بسم الله الرحمن الرحيم

**Sudan University of Science and Technology**

**College of Graduate Studies**

# Implementing the Hazard Analysis Critical Control Points (HACCP) System in Fish Markets in Khartoum State, Sudan

# تطبيق نظام تحليل المخاطر ونقاط المراقبة الحرجة في اسواق الاسماك بولاية الخرطوم، السودان

AThesis Submitted in Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Food Safety


**By:**

**Hisham Mohamed Ahmed Omer**

B.Sc. (honors) Fish Sciences Al Neelain University College of Agricultural Technology and Fish Sciences

M.Sc Degree of Science in Fish Technology Sudan University of Sciences and Technology

**Supervisor:**

**Prof. Mohamed Abdulsalam Abdullah**

Department of Preventive Medicine and College of Veterinary Medicine, Sudan University of Sciences and Technology


**April, 2017**

# الآيــــة

قال تعالى :

{يَا أَيُّهَا الَّذِينَ آمَنُوا إِذَا قِيلَ لَكُمْ تَفَسَّحُوا فِي الْمَجَالِسِ فَافْسَحُوا يَفْسَحِ اللَّــهُ لَكُمْ وَإِذَا قِيلَ انشُزُوا فَانشُزُوا يَرْفَعِ اللَّــهُ الَّذِينَ آمَنُوا مِنكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ وَاللَّــهُ بِمَا تَعْمَلُونَ خَبِيرٌ ﴿١١﴾}

**سورة المجادلة الآية** (11)

## Dedication

To my father

To my mother

To my brothers and sisters

To all persons who support me

**Hisham**

# Acknowledgments

Firstly, thanks for almighty God whom without his help this work could not have been done, a lot of respect to my supervisor Prof. Mohamed Abdulsalam for his constant support, valuable guidance and encouragement during the preparation of this research.

I would like to thank our teaching staff for their great help.

Last but not least; I would like to thank my family and friends for their love and support.

# Table of contents

## List of tables

## List of figures

# Abstract

This study was carried out to isolate and identify the bacteria which contaminate the fish market in Khartoum state which may cause food poisoning. The total number of swab samples was 240, Central market 120 sample and 120 samples from Almorda market. The swab samples were collected randomly in two these fish market from different sites of the fish, workers hands, deportation cars, Porters and Aldrdaqat, Alnnazafin and Equiepment, Wholesaler and Retailer. Therefore, this work was carried out to detect some bacterial microorganisms from fish market such as *Staphylococcus aureus, Salmonella spp and Escherichia coli.* Also the questionnaires was done were filled by 50 workers responders, 25 respondents Central market and 25 respondents from Almorda market. Isolation and identification of bacteria were done by using different biochemical tests. The Total Viable count (TVC) of isolated bacteria was from Central market in Fish 2.59 ± 0.08 log10 CFU /cm², and Almorda market in Wholesaler 2.50 ± 0.10 log10 CFU /cm². It's found in Almorda market the highest percentage of *Salmonella species* was (80.9%), followed by *Escherichia coli* (70%) in Central market and then *Staphylococcus aureus* (41%) from Central market difference significant ($P \leq 0.05$). The results of food hygiene practices, all workers never used prevention to reduce the contamination (84%), also they did not knowing (56%), the transmission of fish diseases and they (96%) use ice or refrigerator and sawdust as preservation materials, while their attitudes in health status (88%) for continuous investment difference significant ($P \leq 0.05$). The all data in this study were statistically analyzed using the statistical package for social science (SPSS) Computer Software Version 11.5. Pathogenic bacteria of public health importance in fresh fish and found in two fish markets and revealed the problem of Knowledge, Attitude of Food Safety Practice of Food Handlers of workers of fish markets. From this study, education program for proper food safety hygiene and improvement of preservation and handling must be applyied.

# ملخص الدراسة


اجريت هذه الدراسة للتعرف علي البكتريا التي تلوث الاسماك في اسواق الاسماك في ولاية الخرطوم والتي قد تتسبب في التسمم الغذائي. وكان العدد الاجمالي للعينات 240 مسحة ، 120 مسحة من السوق المركزي، 120 مسحة من سوق الموردة. وجمعت العينات عشوائياً من الأسماك، ايادي العاملين، سيارات الترحيل ، الحمالون و الدر داقات، النظافين وادواتهم، تجار الجملة وتجار التجزئة. واسهتدفت الدراسة الأنواع التالية :

*Staphylococcus aureus, Salmonella spp and Escherichia coli*

وفي الجزء الثاني من هذه الدراسة لمعرفة سلوك العاملين في اسواق الاسماك بين مجموعة من العاملين 50 عامل: 25 عامل في السوق المركزي و25 عامل في سوق الموردة. بعد الجمع تم عزل وتعريف انواع البكتريا بإستخدام إختبارات كيميائية مختلفة. وتم جمع معلومات الدراسة عن طريق الاستبيان عن طريق اخذ العينة العشوائية للعاملين في اسواق الاسماك.

من هذه الدراسة أنه وجد أن العدد البكتيري في عدة اماكن مختلفة في السوق ولقد وجد ان الاسماك في السوق المركزي $2.59 \pm 0.08$ log10 CFU /cm² وفي الموردة في منطقة تاجر الجملة $2.50 \pm 0.10$ log10 CFU /cm². وجد ايضا ان اكبر نسبة من *Salmonella spp* (80%) في سوق الموردة يليها *Escherichia coli* (70%) في السوق المركزي ثم *Staphylococcus aureus* (41%) في السوق المركزي . كما اظهرت النتائج ان هناك اختلاف احصائي ($P \leq 0.05$) بين السوقين. وقد اوضحت نتائج الدراسة : ان كل الردود للممارسات الصحية الوقائية لتقليل التلوث ان جميع العاملين لا يستخدمون الاجراءات الوقائية لتقليل التلوث (84%) ، وايضا جميع العاملين لايعلمون شئ عن الامراض المنقولة بواسطة الاسماك الملوثة (56%) ولكن اغلب العاملين (96%) يستخدمون الثلج ، الثلاجات ونشارة الخشب في حفظ الاسماك ، بالنسبة للمواقف الصحية (88%) الصحة العامة مهمة

لاستمرار التاجر في السوق. كما اوضحت الدراسة ان هنالك اختلاف احصائي ($P \leq 0.05$) بين السوقين. تم تحليل جميع البيانات في هذه الدراسة إحصائيا باستخدام برنامج الحزمة الإحصائية للعلوم الاجتماعية (SPSS) برامج الكمبيوتر النسخة 11.5. و قد أكدت النتائج أيضا وجود البكتيريا المسببة للأمراض ذات الأهمية في الصحة العامة في الاسماك الطازجة في السوقين. كما اظهرت ايضا ان هنالك مشكلة في صحة وسلامة الغذاء في اسواق الاسماك. التوصية من هذه الدراسة هي تقديم برنامج تعليمي لنظافة أمثل وتحسين وسائل حفظ الاسماك وطرق التداول. وان يتم تدريب وتثقيف العاملين بسلامة الغذاء واتباع الممارسات الصحية السليمة اثناء تداول الاسماك في اسواق الاسماك.

# Chapter One

# Chapter one

## 1.0 Introduction

Fish are the most numerous of vertebrate, with at least 20,000 known species and more than 58% are found in marine environment (Thurman *et al*, 1984). Fish are one of the most important sources of animal protein available in the tropics and has been widely accepted as a good source of protein and other vital nutrients for the maintenance of a healthy body (Andrew, 2001). Fish meats are very important sources of proteins, minerals, vitamin ….etc. However, fish meat spoil more quickly than other muscle foods, particularly when poor handled and such spoilage is primarily bacterial in nature; about 30% of landed fish are lost through microbial activity alone (Ghaly *et al.*, 2010). Developing countries captured 50% of the world harvest and a large proportion of the catch are consumed internally (FAO, 1985). In the Sudan fish distributed over an area that amount to 100,000 km of fresh water and 760 km of marine, the total sustainable production amount estimated to be about 114,100 tonnes/year and human consumption is estimated at only 1.4 kg/ year (Meske, 1985). Freshness is the most important criteria for judging the quality with the pH of 7 (Rodriquez-Jerez *et al.,* 2004). These characteristics make the seafood as a suitable living and proliferation place for bacteria under unhygienic storage conditions. This may harmful for human health by causing infection and intoxication (Liston, 1980). Contamination of fish with microorganism reflected environment pollution (Adeyemo, 2003). Contamination of fish from enteric bacteria of human and animal origin may also be responsible for various food spoilages (Geldreich *et al.*, 1966). Consumer's greatest concern is the quality and safety of food they eat. To achieve these, it is important to popularize good hygienic practices. The post-harvest handling of catch is the most important step in the production of a high quality finished product (Balasubramaniam *et al*, 2009). Sudan fishery in general

depending on traditional fishery (Khalid, *et al* 2008). In Sudan fresh fish transported chilled or refrigerated (FAO, 2002). Ice storage has been the most widely used method for the preservation of fresh fish on sea and on land. After being caught, fishes are placed in fish boxes or mixed with crushed ice and kept in fish carrying crates during trips between the fishing grounds and fish piers or markets. In cold regions, thick layers of slime containing a high bacterial load of diverse organisms often accumulate on the bottom of fish holds. In warm weather or in tropical areas, fish holds are usually filled with an ice water mixture that also accumulates fish secretions. The bacterial flora in ice melt from fish containers is important for economic reasons and for fish quality control (Chen and Chai, 1982). Proper preservation begins at the moment the fish is harvested from the water. Fish should be chilled quickly to the temperature of melting ice soon after capturing and maintained at that temperature, but micro-organisms found in fish from cold water can easily adapt to the low temperature of ice (Shewan, 1977). Food handlers play an important role in food safety and in the transmission of food poisoning, because they may introduce pathogens into foods during production, processing, distribution and even presentation (Angelio *et al*., 2000). An understanding of food safety procedures and potential factors that cause food born illness is very important for all food handlers (Cohen *et al*, 2001). Bacteria have been found to cause disease subsequently leading to low production rate of fish. Most of these bacteria that cause diseases are considered to be saprophytic in nature. These bacteria only become pathogens when they are physiologically unbalanced, nutritionally deficient, or there are other stressors such as poor water quality and overstocking, which allow opportunistic bacterial infections to proceed (Egbere *et al*., 2008). Conscious of the requirements of the importing countries and the regulations for production and placing on the market of fishery products, the processing sector has undertaken all necessary precautions through safety management systems to ensure seafood safety and quality. The production and processing industries are responsible for the

production of good quality and safe seafood using own check mechanisms which based on HACCP principles. The production is consumer oriented and most of their production is meant for export. Production practices, including hygiene and sanitation are controlled and monitored accordingly by the industry. However, the inspection body carries out checks for insight verification and on implementation of the regulations (Directorate of Fisheries, 2003).

## 1.1 Objectives:

- To isolation and identification contamination bacteria in fresh fish in different markets in Khartoum state.
- To assess the knowledge, attitudes and practices of the fresh fish markets in the Khartoum state.
- To evaluate the hygienic handling of fish and various materials used for fish preservation and transportation including, ice, cars, equipments etc.

