on indoor substrates can be difficult in the presence of normal background levels of outdoor bioaerosols (Willeke and Baron, 1993). According to Eickhoff (1994), a substantial number of fungi are capable of spreading via airborne routes. Notwithstanding, *Aspergillus* and *Zygomyces* are the only fungal genera that have been implicated as major airborne hazards. It should be noted that documented outbreaks of airborne fungal infections have occurred mainly in hospitals. Benenson (1990) and Mullins *et al.* (1976) indicated that the most common infectious agents of aspergillosis are *Aspergillus fumigatus*, *A. niger* and *A. flavus*. The mode of transmission is through inhalation of airborne spores whereas no person-to-person transmission has been reported and no feasible preventative measures documented. According to Ingold and Hudson (1993), the ability of many fungi to grow at human body temperatures add to their potential to become pathogenic. Furthermore, a stressed or in some way predisposed person becomes an even more likely host. According to Ahearn and Crow (1994), mycotoxins produced by fungi may induce dermatitis, respiratory irritation and distress, cardiovascular effects including lowered blood pressure, and immunosupressive effects. Von Eiff *et al.* (1994) named the following features as suggestive of underlying fungal disease: 1) fever unresponsive to broad-spectrum antibiotics, 2) underlying haematological malignancy, 3) two or more febrile episodes, and 4) radiologically diffuse bilateral pulmonary infiltrates.

Detection and isolation of several fungal species from household materials have been shown to produce trichothecene mycotoxins, and this was an important step in the characterisation of airborne toxicosis. However, Smoragiewicz *et al.* (1993) stated that no direct evidence was found to link the pathological symptoms of the occupants' illness with airborne mycotoxicosis. Only fungi that are able to grow at body temperature can invade living cells and cause infection in humans. These infections usually occur in people with some immune system dysfunction, in other words, fungi are usually opportunistic. Most fungi produce proteins that are highly antigenic and can cause hypersensitivity diseases in susceptible hosts (Smoragiewicz *et al.*, 1993). Fungi also produce secondary metabolites that are toxic to humans (ACGIH, 1989). According to Von Eiff *et al.* (1994), mixed fungal infections and mixed fungal-bacteria-viral infections are associated with a higher mortality rate than infections caused by a single organism.

Health problems related to buildings

It has long been believed that an indoor environment provides a refuge from air pollution (Burge, 1990). It seems, however, that indoor environments can provide occupants with a unique exposure to pollutants. Building-related diseases are a complex, chronic set of health problems, possibly related to air-conditioning systems in occupational environments (Burge, 1990). Air contaminants, like aero-allergens, organic and inorganic dust, fibres, tobacco smoke, gases and microbes are all suspected of causing these health problems (Smoragiewicz *et al.*, 1993). According to Truter *et al.* (1994), there is little information available on the prevalence of this phenomenon amongst the general population of South Africa. The non-specific nature of these symptoms and difficulty in quantifying the responses of occupants, also contribute to clearly defining the problem (Burge, 1990).

The WHO describes building-related sickness as general, non-specific symptoms of malaise, in particular irritation of the eyes, nose and throat, lethargy, headaches, nausea and dizziness (Truter *et al.*, 1991; Wilson, 1987). Furthermore, the disease and symptoms associated with air-

conditioning systems fall into one of t.... ...., physical, allergic (asthma, humidifier fever, and extrinsic allergic alveolitis) and infections (bacterial, fungal and viral). Based on several investigations, NIOSH categorised the causes of complaints as follows: inadequate ventilation 52%, inside contamination 17%, outside contamination 11%, microbial contamination 5%, building fabric contamination 3%, and unknown causes 12% (Truter *et al.*, 1991).

The SMACNA (1993) as well as Nagda and Harper (1989) and Morey *et al*. (1990), reported that occupants of office buildings and other structures can experience symptoms of ill health as a result of exposure to certain irritants. Complaints from occupants can include itching, burning or watery eyes, dry nose and throat, sore throat, sneezing, coughing, and tightness of the chest. Substantial variability occur in people's sensitivity to irritants, and variations over time also occur with regard to the same individuals (SMACNA, 1993). Humidity is not usually regarded as a contaminant, but a low relative humidity can enhance the effects of irritants. Environmental cigarette smoke can also be an irritant to occupants. Allergic reactions, which manifest as a cough, watery eyes and breathing problems, can be caused by exposure to these pollutants. In addition, bronchitis and other long-term illnesses can be related to biological contaminants.

According to Hirai (1991), airborne transmission of opportunistic pathogens is the most difficult to control. The viability of micro-organisms under dry conditions is a major factor determining their ability to be transported through air. Mullins *et al.* (1976) reported in their study that the majority of potentially allergic subjects are exposed only to low levels of *Aspergillus fumigatus* in the air, unless they are close to decaying organic matter like compost heaps. However, this organism is small and can penetrate the bronchi, while its optimum temperature requirement gives it an advantage over other moulds in allowing it to germinate at body temperature.

Hosein *et al*. (1989) stated that problems contributing to inadequate air supply in poorly ventilated areas, include smoking by workers which can be an irritant that may affect co-workers with respiratory problems. Workers may also experience respiratory problems caused by odours and
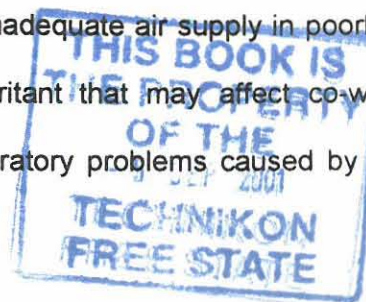
vapours from glues and artificial fibres used in the decor and construction of buildings. High-efficiency air cleaners and carbon filters may be used if properly maintained. Increased ventilation is effective in diluting smoke in general spaces. Hosein *et al.* (1989) found that passive smoking has generally been associated with increased respiratory symptoms, infections, illnesses and chronic disease.

According to Morey *et al.* (1990), Gram-negative bacteria have been associated with two types of respiratory disease and symptoms where exposure involved HVAC systems. The first is respiratory infections such as pneumonia, where the bacteria enter the respiratory tract, grow, colonise and establish an infection. The second respiratory symptom involves an allergic type or immune reaction. Furthermore, many Gram-negative bacteria are able to survive and grow well in aquatic environments, which makes them ideal inhabitants of HVAC systems where water accumulates and/or is aerosolised. Burge (1990) stated that certain bacteria and most fungi produce spores that can persist in indoor environments for long periods of time. These spores penetrate indoor environments freely through open windows, air intakes and doors and are carried in by the occupants.

Stagnant water is always a good reservoir for microbial growth and a potential source of microbial contamination (Willeke and Baron, 1993). In addition to water, micro-organisms only need minute amounts of nutrients, which may be available in the water present in the building or building materials like cellulose, wood or concrete. Thus, spores or other bioaerosols may develop wherever water is leaking or condensing inside a building. According to Brooks and Davis (1992), many pollutants found in indoor air were similar to pollutants found in outdoor air, and in some cases these pollutants actually come from outdoor sources. On the other hand, those pollutants measured in the highest concentrations indoors are those that arise from the structure itself, furnishings or the activities inside the structure. Godish (1989) reported that for allergens to be of much importance and/or harm in inducing sensitivity, they must be abundant in the air for fairly long periods of time.

According to SMACNA (1993), the tota[l] ...les of all sizes may vary from 300 to 1000 micro-organisms per cubic meter ($\mu m/m^3$) over a 24-hour period. However, particulate readings at any given time can be as much as 600 micro-organisms per cubic meter. The indoor/outdoor particulate ratio varies from 0.3:1 to 0.4:1. The following standards for indoor particles have been established:

♦ 10 $\mu m/m^3$ [or nuisance dust, 8-hour time weigh average (TWA)] according to the ACGIH;

♦ According to the ASHRAE 260 micrograms per cubic meter ($\mu g/m^3$) (24-hour continuous exposure).

The manufacturer of the microbiological air sampler used in this study suggests evaluation of microbial growth found on agar surfaces as indicated in the following table:

Table 1 Classification of microbial growth from air samples

Colony count	Description of growth
1 - 29	Very slight growth
30 – 60	Slight growth
61 – 300	Moderate growth
>301	Heavy growth
confluented	Very heavy growth.

Walsh *et al.* (1990) reasoned that it is important to know when health problems in the building started among the occupants, to investigate whether changes in the work processes, office machines, or maintenance to the ventilation system, preceded the complaints. Furthermore, some symptoms that are building-related have an onset when an employee enters the work environment

and are alleviated when the employee leaves the work environment. Symptoms like humidifier fever may occur after work, several hours after the employees have been exposed to building-associated antigens. An onset of symptoms in new employees may provide clues to the incubation period of the disease caused by these antigens.

In non-industrial indoor environments, humans are the most important source of airborne bacteria. Air with a high concentration of human bacteria is not necessarily a health hazard, but indicates bacterial activity because of insufficient ventilation (Willeke and Baron, 1993). According to Meyer (1983), several human activities produce biologically active dust, the spread of which may cause them to accumulate and cause health problems in humans. The variations of symptoms during the day, work-week and year are also important (Walsh *et al.*, 1990). According to Hering (1989), outbreaks of respiratory complaints amongst the occupants of buildings with sealed windows and a high percentage of recycled air have become fairly common, since economical factors have made energy conservation measures necessary. An unknown proportion of the cases is as a result of to exposure to micro-organisms and not chemicals or non-biological particulate matter. Individuals or groups of workers have suffered from allergic diseases and hypersensitivity pneumonitis after repeated exposure to aeroallergens (Hering, 1989).

Overpopulation of offices may also cause health problems. According to Hosein *et al.* (1989), domestic crowding appears to be associated with increased reporting of respiratory symptoms. When symptoms like eye, nose, and lip dryness and irritation, itching, headaches and fatigue occur in a group of people sharing the same indoor environment, and appear to be aggravated by spending time in the building, a health problem associated with the building may be diagnosed. Although the exact pollutants causing a problem may not have been identified, measures taken to remove them from the air or from the mucous membranes could reduce symptom intensity (Brune and Edling, 1989).

Robertson (1993) stated that the symptoms of building related illness usually disappear when the inflicted workers leave the building. This is in contrast to the classical symptoms of Legionnaire's disease caused by *Legionella pneumophila*. The symptoms associated with Legionnaire's disease become more severe with time, even sometimes leading to death. According to Kusneitsov *et al.* (1993), Maraca *et al.* (1988) Tobin *et al.* (1986), Witherell *et al.* (1986), and Kurtz (1985), a major manifestation of Legionnaire's disease is pneumonia. Pontiac fever is a mild, influenza-like illness, also caused by *L. pneumophila*. The reasons why some people develop legionellosis and others Pontiac fever, both following exposure to the same source, are not known. Pontiac fever occurs as an allergic reaction and heals spontaneously (Parkes, 1994; Wilson, 1991).

Truter *et al.* (1991) stated that the investigation of complaints can be very frustrating for the occupants of the work environment involved. These problems can be complicated due to charged emotions, complexity of the buildings themselves and the fact that standard epidemiological and occupational hygiene evaluation techniques may be inconclusive. The NIOSH developed an approach based on the elimination of potential problems. First a background assessment of the building is done, followed by an initial site visit, and then a follow-up site visit is done, if necessary. According to Nagda and Harper (1989), reduction of the ventilation rate increased the symptoms of the occupants. Awareness of the change in ventilation intensified the effect, but did not eliminate the change in the symptoms.

According to the ACGIH (1989), airborne bacteria cause infectious diseases by entering the respiratory system of humans. Legionnaire's disease, pneumonia and tuberculosis are some common airborne infections known to man. Bacterial cells and their products can also cause humidifier fever and pneumonitis. The quality of the indoor air in a large commercial building is dependent on the operation and maintenance of its HVAC system. Most of these systems mix outdoor air with recycled air, heat or cool it and distribute it to the occupied space through a filter system. Inadequate amounts of fresh outdoor air often lead to building-related symptoms and complaints. Outdoor air intakes, when present, should always be open, at least at the minimum

fresh air setting. According to Stewart et al. (1995), airborne micro-organisms in indoor and outdoor environments, from either natural or industrial sources, may produce symptoms in humans, ranging from mild irritation to more servere diseases. To ensure the health of workers, it is important to evaluate the composition and concentration of airborne micro-organisms in contaminated environments. In a study by Ganier et al. (1980), more than 50% of the employees of a large factory suffered from hypersensitivity pneumonitis. The symptoms would occur on a Monday night, for example, the first working day after the weekend. Thereafter, no one would suffer any symptoms during the rest of the week, until the next Monday after work.

Hering (1989) suggested that allergy may be defined broadly as acquired hyperreactivity to a specific substance like pollen or dust, or it could be a physical factor like heat or cold, which on similar exposure is harmless to most people. According to the ACGIH (1989), hypersensitivity diseases result from exposure to materials in the environment that stimulates an immunologic response. Hypersensitivity pneumonitis (allergic alveolitis) is characterised by acute, recurrent pneumonia with fever, cough, chest tightness and lung infiltrates, or even by a progression of cough, shortness of breath, fatigue, and chronic lung fibrosis, or by an intermediate pattern of acute and chronic lung disease. Difficulty in isolating the antigens responsible for hypersensitivity pneumonitis limits its use in situations in which challenge with the untreated water would be unethical (Walsh et al., 1990). According to Garrison et al. (1993), Brooks and Davis (1992) and Ganier et al. (1980), hypersensitivity pneumonitis is one of the best-known examples of a building-related illness. The cause of hypersensitivity pneumonitis has been identified as moulds, bacteria, organic dusts, organic chemicals, metallic fumes and dust, animal dander and aerosolised proteins.

A second building-related illness is humidifier fever. This is a type of hypersensitivity pneumonitis characterised by an acute febrile attack accompanied by malaise, cough and dyspnea (the chronic form is humidifier lung of which the attack rate can be influenced by genetics). Fungi, bacteria, protozoa, microbial endotoxins and arthropods are believed to cause humidifier fever. The

investigators in the study of Ganier e. a.. (1980) observed protozoa as the possible responsible agent in humidifier fever.

Hypersensitivity pneumonitis, according to Burge (1990), is a serious disease that can lead to permanent lung malfunction. Through case studies, it has been associated with contaminated water from ventilation systems or humidifiers. According to Godish (1989), common sources of microbial contamination of buildings that lead to outbreaks of hypersensitivity pneumonitis, include microbial slime developing on cooling-coil condensate drip pans, spray air washers associated with some HVAC systems and water incursions.

Indoor airborne micro-organisms

According to Truter *et al*. (1991), little research has been done on bacteriological sampling associated with building-related illness. A standard method to quantify microbes in indoor air is not available. Micro-organisms are usually quantified in the water from cooling towers and not in the air breathed by the occupants of the buildings. Hering (1989) reported that considerable emphasis has been placed on monitoring gaseous and particulate chemical contaminants. The presence of micro-organisms, pollens and biologically active fragments in the air may also be a cause for concern. There has been even more concern about air quality in indoor environments, including hospitals, drug production areas and also classrooms, offices and other workplaces where infections and allergies are increasingly being recognised as causes for absences and lowered productivity. According to Brooks and Davis (1992) indoor concentrations of outdoor contaminants are determined by outdoor air contaminant concentrations, the rate of outdoor air infiltration through the exterior, and the efficiency of the mechanical ventilation system.

Hering (1989) as well as Hirsch *et al*. (1978) reported that the indoor environment usually contains a smaller variety of micro-organisms than found outdoors, but conditions for the survival of airborne microbes are more favourable indoors. A greater number of airborne flora from human activities is found indoors than outdoors, and indoor samples are generally collected from still or low-velocity air

masses. Outdoor aerosols are also generated by a wide variety of sources. Willeke and Baron (1993) as well as Brook and Davis (1992) stated that many indoor bioaerosols originate outdoors. The surfaces of living and dead plants are probably the most important sources of airborne fungal spores and bacteria. All natural water masses contain large amounts of micro-organisms. Therefore, water or liquid droplets resulting from rain, splashes or bubbling processes may contain bioaerosols that may remain airborne after the water have evaporated. Strong sources of bioaerosols may exist in many work environments when organic material is handled. In non-industrial situations, bioaerosol sources may develop due to microbial growth in a building's HVAC systems or in the structure itself.

According to Smoragiewicz et al. (1993), many of the health problems experienced by building occupants may be ascribed to mycotoxins, but they stressed that the sick building syndrome is a multifactorial phenomenon, possibly including airborne toxicosis. The combination of aero-allergic reactions related to the presence of foreign allergenic proteins in response to the characteristics and contamination with other organic material or chemicals, could produce the complex reaction of the sick building syndrome. Screening of dust from ventilation systems for the presence of trichothecene mycotoxins, in addition to other parameters such as relative humidity and temperature, can be considered as a general test for contamination with other microbes, such as yeasts and bacteria. An increase in relative humidity, including water infiltration and condensation, can produce optimal conditions for the growth of micro-organisms. According to Smoragiewicz et al. (1993), this indicates the need for new legislation to establish quality control and norms for biological contamination of air. He also stated that involvement of a microbial factor, such as mycotoxins, in airborne toxicosis, could not be established beyond a doubt without detailed dose-effect analysis using relevant air-detectable concentrations of fungi.

Aeroallergens, according to Hering (1989), exert their effects as a result of airborne dispersion and, therefore, primarily affect the respiratory system. The respiratory reactions to aeroallergens range from rhinitis to bronchial asthma and extrinsic allergic alveolitis. Pollens are well-known

aeroallergens, which are relatively easy to collect and identify. As these pollens are produced frequently during various seasons, the symptom/exposure relationship is quite apparent. Pollen induces hay fever and asthma in sensitive people. Fungus spores are just as abundant in outdoor air, and contaminate indoor air as well. Nevertheless, as far as fungal aeroallergens are concerned, collection and identification are more difficult and seasonal association is less apparent. In sensitive persons, fungal spores cause symptoms similar to those caused by pollen. Hypersensitivity pneumonitis may also occur following inhalation of very small particles into the lower airways. Furthermore, some bacteria can also induce hypersensitive pneumonitis. Other micro-organisms like slime moulds, protozoas and algae can induce an allergic response. In addition to pollen and micro-organisms, a range of other substances can become airborne, especially in indoor environments, and they can be the cause of allergic reactions. According to Hering (1989), aeroallergens can vary in size from well below 1 µm to in excess of 100 µm. Most wind-borne pollen types range from 15-30 µm, while fungal spores are often between 2 µm and 30 µm. However, some airborne antigens such as those washed from microbial growth in humidifying systems, can be of molecular size (Hering, 1989).

According to Vincent (1995), aerosol measurement in the workplace is carried out for various reasons, which include 1) the assessment of the workplace atmosphere in order to compare concentrations of aerosols to exposure limits that will insure occupants' health and the provision of, 2) to provide valid measurements of the occupants' exposure for epidemiological purposes. According to Willeke and Baron (1993), the objective of bioaerosol sampling is most often to verify and quantify the presence of bioaerosols for exposure assessment, or to identify their source for control. Dose/response relationships are poorly known. Consequently exposure guidelines for acceptable healthy levels of any bioaerosol have not been established.

In order to obtain convincing proof of microbial contribution to hypersensitivity pneumonitis and humidifier fever, culturing of air and water samples from suspected sources like a humidifier, air-cooling system, vacuum pump or filters is required. In most buildings in which hypersensitivity

disease has occurred, trials of antimicrobial agents added to the implicated water source have been ineffective and removal or modification of the contaminated appliance has been required. Furthermore, clean up of the contaminated buildings has been ineffective in preventing recurrent illness in some instances (Walsh *et al.*, 1990). According to Ganier *et al.* (1980), the majority of people suffering from acute hypersensitivity pneumonitis present with sudden chills, fever, cough, dyspnea without wheezing and myalgia, occurring four to six hours after exposure to the offending antigens.

Complaints, according to Truter *et al.* (1991), usually stem from the basis of an investigation into poor health associated with buildings. Normally complaints from occupants are investigated by measuring the prevailing office temperature and humidity. The airflow may also be measured, using a swing hygrometer. These techniques have often failed to reduce the complaints, and building management need to consider different approaches. According to Brooks and Davis (1992), biological sampling should only be performed when the occupants' symptoms indicate a possible relation with biological agents.

In the absence of guidelines, it has become necessary to decide in advance on the criteria to be used in order to determine whether or not an environment is contaminated. Usually it can be determined if an unusual exposure situation exists in the complaint environment as compared to a symptom-free environment. This means that sampling should take place in both the complaint environment and the control environment. The following example by the ACGIH (1989) illustrates that the demonstration of 1000 *Penicillium* spores/m^3 of air in a complaint environment with no *Penicillium* outdoors or in the noncomplaint environment, only constitutes an observation of contamination and is not proof of adverse health effects. *Penicillium* is a common fungus and often reaches these levels in noncomplaint environments. However, when the exposed people are experiencing symptoms of allergic asthma and have positive skin test to the isolated *Penicillium* species, one can conclude with some degree of certainty that the bioaerosol exposure is contributing to the symptoms (ACGIH, 1989).

According to Burge (1990), an inspection like this must focus on environmental determinants for bioaerosols, like the outdoor aerosols, ventilation mode and rate, and the intrusion or accumulation of moisture indoors. Brooks and Davis (1992) described two types of evaluating criteria. The first indicates the indoor-outdoor air ratio. The ratio should be less than 3:1 to prove that the probable microbial sources are affecting the occupants. The other guideline for evaluating biological samples is that if the total average number of colony forming units for fungi, bacteria and thermophilic actinomycetes exceeds 10 000, remedial action should be taken to reduce the concentration of biological contaminants.

According to Hering (1989), depending on local conditions, the concentration of viable particles in the air can range from a few to many thousands or even millions per cubic meter, most of which are non-pathogenic. During a 24-hour day, a person inhales about $20m^3$ of air and with it whatever micro-organism is present. At the concentrations usually found in indoor and outdoor environments, non-pathogenic microbes are seldom of concern for healthy people. However, according to Hering (1989), a high concentration of some species can overwhelm the body's defence mechanisms. Repeated exposure to viable or dead organisms may also cause the development of hypersensitivity reactions. In general, it should be assumed that pathogenic micro-organisms are potentially harmful where and when they are present. When compared to the amount of other particulate matter needed to produce a harmful effect, the volume of material in an infectious dose of a pathogenic micro-organism can be very small. Mullins *et al*. (1976) stated that in the majority of surveys, either no seasonal spread of incidence was found or it was described as low, sporadic or relatively constant throughout the year. When seasonal variations were observed, the periods of maximum incidence included all the seasons, depending on geographical location and the climate of the sampling location.

