STUDY ON THE MICROBIOLOGICAL HAZARDS PRESENT IN SHRIMP AND SHRIMP CULTURE WATER FOR THE IMPROVEMENT OF SEA FOOD QUALITY OF KHULNA REGION, BANGLADESH

BY

MD. ABBAS ALI

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of
Master of Philosophy
in the Department of Chemistry

Khulna University of Engineering &Technology
Khulna 9203, Bangladesh

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Declaration

This is to certify that the thesis entitled, **"Study on the Microbiological Hazards Present in Shrimp and Shrimp Culture Water for the Improvement of Sea Food Quality of Khulna Region, Bangladesh"** has been carried out by Md. Abbas Ali in the Department of Chemistry, Khulna University of Engineering & Technology, Khulna, Bangladesh. The above thesis work or any part of this work has not been submitted anywhere for the award of any degree or diploma.

Signature of the Supervisor
(Prof. Dr. Md. Abdul Aziz)

Signature of the Candidate
(Md. Abbas Ali)

Approval

This is to certify that the thesis work submitted by Md. Abbas Ali entitled **"Study on the Microbiological Hazards Present in Shrimp and Shrimp Culture Water for the Improvement of Sea Food Quality of Khulna Region, Bangladesh"** has been approved by the board of examiners for the partial fulfillment of the requirements for the degree of Master of Philosophy in the Department of Chemistry, Khulna University of Engineering &Technology, Khulna, Bangladesh in November 2009.

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Abstract

Sea food quality and safety is mostly dependent on the microbiological parameters present in the shrimps. Microorganisms can reduce the quality of foods in various ways. They can change the food organoleptically so as to defer the potential consumer or they can make it capable of causing disease. There is no doubt that food related disease is extremely important throughout the world. In developed countries , the people are much more aware about the food borne disease, such as in UK in one year as much as 10% of population will suffer from a food- related disease and some death will result, usually amongst the most valuable the young and the infirm. However, it is known that throughout the world in one year millions of young children die as a result of diarrheas much of which has been caused by organisms contracted from water or food. Shrimps are used as one of the most important choseable and delicious food item in the global market. To improve the quality, to fulfill the requirements of the consumer, to earn more foreign currency and to develop our socio-economic condition, the study has been taken. Shrimps (Penaeus Monodon) and shrimps culture water were collected from different locations of Bagerhat, Satkhira and Khulna to perform microbiological analysis. It has been shown from the investigation that Standard Plate Count (SPC) was found within standard limit at Koyra (pond nos.1,2), Dumuria (pond nos.4,6), Paikgacha (pond nos.7,9), Rampal (pond nos.1,3), Mongla (pond nos.4,6), Bagerhat city area (pond nos.8,9), Shamnagor (pond nos.1,2,3), Ashasuni (pond no.5) and Satkhira city area (pond no.8) of Khulna region for shrimps. It has also been shown for shrimps that Total Coliform (T.Coli) was within standard value at Koyra (pond nos.1,3), Dumuria (pond nos.5,6), Paikgacha (pond nos.7,8), Mongla (pond nos.4,5,6), Bagerhat city area (pond nos.7,8,9), Shamnagor (pond nos.2,3), Ashasuni (pond no.5) and Satkhira city area (pond nos.7,8,9). The Faecal Coliform (F.Coli) was found within standard value at Koyra (pond no.1), Dumuria (pond nos.5,6), Mongla (pond nos.4,6), Bagerhat city area (pond nos.7,9), Shamnagor (pond no.2), Ashasuni (pond no.5) and Satkhira city area (pond no.8). Salmonella were found at Dumuria (pond no.4), Paikgacha (pond no.7), Rampal (pond nos.1,2), Mongla (pond no.6), Bagerhat city area (pond no.9) and Ashasuni (pond no.4) but Vibrio Cholerae were not found anywhere. It was found that Standard Plate Count (SPC) and Faecal Coliform (F,Coli) for shrimp culture water were found above the standard value in all the area. The Total Coliform (T.Coli) were found within standard value at Koyra (pond nos.1,3), Dumuria (pond nos.4,5,6), Rampal (pond no.3),

Mongla (pond nos.4,5,6), Bagerhat city area (pond no.8) and Ashasuni (pond nos.4.5,6). Salmonella was found at Rampal (pond no.1) but Vibrio Cholerae in shrimp culture water were not found in any pond.

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CHAPTER 1

INTRODUCTION

1.1 General

Frozen shrimps are most important of all Bangladeshi export fishery products. Annual exports have increased from 180 M.T. in 1968 to 86840 M.T. in 2007 (BFAR –2007) .This increase corresponds with the expansion of brackish water cultivation of shrimps. The export value in 1972 was approximately less than 3 million US$ but in 2007 it was 515 million which was the 2nd largest export item in our country. EU is the major export market importing 49%in 2007and USA importing 40%in 2007of the total quantity (BFAR-2007). In order to compete in the international market shrimp exporters must meet microbiological standards set by importing countries.

Shrimps are highly perishable foods and should be handled with care at all times and in such a way to inhibit the growth of microorganisms. It is extremely important to chill shrimp as soon as possible after catching if losses in quality are to be avoided. Shrimps are freshest when they are alive and fresh prime quality can be maintained when the time between landing and processing is kept to a minimum.

