A comparative analysis of bacterial flora and proximate composition of mud crab (*Scylla serrata*,Forsskal 1775) collected from different local markets of Dhaka city

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Certificate

This is to certify that this thesis entitled **"A comparative analysis of bacterial flora and proximate composition of mud crab (*Scylla serrata*, Forsskal 1775) collected from different local markets of Dhaka city"** submitted by FarhanaKarim, Roll NO.: Curzon-613, Session: 2012-13, Registration NO.: Ha-1490; has been carried in the laboratories of the Department of Microbiology, University of Dhaka and the Department of Fisheries, University of Dhaka under our supervision.

This is further to certify that it is an original work and suitable in partial fulfillment for the degree of Master of Science (M.S.) in Fisheries from University of Dhaka.

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Dedicated to my father

Md. Rezaul Karim

Without whom my journey to this stage would not have been possible

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The Author

Abstract

The present study was carried out to investigate the presence of bacterial flora and proximate composition of mud crab (*Scylla serrata*) meat from the local markets (Mohakhali, Karwan bazar, Farmgate, Basabo and Agora super shop) of Dhaka city. The densities (cfu/g) of total bacterial count (TBC), total coliform (TC), fecal coliform (FC), total *Salmonella* spp. and *Shigella* spp. (SS), total *E. coli*, total *Staphylococcus*spp. and total *Vibrio*spp. like colonies were counted. Moisture, ash, protein and lipid contents were also determined for the samples of each market.

TC, FC, SS, *E. coil,* and *Vibrio* spp. were detected in most of the samples except the frozen ones from Agora super shop. The samples from Basabo and Karwan bazar had much higher bacterial load than the others. The highest TBC counts were observed in the samples from Karwan bazar (38.1 ± 1.20 $x10^4$cfu/g) and the lowest were from Agora super shop (4.48 ± 0.26 $x10^4$cfu/g). The total *Vibrio*spp. counts were found only in two markets; Farmgate and Basabo. The total *E. coli* countsranged from 1.25± 0.02 $x10^4$cfu/gto 4.33± 0.56$x10^4$cfu/g. The *Salmonella, Shigella* (SS) counts variedbetween3.95± 0.28 $x10^4$cfu/g for Karwan bazarand 16.76±1.26$x10^4$cfu/g for Basabo.Total *Staphylococcus* spp. counts ranged between 14.4± 1.1 $x10^4$cfu/gand 20.01± 1.94 $x10^4$cfu/g for normal crab samples and 0.37±0.025$x10^4$cfu/g for frozen samples. A total of 47 isolates were recovered from the samples and 10 generawere identified as*Bacillus*, *Citrobacter,Alcaligens,Salmonella,Shigella,Vibrio,Escherichia,Klebsiella,Staphylococcus* and *Enterobacter*. Nearly similar nutritional compositions were found in the live crab samples. Whereas,much lower moisture content with higher ash, protein and lipid contents were observed in frozen samples.

The results of this study showed that the bacterial load was significantly higher in live crab samples than the frozen ones which arevery useful to evaluate the safety of mud crab for human consumption.Consumption of normal crabs from above mentioned markets can lead to food borne diseases of human, whether the frozen samples can be considered as much safer. This study also revealed that mud crabs can be an alternative source of protein(excluding the bacterial hazards) to the traditional protein sources as its protein content is high.

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List of Abbreviations

Symbols	**Details**
°C	Degree celcious
ANOVA	Analysis of Variance
cfu	Colony Forming Unit
cm	Centimeter
DoF	Department of Fisheries
EC	European Commission
EMB	Eosine Methylene Blue
EU	European Union
E.	*Escherichia*
eg	For Example
et al.	and others
FC	Fecal coliform
GDP	Gross Domestic Product
GMP	Good Manufacturing Practice
h	Hour
H_2S	Hydrogen Sulphide
HACCP	Hazard Analysis Critical Control Point
i.e.	That is
Kg	Kilogram
L	Litre
ml	Mililitre
mg	Miligram
M	Molar

mm	Millimeter
MSA	Minitol Salt Agar
µg	Microgram
µl	Microlitre
NA	Nutrient Agar
NaCl	Sodium Chloride
NaOH	Sodium Hydroxide
NS	Normal Saline
pH	Negative logarithm of hydrogen ion concentration
ppm	Parts per million
S.	*Staphylococcus*
spp.	Species
SSC	*Salmonella* and *Shigella* count
TBC	Total bacterial count
TCBS	Thiosulphate Citrate Bile Salt Sucrose
TC	Total coliform
TEC	Total *E. coli* count
TVC	Total *Vibrio* spp. count
V.	*Vibrio*
(+)	ve Positive
(-)	ve Negative
<	is less than
>	is greater than

Chapter 1

Introduction

Bangladesh is a land of water resources. Fisheries play a major role in the country's economy contributing 2.73% (DoF, 2011-12) of total export income. But very often due to small size, bacterial load or the presence of chemical residue, fishery products are rejected by the importer countries. So the production and identification of quality fishery products have become a major concern.

Scylla serrata (often called giant mud crab/mud crab or mangrove crab, although both terms are highly ambiguous) is an economically important species of crab found in the estuaries and mangroves of Africa, Australia and Asia. In their most common form, shell colour varies from a deep mottled green to very dark brown. The mud crab (*Scylla serrata*) is one of the most important coastal aquatic species of Bangladesh due to its high demand and price in the international market. Mud crabs occur abundantly in the coastal region of Bangladesh, especially in the coastal areas of Chakoria, Moheskhali and Kutubdia Island in Cox's Bazar district and Khulna, Satkhira and Bagerhat in the Sundarbans regions. Crabs play a vital role in the ecological balance. It offers excellent food for estuarine crocodiles, sharks, estuarine turtles and fishing Eagles (Lee, 1992).

Continued increase in export of live mud crab plays an important role to the foreign exchange earnings of Bangladesh (Azam *et al.,* 1998). Among the 16 marine species only mud crab is commercially important. Bangladesh earns considerable amount of foreign exchange through exporting this animal in live condition. Its fishery supports the livelihood of millions of poor fishers, traders and transporters. More than 50,000 fishers, traders, transporters and exporters are found to be involved in this sector.

The mud crab fishery is absolutely based on wild catch mainly from the swamps of the Sundarbans and vast areas of the traditional shrimp ghers along the coastal region of Bangladesh. People of the coastal region follow traditional fattening process to grow crab. Simple small ponds are used in coastal areas for crab fattening in Bangladesh. Women involvement in fisheries and aquaculture is an old practice in many Asian and African countries. From fish fry collection to grow-out production and management including on farm and on shore post-harvest management, marketing and processing of the fisheries products they are directly or indirectly involved (Kevane and Wydick, 1999;

Shaleesha and Stanley, 2000; Sharma, 2003; Song, 1999). The mud crab fishery has helped these poor women to become self-dependent. In China, Thailand and Philippines the high literacy rate and comparative liberal value system in society placed the skilled women as fish farmers, technicians, extension workers and professionals in various activities of fish production through aquaculture (FAO, 1987). Meanwhile, in Bangladesh, only 3% of working women found to be involved in the fishery which is the second most important occupation in the non-farm sector (BBS, 1996).

In Bangladesh, mud crab is an export fishery that is playing an important role in national and international markets. It generates employment directly and indirectly in terms of people employed in the production, marketing and other associated business. Bangladesh began exporting mud crabs around 1997-98 and since then the value of export earnings has been steadily increasing. In 2002, mud crabs ranked third in terms of frozen food export items. During 2009-10, Bangladesh exported 580 tons crab of 3 million $ (BEPB, 2009-10).

As it has a huge potential in export market, scientific studies are necessary for its quality maintenance.China, Hong Kong, Malaysia, Korea and Taiwan are ranked as the top five consumers of crab. Especially female crabs are playing an important role in marketing, particularly in Asian countries such as Japan, Taiwan, Hong Kong and Singapore. Also, there is a growing market for mud crab meat as a value added product and for frozen soft-shelled mud crab in the USA.Figure1.1shows the export trend and Figure1.2 shows the export quantity of mud crab made in 2009-10 in theimport country wise (BEPB, 2010).

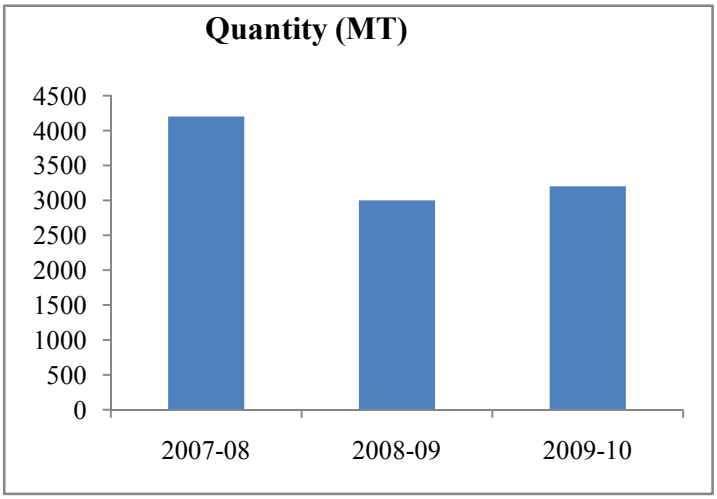


Figure 2.1Export trend of mud crab quantity year wise

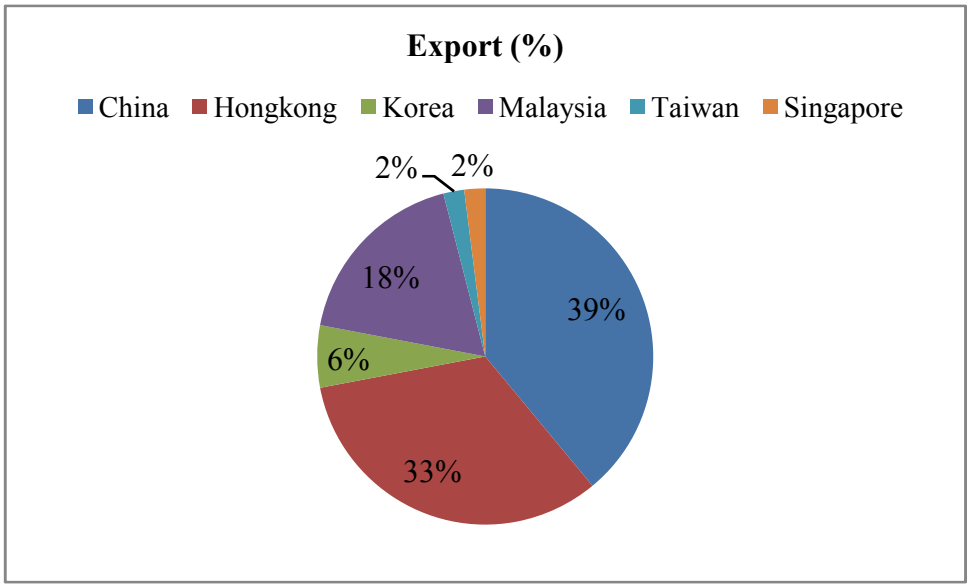


Figure 1.2 Export trend of mud crab country wise

1.1 Background

Fish allows for improved nutritionin that it has a high biological value in terms of highprotein retention in the body, low cholesterol level andpresence of essential amino acids (Emikpe *et al*.,2011). Fish are generally regarded as safe, nutritiousand beneficial but aquaculture products havesometimes been associated with certain food safetyissues (WHO, 2007). Fish take a large number of bacteria into theirgut from water, sediment and food (Emikpe *et al*.,2011). It has been well known that both fresh andbrackish water fishes can harbor human pathogenicbacteria particularly the coliform group (Emikpe *et al*.,2011). Fecal coliform in fish demonstrates the levelof pollution in their environment because coliform arenot named flora of bacteria in fish (Emikpe *et al*.,2011).Fish contamination can also be linked to rawmaterial, personnel, processing tools storage in polluted environment and pests. Somepathogens may even become established in theprocessing plants from niches where they can survivefor a long period of time (Adebayo-Tayo *et al*.,2012b). The tissue of a healthy fish is normallyconsidered sterile until bacterial inversion that leads tospoilage. According to Adams and Moses (2008), thenormal bacterial load of the surface slime of fish canrange from 10^2 – 10^7 cfu/cm^2 and the gills andintestines can range up to 10^3 and 10^7cfu/grespectively.

Shellfish is a food substrate for some zoonoticvibrios of which these microorganisms, cause foodpoisoning and diarrhea in human (Merwad *et al*.,2011). Shellfish make an excellent substrate for themicroorganisms to live in the aquatic habitats due toloose

texture of their flesh (Merwad *et al*., 2011).When the aquatic system is contaminated withpathogenic *Vibrio*spp., these bacteria become part of shellfish microflora (Colakoglu *et al.*, 2006). Concerning the zoonotic aspect, the hazardous pathogenic *Vibrio* spp. causes life threatening food borne infections and poses a considerable public health threat as agents of sporadic and epidemic human infections to be represented an important microbial group in the field of food safety (Espineira *et al*., 2010; Merwad *et al*., 2011**).**So there is a number of food borne diseases related to mud crab consumption.

1.2 Rationale

Quality assessment of raw and frozen mud crab meat is very important as it is related to public health. Microbiological and nutritional quality determination is very useful for both export and country consumption. In Bangladesh, there have been some studies on nutritional assessment of mud crab but microbial analysis is too few to be mentioned. That is why the present study was carried out to investigate the quantitative bacterial analysis and nutritional assessment of raw and frozen mud crab (*Scylla serrata*) meat.

1.3 Problem statement

Fish and shellfish can become contaminated from their natural environment or from subsequent handling or processing. Water polluted with sewage is a particular problem with filter-feeding molluscan shellfish such as oysters and mussels which are often eaten raw or after only light cooking. These feed by filtering nutrients from large volumes of their surrounding water, at the same time accumulating microorganisms from their environment. Since they are mainly harvested from shallow coastal waters where sewage contamination is likely to be greatest, they are commonly contaminated with pathogenic bacteria and viruses of human (and animal) enteric origin.

Seafood related disease outbreaks have been reported almost throughout the world including countries like Japan, U.S, India and U.K. International Committee for Microbiological Food Safety (ICMFS) has devised permissible counts for various pathogens in different food products. Presence of these pathogens above the acceptable level is usually rejected by the importing country as unfit for human consumption, so to assess the microbiological quality of seafood in any part of the world has become significant to avoid health hazards and also economic losses. The economic losses due to spoilage are rarely quantified but a report by the US National Research Council

Committee (FND/NRC) estimated that one-fourth of the world food supply is lost through microbial activity alone (EEC, 1992).

1.4 Microbes associated with mud crab

The aerobic mesophilic flora of mud crab is dominated by gram-negative bacteria belonging to genera *Vibrio, Moraxella, Acinetobacter* and *Pseudomonas/Shewanella* and family Enterobacteriaceae. Among Gram-positive bacteria, *Micrococcus, Staphylococcus* and *Bacillus* are found to a lesser extent (Faghri *et al*., 1984).Bacteria belonging to the genera *Vibrio*, *Aeromonas*, *Pseudomonas*, *Alteromonas*, *Flavobacterium*, *Spirillum*, *Moraxella*, *Pasteurella* and *Photobacterium* are all reportedas probable agents involved in shell disease syndrome incrustaceans (Getchell, 1989). *Vibrio* spp. and aeromonads were primarilyresponsible for progressive infections in blue crab, *Callinectes sapidus* Rathbun and were the predominantbacterial type in heavily infected crabs with shell disease(Davis and Sizemore, 1982; Welsh and Sizemore, 1985). *V. fluvialis, V. hollisae* and *V. mimicus* accounted for gastroenteritis associated infections in humans due to consumption of contaminated raw or undercooked shellfish such as oysters, clams, mussels, or crabs (Huss, 1994).*A. hydrophila,A. sobria* and *A. caviae* have been described as emergent food-borne pathogen of increasing importance causing gastroenteritis (Kirov, 1997). Potentially, *Aeromonas* spp. can become a serious food problem as many of them can grow at refrigeration temperatures. *A. hydrophila* is also often found in association with disease outbreaks in aquaculture production (Nielsen *et al*., 2001). Fang *et al.*(2008) reported pathogens such as *A. veronii, V. mimicus, V. parahemolyticus, Aeromonas trota* and *A. hydrophila* in crab culture. Handling and cross contamination might be a health hazard, particularly with susceptible populations.

1.4.1 Contamination with Enterobacteriaceae

Enterobacteriaceaeare a large, diverse heterogeneous group of rod shaped gram negative bacilli that survive under aerobic conditions and normally inhabit the intestine of man and animals; some are motile while some others are not (Udeze *et al*., 2012b). Enterobacteriaceaewere isolated from gills, skin, muscles and the intestine of randomly collected fishes. Thampuran *et al*. (2005) reported that the microbial quality of the tilapia indicated that all tissue samples except muscle tissues were contaminated with fecal coliform where *Escherichiacoli* was the most common contaminant and is often encountered in high numbers.

1.4.2*Salmonella*spp. contamination

Salmonella species are gram-negative, rod-shaped, usually motile, members of thetaxonomic family, Enterobacteriaceae. Contamination of seafood with *Salmonella* is a major public health concern. The presence of *Salmonella* in seafood has been reported in Vietnam, India, Sri Lanka, Thailand, Taiwan and Japan (Ponce *et al.* 2008; Wafaa *et al.* 2011; Heinitz *et al.* 2000) During a 9-year study (1990–1998), the Food and Drug Administration noted an overall incidence of *Salmonella* in 7.2% of 11,312 samples from imported and 1.3% of 768 samples from domestic U.S. seafood (Wafaa *et al*., 2011).

