A SURVEY OF THE BIOAEROSOL COMPOSITION AND RELATED ENVIRONMENTAL PARAMETERS IN A HIGH-THROUGHPUT CHICKEN PROCESSING FACILITY

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DECLARATION OF INDEPENDENT WORK

I, **MPELINYANA HOPEMORE RETHABISENG RASEPHEI**, do hereby declare that this research project submitted to the Central University of Technology, Free State for the degree MAGISTER TECHNOLOGIAE: ENVIRONMENTAL HEALTH is my own independent work and has not been submitted before to any institution by myself or any other person in fulfilment of the requirements for the attainment of any qualification.

.....

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02 March 2

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SUMMARY

A SURVEY OF THE BIOAEROSOL COMPOSITION AND RELATED ENVIRONMENTAL PARAMETERS IN A HIGH-THROUGHPUT CHICKEN PROCESSING FACILITY

Bioaerosols have been found in the majority of occupational environments, including poultry processing facilities, animal feeding houses and other meat processing plants. Due to the notable concentrations of bioaerosols indicated in such food processing locations, this study set out to investigate the presence of airborne microorganisms together with related environmental parameters in six processing areas (receiving/killing, defeathering, evisceration, air-chilling, packaging and dispatch) in a chicken abattoir. The aims of the study were to quantify and identify the microbial bioaerosols as well as to evaluate the influence of the selected environmental parameters thereon. Samples were collected at different localities as well as over various time intervals at a Grade-A abattoir situated in the city of Kroonstad, Free State Province. Microbial analyses were done using a SAS Bioaerosol Sampler (PBI International, Milan, Italy) through selective culturing procedures whereas the environmental parameters were analysed using calibrated direct reading instruments. Statistical analyses were performed using the StatSoft (Version 7.1) software. Results showed mean counts of 2.2×10^4 cfu.m^{-3} for

Staphylococcus aureus, 2.1 x 10^4 cfu.m^{-3} for fungi, 8.8 x 10^2 cfu.m^{-3} for total coliforms and 3.4 x 10^2 cfu.m^{-3} for *Escherichia coli* respectively. *Pseudomonas aeruginosa* and *Listeria monocytogenes* reached 2.4 x 10^3 cfu.m^{-3} and 1.7 x 10^3 cfu.m^{-3} respectively, and finally, *Bacillus cereus* and presumptive *Salmonella* spp presented counts of 4.0 x 10^3 cfu.m^{-3} and 3.5 x 10^3 cfu.m^{-3}. These counts were without exception within the infective dose limit and the legislative guidelines used as reference in this study, although explicit guidelines for bioaerosols in food processing areas have not yet been set in South Africa.

Relative humidity showed an average of 71% at a temperature of 18°C, and wind velocity and airborne dust particles were recorded as 7 m.s^{-1} and 3.76 mg.m^{-3} respectively. The inter-relationships between the bioaerosolised micro-organisms and the environmental parameters were additionally determined and strong correlations were noted between the occurrence of specifically *Bacillus cereus* and airflow, between *Pseudomonas aeruginosa* and relative humidity, and between the total coliforms and temperature respectively. Based on the findings of this study a number of recommendations were made to industry which include physical separation of the processing sections by material such as plastic curtaining to minimise the spread of micro-organisms, control of the environmental parameters that had a significant influence on the bioaerosols and colour categorisation as a means of representing the extent of the risk in each location.

OPSOMMING

'N ONDERSOEK NA DIE BIO-AËROSOL SAMESTELLING EN VERWANTE OMGEWINGSPARAMETERS IN 'N HOË-DEURSET HOENDERPROSESSERINGSFASILITEIT

Bio-aërosols is al in die meeste werksomgewings gevind, insluitende prosesseringsfasiliteite, dierevoedingshuise en ander vleis prosesseringsaanlegte. Vanweë die hoë konsentrasies van bio-aërosols in sodanige prosesseringslokaliteite, het hierdie studie ten doel gehad om die teenwoordigheid van luggedraagde mikro-organismes saam met verwante omgewingsparameters in ses prosesseringslokaliteite (ontvangs/doding, ontvering, die verwydering van ingewande, verkoeling van lug, verpakking en versending), te bestudeer. Die studie het ten doel gehad om die luggedraagde mikro-organismes te kwantifiseer en te identifiseer sowel as om die invloed van geselekteerde omgewingsparameters daarop te evalueer. Monsters is by verskillende prosesseringslokaliteite en oor verskeie tydsintervalle by 'n pluimvee-abattoir in Kroonstad, Vrystaat Provinsie versamel. Mikrobiologiese analises is met behulp van 'n SAS Bio-aërosol Monsternemer (PBI Internationaal, Milaan, Italië) uitgevoer deur middel van selektiewe kultiveringstegnieke. Die omgewingsparameters is met behulp van direkte leesintrumente bepaal. Statistiese verwerkings is met StatSoft (Weergawe 7.1) sagteware uitgevoer. Resultate het gemiddelde tellings van

2.2 x 10^4 cfu.m^{-3} vir *Staphylococcus aureus*, 2.1 x 10^4 cfu.m^{-3} vir totale fungi en 8.8 x 10^2 en 3.4 x 10^2 cfu.m^{-3} vir totale kolivorme en *Escherichia coli* onderskeidelik, getoon. *Pseudomonas aeruginosa* en *Listeria monocytogenes* het 2.4 x 10^3 cfu.m^{-3} en 1.7 x 10^3 cfu.m^{-3} onderskeidelik bereik en laastens het *Bacillus cereus* en waarskynlike *Salmonella*-tellings, 4.0 x 10^3 cfu.m^{-3} en 3.5 x 10^3 cfu.m^{-3} getoon. Die tellings was sonder uitsondering binne die infektiewe en wetlike riglyne wat as verwysings in die studie gebruik is, hoewel spesifieke riglyne vir bio-aërosols nog nie in Suid Afrika bestaan nie.

Relatiewe humiditeit het 'n gemiddeld van 71% bereik by 'n temperatuur van 18 ºC, en windsnelheid en luggedraagde stofdeeltjies, 7 m.s^{-1} en 3.76 mg.m^{-3} onderskeidelik. Die onderlinge verhoudings tussen mikro-organismes en die omgewingsparameters is ook bepaal en sterk korrelasies is aangeteken tussen *Bacillus cereus* en lugvloei, *Pseudomonas aeruginosa* en relatiewe humiditeit, en totale kolivorme en temperatuur onderskeidelik. Gebaseer op die bevindinge van hierdie studie is aanbevelings vir die bedryf gemaak en wel dat elke prosesseringslokaliteit fisies van die ander geskei moet word deur middel van byvoorbeeld 'n plastiekgordyn om sodoende die verspreiding van verskillende mikro-organismes te verminder, die beheer van omgewingsfaktore wat 'n beduidende invloed op die bio-aërosols het en dat kleurkodering in die verskillende prosesseringsopsette gebruik moet word as 'n manier om die omvang van die risiko in elke afdeling aan te toon.

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CHAPTER 1

INTRODUCTION

1.1 BACKDROP TO THE POULTRY INDUSTRY

During the first part of the 20th century, mechanical incubation made it possible to raise large numbers of chickens without having to depend entirely on hens (Menna and Mortola, 2002; Steve *et al.*, 2003; Moraes *et al.*, 2004). The discovery of Vitamin D in cod liver oil further made it possible to add Vitamin D in the chicken feed, rather than relying on sunshine. This allowed chicks to be grown in poultry houses for lengthy periods (Chennaiah *et al.*, 2004; Sanches *et al.*, 2004). The intense demand for poultry meat during World War II made poultry nutrition and the improvement of feed efficiency a highly emphasised research theme and as a result much attention was given to matters contributing to the poultry processing industry. This led to huge strides in the poultry industry, especially in improving management and slaughtering practices (Wilson *et al.*, 1987; Mekker, 1999; Brown and Adjei, 2001, South African Poultry Association, 2003).

In recent years, large facilities producing a variety of ready-to-cook poultry products have dominated the poultry slaughtering industry (Thaler, 1999). Thaler (1999) further mentioned that in 1967 less than one-third of poultry was produced in large plants whereas today more than four-fifths of poultry products originate from plants employing more than four hundred workers. The production of chickens has changed from mainly whole birds for domestic consumption, to cut and deboned poultry packed in bulk containers for both the domestic and export markets (Raj *et al.*, 2001; Turcsan *et al.*, 2003).

Import and export control of poultry products has become vital to the prevention of the spreading of microorganisms to or from other countries, and the export control of products from South Africa has to comply with the import conditions of the specific country or group of countries to which products are exported (Department of Agriculture, 2001). Export and import regulations states that the owner of an abattoir must apply to the national executive officer for the approval of the abattoir and the meat intended for export must be marked according to the requirements of the importing country. The manner in which meat is to be exported must be sealed with a seal containing a seal number. Imported meat must be stored in such a way as to ensure that no contamination, deterioration or soiling may take place in anyway (South Africa, 2004).

The poultry production industry in South Africa remains a traditional and continuous activity and is keeping up with increasing urban demand (Directorate Veterinary Services, 1985). Despite technical production problems, poultry production in South Africa has increased by nearly 50% since 1995 (Department of Agriculture, 2001). Compared to the mainly extensive cattle and sheep industries, the poultry industries are more intensive and are predominantly located on farms near metropolitan areas. South Africa's poultry meat production exceeded that of red meat for the first time in 1998 and it is continuously increasing.

Red meat abattoirs in South Africa are divided into municipal, private and abattoir corporations, while the poultry processing plants are without exception privately owned (Directorate Veterinary Services, 1985; Department of Agriculture (2001). According to (Nel, 2005, Meat inspector: Veterinary Public Health, personal communication) there were 63 functional poultry abattoirs in 2004 (Free State Province) out of a total of 79 registered. From these abattoirs only three scored below 50% on the Hygiene Assessment System (HAS). To date there are 64 functional poultry abattoirs in the Free State province and 15 of the abattoirs are not in use while only 1 abattoir scored below 50% on HAS. Previously poultry abattoirs were graded as AP (Grade A Poultry), BP (Grade B Poultry), CP (Grade C Poultry), DP (Grade D Poultry), and EP (Grade E Poultry) and the regulation has now classed abattoirs as high and low throughput poultry abattoirs (Nel, 2005, Meat inspector: Veterinary Public Health, personal communication).

1.2 FACTORS INFLUENCING SAFETY AND QUALITY

As far as poultry abattoirs are concerned it may be stated that good co-operation regarding compliance with regulations has generally been obtained and relatively few problems have been reported with private abattoirs with regard to compliance with health and safety requirements (Jeeves and Crush, 1997; Kirsten *et al.*, 1998; Statistics South Africa, 2000; South African Poultry Association. 2003, South African Meat Industry Companies, 2003; Nel, 2005, Meat inspector: Veterinary Public Health, personal communication).

1.2.1 Factors influencing product quality at chicken processing plants

Figures 1.1 and 1.2 represent the processes that are conducted at a typical chicken slaughtering facility. A great deal of research has been done on microbial contaminants associated with the various stages of processing of poultry meat and red meat products (Nortjé *et al.*, 1990; Buys *et al.*, 2000, Borch and Arinder, 2002). Although a number of studies have evaluated indoor air quality in food establishments, hospitals and offices, few investigators have examined indoor air quality in poultry processing plants (Sverdrup and Andersen, 1990; Rylander *et al.*, 1994). Numerous factors have been recognized as sources for microbial contamination of poultry meat at the various stages of slaughtering and processing. Air has been recognised as an important source of microbial contamination in a range of food processing plants. The potential impact of such airborne contamination in these operations includes public health risks should airborne pathogens contaminate meat products, together with the risk that airborne spoilage bacteria may reduce product quality and shelf life (Kang and Frank, 1989; Knudtson and Hartman, 1993; Whyte *et al.*, 2001). Because air plays a significant role as a vehicle for the transmission of pathogens in all food production and processing environments, it is important to obtain accurate information on the numbers and types of bacteria found in the air of such plants (Worfel *et al.*, 1996).