The main challenge that the developing countries fish industry faces is to comply with foreign consumer expectations, particularly quality. A lack of adequate infrastructure and technical expertise often translates into quality defects. These, in run, result in significant financial losses because of rejection and low prices for exports .The raw material quality is the key factor governing the final product quality .Therefore, the first critical point for a fish plant's quality assurance system is the control of raw material.

One of the most common defects in shrimp products is decomposition, which results from the growth of microorganisms and autolysins or "self decomposition" by enzymes naturally present in the shrimp. Bacteria are present in high numbers in the gut of living shrimp and also present over the shell and legs. In healthy living shrimp bacteria are harmless but as soon as they die their natural defense mechanisms to microbial invasion cease to function.

The number and type of bacteria which contaminate shrimps at time of landing depend on the feed water quality and temperature from which they are harvested .The numbers of bacteria are measured by a Standard Plate Count (SPC),which can be carried out at different temperatures and using different microbiological media. The (SPC) is most commonly employed to indicate the sanitary quality of seafood's. Enumeration of mesophiles (bacteria which have an optimum growth temperature of 30-40^{0}C) is carried out at 37^{0}C. These bacteria are active in post harvest shrimp spoilage at ambient tropical temperatures

The main sources of contamination of bacteria on shrimps are: **i)** Natural habitat: This will depend on the bacteriological quality of the water from which they were harvested and the quality of supplemented foods. When shrimps are harvested from farm waters, which are polluted, they may be contaminated with pathogens from land runoff. The possibility of contamination of pond culture shrimps by pathogens associated with raw animal manures used to fertilize ponds and the risk of pollution by domestics and animal sources.

ii) Primary landing: Primary landing with pond cultured shrimps bacterial contamination will occur from mud at the pond site .from dirty fish boxes andice and from handling. **iii)** Secondary handling: During processing shrimps will pack up bacteria from all unclean surfaces with which they come in contact. Unless processing times are kept short and temperatures are kept below 10^{0}c during washing, grading, etc. bacteria will rapidly decompose products. **iv)** Storage: Storage post process contamination can occur when processed products come in contact with unprocessed raw material and unhygienic storage areas.

All members of the genus salmonella are potentially pathogenic for humans. The transmission of the disease is through the food chain. The USFDA standards *for* salmonella in sea foods are "absence in 50g or 25g when analyzed by standard methods". The standard applies for both raw and cooked shrimps. V.Cholerae is the organism responsible for cholera, a severe diarrhea disease which frequently results in the death of infected persons. Consumption of uncooked shrimp resulted in an outbreak of cholera in various parts of the world.

The main sources of contamination of shrimp by pathogens are: **I**) Pollution of ponds by untreated human or animal sewage; **ii**) Use of contaminated water during processing; iii) Poor hygiene; and iv) Exposure of shrimp or processing equipment

The presence of Faecal Coliforms (FC) in shrimp products implies the possible presence of enteric pathogens .This group of bacteria is used by the food industry and authorities as a rapid test to indicate if faecal contamination has occurred. The natural habitat of faecal coliforms ,particullarly E.Coli is the gut of warm blooded animals. Not all members are pathogenic but they do provide a good measure of process sanitation. In cooked shrimp products high faecal coliform counts usually indicate inadequate cooking or post process contamination as they will be destroyed by normal cooking temperatures. High count may also indicate high storage temperatures.

The purpose of this research is to discuss the sources of microorganisms which spoi shrimp products and those pathogens which will cause food poisoning, resulting in products being rejected by international and local markets.

1.2 Background of Research Topic:

Shrimp farming is a highly profitable and a fast growing sector with enormous scope to increase foreign exchange and general employment in a developing country like Bangladesh. However shrimp farming needs to be conducted in a way that is socially acceptable, economically viable, technically appropriate and environmentally sound. In modern world human beings take his/her daily meal with health consciousness. Fishes are thought to be the best food to fulfill the demand of protein. Bangladesh earned about Tk.3600 core in the fiscal year 2006-2007 by exporting fish and fish like items of which about Tk.2295.94 core from Khulna region. About 43000M.T. shrimps were exported from Khulna region, which is about 64% of the total export from Bangladesh. It was known from different News Papers (FIQC) that 2007 year about 19 containers of exported fishes were returned from USA for Salmonella and Filth contamination, whose exported value was about Tk.50 core. To develop the quality of shrimps at Khulna, the microbiological parameters of shrimps and shrimps culture water were determined

1.3. Objective of the Study:

The object of this project was to measure Standard Plate Count (SPC), Total Coliforms (TC), Faecal Coliform(FC), Salmonella and Vibro Cholerae in shrimps and also in shrimps culture water of Khulna region and demonstrate for the development of sea food quality, so that the quality shrimps can be exported from Khulna region.

LITERATURE REVIEW

The survival of farming system can be relatively high with higher anticipated profit [1, 2, 3, 4, and 5]. Used appropriately nursery system can also reduce the spread of disease .A significant improvement in yields has been reported [6, 7, 8].Use intensive nursery system in commercial shrimp production was investigated by Samocha et.el. [9]. The microbiological quality of row shrimps processed in sea food processing plants of Tutocorin, Tamil Nadu, India was investigated by M.M. Antony et.al. [10].