The infectious dose appears to be very low as evidenced by some foods implicatedin foodborne disease with only a few cells recovered. An example of this occurred in USA in 1994 frozen dessert-associated outbreak wherein the level of *Salmonella* serotype *enteritidis* was reported to have a most probable number (MPN) range of 4 cells per 1,000 g to 46 cells per 100 g with a median of 93 cells in 1,000 g (Vought and Tatini, 1998). Evidence from other studies indicates that from 1 to 10 cells may constitute an infectious dose in some circumstances (D'Aoust *et al*., 1985 and Kapperud *et al*., 1990).

1.4.3Contamination with *Escherichia coli*

E. coli is a gram-negative, non-spore-forming short rod-shaped bacterium capable of growth and gas production at 45.5◦C (except when testing water, shellfish, and shellfish harvest water, which use 44.5◦C) in lactose-containing medium (Kornacki and Johnson, 2001). Most *E. coli* strains are harmless inhabitants of the gastrointestinal tract of man and animals. However, several foodborne pathogenic strains of *E. coli* are known to exist(Kornacki and Marth, 1982, Doyle *et al*., 1997b).*Escherichia coli* cause dysentery. Mead and Griffin (1998) reported doses as low as50 cells can be infectious and in one reference the FDA has indicated that as few as 10 cells may be adequate to cause illness (FDA, 2001).

1.4.4Contamination with *Staphylococcus* spp.

Staphylococci belong to the family Micrococcaceae. They are gram-positive sphericalbacteria about 1μm in diameter that appear as grape-like clusters under the microscope. The grape-like configuration of staphylococci helps to distinguish staphylococci from streptococci that usually form chains because they divide in one plane only. Staphylococci are catalase-positive, oxidase-negative, facultative anaerobes that grow by aerobic respiration or fermentatively with the principal end product being

lactic acid. The catalase test is important in distinguishing streptococci (catalase negative) from staphylococci, which are strong catalase producers.

Staphylococcus aureus, a mesophile have been implicated in food poisoning outbreak of some food material (Adebayo-Tayo *et al*., 2009). Odunfa (1988 cited by Adebayo-Tayo *et al*., 2006, 2009) reported that *S. aureus* levels of 10^8/ml are considered potential hazardous to consumers. The presence of *S. aureus* is an indication of contamination by food handlers and 80% of them are being harbored by man as normal micro flora (Adebayo-Tayo *et al*., 2009). *S. aureus*known for production of heat stable enterotoxin and potentials for multiple antibiotic resistances when they get into the living tissue makes the product of immense epidemiological danger (Adebayo-Tayo *et al*., 2009).

1.4.5 Contamination with *Pseudomonas* spp.

Pseudomonas spp.is prevalent among patients with wounds, burns, cystic fibrosis are likely to have introduced into the environment by swimmers and infected individuals. The contamination may be as a result of human activities such as deposition of fecal matters, washing, bathing, discharge of effluents into the rivers were these fish and crabs are harvested from.

1.4.6 Contamination with *Bacillus* spp.

Bacillus spp.causes a toxin-medicated disease rather than infection such as diarrhea and emetic illness characterized by nausea and vomiting (Adebayo-Tayo *et al*., 2006, 2009). The occurrences of *Bacillus* spp. in fish can be said to be as a result of prevalence of their spores in the environment (Adebayo-Tayo *et al*., 2009). *Bacillus* species are spore formers whose spores could survive high temperatures of processing. The organisms are present in most raw materials used in food manufacturing atconcentration of 10^3/g or less. The infectious dose has been estimated to be 10^5/g (Adebayo-Tayo *et al*., 2009)

1.4.7Contamination with *Klebsiella* species

Bacteria from the genus *Klebsiella* causes numerous infections in human, which are often treated with β–lactam antibiotics (Amin *et al*., 2009). A variety of nosocomial and community acquired (food borne) infections are caused by *K. pneumoniae*, one of the most deadly pathogens of Enterobacteriaceae(Amin *et al*., 2009, Udeze *et al*., 2012b).

K. pneumoniae is an enteric Gram negative bacillus causing hospital-acquired infections and infections in debilitated or immuno-compromised patients accounting for up to 10% of all nosocomial bacterial infections(Amin *et al*., 2009; Udeze *et al*., 2012b).

1.4.8Pathogenic *Vibrio*spp.

Pathogenic vibrioshave been a public health concern for seafood consumers and have been cause of import bans, detentions and rejections in international fish trade (Wafaa *et al*., 2011). The family Vibrionaceae is autochthonous to aquatic environments including estuarine, coastal waters and sediments worldwide, and some species are well-knownpathogens of marine organisms including fish and shellfish (Merwad *et al*., 2011). Species such as *V. cholerae, V. parahaemolyticus, V.vulnificus, V. alginolyticus, V. mimicus, V. fluvialis, V. furnissii, V. metschnikovii, V. hollisae* and *V. damsel* are human pathogens (Adeleye *et al*., 2010). They, in estuarine or marine waters throughout the world, are part of the natural flora of zoo-plankton andcoastal fish and shellfish. Their numbers are dependent on the salinity and temperature of the water.Theyaccount for a significant proportion of humaninfections such as gastroenteritis, usually associatedwith consumption of raw or undercooked seafood,wound infections, septicemia and ear infections(Adeleye *et al*., 2010). Most of these vibrios secreteenterotoxins in foods, water or in the gastrointestinaltract.The presence of other species of *Vibrio* (*Vibrio parahaemolyticus, Vibrio fluvialis,* and *Vibrio mimicus*) has been repeatedly reported on shellfishesby Colakogu *et al.* (2006). Although thevast majority of environmental *V. parahaemolyticus* isolates are non-virulent, it is a leading cause ofgastroenteritis linked to seafood consumption in theUnited States (U.S.) (Iwamoto *et al.* 2010; Wafaa *etal*., 2011). It enters human hosts via wound infections orconsumption of raw shellfish (primarily oysters), and infections frequently progress to septicemia and deathin susceptible individuals.

*V. parahaemolyticus*occurs in a variety of fish and shellfish. The latterinclude at least 30 different species such as clams, oysters, lobster, scallops, shrimp and crabs. Since *V. parahaemolyticus*is more frequently found incoastal waters, it is not surprising this food from that environment is most often incriminated in food poisoning. Clams, oysters, lobsters, scallops, shrimp, and crabs have been involved in confirmed outbreaks. In addition, the microorganism has been detected in a wide variety of marine species including eels, octopus, squid, sardines, tuna, mackerel, perch, flounder, rockfish, red snapper, pompano, etc. (Beuchat, 1982).

In a study carried out by the FDA, 86% of the seafood samples examined were positive for*V. parahaemolyticus*. Counts have been reported as high as 1300 cfu/g of oyster tissue

and 1000 cfu/g of crab meat, although levels of 10 cfu/g are more typical for seafood products.

1.5 Proximate composition of mud crab

Biochemical analysis provides information on the nutritive value of a particular organism used as a source of food (Sayed, *et al*., 2013).Though the increasing fishery and as well as its popularity as food item (Ahmed, 1991), the information on the nutritive value of *S. serrata* is scanty in Bangladesh. Biochemical composition and mineral content of some crabs namely *S. serrata* and *Portunus pelagicus*, were reported by Das *et al.* from Bangladesh. There are some researches also available on *S. serrata* and *P. pelagicus* from coastal waters of India and Pakistan. Studies on the biochemical composition of edible organisms are important from the nutritional point of view. But very limited study has been done so far on the nutritional composition of crab. Hence in the present study the proximate composition namely, moisture, protein, lipid and ash contents in meat of mud crabs have also been studied.

1.6Research needs

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 (Emikpe *et al*., 2011). One of the major risks involves the consumption of raw or undercooked crab that may be naturally contaminated by foodborne pathogens present in the marine environment. Such risk is further increased if the food is mishandled during processing where pathogens could multiply exponentially under favourable conditions. Sea foods are prone to bacterial contamination, especially filter feeders such as mussels, and oysters, which concentrate these bacteria in their filtration systems and, therefore, are ideally suited to trap all bacteria and viruses, pathogenic or otherwise, that live in the water (Popovic *et al.* 2010; Wafaa *et al*., 2011).

The environment has a great influence on the bacterial flora of freshly caught crabs. During handling, bacteria present on the body surface or in the intestine can be entered the crab meat and cause contamination. Aquatic crustaceans always exercise large number of microbes into their body parts from water, sediments and food. There are several reports on bacterial infection of the mud crab,*Scylla serrata* and blue swimming crab,*Portunus pelagicus*. The increase demand of mud crab production and their value for export, attention should be given to their microbiological quality and concerning food safety for human consumption.

The importers are much concerned about the nutritional value and microbial quality of fish products. The quality of processed products largely depends on the raw materials and method of handling. Along the entire production chain maintenance of hygienic condition and proper handling is very important. That's why the study of microbial (especially bacterial) flora and proximate composition is necessary to ensure the quality of the product. The presence of indicator organisms will prove the low quality of the product.

1.7Objectives

The specific objectives of studying bacterial flora and proximate composition of mud crab (*Scylla serrata*) are-

- Microbial (especially bacterial) quality assessment of normal and frozen crab
- Determination of different bacterial load such as *Vibrio* spp., *Salmonella*spp., *Shigella* spp., coliform, faecal coliform etc.
- Comparison between the bacterial load in fresh and preserved conditions of crab
- Determination of the percentage of nutrient in crab meat
- Assurance about the quality and the safety of crab meat

Chapter 2

General features of *Scylla serrata*(Forsskål, 1775)

2.1 *Scylla serrata* classification

Kingdom:	Animalia
Phylum:	Arthropoda
Subphylum:	Crustacea
Class:	Malacostraca
Order:	Decapoda
Family:	Portunidae
Genus:	*Scylla*
Species:	***S. serrata***
Binomial name	***Scylla serrata***(Forsskål, 1775)

Plate1*Scylla serrata* (Forsskål, 1775)

2.2 Identifying features(The Mud Crab.Fisheries Information Leaflet. DPI Queensland)

I. Elbow has more than one prominent sharp spine.
II. Dark green colored
III. Claw spines are distinct and prominent
IV. Long narrow lobes between the eyes
V. Obviously patterned(molted) walking legs

2.3 Ecology

Mud crabs prefer estuaries and mangrove areas. They are highly tolerant of variations in water salinity and temperature. Although many occupy burrows in the intertidal zone (where the land is exposed at low tide), most adults live in areas that are below the lowtide mark but are still shallow, where they bury themselves in the mud during the day

2.4 Diet

Mud crabs emerge from their burrows at night to search for food; they eat almost anything. However, they mainly eat slow-moving or stationary bottom-dwelling animals such as molluscs, smaller crabs or worms. They also eat plant material.

They use a range of senses to find food. Their eyes are set on stalks to give them 360 degree vision, in and out of water. They also have a pair of antennae between their eyes that can determine minute changes in water movement and chemistry. In addition, the tips of the legs (known as dactyls) are covered with tiny hairs that are highly sensitive to touch and taste.

2.5 Male and female differentiation

As juveniles, male and female mud crabs are difficult to differentiate butit gets easier as they mature. The abdominal flap of females is much broader than that of males and becomes heavily pigmented when the female reaches maturity. Another difference is the claws, which are much larger in males.

2.6 Crab collection techniques

*Scylla Serrata*is common mud crab of the littoral and inter-tidal zones of the Bay of Bengal. The species hardly occurs in sandy and rocky areas over a wide range of salinity, from 2 ppt. to oceanic waters, from the coast to the interior brackish water. Though the crabs prefer mangrove swamp, they also exist in large number in shrimp ponds and in the burrows of the peripheral dikes. It is observed that 45% of the crab collectors collected

crabs from the Sundarban mangrove forest, whereas 40% and 15% collected crabs from ghers and rivers/canals, respectively. The collectors use various types of gears to harvest the crabs such as trap, bait, hook, and by hand picking. Besides these, about 50% of the collectors use bait and hook, and nearly 25% each of the collectors use trap (bamboo made) and hand picking (Zafar, 2004; Patterson and Sainuel, 2005).

Chapter 3

Materials and Methods

3.1 Laboratory of investigation

The whole process was carried out in two different laboratories of University of Dhaka. The laboratory of the Department of Microbiology was used for microbial analysis and for nutritional analysis the laboratory of theDepartment of Fisheries was used.

3.2 Period of investigation

The experiment was carried out from September 2013 to March,2014.

3.3 Experimental specimen

Mud crab (*Scylla serrata*) was selected for this experiment.

3.4 Condition

Both normal and frozen conditions were considered for study.

3.5 Collection of specimen

Crabs were collected from five different locations (Mohakhali, Karwan Bazar, Farmgate, Basabo and Dhanmondi Agora super shop). Live samples were collected from Mohakhali, Karwan Bazar, Farmgate and Basabo market and frozen samples from Dhanmondi Agora super shop. Frozen crabs were brought to the laboratory in ice box. Live samples were collected in sterile polythene bags. Sampleswere washed several times with distilled water to prevent contamination from shell surface and mantle fluid.

3.6 Sterilization

Before working all the equipments; glasswares, needles, scissors were autoclaved at 121ºC for 15 minutes.

3.7 Media used for isolation

i) **Nutrient Agar (NA):** For total aerobic mesophilic plate count

ii) **mFC Agar:** Total and fecal coliform that gives blue colonies

iii) **MacConkey Agar:** Enterobacteriaceae. Lactose fermenters give pink colonies.

iv) **Thiosulphate Citrate Bile Salt Sucrose (TCBS) Agar:** Pathogenic *Vibrio*spp. gives yellow colony while the non-pathogenic is green.

v) **SSAgar:** *Salmonella*spp. gives dark black centered colony and *Shigella*spp. gives clear colonies.

vi) **Eosin Methylene Blue (EMB) Agar:** For *E. coli* that gives green metallic sheen

vii) **Minitol Salt Agar (MSA):** For *Staphylococcus*spp.

Table 3. 1 Microbial response at different medium

Serial NO.	Medium	Microorganisms	Response	Colony colour
1.	mFC	*Enterobacter aerogenes* ATCC 13048	Growth	Reddish grey to blue grey
		Escherichia coli ATCC 11775	Growth	Dark blue
		Escherichia coli ATCC 25922	Growth	Dark blue
		Salmonella typhimurium ATCC 14028	Growth	Reddish grey
2.	MacConkey	*Escherichia coli* ATCC® 25922	Good to excellent	Pink colonies w/ bile ppt
		Escherichia coli ATCC® 8739	Good to excellent	Pink colonies w/ bile ppt
		Proteus mirabilis ATCC® 12453	Fair to excellent	Colorless colonies; partial inhibition of swarming
		Salmonella typhimurium ATCC® 14028		Colorless colonies
3.	TCBS	*Escherichia coli* ATCC® 25922	Inhibited	

		Vibrio alginolyticus ATCC® 17749	Growth	Yellow
		Vibrio cholerae ATCC® 9459	Growth	Yellow
		Vibrio parahaemolyticus ATCC 17802	Growth	Green
4.	SS	*Enterococcus faecalis* ATCC® 29212	Complete inhibition	-
		Escherichia coli ATCC® 25922	Partial to complete inhibition	Pink to rose-red colonies,
		Salmonella typhimurium ATCC® 14028	Fair to good	Colorless colonies with black centers
		Shigella flexneri ATCC® 12022	Fair to good	Colorless colonies
5.	EMB	*Enterococcus faecalis* ATCC® 29212	Partial inhibition	
		Escherichia coli ATCC® 25922	Growth	Blue-black bullseye; may have green metallic sheen
		Pseudomonas aeruginosa ATCC® 27853	Growth	Colorless
6.	MSA	*Staphylococcus aureus* ATCC® 6538	Fair to good	Yellow colonies; may have yellow halo around colonies.
		Staphylococcus aureus ATCC® 25923	Fair to good	Yellow colonies; may have yellow halo around colonies.
		Staphylococcus epidermidis ATCC® 12228	Fair to good	Colorless to pink colonies

3.8Sample preparation

Samples were homogenized separately in normal saline using homogenizer for bacteriological analysis.

3.9Serial dilution

At first 10g of homogenized samples were transferred to the conical flasks containing 100ml saline solution which gave 10^{-1} dilution. Serial dilutions were done in sterile test tubes each containing 9ml of sterile normal saline. 1ml of solution from 10^{-1} dilution was taken in another test tube containing 9ml saline which gave 10^{-2} dilution.In this way 10 fold dilutions of the samples were made up to 10^{-5}. These dilutions were mixed thoroughly using vortex mixer.

3.10Inoculation

0.01ml of each dilution was taken aseptically with sterile micropipptte and inoculated on the selected media. Spread plate technique was used for isolation and enumeration of the microorganisms present in the collected samples.

3.11Incubation

All the plates were transferred to the incubator at 37ºC (mFC plates at 44ºC) for 24 hours to grow target bacteria.

3.12Colony counting

The countable range of bacterial colonies is 30-300 in a plate. The counts were expressed as colony forming unit (cfu) per gram.

3.12.1 Total bacterial count (TBC)

All the colonies within the countable range on nutrient agar plate were counted as TBC. Thus, cfu/g for 2 replicates were initially averaged and used for final calculation.