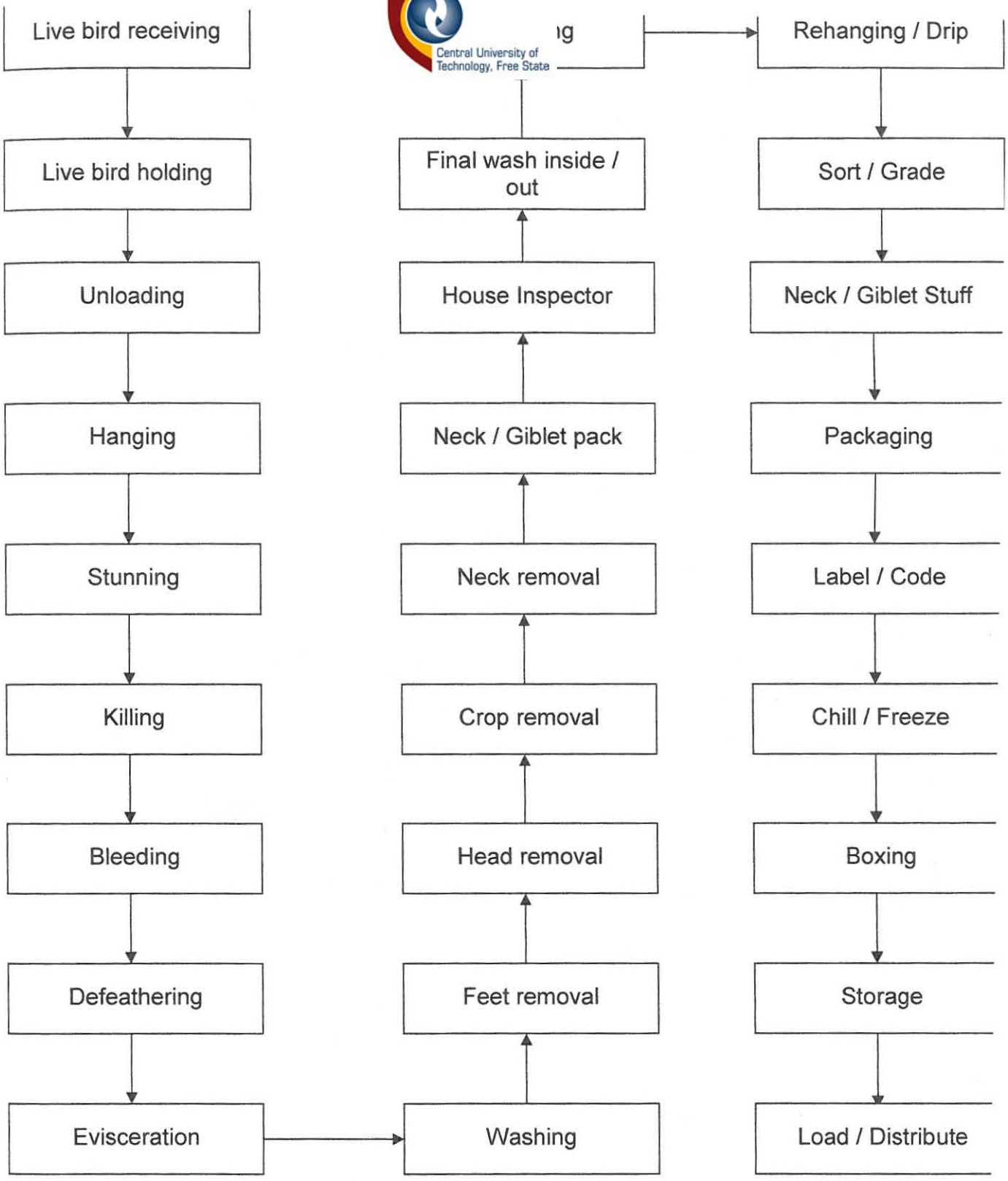

Figure 1.1 Schematic representation of the 27 steps in the slaughtering process (Van Z 1995)

Figure 1.2 Selected processes undertaken at a chicken processing facility (A: Defeathering process and B: Bleeding process)

Chicken feeds are known to affect the appearance and texture of poultry meat, thus reducing product quality. Faecal contamination of carcasses due to *Escherichia coli* continues to be a sporadic problem at abattoirs, the main reason being the rupture of the lower intestinal tract during processing, and the release of contaminating faecal material. However, birds should have a minimum of waste material in their digestive tracts if handled in the correct manner (Summer, 2004).

Listeria monocytogenes is a bacterium that has recently emerged as a prominent human foodborne pathogen. It can cause sporadic cases of disease or full-blown outbreaks after consumption of contaminated meat. *Listeria monocytogenes* has also been implicated in illnesses caused by poultry products and fish (Donnelly, 1994). *Bacillus cereus*, a spore-forming bacillus is another recognised pathogen associated with poultry products. This organism is capable of growing under a wide range of temperatures, varying from 10°C to 48°C, with an optimum range of between 28°C and 35°C (Forsythe, 2000). Although raw poultry products are commonly contaminated by large numbers of microorganisms, *Staphylococcus aureus* is found extensively in the slaughtering environment and originates particularly from employees' hands, noses or hair (Bennet and Lancette, 1995). Depending on the sub-species involved, this organism is generally used as an indicator of food-handler hygiene (Shale, 2004). Spoilage organisms such as *Pseudomonas aeruginosa*

also occur liberally in food processing environments, and are able to survive at low temperatures (Frazier and Westhoff, 1988).

Bacteria detected in the indoor air of processing plants have shown to be mainly derived from humans, and high concentrations of bacteria normally reflect insufficient ventilation in relation to the number of persons and activities involved (Otten and Burge, 1999). The presence of viable airborne microorganisms in indoor air is thus not solely a result of the transport of the outdoor microbes, but may also emanate from intramural sources (Verhoeff *et al.*, 1992). A high air exchange rate or the use of mechanical ventilation usually decreases the concentrations of microbial aerosols (Bartlett *et al.*, 1999), partly due to filtration of incoming air, and also to the removal of particles from intramural sources via the exhaust air. In rooms with low air exchange rates, fungal and bacterial concentrations have been reported to be twice as high as in rooms with a higher exchange rate (Barlett *et al.*, 1999).

1.2.2 Issues relating to occupational health and safety

The operations area in chicken abattoirs pose various risk to employees as a result of slippery floors and surface caused by oil and fat deposits as well as through the use of sharp utensils and equipment. According to the Department of Labour (2000), one in every six workers at a poultry plant suffers a work-related injury daily. This is twice the rate of that found in manufacturing industries. As the demand for chicken rises, labourers work extra-long hours to

meet this demand, and the amount of money made by the employees depends on how fast they get the work done (Linder, 1995; Wethli, 1999). Line speed in poultry processing plants is monitored only for food safety and not for worker safety and as a result, accidents and serious injuries have been reported to occur in many abattoirs (Department of Labour, 2000).

Injuries sustained by food handlers in chicken abattoirs range from deep cuts made by the claws of live chickens, to torn ligaments from repetitive lifting of the birds. Exposure to contaminated air is a further hazard to worker safety because at abattoirs workers spend at least 80% of their time indoors. Mean concentrations of 100 $cfu.m^{-3}$ viable airborne bacteria have been reported as normal findings in the indoor air of slaughtering plants and storage facilities at schools (Smedje *et al.*, 1997b; Barlett *et al.*, 1999; Liu *et al.*, 2000; Scheff *et al.*, 2000). Even levels of up to 1000 $cfu.m^{-3}$ may occur when the air exchange rate is low and when indoor air quality problems prevail due to ineffective ventilation, high temperature and high relative humidity (Liu *et al.*, 2000). In abattoirs the exposure to bioaerosols may occur in many different environments, especially where stored products are handled or where aerosols are produced by leaks from equipment. This may happen intentionally or accidentally or during particular operations (Jay, 2000; Bornehag *et al.*, 2001; Haverinen, 1999a; Douwes *et al.*, 2003; Gora *et al.*, 2004).

A high prevalence of respiratory infections and other symptoms such as eye irritation and fatigue have been reported among employees in the offloading and receiving areas of slaughter houses (Haverinen *et al.*, 1999a; Savilahti *et al.*, 2000). Apart from its obvious effects on worker health, bioaerosols can also have an effect on the shelf life of products (Shale, 2004).

1.3 RATIONALE

This study concentrates on the isolation and enumeration of selected microbiota in the air as well as on the monitoring of environmental factors in a chicken processing facility situated in the Free State Province, South Africa. The aims of this study were:

- to determine the occurence of airborne microorganisms in six areas (receiving/killing, defeathering, evisceration, air-chilling, packaging and dispatch) within the chicken abattoir during processing in order to cast light on the possible sources of contamination as well as the distribution of bioaerosols throughout the facility;

- to monitor selected environmental factors (relative humidity, temperature, airborne particulates and wind velocity) in the selected areas (receiving/killing, defeathering, evisceration, air-chilling, packaging and dispatch) over a specific period in order to evaluate

their influence on the microbial population associated with the bioaerosols, and

- to assess the relationships between the environmental factors and the bioaerosols using inferential statistics in order to predict and control the predomination of selected microorganisms.

In general, the study endeavors to cast light on the above variables in an attempt to contribute to both product quality and worker well-being. By assessing the environmental factors that influence airborne microbiota, specific recommendations can be made to the management of the plant towards rectifying shortcomings and improving safety.

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CHAPTER 2

A SURVEY OF MICROBIAL BIOAEROSOLS IN A HIGH-THROUGHPUT CHICKEN SLAUGHTERING FACILITY

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2.1 ABSTRACT

An investigation was undertaken into the prevalence of microorganisms in the air of various slaughtering areas in a high-throughput chicken slaughtering facility in Kroonstad, South Africa. Air samples were analysed for the presence of *Staphylococcus aureus*, fungi, total coliforms, *Escherichia coli, Pseudomonas aeruginosa, Listeria monocytogenes, Bacillus cereus* and presumptive *Salmonella* spp. Six areas (receiving/killing, defeathering, evisceration, air-chilling, packaging and the dispatch area) were sampled during a period of four months (August to November). The highest concentrations of microorganisms were recorded during the initial stages of processing in the receiving/killing area and in the defeathering area, with counts decreasing towards the evisceration, air chilling, packaging and dispatch areas. Maximum total coliform counts were 8.8×10^2 cfu.m^{-3}, while *E. coli* reached counts of 3.4×10^2 cfu.m^{-3}. *Bacillus cereus* and *Staphylococcus aureus* reached counts of 4.0×10^3 cfu.m^{-3} and 2.2×10^4 cfu.m^{-3} respectively, *P. aeruginosa* 2.4×10^3 cfu.m^{-3} and presumptive *Salmonella* spp 3.5×10^3 cfu.m^{-3}. The highest fungi and *L. monocytogenes* counts were 2.1×10^4 cfu.m^{-3} and 1.7×10^3 cfu.m^{-3} respectively. Because airborne microorganisms that could impact negatively on the quality of the product as well as on the health of workers were isolated, it was suggested that tangible measures be put in place to reduce the airborne microbial load. Such measures would include effective ventilation as well as physical segregation of each of the processing areas in order to minimise the entrance and cross-

contamination of undesirable microorganisms throughout the entire processing environment.

Keywords: Microbial bioaerosols; chicken abattoir

2.2 INTRODUCTION

Several studies report on the sources, incidence and extent of microbial contamination associated with the processing of poultry carcasses (Walker and Ayres, 1956; Patterson, 2001). These studies have focused on the intrinsic contamination originating from the carcass itself during slaughtering or from contact with surfaces, utensils and food handlers. Although limited information is available on the topic, bioaerosol monitoring is, however, rapidly emerging as a tool for characterising microorganisms in the processing environment and is becoming important for identifying microbiological contamination due to product contact with the air (Mead, 1982; Heber *et al.*, 1997).

Air has been reported as a probable source of microbial contamination in various food processing environments, including those that process poultry (Ellerbroek, 1997; Whyte *et al.*, 2001) and beef products (Rahkio and Korkeala, 1997; Sofos *et al.*, 1999). Whyte *et al.* (2001) report that contamination of food products can be caused by airborne microorganisms and is influenced by the period of exposure of the product to the air. May (1962) and Georgnaras *et al.* (1996) reported that airborne microorganisms can contaminate the working surfaces, equipment and the hands of workers, which could lead to cross-contamination of the carcasses.

The airborne microorganisms associated with bioaerosols originate from a variety of sources in the processing plant (Wathes, 1994). Mead (1982) and Prendergast *et al.* (2004) showed that contaminants enter the processing plant in various ways, such as on the clothes, hair or skin of the workers, on feathers and feet of the live birds, and via the water supply.