The most important means of preserving fresh shrimp is by chilling to about 0-40^{0}C.The most common chilling media are wet ice, mixture of ice and sea water or refrigerated sea water [11].At a temperature of 0^{0}C merely slows down microbiological activity .An increase in temperature of 1 or 2^{0}C will markedly increase the rate of bacterial growth and reduce the self life [12]. There are several reports on the storage stability of shrimp of ice [13, 14, 15, and 16].

Microbiology of fresh and spoiled fish has been reviewed extensively [17-20]. Microbial proliferation begins with death of fish, at which time each natural defense mechanisms are destroyed .The rate of growth will depend on the number of types present on the fish and the temperature at which the fish are held. The chemical changes take place is mainly due to bacterial enzymes [21, 22].

The freshness of quality of a particular food refers to the degree of excellence [23]. Freshness quality is considered to be an extremely important factor in determining overall quality [24].The location of harvest [25], time of the year [26], method of catching [27, 28] and the manner in which seafood handled [29] seriously affect the degree of excellence .Freshness of seafood has a considerable influence on its quality and is the most important criteria for judging the quality [30]. Many methods have been tested for freshness quality i.e.; organoleptic [31], TVB-N [32], TMA-[33]. Sensory/Organoleptic evaluation is the most satisfactory and acceptable method to access the quality of fish and selfish [34- 36]

TMA (Tri Methyl Amine) is the compound produced from TMAO (Tri Methyl Amine Oxide) by bacterial enzymatic process. TMA has been considered as useful index for some species. TVB (Total Volatile Base) is used as an alternative to measuring TMA content. Seafood, when closed to spoilage, contains several bases that are volatile. Volatile bases in TVB mainly contain TMA and ammonia. TVB content during spoilage are very similar to those of TMA in the same species except that the initial of TVB is much higher [37].

Detailed bacteriological evaluation of shrimp from cold waters has been attempted by Harrison and Lee [38], Cann et, al., [39]; Novak [40] and lee and Pfeifer [41]. Such data regarding tropical shrimp is rather scanty [42, 43].The self life of shrimp in ice has been studied by many workers [44, 45]. Samples collected from a commercial processing plant had higher initial counts than those processing in the laboratory [46].With proper washing (use of clean, potable, chilled water), the bacterial load of shrimp can be reduced by as much as 65% [47- 49].

Studies on farmed brakishwater shrimp aimed to reveal whether the microbiological load of tropical shrimps was significantly higher than those from temperate waters and whether salmonella is a natural or commensally contaminant of farmed shrimp [50].
The tropical shrimp have higher bacterial load compared to temperate species [51].Cold water shrimp frequently have bacterial loads of $10\text{-}10^3$/g,although the range sometimes very greatly and on certain occasions has been reported up to 10^7/g [52].In most cases warm water shrimp has counts of 10^6/g on capture [53,54]. Coliform, Escherichia Coli and total bacterial count, Vibrio Cholera, Salmonella and qualitative microflora are also reported for shrimps [55]. Five treated and untreated samples were subjected to chemical and microbiological analysis together with organoleptic evaluation using hedonic score sheet (appearance ,colour, texture,taste, flavor) [56].

The incidence of human pathogenic vibrios in seafood harvested along the cost of Karnataka State (India) was studied by enrichment and direct plating methods [57]. Microbiological evaluation of shrimp processing was made once a month, from samples obtained at four processing stage in a factory, over a period of six months [58]. Microbiological quality indices namely standard plate count (SPC),presence/count of vibrio cholera, salmonella and faecal coliforms were determined at specific points in the

preparation of frozen shrimps (Penaeus Monodon) [59]. The physical and chemical change were determined in shrimp penaeus semisulcatus stored at 0^0C,10^0C,20^0C and 30^0C in order to estimate the effect of temperature and time on quality indices and determine their inter-relationships [60].

The importance of cool chain food products in the international market had stimulated many studies of the effects of freezing and low temperature storage in the bacteria associated with food. From the public health point of view, particular interest has been shown in reports indicating the survival of bacteria that have been frequently implicated, directly or indirectly with potential food poisoning hazard [61-63].

In general, the effect of reducing temperature on bacteria depends on various factors such as rate and type of freezing [64], initial numbers types and stages of growth phase of microorganisms involved [65] thawing process, number of cyclical defrosting [66] and the physical protection offered by the food itself its components [67]. Fish taken from open waters usually free from salmonella ,[68] but fish caught in sewage polluted waters have indicated these organisms [69].

Large number of V. Cholerae must usually be ingested to cholerae, thus problems often occur when poor handling and inadequate refrigeration have allowed the organism to multiply. In order to compete in the international market, prawn/shrimp exporters must meet microbiological standards set by importing countries [70]. Problems faced by Asian prawn/shrimp producers have been reviewed [71].