# Chapter Two

# Chapter Two

## 2.0 Literature Review

Important attributes makes the commodity readily susceptible to microbial attack particularly bacteria. Fish flesh naturally contains very low levels of carbohydrates and these are further depleted during the death struggle of the fish (Adams and Tobias., 1999). Fish and fishery products are highly perishable food, and its quick perishability has been the main hurdle in its preservation (Okoro *et al*., 2010; Dewi *et al*., 2011; Musa *et al*., 2010). Is most important source of high quality protein, providing approximately 16% of the animal protein consumed by the world's population (FAO, 1997). In Africa, fish supplies 17% of protein and it is one of the cheapest sources of protein in Africa (Claucas and Ward, 1996). The microbiological diversity of fresh fish muscle depends on the fishing grounds and environmental factors around it (Cahill, 1990). Nutritionally, fish is a very important source of easily digestible, high quality protein, vitamins and fats not obtainable in such high concentrations elsewhere. Nonetheless, fish is a highly perishable foodstuff and spoils very fast unless appropriate preservation and processing techniques are applied. In addition, fish as any other foodstuff pose the risk of becoming unsafe due to hazards or contamination along the food chain. Fish in their natural environment is relatively safe food but food-borne disease and illness may occur due to infections or intoxications. These food safety hazards are closely linked to hygiene, sanitation, processing or preparation operations and marketing of fish. Thus, there is the element of risk in consumption of processed fish (Ahmed, 1991). Fish are susceptible to a wide variety of bacterial pathogens. The organisms especially bacteria are already present on the skin and in the alimentary tract of the living seafood's such as fish (Ampofo and Clerk, 2010). The bacteria are transmitted by fish that have made contact with other diseased fish. Bacterial fish disease and infections are very common and are one of the most difficult health problems to deal with (Douglas,

2007). Recent investigations indicate that the fish caught in unpolluted waters carry only a few micro-organisms on their surfaces and in the gut. Most of the bacterial contamination found on the landed fish appears to be related to the handling practice of the fish and bacterial growth during storage (Elinor *et al.*1985). It has been suggested that the type of micro-organisms that are found associated with particular fish depends on its habitat (Claucas and Ward, 1996). The bacterial pathogens associated with fish have been classified as indigenous and non-indigenous (Kvenberg, 1991). Pathogenic and potentially pathogenic bacteria associated with fish and shellfish include *mycobacteria, Streptococcus iniae, Vibrio vulnificus, Vibrio spp., aeromonads, Salmonella spp*. and others (Lipp and Rose, 1997; Zlotkin *et al*., 1998; Chattopadhyay, 2000). Much variation exists in the patterns of spoilage of fish species. From the small amount work published on spoilage characteristics of fresh waterfish it appears that they have longer shelf lives on ice than those from marine water (Disney 1976, Shewan, 1977 in Gelman *et al*. 1990). Several studies have demonstrated many bacteria species encountered in different fish which are potentially pathogenic under certain conditions as reported for *Pseudomonas angulluseptica, Streptococcus sp* (Wiklund *et al*, 1994). It has been highlighted that fish consumption can be an important avenue for human pathogenic bacteria and other food borne diseases exposure to man (Christopher *et al*., 2009). Human infections caused by pathogens transmitted from fish or the aquatic environment are quite common depending on the season, patients'contact with fish and related environment, dietary habits and the immune system status of the exposed individual. They are often bacterial species facultatively pathogenic for both fish and man and may be isolated from fish without apparent symptoms of disease. The infection source may be fish kept either for food or as a hobby (Acha and Szyfres, 2003). Pathogens from fish can be transmitted to humans through both active and passive contact and may cause food borne diseases such as, dysentery, typhoid, fever, salmonellosis and cholera. The practice of livestock-fish farming

needs to be placed in perspective with the likely health risks (FAO, 2003). The non-indigenous contamination of the fish or the habitat and examples include *Escherichia coli, Clostridium botulinum, Shigella dysenteriae, Staphylococcus aureus , Listeria monocytogens and Salmonella*. The indigenous bacterial pathogens are found naturally living in the fishs habitat for example *Vibrio* species and *Aeromonas* species (Rodricks, 1991). Pathogenic and potentially pathogenic bacteria associated with fish and shellfish include *Mycobacteium, Streptococcus spp., Vibrio spp., Aeromonas spp., Salmonella spp.* and others (Lipp and Ross, 1997). E. coli is a classic example of enteric bacteria causing gastroenteritis. E. coli including other coliforms and bacteria as *Staphylococcus spp*. and sometimes enterococci are commonly used as indices of hazardous conditions during processing of fish. Such organisms should not be present on fresh-caught fish (Chattopadhyay, 2000). The contamination of food of fish origin with pathogenic E. coli probably occurs during handling of fish and during the production process (Ayulo *et al*., 1994; Asai *et al*., 1999). Fish and shellfish appear to be passive carriers of *salmonella*, demonstrate no clinical disease and can excrete *Salmonella spp*. without apparent trouble. The contamination of this organism derives from terrestrial sources and fish may serve as a vector for *Salmonella spp*. (Metz, 1980; Minette, 1986; Chattopadhyay, 2000). The use of different kinds of livestock manure in fish production may increase the level of pathogenic bacteria causing a public health risk to the rural community (Musaiger and Souza, 2008). Fish spoilage is a complex process in which physical, chemical and microbiological mechanisms are implicated (Hozbor *et al*., 2006). Some reports on the storage quality of frozen/chilled tilapia were still not comprehensive on spoilage mechanism and quality assessment (Arannilewa *et al*., 2005). The fresh water or rivers and lakes have a complex flora of microorganisms which include genuinely aquatic species as well as component introduced from terrestrial, animal and plant sources. The speed with which a product spoils is also related to the initial microbial load on the product: the

higher the count, the sooner spoilage occurs. Shell fish such as Tilapia have a particular large pool of nitrogenous extractives and are even more prone to raid spoilage, a factor which accounts for the common practice of keeping them alive until immediately prior to consumption (Adams *et al*., 1999). The scale of human activities determinative effect on coastal waters. Many shell fish used for food out particles from large volume of waters. If these waters have been contaminated with sewage, there is always the risk that enteric organisms from infected individuals may be present and concentrated by the filter feeding activities of shell fish (Adams *et al*., 1999). Also during handling of the commodity, the natural flora of the environment may be contaminated with organisms associated with man such as members of the *Enterobacteriaceae* and *Staphylococcus aureus* which can grow well at 30-37°c (Miceal *et al*., 2007). Bacterial contamination in food often results in food spoilage as well as life-threatening health hazards like food poisoning (Prescott *et al*., 1999). Their high water content, bacteria flora able to live at low temperatures and its high enzymatic activity, mainly autolitic, are responsible of the susceptibility of fish muscle. Among these changes, lipid oxidation is one of the most important. It leads to rancid flavours and reduces the shelf–life of fish products especially during storage (Flick and Martin, 1992). However fish are susceptible to a wide variety of bacterial pathogens, most of which are capable of causing disease and are considered by some to be saprophytic in nature (Lipp and Ross, 1997). Contamination of these seafoods is usually through the fecal-oral route. Fecal contaminated water and unsanitary handling by food handlers are the most common causes of contamination (Walderhaug, 1992). Most seafood is still extracted from a wide population, and the fishermen are hunters with no influence on handling of their prey before it is caught (Adebayo-Tayo *et al*., 2012). Treatment of products with chlorinated water reduces populations of pathogenic and other microorganisms on fresh products but cannot eliminate them. Reduction of risk for human illness associated with raw products can be

better achieved through controlling points of potential contamination in the field; during harvesting, processing or distribution or in retail markets, food-service facilities, or the home (Phpp, 1997). The use of ice for chilling is still one of the most important methods of preserving fish, though ice merely slows down microbial activity since fish can support a population of cold-tolerant bacteria (Liston 1982; Alberto *et al.* 1988). These bacteria are predominantly at the surface of the fish but secrete enzymes into the tissues, bringing about a complex series of chemical changes (Shewan, 1977). USFDA (2007) recommends that seafood be purchased only from reputable sources. These recommendations include: be wary, for example, of vendors selling fish out of the back of their pick-up trucks. buy only fresh seafood that is refrigerated or properly iced, do not buy cooked seafood, such as shrimp, crabs or smoked fish if displayed in the same case as raw fish. Cross-contamination can be occur, do not buy frozen seafood if the packages are open, torn or crushed on the edges, avoid packages that are above the frost line in the store's freezer. If the package cover is transparent, look for signs of frost or ice crystals. This could mean that the fish has either been stored for a long time or thawed and refrozen. Put seafood on ice, in the refrigerator or in the freezer, immediately after buying it. Recreational fishers who plan to eat their catch should follow state and local government advisories about fishing areas and eating fish from certain areas (USFDA, 2007). Usually, the bacteria inhabit the gill, skin and intestines during the life span of the fish without attacking the muscles and the later will only be possible when the fish died. When fish is preserved by icing the rate of bacterial penetration into the flesh muscle is much slower. It was reported that the number of bacteria in fish caught in temperate waters can develop even when in ice, while in tropical water they grow slowly for one or two weeks in icing preservation (Nguyen, 2005).

There have been several reports on the health risks associated with the consumption of processed seafood, ranging from allergic reactions, stomach and intestinal cancerous growths, a general degeneration of peripheral cellular tissues,

to gradual breakdown of the digestive and excretive systems in a statistically high percentage of people examined. Few of these reports however, have looked at the likely risks from a microbiological food safety point of view (Edema *et al*., 2005). The microbiological diversity of fresh fish muscle depends on the fishing grounds and environmental factors around it (Cahill, 1990). It has been suggested that the type of micro-organisms that are found associated with particular fish depends on its habitat (Claucas and Ward, 1996). The bacterial pathogens associated with fish have been classified as indigenous and non-indigenous (Kvenberg, 1991). The bacteria from fish only become pathogens when fish are physiologically unbalanced, nutritionally deficient, or there are other stressors, i.e., poor water quality, overstocking, which allow opportunistic bacterial infections to prevail (Austin, 2011). One of the risks involved in livestock integrated fish farming is possible transfer of pathogens between livestock and humans. Previous research has shown that, different kinds of livestock manure are contaminated with pathogenic bacteria such as *Salmonella, Shigella, Pseudomonas, Vibrio, Streptococcus, and E. coli* (Abdelhamid *et al.*, 2006). The microbial association with fish compromises safety and the quality for human consumption; particularly critical is when the micro-organisms are opportunistic and / or pathogenic in nature (Mhango *et al.*, 2010). The health of fish is dependent on the quality of water. Enteric bacteria such as *Escherichia coli, Staphylococcus aureus, Salmonella spp.* and *Vibrio spp*. are likely to accumulate in fish found in waters polluted with human wastes, located in polluted areas where hygienic standards are not maintained or in fish ponds supplied with water from polluted rivers. Therefore microbial quality of farmed fish is largely determined by the quality of water in which they are farmed (Fafioye, 2011). The speed of spoilage is related to the initial bacterial load: the higher the count, the sooner spoilage occurs (Adam and Tobaias 1999). Spoilage bacteria differ somewhat for freshwater and marine fish and for temperate and tropical water fish. Storage and processing conditions also affect microbial growth (Doyle,

2007). During handling and storage, quality deterioration of fresh fish rapidly occurs and limits the shelf life of the product (Sallam, 2007; Adebayo-Tayo *et al*., 2012d). Kvenberg,(1991) and Rodrick,(1991) classified bacteria pathogen of fish into two: the indigenous bacteria, those living naturally in fish and its habitat and nonindigenous bacteria, which are contaminant of fish or its habitat. Many bacteria that are potential spoilers abound in the surface slime, gill and intestine of live fish but the natural defenses prevent invasion while the fish is alive. Multiplication and invasion occurs soon after death of fish. Contamination of fish from enteric bacteria of human and animal origin may also be responsible for various food spoilages (Emikpe *et al*., 2011). (Agbolagba and Uwagbai, 2011). Agbolagba and Iyeru (1998) reported that improper smoking and unhygienic handling of smoked fish results in high microbial infestation and that storage temperature close to 37°C are ideal for the growth of pathogenic bacteria. They also stated that high humidity and high to moderate temperature support mould growth in stored food. Moulds produce mycotoxins some of which are carcinogenic while fungal groups may cause mycoses and allergies in man. The WHO (2006) has acknowledged that although interventions such as increased education and awareness about safe food handling practices are necessary, they are insufficient bases for personal action to get us the behavioral impact we desire as our end-goals. Essentially, achieving behavior change in relation to food safety implies encouraging individuals to assess their prevailing knowledge and practices, supporting safe and hygienic practices and, when necessary, providing incentives to facilitate the adoption of new, hygienic and healthy practices (WHO 2006; Ehiri and Morris 1996; Foster and Kaferstein 1985). Food safety, an increasingly important public health issue (WHO, 2004), refers to the conditions and practices that preserve the quality of food to prevent contamination microbes or toxic chemicals resulting in food borne illness. Food can be already contaminated with microbes, during the packaging process or mishandled, improbably cooked or stored (Medeiros *et al*, 2004). People most often get

infected as follows (Acha and Szyfres, 2003), through contact with infected fish while handling them, water or other constituents of fish life environment; the following cases of transmissions have been recorded so far; after injury by cleaning aquarium with bare hands (Alinovi *et al.*, 1993), through processing fish in the food industry and preparation of dishes (Notermans and Hoornstra, 2000) orally by consumption infected fish or related products or food contaminated with water or other constituents of water environment. Apart from factors relating to the living environment (exposure), the development of an infectious disease is markedly affected by internal factors such as the physiological status of consumer, particularly by immunosuppression and stress as in the case of HIV/AIDS (Von Reyn *et al.*, 1996). Therefore, food handlers' food safety knowledge is critical when attempting to improve food safety and hygiene practices. However, it is noted that knowledge alone is not sufficient. Several studies have found food handlers fail to implement appropriate practices despite having the requisite food safety knowledge (Clayton, *et al.*, 2002; Subratty, *et al.*, 2004). Subratty *et al.*, (2004) found that food vendors are quite aware of hygiene conditions; however, they do not translate their knowledge into practice. Food borne diseases are responsible for the majority of mortality and morbidity worldwide with up to 30% of population in industrialized countries suffering from foodborne illness annually (WHO, 2014). For more than a century, hand washing has been recognized as an essential component in the prevention of the spread of microbial infection (Fendler *et al.*, 1998). Poor personal hygiene, including inadequate hand washing among food handlers, is a common practice that contributes to foodborne illness in retail establishments (De Waal, 1996; Lynch, *et al.*, 2003; Food and Drug Administration, 2004). Numerous studies have highlighted the need for food safety training and education for food handlers, due to lack of knowledge on microbiological food hazards, optimal food storage temperatures, risks of cross contamination and the importance of personal hygiene (Bas *et al*, 2006; Mudey *et al.*, 2010; Nuchprayoon, *et al.*,