These airborne microorganisms have been indicated in causing allergic reactions and irritation of the upper respiratory systems especially in individuals working in the receiving and defeathering areas (Haglind and Rylander, 1987; Chang *et al.*, 2001; Huang *et al.*, 2002). Gram-positive bacteria, for example *Listeria monocytogenes*, *Bacillus cereus, Staphylococcus aureus* and Gram-negative bacteria, such as *Escherichia coli*, total coliforms, *Pseudomonas aeruginosa* and *Salmonella* spp. are some of the organisms reported as being able to exist in a bioaerosolised state (Lutgring *et al.*, 1997). Once these organisms disperse into the air of processing plants, the risk of food contamination increases and may necessitate alteration to the processing and storage protocol (Geornaras *et al.*, 1995; Eisel *et al.*, 1997; Forsythe, 2000). For example, the presence of *B. cereus* will necessitate in particular the thorough cooking of food before it is consumed (Vorster, Greebe and Nortjé, 1994; Notermans *et al.*, 1997). *L. monocytogenes,* for example, can grow at temperatures ranging from 0°C to 45°C and is more resistant to heat than the majority of non-spore-forming bacteria. Stretch and Southgate (1991) report that *L. monocytogenes* can be

killed by pasteurisation only if temperature and time are strictly adhered to (63°C for 30 minutes or 71.7°C for at least 15 seconds).

This study was primarily undertaken to determine the distribution of airborne microorganisms in various processing areas in a high-throughput chicken abattoir during processing. The data obtained from this study could contribute to the existing information regarding airborne dispersion of microbiota throughout the slaughtering process, with emphasis on the chicken industry. Based on the results, recommendations are made regarding the control of undesirable microbiota in the various areas via the implementation of physical structures and the improvement of process hygiene.

2.3 MATERIALS AND METHODS

2.3.1 Sampling site

Air samples were taken from a high-throughput (± 32 000 birds slaughtered per day) chicken processing facility situated in the industrial area of Kroonstad, Free State Province, South Africa. This facility employs approximately 400 workers and operates 16 hours per day. The operations carried out at this plant include the receiving and killing of birds, scalding, defeathering, evisceration, head and feet removal, spin-chilling and air-chilling of carcasses, whole bird packaging, and portion packaging of the final product.

2.3.2 Sampling protocol

Air samples were collected in duplicate at monthly intervals over a period of four months (August to November) at six processing areas. These areas included receiving/killing, defeathering, evisceration, air-chilling, packaging and dispatch. The samples were analysed for the presence of *Staphylococcus aureus,* fungi, total coliforms, *Escherichia coli, Pseudomonas aeruginosa, Listeria monocytogenes, Bacillus cereus* and presumptive *Salmonella* spp.

2.3.3 Bioaerosol sampling

Bioaerosols were collected by impaction on agar plates using an SAS Super 90 air sampler (PBI International, Milan, Italy) that collects airborne microorganisms onto 55mm RODAC plates (MERCK). The air sampler was pre-calibrated and adjusted to a flow rate of 28 l/min. All removable parts of the air sampler were pre-autoclaved before a sampling run and disinfected with 70% ethanol between samples. Petri dishes containing the samples were labelled to identify the sampling areas.

2.3.4 Isolation of microorganisms

Staphylococcus aureus

Enumeration of *Staphylococcus aureus* was done on Baird Parker Agar (MERCK, South Africa). The agar was incubated at 36ºC for 48 hours and typical *S. aureus* colonies were grey-black and shiny, with clear zones surrounding the colonies. *S. aureus* ATCC 25923 was used as a positive

control, while a blank Baird Parker Agar plate was used as a negative control by inserting it into the air sampler without performing a run.

Fungi

Potato Dextrose Agar ((PDA), MERCK, South Africa) acidified with tartaric acid to pH 3.5 was used for growing and enumerating fungi. Plates were incubated at 25°C for 5 days and yellowish-white colonies were identified as yeasts while mould growth was recognised by its mycelial appearance (Beever and Bollard, 1970; Frazier and Westhoff, 1988; Atlas and Parks, 1993).

Escherichia coli and total coliforms

Violet Red Bile MUG Agar (VRB, MERCK, South Africa) was used to isolate *E. coli* and total coliforms (Manafi and Kneifel, 1989; Stevenson and Segner, 1992; International Standards Organisation, 1993). The plates were incubated at 37°C for 24 hours. Typical *E. coli* colonies were dark red and 2 to 5mm in diameter while total coliforms appeared as small pink colonies. *Escherichia coli* ATCC 25922 was used as positive control while a blank plate containing Violet Red Bile MUG agar was used as negative control.

Pseudomonas aeruginosa

Cetrimide Agar (MERCK, South Africa) with added glycerol was used for culturing and enumerating *Pseudomonas aeruginosa*. Incubation was at 25°C for 48 hours and typical *Ps. aeruginosa* were identified as yellowish colonies.

Blank agar plates with Cetrimide agar were used as negative control while *Ps. aeruginosa* ATCC 27853 was used as positive control.

Listeria monocytogenes

Listeria Selective Agar (MERCK, South Africa) was used for the enumeration of *L. monocytogenes* (Forsythe, 2000). Incubation for *Listeria* was done at 37ºC for a duration of 48 hours and *L. monocytogenes* colonies were 2mm in diameter and greenish-grey with a black core and halo. *Listeria monocytogenes* ATCC 19117 was used as positive control with a blank plate as negative control.

Bacillus cereus

A blank *Bacillus cereus* selective agar plate was used as negative control, while *B. cereus* ATCC 14579 was used as positive control. *Bacillus cereus* Selective Agar (Scharlau, 2000, South Africa) was used to culture and enumerate *B. cereus.* Plates were incubated at 30ºC for 18-24 hours and examined for typical *B. cereus* colonies that occurred as large (approximately 5mm in diameter) colonies with a turquoise to peacock-blue colour and small zones of egg yolk precipitate (Nortjé *et al.*, 1999).

Presumptive *Salmonella* spp

Brilliant Green Agar (BGA) was used for culturing and enumerating *Salmonella* spp. (Higgins *et al.*, 1982; Frampton *et al.*, 1988; Davies and Wray, 1994) and

this was incubated at 42°C for 2 days. *Salmonella* colonies were reddish-pink opaque surrounded by brilliant red zones. *Salmonella enteritidis* (ATCC 13076) was used as a positive control while blank BGA plates (Scharlau, 2000, South Africa) were used as negative controls.

2.3.5 Statistical analysis

General ANOVA was used to measure distribution of bioaerosols in various processing locations (StatSoft, *Statistica* Version 7.1).

2.4 RESULTS AND DISCUSSION

2.4.1 *Staphylococcus aureus*

Staphylococcus aureus is known to be a normal inhabitant of the human body, and due to its clump-like structure, has been found to adhere easily to surfaces (Rahkio and Korkeala, 1997; Plaatjies, 2004). Lutgring *et al.* (1997) as well as Neumann *et al.* (2002), reported *S. aureus* to constitute the majority of the airborne microbiota.

The numbers of *S. aureus* found in the various areas are presented in Figure 2.1. Concentrations of 10^5 cfu.m^{-3} were recorded. These concentrations suggest that from the start of the processing of the poultry in the receiving/killing and the defeathering areas, contamination of the air by microorganisms increases as the processing progresses. This observation corresponds with a study undertaken by Lutgring *et al.* (1997), who attributed

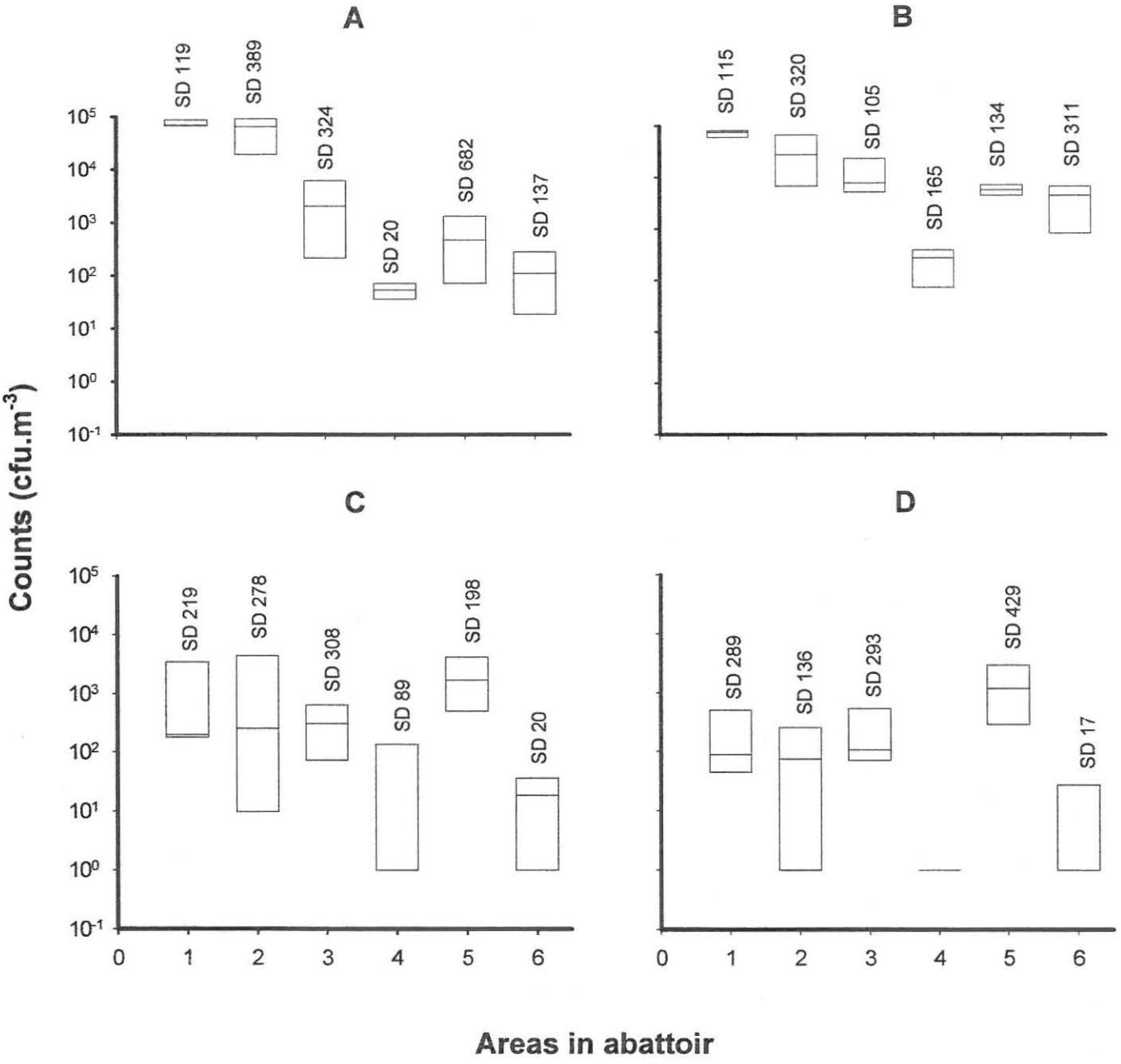

Figure 2.1 The distribution of *Staphylococcus aureus* (A), Fungi (B), Total coliforms (C) and *Escherichia coli* (D) in the air of various areas of a chicken abattoir. X-axis values: 1 represents the receiving/killing; 2, defeathering; 3, evisceration; 4, air chilling; 5, packaging and 6, dispatch areas respectively. SD = Standard deviation

the presence of airborne microorganisms in the receiving/killing area to the flapping of wings and the excessive movements of the birds. Earlier research has also indicated that the microbiota from the receiving area consist mainly of *Staphylococcus* spp. (May, 1962; Holt *et al.*, 1994; Ellerbroek, 1997). The counts of airborne *S. aureus* in the air-chilling area were *circa* 2.1 x 10^2 cfu.m^{-3} (Figure 2.1 (A)). Low counts were expected in this area, which was expected in the presence of a well functioning air-chiller. The packaging and the dispatch areas showed counts within the same range.

2.4.2 Fungi

Similar to *Staphylococcus aureus*, the highest counts of airborne fungi were detected in the receiving/killing and defeathering areas (2.9 x 10^5 cfu.m^{-3} and 1.3 x 10^5 cfu.m^{-3}) as indicated in Figure 2.1 (B), while in the evisceration, packaging and dispatch areas the counts for fungi were 4.8 x 10^4 cfu.m^{-3}, 2.3 x 10^4 cfu.m^{-3} and 1.6 x 10^4 respectively. Adhikari *et al.* (2004) reported that fungal counts constituted the predominant part of microorganisms in bioaerosols in almost all the sampling areas. Previous research has shown that mould spores are very small in size, as well as relatively resistant to drying, and these features contribute to their aerosolisation (Frazier and Westhoff, 1988). Bornehag *et al.* (2001) and Burge (2001) reported that although a clear correlation between airborne fungi and health impacts has not been unambiguously shown in epidemiological studies, they do suggest that indoor fungi should be regarded as a potential health hazard.