Microbiological studies are useful in determining the build- up and movement of pathogens and other organisms throughout the facility during processing. .Microbiological testing has an indispensable role in the HACCP system. The danger from microorganisms is greater in tropical countries where the surrounding conditions are favorable for their rapid growth [72]. It has long been established that rapid fish spoilage even at cold temperature is preliminarily due to bacteria action [73]. It is generally agreed that at 0^0C fish and shrimp become unacceptable organoleptically, biochemically and microbiologically within two weeks [74]. During processing the raw material may be contaminated by bacterial in various ways. If adequate precautions are not adopted soon

after catching to prevent such entry, the fish may be become contaminated by heavy bacterial load within a very short period [75].

Quality problem are also caused by particular nature and variability of raw materials due to the influenced of environment, feed and season .Quality control or the war against spoilage can be defined as "all methods, procedures, techniques and process employed to inhibit, delay or prevent deteriorative changes in freshly killed fish and in fish products and to prevent contamination or adulteration". More simply quality control is the application of technology to the handling, production manufacture, storage and distribution of fish and fish products in order to meet established or acceptable forms [76-77]. Smoke et. al., [78] carried out rapid detection of food borne pathogens and spoilage microorganisms is becoming increasingly important in modern food production. Howgate, [79] worked on the handling and processing of fish in Pakistan with special reference to the quality and quality control of fish and fish products.

Pokern [80] worked on microbiology in food processing and provides an analysis of microbiological control of the food quality, role of microbiologists in conducting the production ,microbiologic criteria and food legislature, source of microorganisms in food, development in microbial association ,in processed article, ecology of microorganisms growth, physiology of food spoilage, inhibition of microorganisms by technologic procedures, thermal processing ,freezing ,dehydration, preservation with preservatives and strategy of microbiologic control in food industry.

Fresh fish and shellfish are highly perishable food commodities due to their biological composition. The muscle tissue of fish and shellfish spoils fast. This can be explained by the higher water content of shrimp, the high free amino acid content and the lower content of connective tissue as compared to other flesh foods .This is a good condition for activities of microorganisms [81]. Cannon-Bonventure [82] reported "freshness'' defined in term of specific characteristics (appearance, order, flavored texture) is usually much more consistent and less confusing. However, texture measurement is most widely used since texture has become a new important and desirability for today's consumer [83]. Gill et. al.,[84] pointed out that texture deterioration of red hake and haddock muscle in frozen

storage and chemical parameters can give significant correlation if carefully controlled conditions are maintained

Several authors [85] have reported on the extended shell life of iced tropical fish species compared with iced fish from cold or temperate water. The data available on the biochemical changes in temperate and warm water fish and shrimp are rather scanty and often inconclusive [86]. Temperature, humidity and time of storage are the most important factors influencing the quality of fish [87]. The keeping quality of fish depends on the species of fish, fishing methods and temperature of storage, storage times, as well as various other conditions, which influence the action of micro-organism [88].

The organoleptic method may be influenced by the physiochemical and psychological state of the judges. Considerable variations are often observed in the sensitivity and the test of the judges of the different times and the different circumstances under which the examinations are performed [89-90]. Certain chemical changes in spoiling fish and shrimp appear to run parallel with changes in door, texture, appearance, etc. Various attempts have been made to measure freshness by estimating the quality of some of these end products as a result of enzymatic and bacterial activity [91], Paryam & Pilgrimhas [92] has developed a useful method for assessing overall acceptability of food products.

Biochemical (TVB-N, TMA-N) test is quite generally applicable; it usually gives reasonable good correlation with organoleptic freshness and can be carried out rapidly without the use of special equipment [93-94]. Jenson ey. al.[95] studied the pattern of increase in TVB-N during the storage of cod muscle at 2^0C and compared there results with sensory method .A good correlation obtained using the total volatile base a quality index does not distinguish the origin or component of these volatile compounds. Hence its use is more general.

DESCRIPTION OF MICROBIOLOGICAL PARAMETERS

3.1 Standard plate count (SPC)

Enumeration of Standard Plate Count (SPC) is designed to provide an estimate of the total number of Standard organisms in particular food. It reflects the microbiological quality of the food and is useful for indicating the potential spoilage of the perishable food products. It is also an indicator of the sanitary conditions under which the food was produced and /or processed and also of the level of Good Manufacturing Practices (GMP) adopted during processing, but in raw frozen food, uncontrolled destruction of organisms might have taken place during freezing which makes the above assumption baseless to a certain extent. In spite of above limitations still SPC can be taken as a valuable indication of the effectiveness of any type of processing or chemical disinfect ion such as cooking, freezing, and chlorination.

The international accepted limit of SPC is 10^6 cfu/g (ICMSF, 1998). All the results showed that the SPC values were lower than accepted limit for both practices. SPC of freshly harvested shrimp ranged from 6.8×10^4 to 1.5×10^5 as observed by Lobrerra et. al., 1990. These counts are within the range of reported values (10^3-10^3) of shrimp from temperate environments by Lillard et. al., [96]; Matches and Layrisse, [97] although Lannelongue et. al. [98] mentioned counts as high as 10^6-10^7. Counts reported from tropical countries also ranged from 10^3 to 10^6 by Varma et. al., [99]; Surendran et. al., 1985). A report by De Silva (1985), however, indicated counts as high as 10^8/gm. Result of this presents study showed that freshly harvested shrimps could meet the existing standards for SPC which is 10^6/g (ICMSF, 1974). According to Azam, [100], shrimp collected from all the three districts (Bagerhat, Khulna and Satkhira) and from the points (ghers) indicated value within 10^5 cfu/g, is acceptable limit even when being practiced normally. The mean (log10 value 1.26) was higher than the acceptable limit as recommended, by Paul [101]. Lyer et. al. [102] reported that 1×10^6/gm to 6×10^6/gm of bacteria which can not meet the present study.