The SMACNA (1993) stated that temperature and humidity warrant checking at various times and places throughout the day, and airflow at air outlets and return air-grilles should be checked as well.

Measurements for airflow are intended to ensure that all outlets are functioning and to determine if the airflow is properly directed. Stewart *et al.* (1995) as well as Boleij *et al.* (1995) reported that a wide range of samplers are available for bioaerosol monitoring. Filter samplers, agar collectors, particle sizing samplers and liquid collectors are some of the measurement instruments available for microbiological monitoring. According to Willeke and Baron (1993), no single sampling method can collect, identify and quantify all of the bioaerosol components present in any environment. In industrial exposure situations, the type and location of sources are frequently evident. In non-industrial environments, the sources are often less obvious and difficult to sample.

Methods of microbial sampling

According to the ACGIH (1989), the primary objective of air sampling is to identify the source of bioaerosol components so that effective corrective action may be taken. Air sampling may also be useful for documenting the contribution to the bioaerosol of previously identified sources and should only be used as a last resort.

According to the ACGIH (1989), no single sampling method allows recovery of all potential harmful bioaerosols. Sampling equipment that is most effective for collection of the bioaerosol of interest must be chosen. If infectious agents are suspected, or if they are of concern, sampling equipment must protect the viability of the sample and allow subsequent growth for identification. However, viable particle sampling will usually underestimate allergen loads and should be done in association with particle sampling when hypersensitivity disease is suspected. According to Hering (1989) the sampling and culturing of cfu's are the simplest method of detecting the number of particles carrying micro-organisms. These samplers usually function through impaction or settling of particles directly onto a solid nutrient medium on which the micro-organisms grow and form colonies. When airborne micro-organism concentrations are measured to calculate respiratory ineffective dosage, it is desirable to determine the total number of viable organisms in a volume of air.
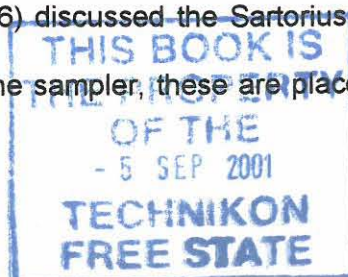
Stewart *et al.* (1995) stated that ideally, the collected micro-organisms should be undamaged and the number of collected organisms should be representative of the airborne concentration. Boleij *et al.* (1995) also found that there are some limitations to bioaerosol monitoring methods, such as the selection of certain species, due to the culture media, resulting in over- and underestimation of these species or conditions like temperature and humidity. It was found that low reproducibility was often caused by the too short sampling times. Only viable micro-organisms and spores can be measured. Gravity plates (settle culture plates) as used by Mullins *et al.* (1976) exposed for periods of 10 minutes, not only present the problem of only viable organisms being counted, but also mean that only organisms big enough to settle will be found on the plate.

According to the ACGIH (1989), the on-site investigation begins outdoors. Fungus spores usually dominate the outdoor air, although pollen, bacteria, algae and insect fragments also are present. Disturbance of natural environments, where fungi and bacteria live, causes levels of these particles to increase dramatically. When investigating any indoor problem, it is essential to examine the adjacent outdoor environment carefully for potential sources and to sample from these sources as controls for all indoor samples. Hering (1989) stated that an investigator must consider the proposed locations of samplers, the number of samples that will be collected, sampling time, the effect of temperature variations, the techniques and logistics of the analysis system to be used, and the required quantities and identification of the isolated organisms. According to Morey *et al.* (1990), after a walk-through inspection to identify possible reservoirs of microbial aerosols, air sampling is recommended if all other factors have been eliminated.

Bacteriological sampling can be done by sampling the cooling tower water (Truter *et al.*, 1991). Water is sampled and tested for the total cfu's. As a rule, counts should be less than 1000 cfu obtained from one cooling tower. Given suitable conditions, viable bacteria and fungi multiply on an agar surface into colonies, and each colony consists of millions of cells. Each particle that contains at least one viable bacterial or fungal cell produces one colony and is counted as a colony forming unit (Hering, 1989).

Brooks and Davis (1992) suggested that whenever bioaerosols are being sampled, duplicate samples should be collected. Counts of the samples should be compared to determine the variability. Where occupants of an entire building show symptoms that warrant biological monitoring, samples should be collected immediately upstream of the outdoor air intake. The supply and return air may be sampled to identify the location of the source. The supply and return air ducts can be sampled for both affected and non-affected areas to determine if a difference exists. Normally two sets of samples should be collected if possible: one when the occupants of the environment are present and the other when there is no activity in the room. This will prove if the activities of the occupants disturb the biological components. According to Brooks and Davis (1992), bulk samples may also be collected from water-damaged furnishings, water reservoirs from humidifiers, cooling-coil condensate pans, and similar sources.

The next step in the on-site investigation is to examine the immediate environment for potential sources that could allow the drawing of excessive contaminated air into the indoor environment. Important data associated with the building itself should also be obtained. When considering biological sampling, a walk-through has to be performed to identify the areas that could be subject to microbial growth. There are no direct-reading instruments that indicate the presence of viable organisms, and samples often require fairly elaborate processing after collection. Aseptic handling of equipment and samples during collection and analysis is essential and requires training in sterile techniques (Hering, 1989). Detection of viable micro-organisms usually requires that the collected cells be allowed to multiply to readily observable numbers. Particles can be impacted directly onto semi-solid nutrient agar and then placed in an incubator until growth appears on the surface. Particles can also be collected on a filter or in a liquid and then transferred to nutrient agar in Petri dishes for growth and possible isolation of bacteria and fungi, or be transferred to a cell culture for isolation of viruses (Hering, 1989). Parks *et al.* (1996) discussed the Sartorius MD8 air sampler using gelatine filters. After removing the filters from the sampler, these are placed onto a nutrient

agar plate where they merge and dissolve. Heavily loaded filters were dissolved in a solution of phosphate buffer, manucol and antifoam.

Collected micro-organisms should be undamaged and the number should be representative of the airborne concentration. Microbial stress occurs as a result of aerosolisation and collection. During aerosolisation, microbial stress was found the greatest at low relative humidities and small droplet size (Stewart *et al.*, 1995). According to the SMACNA (1993), particulate samples can be collected on filters or impactors and incubated for visual examination of growth. Microscopic examinations of collected dust can be used to identify moulds and pollen.

When a bioaerosol sample is withdrawn from the air for collection by impaction, the physical collection process will unequally affect every organism. Depending on the density, size and shape of the micro-organisms, they may or may not be collected. At a low impact velocity the micro-organisms tend to travel with the air streamlines and are not collected when the sampler is operated at a low impact velocity. At a higher critical impact velocity, micro-organisms will carry out a soft landing and are collected by the agar by attaching themselves to the agar surface. At a high impact velocity, micro-organisms may penetrate into the agar surface and remain embedded in the agar (Stewart *et al.*, 1995). According to Marthi *et al.* (1990), the survival of aerosolised bacteria is effected by 1) growth condition before aerosolisation, 2) environmental conditions during aerosolisation, 3) methods of collection, and 4) temperature and relative humidity during aerosolisation. According to Willeke and Baron (1993), particles can bounce when they strike the impaction surface or other previously collected particles. This often occurs when overloading the sample. The bouncing of large particles can lead to under-sampling resulting in underestimation of bioaerosols.

Willeke and Baron (1993) reported that bioaerosol particles should b e collected from the ambient air in an unbiased manner. For the collection to be effective over a broad particle size range, aspiration sampling should be performed under isokinetic conditions, which means sampling

conditions in which the air flowing into .... ve the same velocity and direction as the ambient airflow. An essential part of the sampling is to define the sampling times. Bioaerosol concentrations vary considerably over time. The concentrations rarely remain stable in a narrow concentration range unless the time period is relatively short, or the air is undisturbed like in a closed unpolluted room. Since sampler flow rates are normally not controlled by the investigator, the usual way to mitigate the problems of non-representative or overloaded samples is to control the sampling period. An optimal sample should integrate the change in the sample population it will represent (Willeke and Baron, 1993).

Aspects of building construction

According to the ACGIH (1989), most environments contain a wide variety of bacteria, with human-source bacteria being dominant indoors. Bacterial components are, for the most part, naturally occurring flora and do not usually cause human illness or complaints. Risk of illness increases only when bacteria that can produce disease are present in sufficient numbers and/or become airborne and successfully reach the breathing zone of susceptible humans. According to Willeke and Baron (1993), pathogenic bacteria are often specific and cause disease only to a certain species of animal or plant. Furthermore, most animal and plant pathogens are different from human pathogens. Environmental or saprophytic bacteria are found everywhere and their nutritional and temperature requirements vary. Only few environmental bacteria are opportunistic pathogens that may attack an individual with a weakened immune response. In air, bacteria may occur alone or carried by other particles. Bacteria tend to grow in colonies in their natural habitats, like water and soil. Whenever they become airborne they often occur as aggregates or micro-colonies attached to other materials. According to Brooks and Davis (1992), numerous chemical and biological agents contribute to indoor air pollution - more than 900 compounds have been identified in indoor air. Certain common indoor air contaminants form reaction products as they combine with other contaminant species.

According to Walter (1969), the removal of micro-organisms by ventilation is related to the rate of air change. A ventilation rate of six changes per hour can control odours, but this rate contributes little

to the control of micro-organisms. Furthermore, the removal of airborne bacteria in a ventilated space depends on the pattern of distribution of air in the room. The criteria for assessing these problems must be analytical and scientific. Often quite straightforward mechanical faults are discovered or situations such as overcrowding are found to be the cause of complaints. Study methods should be developed for the checking of the quality of indoor air, and for auditing work areas with regard to light, noise and office space. Design engineers should consider the sterilisation of return air and even fresh air, according to Truter *et al.* (1991).

Smoragiewicz *et al* (1993) stated that poor ventilation in work environments has many effects on the occupants such as drowsiness, coughing, headaches, and the circulation of organisms which may cause colds or influenza, all which may result in low productivity. Smoragiewicz *et al.* (1993) also stated that symptoms of airborne toxicosis include headaches, chronic fatigue, cold- and influenza-like reactions, as well as dermal irritation and others. Other parameters, such as relative humidity and temperature, can be considered as a general test for contamination with microbes like yeasts and bacteria (Smoragiewicz *et al.*, 1993; Hirai, 1991). Thus, an increase in relative humidity, including water infiltration and condensation, can produce conditions optimal for growth of other micro-organisms. The need is also indicated for new legislative efforts or standards to enable quality control and norms for biological contamination of the air in work environments. The National Building Regulations Part O and the SABS Code 0400 empower local authorities to require that buildings using artificial ventilation provide air that is regarded as adequate, safe and healthy to the occupants (Truter *et al.*, 1991). According to the SMACNA (1993) and Wilson (1987), dirty air-conditioning equipment, humidifiers, condensate-drains and ductwork can incubate bacteria and moulds. Areas with high humidity promote the growth of these organisms.

Beachler *et al.* (1991) and Evans and Brachman (1991) reported that the most severe indoor biological contamination resulted from growth of the offending organisms on surfaces within structures, such as cooling towers and condensate drip pans. Many buildings with biological

contamination can trace the probable cause back to the lack of proper maintenance of the HVAC system.

Further factors that may contribute to the indoor air quality, are the overpopulating of offices and exceeding the coping capacity of the building (Larsen, 1995). Larsen (1995) also quoted an architect's associate in saying that research has shown that fresh air requirements are double the norm set during the 1970s. According to Burge (1990), indoor sources of bacteria are controlled by the density and shedding rates of occupants as well as the air replacement rate. Larsen (1995) also states the importance of individual temperature controls in every office to maximise the occupants' comfort. Nagda and Harper (1989) support this statement by saying that temperature seems to be the most important variable in indoor climate and it should be individually controlled.

According to the SMACNA (1993), the effect of the thermal environment of the building on the occupants depends on the thermal radiation of the building and the temperature, velocity and relative humidity of the indoor air. Occupants also have different body temperatures when entering the building as well as different clothing. In general, the recommended range of relative humidity for comfort for most individuals is between 30% and 60% for summer and winter. Relative humidity below 30% may produce discomfort from dryness. When the indoor environment is slightly too cool or too warm for an individual, a cumulative change in internal body temperature is likely to occur, which will result in experiencing an unacceptable thermal environment. There may be interactions in the reports given by occupants about their particular environment. It is unlikely that reports of a too cold environment will coincide with reports of sleepiness or exhaustion, just as it is unlikely that reports of a too warm environment will coincide with reports of high work stress. According to Kodama and McGee (1986), it is ironic that the air-conditioning system that was intended to improve occupational comfort and the quality of indoor air, may be a potential source of micro-organisms implicated as a health concern.

According to Burge (1990), the following environmental factors contribute to the indoor bioaerosol problems: the micro-organisms in the outdoor air, ventilation mode, indoor occupant density and ventilation rate, as well as the moisture in the indoor environment. Brooks and Davis (1992) found that the development of an energy efficient building usually underestimates the effects of recycled air on the health and comfort of the occupants of the building. Garrison *et al.* (1993) suggested that heating-ventilation and air-conditioning sanitation might be an effective way of reducing airborne fungal populations in residential and commercial environments.

The SMACNA (1993) found that the occupants of a building are a source of information as to how a building is working. Occupants are sensitive to all forces and factors found in a building, so that they can tell exactly what they are experiencing, while instruments are sometimes not able to. However, stress and other factors that affect occupants' health or mood outside the building may cause a noticeable reaction to defects in the building. Thus, complaints can be perceived to be either real or imagined. According to the ACGIH (1989), air sampling may document that a source is present or verify that an identified source is contaminating the environmental air. Sampling plans should recognise factors that could have aerosolised material from particular sources. For example, if the filters of the air-conditioning system are apparently contaminated, sampling should be done before, during and after replacement of the filters.

When the initial evaluation of symptoms and complaints in a building leads to the suspicion of bioaerosol-induced illness, an investigation aimed at assessment of the bioaerosol status of the building should be undertaken (ACGIH, 1989). Although proof of the connection between specific exposure and symptoms is often impossible to obtain, it is possible to prove a bioaerosol reservoir during an on-site inspection. During this inspection the investigator studies the structure, maintenance and occupancy patterns of the building, looks for potential sources, and formulates plans either for continued in-depth investigation or for remedial action. Building blueprints, including those for remodelling, should be obtained, while maintenance records can also provide useful information (ACGIH, 1989).

An inspection of the building and HVAC system can reveal the presence of bioamplification, and should be performed before collecting air samples for bioaerosols to determine whether air sampling is necessary. Areas of the ventilation system exposed to high humidity or areas where water may accumulate, should be inspected for signs of slime, and fungal and bacterial growth. Places where water may drip are condensate drip pans for cooling-coils, and spray and steam insulation in an area of high humidity and moisture. Areas inside of the building can also act as sites where microbes can grow. The classical situation may occur when there is a water leak that dampens carpets, furniture, overhead ceilings or structural insulation, allowing the growth of micro-organisms. Damp areas of carpet around plants that have been over-watered can also act as a breeding-ground of micro-organisms (Brooks and Davis, 1992).

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1.3 Rationale

Aim of this study

The aim of this study was to monitor certain species representing pathogenic or opportunistic pathogenic micro-organisms that accumulate in the water and/or surrounding air of the water-based air-conditioning system in the four buildings mentioned. In order to do this, the chosen species were monitored with a microbial air-sampler (SAS Super 90) on a two-weekly basis and the results were compared to data acquired through questionnaires, completed by certain selected workers in the same buildings. The null hypothesis for this study was that a significant correlation exists between the presence of pathogenic or opportunistic pathogenic micro-organisms found in the water and/or surrounding air of the water-based air-conditioning systems of the four buildings relevant to this study, and the prevalence of air-conditioning-related illnesses as determined through questionnaires completed by certain selected workers in these buildings during the same period of time.

CHAPTER 2

MATERIAL AND METHODS

2.1 Study area

Four buildings in the city of Bloemfontein constituted our area of study. Each building was chosen based on the heating, ventilation and air-conditioning systems used in it, as illustrated in Figures 2-5 (Appendix B). Another factor in selecting sampling buildings was the age of HVAC systems. An effort was made to include buildings with HVAC systems from different time periods as new technology influenced the design of the systems over the years. Buildings A and C are much older and longer in use than building D, while building B is the oldest of all four. According to a study by Wilson (1987), older buildings are associated with higher rates of complaints, either suggesting that modern HVAC systems are improving or that with time systems decrease in effectivity and allow the accumulation of contaminants. (Building D were only included later during the study period, therefore, samples were only collected over a period of 10 months) Buildings A, B and D are located in the central city area, within a two-kilometre radius of each other, whereas building C is located in the industrial area of the city, approximately five kilometres from the other three buildings (Figure 1) (Appendix A). To ensure seasonal coverage, samples were collected on a two-weekly basis over a 12-month period (February 1996 through January 1997).

The configuration of the air-conditioning systems involved in this study was as follows:

Building A employs a 100% fresh air system (Figure 2) (Appendix B). All the air is ventilated, from outside the building, through the filters and the heating- or cooling-coils, through the duct system and the office units into the offices. As shown in Figure 2, every office unit has its own cooling-coil, enabling occupants to control the temperature to a certain extent, mostly in summer. No additional heat setting is under control of the office occupants. During winter, the air is pre-heated by the pre-heating coil to 10°C, after which the air is then heated to approximately 21°C before entering the offices (Van Wyk, 1996).

The cooling tower of the system in building A operates by fanning air over water in the sill, thus cooling the water inside. The cold water then circulates through a closed pipe system to the main as well as secondary cooling-coils in the office units. Air is blown over these coils by fans, thus effectively cooling the air (Anderson, 1973). Four offices were selected for sampling in the aforementioned 25-story building: two on the fifth floor and two on the seventeenth floor, on the north and south sides respectively. The filters at the air inlet are specified for replacement every three months. The air outlets of offices in building A are situated in the exterior wall, directly below the windows.

The system in building B uses more or less the same principle, the fundamental difference being the use of return air, as shown in Figure 3 (Appendix B). The heating and cooling of air takes place in the same manner as in building A, however, using extractor fans in every office, through which air is extracted from the office to an air chamber. Here 80% of the returned air is mixed with 20% fresh air and passed over the heating or cooling-coils. Only the fresh air passes through the filters prior to mixing with the return air (Van Wyk, 1996). Similar to building A, offices were selected to represent low and high levels as well as north and south sides. This methodology enabled us to determine the effect of office placement on the microbial populations inhabiting the offices. Air inlets of every office in building B are situated in the interior wall, approximately 10 cm below the ceiling.

Building C is fitted with an older and less complex cooling system. Water and air is brought into direct contact with one another, allowing particles in the water to be blown into the air (Figure 4) (Appendix B). In this system, water is sprayed over a mesh while a fan blows air through the moisturised mesh, thus cooling the air through evaporation. The cooled air is then ventilated through the building. The filters are replaced approximately every 3 months. The water pans, into which the water accumulates after passing through the mesh, are drained and cleaned twice a year. The cooling unit is situated approximately 3.5 m from the floor. The heating system in this building operates entirely independent from the cooling system.

Building D utilises a combination of the systems in buildings A and B (Figure 5) (Appendix B). Using the same principle as building B, 10% return air is utilised in winter. This is done to conserve heat in the building and consequently save energy. In summer, 100% fresh air is used. This is done in the same manner as in building A. An important difference regarding the system used in building D is that water is not cooled by a cooling tower but by fenone, a highly effective cooling agent. The water used by the system is also cleaned of algae by applying 24 volt electrical shock to the water, before the water is let into the coils (Beyer, 1997). Two offices were selected for sampling in this 12-story building, again representing the north and south sides of the building to establish the influence of direct sunlight on the north side.

The boilers in buildings A, B and D operate similarly to one another. Water is let into the boiler from a water tank, where it is heated and pumped to the storage tanks, in which the water is kept under pressure at a temperature of $\pm 130°C$. From here the water is either let through to the pre-heating and heating coil, or back to the boiler to be re-heated if the water temperature drops too low (Anderson, 1973).

2.2 Health profile

Questionnaires (Appendix C) were handed to all the occupants of the offices that were selected for air sampling. Furthermore, questionnaires were also handed to a number of occupants on the same floor in different offices not used for air sampling. The researcher briefly explained the purpose of the questionnaire and the study to each respondent, to set them at ease. The questionnaires were as concise and simple as possible to ensure that the minimum time was required for completion. It was compiled in a manner that made it easily understandable, ensuring a good relative response. The researcher was always at hand to explain a question if there was any uncertainty. The questionnaires were anonymous, ensuring confidentiality and thereby ensuring complete honesty. (Katzenellenbogen et al., 1991; Vaughan and Morrow, 1989).

Questionnaires were distributed twice during the sampling period. The respondents were questioned with regard to their work environment, state of health and respiratory-related symptoms. Symptoms associated with "sick buildings", as defined by the WHO, include: eye, nose, and throat irritation, sensation of dry mucous membranes, mental fatigue, headaches, high frequency of airway infections and cough, hoarseness and wheezing (Truter *et al.*, 1991). The questionnaires were compiled with the objective of covering these symptoms. A total of 41 questionnaires were completed in building A, 32 in building B and, 15 in building D. In building C almost all the occupants of the building completed questionnaires, totalling 13.

The first section of the questionnaires covered general information, like the location of the office and the age and sex of the respondent. Section two, covering the work environment, required more detailed information about the office situation and the comfort of the occupant. Section three (the state of health) inquired about respiratory-related symptoms the respondent may or may not have experienced in his/her work environment. The final section included questions about the respondents' health history over the past six months.

The questionnaires were distributed in the official languages (English and Afrikaans), thus the respondents had a choice of language, as recommended by Katzenellenbogen *et al.* (1991). Only selected information from the questionnaires was used. Questions related to the rest of the study were selected and the responses of the occupants were analysed by computer. Additional information about the comfort of work environments and the number of days lost due to illness of workers were also obtained from the completed questionnaires.

2.3 Air analysis

Air sampling was done twice a month with the use of a microbiological air-sampler (SAS Super 90; PBI International, Milan, Italy). In two multi-storey buildings, A and B, samples were collected on two different floors and on both north and south sides of the buildings. In building D, samples were

collected on one floor only on the north and south sides. In the single-storey building (building C), samples were collected in two sampling locations on the north and south side. Thus, there were four sampling areas in buildings A and B, and two sampling areas in each of buildings C and D.