2.4.3 Total coliforms

Total coliform counts are useful indicators for assessing microbial proliferation in foods, as well as for gauging the level of processing or post-processing recontamination by faecal sources (Department of Health, 2000). The average total coliform concentrations throughout the various sections are shown in Figure 2.1 (C) which further shows the average counts of 1.8×10^2 cfu.m^{-3} in the packaging area. Similar to the previously mentioned microorganisms, total coliform counts were lowest in the fourth area (air-chilling) (7.4×10^1), while the counts in the receiving/killing, defeathering and the evisceration areas were 5.1×10^3, 6.2×10^3 cfu.m^{-3} and 1.4×10^3 cfu.m^{-3} respectively (Figure 2.1 (C)).

2.4.4 *Escherichia coli*

Escherichia coli predominated in particular amongst isolates of air samples from the packaging area, with mean values of 5.9×10^3 cfu.m^{-3}, compared to counts in the receiving/killing area of 8.6×10^2 cfu.m^{-3} and in the evisceration area 9.6×10^2 cfu.m^{-3} (Figure 2.1 (D)). The results regarding *E. coli* agreed with those reported by Geornaras *et al.* (1995) and Wilcock *et al.* (2004). Geornaras *et al.* (1996) also reported *E. coli* presence of between 95.6% and 93.7% in the defeathering and packaging areas respectively of a similar processing plant. In the receiving/killing and the defeathering areas, the relatively high counts of *E.coli* were likely to be due to faecal particulates spread by the flapping of wings.

2.4.5 *Pseudomonas aeruginosa*

Psychrotrophic spoilage bacteria such as members of the genus *Pseudomonas* represent some of the Gram-negative rods that exist in poultry meat. This bacterium is classified as one of the most important in the group of bacteria that cause spoilage of refrigerated foods (Barnes *et al.*, 1979; Buys *et al.*, 2000). *Pseudomonas aeruginosa* concentrations were found to be highest in the receiving/killing area (7.6 x 10^4 cfu.m^{-3}) (Figure 2.2 (E)). These high numbers of organisms could have been introduced into the above-mentioned areas upon arrival of the birds. The airborne *Ps. aeruginosa* in the evisceration and the packaging areas showed counts of 6.1 x 10^2 cfu.m^{-3} and 2.2 x 10^2 cfu.m^{-3} respectively as depicted in Figure 2.2 (E). In studies done by Geornaras *et al.* (1996) and Ellerbroek (1997), it was suggested that the organism originates predominantly from water and/or soil on the equipment.

2.4.6 *Listeria monocytogenes*

Twenty-two *Listeria* species have been described and the majority of these are found in food processing plants. All *Listeria* species are psychrotrophic, Gram-positive, non-spore-forming, facultative anaerobic rods. In addition, this organism is notably resistant to pH fluctuations, high salt concentrations and desiccation (Ryser and Marth, 1991; Miller, 1992). Rammel (2003) reported that normal refrigeration did not stop the proliferation of *Listeria monocytogenes.* In Figure 2.2 (F) levels of airborne *L. monocytogenes* are

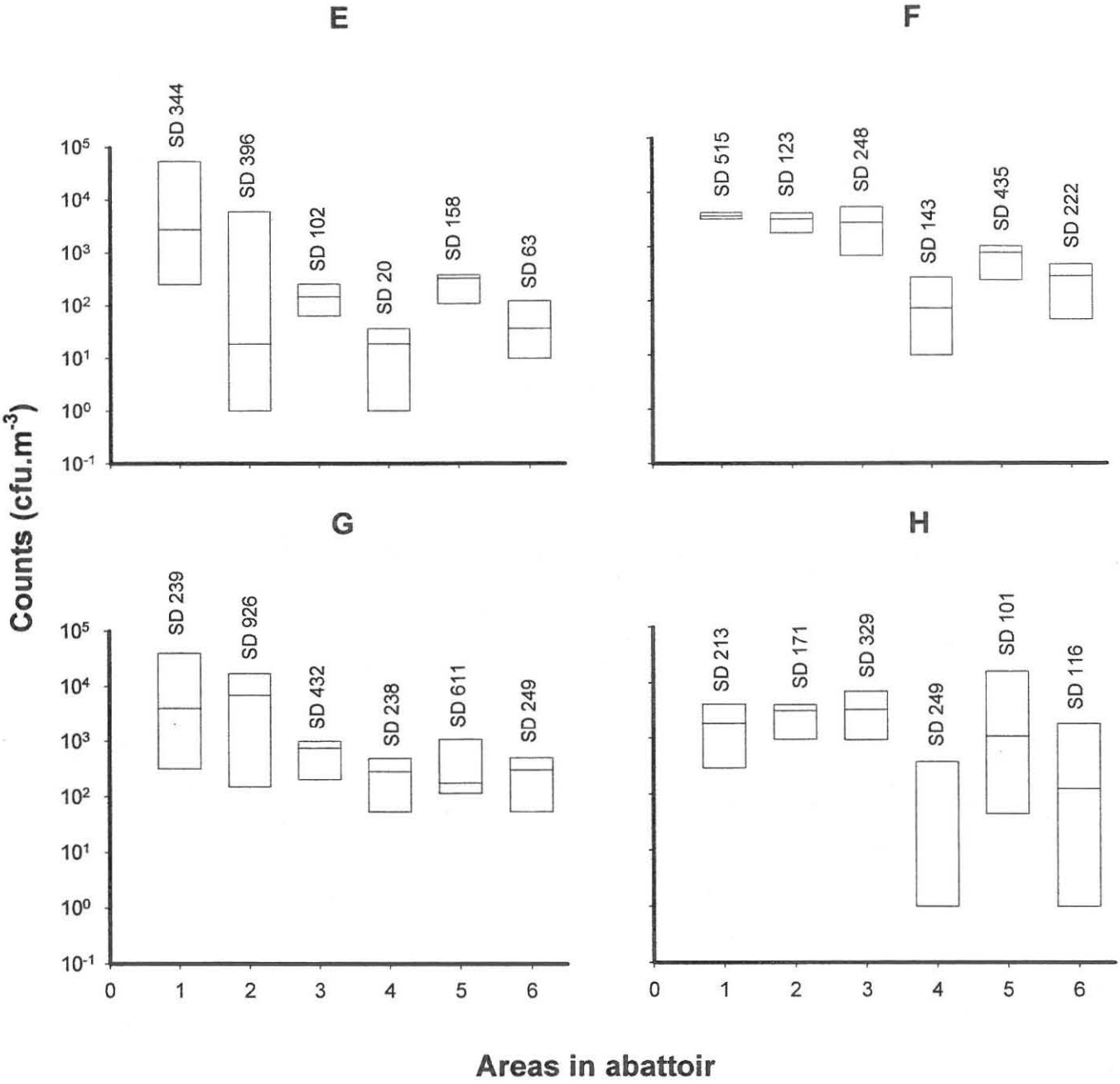

Figure 2.2 The distribution of *Pseudomonas aeruginosa* (E), *Listeria monocytogenes* (F), *Bacillus cereus* (G) and presumptive *Salmonella* spp (H) in the air of various areas of a chicken abattoir. X-axis values: 1, represent receiving/killing; 2, defeathering; 3, evisceration; 4, air-chilling; 5, packaging and 6, dispatch areas respectively. SD = Standard deviation

depicted, with the receiving/killing, defeathering and the evisceration areas showing average counts of 1.5 x 10^4 cfu.m^{-3}, 1.2 x 10^4 cfu.m^{-3} and 1.1 x 10^4 cfu.m^{-3} respectively.

2.4.7 *Bacillus cereus*

The findings of this study regarding the airborne *Bacillus cereus* in the receiving/killing areas, agree in particular with those found in other studies done in similar processing environments. *Bacillus cereus* counts in the receiving/killing and the defeathering areas reached mean counts of 5.9 x 10^4 cfu.m^{-3} and 3.2 x 10^4 cfu.m^{-3} respectively (Figure 2.2 (G)). However, it was evident that the *Bacillus cereus* counts decreased in the evisceration, air-chilling, packaging and dispatch areas. The relatively high numbers of this organism in the receiving/killing and defeathering areas were not unexpected as much of wing flapping occurs in these areas, causing the spread of particulates. *Bacillus* spp. are able to withstand unfavourable conditions such as low temperatures and heat due to their ability to form resistant spores (Stevenson and Segner, 1992; Whyte *et al.*, 2001). A study done by Nel *et al.* (2003) showed that *B. cereus* levels increased rapidly even when a product was exposed to poor handling and processing procedures.

2.4.8 Presumptive *Salmonella* spp

Based on the process flow, presumptive *Salmonella* spp reached the highest counts in the first three areas with averages of 8.5 x 10^3 cfu.m^{-3}, 1.1 x 10^4

cfu.m^{-3} and 1.5 x 10^4 cfu.m^{-3} respectively. Area 4 (air-chilling) had the lowest counts for this organism. The last two areas downstream (packaging and dispatch) revealed counts of 2.3 x 10^4 cfu.m^{-3} and 2.6 x 10^3 cfu.m^{-3} (Figure 2.2 (H)). Gallo *et al.* (1988) reported false positive counts of *Salmonella* found in the receiving/killing area, and although other authors have actually isolated *Salmonella* in this area, it has only infrequently been found in other areas (Lutgring *et al.*, 1997).

2.4.9 Changes of the various microbiota concomitant to the product flow

When considering the trends displayed in Figures 2.1 and 2.2, it is evident that the prevalence of the various microbiota followed the same pattern throughout the various processing areas. Firstly, there was a definite decline in the numbers from the receiving/killing area downstream towards the dispatch area. As was expected, the air-chilling area had the lowest microbial counts due to low temperature of a well functioning air-chiller. The total decline in microbiota numbers were 0.18% (*Staphylococcus aureus*), 5.52% (fungi), 3.53% (total coliforms), 4.53% (*Escherichia coli*), 0.29% (*Pseudomonas aeruginosa*), 7.33% (*Listeria monocytogenes*), 1.83% (*Bacillus cereus*) and 30.59% (presumptive *Salmonella* spp). In cases where the decline in counts was less pronounced (0.18% and 0.29%) the carry-over of such organisms along the processing line was likely to be the highest and *vice versa*. The

reported microorganisms should further be highlighted in particular to curb the microbial proliferation in the plant.

2.4.10 Conformance to guidelines and infective doses

In order to obtain an indication of the levels of the various organisms in terms of their risk and legal conformance the microbial counts were compared to infective dose limits as specified in literature as well as guidelines stipulated in national legislations (Figure 2.3) (Department of Health, 2000). If no infective dose or national guideline limits were indicated, they have not yet been established or reported in literature for the specific environment and/or the organisms found here. Although the guidelines and limits shown in the graph were for the products while the actual numbers were for bioaerosols, they were included in the graph to present an assessment of the levels of microbiota in the air and those perceived to be unacceptable in the product itself. It must, however, be reiterated that no guidelines currently exist in South Africa for bioaerosols in food processing plants.

Although no stipulated guidelines or infective doses for fungi could be obtained, this organism showed counts between 10^4 cfu.m^{-3} and 10^5 cfu.m^{-3}. Germination of fungal spores in the receiving/killing and the defeathering location was made possible by high relative humidity as fungi need water activity (A_w) of at least of 0.70.