2009). According to Higgins (2007), anyone who work in food safety sooner or later discover that one of the most valuable tools for prevention is simply reading about and understanding how past outbreak have occurred. Using major and frequently famous or at least newsworthy outbreaks, Phyllis (2007) illustrate critical factors come together to produce tragic and largely preventable results modern microbes often team up with old practice, short sighted decision or current consumer tend to produce an outbreak. Bacteria may be found on the skin, chitinous shell, gills as well as the intestinal tracts of fish or shellfish. Human infections that may be caused by fish bacteria include food poisoning and gastroenteritis. Consumption of fish is responsible for 5-8% of food-borne disease outbreaks. The risk to public health arises if toxigenic strains multiply to high number during improper handling and storage leading to auto-enzymatic action and bacterial degradation. The microbial population of the ice obviously has a bearing on public health. The investigation of the microorganisms in the drainage of fish holds can provide clues for the improvement of sanitation in fishing boats, the optimization of fish processing, and the extension of fish shelf life (Chal and Levin, 1975). The less developed countries capture 50% of the world harvest and a large proportion of the catch are consumed internally (FAO, 1985). Disease cause economic losses not only from mortality but also treatment expenses, postponement or loss of the opportunity to sell the fish and contraction of zoonotic diseases by the handler and final consumer of the affected fish. Contamination of hands and surfaces during cleaning and evisceration of fish is a common route of pathogen infection through contamination of other food (Buras, 1993). Fish and Shellfish not only transmit disease to man but are themselves subject to many diseases and capable of transmitting many of the established food borne microbial infections and intoxications (FAO/WHO, 1974). The HACCP system was introduced in 1971 during the National Food Protection Conference. Seafood processors must keep in mind that HACCP does not replace GMPs or guard against fraudulent practices (Russell *et al*., 2004).

# Chapter Three

# Chapter Three

## 3.0 Material and Methods

### 3.1 Area of survey

This survey was conducted to assess the effect of personal hygiene microbial contamination of fishs before and after landing in Centeral market and Almorda market fish markets in Khartoum state from June 2014 to March 2016.

### 3.2 Sampling

A total of 240 swab samples were randomly collected (120 samples) from Centeral market and Almorda market (120 samples) from different site (Deportation cars, Porters and Aldrdaqat, Alnnazafin and Equiepment, Wholesaler, Retailer, Fish in every market).

### 3.3 Bacteriology

The swab samples (240 samples) were transported under ice in an ice box to microbiology laboratory of collage of Veterinary Medicine, Sudan University, within two hours. All swab samples were kept frozen before processed for bacterial diagnosis within 72 hours after collection.

### 3.3.1 Bacteria and culture media

The samples were streaked on blood agar, nutrient agar and macConkey agar and incubator at 37°C for 24 hrs. All culture and media were prepared according Oxoid (2006).

#### 3.3.1.1 Solid Media

##### 3.3.1.1.1 Blood agar

The prepare blood agar, the base medium used to make a selective medium. Preparing a batch of blood agar plates, a few plates should be prepared first to make sure the blood were sterile. Sterilization by autoclaving at 121°C for 15 minutes. When the agar cooled to 50°C, add of sterile blood aseptically and mix

gently but well. Avoiding formation of air bubbles. The media Dispensed aseptically in 15 ml amounts in sterile Petri dishes and Stored at 2–8°C, preferably in sealed plastic bags to prevent loss of moisture.

#### 3.3.1.1.2 MacConkey agar

It is differentiated media and usually used at a concentration of 5.2 g in every 100 ml distilled water. Sterilized by autoclaving at 121C° for 15 minutes. Then the medium cooled to 50–55° C, mixed well and dispensed aseptically in sterile Petri dishes (Cheesbrough, 2000).

#### 3.3.1.1.3 Nutrient agar

Nutrient agar prepared at a concentration of 2.8 g in every 100 ml distilled water (concentration may vary depending on manufacturer). Sterilization by autoclaving at 121°C for 15 minutes (Cheesbrough, 2000).

#### 3.3.1.1.4 Modified New York City (MNYC)

The media was prepared by mixing 2.5 ml sterile 10% w/v saponin solution with 50 ml sterile blood (human, bovine, or horse but not sheep). Sterilization of spooning solution by autoclaving at 115°C for 10 minutes dissolving of the GC agar base and yeast extract powder in the water by heating to 100 C°. Sterilization by autoclaving at 121° C for 15 minutes. Allow to cool to 50–55C° and then added aseptically the sterile saponin lyzed blood, the glucose solution, and the antibiotic supplement. Mixing well after each addition. Avoiding formation air bubbles (Cheesbrough, 2000).

#### 3.3.1.1.5 Xylose lysine deoxycholate (XLD) agar

This selective medium recommended for the isolation of salmonellae and particularly shigellae from faecal specimens. The medium prepared at a concentration of 5.3 g in every 100 ml distilled water. Heating the medium with care no overheating or autoclaving. As soon as the medium cooled to about 55°C, mixed well, and dispensed aseptically in sterile Petri dishes (Cheesbrough, 2000).

## 3.3.2. Pure cultures

Pure cultures produced for identification and sensitivity testing. The colonial appearances and changes in the media made by colonies may provide valuable identification information (Cheesbrough, 2000).

### 3.3.3 Gram stain

The Gram staining reaction was used to help identify pathogens in specimens and cultures by their Gram reaction (Gram positive or Gram negative) and morphology. Pus cells were also identified in Gram smears. Following staining with a triphenyl methane basic dye such as crystal violet and treatment with iodine, the dye–iodine complex is easily removed from the more permeable cell wall of Gram negative bacteria but not from the less permeable cell wall of Gram positive bacteria. (Cheesbrough, 2000).

### 3.3.4 Biochemical tests

#### 3.3.4.1 Urease test

The test organism was cultured in a medium which containsed urea and the indicator phenol red. Incubated at 35–37°C (preferably in a water bath for a quicker result) for up to 4 hours or overnight. Red/purple colour Positive urease test Yellow/orange  Negative urease test (Cheesbrough, 2000).

#### 3.3.4.2 Indole test

The test organism was cultured in a medium which contained tryptophan. Indole production was detected by Kovac's or Ehrlich's reagent full slop. This reaction with the Indole to produce a red coloured Examination for a red colour in the surface layer within 10 minutes was considered a positive test (Cheesbrough, 2000).

#### 3.3.4.3 Citrate utilization test

The tested Organism in Simmon's citrate agar cultured slopes in the medium. Using a sterile straight wire, first streak or the slope with a saline suspension of

the test organism and then stab the bottom. Incubating at 35°C for 48 hours. Changing of the medium to bright blue on the Simon's agar indicated a positive citrate test and No change in colour of Negative citrate test of medium. (Cheesbrough, 2000).

### 3.3.4.4 Oxidase test

Preparation of 10 ml Tetramethyl-p phenylenediamine, 0.1 g of dihydrochloride was added 10 ml D.W. it is a piece of filter paper was soaked with a few drops of oxidase reagent. A colony of the test organism was then smeared on the filter paper. Positive test was deep purple colour. (Cheesbrough, 2000).

### 3.3.4.5 Kligler iron agar (KIA)

the medium different organisms, prepared from ready to use dehydrated powder. The medium was used at a concentration of 5.5 g in every 100 ml distilled water and bubbles in the medium indicated gas production from glucose fermentation. Gas was production of fermentation of lactose and glucose (dextrose) and the production of hydrogen sulphide . Organism that can fermented glucose produced a red slant. (Cheesbrough, 2000).

### 3.3.4.6 Motility

The movement of small motile bacteria must be distinguished from the on-the-spot vibratory movement (Brownian movement) which is shown by all microorganisms and particles when suspended in a fluid. True bacterial motility is the ability of an organism to move itself in different directions or a single direction. (Cheesbrough, 2000).

### 3.3.4.7 Catalase test

These organism was bringing into contact with hydrogen peroxide. Bubbles of oxygen were released if the organism was a catalase producer. Active bubbling was Positive catalase test, No bubbles were a Negative catalase test. (Cheesbrough 2000).

### 3.3.4.8 Coagulase test

Free coagulase which converted fibrinogen to fibrin by activating a coagulase-reacting factor present in plasma. Free coagulase was detected by clotting in the bound coagulase (clumping factor) which converted fibrinogen directly to fibrin without requiring a coagulase reacting factor. It can be detected by the clumping of bacterial cells in the test. ( Cheesbrough 2000).

### 3.3.5 Isolation and Identification Procedures

Isolated bacteria were identified based on morphological characteristics, Gram's stain reaction and biochemical test. Result were confirmed using manual for determinative bacteriology Identification media: These include media to which substrates or chemicals are added to help identify bacteria isolated on primary cultures. Organisms are mainly identified by a change in the colour of the medium and or the production of gas. (Cheesbrough, 2000).

### 3.3.6 Total viable count

The total viable count (TVCs) of the isolated microorganism was carried out according to the method of Harrigan (1998), aseptically the appropriate serial dilutions of both parts of fishes were spread on poured counting agar, plates Baird-Parker agar and Potato Dextrose Agar for counting of total viable bacteria count. All inoculated plates were incubated at 37 ℃ for 24-48 hours except Potato dextrose agar palates, which were incubated for 72 hours at 25 °C as described by (Harrigan, 1998).

## 3.4 Questionnaire

Questionnaires were filled by 50 workers in Two fish markets in Khartoum state. Each Questionnaire was comprised three distinct parts: food hygiene knowledge, attitudes and practices. In the knowledge part, there were close-ended questions emphasizing personal hygiene. Each question was provided by three possible answers (true, false and don not know ). Subsequent part of the questionnaire was dealing with the attitudes of the responders about various hygiene measures for food safety. The handlers were asked to indicate their level of agreement to the statements using a three-point rating scale (agree, disagree and no idea). Practices of food workers assessed by their self-reported hygienic behaviors in the last part of the questionnaire . In this part question were provided with five-point rating scale (never, rarely, sometimes, often and always). Although the questionnaire was intended to be self-administered, some illiterate workers needed help in filling it.

### 3.5 Statistical analysis

Statistical analysis was conducted using SPSS software for windows, version 11.5. Descriptive statistics crosstabulion were provided and spearman's correlations coefficient was used to test the association between knowledge, attitudes and practices scores. P-value less than 0.05 were considered as statistically significant.