Microbiological media were prepared for isolation of total mesophilic organisms (plate count agar, Oxoid), yeasts and moulds (Potato dextrose agar, Oxoid), and members of the genus *Pseudomonas* (*Pseudomonas* selective agar, Oxoid) according to the manufacturer's specifications. The media were poured into sterile plastic Petri dishes (65 mm) for use in the SAS Super 90 microbiological air sampler and stored at ±8°C for 2-3 days before use, to rid the media of excessive moisture.

All experiments were done in duplicate. At each sampling location, 500 dm^3 of air was collected for analysis with the aid of the SAS Super 90. Sampling of this volume of air onto one Petri dish took approximately 5 minutes. The sampler was placed on a tripod, approximately 1.5 meter from the floor and 2-2.5 meters from the wall, where the inlet of the HVAC systems was situated. The sampler was placed in this manner to standardise height and distance and because this is the approximate height of the breathing zone of a sitting person. The Petri dishes were handled aseptically at all times and the head of the instrument was wiped with a alcohol cloth. The operator's hands were also periodically wiped with the damp alcohol cloth. Air was ventilated directly onto the agar surface of the Petri dish. After collection of each sample, the Petri dish was removed from the sampler, the lid replaced, sealed and marked. All the samples were transported back to the laboratory in a coolbag at ± 0°C. The Petri dishes were placed in sealed plastic bags to prevent drying and incubated at 35°C for 48 hours.

In addition to the air samples, the temperature was measured at each location by means of a microprocessor thermometer (Microcomputer HI 9043; Hanna). This was done not only to establish the relationship between temperature and the prevalence of organisms, but also to determine the

difference in temperature between the north and south sides of the buildings as well as the upper and lower levels (Keith 1992).

After incubation, the various colonies morphologies were caricaturised according to the specifications recommended by the manufacturer of the media. In the case of the potato dextrose agar, (PDA) a distinction was made between mould and yeast colonies and these were quantified separately. Colonies were quantified with the aid of an electronic colony counter (Gerber Instruments). Figures 6 and 7 (Appendix D) show the variety of the colonies found on plate count agar (PCA) and PDA media after incubation of air samples. Consequently the averages of the colony counts were calculated and converted with the aid of a conversion table 2 (Appendix E) to the most probable number. From table 2 (Appendix E) it was apparent that even colony counts of one colony per plate should be taken into account. The number of colony forming units (cfu) per cubic meter of air was calculated as follows (SAS Super 90: Microbiological Monitoring of the Environment; Milan):

$$X = \frac{Pr \times 1000}{V}$$

Where: X = Colony forming units per 1000 dm^3 (1 m^3) of air

Pr = Probable count obtained from conversion table

V = Volume of sampled air (500 dm^3)

r = Colony forming units on Petri dish

During the analysis of the data no significant (P < 0,05) differences occurred in microbial counts from lower and higher levels of building A and B. Thus, it was decided to calculate averages for the north and south sides of these two buildings, for future evaluation.

2.4 Water analysis

For water sampling, the same culture media as used for air sampling, were prepared using 90 mm Petri dishes. In preparation, sampling containers were also sterilised for the collection of water samples at building C. Samples were collected in sterile glass containers of 250 ml each. Four samples were collected on a monthly basis at this building. The water samples were collected from two air-conditioning units on the outside of the building, one unit on the north side and the other on the south side. The unit situated on the south side was in the shade for most of the day, resulting in a difference in the temperature of re-circulated water between the northern and southern units.

Care was taken to limit changes in micro-organism proliferation during transport to the laboratory by maintaining the sample temperatures at below 10°C. Duplicate plates were inoculated using the spread-plate method and incubated at 35°C for 48 hours (Gaudy and Gaudy, 1981; Singleton, 1981; Penn, 1991). After incubation, the colonies were counted with the aid of an electronic colony counter (Gerber Instruments). Figures 8 to 11 (Appendix F) is a visual representation of the colonies obtained from the water samples.

2.5 Comparative analysis

The results of the microbial counts obtained through the methods described in paragraph 2.3, were calculated as averages for every month. The questionnaires (paragraph 2.2) investigating respiratory-related symptoms suffered by the occupants during the 12-month period, were analysed. By calculating a microbial average for every month, a profile for the 12-month period could be set up, enabling comparison of the two sets of data.

Statistical calculations included the determination of the statistical correlation between the average total counts, temperatures, the occurrence of respiratory-related symptoms and the averages of the sampled organisms. This included the occupants who open their windows, as found in every

sampling location. Additionally, correlations were drawn between the data of the water collected from building C and the data obtained from air samples in this building. By using this data, the relationship between organisms in the water and organisms in the air could be assessed.

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CHAPTER 3

RESULTS AND DISCUSSION

3.1 Health profile

3.1.1 Results of the health profile analysis

The responses obtained from occupants to the question regarding cold and influenza symptoms suffered by the respondents are shown in Figure 12. On average buildings A and C, had the highest positive response, with building C the highest occurrence in a single month, namely June (Figure 12). Reports of symptoms in buildings A and B reached a peak during the winter months of May and June. In building D, the symptoms peaked during the months of April and July, however the peak of symptoms in this building was not as high as in the other buildings (Figure 12).

The response to the question regarding symptoms associated with a sore throat and a runny nose (hereafter, hay fever symptoms) is displayed in Figure 13. Building C showed the highest positive response, which reached a maximum during the months of June, July and August. In the other buildings no prominent peaks of complaints during specific months were observed for these symptoms. Building B had the highest positive responses in the months of May and June, while the respondents from building A showed a constant occurrence of the specific symptoms throughout the study period. Respondents of building D experienced a relatively low occurrence of hay fever symptoms (Figure 13).

Responses to selected questions from the respondents, expressed in terms of percentile positive responses of every building to a specific question are shown in Table 3. It was clear from the results that building D had the least positive responses to the selected questions (16.5%). Only the question regarding dry or gritty eyes provoked a relatively high positive response from occupants in this building. This building utilised the newest HVAC system of the four sampled buildings. Building B, on average, had the highest positive response (47.2%) (this building uses 80% return air in its HVAC system). The question about blocked noses in particular, provoked a high positive response.

From the data it was evident that occupants from building C suffered mostly from dry or gritty eyes, with an average positive response to respiratory-related symptoms of 24.0%. Responses from building A revealed a high positive response to the questions regarding dry noses and an average positive response to all respiratory-related related symptoms of 34.3%.
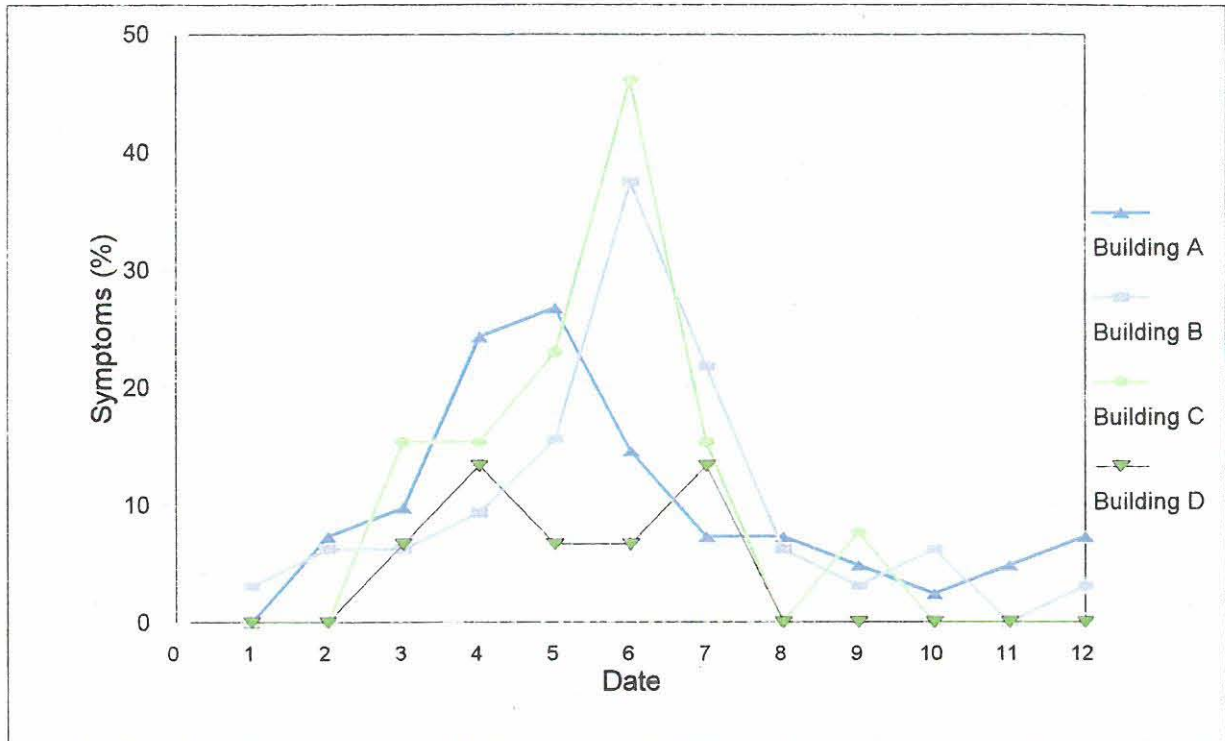
Figure 12. Flu symptoms, experienced by building occupants in all four buildings, during a 12 month period.
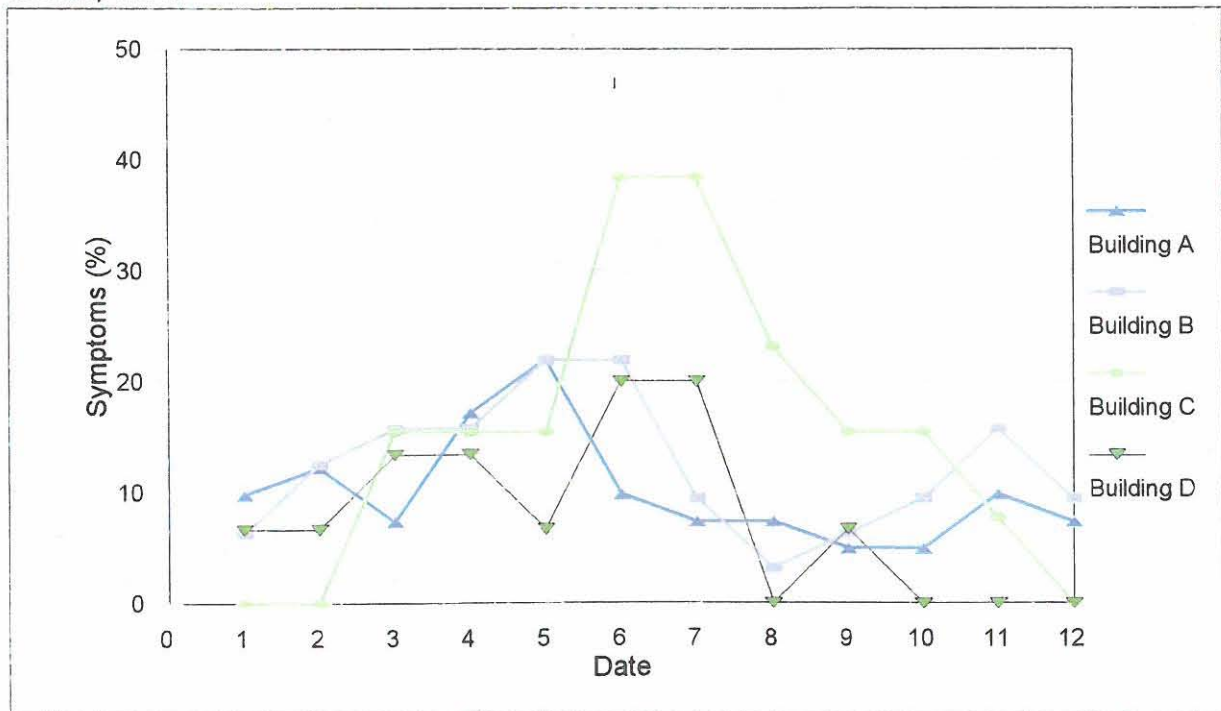

Figure 13. Hayfever symptoms experienced by building occupants for all four buildings, during a 12 month period. [X - asse values represent a 12 month period, ranging from February 1996 to January 1997.]

Table 3 Positive responses (%) from the four buildings, related to nasopharyngeal symptoms

NASOPHARYNGEAL SYMPTOMS	BUILDING			
	A	B	C	D
Symptoms of influenza	31.7	28.1	30.7	6.7
Hay fever or allergies	21.9	40.6	30.7	6.6
Sore throat	24.4	46.9	7.7	6.6
Blocked nose	43.9	62.5	7.7	6.6
Dry nose	46.3	53.1	23.1	20
Runny nose	41.4	56.2	30.7	13.3
Dry or gritty eyes	31.7	56.2	38.4	46.6
Watery eyes	34.1	34.3	23.1	26.1
Average respiratory-related symptoms	**34.3**	**47.2**	**24.0**	**16.5**

3.1.2 Discussion of health profile analysis

From the questionnaires it was evident that only 61% of the occupants of the four buildings experienced comfortable temperatures in their work environment during the summer months. During winter, 57% of the occupants found the temperature comfortable. In building A, 6% of the occupants covered the air inlets in their offices. The reason given for this was mostly because they felt that the air made them sick or the air was too cold. Although not shown graphically, the loss of working hours was also tested in the questionnaires. In building A, 64 working days were lost due to the absence of workers with colds, influenza or related illnesses. According to the responses obtained from building B, 38 working days were lost, while buildings C and D lost 8 and 2 working days, respectively, as a direct consequence of respiratory-related illnesses or symptoms. In building C, surprisingly few working days were lost, probably because the small work force in this building experienced more pressure to complete their tasks and, therefore, did not easily stay away from work.

Traditionally, cold and influenza symptoms occur more frequently in winter among the public in the study area (Bloemfontein). Therefore, this trend was also expected in the sampled buildings. In building A, most of the respondents suffered from influenza symptoms during April and May, coinciding with the season getting colder. In buildings B and C, the highest number of positive responses to influenza symptoms were received during June, in the heart of winter (Figure 12). It is difficult to explain why occupants from building A started experiencing influenza symptoms earlier in winter than the occupants of the other buildings. It could possibly be explained through the fact that some offices are shared by workers, which could cause workers to infect each other more easily. As well as the fact that some workers live in disadvantage areas and have to travel long distances to work, this may lower their resistance to influenza. Respondents from building D generally did not show a high positive response to the question regarding influenza symptoms, which could indicate effective filtration of air in this building and possible removal of the agents causing influenza and cold symptoms from the surrounding air.

The results show that the highest positive response to the question regarding hay fever symptoms was reported in building C (Figure 13). All the buildings were associated with higher positive responses during the colder months. Buildings A and C showed a positive response to the question regarding hay fever symptoms during every month of the test period. It could therefore be suggested that the responsible agents were present throughout the 12-month period. No hay fever symptoms occurred among occupants from building B during the month of December. During the months of January, August, October, November as well as December, the occupants of building D reported no hay fever symptoms.

According to the results portrayed in Table 3 the respondents of building B had the highest number of positive responses to most of the questions, and thus, these occupants suffered from various respiratory symptoms. A total of 32 occupants were questioned and amongst them 38 working days were lost due to respiratory-related illnesses. Respondents complained mostly about runny noses and dry eyes. The respondents from building A also had relatively high responses to the questions listed in Table 3. A total of 41 occupants were questioned, and among them a total of 64 working days were lost. Respondents from building A complained mostly of dry or blocked noses, which could be related to a high airflow rate, resulting in dry conditions. In building C, only eight working days were lost due to illness during the 12 months of analysis. Positive responses regarding influenza and hay fever symptoms from the occupants of this building were very high. This could indicate a low airflow rate and/or insufficient dilution of airborne particles and organisms by fresh air. The respondents of building D had relatively few complaints, but almost 50% of them were troubled by blocked noses and gritty eyes. As in building A, this could be ascribed to a high airflow rate, or low humidity, which could also influence the microbial counts.

3.2. Air sampling

3.2.1 Results of air sample analysis

Table 4 represents the average counts of the various organisms isolated from air samples collected in the four buildings. From this table, it was apparent that building C produced the highest average counts for all sampled organisms. Furthermore, results from building A also showed a high total count, and building B a high yeast count. Building D in general showed relatively low counts.

Table 4 Average colony forming units per cubic meter of air obtained during the sampling period of 12 months, in four buildings

	Total count	Moulds	Yeasts
Building A	100	4	20
Building B	66	4	21
Building C	171	9	42
Building D	34	2	10

■ *Building A*

In building A, an average of 130 cfu/m^3 air was obtained during the sampling period on the north side of the building (Figure 14a). These counts represented moderate growth. At times heavy growth was found in samples from this building. In the same building, (south side), an average count of 69 cfu/m^3 was found. Six persons shared the office on the lower level south side, whereas eight people shared the office on the north side lower level, where a higher average count was observed. Figure 14a shows that the lowest total counts were during the months of May, June and July, while the highest counts manifested during October, December and January. The highest counts were predominantly found on the north side of the building throughout the sampling period.

The largest number of moulds (5 cfu/m³ air) was obtained on the north side of the building (Figure 14b), whereas an average of 4 cfu/m³ air was calculated on the south side. The highest mould count was observed during the months of February, March, April and October (Figure 14b), while the lowest mould counts were found during May through September (mostly winter months).

From the data it is apparent that the largest number of yeast (23 cfu/m³ air) was found on the north side, and the lowest number (16 cfu/m³ air) was counted on the south side. Figure 14c shows that during the months of June, July and August (cold winter months) the lowest counts of yeast were found in this building. The highest counts were obtained during October, through January, as the temperatures increased. Again, the north side revealed the highest number of yeast counts.

■ Building B

An average total count of 51 cfu/m³ air was obtained on the north side of building B (Figure 15a). On the south side, an average total count of 81 cfu/m³ was counted. The sampled office on the north side lower level was used by only one person and revealed lower counts than the office on the same level on the south side that was used by four people. The office on the north side was also exposed to direct sunlight, which could influence the organism counts. Total counts for building B, which revealed moderate growth, are shown in Figure 15a. The highest counts were found during the months of November, December and January, whereas the lowest counts in this building were found during April, May and July.

Mould count of 4 cfu/m³ was measured in building B on the south and north sides (Figure 15b). In this building, the highest mould counts were found in the months of February, March, June and September, while the lowest counts were found in August, October and November.
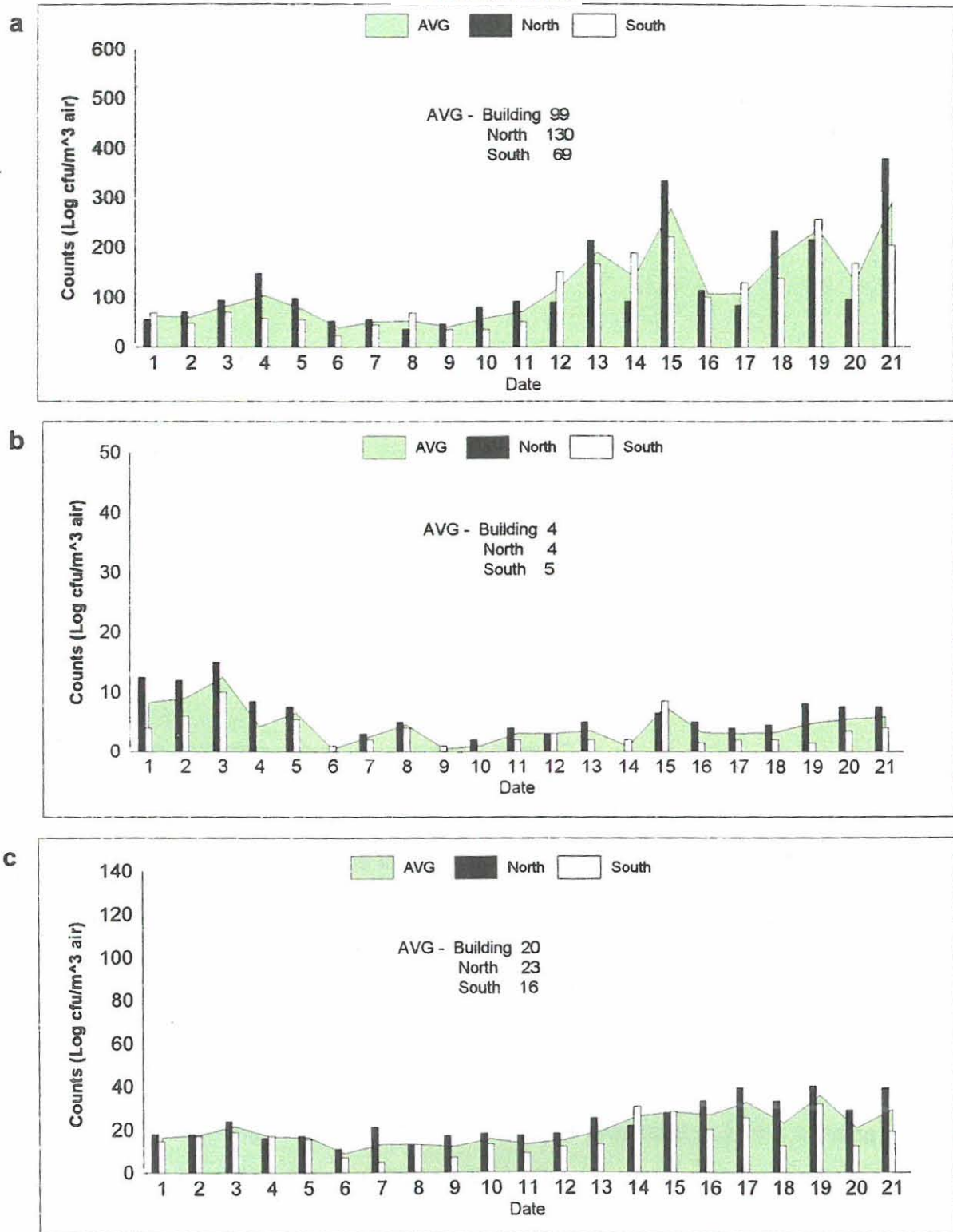
Figure 14 a) Total counts, **b)** mould and **c)** yeast colony forming units per cubic meter of air, sampled in building A on the north and south side over a 12- month period [X-axis values represent 21 samples collected consecutively with 14 day intervals - approximately every 2 weeks ranging over a period from February 1996 to January 1997]

Key to X-axis: 1- Feb. 1996; 2- March 1996; 3- April 1996; 4 & 5- May 1996; 6 & 7-June 1996; 8 & 9- July 1996; 10 & 11- Aug. 1996; 12 & 13- Sept. 1996; 14 & 15 Oct. 1996; 16 & 17- Nov. 1996; 18 & 19- Dec. 1996; 20 & 21- Jan. 1997.