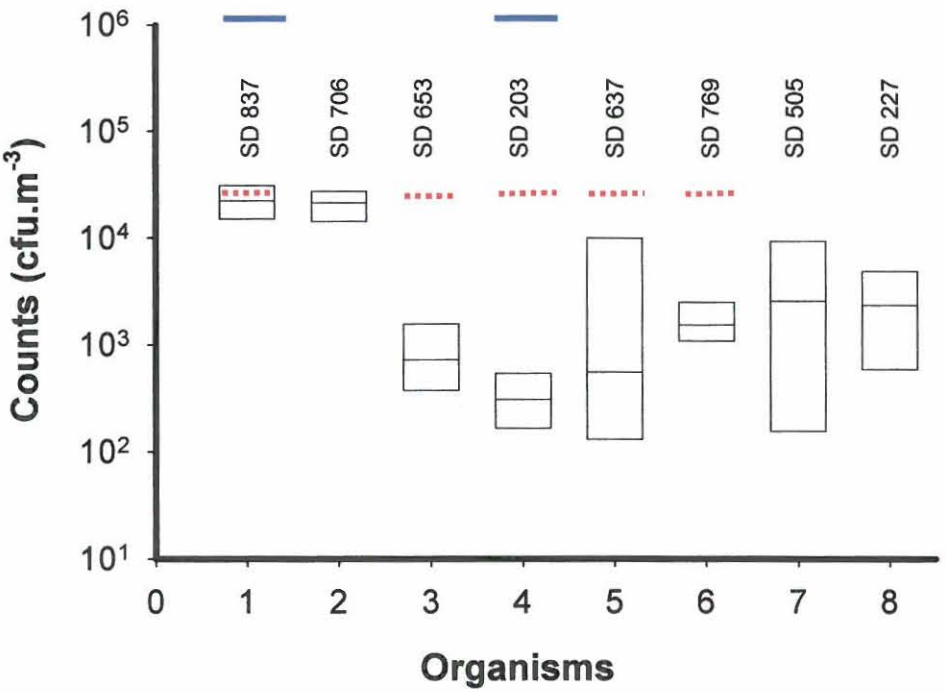

Figure 2.3 The distribution of various microorganisms associated with chicken processing over the four sampling months with 1: *Staphylococcus aureus*, 2: Fungi, 3: Total coliforms, 4: *Escherichia coli*, 5: *Pseudomonas aeruginosa*, 6: *Listeria monocytogenes*, 7: *Bacillus cereus*, 8: Presumptive *Salmonella* spp.

..... Guideline limit

——— Infective dose limit

SD = Standard deviation

The South African Department of Health (2000) proposes a guideline of 10^1 cfu.m^{-3} for *E. coli* and an infective dose of 10^6 cfu.g^{-1} (Figure 2.3). In this study, *E. coli* had mean counts of between 10^2 cfu.m^{-3} and 10^3 cfu.m^{-3}. Total coliforms showed mean counts between 10^2 cfu.m^{-3} and 10^3 cfu.m^{-3}, exceeding neither the guideline limit of 10^2 cfu.g^{-1} nor the infective dose limit (Department of Health, 2000; Nel *et al.*, 2003). Similarly, the mean counts for *Salmonella* spp, *Listeria monocytogenes*, *Bacillus cereus* and *Pseudomonas aeruginosa* were lower than the national guideline as well as the infective dose limit.

2.5 CONCLUSION

Results obtained from this study indicate that counts of airborne microorganisms were, without exception, higher in the receiving/killing and the defeathering areas than in the rest of the sampling areas. This observation further showed the importance of controlling the levels of airborne microorganisms that may affect poultry prior to processing, in addition to implementing control measures in the processing facility. This could be achieved by ensuring that the pathogens that are likely to be airborne are limited to levels that will not pose a risk to the employees. It is furthermore suggested that the carry-over of airborne microbiota downstream from areas that are likely to generate high counts to the areas where the product is exposed to air and surface contamination is limited. This can be achieved by ensuring a positive air pressure inside the plant that would generate a flow of air from inside to outside and not *vice versa*. SANS 10049/ SABS 049 (2001)

further suggests that high-risk areas be strictly controlled with the aid of air and/or plastic curtains or with walls and doors.

Poultry products are prone to bacterial contamination and the need for guidelines in airborne microorganisms are becoming increasingly important, together with the need for the implementation of quality control and monitoring systems such as the Hazard Analysis Critical Control Point (HACCP) system. To address the problem of airborne microorganisms in a food processing plant, the management at the plant should not only focus their energy on surface cleaning and disinfecting of utensils, but should also implement and apply general preventative measures such as Pre-requisite Programs (PRPs) to HACCP and good manufacturing practices (GMPs) in order to reduce or eliminate air contamination.

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CHAPTER 3

A SURVEY OF BIOAEROSOL-RELATED ENVIRONMENTAL PARAMETERS IN A HIGH-THROUGHPUT CHICKEN PROCESSING FACILITY

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3.1 ABSTRACT

Environmental factors in food processing facilities are gaining increasing importance as indirect determinants of spoilage and in view of problems that could arise in these facilities if such factors are not adequately controlled. Relative humidity, temperature, wind velocity and airborne particulates were investigated in six areas (receiving/killing, defeathering, evisceration, air-chilling, packaging and dispatch) in a chicken processing facility. The aim of the study was to evaluate the levels of these parameters across the various sections of the abattoir, as well as to cast light on the changes of these parameters over a four-month period, in order to ensure that the quality of the product is not affected by unfavourable environmental parameters. To promote suitable processing conditions, food production facilities should implement measures to ensure sanitary processing conditions, reduction of environmental impacts and promote employee safety. Across the six processing areas, the environmental parameters showed respective average values of 71% (min 7%, max 89%) relative humidity, 18°C temperature (min 2°C, max 24°C), 7 $m.s^{-1}$ wind velocity (min 0.1 $m.s^{-1}$, max 13 $m.s^{-1}$) and 3.76 $mg.m^{-3}$ for airborne particulates (min 0.1 $mg.m^{-3}$, max 7.8 $mg.m^{-3}$). The environmental parameters varied notably concomitant to the process flow, while a relationship was noted between temperature and relative humidity. With regard to the changes in the environmental parameters over the four-month sampling period, relative humidity showed some variation between the first

and the last sampling run (30% increase) while temperature showed considerable variation (63% decrease), wind velocity decreased by 70% and airborne particulates increased from 0.28 mg/m^{-3} to 6.1 mg/m^{-3}. It was suggested that occupational health and safety control measures such as the use of personal protective equipment approved by the National Institute for Occupational Safety and Health be used in order to protect employees and to curb the spread of undesirable microbiota. Physical segregation of the processing areas supported by effective ventilation should go a long way in addressing the continuous fluctuation amongst the mentioned environmental parameters.

Keywords: Environmental parameters, chicken abattoir, occupational health and safety.

3.2 INTRODUCTION

Although research on the environmental factors impacting on food quality has intensified, updated literature on the relevance of these environmental factors is still lacking (Ahearn, 1991). Previous studies have shown the potential threat environmental factors can pose to the health and well-being of the employees in food processing plants. It has furthermore been shown that indoor contaminants originate from the outdoor environment and are normally higher than pollutants encountered in the outdoor environments (Homes *et al.*, 1996; Baek *et al.*, 1997; Jones *et al.*, 2000; Lee and Chang, 2000, Lee *et al.*, 2001). The presence of contaminants in the indoor environment would often be attributed to ventilation systems not being maintained properly and generating excessive moisture and condensation (Law *et al.*, 2001; Lee *et al.*, 2001; Li *et al.*, 2001; Lee *et al.*, 2002; Guo *et al.*, 2004).

According to Godish (1995) airborne particle concentrations in various processing environments differ due to a number of factors that include external temperature, manure management methods and the sampling method used for sample collection. Godish (1995) further mentions that airborne particulates containing spores and environmental bacteria may enter from the outdoor environment through the inlet or infiltration systems, through employees' clothes and skin, and from building materials (Fanger *et al.*, 1988; Fischer and Dott, 2002; Mezzaria *et al.*, 2002).

Poultry processing plants are prone to indoor air contamination that originates from various sources such as smoking, manure, cleaning of the processing areas as well as from birds' feathers (Whyte, 2002). The design of the processing facility can further contribute to contamination. Several authors have indicated that poultry dust may present a respiratory hazard and excessive exposure therefore may cause acute and chronic respiratory diseases (Samson, 1994; Rylander *et al.*, 1999; Douwes *et al.*, 2000; Whyte, 2002). In a study conducted by Verhoeff *et al.* (1995), respiratory complaints were associated with airborne particulates occurring due to scattering of particulates as well as dirt and feathers being knocked out from the coops during offloading of the birds.

The importance of the airflow pattern in food processing plants was often overlooked and ventilation systems were designed without taking the settling of particles into account (Holmberg and Chen, 2003). Jolly (1996) and Nielsen (2003) report that air distribution is predominantly affected by the area of the inlets and outlets, as well as by wind velocity at different points within the ventilation system. These factors can further affect the relative humidity and the temperature. The implications that parameters such as relative humidity, temperature, wind velocity and airborne particulates may have regarding the occupational milieu of employees and the relationship that these parameters might have on the airborne microbial load merits further investigation. Venter *et al.* (2004) indicate clear relationships between environmental factors and

bioaerosol composition in chicken egg production plants, whilst the latter in turn, affects product quality. The aim of this study was firstly to evaluate selected environmental factors (relative humidity, temperature, airborne particulates and wind velocity) in six areas (receiving/killing, defeathering, evisceration, air-chilling, packaging and dispatch) at a chicken processing facility and secondly to cast light on the changes that these parameters presented throughout the slaughtering process, over a four-month sampling period.

3.3 MATERIALS AND METHODS

3.3.1 Sampling Protocol

Samples were collected from a South African high-throughput chicken abattoir (processing ± 32,000 chicken slaughtering units per day) situated in the industrial area of the city of Kroonstad, Free State Province. Temperature, relative humidity, wind velocity and airborne particulates were evaluated from August to November (winter to spring). Samples were collected in six areas (receiving/killing, defeathering, evisceration, air-chilling, packaging and dispatch) as shown in Figure 3.1. All measurements were taken at a height of 1.5m above the floor (Venter *et al.*, 2004). In order to obtain a representative sample, the selected parameters were measured in triplicate in each sampling area. The following direct reading instruments were used for monitoring: 1)

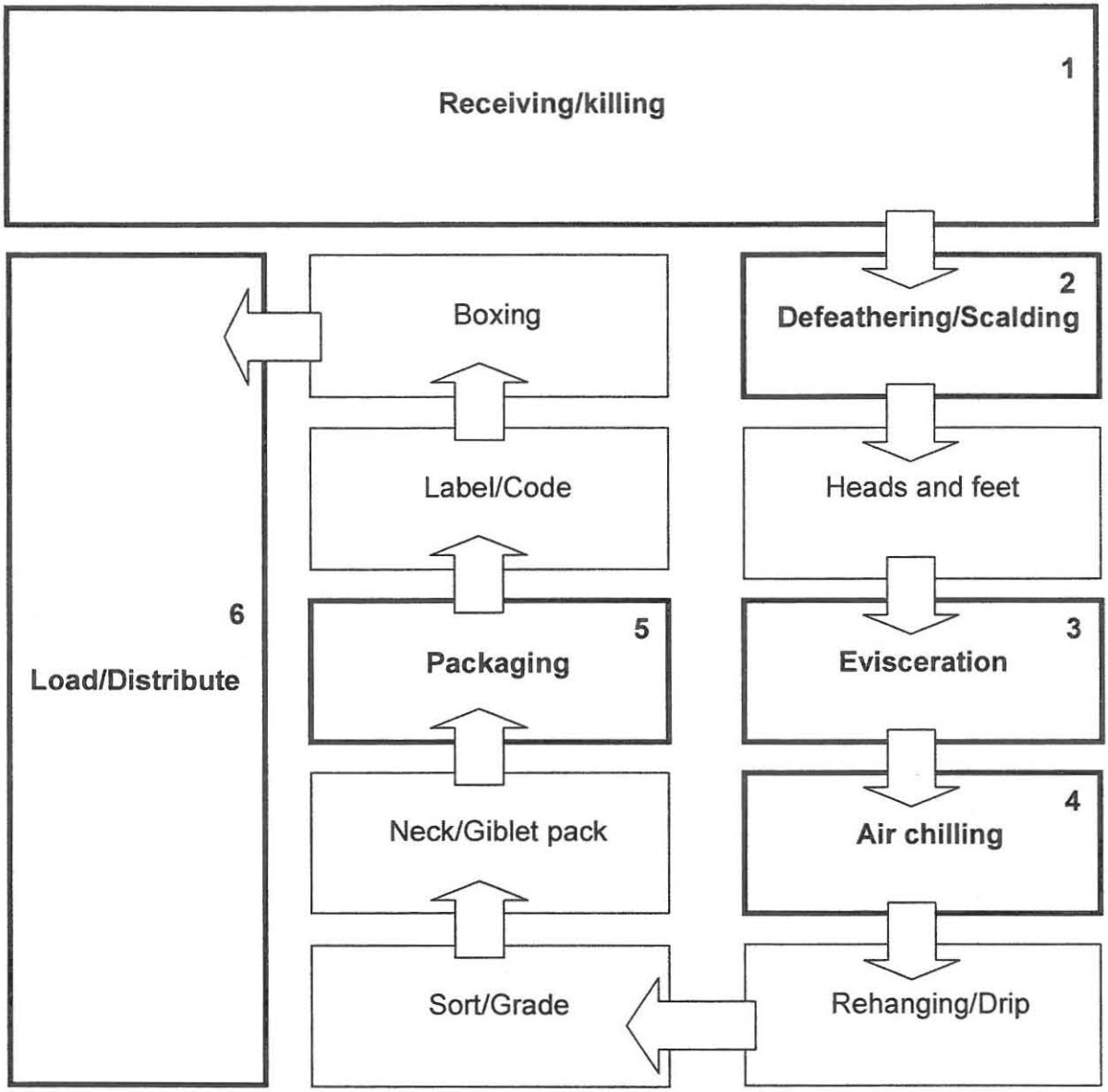

Figure 3.1 Schematic representation of the slaughtering processes at a chicken processing plant with numbers 1-6 representing sampling areas

Area Heat Stress Monitor (Questemp°15; Questemp, South Africa) for measuring temperature as well as relative humidity; 2) airflow Anemometer (LCA 6000 VT; Airflow Instrumentation, South Africa) for measuring wind velocity and 3) handheld Aerosol Monitor (1005/1060; PPM Enterprises, Inc., South Africa) for determining airborne particulates (Venter *et al.*, 2004). All equipments used were calibrated.