# Chapter Four

# Chapter Four

## 4.0 Result

The mean and standard error of total viable count (TVC), of bacteria on two fish markets were 2.39 log10 CFU/cm² in the Almorda market and in central market 2.48 log10 CFU/cm², over all mean 3.94. Both fish markets showed high significant difference ($P < 0.001$) in Table 1. The mean and standard deviation of total viable count of bacteria in Almorda market deportation cars 2.43 ± 0.14, porters and Aldrdaqat 2.29 ± 0.09, alnnazafin and equipment 2.44 ± 0.11, Wholesaler 2.50 ± 0.10, Retailer 2.48 ± 0.09 and Fish in every market 2.25 ± 0.13. Bacterial contamination was found in all places of sampling of the market, which Significant difference ($P < 0.05$) (Table 2).

As shown in Table 3 mean and standard deviation of total viable count of bacteria on Place of sampling of Central market Deportation cars 2.37 ± 0.19, porters and Aldrdaqat 2.43 ± 0.18, Alnnazafin and Equiepment 2.47 ± 0.15, Wholesaler 2.54 ± 0.08, Retailer 2.51 ± 0.21 and Fish in every market 2.59 ± 0.08. The Significant difference was high ($P < 0.001$) of overall mean between place of sampling fish markets.

In table 4 deportation cars in both fish markets were significantly difference ($P < 0.05$). Where as Wholesaler in two fish markets were showed no significant difference.

Table 5 showed the types of bacteria isolated from two fish markets. The high rate of contaminated of *E. coli* was 70 % in Central market, while *Salmonella species* 80 % in Almorda market.

The results of the questions regarding food safety and hygiene practices were shown in Table 9. The questions were divided into personal hygiene practices Overall (84.0%) of participants ‘never’ using mask during work in Almorda market. Implemented personal hygiene practices expected of food handlers. More

than half of the respondents (96.0%) they always wearing gloves during work in Central market.

The results at the questions regarding food safety knowledge were presented in Table 10. The knowledge was evaluated in relation to disease transmitted by fish contamination and regulations for food safety and workers' health. The majority of participants selected the false answer or identified that they did not know the answer for most of the food safety topics (56%) in Almorda market and answer (96%) to using ice or refrigerator reducing fish spoilage, using sawdust to save fish the participants selected the true (68%). The data relating to participants' attitudes to food safety were shown in Table 11. It Participants believed food safety was an important responsibility in their job. the majority disagreeing with this survey or did not awareness of food safety.

## 4.1 Bacterial Viable Count

**Table (1): Over all mean and standard Error of Total viable count of bacteria on two fish markets in Khartoum state.**

| n.o | Area | (CFU/cm²) Log10 | Sig. |
|---|---|---|---|
| 1 | **Almorda market** | 2.39 | * |
| 2 | **Central market** | 2.48 | * |
| 3 | **Over all mean** | 3.94 | *** |
| 4 | **S.E** | 0.002 | NS |

**S.E:** Stand Error. **Sig:** Significance. *: Significant difference at level (**P** < 0.05).
***: Hig significant difference at **P** value of <0.001. **NS.** Not significant (**P** > 0.05).

**Table (2): Mean and Standard deviation of Total viable count of bacteria on Place of sampling of Almorda market in Khartoum state.**

| No | Place of sampling | Std. Deviation | (CFU/cm2) Log10 | Sig. |
|---|---|---|---|---|
| **1** | **Deportation cars** | ± 0.14 | 2.43 | ** |
| **2** | **porters and Aldrdaqat** | ± 0.09 | 2.29 | |
| **3** | **Alnnazafin and Equiepment** | ± 0.11 | 2.44 | |
| **4** | **Wholesaler** | ± 0.10 | 2.50 | |
| **5** | **Retailer** | ± 0.09 | 2.48 | |
| **6** | **Fish in every market** | ± 0.13 | 2.25 | |

**Sig:** Significance. ****:** significant difference at P value of <0.05

**Table (3): Mean and Standard deviation of Total viable count of bacteria on Place of sampling of Central market in Khartoum state.**

| N.o | Place of sampling | Std. Deviation | (CFU/cm2) Log10 | Sig. |
|---|---|---|---|---|
| **1** | **Deportation cars** | ± 0.19 | 2.37 | *** |
| **2** | **porters and Aldrdaqat** | ± 0.18 | 2.43 | |
| **3** | **Alnnazafin and Equiepment** | ± 0.15 | 2.47 | |
| **4** | **Wholesaler** | ± 0.08 | 2.54 | |
| **5** | **Retailer** | ± 0.21 | 2.51 | |
| **6** | **Fish in every market** | ± 0.08 | 2.59 | |

**Sig**: Significance

******* : Hig significant difference at **P** value of <0.001

**Table (4): Comparison of mean total viable count of bacteria (log10 CFU/cm2) ± Standard deviation at different Place of sampling of fish markets in Khartoum state.**

| N.o | Place of sampling | (CFU/cm²) Log10 ± std deviation | | Sig |
|---|---|---|---|---|
| | | Almorda market | Central market | |
| 1 | **Deportation cars** | 2.43 ± 0.14 | 2.37 ± 0.19 | * |
| 2 | **porters and Aldrdaqat** | 2.29 ± 0.09 | 2.43 ± 0.18 | * |
| 3 | **Alnnazafin and Equiepment** | 2.44 ± 0.11 | 2.47 ± 0.15 | NS |
| 4 | **Wholesaler** | 2.50 ± 0.10 | 2.54 ± 0.08 | NS |
| 5 | **Retailer** | 2.48 ± 0.09 | 2.51 ± 0.21 | NS |
| 6 | **Fish in every market** | 2.25 ± 0.13 | 2.59 ± 0.08 | * |

**Sig:** Significance. ***** Significant difference at level (**P** < 0.05). **NS.** Not significant (**P** > 0.05)

**Table (5): Bacteria isolated and identified (N %) at two fish markets in Khartoum state.**

| N.o | Area | *Escherichia coli* | | *Salmonella* | | *Staphylococcus* | |
|---|---|---|---|---|---|---|---|
| | | Negative | Positive | Negative | Positive | Negative | Positive |
| **1** | **Almorda market** | 40 % | 60% | 20% | 80% | 61% | 39% |
| **2** | **Central market** | 30% | 70% | 48% | 52% | 59% | 41% |

## 4.2 Biochemical reactions of bacteria

### Table (6): Biochemical reactions of *Salmonella* spp. isolated from the fish samples collected from the fish markets

| **Test/substrate** | **Results** | | ***Salmonella* spp. reaction** |
|---|---|---|---|
| | **Positive** | **Negative** | |
| Urease | No color change | Change to pink | _ |
| Indole | color change | No color change | + |
| Hydrogen sulphide | Blackening | No blackening | + |
| Citrate | Chang color and turbid medium | No chang color and turbid medium | + |
| Kliger iron agar (KIA) | color change | No color change | + |
| Catalase | Release of oxygen bubbles | No release of oxygen bubbles | + |
| Oxidation fermentation | Fermentative (F) | Oxidative (O) | F |
| Motility | Turbidity | No turbidity | + |
| Oxidase | Color on reagent paper | No color on reagent paper | _ |

KEY: + Positive
- Negative

**Table (7): Biochemical reactions of *Staphylococcus aureus* isolated from the fish samples collected from the fish markets**

| **Test/substrate** | **Results** | | ***Staphylococcus aureus* . reaction** |
|---|---|---|---|
| | **Positive** | **Negative** | |
| Urease | No color change | Change to pink | _ |
| Indole | color change | No color change | _ |
| Hydrogen sulphide | Blackening | No blackening | + |
| Citrate | Chang color and turbid medium | No chang color and turbid medium | _ |
| Kliger iron agar (KIA) | color change | No color change | + |
| Catalase | Release of oxygen bubbles | No release of oxygen bubbles | + |
| Oxidation fermentation | Fermentative (F) | Oxidative (O) | F |
| Motility | Turbidity | No turbidity | _ |
| Oxidase | Color on reagent paper | No color on reagent paper | _ |

KEY: + Positive
- Negative

**Table (8): Biochemical reactions of *Escherichia coli* isolated from the fish samples collected from the fish markets**

| Test/substrate | Results | | *Escherichia coli* . reaction |
|---|---|---|---|
| | **Positive** | **Negative** | |
| Urease | No color change | Change to pink | _ |
| Indole | color change | No color change | + |
| Hydrogen sulphide | Blackening | No blackening | _ |
| Citrate | Chang color and turbid medium | No chang color and turbid medium | _ |
| Kliger iron agar (KIA) | color change | No color change | + |
| Catalase | Release of oxygen bubbles | No release of oxygen bubbles | + |
| Oxidation fermentation | Fermentative (F) | Oxidative (O) | F |
| Motility | Turbidity | No turbidity | + |
| Oxidase | Color on reagent paper | No color on reagent paper | _ |

KEY: + Positive
- Negative

**Table(9): Responses for assessment of respondent's food hygienic practices of food workers in fish markets in Khartoum state.**

| Statements | Area | Responses, | | | | | Sig |
|---|---|---|---|---|---|---|---|
| | | Never | Rarely | Sometimes | Often | Always | P-value |
| **Do you use gloves during work?** | **Central market** | 4.0% | 0% | 0% | 0% | 96.0% | .000 |
| | **Almorda market** | 76.0% | 16.0% | 4.0% | 0% | 4.0% | |
| **Do you wash your hands before using gloves?** | **Central market** | 36.0% | 24.0% | 8.0% | 4.0% | 28.0% | .33 |
| | **Almorda market** | 48.0% | 20.0% | 20.0% | 4.0% | 8.0% | |
| **Do you wear apron during work?** | **Central market** | 44.0% | 16.0% | 12.0% | 4.0% | 24.0% | .35 |
| | **Almorda market** | 56.0% | 16.0% | 20.0% | 4.0% | 4.0% | |
| **Do you use mask during work?** | **Central market** | 68.0% | 8.0% | 4.0% | 4.0% | 16.0% | .22 |
| | **Almorda market** | **84.0%** | 12.0% | .0% | 4.0% | .0% | |
| **Do you use cap during work?** | **Central market** | 48.0% | 4.0% | 36.0% | 8.0% | 4.0% | .01 |
| | **Almorda market** | 68.0% | 24.0% | 4.0% | .0% | 4.0% | |
| **Do you wash your hands before you touch raw meat?** | **Central market** | 20.0% | 12.0% | 24.0% | .0% | 44.0% | .04 |
| | **Almorda market** | 20.0% | 20.0% | 48.0% | 4.0% | 8.0% | |
| **Do you wash your hands after you touch raw meat?** | **Central market** | 8.0% | 8.0% | 4.0% | 0% | 80.0% | .46 |
| | **Almorda market** | 12.0% | 4.0% | 16.0% | 0% | 68.0% | |
| **Do you eat or drink in your work place?** | **Central market** | 48.0% | .0% | 24.0% | 4.0% | 24.0% | .05 |
| | **Almorda market** | 32.0% | 12.0% | 4.0% | 4.0% | 48.0% | |
| **Do you smoke in your work place?** | **Central market** | 52.0% | 4.0% | 12.0% | 8.0% | 24.0% | .10 |
| | **Almorda market** | 28.0% | 24.0% | 4.0% | 4.0% | 40.0% | |

## Table (10): Food safety knowledge in fish markets in Khartoum state.

| Statements | Area | Responses, n (%) | | | Sig P-value |
|---|---|---|---|---|---|
| | | True | False | Do not know | |
| **Typhoid can be transmitted by fish contamination** | **Central market** | 4.0% | 52.0% | 44.0% | 1.00 |
| | **Almorda market** | 4.0% | 52.0% | 44.0% | |
| **Diarrhea can be transmitted by fish contamination** | **Central market** | 20.0% | 52.0% | 28.0% | .09 |
| | **Almorda market** | 4.0% | 44.0% | 52.0% | |
| **AIDS can be transmitted by fish contamination** | **Central market** | 0% | 52.0% | 48.0% | .57 |
| | **Almorda market** | 0% | 44.0% | 56.0% | |
| **Jaundice can be transmitted by fish contamination** | **Central market** | 0% | 52.0% | 48.0% | .57 |
| | **Almorda market** | 0% | 44.0% | 56.0% | |
| **Abortion in pregnant women may be induced by food borne disease** | **Central market** | .0% | 56.0% | 44.0% | .59 |
| | **Almorda market** | 4.0% | 52.0% | 44.0% | |
| **bacteria is among the causes fish spoilage** | **Central market** | 48.0% | 36.0% | 16.0% | .51 |
| | **Almorda market** | 64.0% | 24.0% | 12.0% | |
| **Remember the fish well with ice or use the refrigerator reduces fish spoilage** | **Central market** | 96.0% | 4.0% | .0% | 1.00 |
| | **Almorda market** | 96.0% | 4.0% | .0% | |
| **Use sawdust to save the fish increases pollution and fish contamination** | **Central market** | 68.0% | 32.0% | .0% | .76 |
| | **Almorda market** | 64.0% | 36.0% | .0% | |