On the south side of building B an average yeast counts of 22 cfu/m^3 was counted, and on the north side an average of 20 cfu/m^3 (Figure 15c). The highest yeast counts were obtained during the months of April, September, October and November. The lowest yeast counts were obtained in June, July and August. The offices on the south side showed the highest counts during the sampling period (Figure 15c).

■ Building C

In building C, an average total count of 168 cfu/m^3 was found in the south side of the building. On the north side, a slightly higher count of 174 cfu/m^3 was found, as illustrated in Figure 16a. Moderate and sometimes heavy growth were obtained from air samples. During the months of May, October and November, relatively high total counts were observed, while the lowest counts were recorded in March and July. In general, the north side of the building produced the highest counts (Figure 16a).

In Figure 16b, the collective mould counts for building C is shown. On both the north and south sides of this building, 9 cfu/m^3 moulds were counted. The highest numbers were found during May and July as well as October, whilst the lowest numbers were found in June, August and September. The south side of the building generally had the highest mould counts. In general higher yeast counts than mould counts were obtained in this building.

From the south side of the building, an average of 37 cfu/m^3 yeast was found, and on the north side 46 cfu/m^3. As shown in Figure 16c, the highest counts of yeast counts in this building were during June, July, August and November. The lowest numbers were in February, March, April and January. As with the total counts, the highest yeast counts were found in the north side of the building.
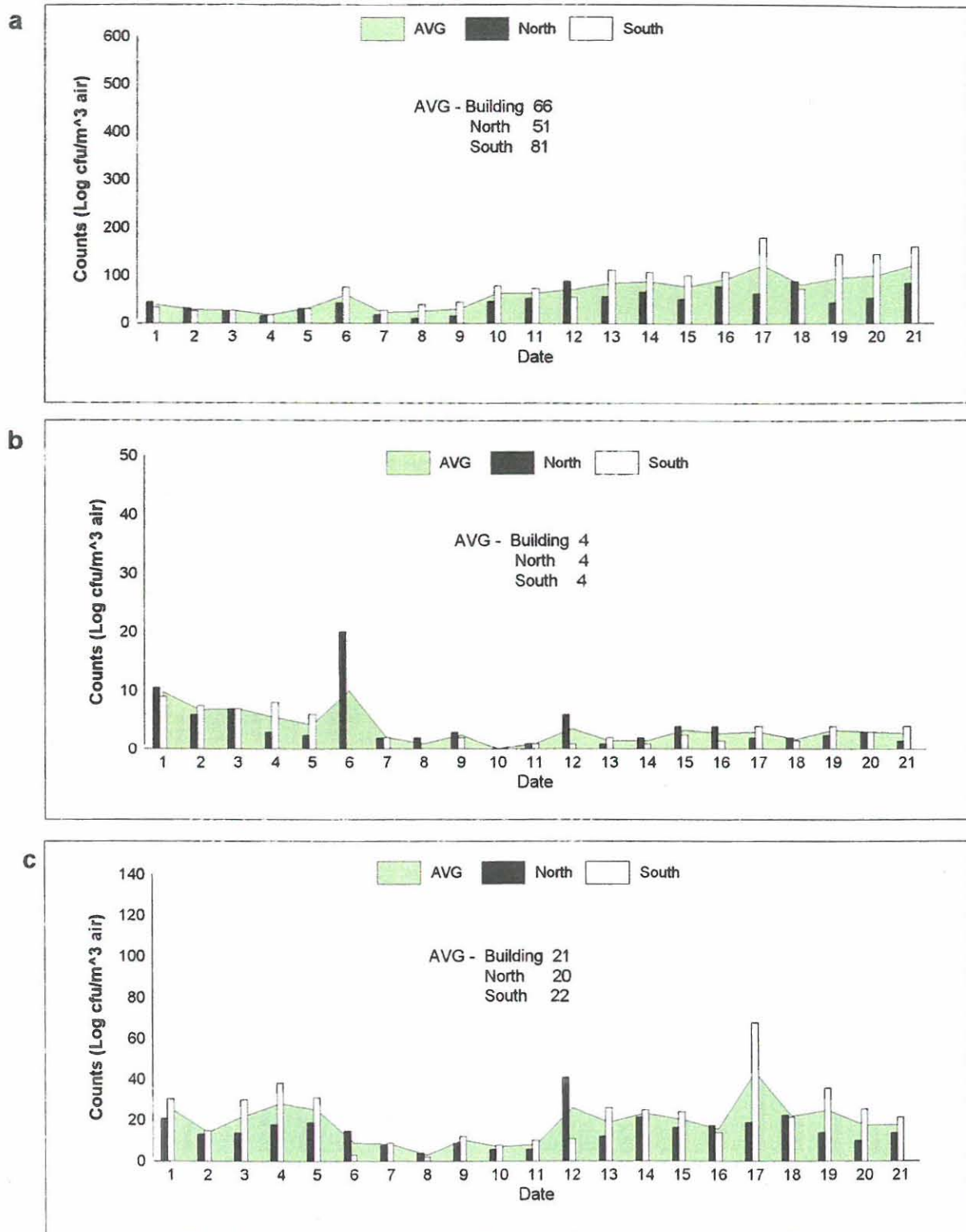
Figure 15 a) Total counts, **b)** mould and **c)** yeast colony forming units per cubic meter of air sampled in building B on the north and south side over a 12- month period. [X-axis values represent 21 samples collected consecutively with 14 day intervals - approximitaly every 2 weeks ranging over a period from February 1996 to January 1997]

Key to X-axis: 1- Feb. 1996; 2- March 1996; 3- April 1996; 4 & 5- May 1996; 6 & 7- June 1996; 8 & 9- July 1996; 10 & 11- Aug. 1996; 12 &13- Sept. 1996; 14 & 15- Oct. 1996; 16 & 17- Nov. 1996; 18 &19- Dec. 1996; 20 & 21- Jan. 1997.
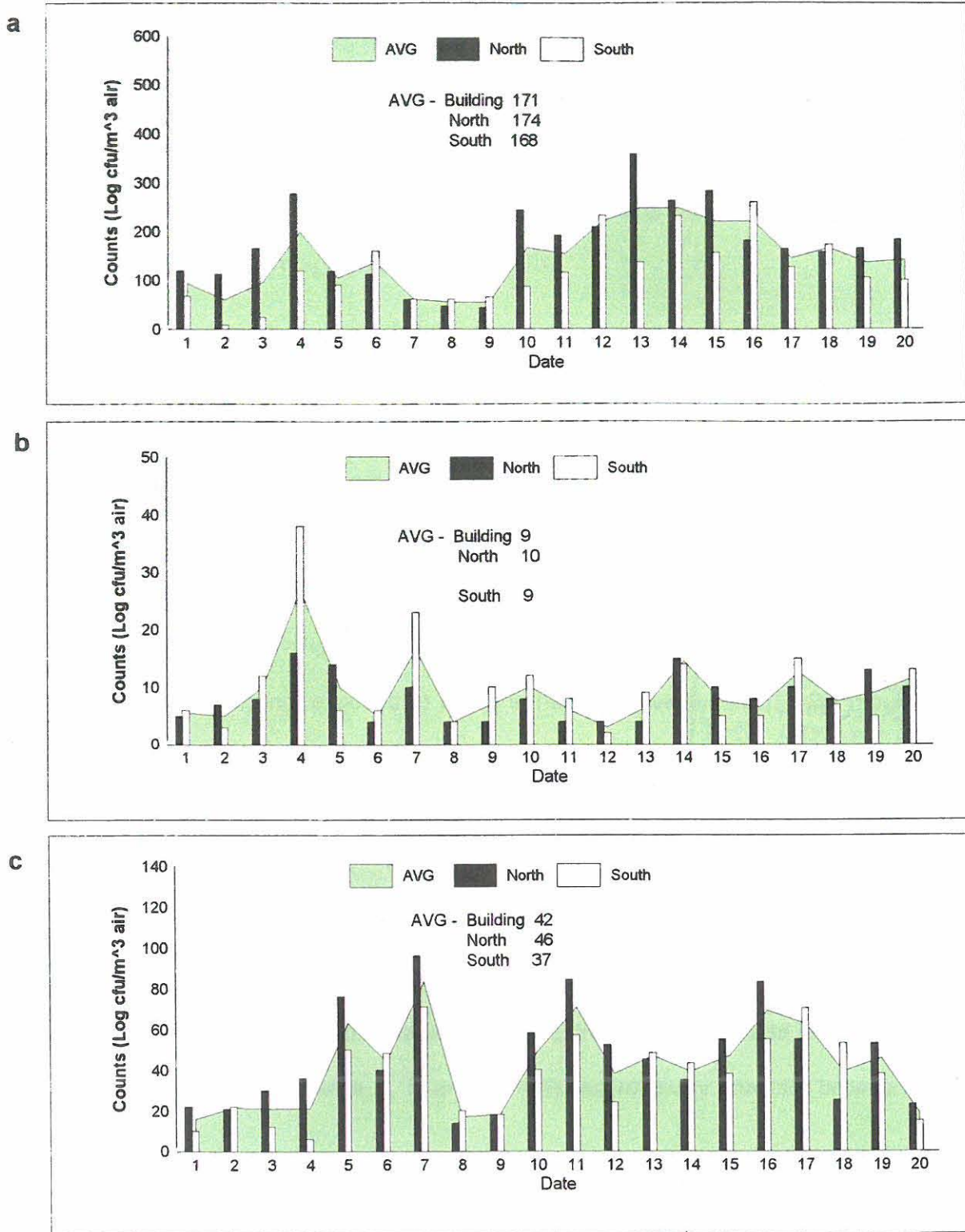
Figure 16 a) Total counts, b) mould and c) yeast colony forming units per cubic meter of air, sampled in building C on the north and south sied over a 12-month period. [X-axis values represent 21 samples collected conseuitively with 14 day intervals - approximately every 2 weeks ranging over a period from February 1996 to January 1997]

Key to X-axis: 1- March 1996; 2- April 1996; 3 & 4- May 1996; 5 & 6- June 1996; 7 & 8- July 1996; 9 & 10- Aug. 1996' 11 & 12- Sept. 1996; 13 & 14- Oct. 1996; 15 & 16- nov. 1996; 17 & 18- Dec. 1996; 19 & 20- Jan. 1997.

■ Building D

In building D, two people shared the office sampled on the south side and an average total count of 35 cfu/m^3 was found, while an average total count of 33 cfu/m^3 was found on the north side (only one person used this office). Only slight growth was obtained from the air samples in this building. In June, October, November and January the highest total counts were recorded in building D. The north side had the highest average counts (Figure 17a).

Low numbers of moulds were counted in this building, the highest counts being during April, May and January. No moulds were found during August and early September (Figure 17b). Yeasts were found in slightly higher numbers than moulds during the sampling period (Figure 17c). An average of 9 cfu/m^3 yeast was counted on the south side, whereas on the north side an average of 10 cfu/m^3 yeast was found. The highest numbers of yeast were found during October, December and January. As illustrated in Figure 17c, the lowest counts were found during April, May and July. The north side of the building, exposed to sunlight, had a higher average yeast, mould and total count than the south side.

■ Temperature

As shown in Figure 18, it is illustrated that the temperatures did not fluctuate markedly within the buildings. The temperatures of building C were generally more extreme than those of the other three buildings. Only in building C did the temperatures drop below 10°C and rise above 25°C. The temperature readings from buildings A, B and D remained relatively constant between 20°C and 25°C.
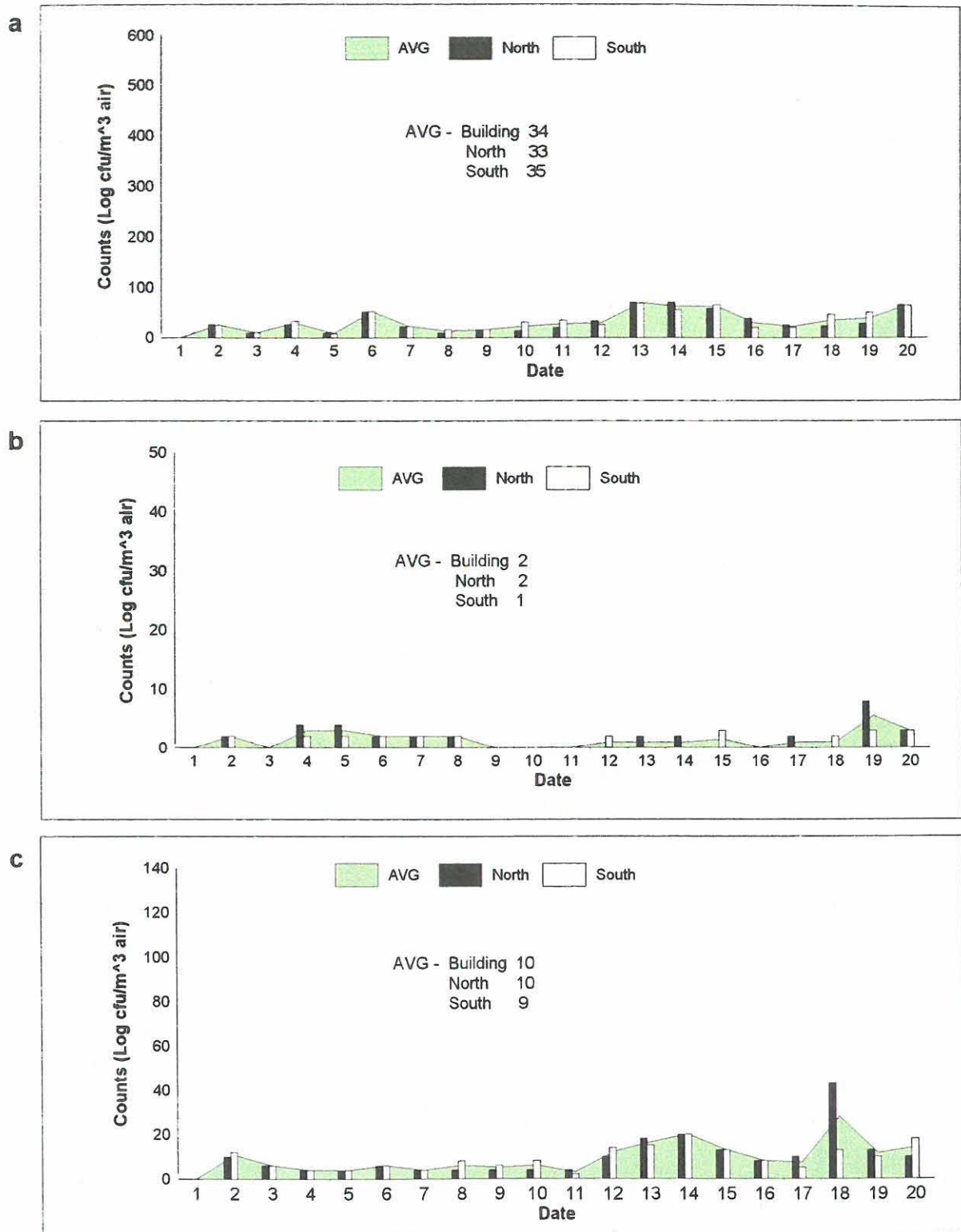
Figure 17 a) Total counts, **b)** mould and **c)** yeast colony forming units per cubic meter of air, sampled in building D on the north and south side over a 12- month period. [X-axis values represent 20 samples collected consecutively with 14 day intervals - approximately every 2 weeks ranging over a period from February 1996 to January 1997]

Key to X-axis: 1- March 1996; 2- April 1996; 3 & 4- May 1996; 5 & 6- June 1996; 7 & 8- July 1996; 9 & 10- Aug. 1996; 11 & 12- Sept. 1996; 13 & 14 Oct. 1996; 15 & 16- Nov. 1996; 17 & 18- Dec. 1996; 19 & 20 Jan. 1997.
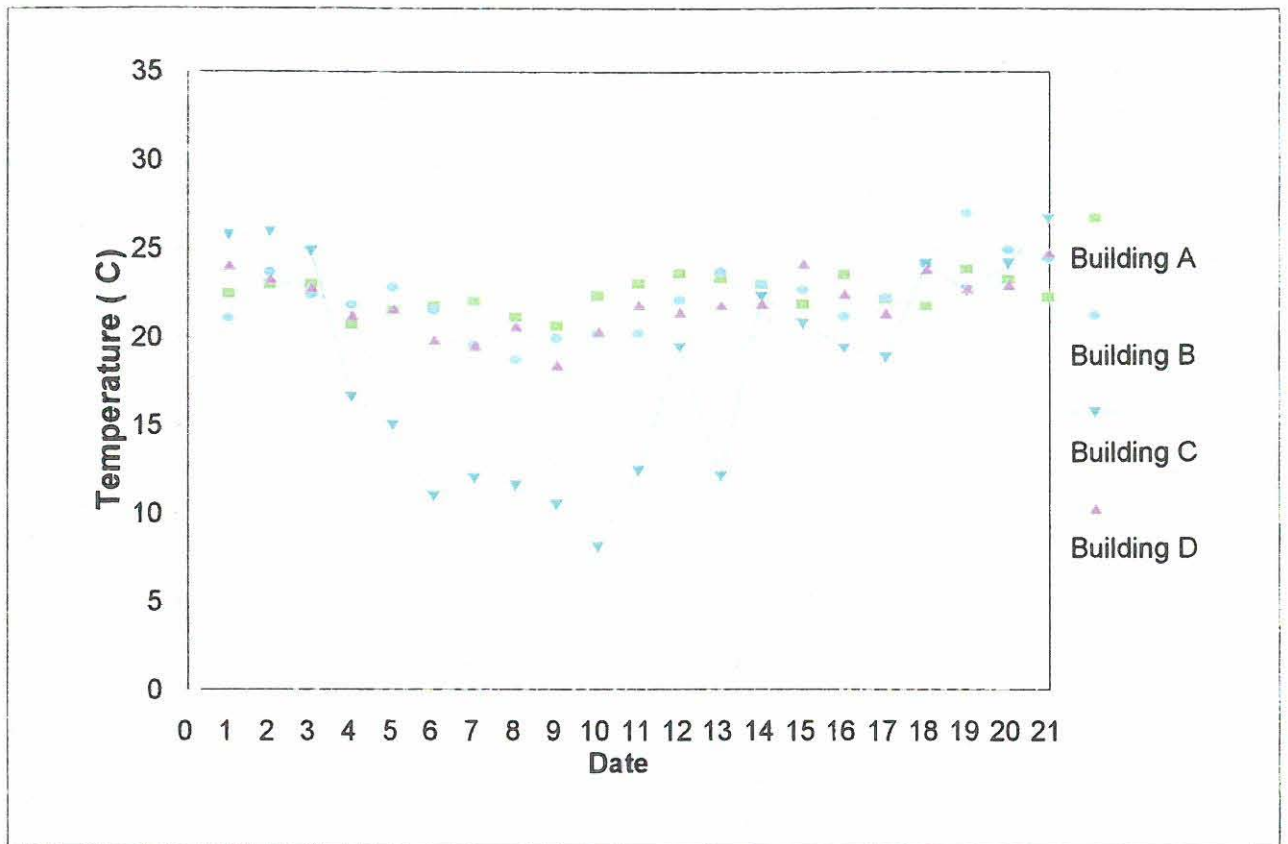
Figure 18 Temperature readings obtained from the four sampled buildings, during the 12 month sampling period. X -axis values represent 21 consecutive readings taken every two weeks, ranging over a period from February 1996 to January 1997.

3.2.2 Discussion of air sample analysis

Organisms including yeast and moulds are generally associated with transmission of diseases through air. These organisms are also known to cause illness or allergic reactions in humans (Evans and Brachman, 1991; Walsh *et al.*, 1990; Walter.1969). The microbiological air sampler (SAS Super 90) used in this study has a 100% sampling efficiency for aerosols of a known particle size over 4 microns, and an efficiency of 50% for aerosols between 2 and 4 microns in size.

Results obtained in this study showed that bacteria predominated during the warmer summer months. This corresponds with a study conducted by Hirsch *et al.* (1978), who stated that seasonal distribution could be reflected in the growth of bacteria. The highest total counts during the sampling period was found in building C, which utilises an air-conditioning system where small water droplets (which may contain micro-organisms) enter the indoor environment, similar to the findings of Burge (1990). According to the results found in this study, building D appeared to be the "cleanest" in this respect. This building utilises the newest HVAC system of all the systems surveyed in the study. The office in building A (shared by eight people) showed the highest average total count during the sampling period. These high counts could be explained by the high frequency of human activity resulting from possible overpopulation of the mentioned office. The other offices sampled in the same building did not show such high counts, and therefore, human activity and not the HVAC system, is suspected for causing the high counts in the mentioned office. (The ideal would have been to only sample offices with the same number of occupants, but this was not possible. Not all offices were available for sampling, the number of occupants in an office varied greatly on one floor, and the number of occupants was moreover not constant throughout the sampling period.) It appeared that, in general, the offices on the northern side of the buildings showed higher microbial counts than those on the southern side. This could be attributed to the temperature on the north sides being higher due to direct exposure to sunlight (Figure 18). It was notable that the offices situated on higher levels in buildings A and B showed slightly lower average temperatures.

Airborne mould spores were found in abundance in all the sampled environments, also reported by Walsh *et al.* (1990), which indicates that these organisms can be found in all of these environments during all seasons. However, in general, smaller numbers of moulds were found during the winter months, indicating the sensitivity of these organisms to the lower temperatures. The mould counts (4 cfu/m^3) obtained from the north and south side of building B revealed that direct sunlight on the northern side had no influence on the organism numbers in this building. Building C showed the highest mould counts in comparison to the other three buildings (Figure 8b). This could be attributed to the type of air-conditioning system used in this building, where the storage area could supply an ideal, undisturbed environment for mould growth because of the water droplets from the HVAC system. Garrison *et al.* (1993) reported that moist environments provide suitable substrates for mould growth. Building A had the second highest average mould count of the four buildings. The age and condition of the building and HVAC system could explain this phenomenon as dust and other particles are probably accumulating in the air-ducts and filters. Furthermore, filters are specified for replacement every three months, but because of high costs, this period is sometimes extended. In buildings A, B and D, a clear decline in mould counts were found during the middle of the sampling period (the months of June, July, August and October, which are generally cooler months). In building C, no decline in mould counts could be observed during the winter months. This was an unexpected observation, as very low temperatures were experienced during the winter months in this building.