There is evidence to support the fact that surface water carries fewer bacteria compared to bottom mud about 50th below the surface Williams et. al., [103]. As shrimps are bottom dwelling animals, the likelihood of their becoming contaminated with bacteria from the muddy substrate is always a possibility. Beheading if shrimps has lead to reduction of bacterial counts by 75% and effect of through washing on the reduction of microbial load of rowans has been documented by Green [104].These results demonstrate that a wash with clean water and proper handling techniques will reduce high SPC.

According to Castell et. al. [105], Georgala [106] and lyer and Chouduri [117] icing with inadequate quality water introduced bacteria into fish, causing higher bacterial counts. The present study result did differ from the statement. According to Shewan [19] most of the bacteria adhering to the slime and skin surface could be away with water. Shewan [19] reported the bacterial count of shrimp to be in the range of 104-105/g, 105 being very common. Similar values were also reported by Harrisan and Lee [38]; and Lee and Pfeier [41].

Cann [42] on the other hand, states that based on studies in Thailand and USA, the differences in bacterial counts between whole and beheading shrimps was not significant. The beheading of shrimps in the present study was carried out manually. The effect of washing on the bacterial load of the processed produced was evident as a majority (58.3%) of counts fell within the 105-106/g range and this reduction was statically non significant. The co-efficient of correlation between the organoleptic score and the present investigation was in agreement with Azam, [100].

3.2 Total Coliform (TC)

Total Coliforms (TC) group consists of aerobic and facultatively anaerobic Gram-negative, nonsporing rods that ferment lactose in Brilliant green Lactose Bile Broth or Mac Conkey Broth within 48hrs at 37^0C.The predominant aerobic bacterial flora of the large intestine of human beings and animals is composed of nonsporing, non-acid fast, and Gram-negative bacteria. They exhibit general morphological and biochemical similarities and are grouped together in the large and complex family of Enterobacteriaceae. Members of the coliform,

including faecal coliforms are referred to as indicator organisms, since their presence in certain numbers, may indicate the potential presence of pathogens in foods.

TC values were very low in CS compared to normal practice. The acceptability limit of TC is 10 and 100 MPN/g. At all points, their values were lower and were acceptable. This also indicated a better status of shrimp quality. The Coli form contents of farm shrimp varied between 460-1100 (Putro et. al., [50]. In the present study, Coli form contents were not very high in CS. The counts varied between 2 to 28/gm in CS. However, shrimp collected in normal practice showed relatively higher coli form count. The higher counts were observed in pond (gher) (53MPN/gm). According to Fonseka [55], 50% of the shrimp samples had coli form less than 20 (MPN/g) while the rest of the samples carried coli forms below 75 (MPN/g). The present study showed similar result.
Detailed bacteriological evaluation of shrimp from cold waters has been attempted by Harrison and Lee [38]; Cann, [39]; Novak [40] and Lee and Pfeifer [41]. Such data regarding tropical shrimp is rather scanty Cann, [42]; Zuberi et. al., [43]. Jayaweera et. al. [58] observed coli form counts of raw material ranging from <3/ to 1, 100/g, with 50% of the counts in the 3-102/g range. Shrimp caught in deeper waters generally do not carry coli from, but those caught close to the shore and from inshore waters have been found to be contaminated frequently with coli form Cann, [42].

The changes in the bacterial flora of shrimps have been studied previously, but not as extensively as that of fish. Various authors have studied the coliform counts in shrimp Campbell and Williams, [104], Cann, [53]; Matches, [12] and [97] during storage in ice. Their result indicated relatively lower coliform counts. In the present investigation, it was also observed that the coliform counts were reduced during ice storage.

3.3 Faecal Coliform(FC)

An elevated temperature is used to differentiate coliforms of faecal and non-faecal origin. Faecal Coliforms ferment lactose within 48 hrs at 45°C whereas non-faecal coliforms cannot do. So Faecal Coliforms, normally grow in the gastero-intestinal tract of humans and other warm blooded animals. They include members of three genera-Escherichia, Klebsiella and Enterobacter.

Shrimp collected from Khulna region showed a very lower count of Faecal Coliform. The recommended value of Faecal Coliform is $\log_{10}$ value 1.00 Paul, [101]. The acceptable limit of FC is 10MPN/g. The present investigation showed FC count was within acceptable limit. A slightly higher count was observed in normal practice (15 MPN/gm) but in most FC was not detected. The relatively lower counts were observed in CS, which varied between 0 to 9 MPN/gm.