**Table (11): Food safety attitudes of food workers in fish markets in Khartoum state.**

| Statements | Area | Responses | | | Sig P-value |
|---|---|---|---|---|---|
| | | Agree | Disagree | No idea | |
| **Using gloves is important in reducing risk of food contamination** | **Central market** | 52.0% | 48.0% | .0% | .59 |
| | **Almorda market** | 48.0% | 48.0% | 4.0% | |
| **Using apron is important in reducing risk of food contamination** | **Central market** | 52.0% | 48.0% | .0% | .23 |
| | **Almorda market** | 36.0% | 56.0% | 8.0% | |
| **Using masks is important in reducing risk of food contamination** | **Central market** | 52.0% | 48.0% | .0% | .09 |
| | **Almorda market** | 24.0% | 72.0% | 4.0% | |
| **Using caps is important in reducing risk of food contamination** | **Central market** | 48.0% | 52.0% | .0% | .00 |
| | **Almorda market** | 8.0% | 92.0% | .0% | |
| **Food hygiene training for workers in fish markets is an important issue in reducing risk of food contamination** | **Central market** | 88.0% | 4.0% | 8.0% | .00 |
| | **Almorda market** | 80.0% | .0% | 20.0% | |
| **Health status of the workers should be evaluated in fish markets** | **Central market** | 88.0% | 4.0% | 8.0% | .30 |
| | **Almorda market** | 80.0% | .0% | 20.0% | |
| **Food borne illnesses can have deleterious health and economic effects on the society** | **Central market** | 36.0% | 56.0% | 8.0% | .07 |
| | **Almorda market** | 12.0% | 64.0% | 24.0% | |

# Chapter Five

# Chapter Five

## 5.0 Discussion

This is the first study on the microbial quality of market fishes in Centeral market and Almorda market, Khartoum state Sudan.

The result from this research shows that the bacterial load varies in the three segments of the fishes analyzed, since the markets and in the stages of production was found by the proportions of the overall mean and standard error of total viable count of bacteria on two fish markets (Table1) and high rates bacterial contamination in the transportation to a direct sales of retailers of (Table 1). These results are agreed results of (FAO, 1983). Generally sterile immediately after catching, however, it may become contaminated with different microorganisms during subsequent handling as these microorganisms can penetrate from skin and the gut to the flesh. The microbiological diversity of fresh fish muscle depends on the fishing grounds and environmental factors around it (Cahill, 1990). These results showed (Table 2) total viable count of bacteria at different Places of sampling and in Almorda market the bacterial contaminationed all stages of the market, which led a significant difference at level ($P < 0.05$) and this in agreement with Disease breaks out in fish tank very quickly and having the first identification of the type of disease before the action take place. The bacteria are transmitted by contact with other diseased fish. Bacterial fish disease and infections are very common and are one of the most difficult health problems to deal with (Douglas, 2007). It has been suggested that the type of micro-organisms that are found associated with particular fish depends on its habitat (Claucas and Ward, 1996). The quality of fish continuously changes during different stages from harvesting to marketing. After death, the fish has to cross rigor mortis and the body of fish acts as a suitable medium for the growth and multiplication of bacteria. and this in agreement with our results

(Tanle 3) with Jayasinghe (2006) investigated the quality of tap water, sea water, ice, fish surface and landing centre and found them contaminated by fecal bacteria like *E. coli* and *Salmonella sp* and this in agreement with our results (Table3). Spencer (1959) emphasized on the sanitation of fish boxes and has made investigations on their quantitative and qualitative bacteriological studies. Comparison of total viable count of bacteria at different Place of sampling of fish markets (Table 4) showed highest contamination and this was in agreement with fish flesh (FAO,1983). These results in agreement with some reports on the storage quality of frozen/chilled tilapia were still not comprehensive on spoilage mechanism and quality assessment (Arannilewa *et al.*, 2005). The Percentage of bacteria isolated from two fish markets (Table 5) in these results indicated unhygienic condition and high contamination both places. The isolating of *Staphylococcus aureus* in this results may be due to improper handling, improper storage and cross contamination. In the high incidence of *Staph aureus* in the examined samples could indicate unhygienic conditions because the product contamination could be the result of a combination of improper handling, improper storage and cross contamination (Simon and Sanjev, 2007). *Salmonellosis* can manifest in a number of disease syndromes including gastroenteritis, bacteremia, typhoid fever and focal infections (Darwin and Miller, 1999). Most fish related food borne illness are traced to *Salmonella, Staphylococcus spp., Escherichia coli., Vibrio parahemolyticus ,Clostridium perfringens ,Clostridium botulinum E and Entero viruses* (Center for food safety and nutrition, 2001). Hess (1934), Kiser (1944), Ingram (1958) and Redfort (1932) who have reported that bacterial multiplication occurred in frozen fish, although the speed of development might be strikingly altered. Some bacteria suffer death while freezing however, freezing and storage under frozen conditions have virtually no action on bacterial spores and yeasts. Ingram (1951), Lochhead and Jones (1938), Lund and Halvorson (1951), Gunderson and Rose (1948) and Hartsell (1951) stated that freezing did not appear to have practically

any effect on the cultural or other characteristics of most psychrophilic bacteria after thawing. They have also reported that frozen fish spoil quickly than slow frozen fish. Chistyakov and Noskova (1955) have observed that some micro-organisms have adapted to freezing temperature.

The questionnaire included three part of the practical extent of the workers and their knowledge, attitudes and hygienic practices of workers (Table 9).These results disagree with consumer's greatest concern is the quality and safety of food they eat. To achieve these, it is important to popularize good hygienic practices. The post-harvesting and handling of catching is the most important step in the production of a high quality finished product (Balasubramaniam *et al.*, 2009). The observation through the answers especially knowledge were varied in preventive operations to reduce pollution. For practice, the reducing or increasing to limit pollution most of answers were false. These results demonstrate the lack of knowledge of the health and food safety for workers in the fish markets (Table 10). These results agreed with (Poyoung, 2007) food handlers which are not attend food hygiene training programs as in another study in which food handlers were negligent or lacked food sanitation and hygiene training. This result was similar to the results of (Wilcock *et al.*, 2004) who found that there was an association between knowledge and current practice. It was also mentioned that awareness of food handlers would improve their practice. All employees agreed that diseases of borne contamination unrelated to the fishing grounds but transmitted by fish contamination such as Typhoid, Diarrhea, AIDS, Jaundice, abortion in pregnant women may be induced by food borne disease (Table 10). Pathogens from fish can be transmitted to humans through both active and passive contact and may cause food borne diseases such as, dysentery, typhoid, fever, salmonellosis and cholera. The practice of livestock-fish farming needs to be placed in perspective with the likely health risks (FAO, 2003). One of the risks involved in livestock integrated fish farming is possible transfer of pathogens between livestock and humans. Previous study showed that different kinds of

livestock manure are contaminated with pathogenic bacteria such as *Salmonella, Shigella, Pseudomonas, Vibrio, Streptococcus, and E. coli species* (Abdelhamid *et al*., 2006). Well of fish with good ice or using the refrigerator reduces fish spoilage rot and this is not knowing by all workers in the field and also they agreed that the bacteria increase pollution and lack of sawdust to save the good fish (Table 10). It is believed that many consumers do not adhere to safety guidelines of prompting refrigeration after purchasing perishable foods, separation of raw and ready to eat foods, proper dairy storage, keeping perishable foods at or below 4°C and following the correct procedures when thawing frozen foods (Li Cohen *et al*, 2002). Another part of the questionnaire the attitudes of food workers in fish markets and their answers are sandwiched between agree or disagree, or no idea such as using gloves, apron, masks, caps are important in reducing risk of food contamination (Table 11). Food hygiene training for workers in fish markets is an important issue in reducing risk of food contamination. In addition, Kraus (1995) suggested that attitude was an important psychological factor which could predict food safety behavior. Health status of the workers should be evaluated in fish markets, food borne illnesses can have deleterious health and economic effects on the society. Behaviors such as these help to determine the food safety attitude of consumers and can also contribute to an understanding of their actual food safety behavior (Redmond and Griffith, 2003).

## 5.1. Conclusion

This study showed that food handlers had less understanding of the risk of microbial and chemical contamination of food or did not they know how to manage the increase contamination. Food handlers should be attended proper training in the basic principles of food safety and rules of personal hygiene in order to improve their practices in food handling. Food handlers should pay attention to the importance of cross contamination, cleaning raw material, and the factors determining the growth of pathogenic organisms in food and indicated that food safety education must be applied in this target group.

## 5.2 Recommendations

1 Do not use traditional methods of display gabions and use plastic utensils.
2 Do not display fish products on the ground and exposed to direct sunlight.
3 Improving the means of storage central refrigerators to maintain quality of the fish.
4 Proper processing, storage, and handling procedures should be cultivated.
5 Training and qualification of workers in the field of fish in the health and food safety.
6 More studies should be done in this topic.

## 5.3 References

**Abdelhamid, A.M., Gawish, M.M., Soryal, K.A. (2006).** Comparative study between desert cultivated and natural fisheries of mullet fish in Egypt. IIMicrobiological concern. J. Agric. Scie., Mansoura Univ., 31: 5681- 5687.

**Acha P.N., Szyfres B. (2003).** Zoonoses and communicable diseases common to man and animals. Vol. I. Bacterioses and mycoses. 3rd ed. Scientific and Technical Publication No. 580, Pan American Health Organization, Regional Office of the WHO, Washington, USA, ISBN 92 75 31580 9, 384 pp.

**Adams, A, J., and Tobias,W.J. (1999).** Red mangrove prop-root habitat as fin fish nursery area: A case study of Salt River Bay, St Croix, U.S.V.I. Proceedings of the 46th. Gulf and Carribean. Fisheries Institute, 46, 22-46.

**Adeyemo OK (2003).** Consequences of Pollution and Degradation of Nigerian Aquatic environment on Fisheries Resources. The Environmentalist. 23(4): 297-306.

**Adebayo-Tayo AC; Odu NN; Michael MU;Okonko IO.( 2012)**. Multi-Drug Resistant (MDR) Organisms isolated from Sea-foods in Uyo, South-Southern Nigeria. Nature and Science; 10(3): 61-70.

**Andrew, A. E. (2001).** Fish processing technology. University of Ilorin press, Nigeria. pp. 7-8.

**Angelio IF, Viggiani NM, Rizzo L, Bianco. A (2000).** Food handlers And food borne diseases; Knowledge, attitudes and reported behaviour in Italy. J. food Prot. 63: 381 – 383.

**Arannilewa ST, Salawu SO, Sorungbe AA, Ola-Salawu BB (2005).** Effect of frozen period on the chemical, microbiological and sensory quality of frozen tilapia fish (Sarotherodun galiaenus). Afr. J. Biotechnol. 8: 852-855.

**Austin, B. (2011).** Taxonomy of bacterial fish pathogens. Vet. Res., 42 (1): 20. Cahill, M.M. 1990. Bacterial flora of fishes: a review. J. Microb. Ecol., 19(1): 21-41.

**Agbolagba, O.J and Iyeru, O.A.(1998).** Study of insect pest infecting traditionally processed fish sold in Benin City metropolis. Nigerian Journal Applied Science, 16, 25-29.

**Agbolagba O.J. and Uwagbai, E.C. (2011).** A comparative analysis of the microbial load of smoked dried fishes (Ethmalosa fimbriata and Pseudotoliyhus elongates) sold in Oba and Koko markets in Edo and Delta states Nigeria at different seasons. Australian Journal Basic and Applied Sciences, 5(5), 544-550.