According to Evans and Brachman (1991), and Tietd (1979), *Pseudomonas aeruginosa* is a pathogen frequently reported as a cause of respiratory infections in humans. In building C, the occurrence of this organism was expected in association with the water droplets from the air-conditioning system in this building. Leakage of water or droplets would provide organisms with much-needed moisture for survival (Garrison *et al.*, 1993). Apparently, this organism could not survive in the surrounding air of the sampled environments.

3.3 Water sampling

3.3.1 Results of water sample analysis

All four organism groups were found in the water samples taken from the cooling units at building C. High total bacterial counts were obtained from the water throughout the sampling period (Table 5). An average total count of 9563 cfu/m^3 was found in the water from the south side of the building, and on the north side an average of 9368 cfu/m^3 was obtained, indicating that water on both sides were heavily contaminated, with little difference between the counts from the north and south sides (Figure 19).

Moulds were constantly present in the water during the sampling period, with an average of 64 cfu/m^3. Higher numbers of moulds were found in the water from the north side than in the water from the south side of the building (Figure 20). Smaller numbers of moulds were found during September, October and December.

An average of 974 cfu/m^3 yeasts was found in the water during the sampling period (Table 5). The lowest counts were found during August and October (Figure 21). On average, the highest counts of yeasts were found in the water taken from the south side of the building. The highest counts of yeasts were obtained during the months of November, January and February (warm summer months).

Small numbers of *Pseudomonas* species were found in the water. An average of 27 cfu/m^3 was calculated in the water from the south side and an average of 244 cfu/m^3 in the water from the north side (Figure 22). The north side was exposed to sunlight for the most part of the day, presumably causing higher water temperatures and consequently higher *Pseudomonas* counts. The highest counts were found during January, February and March. No *Pseudomonas* spp. were found during October.

Table 5 Average counts obtained from water samples at building C

Organism	Counts cfu/m^3 water
Total bacterial count	9466
Moulds	64
Yeasts	974
Pseudomonas	136

Relationships between the microbial results from air samples and water samples taken in building C, were investigated by calculating the statistical correlation. As mentioned previously, the cooling system in this building allows water droplets into the indoor environment. This study attempted amongst others, to determine whether organisms present in the water are also found in corresponding numbers in the indoor air.

Table 6 Relationships between the air and water obtained in building C

Organisms	Correlations (r)
Total counts	-0.16008
Moulds	0.32093
Yeasts	-0.28003

The correlation (0.32093) between mould counts obtained from water and air, shows the only positive correlation, although low (Table 6). This may indicate more or less the same trend in the occurrence of moulds in the water and surrounding air. Correlations of -0.16008 for total counts and -0.28003 for yeasts were found between water and the surrounding air sample, indicating no relationship.
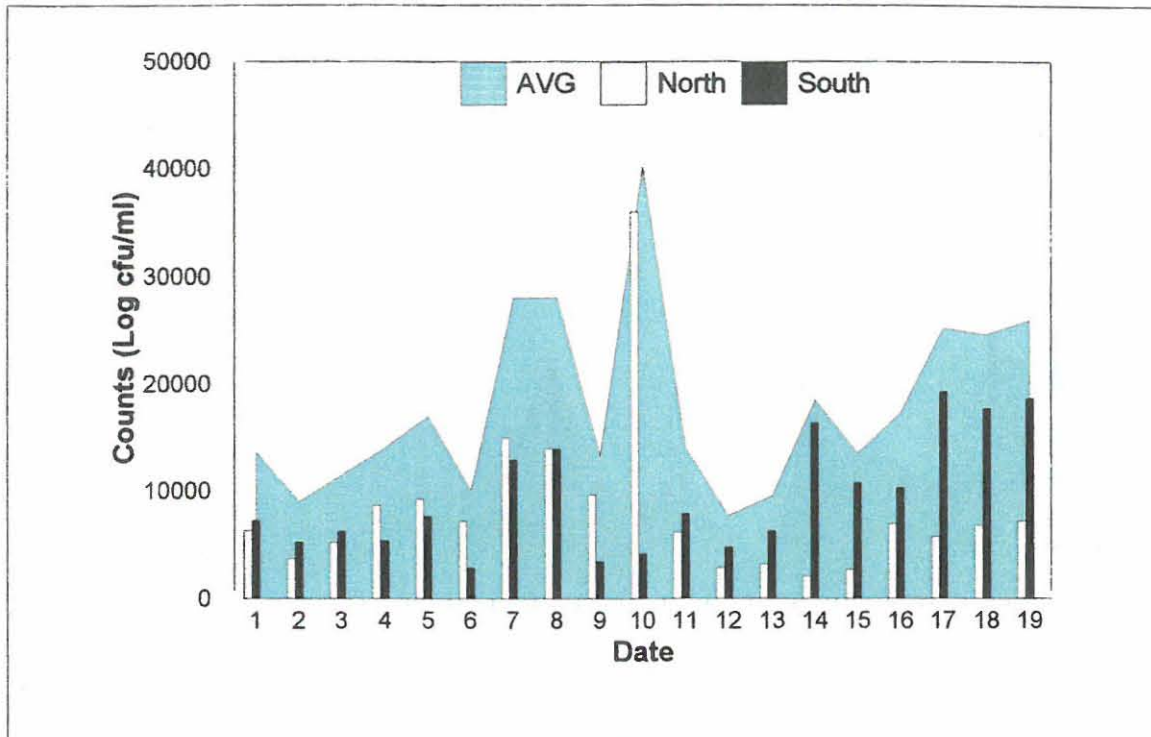
Figure 19 Total counts obtained from air cooling unit water, during a 12-month period at buildingC
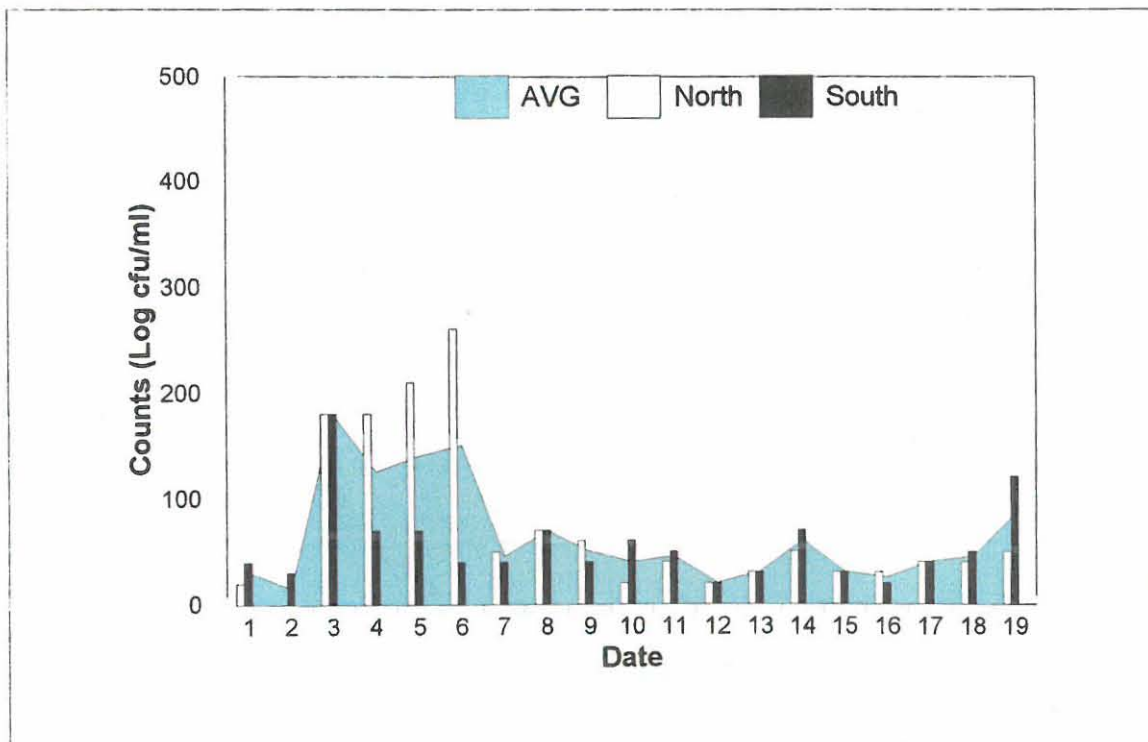

Figure 20 Mould counts obtained from air cooling unit water, during a 12-month period at building C

Key to X-axis of figure 19 and 20: 1- March 1996; 2- April 1996; 3- May 1996; 4 & 5- June 1996; 6 & 7- July 1996; 8 & 9- Aug. 1996; 10 & 11- Sept. 1996; 12 & 13- Oct. 1996; 14 & 15- Nov. 1996; 16 & 17- Dec. 1996; 18 & 19- Jan. 1997.

Figure 21 Yeast counts obtained from air cooling unit water, during a 12-month period at building C

Figure 22 *Pseudomonas* counts obtained from air cooling unit water, during a 12-month period, at building C

Key to X-axis of figure 21 and 22: 1- March 1996; 2- April 1996; 3- May; 4 & 5- June 1996; 6 & 7- July 1996; 8 & 9- Aug. 1996; 10 & 11- Sept. 1996' 12 & 13- Oct. 1996; 14 & 15- Nov. 1996; 16 & 17- Dec. 1996; 18 & 19- Jan. 1997.
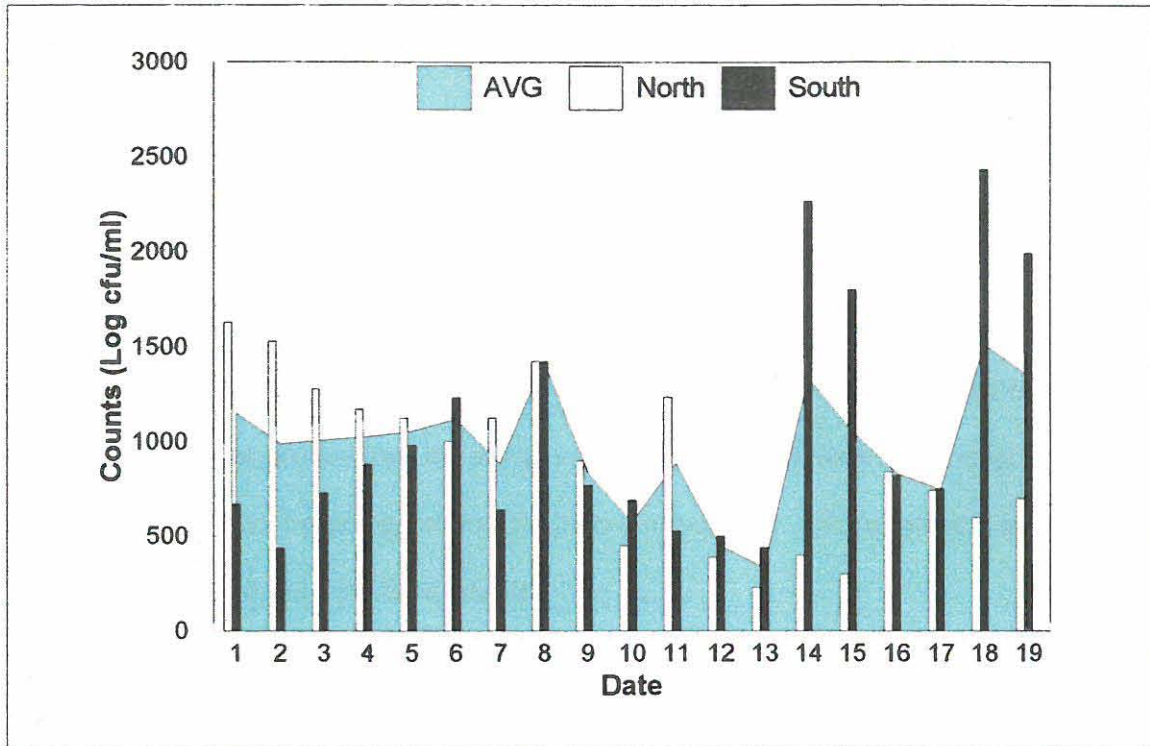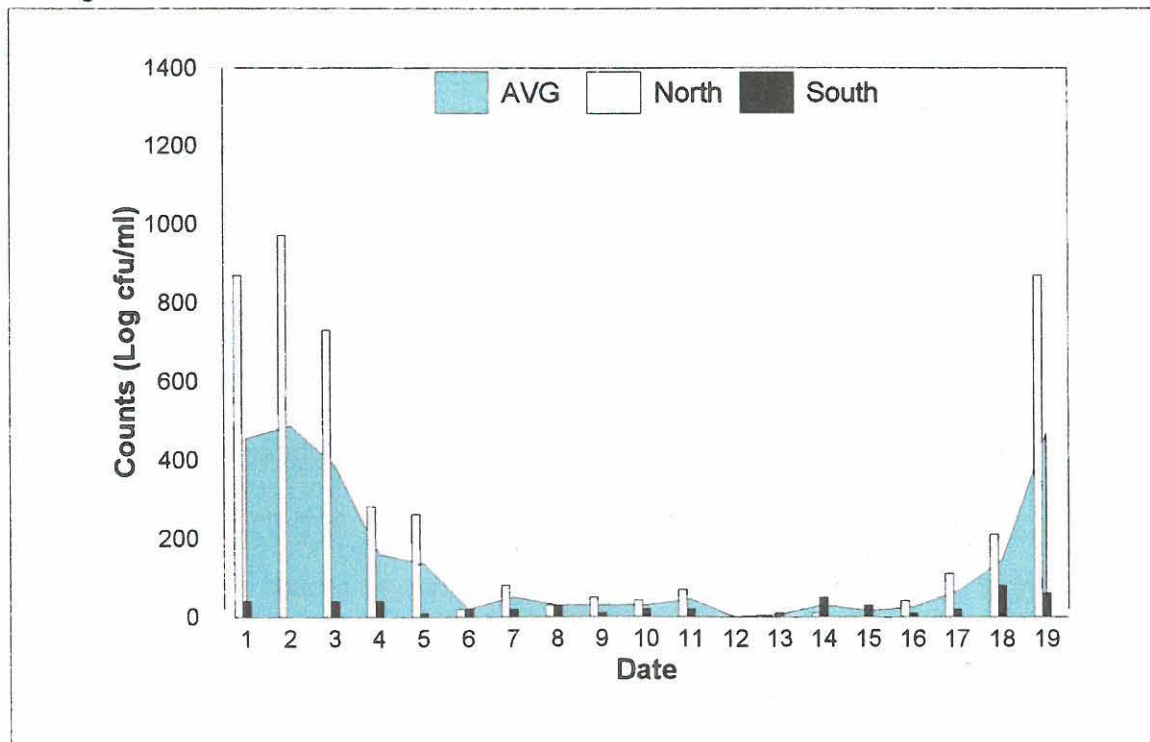
3.3.2 Discussion of water sample analysis

High total mesophilic counts were found in the water from building C during the sampling period. The lowest total counts were observed during the months of February and October. The south side of the building showed only a slightly higher average count than the north side. These high counts were expected because the water was exposed to the outdoor environment and could easily be contaminated by a variety of organisms. The combination of the outdoor temperature, as well as the abundance of water, provided an ideal environment for the accumulation of micro-organisms. Sediment in the water proved that the water was indeed contaminated with dust and other particles that rendered it a rich breeding-ground for micro-organisms. No apparent seasonal distribution could be distinguished from the total counts.

Moulds were isolated from both the north and south sampling locations throughout the sampling period. Again, no seasonal distribution was prevalent. Yeasts were also found in abundance in the water, which was expected because of the contamination level of the water due to the sediment. A slight seasonal distribution was observed, reflected by the yeast counts declining slightly during the winter. This is in accordance with results obtained by Willeke and Baron (1993).

Pseudomonas spp. were found in relatively small numbers in the water obtained from the HVAC systems in building C. Most of these organisms were also found in the water from the northern side of the building. This could indicate the organism's requirement for warmer temperatures. A very distinct seasonal distribution could be observed in the numbers of organisms found during the sampling period. Low counts were found during the winter months, opposed to increased counts in the summertime. Correlations between organisms obtained from air and water samples revealed that organism numbers found in the air did not correspond with organism numbers found in the water. The only positive correlation, although insignificant was for mould counts observed in the air and water samples.

3.4. Comparative analysis

3.4.1 Results of comparative analysis

Results obtained from respondents who completed the health questionnaire were compared to the microbial sampling data of the corresponding buildings.

In building A, an average total microbial count of 99 cfu/m^3 air was observed during the 12-month period. The highest total count (175 cfu/m^3) was found during October. The distribution of these organisms throughout the period in relation to influenza and hay fever symptoms, is illustrated in Figure 23. Apparently, the influenza symptoms reported by the occupants (Figure 23a) increased during the colder months as the total microbial counts decreased. Influenza symptoms of 24.3% and 26.8% were experienced during May and June respectively. The questionnaires also revealed that 21.9% of the occupants suffered from a sore throat or runny nose during the month of June (Figure 23b). An average mould count of 4 cfu/m^3 was found during the 12-month period in building A, with the highest count of 12 cfu/m^3 found in April. An average yeast count of 20 cfu/m^3 was observed during the 12-month sampling period. The highest yeast count (29 cfu/m^3 air) was found during November. Hay fever symptoms, as illustrated in Figure 23b, occurred constantly throughout the 12-month period.

In building B, 38.0% of the respondents suffered from influenza symptoms during the month of July. A high occurrence of the aforementioned symptoms was also noted during June (15.6%) and August (21.8%). This incidence of symptoms in association with the distribution of the micro-organisms sampled in building B during the 12-month period, is showed in Figure 24a. In this building, the influenza and cold symptoms peaked during the colder winter months, when the organism counts declined. During June and July, 21.8% of the respondents complained of a sore throat or runny nose (Figure 24b). A total count of 59 cfu/m^3 was found, with the highest count (110 cfu/m^3) during January. An average mould count of 6 cfu/m^3 and yeast count of 18 cfu/m^3, were

found during the sampling period. The highest mould and yeast counts (30 cfu/m^3 each) were observed in May and November, respectively.