Putro et. al. [50] could not detect FC either for shrimp of surrounding waters. According to Fonseka, [55] FC were not detected in 82.5% samples and counts of FC in pond water also found to be less than those in shrimps. Cann [42] reported that freshly caught penaeid shrimp did not carry FC but smaller samples were observed for sea water contaminated with FC.

Coliform organisms are primarily used to indicate some degree of potentially hazardous contamination based on the assumption that the natural habitat of the family (Enterobacteriaceae) to which these bacteria belong is the fasces of man and other mammals, thereby indicating faecal contamination. Jayaweera et. al. [58] found FC counts in the raw material ranging from <3 to 10^3/g but were reduced between <3/g (66.7%) and 3 to 10^3/g (33.3%). The FC counts did not vary significantly during the processing stage of beheading and grading, but the counts declined slightly during the next stage i. e., just before storage in ice, but significantly after keeping ice for a prolonged period.

Surendran et. al. [44] found Moraxella and Acinetobacter to be the predominant group at spoilage in P. indicus, M dobsoni and M affinis. Sure dram and Gopakumar [107] also reported that Moraxella and Acinetobacter comprised 74% of the spoilage flora in shrimps. Reilly et. al. [45] working on the spoilage of shrimp at different temperature, found the initial micro flora to be mesophilic. The rate of survival of different types of faecal organisms like E. coli occurring along with normal flora of iced fish at subsequent storage at- 23.3^{0}C had been studied, by Pillai et. al. [108]. However, the present study indicated that cold storage resulted in decreased number of bacterial load.

Rose et. al. [59] observed counts of the incoming product to be high, i. e., more than 20/g. According to Azam, [100], no FC was detected in shrimp distribution channel in Khulna

region, However, in the present study, value of FC count showed a different trend in Khulna region. Although there were variations at different points with regard to FC count, there were no significant differences ($P \leq 0.05$) within the points and value lower than the acceptable limit recommended in International market.

3.4 Salmonella

Salmonella is Gram-negative, rods, motile with the exception of S.gallinarum-pullorum which is non-motile .It is cataloes-positive, oxidize-negative and facultative anaerobic. It attacks sugars by fermentation with production of acid and gas, citrate usually positive. Important exception is salmonella paratyphi-A which does not produce gas and citrate-negative.

According to Azam, [100] salmonella were not detected in the sample of shrimps. The present investigation is in absolute agreement with the findings of Azam, [100]. Salmonella was not isolated from freshly harvested shrimp Lobrerra et. al., [46]; Putro et. al.,[50]. However salmonella contamination was observed in freshly harvested shrimp as found by Rattagool et. al.[56]. The results of the microbial parameters in this investigation indicated a similar pattern as observed by Fonseka [55]. The author reported that salmonella was not detected from shrimp.

The present study indicated no detection of Salmonella Sps in 25g samples of shrimp collected from any points of distribution channel in Khulna reason. The acceptable limit of Salmonella is zero in 25gm of shrimp samples. Salmonella were detected in prawn samples in the storage stability of brackish prawn during processing for export by Dangla, [70] particularly after harvesting but was not found during or after processing.

Rose [59] found the presence of salmonella on the frozen marine prawn immediately after processing. The absence of salmonella in the frozen farmed prawn might have been caused due to the absence of these bacteria in the raw material rather than the effect of processing measures. Rattagool et. al. [56] found salmonella sp. Associated with raw peeled salmonella shrimp and factory peeled shrimp. Jayaweera et. al. [58] could not detect in 25g samples from any stage of processing. The present study is in absolute agreement. Rose et.

al. [59] salmonella) state that salmonella was not found in the marine shrimp naturally and any in the marine shrimp was probably due to contamination during handling, transportation or processing.

3.5 Vibrio Cholerae

Vibrio Cholerae is the type species of the genus Vibrio.It is non-spore former, Gram-negative, shirt rod, often curved, motile by single polar flagella.
Vibrio Cholerae were detected in prawn samples in the storage stability of brackish water prawn during processing for export by Dangla, [70] particularly after harvesting but was not found during processing. In the present investigation, on Vibrio Cholerae were detected in any samples throughout the laboratory experiment for shrimps at any points in distribution channel (ghers).

In a study of microbial flora of pond cultured shrimp, only two samples were positive for vibrio sps. By Fonseka,[55]. The presence of vibrio in pond reared shrimps Christopher et. al., [109]; Vanderzant et, al., [110] and fish and shellfish from estuarine and marine waters is well documented Durairaj et. al., [111]; Simidu et. al., [112]; Surendran and Gopakumar,[113].

Rosmaewaty found Vibrio parahaemdytius being not detected throughout the processing lines. Reilly et. al. [114] working on brackish water prawns purported that Vibrio parahaemdyticus was present commonly as part of the natural flora, but not isolated after processing. According to Azam, [100], Vibrio sps, were not detected in 25g samples of shrimp sample collected from any points of distribution channel in Bagerhat, Khulna and Satkhira district. The acceptable limit of Vibrio Cholerae is zero in 25gm of shrimp samples. The present investigation did not differ from those observed by Azam, [100]. However, V. cholerae strains have been reported to be widely distributed in the environment in Europe, Asia and United States by Karunasagar et. al., [57]. Black et. al. [115] summarized that V. cholerae is an autochithonous speices of the estuarine ecosystem in as much as the occurrence of V. cholerae was not correlated with other microbial indicators of faecal pollution.