3.3.2 Statistical processing

Data represented are the means of samples collected during the four sampling months at three different points in each area. Box plots were used to show the 25th and 75th percentiles as well as the means (StatSoft, *Statisica* Version 7.1).

3.4 RESULTS AND DISCUSSION

3.4.1 Relative humidity

The overall relative humidity in area one (receiving/killing) reached an average of 71% as shown in Figure 3.2 (A). The measured relative humidity in this area was likely to be due to rainy weather conditions during the second sampling interval. A lower relative humidity (42%) was recorded in the dispatch area which was situated towards the exterior, which fact could have contributed to the lower levels of water vapour in the area. The evisceration and the packaging areas had the highest mean relative humidity of 77% and 64% respectively.

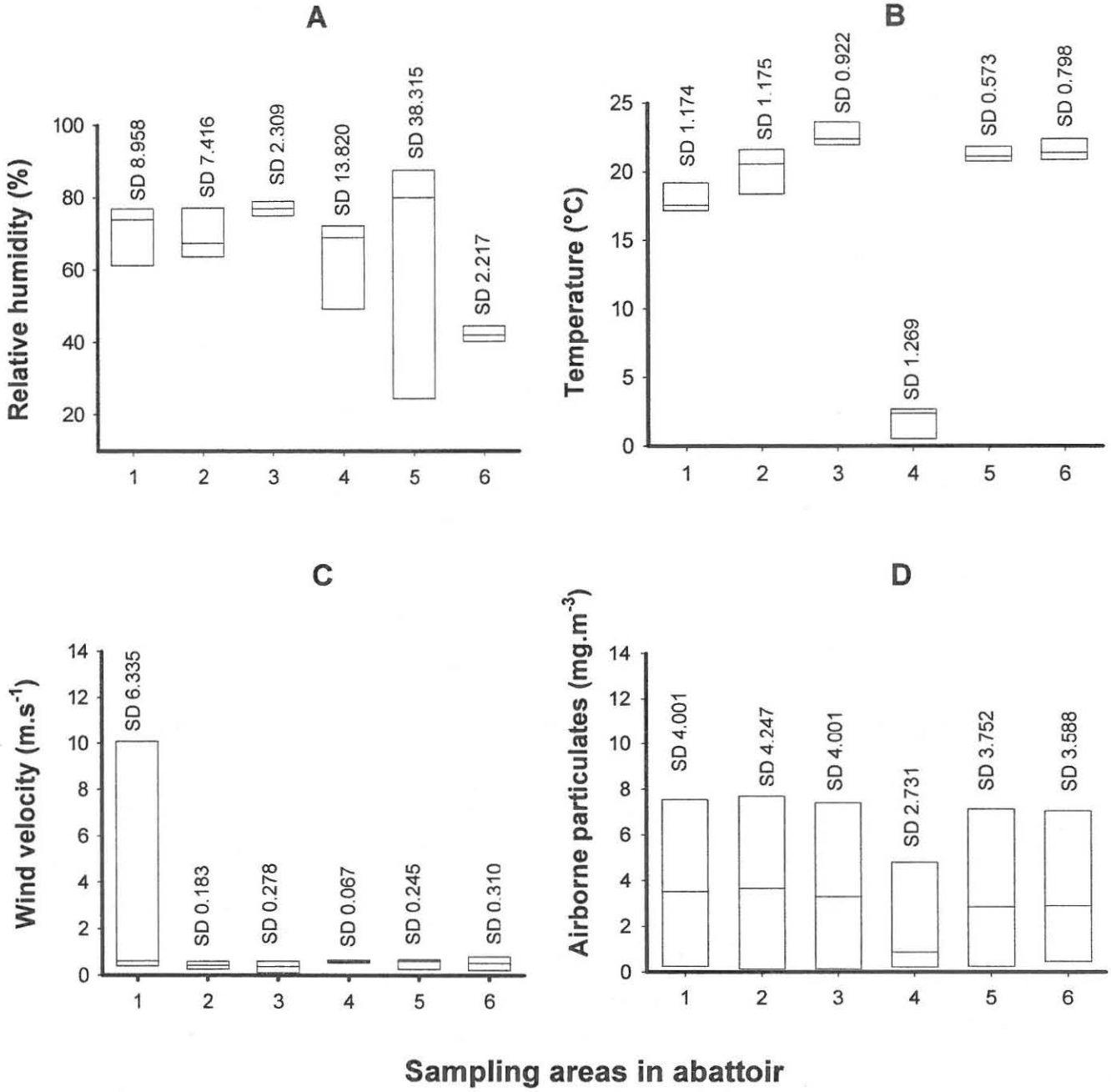

Figure 3.2 Environmental factors monitored in the six processing areas (1-6) during a four-month sampling period, where A = Relative humidity (%), B = Temperature (°C), C = Wind velocity ($m.s^{-1}$), D = Airborne particulates ($mg.m^{-3}$)

Multiple processes, including the final washing of the carcasses using chlorinated water, packaging and weighing of livers, feet and heads, were carried out in the relatively small evisceration area which probably contributed to the elevated relative humidity due to condensation of water vapour. Wang *et al.* (2001), Cappella (2004) and Williams (2004) state that relative humidity should not exceed 100%, as this would elevate condensation processes and lead to high levels of discomfort. The South African National Standards (SANS) 10049/SABS 049 (2001) further state that where necessary, the relative humidity should be controlled in order to protect food quality.

3.4.2 Temperature

The temperature measurements in the sampling areas are shown in Figure 3.2 (B). The temperatures observed in the receiving/killing and defeathering areas were generally below 18°C, whereas the average temperature measurements in the packaging and dispatch areas were 21°C and 22°C respectively. The evisceration area had average temperature of 23°C and the air-chiller 4°C throughout the four-month sampling intervals. Beside the fact that low temperatures inhibit microorganisms, Pompermayer and Gaylarde's (2000) indicated that temperature is an important factor influencing the adhesion of microorganisms to surfaces (Czechowski, 1990; Pompermayer, 2000). Poultry regulation states that meat must be subjected to uninterrupted chilling to reduce the core temperature of the meat to 4°C within 12 hours in the case of chilled meat and meat that is being frozen may not be removed from the

freezer before a core temperature of minus 12°C has been reached (South Africa, 2004). Republic of South Africa (1993) states that no employer shall require or permit an employee to work in an environment in which the time-weighted average (TWA) dry-bulb temperature over a period of four hours is less than 6°C, unless the employer takes reasonable measures to protect such employee against the cold. The above-mentioned act further states that no employee shall be permitted to work in a refrigerated environment in which the actual dry-bulb temperature is below 0°C unless employees are provided with protective clothing.

3.4.3 Wind velocity

An important function of airflow or wind velocity is to ensure proper mixing of the temperature and humidity throughout the processing environment (Godish, 1995; Goodman, 1999). In this study the wind velocity in the receiving/killing area reached an average of 3.7 $m.s^{-1}$ (Figure 3.2 (C)). The wind velocity in this area was suspected to be due to the contributory effect of air from the outside environment, thus causing elevated wind velocity in the above-mentioned processing area. This could be a concern in that elevated wind speed results in the dispersal of airborne particles (Holmberg and Chen, 2003). Goodman (1999) reported that low air-exchange affects high air humidity and high prevalence of dust, and the SANS 10049/SABS 049 (2001) adds that the air supply to processing areas with a high prevalence of contaminants should be filtered to at least 2µm.

3.4.4 Airborne particulates

The highest concentrations of airborne particulates were measured in the receiving/killing and defeathering areas (Figure 3.2 (D)), showing similar results of 3.76 $mg.m^{-3}$ and 3.83 $mg.m^{-3}$ respectively. High concentrations of particulates in the defeathering area could be explained by the absence of any physical segregation between the receiving/killing area and the defeathering area. The higher levels of particulates detected in the receiving/killing area could be associated with the flapping of wings and similar findings were reported by Lutgring *et al.* (1997).

3.4.5 The effect of time on the environmental factors

Variations in relative humidity (Figure 3.3 A) throughout the sampling intervals were not as pronounced as had been expected due to changes in ambient temperature. During the four-month sampling period, the relative humidity levels ranged from 58% in August to a maximum of 77% in September, October and November (Figure 3.3 A). Temperature was one of the factors that showed a great variation between the first (Figure 3.3 B) and the last sampling period. There was little noticeable change in wind velocity between the four sampling intervals (Figure 3.3 (C)). It is nevertheless essential to ensure that the building is designed in such a way that proper air distribution is achieved in order to ensure controlled dispersal of microbes, as low air exchange rate influences humidity and the prevalence of dust (Goodman, 1999).

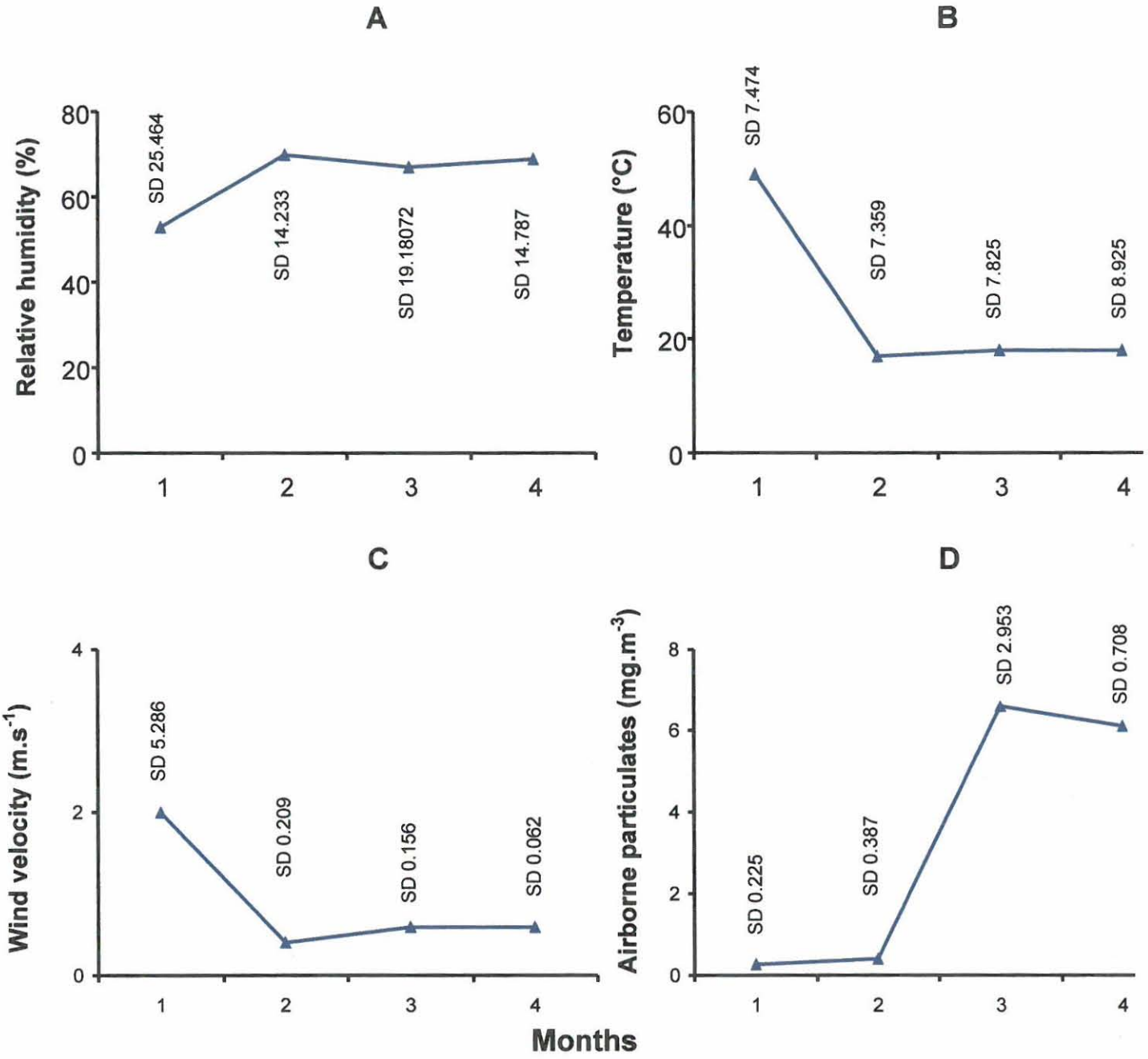

Figure 3.3 Changes in selected environmental parameters during the four months (Aug-Nov).