Figure 23 a) Influenza symptoms **b)** Hay fever symptoms, experienced by building occupants as well as corresponding micro-organisms, found in building A over a 12-month period.
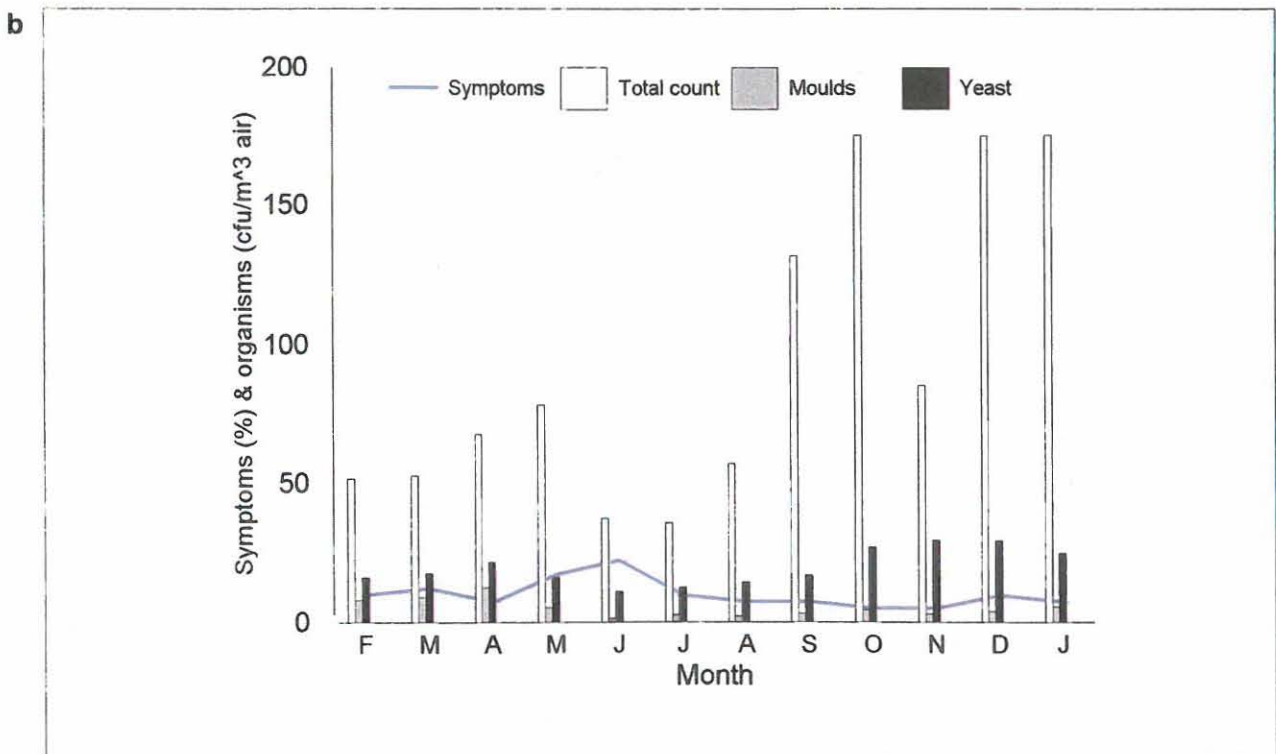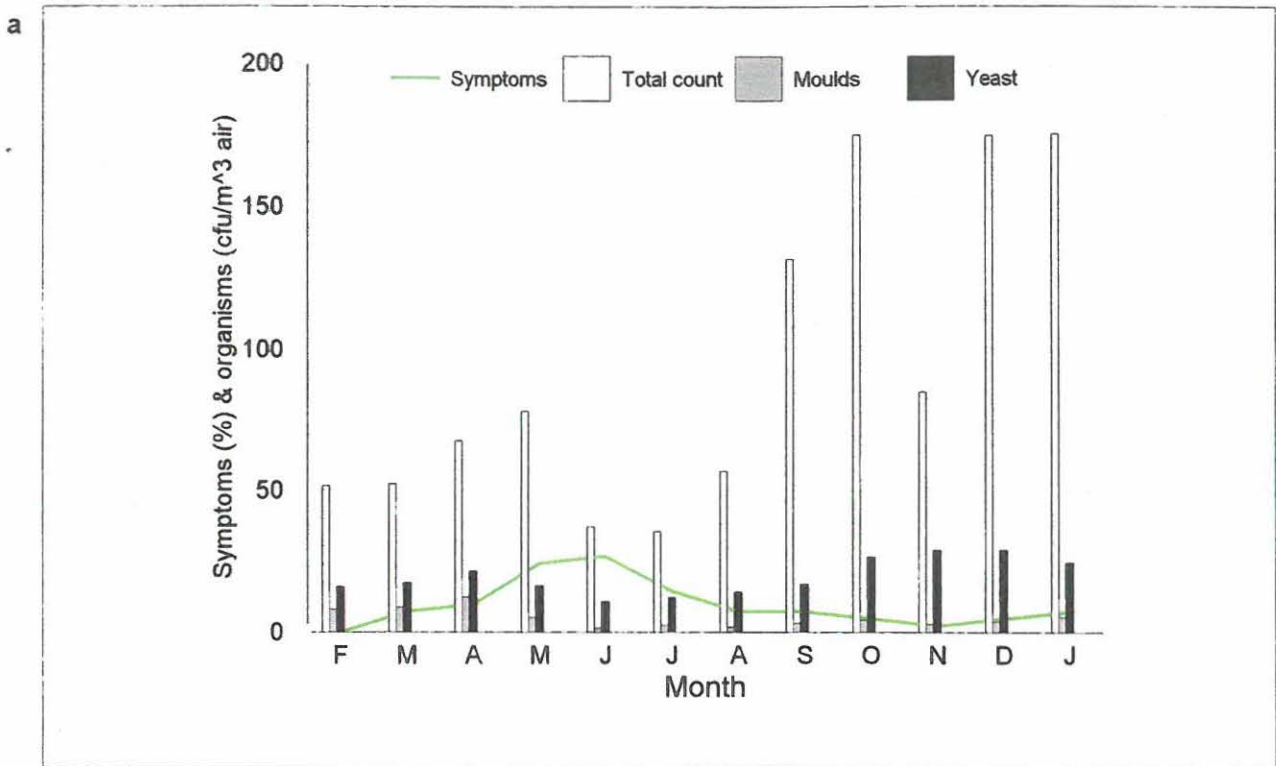
Figure 24 a) Influenza symptoms **b)** Hay fever symptoms, experiemced by building occupants as well as corresponding micro-organisms, found in building B over a 12-month period.
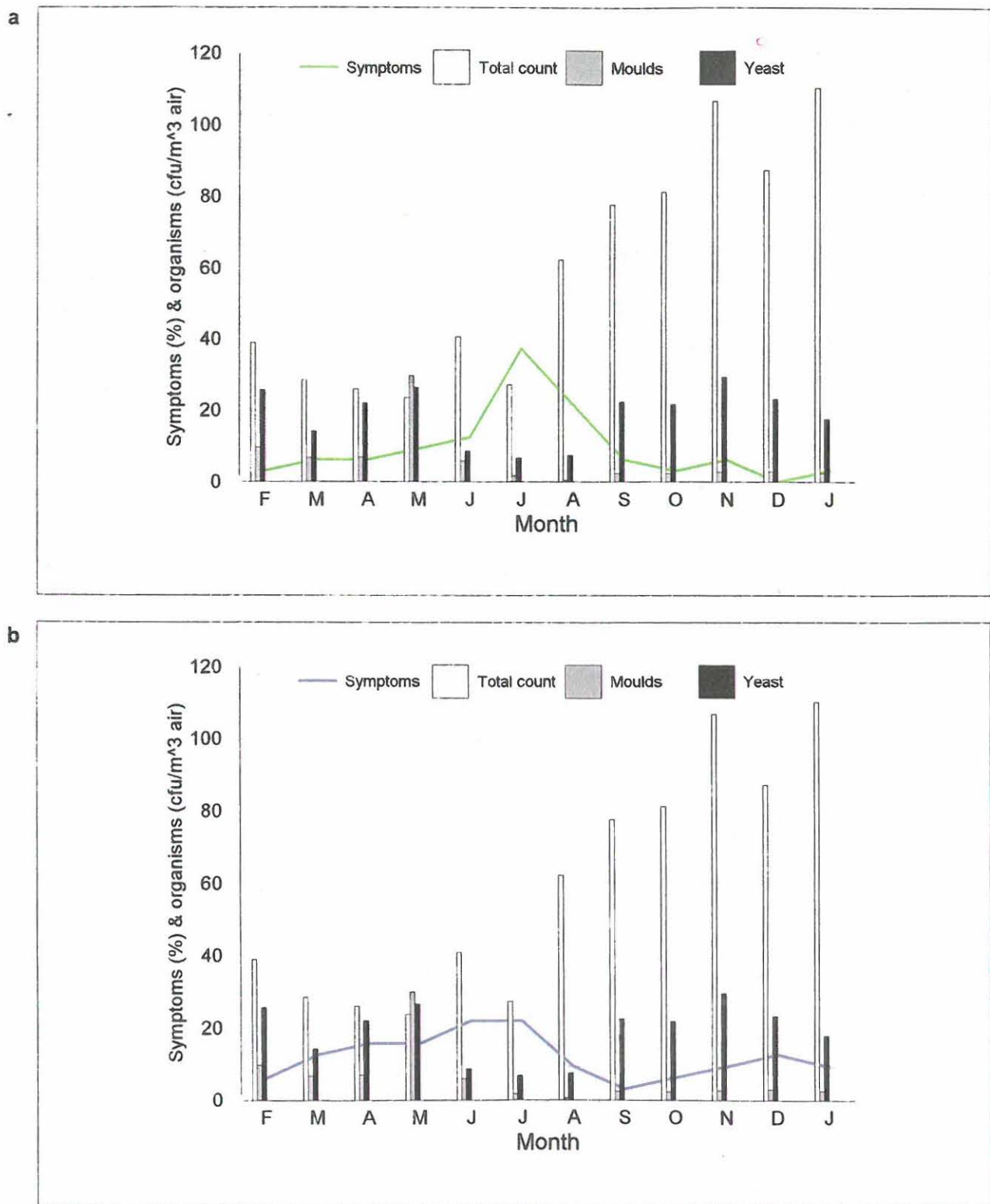
In building C, a total microbial count of 141 cfu/m³ was calculated during the sampling period, with the highest count of 222 cfu/m³ found during November. According to the responses to the questionnaires, 23% and 46% of the occupants suffered from influenza symptoms during June and July, respectively (Figure 25a). In the months of March and October, when mould counts were relatively high, the complaints of influenza symptoms showed a slight increase. During the 12-month sampling period, an average mould count of 10 cfu/m³ was obtained, with the highest count of 11 cfu/m³ found during October, compared to an average yeast count of 37 cfu/m³. The highest yeast count in this building was found during November (58 cfu/m³). No *Pseudomonas* spp. were found in building C throughout the sampling period. During the month of July, when complaints of hay fever symptoms reached a peak, yeast counts were relatively high, similar to the observations made with regard to influenza symptoms. The occurrence of hay fever symptoms, illustrated in Figure 25b, included a runny nose and sore throat. It was reported that 38.4% of the respondents suffered from these symptoms during the months of July and August.

The microbial analyses of data derived from building D revealed total microbial count of 27 cfu/m³ air, while the highest total count of 175 cfu/m³ was obtained during October. In this building, 20% of the respondents complained of a sore throat or runny nose during July and August

(Figure 26a). During May and August, 13.3% of the respondents experienced influenza symptoms. During April, June and July, 6.7% of the respondents suffered from influenza symptoms, while no influenza symptoms were experienced during the other months. (Figure 26b). An average mould count of 2 cfu/m³, and 8 cfu/m³ yeasts, were enumerated during the sampling period in this building, with the highest mould count of 13 cfu/m³ counted during April and November. The highest yeast count throughout the 12-month sampling period, was 30 cfu/m³ (Figure 17c). In October, yeast counts in building D showed an increase, corresponding to an increase in hay fever symptoms. From Figure 26 it is apparent that very few complaints of symptoms were received and that low numbers of the sampled organisms were found.

Figure 25 a) Influenza symptoms **b)** Hay fever symptoms, experienced by building occupants as well as corresponding micro-organisms, found in building C over a 12-month period
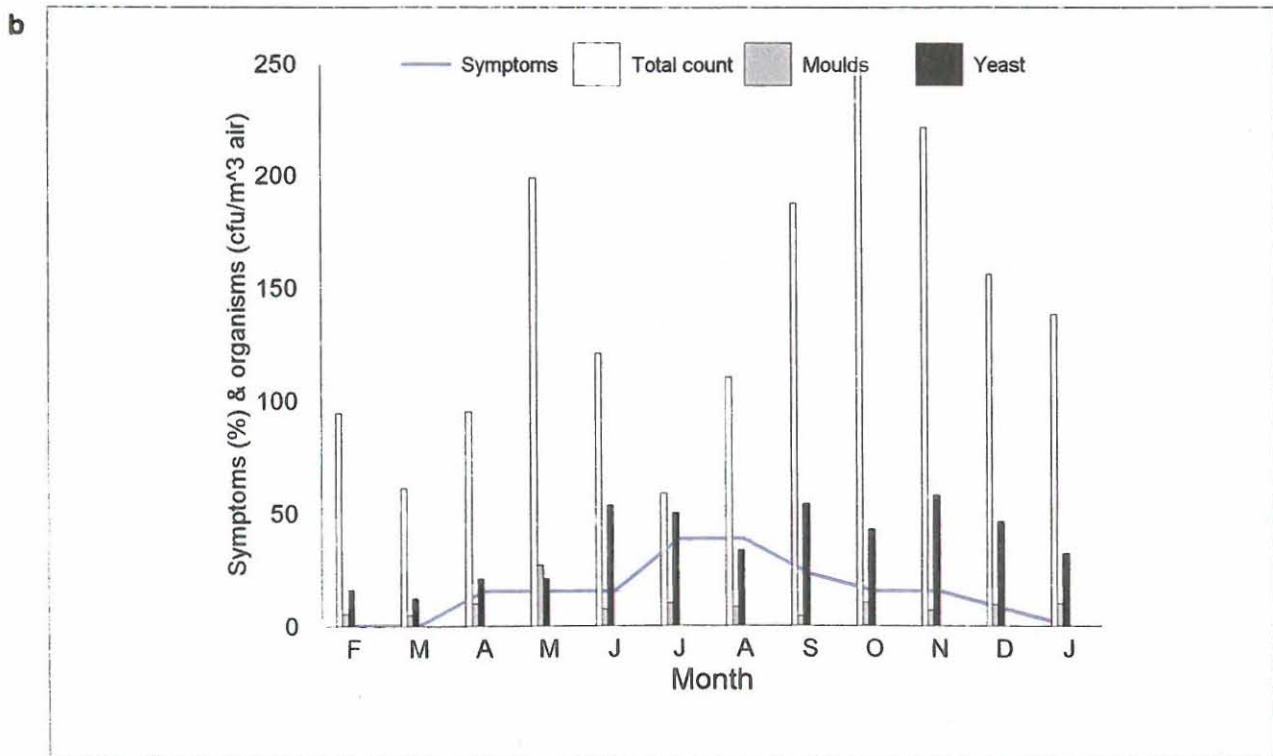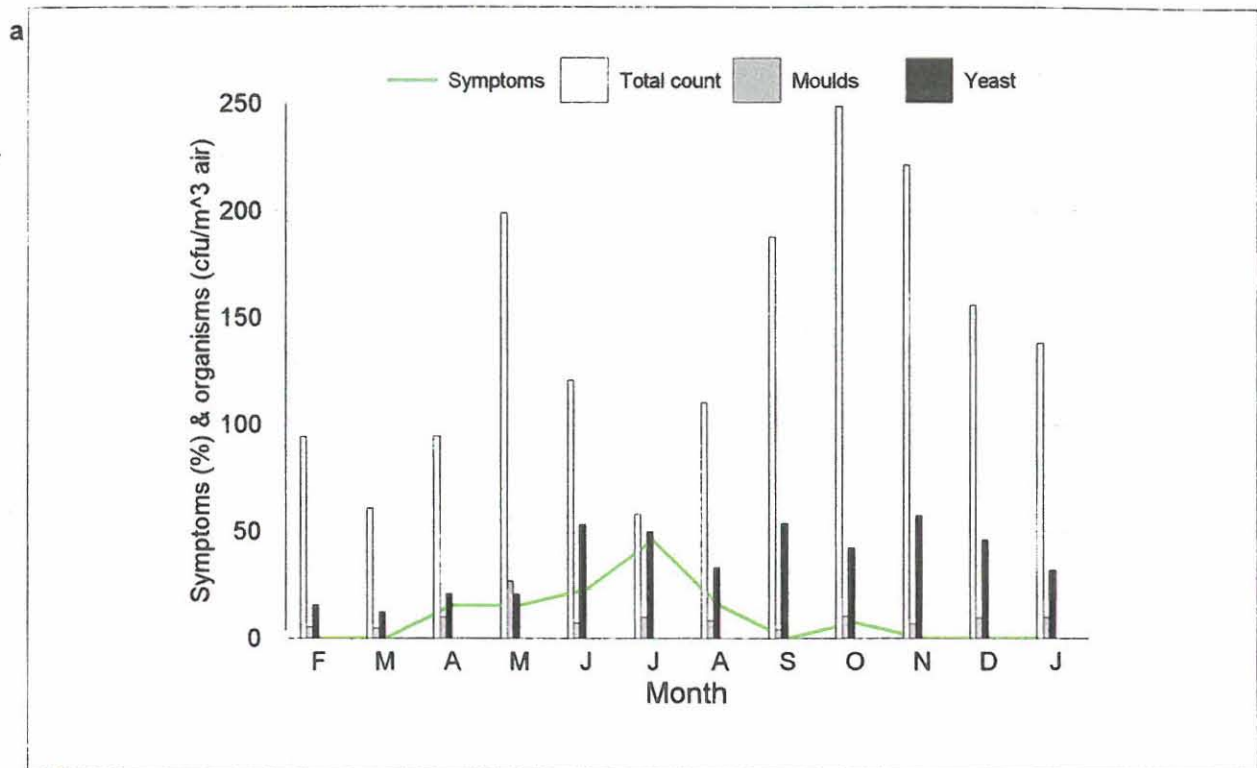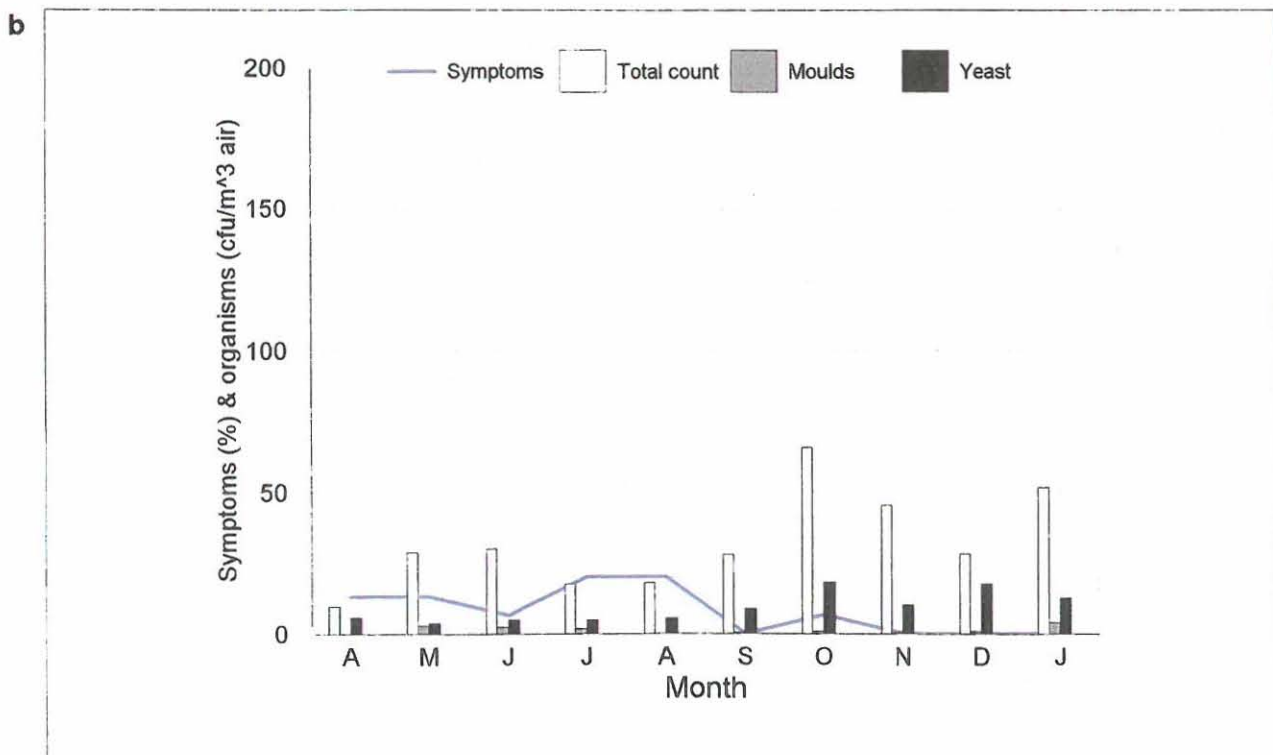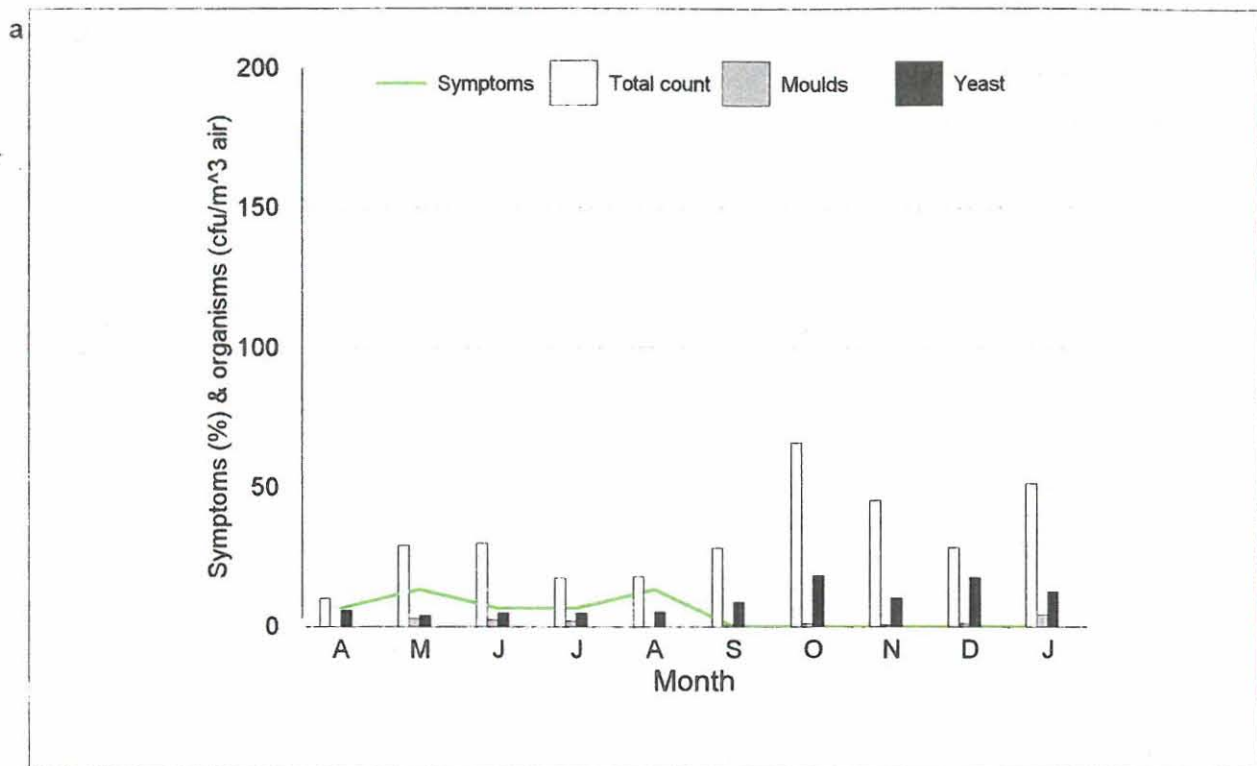
Figure 26 a) Influenza symptoms **b)** Hay fever symptoms, experienced by building occupants as well as corresponding micro-organisms, found in building D over a 10-month period

Correlations were calculated between selected variables in the study to investigate the influence of these aspects (open office windows, organism counts and respiratory-related related symptoms) on each other. Although not shown in the table a correlation of 0.481 was obtained between the average total microbial count (cfu/m^3) and those occupants who opened their office windows. Correlations between temperature readings and organism counts revealed littel useful information because of the very slight fluctuations in temperatures observed in the buildings. Temperatures in buildings A, B and D especially, were very constant, while some notable fluctuations occurred in building C (Figure 18). Table 7 represents the relationship found between the various organisms and selected respiratory-related symptoms experienced by the occupants.

Table 7 Correlations calculated between respiratory-related symptoms as experienced by the office occupants, and the average counts of the various organisms

Respiratory symptoms	Correlation (r)
Total counts	0.3076
Moulds	0.2702
Yeasts	0.2928

All correlations obtained between respiratory-related symptoms and the sampled organisms were positive, though negligible.

3.4.2 Discussion of comparative analysis

According to the results, it appeared that most of the occupants of building A suffered from influenza symptoms during the winter months, as expected. Moulds and yeasts were not found in significant numbers during this period in building A. During June, when the mould and yeast counts were at their lowest, 26.8% of the respondents from building A complained of influenza symptoms. The highest total microbial counts were found during the warmer summer months, when the influenza symptoms experienced by the occupants were very low. It can therefore be concluded that these

organisms did not solely provoke the mentioned symptoms in the occupants of this building. During June, some of the occupants also complained of hay fever. These symptoms decreased during the remaining winter months and showed an increase in the summer months (December through March). It was suspected that some respondents did not clearly distinguish between influenza symptoms and hay fever symptoms, although clearly emphasised in the questionnaires. As expected, hay fever symptoms were reported continuously throughout summer, when higher total microbial counts were observed.