Karunasagar et. al. [57] did not find statistically significant correlation between faecal coliform count and incidence of V. cholerae. Karunasagar et. al. [57] showed that V. parahamdyticus was the most common (69%) Vibrio found in all the samples tested, V. valnificus was present in 25% of samlpes, *V, cholerae* is 9% and V. mimicus in 6.5% of the samples. Jayaweera et. al. [58] showed that V. parahaemolyticus dose not present a problem for the Srilanka prawn industry.

Reilly et. al. [45] working on the spoilage of P. monodon at different temperatures, found the initial micro flora to be mesophilic and dominated by several sp. and Vibrio cholerae (6.7%). According to Rose et. al. [59], V. cholerae was detected twice throughout the pond and plant survey. Reilly [45] reports isolated of V. cholera in brackish water prawn and suspected that the bottom mud is the source. Iyer et. al. [116] found no presence of Vibrio Cholerae as observed in the present study.

EXPERIMENTAL SETUP AND INVESTIGATION

4.1 Determination of Standard Plate Count (SPC)

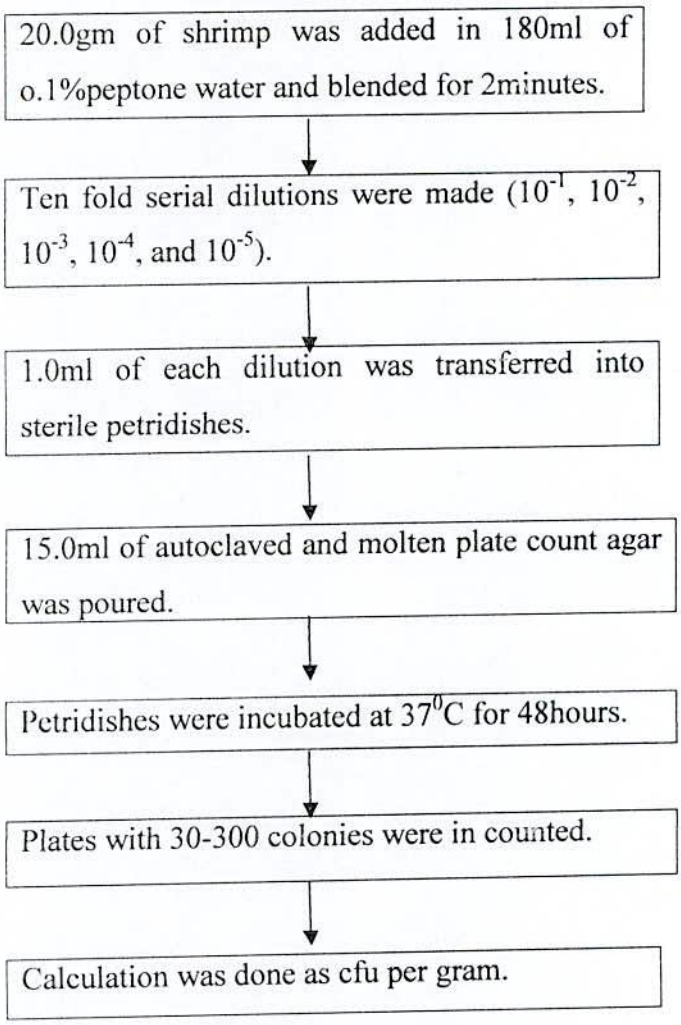
Procedure:

2.35 gm of Plate Count Agar [PCA] was first added in 100ml distilled water and boiled to dissolve the media completely. The media was then sterilized by autoclaving at 15 lbs pressure (121^0C) for 15 minutes. 20 gm of shrimp sample was weight and taken into a sterile blender jar for blending. The sample was added into a flask containing 180 ml. 0.1 % sterile peptone water. The mixture was homogenized for two minutes. This provided a dilution of 10^{-1} . 1.0ml of this suspension which was transferred into $_{Mc}$carty's bottles containing 9 ml of 0.1% peptone water to give dilution of 10^{-2}. The process was repeated for the preparation of10^{-3}, 10^{-4} and 10^{-5} dilutions respectively.

The appropriate dilutions were selected and for every dilutions 1.0 ml aliquot were transferred into a sterile Petri dishes .About 15 ml portion of molten Plate Count Agar (PCA) was poured into each of these sterile Petri dishes .The plates were then rotated 5 times clockwise, 5 times anticlockwise, 5 times back and forward. Care was taken not to splash agar on the lid of dish .Plates were left to solidify and after wards inverted the petridishes up side down and incubated them at 37^0c for 48-72 hours. Plates with 30-300 colonies on the surface were only counted.

The measurement of Standard Plate Count is shown in flow chart 1

Flow Chart 1



4.2 Determination of Total Coliform(TC)

Procedure:

3.56 gm of Laurel Sulphate Tryptose Broth (LSTP) was dissolved in 100ml of distilled water and then was distribute into test tubes with inserting Durham tubes and sterilized by autoclaving at 15 1bs pressure (121^{0}C) for 15 minutes.