**Ahmed F.E, (1991),** Seafood Safety, Food and Nutrition Board Institute of Medicine, National Academy Press, Washington, DC.

**Alberto Pedrosa-Menabrito and Joe M R. (1988).** Shelf-life of fresh fish-A review part 1 – spoilage fish. *Journal of food quality* **11:** 117-127.

**Alinovi A., Vecchini F., Bassissi P. (1993).** Sporothricoid mycobacterial infection – a case-report. Acta Dermato- Venereologica, 73, 146–147.

**Ampofo, J. A. and Clerk, G. C. (2010).** Diversity of bacteria contaminants in tissues of fish cultured in organic waste-fertilized ponds: health implications. *The Open Fish Science Journal.* **3**: 142-146.

**Asai Y., Murase T., Osawa R., Okitsu T., Suzuki R., Sata S., Yamai S., Terajima J., Izumiya H., Tamura K., Watanabe H. (1999).** Isolation of Shiga toxin-producing Escherichia coli O157:H7 from processed salmon roe associated with the outbreaks in Japan, 1998, and a molecular typing of the isolates by pulsed-field gel electrophoresis. Kansenshogaku Zasshi., 73, 20–24.

**Ayulo A.M., Machado R.A., Scussel V.M. (1994).** Enterotoxigenic Escherichia coli and Staphylococcus aureus in fish and seafood from the southern region of Brazil. Int. J. Food Microbiol., 24, 171–178.

**Balasubramaniam, S., J. Charles and Krishna Srinath (2009).** Adoption of hygienic practices at Fish Landing Centres and Markets Fish. Technology. 46: 177-184.

**Bas, M., Ersun, A. S., & Kivanc, G. (2006).** The evaluation of food hygiene knowledge, attitudes, and practices of food handlers in food business in Turkey. Food Control, 17, 317e322.

**Burgess, G.H.D. & Shewan, J.M. (1970).** The bacteriology of fresh and spoiling fish and some related chemical changes. In: Recent Advances in Food Science (edited by J. Hawthorn & M. Leitch). Pp.167–193. London: Butterworths.

**Buras, N. (1993).** Microbial safety of produce from wastewater-fed aquaculture. In: Pullin RVC, Rosenthal H, Maclean J.L eds. Environment and aquaculture in developing countries. Proceeding of the 31st ICCLARM Conference Manila. Internatinal center for Living and Aquatic Resources. 285-295.

**Cahill, M.M. (1990).** Bacterial flora of fishes: a review. J. Microb. Ecol., 19(1), 21- 41.

**Center for food safety and Applied Nutrition (2001).** Food and drug administration .3rd edition, Washington .pp.145-166.

**Chal, T. and Levin, R. E. (1975).** Characteristics of heavily mucoid bacterial isolates from fish pen slime. Appl. Microbiol. 30: 450-455**.**

**Chattopadhyay P. (2000).** Fish – catching and handling. In: Robinson R.K. (ed.): Encyclopedia of Food Microbiology. Vol. 2, Academic Press, London. 1547 pp.

**Cheesbrough, M. (2000).** District Laboratory Practice in Tropical Countries Part 2, Second Edition.

**Chen, H. C. and Chai, T. (1982).** Microflora of Drainage from Ice in Fishing Vessel Fishholds. Applied And Environmental Microbiology. 43: 1360-1365.

**Chistyakov, F. and Noskova, G. (1955).** The adaptation of microorganisms to low temperatures. Proc. Intern. Congr. Refrig. 9th Cong., Paris. 2: 230-235.

**Christopher, A.E., Vincent, O., Grace, I., Rebecca, E., Joseph, E. (2009).** Distribution of heavy metals in bones, gills, livers and muscles of (Tilapia) Oreochromis niloticus from Henshaw Town Beach market in Calabar Nigeria. Pak. J. Nutr., 8: 1209 -1211.

**Claucas, I.J., Ward, A.R. (1996).** Post-harvest fisheries development: A guide to handling, preservation, processing and quality. Charthan, Maritime, Kent ME4 4TB, United Kingdom.

**Clayton, D., Griffin, C., Price, P., & Peter, A. (2002).** Food handlers' beliefs and self-reported pract ices. *International Journal Environment Health Report*, 12(1),25e39.

**Cohen E, Reichel A, Schwartz Z (2001)**. On the efficiency of an in- House food sanitation training programme: Statistical measurement and practical conclusions.

**De Waal, C.S., & Dahl, E. (1996).** *Dine at your own risk: The failure of local agencies to adopt and enforce national food safety standards for restaurants.* Washington, DC: Center for Science in the Public Interest.

**Dewi RS, Huda N, Ahmed R. (2011).** Changes in the physiochemical properties, microstructure and sensory characteristics of shark dendeng using different drying methods. Am. J. Food Technol., 6: 149-157.

**Directorate of Fisheries, (2003),** UNU/FTP lecture notes on the structure of inspection system in Iceland.

**Disney, D. G. (1976).** The spoilage of fish in the tropics. Proc. 1st. Ann. Trop.sub. trop. Technology conf. Americas, Texas. A & M University, Texas. pp 23-39.

**Douglas, D. (2007).** Identifying fres water Aquarium fish disease. Available on line at *http://fishsuite101. com/article.cfm/identifyingfishdisea ses.*

**Doyle EM. (2007).** FRI BRIEFINGS: Microbial Food Spoilage: Losses and Control Strategies. A Brief Review of the Literature. Food Research Institute, University of Wisconsin–Madison

*http://fri.wisc.edu/docs/pdf/FRI_Brief_Microbial_Food_Spoilage_7_07.pdf*

**Edema MO, Atayese AO, Idowu AO (2005).** Microbiological Quality of Microwave-processed foods. In: the Book of Abstract of the 29th Annual Conference and General Meeting on Microbes As Agents of Sustainable

Development, organized by Nigerian Society for Microbiology (NSM), UNAAB, Abeokuta from 6-10th Nov., 2005. p.17

**Egbere, O. J., A'kadir, T., Oyero and Steve, K. (2008).** Bacteriological quality of catfish ponds in Metropolis, Nigeria. University of Jos Nigeria Institutional Repository. pp 1-19.

**Ehiri J, Morris G. (1996).** Hygiene training and education of food handlers: Does it work? Ecol. FoodNutr. 35:243–251.

**Elinor M., Ravesi Josegh J., Licciardello, Bette E., Tuhkunen and Ronald C. Lundstrom. (1985).** The effect of handling or processing treatments on storage characteristics of fresh spiny dogfish. Marine fisheries review 47: 1 48-64.

**Emikpe B.O., Adebisi T. and Adedeji O.B. (2011).** Bacteria load on the skin and stomach of Clarias Gariepinus and Oreochromis Niloticus from Ibadan, South West Nigeria: Public health implications. J. Microbiol. Biotech. Res., 1 (1): 52-59.

**FAO/WHO (1974).** Food and Agriculture Organization of the United Nations and World Health Organization Technical Report series (No. 550) Fish and Shellfish Hygiene report of WHO expert committee convened in cooperation with FAO.

**FAO, (1983).** Zoonotic diseases in the Near East Region .Regional Office.

**FAO, (1997).** Review of the State of World Aquaculture. FAO Fisheries Circular No. 886, Rev. 1. Rome, Italy.

**FAO (2002).** Structure and characteristics of the fishing industry, p.2- 4.

**FAO, (2003).** Recycling of animal wastes as a source of nutrients for freshwater fish culture within an integrated livestock system.

*http://www.fao.org/docrep/field/003/A C526E/AC526E01.htm*.

**Fafioye, O. O. (2011).** Preliminary studies on water and bacterial population in high yield Kajola fish ponds.

**Fendler, E.J., Dolan, M.J., & Williams, R.A. (1998).** Handwashing and gloving for food protection, Part I: Examination of the evidence. *Dairy, Food and Environmental Sanitation*, *18*, 814-823.

**Flick GF, Martin J (1992).** Lipid oxidation in Food, in Advances in seafood biochemistry. Technomic Publishing Company, Inc.

**Food and Agric. Org. (1985).** World catch and trade of fisheries and products in 1984 . info fish marketing digest. No. 25. Hardy R, smith, JGM (1976). The storage of mackerel (scomber).

**Food and Drug Administration. (1997).** Center for Food Safety and Applied Nutrition. *http://www.cfsan.fda.gov/guidance.html*. (Accessed October 2, 2008) Produce Handling and Processing Practices. Emerg, Infect, Dis. 3(4).

**Food and Drug Administration. (2004).** *FDA report on the occurrence of foodborne illness risk factors in selected institutional foodservice, restaurant, and retail food store facility types (2004).* College Park, MD: FDA/Center for Food Safety and Applied Nutrition, Office of Compliance.Retrieved May 10, 2006, from *http://www.cfsan.fda.gov/~dms/retrsk2.html.*

**Foster G, Kaferstein F. (1985).** Food safety and the behavioral sciences. Soc.Sci. Med. 21:1273–1277.

**Gelman, A. R., Pasteur and Rave M. (1990).** Quality changes and storage life of common carp (Cyprinus carpio) at various storage temperatures. Journal of science food agriculture. 52: 231-247.

**Geldreich, E.E and N.A Clarke(1966).** Applied Microbiol.14:429-437.

**Ghaly AE, Dave D, Budge S, Brooks MS. (2010).** Fish spoilage mechanism and preservation techniques review. American Journal of Applied Sciences., 7(7): 859-877.

**Gunderson, M. F. and Rose, K. D. (1948).** Survival of bacteria in a precooked fresh-frozen food. Food Res. 13:254-263.

**Hartsell, S. E. (1951).** The longevity and behavior of pathogenic bacteria in frozen foods; the influence of plating media. Am. J. Public Health. 41: 1072-1077.

**Harrigan WF.(1998).** Laboratory Methods in Food Microbiology. 3rd ed. Academic Press, London.UK.

**Hess, E. (1934).** The effects of freezing on marine bacteria. I. Quantitative studies. J. Biol. Board Can.1: 95-108.

**Higgins Charles (2007).** Food safety: old habits new perspectives in: Book and media Journal of Emerging Infectious Disease 13 (6):960-961.

**Hozbor MC, AI Saiz, MI Yeannes and R Fritz.(2006).** Microbiological changes and its correlation with quality indices during aerobic iced storage of sea salmon (Pseudopercis semifasciata). LWT - Food Science and Technology, 39(2): 99-104.

**Ingram, M. (1951).** The effect of cold on microorganisms in relation to food. Proc. Soc. Appl. Bacteriol. 14: 243-260.

**Ingram, J. L. (1958).** Growth of Psychrophillic bacteria. J. Bacteriol. 76: 75-80.

**Jayasinghe, P. S. ( 2006).** Quality of water and ice used for fish handling in fishing harbours in Sri Lanka, Final Project report, National Aquatic Resource Research and Development Agency Crow island, Colombo, Sri Lanka.

**Khalid, A. Farah, O. M. and Ali, S. M. (2008).** Aquatic Resources of Sudan Possibility of their Utilization. Animal Resources Research Corporation, the Proceedings of the First Scientific Conference. Friendship Hall. P p. 141-148. Khartoum. Sudan.

**Kiser, J. S. (1944).** Effect of temperature approximating 0ȩC upon growth and biochemical activities of bacteria isolated from Mackerel. Food Res. 9: 257-267.

**Kraus, S.J. (1995)** Attitudes and the Prediction of Behavior: A Meta Analysis of the Empirical Literature. Personality and Social Psychology Bulletin,21,58-75 : 927-30. http://dx.doi.org/10.1177/0146167295211007.

**Kvenberg E.J (1991).** Non-indigenous bacteria pathogen: In Microbiology of Marine food products. (Eds) Donn R.W and Cameron H Van Nostrand Reinhold, N York p 263-291.

**Lochhead, A. G. and Jones, A. H.(1938).** Types of bacteria surviving in frozen packed vegetables. Food Research. 3: 299-306.

**Li Cohen, A.E. and Bruhn, C.M. (2002)** Safety of Consumer Handling of Fresh Produce from the Time of Purchase to the Plate: A Comprehensive Consumer Survey. Journal of Food Protection, 65, 1287-1296.