In building B, the occupants also experienced influenza symptoms during the winter months, especially during June, July and August when a high percentage of occupants reported influenza symptoms. During these months, relatively high total microbial counts were observed, but not as high as the total counts during November and January. It would appear, from our data, that higher temperatures are apparently favoured by these organisms as fewer influenza symptoms occurred during the warmer months. Only during September and October did the occupants have fewer complaints of hay fever symptoms. During this time, the mould counts were very low. Thus, it is suspected that moulds contributed to some extent to the hay fever symptoms experienced in the occupants of this building. Yeast counts showed a distinct decline during the summer months.

In building C, high total microbial counts were obtained during May, October and November, with a slight seasonal tendency in the distribution. It could therefore be concluded that these organisms were sensitive to lower temperatures, implicating that less organisms were present during the colder months and consequently the effect on occupants health was reduced. No hay fever symptoms were reported by the occupants of this building during the months of February and January. As expected, a high incidence of influenza symptoms was observed during the winter months of June and July. In late summer, lower yeast counts were detected. The distribution of moulds showed no seasonal tendency.

In building D, all the counts appeared to be relatively lower than in the other buildings. This could mean that the HVAC system in this building was more effective in filtering outside air, or the air replacement rate was higher. The fact that no organisms could enter through open windows in this building might also have contributed to the lower organism counts. High total counts were found during the summer months of October, November and January. Unlike in the other buildings, 13.3% of the occupants, suffered from influenza symptoms in May and August. Mould counts appeared higher during the warmer months, and in general, the yeast count showed the same trend in distribution. As the hay fever symptoms started to increase at the beginning of the year, a corresponding increase in total counts was also observed. No hay fever symptoms were noted during November, December and January, but the incidence of symptoms soared in April, March, July and August.

The correlation between the organism counts and respiratory-related symptoms reported by the occupants revealed a positive, but negligible correlation between the total counts. It would therefore seem that these organisms would only have a very small effect on the health of the building occupants.

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CHAPTER 4
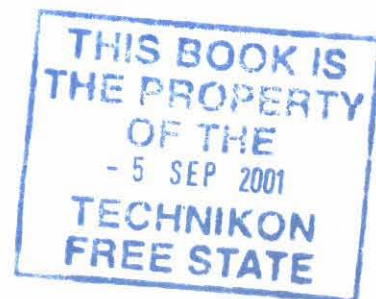
CONCLUSION

4.1 Concluding remarks

Health profile

The health profile obtained from the questionnaires indicated that many of the occupants did not feel comfortable as far as temperatures in their work environment were concerned. Occupants were also regularly absent from work due to respiratory-related symptoms. This resulted in reduced productivity and higher work stress on the remaining work force. It is important to note that the occurrence of these symptoms did not necessarily result in people taking time off from work, but they could leave early or feel incapacitated, leading to lower productivity. The high occurrence of influenza symptoms found among the occupants during the winter months is a normal occurrence found throughout the population of the study area in general. The frequency of complaints regarding influenza symptoms, thus did not justify over-concern. It could be suspected that the agents responsible for these symptoms could very well be distributed primarily by the occupants of the building and not necessarily by the HVAC system.

Relatively high prevalence of hay fever symptoms occurred during the 12-month sampling period, could possibly be explained by the fact that the responsible agents were always present in the study environments. Thus, some workers in the sampled buildings always suffered from these symptoms. Occupants uncomfortable in their work environments because of the room temperature, could probably have been more prone to complaining, and more sensitive to foreign agents such as dust or micro-organisms, making it hard to judge whether complaints about respiratory symptoms were real or just imaginary. Where possible, some occupants tried to prevent air from the air-supply units from entering their offices. They believed that the cold air was responsible for causing these illness. The possibility exists that

contaminated air was allowed to enter ...., but on the other hand, a high airflow rate could have diluted any contamination which might have been present in the indoor environment.

The large number of employees that suffered from dry noses at work could be ascribed to low relative humidity or a high airflow rate in the sampled environment. A high airflow rate could disturb and distribute some of the organisms which accumulated on structures, resulting in a harmful effect on the health of occupants in the environment. A large number of occupants also complained of dry or gritty eyes at work. Again, this could be attributed to dust and other particles in the surrounding air, let in through the HVAC system and affecting the occupants. When it is taken into consideration that each working day consists of eight hours, the loss of several working days due to respiratory-related illness undoubtedly influenced the productivity of workers in these buildings. Although it cannot be stated unequivocally that all of these illnesses or symptoms occurred as a direct result of the micro-organisms present in the indoor environment, they certainly made a significant contribution.

Microbial profile

The sampling period of this study ran over 12 months to establish a seasonal distribution for the sampled micro-organisms. Temperature readings obtained from every sampling location did not reveal great variation, because indoor temperatures were controlled by the HVAC systems. However, some temperature fluctuations were observed in building C, which made the work environment uncomfortable at times. This showed that indoor temperature was not as well controlled in building C as it was in the other three buildings, which resulted in temperatures below 10°C and possibly a decline in micro-organisms during the winter months, as well as occupant discomfort. The higher total counts in three of the sampled buildings, A, B and C, could probably be ascribed to effective maintenance of the HVAC system in building D, the younger age of the building itself, or the use of much less return air (10% in winter)

compared to the other building (building D and C). Thus, the fewer organisms in building D should also have a less pronounced effect on occupants' health.

Higher mould counts in building C could be ascribed to due to water aerosols entering directly into the indoor air from the HVAC system. Moulds could accumulate on the evaporator mesh and/or filter, be dislodged through the fanned air and ventilated into the indoor environment. Although mould counts increased in building D in summer, when no return air was used, this observation could not be attributed to the HVAC system, but rather to the higher temperatures which probably stimulated mould growth in the indoor environment. Yeast counts found in building C could be ascribed to contaminated water droplets being let into the building via the air-conditioning system and accumulating indoors. Building D revealed the lowest yeast counts, indicating effective filtration of outside air as well as proper maintenance of the HVAC system. Another contributing factor might have been the younger age of the building D, resulting in a low accumulation of yeasts in this building. Furthermore, yeast counts showed a distinct seasonal distribution.

Although, *Pseudomonas* spp., which can cause pneumonia in humans, were not found in the surrounding air of any of the buildings, these organisms were isolated from the water samples from the air-supply units at building C. It could therefore be concluded that these organisms, present in the water, were not successfully transmitted to the surrounding air of this building., There existed a possibility of these contaminants being transferred to the surrounding indoor air, via water droplets in building C, as the numbers obtained for total bacteria counts in water exceeded the set guidelines for contamination. Furthermore, high yeast counts were also obtained. Analysis of water samples showed the presence of moulds, yeasts, *Pseudomonas*, as well as bacteria. The high counts obtained for air samples in this building could thus, at least partly, be attributed to contamination of cooling unit water.

Correlations between open windows in the sampled offices and the organism counts, revealed that two of the sampled buildings have possible microbiological problems, as a result of this, which could influence the health of the occupants of those buildings. Calculated correlations suggest that moulds or their spores could be carried over from the water into the indoor environment by the HVAC system used in building C. These organisms might find a suitable environment indoors, accumulate even further, and contaminate the surrounding air. Correlations between total counts and yeasts found in water and air, did not reveal a positive relationship and it would therefore seem that these organisms were not carried over from the water into the indoor environment. Organisms found with air sampling probably accumulated indoors after entering via alternative routes. In buildings A, B and D, where relative effective filtration of outside air occurred, it appeared that opening windows might allow organisms to enter (buildings A and B), while in building D, where no windows could be opened, organism counts were lower. Thus, presuming that filtration was done effectively, that occupants opening windows nullified the filtration of outside air.

Comparative analyses

The sampled organisms were probably not responsible for the influenza symptoms suffered by the occupants of the buildings, because a high incidence of complaints about influenza symptoms was observed during the winter months, while lower organism counts were generally found during these colder months. Total counts decreased during the winter months, revealing the sensitivity of these organisms to lower temperatures and, therefore, these organisms had a smaller influence on the occupants' health. Higher total counts were observed in summer when, in general, less symptoms were reported. The occurrence of hay fever symptoms throughout the 12-month period indicated that the responsible agents were constantly present in the indoor environment. In buildings A and B, the occupants reported hay fever symptoms during every sampled month. This indicated, that agents causing hay fever affected at least one worker every month during the 12 months.

Total bacterial counts found in the surrounding air of building A did not exceed levels regarded as moderate growth but at times, heavy to very heavy growth (>300 cfu/m^3) was found. This could possibly explain some respiratory-related symptoms experienced by the occupants. Total bacterial counts found in building B did not exceed levels set for moderate growth by the manufacturer of the microbiological sampler. Only one count of >300 cfu/m^3 was found. Thus, total bacteria in this building probably did not affect the occupants' health. The total bacterial counts found in the surrounding air of building C did at times exceed levels recommended to be regarded as moderate growth (>300 cfu/m^3). It is possible that these numbers of organisms could affect the health of occupants. Total bacterial counts found in building D never exceeded levels recommended as exceed levels set for moderate growth (61-300 colony forming units), during the sampling period indicating the effectiveness of the HVAC system in controlling indoor air contaminants through filtration, airflow rate and general maintenance. These low counts could also explain the fewer complaints of respiratory-related symptoms in comparison to the other sampled buildings, possibly due to the younger age and effective maintenance of the HVAC system of building D.

Although building B did not reveal the highest microbial counts, occupants of this building reportedly suffered the most respiratory-related symptoms. It could be possible that some other micro-organisms not sampled in this study, were responsible for provoking some of the symptoms, and that the percentage recycled air also played a significant role in the occurrence of respiratory-related symptoms.

According to the standards set by the manufacturer of the SAS Super 90, buildings B and D did not appear to have microbiological problems which contributed to respiratory-related symptoms in occupants. Buildings A and C did show the highest average total count over the 12-month period, ranging from heavy to very heavy growth obtained from the air samples.

This indicate that these two buildings might have microbiological problems, which could have been detrimental to the health of building occupants.

It is possible that organisms entering buildings via the HVAC system may settle in the indoor environment and accumulate on various surfaces and are not inhaled in by occupants (or aspirated by the air-sampler). However, when the accumulated organisms are in some way disturbed and allowed to be released into the surrounding air, respiratory-related symptoms could develop when these harmful organisms are inhaled. This theory could explain the poor correlation found between air and water samples in building C. From the analyses of all the results, it seems apparent that some HVAC systems are more effective in preventing micro-organisms from entering indoor work environments. Factors like HVAC system maintenance and building properties (non-opening windows) could also have an influence on micro-organism quantities.

4.2 Recommendations

Several remedial actions can be recommended, depending on the nature and source of the problem. These must be established through an extensive study targeting the microbiological population to species level, and probably expanding the study area to other cities, with reference to specific pathogenic air-borne contaminants. The nature of the problem, such as low airflow rate, uncomfortable temperatures or biological contamination, will guide the remedial action. General control and effective maintenance of the HVAC system could reduce and even prevent the aforementioned problems to a certain extent, depending on the growth requirements of the microbial contaminants.

The following are suggested methods of improving the indoor air quality and could possibly be applied in at least building C of this study:

a) Access to outdoor contamination should be limited as far as possible in order to prevent biological contaminants from entering the indoor environment, which means that doors and windows should be kept closed as much as possible. In building D no windows can open, and only doors on the ground floor are left open, allowing unfiltered air into the building. Keeping such doors closed with automatic closing mechanisms could improve the situation significantly. Positive air pressure, achieved with properly filtered air, could also be employed.

b) If all the indoor air cannot be replaced, adequately filtered air should be let into the indoor environment to adequately dilute the aerosols present in the indoor air. In practice this may be hard to regulate, because the extent of indoor contamination will determine the quantity of filtered air needed to dilute the aerosols to acceptable levels.

c) Outdoor air allowed into indoor environments should be adequately filtered. The most effective filter for the system should be used according to the specifications, and it should be replaced or washed as frequently and in the manner recommended by the manufacturer.

d) Filters of air supply systems should be cleaned or replaced at regular intervals. Filters not suitable to be cleaned, should not be washed but replaced. Filters used for longer than the specified period of time cannot be considered effective.

e) In addition to preventing contaminants from entering the indoor environment, the multiplication of micro-organisms should be averted or at least controlled. Areas where dust or water may accumulate (window-sills, under pot plants) should be cleaned and disinfected on a

regular basis. Existing contamination should be removed as soon as it is found and the area cleaned with a very effective disinfectant.

f) Humidity should be maintained at < 60% at all times, and the intrusion of moisture should be prevented. Regulating humidity in indoor environments will, to a certain extent, deny micro-organisms much-needed moisture in these buildings. This could be achieved by frequently monitoring humidity levels in buildings.

g) Where biological contamination is suspected as in building A and C, all hard surfaces should be disinfected and dried. This will remove all dust particles and other harmful contaminants, thus preventing these from entering the surrounding air.

h) Carpets and curtains should be removed and effectively cleaned on a regular basis. This should be done in all the buildings especially A, B and C. Although this may be expensive in practice, it will remove contaminants, preventing them from entering the surrounding air and possibly affecting occupants' health.

i) Air inlets into buildings should not be placed in locations close to possible contaminants like dust or gases. Inlets at street level are not desirable. Inlets of buildings A and B are at street level, possibly letting in more particles than inlets on a roof, as in building D. Inlets at ground level may cause filters to clog sooner than anticipated. These inlets and filters should thus be cleaned more frequently.

j) Maintenance of the ventilation system should as far as possible be carried out when the occupants of the buildings are not present, and the system should be cleaned thoroughly. Doing this will prevent particles from escaping from ducts and filters during maintenance causing harm to occupants.

k) According to Wilson (1987) a qualified, well-trained person should be in charge of the day-to-day operation and maintenance of the HVAC system. In buildings A, B and D, personnel are on duty to handle the day-to-day operation of the HVAC system of the respective buildings. At building C, only maintenance when needed and some routine inspections are done.

l) Water leakage should be repaired promptly, because water may provide a suitable breeding-ground for micro-organisms (SMACNA, 1993). This could be more of a problem in building C and should thus be more closely maintained.

m) The air ducts should be cleaned from dust and other particles. Although this may be difficult to do in practice, it would ensure that particles settling in ducts would not be let into the indoor environment. This is especially important where no filtration occurs in the office units as in building D.

n) Overpopulation of offices should be prevented (Larsen, 1995). Offices in building A and B were found to be somewhat overpopulated. According to (Larsen, 1995) this should be prevented as overpopulation would not only result in fewer organisms being dispersed into the air due to human activity, but furniture in an overpopulated office could cause difficulty during cleaning of the office.

o) Housekeeping in all the buildings should be done adequately to remove dust and other dirt.

p) Restructuring of office interiors should be done when the occupants are not present, or should in some way be covered to prevent the distribution of dust and other particles. Dust

and harmful particles may be distur___ ___ ___ ___ into the surrounding air, negatively affecting the health of occupants.

4.3 Future research

Future research could include the following:

1) An assessment of air-borne pathogens associated with the mentioned indoor environments too spesieslevel.

2) An estimation of the effect of overpopulation of offices on the quantity and composition of micro-organism populations and the consequent increase in health problems.

3) An assessment of the effect of frequent and thorough housekeeping on the quantity and composition of microbial populations in office environments.

4) An survey on the economical implications of air-borne micro-organisms on productivity in office buildings.

5) Expanding the study with regard to microbial populations monitored and enlargement of the sample area to include more buildings and possibly other cities.

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Appendix A

Figure 1 Locations of the four studied buildings in the city of Bloemfontein.
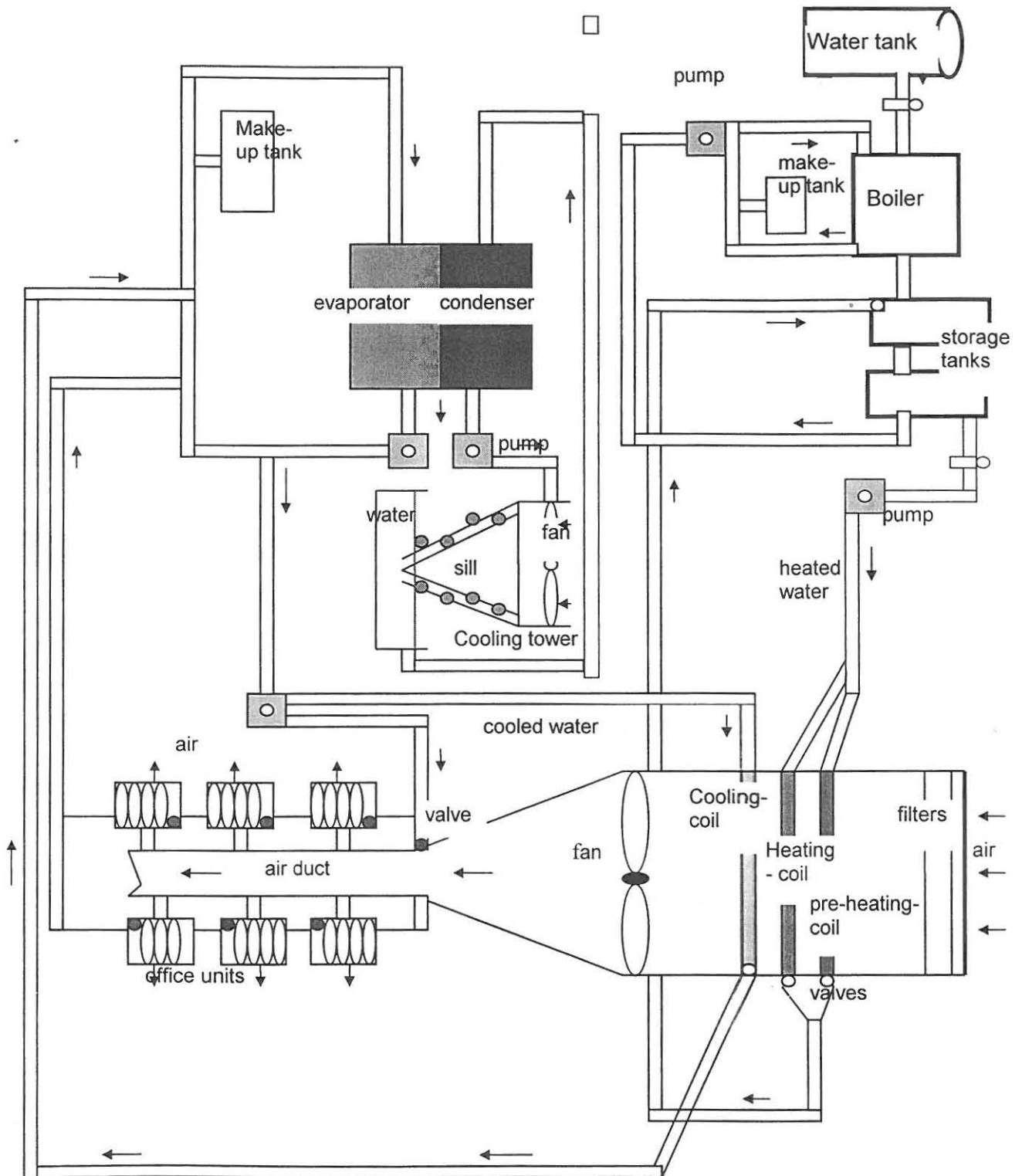
Figure 2 Heating, ventilation and air-conditioning system as used in building A, 100% fresh air.