SANS 1049/SABS 049 states that if forced ventilation is present, it should be designed in such a way that the airflow is in the opposite direction to that of the product flow. Air-intake points should be at least 1m above the internal floor levels and 1m above outside surfaces and they should also be fitted with fly screen and dust filters. Airborne particulates showed more variation than the other three parameters (6.1 mg/m^{-3} and 0.28 mg/m^{-3}) (Figure 3.3 (D)). The concentration of airborne particulates concentration during the first two months was between 0.28 $mg.m^{-3}$ and 0.4 $mg.m^{-3}$ while a notable variation was observed between the second and the third months namely 0.4 mg/m^{-3} and 6.6 mg/m^{-3} respectively.

3.5 CONCLUSION

None of the results observed in this study exceeded the South African National Standards for any of the measured parameters. Although the environmental factors monitored did not exceed the prescribed standards, it is suggested that a ventilation system be properly maintained and/or upgraded in each area to prevent the possible condensation of moisture, to control odours and to ensure sanitary and suitable processing and operating conditions. The ventilation system should be carefully designed and regularly checked and maintained to limit the entrance of dust, dirt, and insects in order to stop other contaminating materials from reaching clean areas of the plant. As one person's ideal humidity level might be uncomfortable for someone else, it is recommended that the indoor temperature be maintained at least below 20ºC

with a relative humidity of between 30% and 50% in order to limit the entrance of dust and proliferation of bacteria.

It is of vital importance that management implements control measures to ensure that airborne contaminants are kept as low as possible and that employee exposure time is limited by rotating the employees so that they do not work for more than three hours at a time in one processing area. Although this measure would benefit worker health and safety, it is likely to be detrimental to product quality as it stands in contrast with proper manufacturing principles dictating that cross-contamination due to excessive entry/exit of food handlers to high-risk areas should be avoided. It is thus recommended that such measures be carefully implemented in accordance with rigorous hygiene measures. The emphasis on clean personal protective equipment and sanitation when entering a clean area in order to reduce chances of cross-contamination should be carefully managed. Ventilation systems are of the utmost importance in every facility in order to obtain a suitable airflow. The location and the size of the ventilation intakes should be well thought through before installation because the design of the abattoir would affect the extent to which airborne particulates containing bacteria can enter the processing facility. Physical segregation from floor to ceiling, or directed air movement, would be effective in reducing dispersal of particulates in the various areas.

Chicken production facilities should be planned as an integral system that reduces environmental impacts while promoting worker safety. The building and its roof should be well designed so that they fit in well with their surroundings. The flooring design has been shown to affect airborne particulates levels and cleaning procedures to a significant extent, and solid floors have been proven to facilitate easier cleaning procedures than open-mesh floors. Any adaptations should take the climate and other environmental characteristics of the region into consideration while addressing the hygiene requirements of the facility.

Management should select an employee to be in charge of evaluating the ventilation system in all the areas of the plant in order to ensure that problems occurring due to failures are dealt with promptly. The particular employee should be given training on the monitoring and evaluation of the ventilation systems prior to commencing with such duties. In the case of a multi-purpose room, the air distribution in the area should also be taken into consideration. In the particular facility investigated, "clean operations" such as packaging are conducted in the same room as "dirty operations" like evisceration, and in such cases efficient air circulation should prevent unnecessary product contamination.

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CHAPTER 4

RELATIONSHIP BETWEEN ENVIRONMENTAL PARAMETERS AND BIOAEROSOLS AT A CHICKEN PROCESSING FACILITY

4.1 INTRODUCTORY REMARKS

Literature articulates several routes of food contamination such as via surface contact, personnel or the air, but whether these routes could be influenced by other contaminating sources is not clear (Lammerding, 1997; Lutgring *et al.*, 1997; Marks *et al.*, 1998; Churchill *et al.*, 2000; Hoffman, 2002; Den Aantrekker, 2003; Prendergast *et al.*, 2004). This chapter investigates the association between airborne microbiota and various environmental factors that have an influence on the dispersal of microorganisms at the chicken abattoir discussed in the previous chapters. Mathematical and statistical models are used to show interactions between dependent variables and such interactions are in turn applied to predict changes in one variable concomitant with changes in another.

Estimations of the nature and extent of relationships between selected airborne microorganisms (*Staphylococcus aureus*, fungi, total coliforms, *Escherichia coli*, *Pseudomonas aeruginosa*, *Listeria monocytogenes*, *Bacillus cereus* and presumptive *Salmonella* spp) and the environmental factors (relative humidity, temperature, wind velocity and airborne particulates) analysed in previous chapters of this study were calculated and inferential statistics were used to indicate possible relationships. This may further cast light on the control of such environmental factors in an attempt to curb the resulting proliferation of microbiota. In contrast, if no clear relationship exists,

additional research will be necessary to find ways and means of controlling the airborne microbial populations.

4.2 STATISTICAL ANALYSIS

For normally distributed data, general ANOVA and correlations were used to measure significant differences amongst means of the environmental factors and bioaerosols. The data consists of four observations (August to November) with sample collections done in duplicate. Stepwise regression in determining the significance of the independent variables (environmental factors) in various regression models was used. In this context the *t* statistics indicated that the independent variable is significant if the related *p*-value is less than 0.05 (StatSoft, *Statistica* Version 7.1).

4.3 RESULTS AND DISCUSSION

4.3.1 Correlations and *p*-values calculated between the environmental factors and the bioaerosols

In the present study, the environmental factors and microorganisms that had significant influences were listed in Table 4.1. Relative humidity and *Bacillus cereus* in the receiving/killing area indicated a strong negative relationship (r=-0.9838) with the p-value representing a significant difference (*p*-value=0.016) (Table 4.1). The results of *Bacillus cereus* in the receiving/killing area further suggested that relative humidity had a negative effect on the dispersal of airborne *Bacillus* spores.

Table 4.1. Correlation and *p*-values calculated between environmental factors and bioaerosols

Variables	*r*	*p-value*
Humidity 1 & *Bacillus cereus* [1]	-0.9838	0.016
Temperature 1 & *Staphylococcus aureus* [1]	0.9738	0.026
Airflow 1 & Total coliforms [1]	0.9997	0
Airflow 1 & *E. coli* [1]	0.9925	0.007
Airflow 1 & *Pseudomonas aeruginosa* [1]	0.9984	0.002
Airflow 1 & *Bacillus cereus* [1]	0.9923	0.008
Airborne particles 1 & *Salmonella* [1]	0.9439	0.056
Airflow 2 & *Bacillus cereus* [2]	-0.9802	0.02
Temperature 3 & Fungi [3]	0.9761	0.024
Airborne particles 3 & *Bacillus cereus* [3]	-0.9737	0.026
Temperature 4 & Total coliforms [4]	-0.9770	0.024
Temperature 4 & *Salmonella* [4]	-0.9773	0.022
Airborne particles 4 & Total coliforms [4]	0.9869	0.013
Airborne particles 4 & *Salmonella* [4]	0.9867	0.014
Humidity 5 & *Pseudomonas aeruginosa* [5]	0.9687	0.031
Airflow 5 *Pseudomonas aeruginosa* [5]	0.986	0.014

1. Receiving/Killing area
2. Defeathering area
3. Evisceration area
4. Air-chilling area
5. Packaging area
6. Dispatch area

The presence of *Pseudomonas aeruginosa*, a prominent spoilage microorganism, showed an increase associated with an increase in relative humidity conditions (r=0.9687; p=0.031). It is known that a microorganism stops growing when its growth medium dries out and that the majority of microorganisms survive better in an environment with high relative humidity. However, *Bacillus cereus* appears to prefer a dry environment (Table 4.1).

Unlike *Bacillus cereus, Staphylococci* spp. prefer humans as primary reservoir and contamination of food occur either by direct contact or indirectly through skin fragments, or respiratory tract droplets (Jablonski and Bohach, 1997; Buchanan, 2000). This organism may further colonise the food processing equipment and especially areas that are difficult to clean such as the ventilation system. The occurrence of *Staphylococcus aureus* in the air of the receiving/killing location was expected to be high due to the organism's widespread and ubiquitous nature (Nagase *et al.*, 2001). *Staphylococcus aureus* counts detected in this study were relatively similar to those described in a study conducted by Shale (2004) on red meat, which reported *Staphylococcus aureus* to be the most abundant organism found. A strong positive correlation (r=0.9738) was observed between the temperature and *Staphylococcus aureus* in the receiving/killing area with a *p*-value showing significance at 0.026.

The parameters, fungi and temperature showed a strong positive relationship in the evisceration area (r=0.9761; *p*=0.024) while the total coliforms and presumptive *Salmonella* spp. both showed a strong negative relationship with temperature in the air-chilling area. The majority of airborne microorganisms observed in this study, showed a strong positive relationship with airflow in the receiving/killing area. In the defeathering area however, *Bacillus cereus* showed a strong negative relationship, as was the case with *Pseudomonas aeruginosa* in the packaging areas. Results confirmed airflow and airborne particulates to be a factor that contributes to the dispersal of *Bacillus cereus* spores.

Airborne particulates and the presence of *Salmonella* spp. in the receiving/killing area correlated strongly (r=0.9439) and showed significance at 0.056. Airborne particulates and aerosols can therefore serve as carriers of bacteria and pathogens particularly in the receiving/killing area. Lutgring *et al.* (1997) similarly isolated *Salmonella* from the receiving/killing area although rarely in other processing areas. Mitchell (2000) also suggested that this organism was likely to be introduced into the facility via dust spread by the birds. The environmental factors monitored in the air-chilling area did not appear to have any significant influence on the dispersal of total coliforms or on airborne presumptive *Salmonella* spp in the air-chilling area. On the other hand, airborne particulates in the air-chilling area correlated strongly (r=0.9869; *p*-value=0.013) with total coliforms as well as with *Salmonella*

(r=0.9867; *p*=0.014) indicating that the dispersal of microorganisms is likely to occur due to particulates carried from one area to another.