3.12.2 Total coliform (TC)

For the enumeration of total coliform membrane fecal coliform (mFC) agar plate was used. Plates were incubated at 37°C for 18 to 24 hours. Blue colonies were considered as total coliform.

3.12.3 Fecal coliform (FC)

For the enumeration of fecal coliform same procedure was followed as the enumeration of total coliform except the incubation temperature; 44°C for overnight.

3.12.4 Total *Vibrio* spp. count

All the colonies of yellow and green color on TCBS plate after 18-24 h of incubation were counted as total *Vibrio* cfu/g.

3.12.5 Total *E. coli* count

All the colonies of blue black colour with green metallic sheenon EMB plate after 18-24 h of incubation were counted as total *E. coli*cfu/g.

3.12.6 Total *Salmonella*spp. and *Shigella*spp. count

Colourless and black centered colonies on SS agar were counted as total *Salmonella*spp. and *Shigella*spp. cfu/g.

3.12.7 Total *Staphylococcus*spp. count

Colonies on MSA plate were considered as total *Staphylococcus*spp. count.

3.13Pure culture

Each type of colony those were morphologically different was picked up using sterile needle from different media and streaked on same type of media to acquire pure culture. Then the colonies were picked up again and streaked on nutrient agar plate to get pure culture.

3.14Storage

For bacterial storage nutrient broth was prepared. 0.85µl of nutrient broth was taken in an eppendorf and inoculated with bacterial colony and incubated for 4 h. Then 0.15µl sterile glycerol was added and stored at - 4ºC.

3.15Observation of colony morphology

Different types of colonies in various media were observed. Morphological characteristics including shape, size, edge, elevation, colour etc. were studied.

3.16Gram staining

Gram-positive bacteria have a thick mesh-like cell wall made of peptidoglycan (50-90% of cell envelope), which are stained purple by crystal violet, whereas Gram-negative bacteria have a thinner layer (10% of cell envelope), which are stained pink by the counter-stain (Gram's staining:Wikipedia). There are four basic steps of the Gram's staining:

- Applying a primary stain (crystal violet) to a heat-fixed smear of a bacterial culture
- The addition of iodine, which binds to crystal violet and traps it in the cell
- Rapid decolorization with alcohol
- Counterstaining with safranin

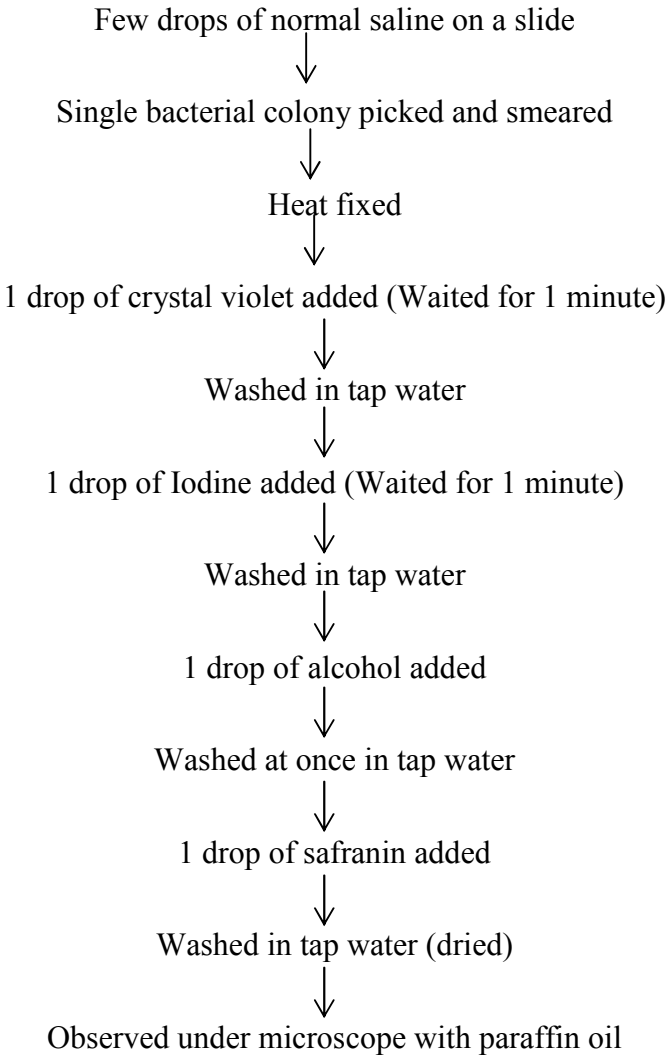


Figure 3.1 Gram's staining method

3.17Biochemical tests

I) Oxidase test

Principle: This test depends on the presence of cytochrome oxidase in bacteria that will catalyze the transport of electrons between electron donors and redox dye. Tetramethyl-

p-phenylene diamine dihydrochloride in the reagent is reduced to deep purple color. This test is used for the screening of *Pseudomonas, Vibrio, Neisseria, Brucella* and *Pasteurella* which give positive test. Enterobacteriaceae are oxidase negative.

Procedure:A piece of filter paper was placed in a petri dish and 3 drops of freshly prepared oxidase reagent (1% solution of tetramethyl-p-phenylene diamine dihydrochloride) was added. Using a sterile glass rod, a colony of test organisms from a culture plate was picked and smeared it on the filter paper.

Interpretation: Oxidase positive organisms gave blue color within 5-10 seconds, and in oxidase negative organisms, colour did not change.

II) Catalase test

Procedure:A small drop of normal saline was placed on a slide.With a sterilized and cooled inoculating loop, a small amount of the culture from the nutrient agar plate was picked. It was smeared to make a smooth suspension.With a Pasteur pipette, one drop of hydrogen peroxide over the test smear was placed. Observe the fluid over the smears for the appearance of gas bubbles.

Interpretation: Catalase positive organisms gave gas bubbles within 5-10 seconds, and in catalase negative organisms, no change occurred.

III) KIA test

Procedure: With needle a colony was picked and stabbed through the center of the agar to the bottom of the tube. Then the surface of the slant was streaked. Kligler slants were incubated for 24 hours.

Interpretation: Lactose (+) organisms yielded a yellow slant and lactose (-) organisms yielded a red slant.Glucose (+) organisms yielded a yellow buttH_2S (+) organisms blacken the lower portion of the tube due to formation of iron sulfide.

IV) Motility detection

Procedure: In a test tube containing MIU mediuma wired loop was used to stab the medium. Test tubes were incubated for 24 hrs.

Interpretation: Non-motile organisms which lack flagella formed a single line of growth that does not spread into the surrounding area while a motile bacterium grewaroundthe stab line producing a hazy zone.

V) Carbohydrate fermentation test

Procedure: Each labeled carbohydrate broth was aseptically inoculatedwith bacterial culture (uninoculated tubes were kept as control tubes). The tubes were incubated for 18-24 hours at 37^{o}C.

Interpretation

Acid production: The medium changed into yellow color- organism fermented the given carbohydrate and produced organic acids there by reducing the pH of the medium into acidic.

Acid and Gas production: The medium changed into yellow color-organism fermented the given carbohydrate and produced organic acids and gas. Gas production was detected by the presence of small bubbles in the inverted durham tubes.

Absence of fermentation: The broth retained the red color. The organism could not utilize the carbohydrate but the organism continued to grow in the medium using other energy sources in the medium.

VI)Indole test (tryptone broth)

Principle: Some bacteria split tryptophan into indole and pyruvic acid using the hydrolase called tryptophanase. Indole can be detected with Kovac's reagent (Indole reagent). This test is very important in differentiating *E. coli* (indole positive) from some closely related enteric bacteria. It also differentiates *Proteus mirabilis* (indole negative) from all other *Proteus* species (indole positive). Tryptone broth is used for this test as it contains a large amount of tryptophan.

Procedure:A loopful of bacteria was inoculated into a tryptone broth and incubated for 48 hours.

Interpretation:A few drops of Indole reagent was added to the broth culture (tryptone broth). A positive result had a red layer at the top. A negative result had a yellow or brown layer.

VII) Citrate test(Simmon's citrate slant)

Principle: Simmon's citrate agar tests for the ability of an organism to use citrate as its sole source of carbon. This media contains a pH indicator called bromthymol blue. The agar media changes from green to blue at an alkaline pH.

Procedure:A loopful of bacteria was streaked onto a citrate agar slantnot stabbing the butt. It was incubated for 24 to 48 hours with loose cap, longer for *Bacillus* species.

Interpretation:A positive reaction was indicated by a slant with a Prussian blue color. A negative slant remained green.

VIII) Methyl red test(MRVP broth)

Principle: Many gram-negative intestinal bacteria can be differentiated based on the products produced when they ferment the glucose in MR-VP medium. *Escherichia, Salmonella*, and *Proteus* ferment glucose to produce lactic, acetic, succinic, and formic acids and CO_2, H_2, and ethanol. The large amounts of acids produced lowers the pH of the medium - Methyl red (a pH indicator) will turn red when added to the medium if the organism was a mixed acid fermenter. Many of these organisms also produce gas.

Procedure:A loopful of bacteria was inoculated into MRVP broth and incubated for 3 to 5 days.

Interpretation:A few drops of methyl red was added to the turbid broth. A positive result had a distinct red layer at the top of the broth. A negative result had a yellow layer.

IX) Voges-Proskauer test

Principle: Organisms that are negative in the methyl red test may be producing 2, 3 butanediol and ethanol instead of acids. These non-acid products do not lower the pH as much as acids do. *Enterobacter, Serratia* and some species of *Bacillus* produce these substances. There is no satisfactory test for determining production of 2, 3 butanediol. A precursor of 2,3 butanediol called acetoin can be detected with Barritt's reagent.

Procedure:A loopful of bacteria was inoculated into MRVP broth and incubated for 3 to 5 days.

Interpretation: The VP A reagent (15 drops) and 5 drops of the VP B reagentwere added to the 1 ml of broth culture. With a positive reaction the medium changed to pink or red indicating that acetone was present. With a negative reaction the broth did not change color or was copper colored. At least 15 minutes waited for color to develop before calling the test negative.

X) Nitrate reduction Test

Principle: Nitrate broth is used to determine the ability of an organism to reduce nitrate (NO_3) to nitrite (NO_2) using the enzyme nitrate reductase. It also tests the ability of organisms to perform nitrification on nitrate and nitrite to produce molecular nitrogen.

Procedure: After incubating the nitrate broth with target colony, a dropperful of sulfanilic acid and α-naphthylamine was added.

Interpretation: The medium turned red after the addition of the nitrate reagents was considered a positive result for nitrate reduction. When the medium did not turn red after the addition of the nitrate reagents, a small amount of powdered zinc was added. If the tube was turned red after the addition of the zinc, it meant that unreduced nitrate was present. Therefore, a red color on the second step was a negative result. If the medium did not turn red after the addition of the zinc powder, then the result was called a positive complete. If no red color forms, there was no nitrate to reduce. Since there was no nitrite present in the medium, either, that means that denitrification took place and ammonia or molecular nitrogen were formed.

XI) Salt tolerance test

Procedure: All isolates of *Vibrio*spp. were tested for their salt tolerance in alkaline peptone water (APW) containing 0, 6.5 and 8% (w/v) sodium chloride. Tubes containing 3.0 ml broth were inoculated with test organism. Growth was observed visually after 24 and 48 hours of incubation at 37^{o}C.

Interpretation: The cloudy appearance of the whole tube after shaking indicated positive growth.

3.18 Identification of the isolates

All the isolates were identified following Bergey's manual of determinative bacteriology.

3.19Proximate composition

3.19.1 Determination of moisture content

- The empty crucibles were dried in the oven for 15 minutes and then transferred to the desiccator to cool.
- The empty foils were weighted.

- About 10g of the prepared sample was transferred to the crucibles and the foil and its contents were weighted.
- The prepared sample with crucibles was kept into oven for drying overnight (16 hours).
- After 16 hours, the crucibles were removed from oven and cooledin desiccators and reweighted.

Calculation:

Weight of the crucibles = W_0

Weight of the crucibles + wet sample = W_1

Weight of the crucibles + dry sample = W_2

Moisture content of sample (%) = $\frac{(W1-Wo)-(W2-Wo)}{W1-W0}$ X 100

3.19.2 Determination of ash

- The crucibles were placed into a muffle furnace for drying and removed dishes after 15 minutes. Then crucibles were cooled in desiccators to room temperature and weighted dry crucibles to the second decimal place.
- About 5g prepared sample was transferred to the crucibles.
- Three crucibles with sample were kept into oven over night (16 hours).
- The crucibles were removed from the oven and cooled in a desiccators and weighted the crucibles and contents as rapidly as possible, to the nearest second decimal place.
- The crucibles were placed inside the muffle furnace, as near to the center as possible and ash overnight at 550 ºC.
- The crucibles were removed from the muffle furnace and placed in a desiccators and allow to cool to room temperature.

Calculation:

Weight of dry, clean crucibl= W_0

Weight of dry, clean crucible + wet sample = W_1

Weight of dry, clean crucible + ash = W_2

Ash content of sample (%) = $\frac{(W2-Wo)}{W1-W0}$ X 100

3.19.3Determination of crude protein

- At first about 0.5 g grinded sample in a filter paper was weighed and transferred to the digestion apparatus flask.
- It was digested with 15 ml. H_2SO_4 (conc.) and 1g digestion mixture and continued heating at 315^0C for 6 hours until the acid become clear.
- The digested products were cooled and after that the digested products were transferred to 100 ml volumetric flask and were made up to 100 ml with distilled water.
- 5 ml from dilute digested mixture was transferred in "Kjeldahl" dilution apparatus and distilled with 10 ml of 40% NaOH.
- The distillate was collected in 5 ml of 2% Boric acid solution in a 100 ml. conical flask with addition of 2 drops of Methyl red indicator.
- It was then titrated against 0.01N HClsolution.

Calculation:

$$\text{Moisture factor (mf)} = \frac{100 - \text{Moisture}}{100}$$

$$\%\ \text{of nitrogen} = \frac{(S-B) \times A \times C \times F \times 100}{\text{Weight of sample}}$$

Here,

S= titration reading for sample

B= titration reading for blank

A= strength of 0.01N HCl

C= digest taken for distillation (dilution factor) ~20

F= factor

3.19.4Determination of lipid content

- Crude lipid was determined by extracting the sample with methanol and chloroform solution (1:2).

- The clear extracted solution was kept at waterbath until the evaporation of chloroform and methanol.
- The percentage of crude lipid content was calculated by the following equation.

Calculation:

Percentage (%) of fat = (Weight of extract/Weight of sample) × 100

3.20 Statistical analysis

The means of bacterial load and the data of proximate composition were compared using SEM and ANOVA followed by Tukey's post hoc for multiple comparisons. Statistical software SPSS version 20.0 was used to analyze the data with the level of significance at $p<0.05$. Microsoft Excel 2010 was used for graphing of the data.