boiler

pump ←

water
tank

make-up
tank

Insulated
storage
tank

pump →

inlet 20%

filters

Air chamber

pre-heating-coil

Heating-coil

Cooling- coil

80% return
air

fan

pump

pump

valve

evaporato   condense

make-up
tank

pump

cooling tower

sill

fan

valve

extractor
fan

valve

office
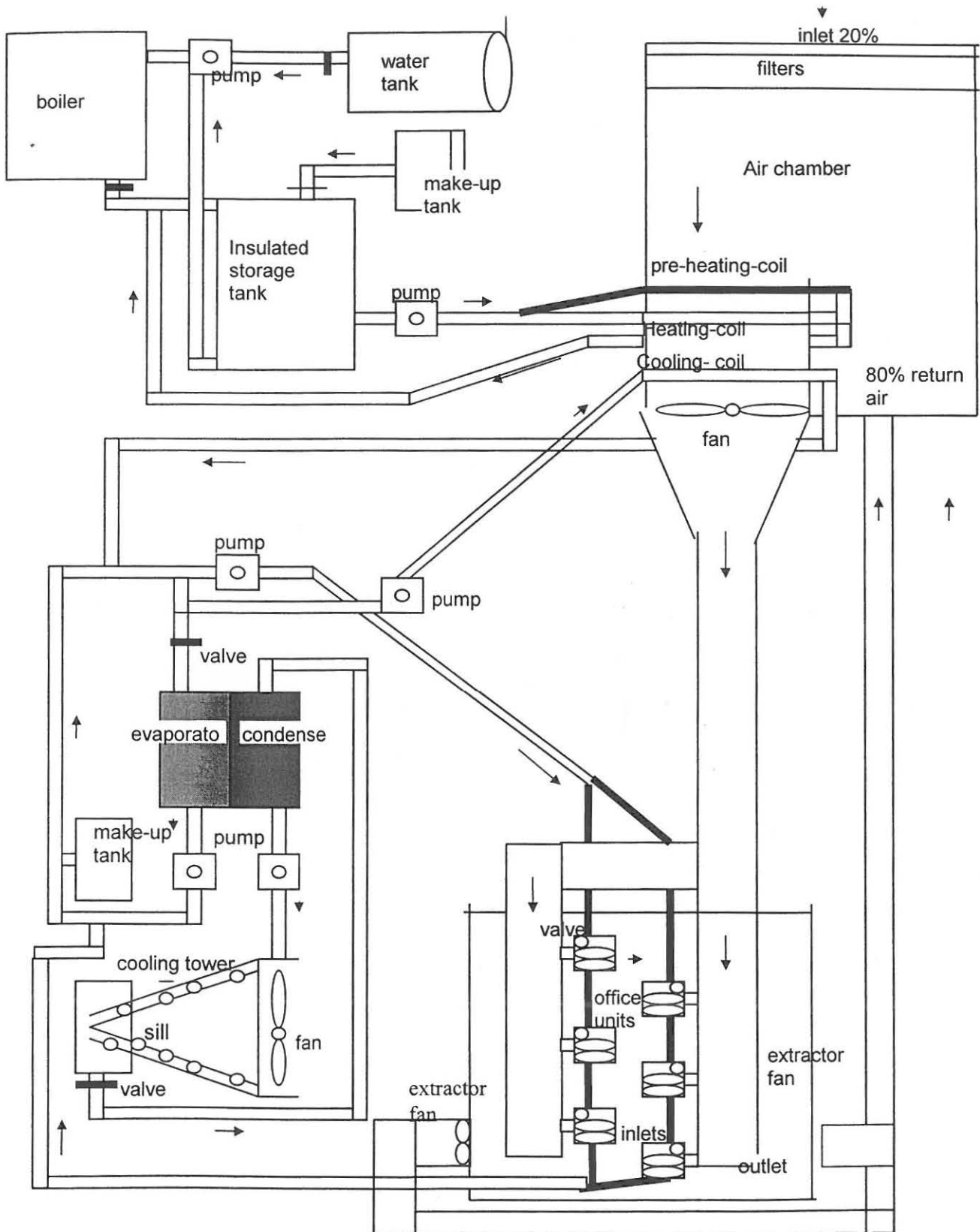units

inlets

extractor
fan

outlet

Figure 3 Heating, ventilation and air-conditioning systems as used in building B, 80% return air
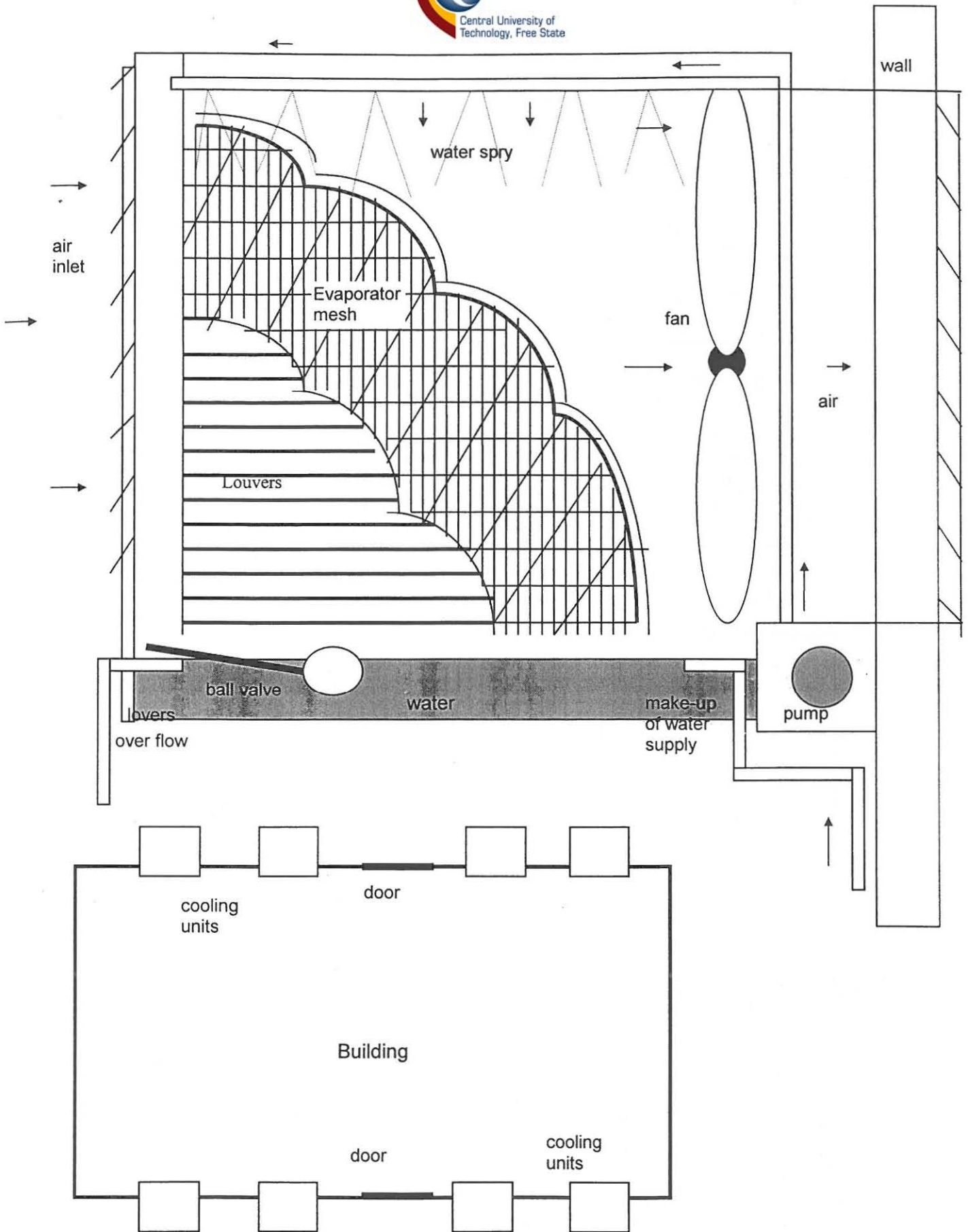
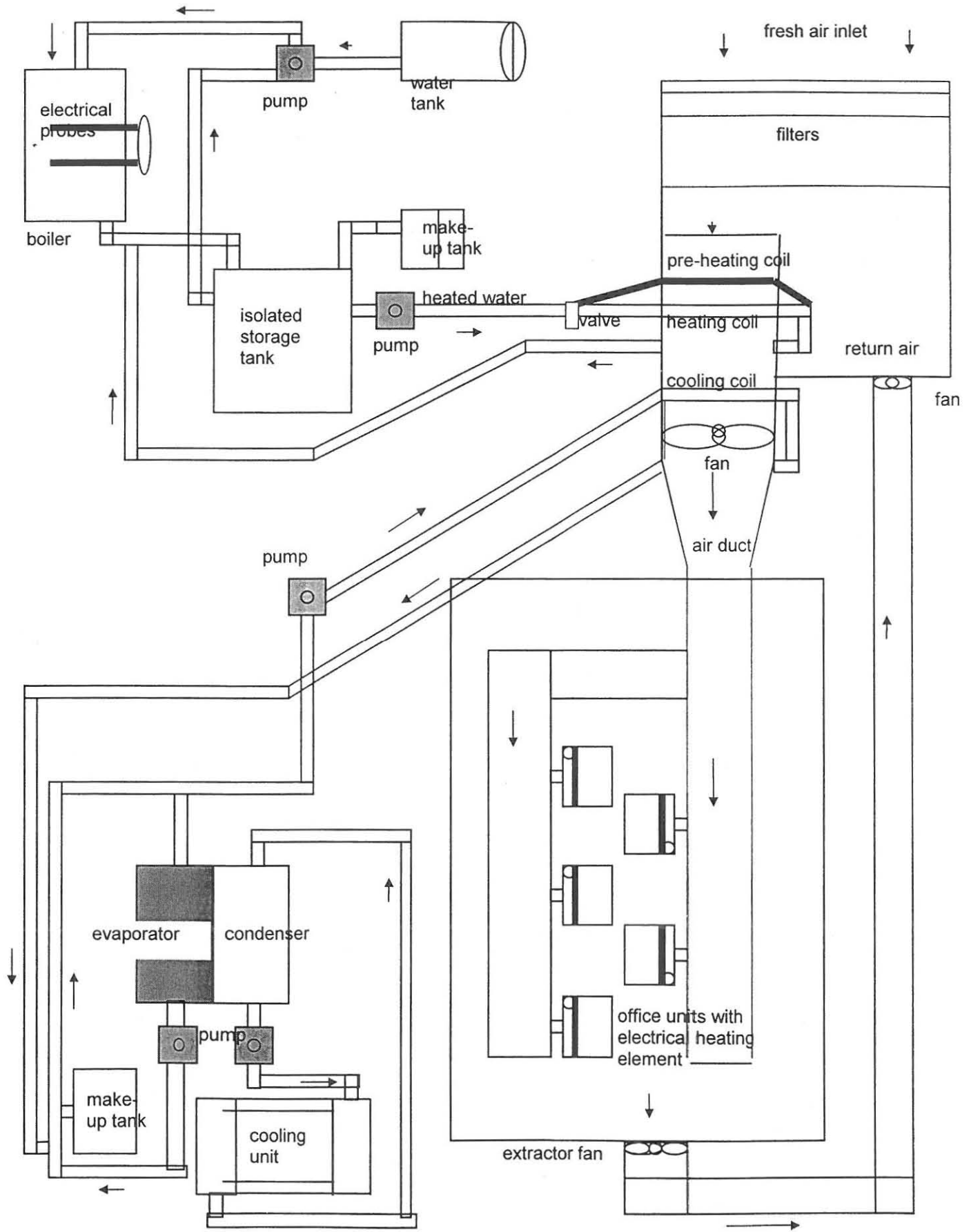**Figure 4** Cooling units as used in building C; bottom drawing, outlay of building

Figure 5 Heating ventilation and air-conditioning system used in building D.

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Appendix C

Occupational Health Questionnaire

INSTRUCTIONS: Mark the applicable with an "X" where applicable.
Answer the other questions as concise as possible.

1. General

1.1 Date

1.2 Name of building?

1.3 On which floor of the building do you work?

1.4 For how long have you been working here?

1.5 Your age?

1.6 Male   Female

2. Work environment

2.1 What are your normal working hours?

2.2 Does the window in your office face   North   or South

2.3 Do you share the office with other persons?   Yes/ No

2.3.1   If you answered yes, with how many do you share?

2.4 Do you open the windows of your office ?

Never

Seldom

Regularly

Always

2.5 Is anything placed over/in front of the air-inlet in your office that may obstruct   Yes/ No
the flow of air?

2.5.1 If you answered yes, what is placed
over/in front of the air-inlet?

And why?

2.6 Do you find the temperature in your office comfortable?

In summer?   Yes / No
In winter?

Yes / No

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3. State of Health

3.1 Do you often suffer from colds and influenza? | Yes / No |

3.2 Did you have a cold since the beginning of this year? | Yes / No | If yes, how often? [   ]

3.3 Do you suffer from Hay fever/allergies? | Yes / No |

3.3.1   How often do you get Hay fever? [   ]

3.4 Do you sometimes have a sore throat at work? | Yes / No |

3.4.1   If yes, how often does it happen? [   ]

3.5 When at work, do you ever feel:

3.5.1   that your nose is blocked? | Yes / No |

3.5.2   that your nose is dry? | Yes / No |

3.5.3   that your nose is running? | Yes / No |

3.6 When at work, do you ever feel:

3.6.1   that your eyes are dry and gritty? | Yes / No |

3.6.2   that your eyes are watery? | Yes / No |

3.7 Were you, during the last 6 months, treated by a medical doctor for a cold, hay fever or

related symptoms? | Yes / No |

3.7.1   If yes, what were the symptoms? [   ]

3.8 Did you take any medication during the last 6 months for colds or hay fever symptoms

(like a sore throat, sneezing or a blocked nose) ? | Yes / No |

3.8.1   If yes, for what symptoms? [   ]

3.9 Were you absent from work during the last 6 months with symptoms of a cold,

sinusitis or allergy? | Yes / No |

3.9.1   If yes, what were the symptoms? [   ]

3.9.2   For what period were you absent from work? [   ]

3.9.3   Were you absent more than once with these symptoms? [   ]

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4. History

4.1 Please mark in the relevant block with a cross if you had a cold during the month

July ☐

August ☐

September ☐

October ☐

November ☐

December ☐

January 1997 ☐

4.2 Mark in the relevant block if you had any hay fever symptoms during the month

July ☐

August ☐

September ☐

October ☐

November ☐

December ☐

January 1997 ☐

Thank you.

Figure 6 Example of total counts on a PCA-medium, obtained from air sampling.

Figure 7 Example of yeast and mould colonies on a PDA-medium, obtained from air sampling

Table 2 Conversion table, for converting counted colonies into a probable count

r	Pr	r	Pr	r	Pr	r	Pr	r	Pr	r	Pr	r	Pr
1	1	32	34	62	73	92	119	122	178	158	278	194	471
2	2	33	36	63	74	93	121	123	180	159	282	195	480
3	3	34	37	64	76	94	122	124	182	160	286	196	489
4	4	35	38	65	77	95	124	125	185	161	289	197	499
5	5	36	39	66	78	96	126	126	187	162	293	198	508
6	6	37	40	67	80	97	128	127	189	163	297	199	519
7	7	38	42	68	81	98	130	128	192	164	301	200	530
8	8	39	43	69	83	99	131	129	194	165	305	201	542
9	9	40	44	70	84	100	133	130	196	166	309	202	554
10	10	41	45	71	86	101	135	131	199	167	313	203	567
11	11	42	46	72	87	102	137	132	201	168	317	204	580
12	12	43	48	73	88	103	139	133	204	169	322	205	595
13	13	44	49	74	90	104	141	134	206	170	326	206	611
14	14	45	50	75	92	105	142	135	209	171	331	207	627
15	15	46	51	76	93	106	144	136	212	172	335	208	646
16	17	47	53	77	95	107	146	137	214	173	340	209	666
17	18	48	54	78	96	108	148	138	217	174	344	210	687
18	19	49	55	79	98	109	150	139	220	175	349	211	712
19	20	50	57	80	99	110	152	140	222	176	354	212	739
20	21	51	58	81	101	111	154	141	225	177	359	213	770
21	22	52	59	82	102	112	156	142	228	178	365	214	807
22	23	53	60	83	104	113	158	143	231	179	370	215	851
23	24	54	62	84	106	114	160	144	234	180	375	216	905
24	25	55	63	85	107	115	162	145	237	181	381	217	978
25	26	56	64	86	109	116	165	146	240	182	387	218	1088
26	28	57	66	87	110	117	167	147	243	183	393	219	1307
27	29	58	67	88	112	118	169	148	246	184	399		
28	30	59	69	89	114	119	171	149	249	185	405		
29	31	60	70	90	116	120	173	150	252	186	412		
30	32	61	71	91	117	121	175	151	255	187	418		
								152	258	188	425		
								153	261	189	432		
								154	265	190	439		
								155	268	191	447		
								156	271	192	455		
								157	275	193	463		

r = colony forming units counted
Pr = probable count

Appendix F

Figure 8 Example of total counts obtained from water sampling
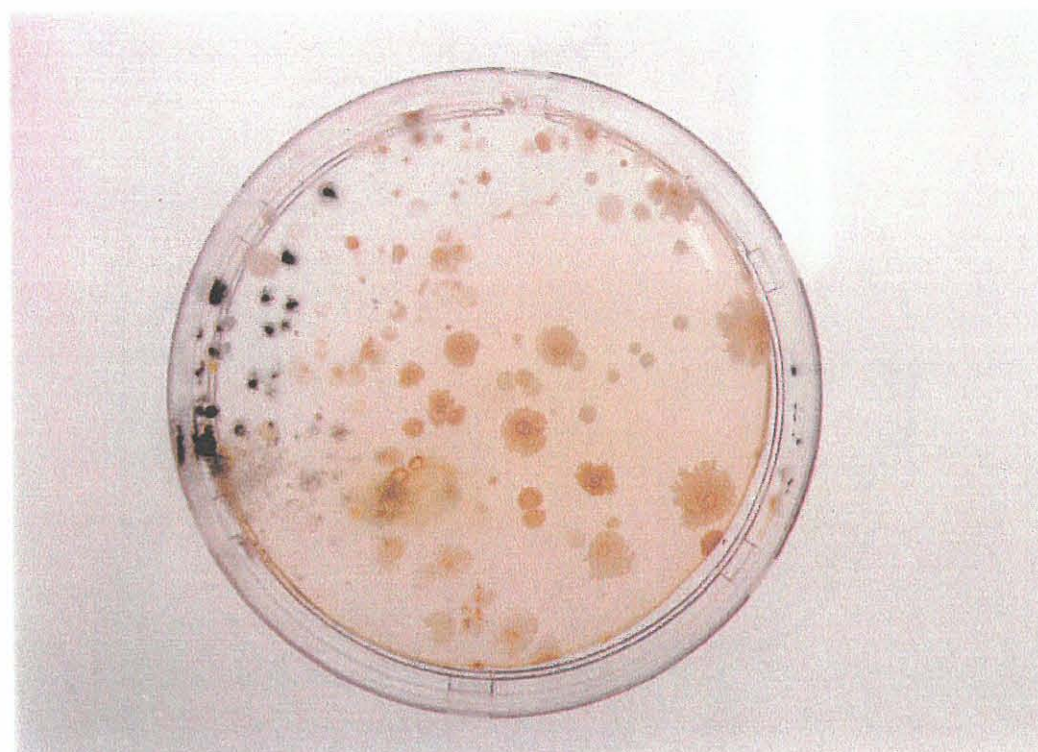

Figure 9 Example of yeast counts obtained from water sampling
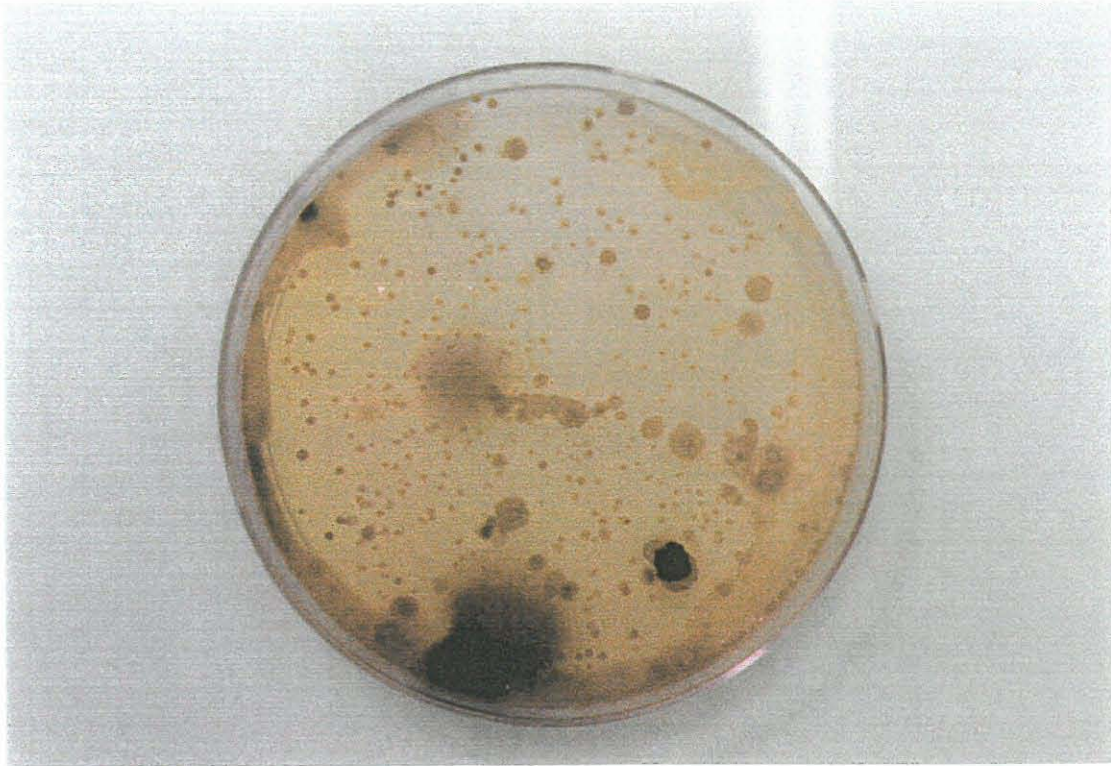
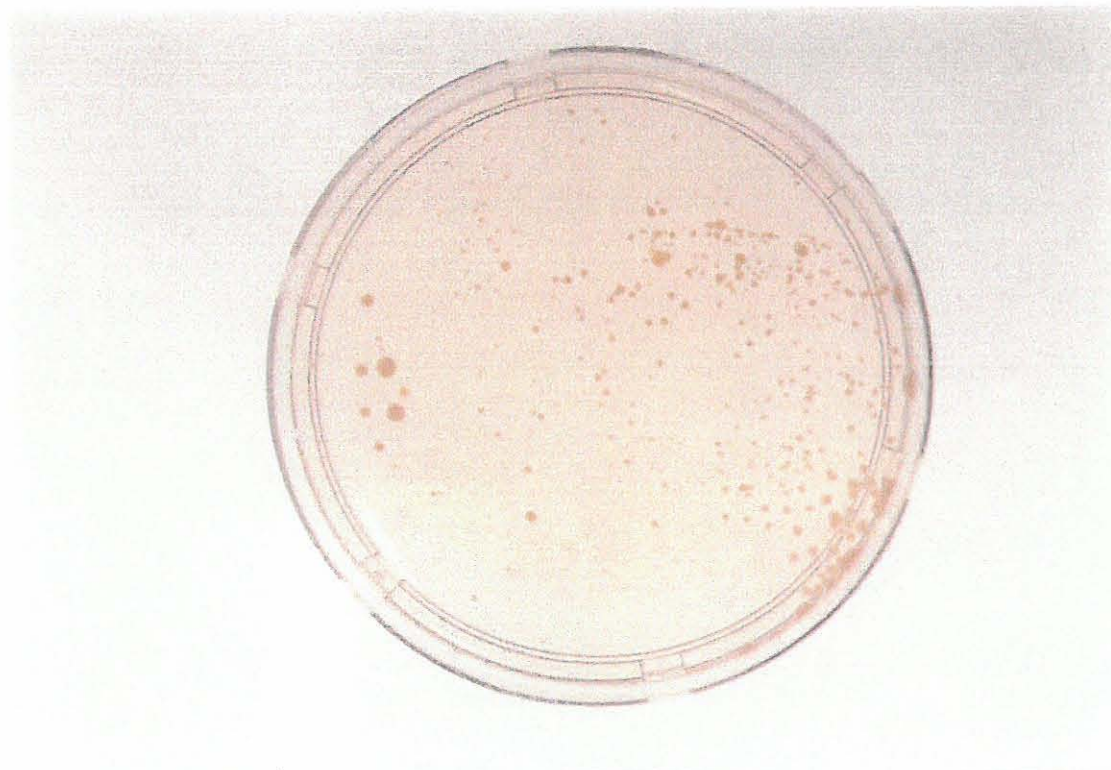**Figure 10** Example of mould colonies, obtained from water sampling

Figure 11 Example of Pseudomonas organisms obtained from water sampling.