**Lipp, E.K., Rose, J.B. (1997).** The role of seafood in food borne diseases in the United States of America. Rev. Sci. Tech. OIE., 16: 620- 640.

**Liston, J. (1980).** Microbiology in fishery science. In: Advances in Fishery Science and Technology (edited by J.J. Connell). Pp. 138–157. Farnham, UK: Fishing News Books Ltd.

**Lund, A. J. and Halvorson, H. O. (1951)**. The effect of low temperature on enzymes and micro-organisms. Proc. Conf. on Reseaech Coun. Ammeat Inst., Univ. Chicago 3rd Conf. p. 59.

**Lynch, R.A., Elledge, B.L., Charles, P.H., Griffith, C.C., & Boatright, D.T. (2003).** A comparison of food safety knowledge among restaurant managers, by source of training and experience, in Oklahoma County, Oklahoma. *Journal of Environmental Health*, *66*(2), 9-14.

**Medeiros LC, Hillers VN, Chen G, Bergmann V, Kendall P, Schroeder M (2004).** Design and development of food safety knowledge and attitude scales for consumer food safety education. *J Am Diet Assoc*, 104(11):1671-7.

**Meske, C. (1985).** Fish aquaculture Technology and Federal Research Center for Fisheries, Institute for Coastal and Inland Fisheries, Hamburg, federal republic of Germany. Edited and translated by Fredrick Vogt Formally of the polytechnic of Central London, U. K.

**Metz, H. (1980).** Water as a vector of infection: waterborne bacteria (in German). Zentralbl Bakteriol Mikrobiol Hyg (B), 172, 255–274.

**Mhango, M., Mpuchane, S.F., Gashe, B.A. (2010).** Incidence of indicator organisms, opportunistic and pathogenic bacteria in fish. Afr. J. Food Agric. Nutr. Dev., 10(10): 4202- 4218.

**Miceal, W., Johan, Suen, F; Carina, K and Tor M. (2007).** Journal of clinical Microbiology published by the American Society for Microbiology. 45:1-7.

**Minette H.P. (1986).** Salmonellosis in the marine environment. A review and commentary. Int. J. Zoonoses, 13, 71–75.

**Musa US., Hati S., Adam YI., Mustapha A. (2010).** Pesticide residues in smoked fish samoles from Notrh-Eastern Nigeria. J. Applied Sci., 10: 975-980.

**Mudey, A. B., Kesharwani, N., Mudey, A. G., Goyal, R. C., Dawale, A. K., & Wagh, V. V. (2010).** Health status and personal hygiene among food handlers working at food establishment around a rural teaching hospital in Wardha district of Maharashtra, India. Global Journal of Health Science, 2, 198e206.

**Musaiger, A.O., D Souza, R. (2008).** Chemical composition of raw fish consumed in Bahrain. Pak. J. Biol. Sci., 11: 55 - 61.

**Nguyen Huy Quang., ( 2005).** Guidelines for handling and preservation of fresh fish for further processing in Vietnam. Quality Assurance Department. Seafood Export and Quality Improvement Program. Vietnam Final Project.

**Notermans S., Hoornstra E. (2000).** Risk assessment of Listeria monocytogenes in fish products: some general principles, mechanism of infection and the use of performance standards to control human exposure. Int. J. Food Microbiol., 62, 223–229.

**Nuchprayoon, S., Sanprasert, V., Kaewzaithim, S.,& Saksirisampant, W. (2009).** Screening for intestinal parasitic infections among Myanmar migrant workers Office of Seafood Retrieved on 14 October, 2007.

**Okoro CC, Aboaba OO, Babajide OJ. (2010).** Quality Assessment of a Nigerian Marine Fish, Mullet (Liza falcipinnis) under different Storage Conditions. *New York Science Journal.,* 3(8): 21-28

**Poyoung A. (2007).** Knowledge, attitude and practice of hawkers in street food and food sanitary condition of street food in Ladkrabang Bangkok [ The degree of Master of Science (Nutrition)]. Bangkok: Faculty of Graduate Studies, Mahidol University.

**Phyllis Entis (2007).** Food safety: Old Habits new perspectives. ASM Press. Herndon, Virginia USA. P 414.

**Produce Handling and Processing Practices (1997).** Emerg, Infect, Dis. 3(4). in Thai food industry: a high-risk transmission. *Journal of Immigrant and Minority Maharashtra, India. Global Journal of Health Science*, 2, 198e206.

**Prescott, L.M., Harry, J.P. and Klein, D.A, (1999)** Food and Industrial Microbiology. Chapter 43, 4th Edition, New york, Mc Graw Hill publication.

**Redfort, A. L. (1932).** Microbiological activities at low temperatures with particular reference to marine bacteria. Quart. Rev. Biol. 9: 460- 466.

**Redmond, E.C. and Griffith, C.J. (2003)** Consumer Food Handling in the Home: A Review of Food Safety Studies. Journal of Food Protection, 66, 130-161.

**Russell Miget. (2004).** The HACCP Seafood Program and Aquaculture.Southern Regional Aquaculture Centre (SRAC) Publication No. 4900, Texas A&M University, February 2004 PR.

**Rodricks, E.G. (1991).** Indigenous pathogen: Vibrionaceae of microbiology of marine food products, Reinhold, New York, Pp. 285- 295.

**Rodriguez-Jerez, J.J., Hernandez-Herrero, M.M. & Roig Sagues, A. X. (2004).** New methods to determine fish freshness in research and industry. *http://216.239.59.104/custom?q=cache:9fhOb4*-H47iOJ:resources.ciheam. org/om/pdf/c.

**Sallam KI. (2007).** Chemical, sensory and shelf life evaluation of sliced salmon treated with salts of organic acids. Food Chem. 2007; 101(2): 592–600.

**Shewan, J. M. (1977).** The bacteriology of fresh and spoiling fish and the biochemical changes induced by bacterial action. In: Proceedings of the

conference on handling, processing and marketing of tropical fish. *Tropical products institute, London*, 51-66.

**Simon, S.S., Sanjeev,S. (2007).** Prevalence of enterotoxigenic Staphylococcus aureus in fishery products and fish processing factory workers. Food control, 18(12): 1565-1568.

**Spencer, R. (1959).** The sanitation of fish boxes, quantitative and qualitative bacteriology of commercial wooden fish boxes. J. Appl. Bacteriol. 22: 73-84.

**Subratty, A. H., Beeharry, P., & Sun, M. C. (2004).** Asurvey of hygiene practices among food vendors in rural areas in Mauritius Nutrition and food science 34(5), 203-205. *http://dx.doi.org/10.1108/00346650410560361*.

**Thurman, H. V. and Webber, H. H. (1984).** Marine biology Charles, E. merril Publishing C. A. Bell and Howell co. Columbus, Ohio U. S. A.

**USFDA, (2007).** The Fish and Fisheries Products Hazards and Control Guidance. Fourth edition, U. S. Food and Drug Administration Center for Food Safety and Applied Nutrition Office of Seafood Retrieved on 14 October, 2007.

**Von Reyn C.F., Arbeit R.D., Tosteson A.N.A., Ristola M.A., Barber T.W., Waddell R., Sox C.H., Brindle R.J., Gilks C.F., Ranki A., Bartholomew C., Edwards J., Falkingham J.O., O′Connor G.T., Jacobs N.J., Maslow J., Lahdevirta J., Buhler S., Ruohonen R., Lumio J., Vuento R., Prabhakar P., Magnusson M. (1996).** The international epidemiology of disseminated Mycobacterium avium complex infection in AIDS. AIDS, 10, 1025–1032.

**Walderhaug, M. (1992).** A handbook of foodborne pathogenic microorganisms and natural toxins. The "Bad Bug Book" prepared by the Division of Seafood Safety in the Center for Food Safety and Applied Nutrition at the U.S.

**WHO (2004).** Surveillance Programme for Control of Foodborne Infections and Toxications in Europe 8th report 1999–2000,Country reports: Turkey.

**Wilcock A, Pun M, Khanona J, Aung M. (2004).** Consumer attitudes , knowledge and be- havior: a review of food safety issues. Trends Food Sci & Technol., 15: 56-66.

**Wiklund, T. and Lonnstrom, L.(1994).** *Aquaculture* 126,211-217.

**World Health Organisation (WHO). (2006).** Five-keys to safer food manual. WHO Press, Geneva, Switzerland.Availableat: *http://www.who.int/foodsafety/publications/consumer/manual_keys.pdf* (Accessed May13 2008).

**World Health Organization (WHO). (2014).** Initiative to estimate the global burden of foodborne diseases: Information and publications. Geneva: WHO.

**Zlotkin A., Eldar A., Ghittino C., Bercovier H. (1998).** Identification of Lactococcus garvieae by PCR. J. Clin. Microbiol., 36, 983–985.