Plate 2 Sample Specimen, *Scylla serrata*

Plate 3 Different media used for bacterial analysis

Plate 4 Bacterial colonies on TCBS agar media

Plate 5 Bacterial colonies on MacConkey agar media

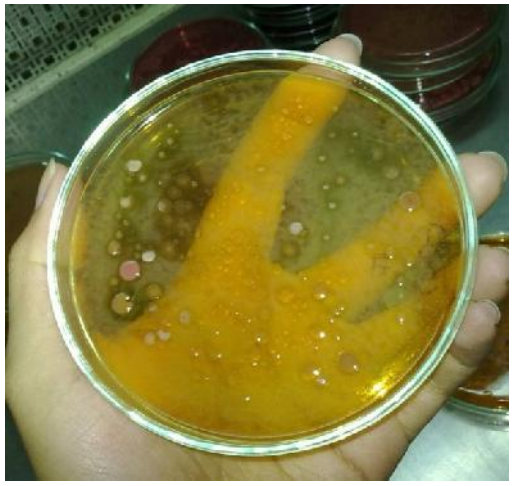


Plate 6 Bacterial colonies on SS agar media

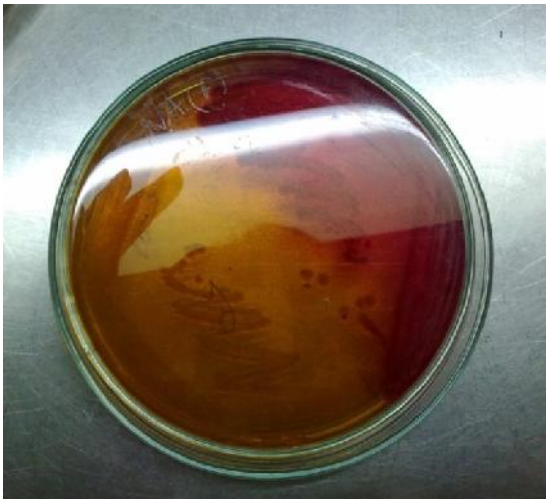


Plate 7 Pure culture of colonies on MacConkey agar media

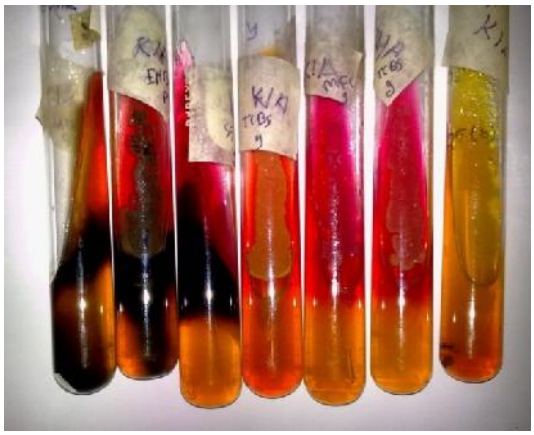


Plate 8 KIA test

Plate 9 Simmon's citrate test

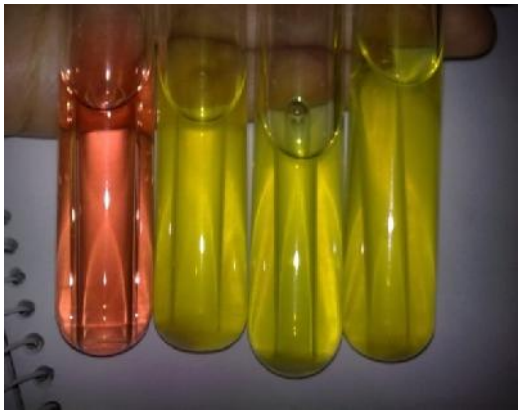


Plate 10 Carbohydrate test

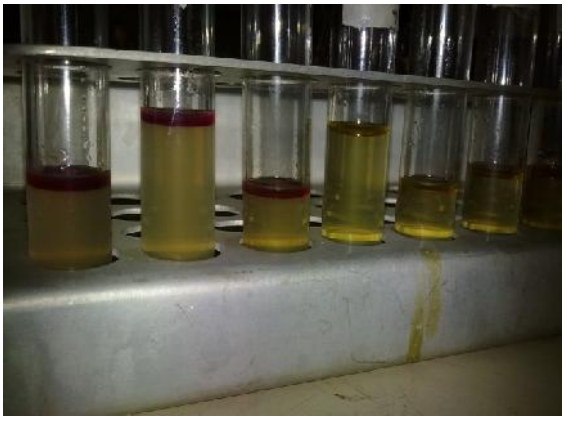


Plate 11 Indole test

Plate 12 Determination of moisture content

Plate 13 Determination of lipid content

Chapter 4

Result

The results of total bacterial counts (TBC), total coliform (TC), fecal coliform (FC), total *Vibrio*spp., total *E. coli*, total *Salmonella*spp. and *Shigella*spp. and total *Staphylococcus* spp. countsof mud crab (*Scylla serrata*) meatare shown in Table 4.1. The moisture, ash, protein and lipid contents of the meat are shown in Figure 4.4.

4.1 Bacterial density in mud crab samples

Table 4.1The density (cfu/g) of TBC, total *Vibrio* spp.,total *E. coli*, TC, FC, SS and total *Staphylococcus*like colonies in mud crab (*Scylla serrata*) meat*.

Sources	TBC (10^4) cfu/g	Total *Vibrio*spp.(10^4) cfu /g	Total *E. coli* (10^4) cfu /g	TC (10^4) cfu /g	FC (10^4) cfu /g	Total SS (10^4) cfu /g	Total *Staphylococcus* spp. (10^4) cfu /g
Mohakhali	23.07±1.24^{b}	0.0 ± 0.0	3.19± 0.21^{ab}	9.85± 0.45^{a}	3.02± 0.01^{b}	12.53±0.6^{b}	14.4± 1.1^{a}
Karwan [illegible]	38.1±1.20^{a}	0.0± 0.0	4.33± 0.56^{a}	9.61± 0.71^{a}	4.47± 0.22^{a}	3.95± 0.28^{c}	16.61± 1.71^{a}
Farmgate	23.83±0.80^{b}	12.54±0.38^{a}	1.25± 0.02^{cd}	8.36± 0.36^{a}	2.25± 0.25^{b}	11.68±0.32^{b}	17.88± 0.85^{a}
Basabo	25.93±0.63^{b}	10.76±0.46^{b}	2.33± 0.31^{bc}	10.55±0.65^{a}	2.82± 0.14^{b}	16.76±1.26^{a}	20.01± 1.94^{a}
Agora	4.48±0.26^{c}	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0±0.0	0.0± 0.0	0.37±0.025^{b}

* Means (± SEM) within row and column for total and column with different letters are significantly different (ANOVA, HSD, $p<0.05$).

The crabs were sampled from different 5 markets in Dhaka city. Samples from agora super shop were in frozen condition and from other four markets were in normal condition.TBC counts ranged from 4.48 ± 0.26 x10^4 cfu/g to38.1 ± 1.20 x10^4 cfu/g. The highest TBCcounts were observed in the samples from Karwan bazar and the lowest were from Agora super market. The statistical differences in the TBC counts are calculated at 5% probability levels.

The total *Vibrio*spp. counts were found in only two markets Farmgate and Basabo; ranged between10.76±0.46x10^4 cfu/g and12.54±0.38x10^4 cfu/g.

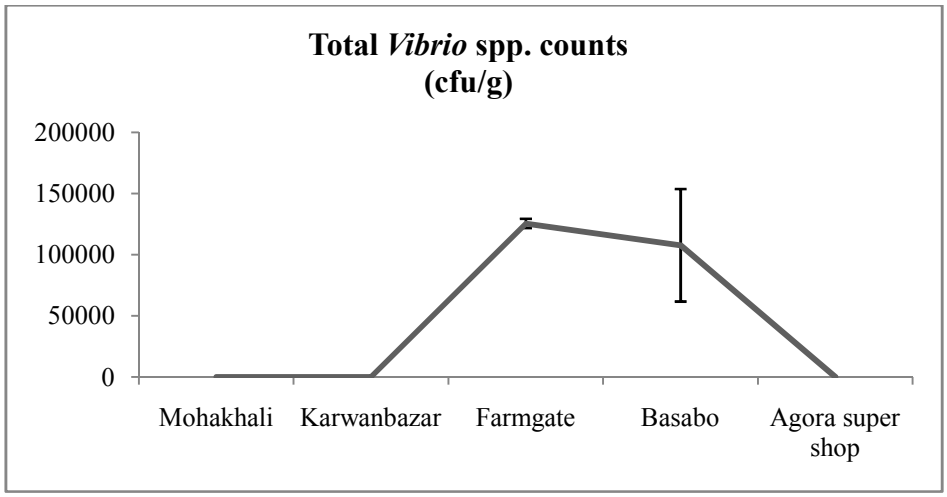


Figure 4.1Thesources of sample collection plotted against total *Vibrio*counts (cfu/g)

The counts of the total*E. coli* were 4.33±0.56x10^4 cfu/g for Karwan bazar,3.19±0.21x10^4 cfu/g for Mohakhali,2.33±0.31x10^4 cfu/g for Basabo,1.25±0.02x10^4 cfu/g for Farmgateand 0.0±0.0x10^4 cfu/g for Agora super shop.

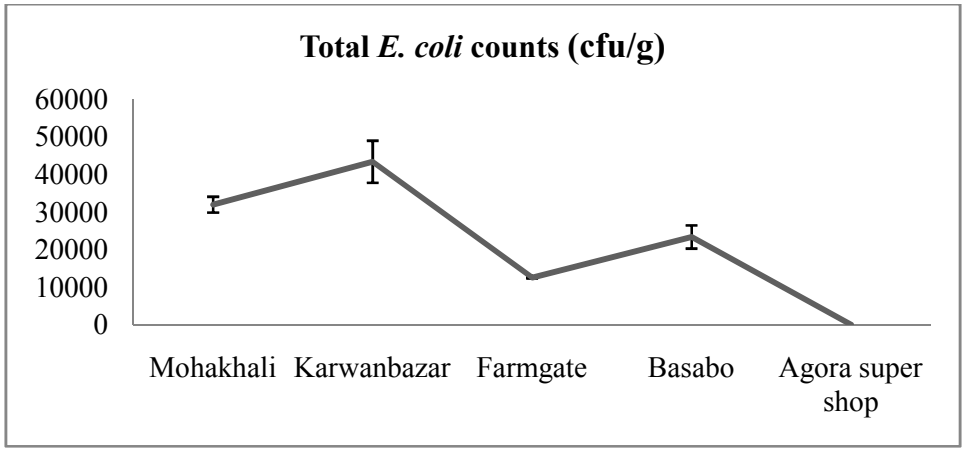


Figure 4.2Thesources of sample collection plotted against total *E. coli* counts (cfu/g)

The counts of the total coliform were $10.55\pm0.65x10^4$ cfu/g for Basabo,$9.85\pm0.45x10^4$ cfu/g for Mohakhali, $8.36\pm0.36x10^4$ cfu/g for Farmgate,$4.33\pm0.56x10^4$ cfu/g for Karwan bazar and $0.0\pm0.0x10^4$ cfu/g for Agora super shop. There were no significant differences ($p>0.05$) in the counts of the total coliforms of the samples obtained from the five markets.

The FC counts ranged from $0.0\pm0.0x10^4$ cfu/g at Agora super shop to $4.47\pm0.22x10^4$ cfu/g at Karwan bazar samples.

The *Salmonella*spp. and *Shigella*spp. counts were found in all four markets except Agora; ranged from$3.95\pm0.28x10^4$ cfu/g at Karwan bazar to $16.76\pm1.26x10^4$ cfu/g at Basabo.

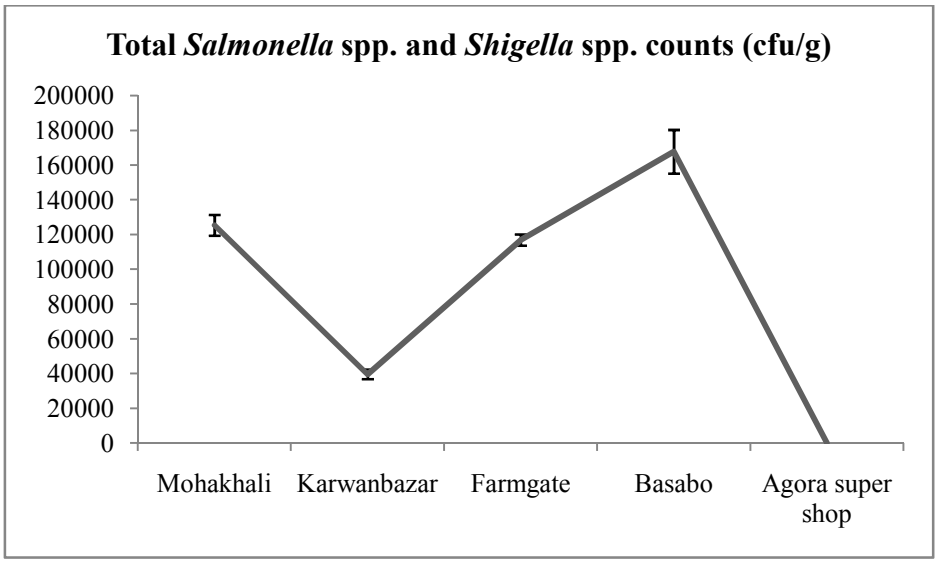
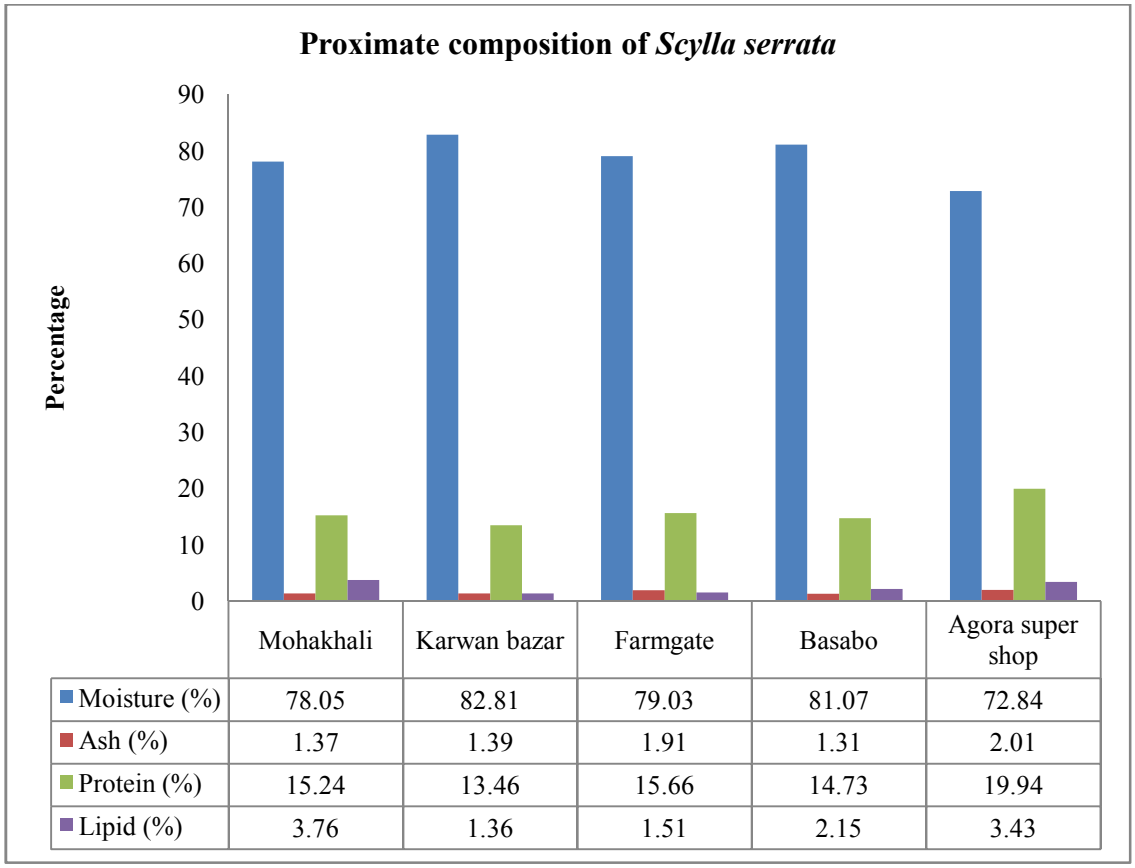


Figure 4.3The sources of sample collection plotted against the total *Salmonella*spp. and *Shigella*spp. counts (cfu/g)

The staphylococcal counts ranged from $0.37\pm0.025x10^4$ cfu/g to$20.01\pm1.94x10^4$ cfu/g.The highest staphylococcal counts were obtained from Basabo market while the least count was obtained from the agora super shop. Significant differences exist in the staphylococcal counts of the crabs obtained from the five markets.

4.2 Proximate composition(moisture, ash,protein, and lipid content) of mud crab meat



	Mohakhali	Karwan bazar	Farmgate	Basabo	Agora super shop
Moisture (%)	78.05	82.81	79.03	81.07	72.84
Ash (%)	1.37	1.39	1.91	1.31	2.01
Protein (%)	15.24	13.46	15.66	14.73	19.94
Lipid (%)	3.76	1.36	1.51	2.15	3.43

Figure 4.4 Proximate composition of mud crab (*Scylla serrata*) meat; sampled from different markets in Dhaka city. Sample from agora super shop was in frozen condition and from other four markets were in normal condition.

The lowest (72.84 ± 0.16^{d}%) and the highest (82.81 ± 0.21^{a}%) moisture content were recorded from Agora and Karwan bazar crab samples respectively (Figure 4.4). No significant variation in moisture content was found regardless of the variation of the sources ($p>0.05$).

Ash content varied between 1.31 ± 0.04^{b}%to 2.01 ± 0.03^{a}%. The frozen crabs showed higher ash content than the other crab samples. The ash contents of Farmgate and Agora were significantly different from theash contents of Mohakhali, Karwan Bazar and Basabo crab samples.

Protein content varied between 13.46±0.44^{c} % and 19.94±0.19^{a}%. Protein content was found comparatively higher in frozen crabsthan the othernormal crab samples. There were no significant difference between the protein content of Farmgate and Basabo.

The maximum (3.76±0.01^{a}%) lipid recorded from Mohakhali samples and the minimum (1.36±0.04^{c}%) from Karwan bazar.The lipid contents of the samples from Mohakhali and agora were significantly different from Basabo samples and those 3 sample sources vary significantly fromKarwan Bazar and Farmgate samples.

4.3 Identification of the isolates

4.3.1 Isolation of bacteria

All the samples except the frozen ones had the load of coliform bacteria. Fecal coliform was also found. MacConkey agar was used to isolate indicator bacteria. There were 3 different types of colonies observed on MAC. Pink colonies were suspected as *E. coli.* Biochemical tests were performed for confirmation.

SS agar was used for isolation of *Salmonella* spp. and *Shigella* spp. Clear colonies on SS agar plate were suspected as *Shigella* spp.and black centered colonies as *Salmonella* spp.

On EMB plates pink colonies were assumed to be *E. coli.* Blue and grey colonies were found on mFC agar plates. Grey colonies were suspected as *Enterobacter.* On MSA media yellow colonies had the characteristic of mannitol fermenting *Staphylococcus*. Those were isolated and biochemical tests were performed.

Three types of colonies were found on TCBS agar plates; yellow, green and dark green. Yellow were suspected as *Vibrio cholerae* as they had the ability to ferment sucrose on TCBS agar. Small green were presumed to be *Vibrio vulnificus* and slightly larger dark green ones as *V. parahaemolyticus*. For confirmation biochemical tests were performed. Salt tolerance test was particularly performed for *Vibrio* spp. identification.