4.3.2 Results of stepwise regression

A linear relationship between *Staphylococcus aureus,* relative humidity and temperature in the receiving/killing area was observed when general ANOVA was used (Table 4.2). A second model in the stepwise regression used fungi in the receiving/killing area as an independent variable, and no significant difference was shown. Temperature, airflow and airborne particulates could not be used to explain the presence of fungi in the receiving/killing area. *Pseudomonas aeruginosa* (p=0.0016) and *Bacillus cereus* (p=0.0633) in the receiving/killing area were significantly different when airflow was used as a parameter estimate, suggesting that the distribution of microorganisms was adequately described by one or more of the parameters relative humidity, temperature, airflow and airborne particulates (Hoffman, 2002). Once again when *Bacillus cereus* was chosen as an independent variable, relative humidity in the receiving/killing area showed significance at p=0.0167 (Table 4.2). In this study, the presence of *Staphylococcus aureus*, fungi and total coliforms in the defeathering area could not be explained with the environmental factors monitored. *Bacillus cereus* was the only organism that displayed a significant relationship with airborne particulates (p=0.0032). In the packaging area *Staphylococcus aureus*, fungi, total coliforms, *Listeria monocytogenes*, *Bacillus cereus* and presumptive *Salmonella* spp. showed no

Table 4.2. Stepwise regression representing signific ous microbial groups and the environmental factors

	S. aur [1]		Fungi [1]	Total C [1]		Ps [1]	*Listeria* [1]	BC [1]		*S. aur* [2]
1. y=bo+b1+b2+	Humi 1	Temp 1								
2. Analysis of variance (Pr>F)		0.0020								
3. Ha B1≠0 (Humidity) (P<0.05)	0.0022									
Ha B2≠0 (Temperature) (P<0.05)	0.0006									
1. y=bo+b1+b2+			Airflow 1							
2. Analysis of variance (Pr>F)			0.1789							
3. Ha B1 ≠0 (Intercept) (P<0.05)			0.0011							
1. y=bo+b1+b2+				Airflow 1	Temp 1					
2. Analysis of variance (Pr>F)				0.0003						
3. Ha B1≠0 (Airflow 1) (P<0.05)				0.00026						
1. y=bo+b1+b2+						Airflow 1				
2. Analysis of variance (Pr>F)						0.0016				
3. Ha B1≠0 (Humidity) (P<0.05)						0.0077				
1. y=bo+b1+b2+							Temp 1			
2. Analysis of variance (Pr>F)							0.0633			
3. Ha B1≠0 (Humidity) (P<0.05)							0.00073			
1. y=bo+b1+b2+								Hum 1	Airflow1	
2. Analysis of variance (Pr>F)								0.0032		
3. Ha B1≠0 (Humidity) (P<0.05)								0.0167		
Ha B2≠0 (Airflow1) (P<0.05)								0.0115		
1. Analysis of variance (Pr>F)										0
2. Intercept										0.0575

1. *S. aur* [1] – *Staphylococcus aureus* in the receiving/killing location
2. Total C [1] – Total coliforms in the receiving/killing location
3. Ps [1] – *Pseudomonas aeruginosa* in the receiving/killing location
4. BC [1] – *Bacillus cereus* in the receiving/killing location
5. *Listeria* [1] – *Listeria monocytogenes* in the receiving/killing location

linear relationship with any of the environmental factors. *Pseudomonas aeruginosa* showed significance at p=0.0077, with airflow in the packaging area with general ANOVA showing a linear relationship between *Pseudomonas aeruginosa* and airflow in the same area. *Staphylococcus aureus* and total coliforms in the dispatch area showed no significant difference because no variables met the 0.500 significance level in this area of the abattoir.

In conclusion, relative humidity and airborne particulates appeared to be the parameters that had the most influence on the presence of microorganisms in the facility. Results further showed that the higher the relative humidity, the better the chances of proliferation of microorganisms. From these observations it is recommended that the relative humidity in the facility be maintained at least at levels below 50%. Airflow in the receiving/killing area proved to have an influence on the dispersal of the selected microorganisms, further evidencing a strong positive correlation and necessitating the regular monitoring of the airflow in the processing areas.

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CHAPTER 5

GENERAL CONCLUSIONS

5.1 INTRODUCTION

The purpose of this study was to evaluate the presence of airborne microorganisms and the influence that environmental factors have on the distribution of microorganisms in a chicken processing facility. Chapter 2 reported on the distribution of airborne microorganisms in six processing areas (receiving/killing, defeathering, evisceration, air-chilling, packaging and dispatch) while the third chapter focused on the environmental factors that were considered the most likely sources of the distribution of airborne microorganisms in the six processing areas. Finally chapter 4 reported on the possible statistical relationship that exists between airborne microorganisms and environmental factors monitored in an attempt to identify the most likely sources of contamination by specific microbiota.

5.2 A SURVEY OF MICROBIAL BIOAEROSOLS IN A HIGH-THROUGHPUT CHICKEN SLAUGHTERING FACILITY

In Chapter 2, the levels of airborne microorganisms were shown to correlate with reports in similar studies undertaken in food processing environments (Cormier *et al.*, 1990; Heedreick *et al.*, 1991), where *Staphylococcus aureus* constituted the largest number of all airborne microbiota (2.2×10^4 cfu.m^{-3}). Fungi showed an average almost similar to that of *Staphylococcus aureus* (2.1×10^4 cfu.m^{-3}). None of the measured bioaerosols exceeded stipulated guidelines. The overall results showed the airborne microorganisms to occur predominantly in the receiving/killing

area. Generally, the microbiological quality of the air in the present study was within acceptable safety limits when compared to infective doses and related local legislation. The presence of certain types of airborne microorganisms in the facility, although within acceptable limits, indicated a need for improving the infrastructure of the facility. The need for proper physical separation of all the processing areas as well as the introduction of guidelines governing airborne microorganisms in South African poultry processing plants need definite consideration.

5.3 A SURVEY OF BIOAEROSOL-RELATED ENVIRONMENTAL PARAMETERS IN A HIGH-THROUGHPUT CHICKEN PROCESSING FACILITY

Environmental factors such as relative humidity, temperature and wind velocity have been reported to be the key parameters related to the survival of bacteria in dust (Ellerbroek, 1997; Alwis *et al.*, 1999). A proper ventilation system is a requirement in almost every area of a food processing plant and one of the primary functions of such a system is to dilute and remove indoor airborne contaminants at a rate dependent on the effective rate of ventilation and outdoor air pollutant concentrations (Done, 1991; Smith *et al.*, 1993).

The results of this study revealed relative humidity at an average of 71%, temperature at 18°C, wind velocity at 7m.s^{-1} and airborne particulates at 3.76 mg.m^{-3}. Based on the results, it was suggested that inadequate ventilation in the facility was among other things a key factor that could

hamper product quality. The design of the facility also affects the extent to which particulates containing bacteria can enter. Directed movement of air from clean to dirty processing areas and the physical separation of different processing areas were regarded as important to avoid cross-contamination. Management at the facility should be acquainted with regulations governing proper processing environments (such as the SANS 049 and R918) in order to produce products meeting consumer demands as well as import and export standards. In order to produce safe products, cross-contamination must be avoided at all times. It was finally concluded from the observations that factors such as airborne particulates and relative humidity have a notable effect on the airborne microbial load.

5.4 RELATIONSHIPS BETWEEN ENVIRONMENTAL PARAMETERS AND BIOAEROSOLS AT A CHICKEN PROCESSING FACILITY

Chapter 4 highlighted the relationships that were observed between the selected environmental factors and airborne microorganisms using inferential statistical methods. The relationship found between airborne microorganisms and environmental factors indicated that the organisms are distributed in the plant to a large extent due to the influence of these environmental factors. The findings of this study indicated that the prevalence of spoilage and pathogenic bacteria increased as relative humidity increased ($r=0.9687$; $p=0.031$). In cases similar to this, air movement needs to be properly controlled in order to reduce the likelihood of contamination of the final product. The presence of *Bacillus cereus* in

the receiving/killing area showed relative humidity to have a negative effect on the dispersal of *Bacillus* spp spores (r=-0.9838; p=0.016) (Table 4.1). In cases where fungi were detected, relative humidity and temperature appeared to be jointly responsible for occurrence of fungi, thus necessitating the monitoring and control of airborne microorganisms. The presence of *Staphylococcus aureus* in the receiving/killing area was also expected due to the nature and ecology of the organism.

5.5 RECOMMENDATIONS TO INDUSTRY

Based on the results of this study, it is clear that the presence of airborne microorganisms together with the monitored environmental factors have the potential to contribute considerably to the spoilage of the product as well as to exert adverse effects on exposed workers. The following recommendations are suggested:

- Each of the processing areas should be colour coded in order to represent the extent of the hazard in that particular area (Figure 5.1). This strategy should go a long way towards sensitising the workers regarding the possibility of contamination in the area.
- Effective air distribution should be improved, rather than simply increasing the overall ventilation rate in all processing areas.
- Regular surveillance, cleaning and servicing ventilation system is essential to ensure that they are in a good operating order.
- Separation of processing areas by physical barriers, such as plastic or air curtains is recommended.

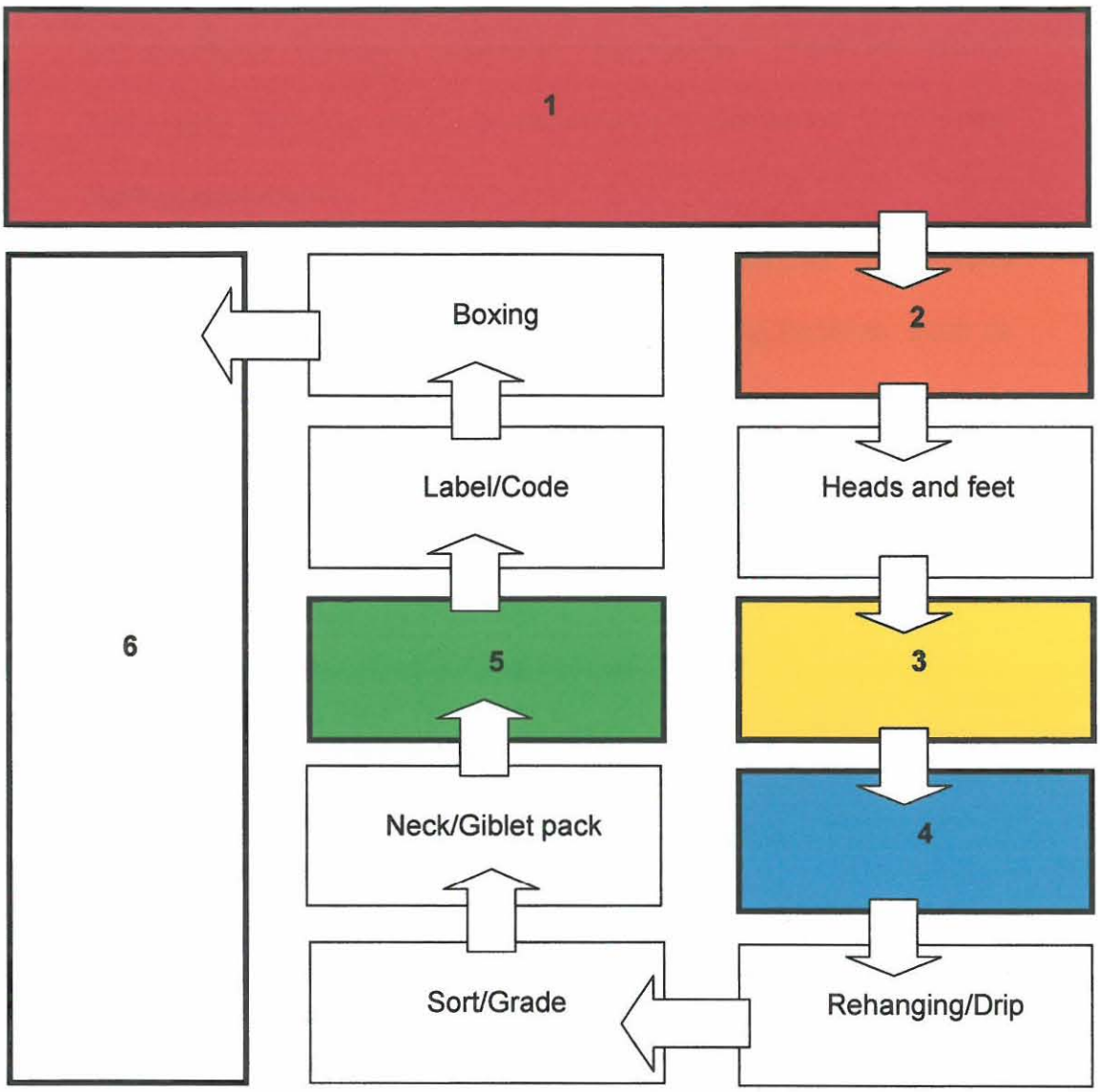

Figure 5.1 A visual representation of the risk categorisation related to airborne contamination in various processing areas of a typical poultry abattoir: 1) high contamination risk (red); 2) relatively high risk (orange); 3) moderate risk (yellow); 4) low risk (blue); 5) minimal/negligible risk; and 6) neutral area (white). This classification should aid management in identifying which areas are the most prone to contamination and which strategies should receive priority towards reducing the bioaerosol contamination.

- Possible sources and modes of contamination of the product should be identified through observing employees' practices during processing. Training should be essentially implemented to minimise such contamination.
- The development and/or refinement of national regulations governing airborne microorganisms in processing facilities, such as poultry plants, should be considered.
- Quality assurance and control programmes such as regular audits by third parties, and the pre-requisite programmes to HACCP or ISO 9000, should be implemented.

5.6 FUTURE RESEARCH

The following possible research projects were identified:

- Assessment of the food-handler practices that contribute to the airborne microbial population through the use of a descriptive survey.

- Comparison of the microbial species distribution and frequencies in the indoor air of the poultry processing plant for use in indoor air quality investigations.

- Investigation of the routes of contamination and intervention measurements to minimise microbial dispersion in the receiving/killing locations.

- Determination of the contribution of airborne contaminants to the actual microbial load on the product and resulting effects on shelf life.

- Identification of economic considerations related to the reduction of shelf-life of the product due to airborne microorganisms.

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