## 5.4 Appendix

### 5.4.1 Appendix 1: Chart of bacteria

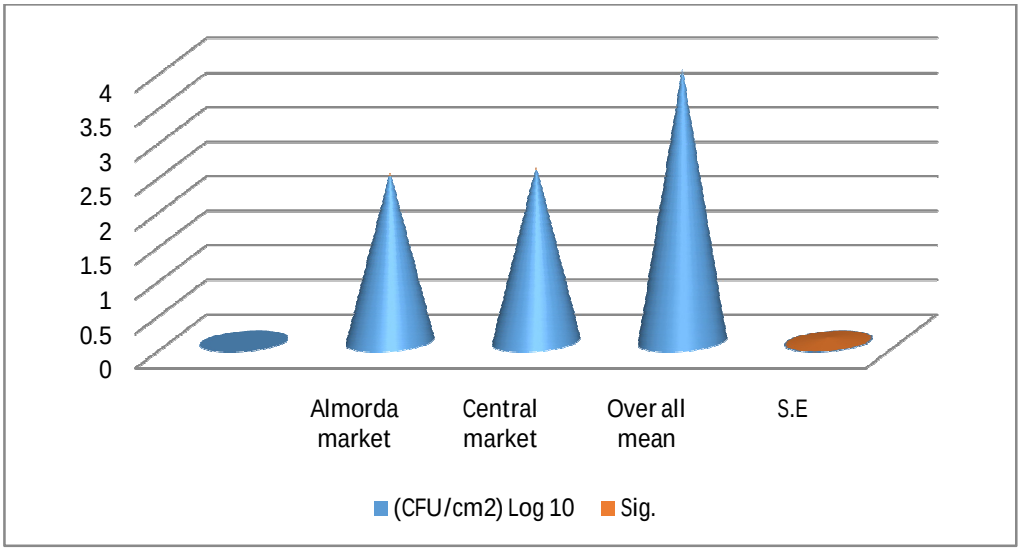


**Fig.1. Over all mean and standard Error of Total viable count of bacteria on two fish markets.**

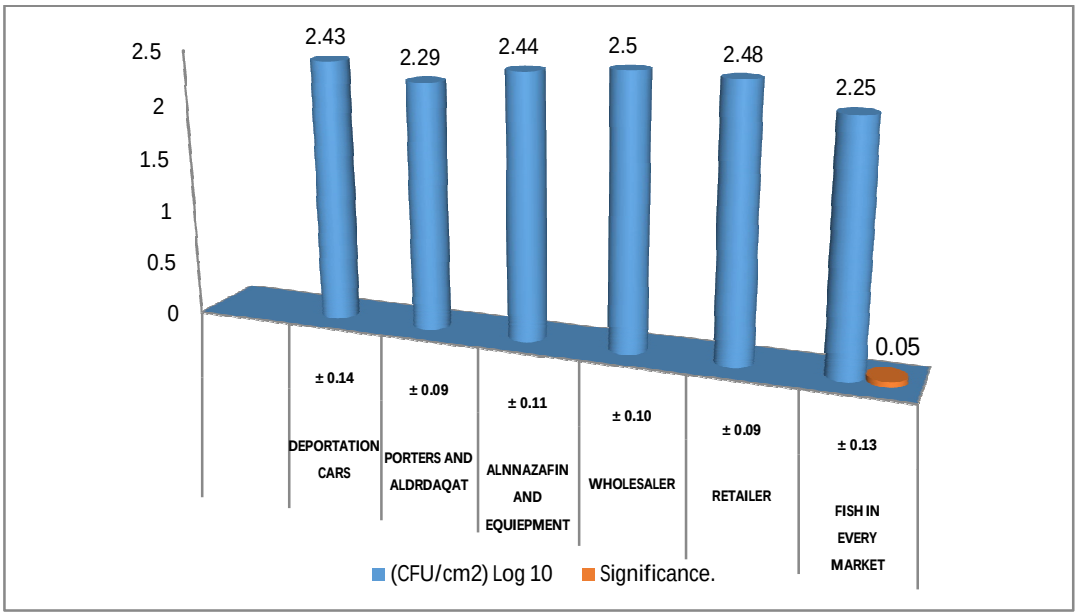


**Fig.2. Mean and Standard deviation of Total viable count of bacteria on Place of sampling of Almorda market**

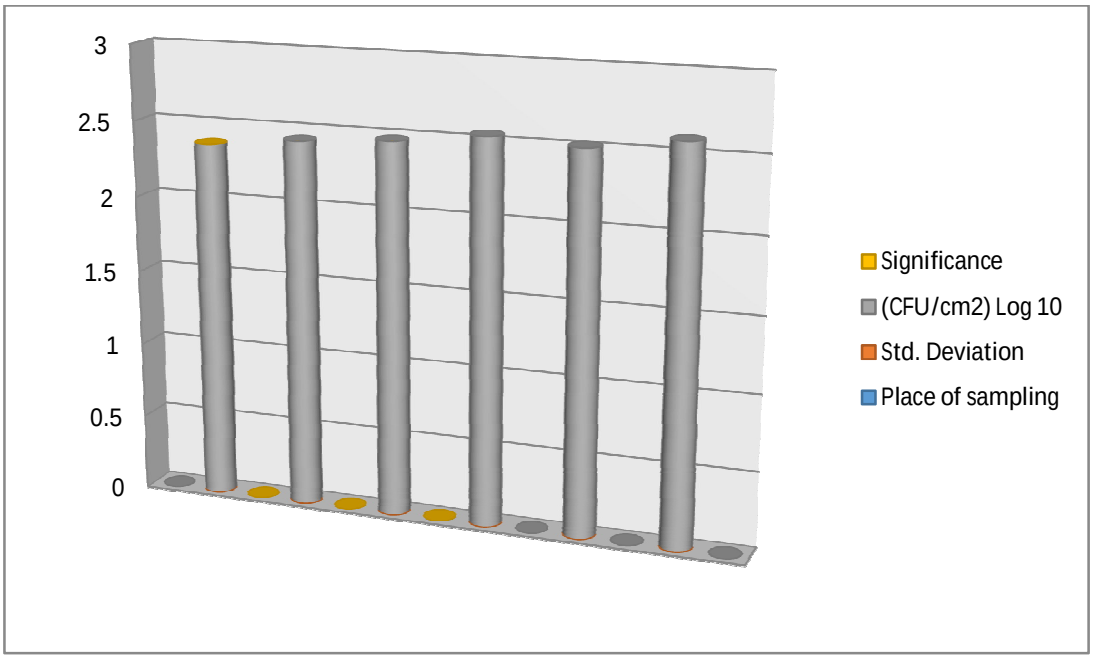


**Fig.3. Mean and Standard deviation of Total viable count of bacteria on Place of sampling of Central market**

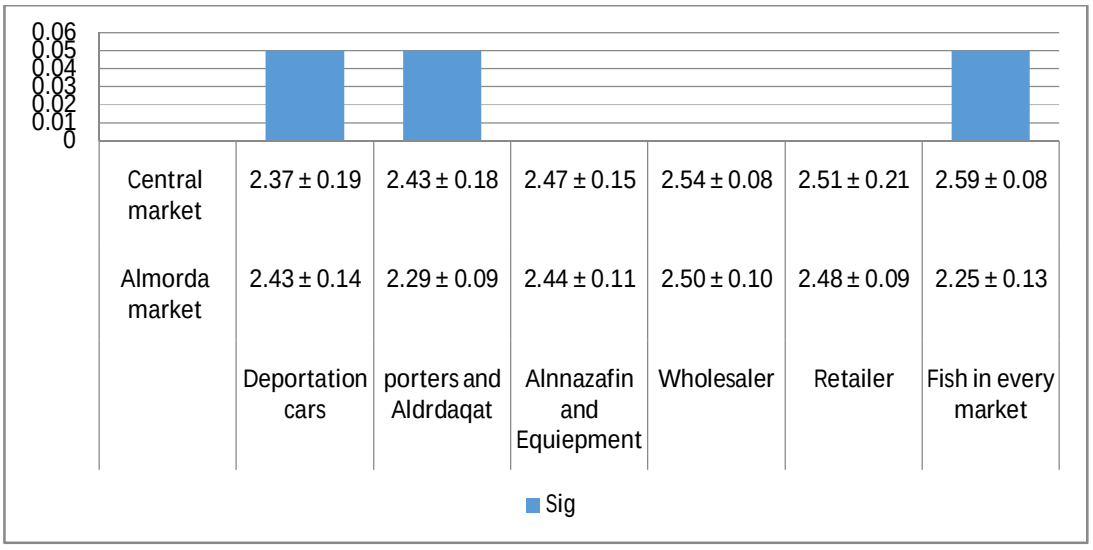


**Fig.4. Comparison of mean total viable count of bacteria (log10 CFU/cm2) ± Standard deviation at different Place of sampling of fish markets**

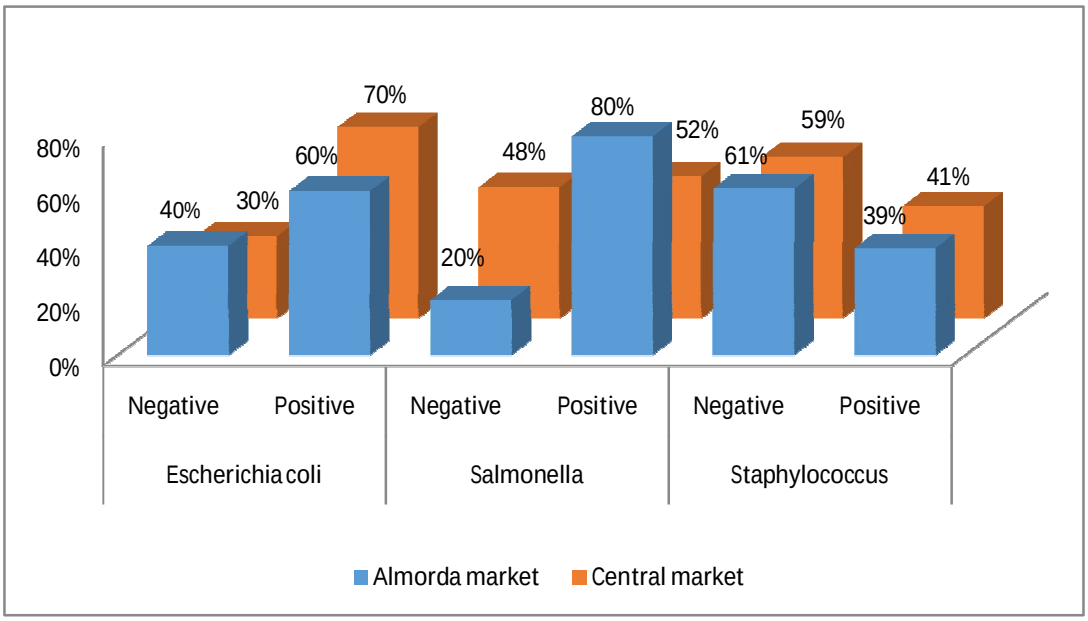


**Fig.5. Bacteria isolated and identified (N %) at two fish markets**

### 5.4.2 Appendix 2 : Growth of bacteria

**Fig.6: Growth of *Salmonella spp***

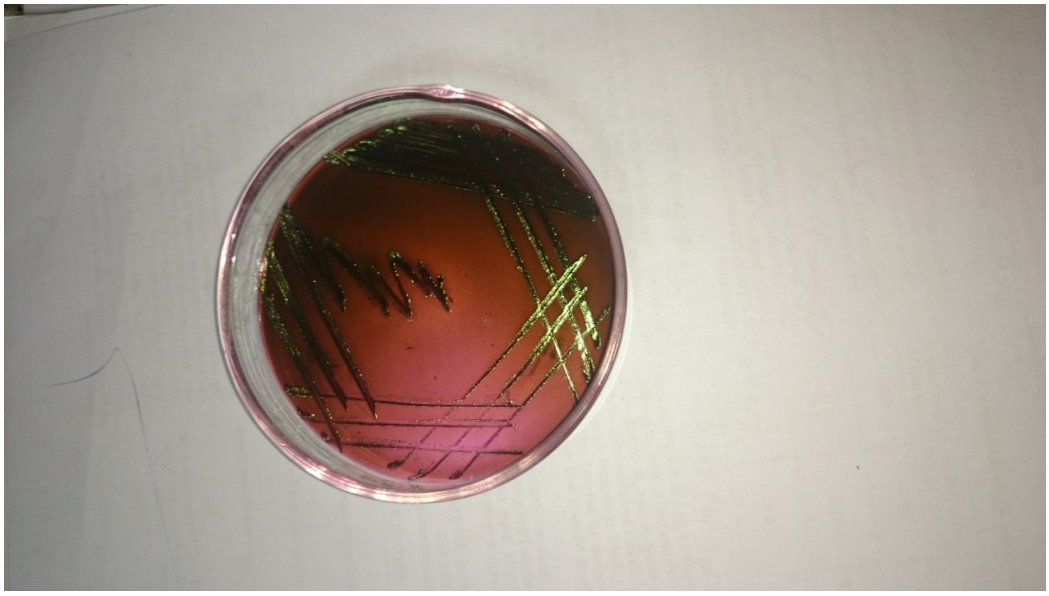

**Fig.7: Growth of *Escherichia coli***

**Fig.8: Growth of *Staphylococcus aureus***

### 5.4.3 Appendix 3 : Summary of questions and responses for assessment of respondent's Food safety knowledge , hygienic practices and attitudes of food workers in fish markets in Khartoum state

**Table (1): Food hygienic practices of food workers in fish markets:**

| Statements | Responses, | | | | |
|---|---|---|---|---|---|
| | Never | Rarely | Sometimes | Often | Always |
| Do you use gloves during work? | | | | | |
| Do you wash your hands before using gloves? | | | | | |
| Do you wear apron during work? | | | | | |
| Do you use mask during work? | | | | | |
| Do you use cap during work? | | | | | |
| Do you wash your hands before you touch raw meat? | | | | | |
| Do you wash your hands after you touch raw meat? | | | | | |
| Do you wash your hands after rest time when you come back to work? | | | | | |
| Do you eat or drink in your work place? | | | | | |
| Do you smoke in your work place? | | | | | |

**Table (2): Food safety knowledge in fish markets:**

| Statements | Responses, n (%) | | |
|---|---|---|---|
| | True | False | Do not know |
| Typhoid can be transmitted by fish contamination | | | |
| Diarrhea can be transmitted by fish contamination | | | |
| AIDS can be transmitted by fish contamination | | | |
| Jaundice can be transmitted by fish contamination | | | |
| **Statements** | **Responses, n (%)** | | |
| | **True** | **false** | **Do not know** |
| Abortion in pregnant women may be incduced by food borne disease | | | |
| bacteria is among the food borne pathogens | | | |
| Remember the fish well with ice or use the refrigerator reduces fish contamination | | | |
| Use sawdust to save the fish increases pollution and corruption fishs | | | |

## Table (3): Food safety attitudes of food workers in fish markets

| Statements | Responses | | |
|---|---|---|---|
| | Agree | Disagree | No idea |
| Using gloves is important in reducing risk of food contamination | | | |
| Using apron is important in reducing risk of food contamination | | | |
| Using masks is important in reducing risk of food contamination | | | |
| Using caps is important in reducing risk of food contamination | | | |
| Food hygiene training for workers in fish markets is an important issue in reducing risk of food contamination | | | |
| Health status of the workers should be evaluated in fish markets | | | |
| Food borne illnesses can have deleterious health and economic effects on the society | | | |