4.3.2Cultural characteristics of the microorganisms

Microorganisms show differences in the appearance of their growth when they grow on variety of media. These particular differences are called cultural characteristics. For differentiation of the bacterial colonies into two wide groups (‘+’ and ‘-‘), Gram’s

staining was used. Only two isolates, one from Nutrient agar and another from MSA, showed Gram positive characteristics and all the rests were Gram negative.

Table 4.2Cultural characteristics of microorganisms with Gram's reaction results.

Medium	Colour	Form	Margin	Elevation	Gram's reaction
NA 1	White	Irregular	Undulate	Convex	+
NA 2	Yellow	Circular	Entire	Flat	-
NA 3	Cream	Circular	Entire	Raised	-
SS 1	Black center	Circular	Entire	Convex	-
SS 2	Clear	Circular	Entire	Raised	-
TCBS 1	Yellow	Circular	Entire	Raised	-
TCBS 2	Green	Circular	Entire	Raised	-
TCBS 3	Dark Green	Circular	Entire	Raised	-
MAC 1	Pink	Circular	Entire	Convex	-
MAC 2	Yellow	Circular	Entire	Flat	-
MAC 3	Light pink	Circular	Entire	Convex	-
MSA 1	Yellow	Circular	Entire	Raised	+
mFC1	Blue	Circular	Entire	Convex	-
mFC 2	Grey	Circular	Entire	Raised	-
EMB	Pink	Circular	Entire	Raised	-

4.3.3 Identified isolates

Among the 15 isolated strains 10 different genera were found;*Bacillus*, *Citrobacter,Alcaligens,Salmonella,Shigella,Vibrio,Escherichia,Klebsiella,Staphylococcus* and *Enterobacter*.

Table 4.3 Isolates from different media and their identified names

Isolates	Identified bacteria
NA 1	*Bacillus* spp.
NA 2	*Citrobacter*spp.
NA 3	*Alcaligens faecalis*

SS 1	*Salmonella typhi*
SS 2	*Shigella* spp.
TCBS 1	*Vibrio cholerae*
TCBS 2	*Vibrio vulnificus*
TCBS 3	*Vibrio parahaemolyticus*
MAC 1	*Escherichia coli*
MAC 2	*Alcaligens faecalis*
MAC 3	*Klebsiella pneumoniae*
MSA 1	*Staphylococcus aureus*
mFC 1	*E. coli*
mFC 2	*Enterobacter* spp.
EMB 1	*E. coli*

Chapter 5

Discussion

Marine invertebrates are constantly exposed to high concentration of microorganisms. Crabs and shrimps have been implicated in *Vibrio parahaemolyticus* food poisoning, Cholera, Salmonellosis, Shigellosis and Yersinia food infection. Deaths from staphylococcal food poisoning have been reported. The normal temperature used in cooking will not destroy the toxins and foods containing staphylococcal enterotoxins and they usually look and taste normal.

Fish of good quality should have bacterial count less than 10^5 per gram. Contamination of fish from enteric bacteria ofhuman and animal origin may be responsible for various food borne diseases (Emikpe *et al*., 2011).

The results of the bacterial analysis offresh crabs bought from four markets in Dhaka city indicate high levels of contamination with pathogenic bacteria. The total aerobic bacterial counts (TBC) exceeded the minimum acceptable limit ($<10^5$ cfu/g) for total aerobic counts (Refai, 1979). Total coliform and staphylococcal counts of the crab samples were also above the acceptable limit (coliform $<10^2$ cfu/g, coagulase positive staphylococcus $<10^2$ cfu/g) according to Whong *et al*.(2003). The results confirmed that the samples of local markets contained high pathogenic bacterial load which are supposed to be a threat to food safety creating food borne diseases (Nilla, *et al*., 2012).The high total aerobic counts is an indication of reduced shelf-life for the crab meats while the high coliform and staphylococcal counts is an indication of potential food infection/ intoxication (Buchanan, 1991).Lalitha *et al*., 2012 found *Vibrio,* Enterobacteriaceae, *Moraxella, Acinetobacter, Pseudomonas/Shewanella, Micrococcus* and *Bacillus* in farmed crab meat. On the other hand, this study showed the presence of *Vibrio,* Enterobacteriaceae, *Bacillus, Alcaligens* and some other bacteria.But the TBC counts of this study were more or less similar to the study of Lalitha *et al*., 2012.

The frozen samples from Agora super shop had significant lower level of bacterial counts. *Vibrio, E. coli*, Total coliform, fecal coliform, *Salmonella, Shigella*were absent in those samples. This indicates the good quality of the frozen product.

Fecalcoliforms and *Escherichia coli* were recovered from all the four normal crab samples. *E. coli* counts in fresh crab meat from all four markets were withinthe

maximum limit for acceptability of crab meat, as recommended by the ICMSF (1998). The levels of fecal coliforms, Staphylococci, and *E. coli* collected from Karwan Bazar samples were very high compared to the other 3 markets. Staphylococcicounts of live crabs were not within the acceptable limit (10^3 cfu g^{-1}) recommended by ICMSF (1998). These microbial groups are important in foods as indicators of hygienic quality of foods. The study revealed presence of pathogenic bacteria such as high numbers of fecal coliform, *E. coli , Salmonella*spp. and *Shigella*spp.inmud crab meat. This knowledge will increase our understanding of the effects of aquaculture operations on bacterial community composition in the crab and provide necessarydata for the development of control measures in crab farms.

The results of the study indicate that good handlingand post-harvest practices should be adopted to improve the microbiological quality of mud crab. While analyzing fresh picked crab meat from twelve different blue crab processing facilities, Reinhard *et al.* (1996) observed coliform and fecal coliform counts in the range of <0.3 to 32.8 MPN g^{-1} and <0.3 to 2.26 MPN g-1, respectively and *Escherichia coli* counts ranged from <0.3 to 0.77 MPN g^{-1}.

The samples of crab analyzed bacteriologically showed presence of *Vibrio* spp. contamination in two markets only; Farmgate and Basabo. In the present study, *Vibrio parahaemolyticus, Vibrio vulnificus*and *Vibrio cholerae*were detected.Inthe study of Aftabuddin,*et al*., 2013 *Vibrio vulnificus* was not found in *Scylla serrata* from Chakoria coast, Bangladesh. *Vibrio parahaemolyticus* were not detected from the findings of Merwad *et al*., 2011 who reported zero sediment worldwide and some species. Adebayo-Tayo *et al.,* 2011 mentionedthat *Vibrio parahaemolyticus, Vibrio vulnificus*were not detected from aquatic crabs which do not go entirely with the result of present study. International Commission of Microbiological Specification for Foods (ICMSF, 1998) recommended that counts of chilled/frozen crab meat should below 10^5 cfu/g and the maximum counts of *V. prahaemolyticus* should be 1 x 10^2. But the result of present study exceeds the level.

Regarding the zoonotic significance, *V. vulnificus* are usually acquired through ingestion of shellfish or through contaminating open wounds during swimming, crabbing, shellfish cleaning and other marine activities as was previously sustained and are implicated in

epidemic human gastroenteritis.In a study by Zahid *et al*., 2010,*V. vulnificus* was frequently isolated from coastal but not freshwater aquaculture in Bangladesh.

This study revealed 3*Vibrio* spp. from shellfish and all of them have a zoonotic importance. Therefore the surveillance of contaminant *Vibrio* in shellfish is crucial for sustenance of public health. In this study, thepresence of contaminating bacteria in crab meat could be attributed to cross-contamination from environment, toxigenic source and handling by the sellers. Most *Vibrio cholerae* found on the samples indicate that the cholera-like diarrhea and blood stream infection in the samples might have been contaminated from handles and due to unsanitary practices and processing condition. Some authors had earlier stated Prevalence of *Vibrio cholerae* and *Vibrio* species are basically used as test organism for *Salmonella* in a major shrimp production area.

The presence of *Escherichia coli* in all the samples except the frozen one may be suggestive of fecal contamination due to poor hygiene and sanitation. *E. coli* has been implicated in human diarrheal particularly type 0157:H7 (Nester *et al*., 2007).

The presence of *Salmonella* spp. and *Shigella* spp. in the crab samples may be due to exposure to prevailing unhygienic and poorsanitary conditions in the markets. *Salmonella* and *Shigella* are well-known important human pathogens. Their presence is unacceptable because of their attendant health risks (Mensah *et al*., 2002). In this regard, all live crab samples were unsuitable for export and human consumption. But the frozen samples were free from *Salmonella* and *Shigella*which assured its safety.

Significant numbers of detections of *Salmonella* spp. in fish and fishery products indicate thatcurrent strategies for *Salmonella* control in the aquaculture production and processing sectors are not adequate. While some crabs caught offshore and handled hygienically and at low temperature according to the Codex Code of Practice for fish and fishery products (CAC/RCP/52-2003) may be suitable for raw consumption, it would be advisable to consume products of aquaculture only after cooking. The *Salmonella* problem should be resolved by the use of good manufacturing procedures and the strict application of sanitary practices. On the other hand, Hazard analysis and critical control point (HACCP) systems should be implemented increasingly by private industry for seafood. These must be rigidly enforced throughout the processing line and require the full understanding and cooperation of plant management and every employee. Investment in new technologies and equipment will also improve the seafood safety.

The high staphylococcal counts in all the crab samples may be due to the fact that crabs are constantly being touched by both buyers and sellers, thus introducing and increasing the population of *Staphylococcus aureus* (Whong *et al.*, 2003). Ezeama (2007) is of the view that staphylococcal counts exceeding 10^2 cells/g in foods may be associated with gross mishandling and contamination. *S. aureus* competes poorly with the native micro flora in most raw foods; however,products containing higher levels of salt provide *S. aureus* with a competitive edge. Consequently, products most frequently incriminated in SFP outbreaks are cooked or otherwise processed high-protein foods that have come in contact withworker's hands and then were either served after being temperature abused or served after improper heating/refrigerated storage. Foods that require considerable handling during preparation and that are kept at slightly elevated temperatures after preparation are most frequently involved in staphylococcal food poisoning. Hence, adherence to stringent hand washing and sanitation practices in food preparation areas along with proper storage temperatures is critical in minimizing contamination along with subsequent growth of *S. aureus* to potentially toxic levels.

It is important to understand the mechanisms and interactions between nutrients and contaminants in seafood if researchers are to give sound scientific advice on the amount and type of seafood that should be recommended to promote health and maximize safety in different groups of the population (Hastein *et al*., 2006). At present, there is no agreed methodology for taking both the risks and benefits of seafood into account in a quantitative way. The organization EFSA advised that a framework should be developed which allows such a quantitative comparison, based on a common scale of measurement (EFSA, 2005).

To protect animal and human health, internationally agreed maximum permittedlevels have been set for several chemical contaminants in both feed and food (Hastein *et al*., 2006). National and international monitoring programmes exist to ensure that the levels present are acceptable. People are continuously made aware on the proper measures to be embarked in sewage and effluents disposal and not to the rivers as this would pollute the water and cause health risk (Adebayo-Tayo *et al*., 2012c; Udeze *et al*., 2012b).In addition, aquaculture industries are using hazard analysis critical control point principles to ensure the acceptable quality of their products (Hastein *et al*., 2006).

If fish and fish products contain values of environmental contaminants above the accepted international levels, this will almost certainly have a significant impact on

international trade. Importing countries will introduce bans on fish and fish products. They have already done so due to contaminants such as cadmium, dioxins and malachite green (Hastein *et al*., 2006). Good manufacturing practices should always be observed by the trade to minimize the risk of food poisoning associated with the consumption of crab meat products.

Biochemical studies are very important for studying the nutritional aspects of the food items. The lowest moisture content of crab samples obtained from Agora super market may be responsible for their negative coliformand low staphylococcal counts compared to the samples from other four markets. However, the moisture contents for all the crab samples were high. This could be responsible for the high bacterial load of the crab samples.

Protein is very much essential for the sustenance of life and growth. It is the most prominent organic compounds. The highest protein content was in frozen crabs with lowest moisture content.This may indicate that those were fattened crabs as they show higher protein values than the natural ones. Thus, fattened are nutritionally more superior to the natural crabs.In the present study, it has also been shown that the protein, lipid, ash and moisture contents of *S. serrata* did not vary much with market variations.

Table 5.1Acomparative table of proximate composition of body meat in *S. serrata* from different published reports with the present findings

Information Source		**Moisture (%)**	**Protein (%)**	**Lipid (%)**	**Ash (%)**
Srinivasagam, 1979		78.38	16.65	F>M	-
George and Gopakumar, 1987		80.19 M	20.92F	F>M	5.09
Das *et al*., 1995		77.45 F	19.98 F	0.48 F	1.23 F
Mannan, 1977		-	7.3	0.5	0.16
Sarower *et al,* 2013		83.64	22.77	1.35	2.09
Present study	Normal	79.03 – 82.81	13.46- 15.66	1.36- 3.76	1.31- 1.91
	Frozen	72.84	19.94	3.43	2.01

Moisture was found to be the major component as it varied from 72.84% to 82.81% in body meat. The results are supported by other such studies such as 78.38% by Srinivasagam, 1979 and 80.19% by George and Gopakumar, 1987. The processed frozen

samples had much lower moisture content, 72.84% than the fresh ones. The percentage of protein (19.94%)in the body meat was alsoextremely close to the study of Das et al., 1995who reported 19.98% protein in meat of *S. serrata* from India. The level of protein in mud crabs from all sources is higher than molluscs (8-13%) reported in Bangladesh (Baby *et al*., 2010). In aspect of lipid percentage, present result showed a much higher value (1.36- 3.76%) than other studies (0.5%, Mannan, 1977; 0.48%, Das *et al.* 1995.But the study was agreed with Sarower *et al*.,2013 who reported 1.35% lipid content. However, the percentage of ash contents was more or less similar to other such studies. George and Gopakumar, 1987 reported 5.09% ash content in crab body meat whereas Das *et al.,* 1995 recorded 1.23%. In the present study, frozen samples had highest ash content, 2.01% which agrees with Sarower *et al,*2013; 2.09%.

Chapter 6

Recommendations and Conclusion

6.1 Recommendations

The safety of the public health depends on the improvement of sanitation within the metropolis by provision of public toilets, and enactment of effective policy for the collection and disposal (management) of municipal solid waste as these would drastically reduce the pollution of running water and rivers with human and domestic waste. Owing to the potential hazard of some pathogens, it is clearly necessary to put more emphasis on food hygiene. Therefore, surveillance of potential contaminant bacteria in harvested seafood is crucial for sustenance of public health. The sanitary conditions under which mud crabs are reared or cultured in ponds should be improved by following standard or good practices; such as use of good quality water, use of feeds with high microbial quality, regular draining of pond water after specific period of time, closure of ponds to the public among other things. The farmers should embrace standard operating practices as applicable to mud crab farming. The workforce should be educated on the maintenance of good hygienic practices, and should be provided with necessary working and safety equipment. Crabs those have shown signs of spoilage should not be sold to the public so as to boost the health standard of the people. The microbial load of mud crabs can also be improved through regular disinfection of catching gears or working equipment, and brief immersion of caught crabs in disinfecting solution such as brine water to reduce the microbial load on the fish before storing at cold temperature or sold to the public.

6.2 Conclusion

The findings of this study show that themud crabs sold in Dhaka are highly contaminated and potentially hazardous if consumed directly or without proper cooking. But the frozen samples sold in super markets are much safer. The high bacterial load of those products may be due to inadequate farming process in addition to poor hygiene practices and sanitary conditions prevailing in the markets or the environment from which they are caught.

The presence of *Vibrio* spp., *Salmonella* spp., *Shigella*spp. and *Staphylococcus*spp. in the sampled crabs indicate the probability of outbreak of food borne diseases through consumption of infected crabs. So long term monitoring and hygienic practices are necessary to avoid such situations.

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Appendices 1

Microbiological media

All the media used were prepared by standard methods using appropriate compositions. Components used were high grade and were produced either by Acumedia or DIFCO, USA. All media were sterilized by autoclaving for 15 minutes. The composition used for different media have been shown below.

Alkaline Peptone Water (APW)

Ingredients	Amount (g/L)
Peptone	10 g
NaCl	10 g
Distilled water	1 L

Adjust pH to 8.5 ± 0.2 after dissolving ingredients.

Oxidase Reagent

N,N,N',N'-tetramethyl-p-phenylenediamine.2HCl	0.1 g
Distilled water	10 ml

Use this reagent on the day of preparation.

0% NaCl solution

Peptone	1g
NaCl	0 g
Dislilled water	100 ml
pH adjusted to	7.4
pH adjusted to	7.4

6.5% NaCl solution

Ingredients for 100mL

Peptone	1g
NaCl	6.5g
Dislilled water	100 ml
pH adjusted to	7.4

8% NaCl solution

Ingredients for 100 ml

Peptone	1g

NaCl	8 g
Dislilled water	100 ml
pH adjusted to	7.4

Normal saline

Ingredients	Amount (g/L)
NaCl (sigma)	8.5
Distilled water	1.0

pH was adjusted to 7.8.

Nutrient Agar

Ingredients	Amount (g/ml)
Powder nutrient agar	5.6
Distilled water	200

mFC Agar

Ingredients	Amount (g/ml)
Powder mFC agar	10.42
Distilled water	200
Rosolic acid	few drops

Rosolic Acid

Ingredients	Amount (g/ml)
Rosolic acid powder	0.5
Sodium Hydroxide	0.2
Distilled water	5.0

Minitol Salt Agar

Ingredients	Amount (g/ml)
Powder MSA	22.2
Distilled water	200

MacConkey Agar

Ingredients	Amount (g/ml)
Powder MacConkey agar	10.4
Distilled water	200

Eosin Methylene Blue Agar

Ingredients	Amount (g/ml)
Powder EMB agar	7.2
Distilled water	200

SS Agar

Ingredients	Amount (g/ml)
Powder SS agar	12.6
Distilled water	200

TCBS Agar

Ingredients	Amount (g/ml)
Powder TCBS agar	17.6
Distilled water	200

Nutrient Broth

Peptone	0.25 g
NaCl	0.25 g
Beef extract	0.15 g
Distilled water	50 ml

Indole Broth

Peptone	0.50 g
NaCl	0.25 g

Distilled water 50 ml

Carbohydrate Broth

Tryptone 0.50 g

NaCl 0.25 g

Sugar 0.25 g

Phenol red 0.001 g

Distilled water 50 ml

MRVP Broth

Peptone 1.05 g

Dextrose 0.75 g

Potassium phosphate 0.75 g

Distilled water 150 ml

Nitrate reduction Broth

Peptone 0.50 g

NaCl 0.50 g

Beef extract 0.30 g

KNO_3 0.50 g

Agar powder 0.10 g

Distilled water 100 ml

Methyl red reagent

Ethyl alcohol 95% 60 ml

Methyl red 0.10 g

Distilled water 40 ml

Nitrate reduction reagents

Solution A

Sulfanilic acid 0.8 ml

Acetic acid 100 ml

Solution B

Alpha Napthalamine 0.5 g

Acetic acid 100 ml

5N Acetic acid

Glacial Acetic acid 63 ml

Distilled water 137 ml

Barrit's reagents

Solution A

Alpha Napthol 5 g

Ethanol 95 ml

Solution B

KOH 40 g

Creatine 0.30 g

Distilled water 100 ml

Appendics 2

Case Summaries[a] of bacterial load of mud crab meat

				TBC	TEC	SSC	TVC	TC	TStaphy	FC
Sources of sample	Mohakhali	1		243100.00	29700.00	131300.00	.00	94000.00	155000.00	30100.00
		2		218300.00	34000.00	119300.00	.00	103000.00	133000.00	30300.00
		Total	N	2	2	2	2	2	2	2
			Mean	230700.0000	31850.0000	125300.0000	.0000	98500.0000	144000.0000	30200.0000
			Std. Error of Mean	12400.00000	2150.00000	6000.00000	.00000	4500.00000	11000.00000	100.00000
	Karwan bazar	1		369000.00	49000.00	42300.00	.00	89000.00	149000.00	47000.00
		2		393000.00	37700.00	36700.00	.00	103300.00	183300.00	42500.00
		Total	N	2	2	2	2	2	2	2
			Mean	381000.0000	43350.0000	39500.0000	.0000	96150.0000	166150.0000	44750.0000
			Std. Error of Mean	12000.00000	5650.00000	2800.00000	.00000	7150.00000	17150.00000	2250.00000
	Farmgate	1		230300.00	12700.00	120000.00	129300.00	80000.00	179700.00	20000.00
		2		246300.00	12300.00	113600.00	121600.00	87300.00	178000.00	25000.00
		Total	N	2	2	2	2	2	2	2
			Mean	238300.0000	12500.0000	116800.0000	125450.0000	83650.0000	178850.0000	22500.0000

			Std. Error of Mean	8000.00000	200.00000	3200.00000	3850.00000	3650.00000	850.00000	2500.00000
		1		253000.00	20300.00	180300.00	103000.00	99000.00	180700.00	29700.00
		2		265600.00	26600.00	155000.00	112300.00	112000.00	219600.00	26800.00
			N	2	2	2	2	2	2	2
	Basabo		Mean	259300.0000	23450.0000	167650.0000	107650.0000	105500.0000	200150.0000	28250.0000
		Total	Std. Error of Mean	6300.00000	3150.00000	12650.00000	4650.00000	6500.00000	19450.00000	1450.00000
		1		47500.00	.00	.00	.00	.00	3500.00	.00
		2		42200.00	.00	.00	.00	.00	4000.00	.00
			N	2	2	2	2	2	2	2
	Agora Super shop		Mean	44850.0000	.0000	.0000	.0000	.0000	3750.0000	.0000
		Total	Std. Error of Mean	2650.00000	.00000	.00000	.00000	.00000	250.00000	.00000
		N		10	10	10	10	10	10	10
	Total	Mean		230830.0000	22230.0000	89850.0000	46620.0000	76760.0000	138580.0000	25140.0000
		Std. Error of Mean		36029.13034	5110.86098	20471.16807	19145.95867	13115.81065	23652.74614	4881.35227

a. Limited to first 100 cases.

ANOVA

TBC

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	116011896000.000	4	29002974000.000	177.509	.000
Within Groups	816945000.000	5	163389000.000		
Total	116828841000.000	9			

Post Hoc tests

Multiple Comparisons

Dependent Variable: TBC

	(I) Sources of sample	(J) Sources of sample	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	Mohakhali	Karwan bazar	-150300.00000*	12782.37067	.000	-201576.5408	-99023.4592
		Farmgate	-7600.00000	12782.37067	.970	-58876.5408	43676.5408
		Basabo	-28600.00000	12782.37067	.299	-79876.5408	22676.5408
		Agora Super shop	185850.00000*	12782.37067	.000	134573.4592	237126.5408
	Karwan bazar	Mohakhali	150300.00000*	12782.37067	.000	99023.4592	201576.5408
		Farmgate	142700.00000*	12782.37067	.001	91423.4592	193976.5408
		Basabo	121700.00000*	12782.37067	.001	70423.4592	172976.5408
		Agora Super shop	336150.00000*	12782.37067	.000	284873.4592	387426.5408
	Farmgate	Mohakhali	7600.00000	12782.37067	.970	-43676.5408	58876.5408
		Karwan bazar	-142700.00000*	12782.37067	.001	-193976.5408	-91423.4592
		Basabo	-21000.00000	12782.37067	.533	-72276.5408	30276.5408
		Agora Super shop	193450.00000*	12782.37067	.000	142173.4592	244726.5408
	Basabo	Mohakhali	28600.00000	12782.37067	.299	-22676.5408	79876.5408
		Karwan bazar	-121700.00000*	12782.37067	.001	-172976.5408	-70423.4592
		Farmgate	21000.00000	12782.37067	.533	-30276.5408	72276.5408
		Agora Super shop	214450.00000*	12782.37067	.000	163173.4592	265726.5408
	Agora Super shop	Mohakhali	-185850.00000*	12782.37067	.000	-237126.5408	-134573.4592

LSD	Mohakhali	Karwan bazar	-336150.00000*	12782.37067	.000	-387426.5408	-284873.4592
		Farmgate	-193450.00000*	12782.37067	.000	-244726.5408	-142173.4592
		Basabo	-214450.00000*	12782.37067	.000	-265726.5408	-163173.4592
		Agora Super shop	-150300.00000*	12782.37067	.000	-183158.1299	-117441.8701
	Karwan bazar	Mohakhali	150300.00000*	12782.37067	.000	117441.8701	183158.1299
		Farmgate	142700.00000*	12782.37067	.000	109841.8701	175558.1299
		Basabo	121700.00000*	12782.37067	.000	88841.8701	154558.1299
		Agora Super shop	336150.00000*	12782.37067	.000	303291.8701	369008.1299
	Farmgate	Mohakhali	-7600.00000	12782.37067	.578	-40458.1299	25258.1299
		Karwan bazar	-142700.00000*	12782.37067	.000	-175558.1299	-109841.8701
		Basabo	-21000.00000	12782.37067	.161	-53858.1299	11858.1299
		Agora Super shop	193450.00000*	12782.37067	.000	160591.8701	226308.1299
	Basabo	Mohakhali	28600.00000	12782.37067	.075	-4258.1299	61458.1299
		Karwan bazar	-121700.00000*	12782.37067	.000	-154558.1299	-88841.8701
		Farmgate	21000.00000	12782.37067	.161	-11858.1299	53858.1299
		Agora Super shop	214450.00000*	12782.37067	.000	181591.8701	247308.1299
	Agora Super shop	Mohakhali	-185850.00000*	12782.37067	.000	-218708.1299	-152991.8701
		Karwan bazar	-336150.00000*	12782.37067	.000	-369008.1299	-303291.8701
		Farmgate	-193450.00000*	12782.37067	.000	-226308.1299	-160591.8701
		Basabo	-214450.00000*	12782.37067	.000	-247308.1299	-181591.8701

*. The mean difference is significant at the 0.05 level.

Homogeneous Subsets

TBC

	Sources of sample	N	Subset for alpha = 0.05		
			1	2	3
Tukey HSD[a]	Agora Super shop	2	44850.0000		
	Mohakhali	2		230700.0000	
	Farmgate	2		238300.0000	
	Basabo	2		259300.0000	
	Karwan bazar	2			381000.0000
	Sig.		1.000	.299	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

ANOVA

TVC

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	32918206000.000	4	8229551500.000	564.519	.000
Within Groups	72890000.000	5	14578000.000		
Total	32991096000.000	9			

Post Hoc tests

Multiple Comparisons

Dependent Variable: TVC

	(I) Sources of sample	(J) Sources of sample	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	Mohakhali	Karwan bazar	.00000	3818.11472	1.000	-15316.3854	15316.3854
		Farmgate	-125450.00000*	3818.11472	.000	-140766.3854	-110133.6146
		Basabo	-107650.00000*	3818.11472	.000	-122966.3854	-92333.6146
		Agora Super shop	.00000	3818.11472	1.000	-15316.3854	15316.3854
	Karwan bazar	Mohakhali	.00000	3818.11472	1.000	-15316.3854	15316.3854
		Farmgate	-125450.00000*	3818.11472	.000	-140766.3854	-110133.6146
		Basabo	-107650.00000*	3818.11472	.000	-122966.3854	-92333.6146

LSD	Farmgate	Agora Super shop	.00000	3818.11472	1.000	-15316.3854	15316.3854
		Mohakhali	125450.00000*	3818.11472	.000	110133.6146	140766.3854
		Karwan bazar	125450.00000*	3818.11472	.000	110133.6146	140766.3854
		Basabo	17800.00000*	3818.11472	.028	2483.6146	33116.3854
		Agora Super shop	125450.00000*	3818.11472	.000	110133.6146	140766.3854
		Mohakhali	107650.00000*	3818.11472	.000	92333.6146	122966.3854
		Karwan bazar	107650.00000*	3818.11472	.000	92333.6146	122966.3854
	Basabo	Farmgate	-17800.00000*	3818.11472	.028	-33116.3854	-2483.6146
		Agora Super shop	107650.00000*	3818.11472	.000	92333.6146	122966.3854
		Mohakhali	.00000	3818.11472	1.000	-15316.3854	15316.3854
		Karwan bazar	.00000	3818.11472	1.000	-15316.3854	15316.3854
	Agora Super shop	Farmgate	-125450.00000*	3818.11472	.000	-140766.3854	-110133.6146
		Basabo	-107650.00000*	3818.11472	.000	-122966.3854	-92333.6146
		Karwan bazar	.00000	3818.11472	1.000	-9814.7763	9814.7763
	Mohakhali	Farmgate	-125450.00000*	3818.11472	.000	-135264.7763	-115635.2237
		Basabo	-107650.00000*	3818.11472	.000	-117464.7763	-97835.2237
		Agora Super shop	.00000	3818.11472	1.000	-9814.7763	9814.7763
		Mohakhali	.00000	3818.11472	1.000	-9814.7763	9814.7763
	Karwan bazar	Farmgate	-125450.00000*	3818.11472	.000	-135264.7763	-115635.2237
		Basabo	-107650.00000*	3818.11472	.000	-117464.7763	-97835.2237
		Agora Super shop	.00000	3818.11472	1.000	-9814.7763	9814.7763
		Mohakhali	125450.00000*	3818.11472	.000	115635.2237	135264.7763
		Karwan bazar	125450.00000*	3818.11472	.000	115635.2237	135264.7763
	Farmgate	Basabo	17800.00000*	3818.11472	.006	7985.2237	27614.7763
		Agora Super shop	125450.00000*	3818.11472	.000	115635.2237	135264.7763
		Mohakhali	107650.00000*	3818.11472	.000	97835.2237	117464.7763
	Basabo	Karwan bazar	107650.00000*	3818.11472	.000	97835.2237	117464.7763
		Farmgate	-17800.00000*	3818.11472	.006	-27614.7763	-7985.2237

	Agora Super shop	107650.00000*	3818.11472	.000	97835.2237	117464.7763
	Mohakhali	.00000	3818.11472	1.000	-9814.7763	9814.7763
	Karwan bazar	.00000	3818.11472	1.000	-9814.7763	9814.7763
Agora Super shop	Farmgate	-125450.00000*	3818.11472	.000	-135264.7763	-115635.2237
	Basabo	-107650.00000*	3818.11472	.000	-117464.7763	-97835.2237

*. The mean difference is significant at the 0.05 level.

Homogeneous subsets

TVC

	Sources of sample	N	Subset for alpha = 0.05		
			1	2	3
Tukey HSD[a]	Mohakhali	2	.0000		
	Karwan bazar	2	.0000		
	Agora Super shop	2	.0000		
	Basabo	2		107650.0000	
	Farmgate	2			125450.0000
	Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

ANOVA

TEC

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2257866000.000	4	564466500.000	30.343	.001
Within Groups	93015000.000	5	18603000.000		
Total	2350881000.000	9			

Post Hoc tests

Multiple Comparisons

Dependent Variable: TEC

	(I) Sources of sample	(J) Sources of sample	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	Mohakhali	Karwan bazar	-11500.00000	4313.11952	.191	-28802.0995	5802.0995
		Farmgate	19350.00000*	4313.11952	.033	2047.9005	36652.0995
		Basabo	8400.00000	4313.11952	.400	-8902.0995	25702.0995
		Agora Super shop	31850.00000*	4313.11952	.004	14547.9005	49152.0995
	Karwan bazar	Mohakhali	11500.00000	4313.11952	.191	-5802.0995	28802.0995
		Farmgate	30850.00000*	4313.11952	.004	13547.9005	48152.0995
		Basabo	19900.00000*	4313.11952	.029	2597.9005	37202.0995
		Agora Super shop	43350.00000*	4313.11952	.001	26047.9005	60652.0995
	Farmgate	Mohakhali	-19350.00000*	4313.11952	.033	-36652.0995	-2047.9005
		Karwan bazar	-30850.00000*	4313.11952	.004	-48152.0995	-13547.9005
		Basabo	-10950.00000	4313.11952	.219	-28252.0995	6352.0995
		Agora Super shop	12500.00000	4313.11952	.150	-4802.0995	29802.0995
	Basabo	Mohakhali	-8400.00000	4313.11952	.400	-25702.0995	8902.0995
		Karwan bazar	-19900.00000*	4313.11952	.029	-37202.0995	-2597.9005
		Farmgate	10950.00000	4313.11952	.219	-6352.0995	28252.0995
		Agora Super shop	23450.00000*	4313.11952	.015	6147.9005	40752.0995
	Agora Super shop	Mohakhali	-31850.00000*	4313.11952	.004	-49152.0995	-14547.9005
		Karwan bazar	-43350.00000*	4313.11952	.001	-60652.0995	-26047.9005
		Farmgate	-12500.00000	4313.11952	.150	-29802.0995	4802.0995
		Basabo	-23450.00000*	4313.11952	.015	-40752.0995	-6147.9005

LSD	Mohakhali	Karwan bazar	-11500.00000*	4313.11952	.045	-22587.2267	-412.7733
		Farmgate	19350.00000*	4313.11952	.006	8262.7733	30437.2267
		Basabo	8400.00000	4313.11952	.109	-2687.2267	19487.2267
		Agora Super shop	31850.00000*	4313.11952	.001	20762.7733	42937.2267
	Karwan bazar	Mohakhali	11500.00000*	4313.11952	.045	412.7733	22587.2267
		Farmgate	30850.00000*	4313.11952	.001	19762.7733	41937.2267
		Basabo	19900.00000*	4313.11952	.006	8812.7733	30987.2267
		Agora Super shop	43350.00000*	4313.11952	.000	32262.7733	54437.2267
	Farmgate	Mohakhali	-19350.00000*	4313.11952	.006	-30437.2267	-8262.7733
		Karwan bazar	-30850.00000*	4313.11952	.001	-41937.2267	-19762.7733
		Basabo	-10950.00000	4313.11952	.052	-22037.2267	137.2267
		Agora Super shop	12500.00000*	4313.11952	.034	1412.7733	23587.2267
	Basabo	Mohakhali	-8400.00000	4313.11952	.109	-19487.2267	2687.2267
		Karwan bazar	-19900.00000*	4313.11952	.006	-30987.2267	-8812.7733
		Farmgate	10950.00000	4313.11952	.052	-137.2267	22037.2267
		Agora Super shop	23450.00000*	4313.11952	.003	12362.7733	34537.2267
	Agora Super shop	Mohakhali	-31850.00000*	4313.11952	.001	-42937.2267	-20762.7733
		Karwan bazar	-43350.00000*	4313.11952	.000	-54437.2267	-32262.7733
		Farmgate	-12500.00000*	4313.11952	.034	-23587.2267	-1412.7733
		Basabo	-23450.00000*	4313.11952	.003	-34537.2267	-12362.7733

*. The mean difference is significant at the 0.05 level.

Homogeneous subsets

TEC

	Sources of sample	N	Subset for alpha = 0.05			
			1	2	3	4
Tukey HSD[a]	Agora Super shop	2	.0000			
	Farmgate	2	12500.0000	12500.0000		
	Basabo	2		23450.0000	23450.0000	
	Mohakhali	2			31850.0000	31850.0000
	Karwan bazar	2				43350.0000
	Sig.		.150	.219	.400	.191

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

ANOVA

TC

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	15228314000.000	4	3807078500.000	74.975	.000
Within Groups	253890000.000	5	50778000.000		
Total	15482204000.000	9			

Post Hoc tests

Multiple Comparisons

Dependent Variable: TC

	(I) Sources of sample	(J) Sources of sample	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	Mohakhali	Karwan bazar	2350.00000	7125.86837	.997	-26235.4549	30935.4549
		Farmgate	14850.00000	7125.86837	.349	-13735.4549	43435.4549
		Basabo	-7000.00000	7125.86837	.853	-35585.4549	21585.4549
		Agora Super shop	98500.00000*	7125.86837	.000	69914.5451	127085.4549
	Karwan bazar	Mohakhali	-2350.00000	7125.86837	.997	-30935.4549	26235.4549
		Farmgate	12500.00000	7125.86837	.482	-16085.4549	41085.4549
		Basabo	-9350.00000	7125.86837	.697	-37935.4549	19235.4549

LSD	Farmgate	Agora Super shop	96150.00000*	7125.86837	.000	67564.5451	124735.4549
		Mohakhali	-14850.00000	7125.86837	.349	-43435.4549	13735.4549
		Karwan bazar	-12500.00000	7125.86837	.482	-41085.4549	16085.4549
		Basabo	-21850.00000	7125.86837	.127	-50435.4549	6735.4549
		Agora Super shop	83650.00000*	7125.86837	.000	55064.5451	112235.4549
		Mohakhali	7000.00000	7125.86837	.853	-21585.4549	35585.4549
		Karwan bazar	9350.00000	7125.86837	.697	-19235.4549	37935.4549
	Basabo	Farmgate	21850.00000	7125.86837	.127	-6735.4549	50435.4549
		Agora Super shop	105500.00000*	7125.86837	.000	76914.5451	134085.4549
		Mohakhali	-98500.00000*	7125.86837	.000	-127085.4549	-69914.5451
		Karwan bazar	-96150.00000*	7125.86837	.000	-124735.4549	-67564.5451
	Agora Super shop	Farmgate	-83650.00000*	7125.86837	.000	-112235.4549	-55064.5451
		Basabo	-105500.00000*	7125.86837	.000	-134085.4549	-76914.5451
		Karwan bazar	2350.00000	7125.86837	.755	-15967.6278	20667.6278
		Farmgate	14850.00000	7125.86837	.092	-3467.6278	33167.6278
	Mohakhali	Basabo	-7000.00000	7125.86837	.371	-25317.6278	11317.6278
		Agora Super shop	98500.00000*	7125.86837	.000	80182.3722	116817.6278
		Mohakhali	-2350.00000	7125.86837	.755	-20667.6278	15967.6278
		Farmgate	12500.00000	7125.86837	.140	-5817.6278	30817.6278
	Karwan bazar	Basabo	-9350.00000	7125.86837	.246	-27667.6278	8967.6278
		Agora Super shop	96150.00000*	7125.86837	.000	77832.3722	114467.6278
		Mohakhali	-14850.00000	7125.86837	.092	-33167.6278	3467.6278
		Karwan bazar	-12500.00000	7125.86837	.140	-30817.6278	5817.6278
	Farmgate	Basabo	-21850.00000*	7125.86837	.028	-40167.6278	-3532.3722
		Agora Super shop	83650.00000*	7125.86837	.000	65332.3722	101967.6278
		Mohakhali	7000.00000	7125.86837	.371	-11317.6278	25317.6278
	Basabo	Karwan bazar	9350.00000	7125.86837	.246	-8967.6278	27667.6278
		Farmgate	21850.00000*	7125.86837	.028	3532.3722	40167.6278

Agora Super shop	Agora Super shop	105500.00000*	7125.86837	.000	87182.3722	123817.6278
	Mohakhali	-98500.00000*	7125.86837	.000	-116817.6278	-80182.3722
	Karwan bazar	-96150.00000*	7125.86837	.000	-114467.6278	-77832.3722
	Farmgate	-83650.00000*	7125.86837	.000	-101967.6278	-65332.3722
	Basabo	-105500.00000*	7125.86837	.000	-123817.6278	-87182.3722

*. The mean difference is significant at the 0.05 level.

Homogeneous subsets

TC

	Sources of sample	N	Subset for alpha = 0.05	
			1	2
Tukey HSD[a]	Agora Super shop	2	.0000	
	Farmgate	2		83650.0000
	Karwan bazar	2		96150.0000
	Mohakhali	2		98500.0000
	Basabo	2		105500.0000
	Sig.		1.000	.127

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

ANOVA

FC

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2117634000.000	4	529408500.000	98.586	.000
Within Groups	26850000.000	5	5370000.000		
Total	2144484000.000	9			

Post Hoc tests

Multiple Comparisons

Dependent Variable: FC

	(I) Sources of sample	(J) Sources of sample	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	Mohakhali	Karwan bazar	-14550.00000*	2317.32605	.008	-23845.9645	-5254.0355
		Farmgate	7700.00000	2317.32605	.098	-1595.9645	16995.9645
		Basabo	1950.00000	2317.32605	.907	-7345.9645	11245.9645
		Agora Super shop	30200.00000*	2317.32605	.000	20904.0355	39495.9645
	Karwan bazar	Mohakhali	14550.00000*	2317.32605	.008	5254.0355	23845.9645
		Farmgate	22250.00000*	2317.32605	.001	12954.0355	31545.9645
		Basabo	16500.00000*	2317.32605	.005	7204.0355	25795.9645
		Agora Super shop	44750.00000*	2317.32605	.000	35454.0355	54045.9645
	Farmgate	Mohakhali	-7700.00000	2317.32605	.098	-16995.9645	1595.9645
		Karwan bazar	-22250.00000*	2317.32605	.001	-31545.9645	-12954.0355
		Basabo	-5750.00000	2317.32605	.232	-15045.9645	3545.9645
		Agora Super shop	22500.00000*	2317.32605	.001	13204.0355	31795.9645
	Basabo	Mohakhali	-1950.00000	2317.32605	.907	-11245.9645	7345.9645
		Karwan bazar	-16500.00000*	2317.32605	.005	-25795.9645	-7204.0355
		Farmgate	5750.00000	2317.32605	.232	-3545.9645	15045.9645
		Agora Super shop	28250.00000*	2317.32605	.000	18954.0355	37545.9645
	Agora Super shop	Mohakhali	-30200.00000*	2317.32605	.000	-39495.9645	-20904.0355
		Karwan bazar	-44750.00000*	2317.32605	.000	-54045.9645	-35454.0355
		Farmgate	-22500.00000*	2317.32605	.001	-31795.9645	-13204.0355
		Basabo	-28250.00000*	2317.32605	.000	-37545.9645	-18954.0355

LSD	Mohakhali	Karwan bazar	-14550.00000*	2317.32605	.002	-20506.8762	-8593.1238
		Farmgate	7700.00000*	2317.32605	.021	1743.1238	13656.8762
		Basabo	1950.00000	2317.32605	.438	-4006.8762	7906.8762
		Agora Super shop	30200.00000*	2317.32605	.000	24243.1238	36156.8762
	Karwan bazar	Mohakhali	14550.00000*	2317.32605	.002	8593.1238	20506.8762
		Farmgate	22250.00000*	2317.32605	.000	16293.1238	28206.8762
		Basabo	16500.00000*	2317.32605	.001	10543.1238	22456.8762
		Agora Super shop	44750.00000*	2317.32605	.000	38793.1238	50706.8762
	Farmgate	Mohakhali	-7700.00000*	2317.32605	.021	-13656.8762	-1743.1238
		Karwan bazar	-22250.00000*	2317.32605	.000	-28206.8762	-16293.1238
		Basabo	-5750.00000	2317.32605	.056	-11706.8762	206.8762
		Agora Super shop	22500.00000*	2317.32605	.000	16543.1238	28456.8762
	Basabo	Mohakhali	-1950.00000	2317.32605	.438	-7906.8762	4006.8762
		Karwan bazar	-16500.00000*	2317.32605	.001	-22456.8762	-10543.1238
		Farmgate	5750.00000	2317.32605	.056	-206.8762	11706.8762
		Agora Super shop	28250.00000*	2317.32605	.000	22293.1238	34206.8762
	Agora Super shop	Mohakhali	-30200.00000*	2317.32605	.000	-36156.8762	-24243.1238
		Karwan bazar	-44750.00000*	2317.32605	.000	-50706.8762	-38793.1238
		Farmgate	-22500.00000*	2317.32605	.000	-28456.8762	-16543.1238
		Basabo	-28250.00000*	2317.32605	.000	-34206.8762	-22293.1238

*. The mean difference is significant at the 0.05 level.

Homogeneous subsets

FC

	Sources of sample	N	Subset for alpha = 0.05		
			1	2	3
Tukey HSD[a]	Agora Super shop	2	.0000		
	Farmgate	2		22500.0000	
	Basabo	2		28250.0000	
	Mohakhali	2		30200.0000	
	Karwan bazar	2			44750.0000
	Sig.		1.000	.098	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

ANOVA

SSC

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	37287980000.000	4	9321995000.000	108.850	.000
Within Groups	428205000.000	5	85641000.000		
Total	37716185000.000	9			

Post Hoc tests

Multiple Comparisons

Dependent Variable: SSC

	(I) Sources of sample	(J) Sources of sample	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	Mohakhali	Karwan bazar	85800.00000*	9254.24227	.001	48676.5631	122923.4369
		Farmgate	8500.00000	9254.24227	.879	-28623.4369	45623.4369
		Basabo	-42350.00000*	9254.24227	.030	-79473.4369	-5226.5631
		Agora Super shop	125300.00000*	9254.24227	.000	88176.5631	162423.4369
	Karwan bazar	Mohakhali	-85800.00000*	9254.24227	.001	-122923.4369	-48676.5631
		Farmgate	-77300.00000*	9254.24227	.002	-114423.4369	-40176.5631

LSD	Farmgate	Basabo	-128150.00000*	9254.24227	.000	-165273.4369	-91026.5631
		Agora Super shop	39500.00000*	9254.24227	.039	2376.5631	76623.4369
		Mohakhali	-8500.00000	9254.24227	.879	-45623.4369	28623.4369
		Karwan bazar	77300.00000*	9254.24227	.002	40176.5631	114423.4369
	Basabo	Farmgate	-50850.00000*	9254.24227	.014	-87973.4369	-13726.5631
		Agora Super shop	116800.00000*	9254.24227	.000	79676.5631	153923.4369
		Mohakhali	42350.00000*	9254.24227	.030	5226.5631	79473.4369
		Karwan bazar	128150.00000*	9254.24227	.000	91026.5631	165273.4369
	Agora Super shop	Farmgate	50850.00000*	9254.24227	.014	13726.5631	87973.4369
		Basabo	167650.00000*	9254.24227	.000	130526.5631	204773.4369
		Mohakhali	-125300.00000*	9254.24227	.000	-162423.4369	-88176.5631
		Karwan bazar	-39500.00000*	9254.24227	.039	-76623.4369	-2376.5631
	Mohakhali	Farmgate	-116800.00000*	9254.24227	.000	-153923.4369	-79676.5631
		Basabo	-167650.00000*	9254.24227	.000	-204773.4369	-130526.5631
		Karwan bazar	85800.00000*	9254.24227	.000	62011.2129	109588.7871
	Karwan bazar	Farmgate	8500.00000	9254.24227	.400	-15288.7871	32288.7871
		Basabo	-42350.00000*	9254.24227	.006	-66138.7871	-18561.2129
		Agora Super shop	125300.00000*	9254.24227	.000	101511.2129	149088.7871
		Mohakhali	-85800.00000*	9254.24227	.000	-109588.7871	-62011.2129
	Farmgate	Farmgate	-77300.00000*	9254.24227	.000	-101088.7871	-53511.2129
		Basabo	-128150.00000*	9254.24227	.000	-151938.7871	-104361.2129
		Agora Super shop	39500.00000*	9254.24227	.008	15711.2129	63288.7871
		Mohakhali	-8500.00000	9254.24227	.400	-32288.7871	15288.7871
		Karwan bazar	77300.00000*	9254.24227	.000	53511.2129	101088.7871
	Basabo	Basabo	-50850.00000*	9254.24227	.003	-74638.7871	-27061.2129
		Agora Super shop	116800.00000*	9254.24227	.000	93011.2129	140588.7871
		Mohakhali	42350.00000*	9254.24227	.006	18561.2129	66138.7871

	Karwan bazar	128150.00000*	9254.24227	.000	104361.2129	151938.7871
	Farmgate	50850.00000*	9254.24227	.003	27061.2129	74638.7871
	Agora Super shop	167650.00000*	9254.24227	.000	143861.2129	191438.7871
	Mohakhali	-125300.00000*	9254.24227	.000	-149088.7871	-101511.2129
Agora Super shop	Karwan bazar	-39500.00000*	9254.24227	.008	-63288.7871	-15711.2129
	Farmgate	-116800.00000*	9254.24227	.000	-140588.7871	-93011.2129
	Basabo	-167650.00000*	9254.24227	.000	-191438.7871	-143861.2129

*. The mean difference is significant at the 0.05 level.

Homogeneous subsets

SSC

	Sources of sample	N	Subset for alpha = 0.05			
			1	2	3	4
Tukey HSD[a]	Agora Super shop	2	.0000			
	Karwan bazar	2		39500.0000		
	Farmgate	2			116800.0000	
	Mohakhali	2			125300.0000	
	Basabo	2				167650.0000
	Sig.		1.000	1.000	.879	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

ANOVA

TStaphylococci

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	48762296000.000	4	12190574000.000	38.373	.001
Within Groups	1588420000.000	5	317684000.000		
Total	50350716000.000	9			

Post Hoc tests

Multiple Comparisons

Dependent Variable: TStaphylococci

	(I) Sources of sample	(J) Sources of sample	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	Mohakhali	Karwan bazar	-22150.00000	17823.69210	.732	-93649.8257	49349.8257
		Farmgate	-34850.00000	17823.69210	.397	-106349.8257	36649.8257
		Basabo	-56150.00000	17823.69210	.116	-127649.8257	15349.8257
		Agora Super shop	140250.00000*	17823.69210	.003	68750.1743	211749.8257
	Karwan bazar	Mohakhali	22150.00000	17823.69210	.732	-49349.8257	93649.8257
		Farmgate	-12700.00000	17823.69210	.945	-84199.8257	58799.8257
		Basabo	-34000.00000	17823.69210	.416	-105499.8257	37499.8257
		Agora Super shop	162400.00000*	17823.69210	.001	90900.1743	233899.8257
	Farmgate	Mohakhali	34850.00000	17823.69210	.397	-36649.8257	106349.8257
		Karwan bazar	12700.00000	17823.69210	.945	-58799.8257	84199.8257
		Basabo	-21300.00000	17823.69210	.756	-92799.8257	50199.8257
		Agora Super shop	175100.00000*	17823.69210	.001	103600.1743	246599.8257
	Basabo	Mohakhali	56150.00000	17823.69210	.116	-15349.8257	127649.8257
		Karwan bazar	34000.00000	17823.69210	.416	-37499.8257	105499.8257
		Farmgate	21300.00000	17823.69210	.756	-50199.8257	92799.8257
		Agora Super shop	196400.00000*	17823.69210	.001	124900.1743	267899.8257
	Agora Super shop	Mohakhali	-140250.00000*	17823.69210	.003	-211749.8257	-68750.1743
		Karwan bazar	-162400.00000*	17823.69210	.001	-233899.8257	-90900.1743
		Farmgate	-175100.00000*	17823.69210	.001	-246599.8257	-103600.1743
		Basabo	-196400.00000*	17823.69210	.001	-267899.8257	-124900.1743
LSD	Mohakhali	Karwan bazar	-22150.00000	17823.69210	.269	-67967.2592	23667.2592
		Farmgate	-34850.00000	17823.69210	.108	-80667.2592	10967.2592

	Basabo	-56150.00000*	17823.69210	.025	-101967.2592	-10332.7408
	Agora Super shop	140250.00000*	17823.69210	.001	94432.7408	186067.2592
	Mohakhali	22150.00000	17823.69210	.269	-23667.2592	67967.2592
	Farmgate	-12700.00000	17823.69210	.508	-58517.2592	33117.2592
Karwan bazar	Basabo	-34000.00000	17823.69210	.115	-79817.2592	11817.2592
	Agora Super shop	162400.00000*	17823.69210	.000	116582.7408	208217.2592
	Mohakhali	34850.00000	17823.69210	.108	-10967.2592	80667.2592
	Karwan bazar	12700.00000	17823.69210	.508	-33117.2592	58517.2592
Farmgate	Basabo	-21300.00000	17823.69210	.286	-67117.2592	24517.2592
	Agora Super shop	175100.00000*	17823.69210	.000	129282.7408	220917.2592
	Mohakhali	56150.00000*	17823.69210	.025	10332.7408	101967.2592
	Karwan bazar	34000.00000	17823.69210	.115	-11817.2592	79817.2592
Basabo	Farmgate	21300.00000	17823.69210	.286	-24517.2592	67117.2592
	Agora Super shop	196400.00000*	17823.69210	.000	150582.7408	242217.2592
	Mohakhali	-140250.00000*	17823.69210	.001	-186067.2592	-94432.7408
	Karwan bazar	-162400.00000*	17823.69210	.000	-208217.2592	-116582.7408
Agora Super shop	Farmgate	-175100.00000*	17823.69210	.000	-220917.2592	-129282.7408
	Basabo	-196400.00000*	17823.69210	.000	-242217.2592	-150582.7408

*. The mean difference is significant at the 0.05 level.

Homogeneous subsets

TStaphylococci

	Sources of sample	N	Subset for alpha = 0.05	
			1	2
Tukey HSD[a]	Agora Super shop	2	3750.0000	
	Mohakhali	2		144000.0000
	Karwan bazar	2		166150.0000
	Farmgate	2		178850.0000
	Basabo	2		200150.0000
	Sig.		1.000	.116

a. Uses Harmonic Mean Sample Size = 2.000.

Case summaries of proximate composition of mud crab

				Moisture	Ash	Protein	Fat
Sources of Samples	Mohakhali	1		78.02	1.35	15.50	3.78
		2		78.09	1.40	14.98	3.75
		Total	N	2	2	2	2
			Mean	78.0550	1.3750	15.2400	3.7650
			Std. Error of Mean	.03500	.02500	.26000	.01500
	Karwanbazar	1		83.02	1.36	13.02	1.32
		2		82.60	1.43	13.90	1.40
		Total	N	2	2	2	2
			Mean	82.8100	1.3950	13.4600	1.3600
			Std. Error of Mean	.21000	.03500	.44000	.04000
	Farmgate	1		79.06	1.97	15.60	1.45
		2		79.00	1.85	15.72	1.57
		Total	N	2	2	2	2
			Mean	79.0300	1.9100	15.6600	1.5100
			Std. Error of Mean	.03000	.06000	.06000	.06000
	Basabo	1		81.84	1.27	14.41	2.00
		2		80.30	1.35	15.05	2.31
		Total	N	2	2	2	2
			Mean	81.0700	1.3100	14.7300	2.1550
			Std. Error of Mean	.77000	.04000	.32000	.15500
	Agora	1		72.68	2.05	20.13	3.47
		2		73.01	1.98	19.75	3.39
		Total	N	2	2	2	2
			Mean	72.8450	2.0150	19.9400	3.4300
			Std. Error of Mean	.16500	.03500	.19000	.04000
	Total	N		10	10	10	10
		Mean		78.7620	1.6010	15.8060	2.4440
		Std. Error of Mean		1.13492	.10037	.73781	.32928

a. Limited to first 100 cases.

Table: Proximate composition of mud crab (*Scylla serrata*) meat; sampled from different markets in Dhaka city. Means (± SEM) within row and column for total and column with different letters are significantly different (ANOVA, HSD, $p<0.05$).

SL no.	Sources	Moisture (%)	Ash (%)	Protein (%)	Lipid (%)
1	Mohakhali	78.05 ± 0.03^{c}	1.37 ± 0.02^{b}	15.24 ± 0.26^{b}	3.76 ± 0.01^{a}
2	Karwan Bazar	82.81 ± 0.21^{a}	1.39 ± 0.03^{b}	13.46 ± 0.44^{c}	1.36 ± 0.04^{c}
3	Farmgate	79.03 ± 0.03^{bc}	1.91 ± 0.06^{a}	15.66 ± 0.06^{b}	1.51 ± 0.06^{c}
4	Basabo	81.07 ± 0.77^{ab}	1.31 ± 0.04^{b}	14.73 ± 0.32^{bc}	2.15 ± 0.15^{b}
5	Agora	72.84 ± 0.16^{d}	2.01 ± 0.03^{a}	19.94 ± 0.19^{a}	3.43 ± 0.04^{a}

Moisture **ANOVA**

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	114.591	4	28.648	107.481	.000
Within Groups	1.333	5	.267		
Total	115.924	9			

POST HOC

	(I) Sources of Samples	(J) Sources of Samples	Mean Difference (I-J)	Std. Error	Sig.
Tukey HSD	Mohakhali	Karwanbazar	-4.75500*	.51628	.001
		Farmgate	-.97500	.51628	.424
		Basabo	-3.01500*	.51628	.011
		Agora	5.21000*	.51628	.001
	Karwanbazar	Mohakhali	4.75500*	.51628	.001
		Farmgate	3.78000*	.51628	.004
		Basabo	1.74000	.51628	.093
		Agora	9.96500*	.51628	.000
	Farmgate	Mohakhali	.97500	.51628	.424

		Karwanbazar	-3.78000*	.51628	.004
		Basabo	-2.04000	.51628	.053
		Agora	6.18500*	.51628	.000
		Mohakhali	3.01500*	.51628	.011
	Basabo	Karwanbazar	-1.74000	.51628	.093
		Farmgate	2.04000	.51628	.053
		Agora	8.22500*	.51628	.000
		Mohakhali	-5.21000*	.51628	.001
	Agora	Karwanbazar	-9.96500*	.51628	.000
		Farmgate	-6.18500*	.51628	.000
		Basabo	-8.22500*	.51628	.000
		Karwanbazar	-4.75500*	.51628	.000
	Mohakhali	Farmgate	-.97500	.51628	.118
		Basabo	-3.01500*	.51628	.002
		Agora	5.21000*	.51628	.000
		Mohakhali	4.75500*	.51628	.000
	Karwanbazar	Farmgate	3.78000*	.51628	.001
		Basabo	1.74000*	.51628	.020
		Agora	9.96500*	.51628	.000
		Mohakhali	.97500	.51628	.118
LSD	Farmgate	Karwanbazar	-3.78000*	.51628	.001
		Basabo	-2.04000*	.51628	.011
		Agora	6.18500*	.51628	.000
		Mohakhali	3.01500*	.51628	.002
	Basabo	Karwanbazar	-1.74000*	.51628	.020
		Farmgate	2.04000*	.51628	.011
		Agora	8.22500*	.51628	.000
		Mohakhali	-5.21000*	.51628	.000
	Agora	Karwanbazar	-9.96500*	.51628	.000
		Farmgate	-6.18500*	.51628	.000
		Basabo	-8.22500*	.51628	.000

Homogeneous subsets

Moisture

	Sources of Samples	N	Subset for alpha = 0.05			
			1	2	3	4
Tukey HSD[a]	Agora	2	72.8450			
	Mohakhali	2		78.0550		
	Farmgate	2		79.0300	79.0300	
	Basabo	2			81.0700	81.0700
	Karwanbazar	2				82.8100
	Sig.		1.000	.424	.053	.093

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

ANOVA

Ash

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.890	4	.223	67.231	.000
Within Groups	.017	5	.003		
Total	.907	9			

POST HOC

	(I) Sources of Samples	(J) Sources of Samples	Mean Difference (I-J)	Std. Error	Sig.
Tukey HSD	Mohakhali	Karwanbazar	-.02000	.05753	.996
		Farmgate	-.53500*	.05753	.001
		Basabo	.06500	.05753	.787
		Agora	-.64000*	.05753	.001
	Karwanbazar	Mohakhali	.02000	.05753	.996
		Farmgate	-.51500*	.05753	.002
		Basabo	.08500	.05753	.614
		Agora	-.62000*	.05753	.001
	Farmgate	Mohakhali	.53500*	.05753	.001
		Karwanbazar	.51500*	.05753	.002

LSD	Basabo	Basabo	.60000*	.05753	.001
		Agora	-.10500	.05753	.451
		Mohakhali	-.06500	.05753	.787
		Karwanbazar	-.08500	.05753	.614
		Farmgate	-.60000*	.05753	.001
	Agora	Agora	-.70500*	.05753	.000
		Mohakhali	.64000*	.05753	.001
		Karwanbazar	.62000*	.05753	.001
		Farmgate	.10500	.05753	.451
	Mohakhali	Basabo	.70500*	.05753	.000
		Karwanbazar	-.02000	.05753	.742
		Farmgate	-.53500*	.05753	.000
	Karwanbazar	Basabo	.06500	.05753	.310
		Agora	-.64000*	.05753	.000
		Mohakhali	.02000	.05753	.742
		Farmgate	-.51500*	.05753	.000
	Farmgate	Basabo	.08500	.05753	.200
		Agora	-.62000*	.05753	.000
		Mohakhali	.53500*	.05753	.000
		Karwanbazar	.51500*	.05753	.000
		Basabo	.60000*	.05753	.000
	Basabo	Agora	-.10500	.05753	.128
		Mohakhali	-.06500	.05753	.310
		Karwanbazar	-.08500	.05753	.200
		Farmgate	-.60000*	.05753	.000
	Agora	Agora	-.70500*	.05753	.000
		Mohakhali	.64000*	.05753	.000
		Karwanbazar	.62000*	.05753	.000
		Farmgate	.10500	.05753	.128
		Basabo	.70500*	.05753	.000

Homogeneous subsets

Ash

	Sources of Samples	N	Subset for alpha = 0.05	
			1	2
Tukey HSD[a]	Basabo	2	1.3100	
	Mohakhali	2	1.3750	
	Karwanbazar	2	1.3950	
	Farmgate	2		1.9100
	Agora	2		2.0150
	Sig.		.614	.451

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

ANOVA

Protein

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	48.186	4	12.047	74.675	.000
Within Groups	.807	5	.161		
Total	48.993	9			

POST HOC

	(I) Sources of Samples	(J) Sources of Samples	Mean Difference (I-J)	Std. Error	Sig.
Tukey HSD	Mohakhali	Karwanbazar	1.78000*	.40165	.034
		Farmgate	-.42000	.40165	.826
		Basabo	.51000	.40165	.719
		Agora	-4.70000*	.40165	.000
	Karwanbazar	Mohakhali	-1.78000*	.40165	.034
		Farmgate	-2.20000*	.40165	.014
		Basabo	-1.27000	.40165	.115
		Agora	-6.48000*	.40165	.000
	Farmgate	Mohakhali	.42000	.40165	.826
		Karwanbazar	2.20000*	.40165	.014

LSD	Basabo	Basabo	.93000	.40165	.276
		Agora	-4.28000*	.40165	.001
		Mohakhali	-.51000	.40165	.719
		Karwanbazar	1.27000	.40165	.115
		Farmgate	-.93000	.40165	.276
	Agora	Agora	-5.21000*	.40165	.000
		Mohakhali	4.70000*	.40165	.000
		Karwanbazar	6.48000*	.40165	.000
		Farmgate	4.28000*	.40165	.001
		Basabo	5.21000*	.40165	.000
	Mohakhali	Karwanbazar	1.78000*	.40165	.007
		Farmgate	-.42000	.40165	.344
		Basabo	.51000	.40165	.260
		Agora	-4.70000*	.40165	.000
	Karwanbazar	Mohakhali	-1.78000*	.40165	.007
		Farmgate	-2.20000*	.40165	.003
		Basabo	-1.27000*	.40165	.025
		Agora	-6.48000*	.40165	.000
	Farmgate	Mohakhali	.42000	.40165	.344
		Karwanbazar	2.20000*	.40165	.003
		Basabo	.93000	.40165	.068
		Agora	-4.28000*	.40165	.000
	Basabo	Mohakhali	-.51000	.40165	.260
		Karwanbazar	1.27000*	.40165	.025
		Farmgate	-.93000	.40165	.068
		Agora	-5.21000*	.40165	.000
	Agora	Mohakhali	4.70000*	.40165	.000
		Karwanbazar	6.48000*	.40165	.000
		Farmgate	4.28000*	.40165	.000
		Basabo	5.21000*	.40165	.000

Homogeneous subsets

Protein

	Sources of Samples	N	Subset for alpha = 0.05		
			1	2	3
Tukey HSD[a]	Karwanbazar	2	13.4600		
	Basabo	2	14.7300	14.7300	
	Mohakhali	2		15.2400	
	Farmgate	2		15.6600	
	Agora	2			19.9400
	Sig.		.115	.276	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

ANOVA

Lipid

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	9.696	4	2.424	195.176	.000
Within Groups	.062	5	.012		
Total	9.758	9			

POST HOC

	(I) Sources of Samples	(J) Sources of Samples	Mean Difference (I-J)	Std. Error	Sig.
Tukey HSD	Mohakhali	Karwanbazar	2.40500*	.11145	.000
		Farmgate	2.25500*	.11145	.000
		Basabo	1.61000*	.11145	.000
		Agora	.33500	.11145	.135
	Karwanbazar	Mohakhali	-2.40500*	.11145	.000
		Farmgate	-.15000	.11145	.680
		Basabo	-.79500*	.11145	.004

LSD	Farmgate	Agora	-2.07000*	.11145	.000
		Mohakhali	-2.25500*	.11145	.000
		Karwanbazar	.15000	.11145	.680
		Basabo	-.64500*	.11145	.011
	Basabo	Agora	-1.92000*	.11145	.000
		Mohakhali	-1.61000*	.11145	.000
		Karwanbazar	.79500*	.11145	.004
		Farmgate	.64500*	.11145	.011
	Agora	Agora	-1.27500*	.11145	.000
		Mohakhali	-.33500	.11145	.135
		Karwanbazar	2.07000*	.11145	.000
		Farmgate	1.92000*	.11145	.000
		Basabo	1.27500*	.11145	.000
	Mohakhali	Karwanbazar	2.40500*	.11145	.000
		Farmgate	2.25500*	.11145	.000
		Basabo	1.61000*	.11145	.000
	Karwanbazar	Agora	.33500*	.11145	.030
		Mohakhali	-2.40500*	.11145	.000
		Farmgate	-.15000	.11145	.236
		Basabo	-.79500*	.11145	.001
	Farmgate	Agora	-2.07000*	.11145	.000
		Mohakhali	-2.25500*	.11145	.000
		Karwanbazar	.15000	.11145	.236
		Basabo	-.64500*	.11145	.002
	Basabo	Agora	-1.92000*	.11145	.000
		Mohakhali	-1.61000*	.11145	.000
		Karwanbazar	.79500*	.11145	.001
		Farmgate	.64500*	.11145	.002
	Agora	Agora	-1.27500*	.11145	.000
		Mohakhali	-.33500*	.11145	.030
		Karwanbazar	2.07000*	.11145	.000
		Farmgate	1.92000*	.11145	.000
		Basabo	1.27500*	.11145	.000

Homogeneous subsets

Lipid

	Sources of Samples	N	Subset for alpha = 0.05		
			1	2	3
Tukey HSD[a]	Karwanbazar	2	1.3600		
	Farmgate	2	1.5100		
	Basabo	2		2.1550	
	Agora	2			3.4300
	Mohakhali	2			3.7650
	Sig.		.680	1.000	.135

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Provisional identification of bacterial colonies through different biochemical tests. '+' indicates positive reaction; '-' indicates negative reaction; 'A' for acid production; 'K' for alkali production.

Medium	**Colour**	**Name of the organism**	**KIA**			**MIU**			OXIDASE	CATALASE	MR	VP	CITRATE	NITRATE	CARBOHYDRATE			NaCl (%)		
			SLANT	BUTT	H_2S	MOTILITY	INDOLE	UREA							SUCROSE	LACTOSE	DEXTROSE	0	6.5	8
NA 1	White	*Bacillus* spp.	K	A	-	+	-	-	+	+	-	-	+	+	+	-	+	ND		
NA 2	Yellow	*Citrobacter* spp.			+	+	+	-	-	+	+	-	+	-	+	-	+	ND		
NA 3	Cream	*Alcaligens faecalis*	K	K	-	+	-	-	+	+	-	-	-	+	-	-	-	-	+	-
SS 1	Black centered	*Salmonella typhi*	A	A	+	+	-	+	-	+	+	-	+	+	+	-	-	ND		

SS 2	Clear	*Shigella* spp.	A	A	-	-	-	-	+	+	+	-	-	-	ND					
TCBS 1	Yellow	*Vibrio cholerae*	K	A	-	+	+	-	+	+	-	+	+	+	ND			+	+	-
TCBS 2	Dark Green	*Vibrio parahaemolyticus*	K	A	-	+	+	+	+	+	+	-	+	+	ND			-	+	+
TCBS 3	Green	*Vibrio vulnificus*	K	A	-	+	+	-	+	+	+	-	+	+	ND			-	+	-
MAC 1	Pink	*Escherichia coli*	A	A	+	+	+	-	-	+	+	-	+	+	ND					
MAC 2	Yellow	*Alcaligens faecalis*	K	K	-	+	-	-	+	+	-	-	-	+	-	-	-	-	+	-
MAC 3	Light pink	*Klebsiella pneumoniae*	A	A	-	-	-	+	+	+	+	-	+	-	ND					
MSA 1	Yellow	*Staphylococcus aureus*	A	K	-	+	-	-	-	+	+	-	-	+	+	-	+	ND		
mFC1	Blue	*E. coli*	A	A	+	+	+	-	-	+	+	-	+	+	ND					
mFC 2	Grey	*Enterobacter* spp.	A	A	-	+	-	+	-	+	+	+	+	+	ND					
EMB	Pink	*E. coli*	A	A	+	+	+	-	-	+	+	-	+	+	ND					