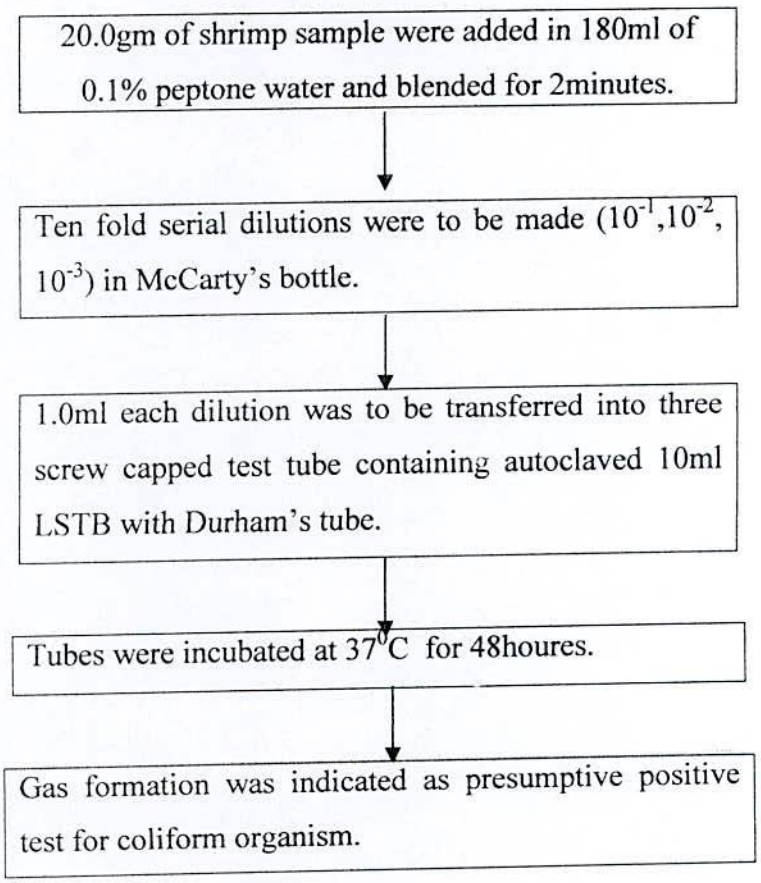
20 grams of shrimp sample was weighted and aseptically taken into a sterile blender jar for blending. After blending, the sample was added into conical flask containing 180 ml of 0.1% peptone water. Ten fold serial dilutions were made with this suspension in McCarty's bottles containing 9.o ml of 0.1% peptone water.

!.0ml of each dilution was transferred into three screw –capped test tubes containing autoclaved Lauryl Sulphate Tryptose Broth (LSTB) (10 ml) with Durham's tubes. The tubes were inverted to ensure that Durham's tubes did not contain gas bubbles .These tubes

were incubated at 35 or37^{0}C for 24h to 48 hours .Gas formation within 24 or 48 hours was consider as evidence for the presumptive positive test for total coli form organisms.

The determination of total coliform is shown in flow chart 2

Flow Chart 2



4.3 The Measurement of Faecal Coliform (FC)

Procedure :

4.0 gm Brilliant Green Lactose Bile Broth (BGLBB) was suspended in 100 ml distilled water .It was mixed well and then it was distributed in fermentation tubes and sterilized by autoclaving at 15 1bs pressure (121^{0}C) for 15 minutes.

1.0 gram trip tone water was suspended in 100 ml distilled water .Then it heated to boiling to dissolve the medium completely .Sterilize by autoclaving for 15 minutes at 15 1bs pressure (121^{0}C).

5.0gm of Kovas reagent dissolve the 4-dimethylabenzaldehyde in 75ml of the 2-mhylbutan-2-ol solution and then add 25 ml of Hcl slowly.

Those tubes of Lauryl Sulphate Tryptose Broth (LSTB) which were positive for gas formation in total coliform (TC) estimation tests were considered. Subcultures were made from all positive tubes of Lauryl Sulphate tryptose Broth (LSTB) into a 10ml volume of Brilliant Green Bile Broth (BGLB) and into 10ml volume of tryptone water. Tubes were incubated in circulating water bath at $44.5\pm0.5^{0}C$ up to 48 hours. Gas formation in BGLB broth confirms the presence of coliform and corresponding tryptone watre tubes were tested with Kovac's reagent. Formation of reddish ring at upper surface of tryptone watre tubes confirms the presence of faecal coliforms.

The measurement of Faecal Coliforms (FC) is shown in flow chart 3

Flow Chart 3

20.0gm of shrimp sample were added in 180ml of 0.1%peptone water and blended for 2minutes.

↓

Ten fold serial dilutions were to be made (10^{-1},10^{-2}, 10^{-3}) in McCarty's bottle.

↓

1.0ml each dilution was to be transferred into three screw capped test tube containing autoclaved 10ml LSTB with Durham's tube.

↓

Tubes were incubated at 37^{0}C for 48hours.

↓

Gas formation was indicated as presumptive positive test.

↓

A loopful of suspension from positive LSTB tubes were transferred into BGLB and tryptone water tubes.

↓

Tubes were incubated at 44.5^{0}C for 48 hours.

↓

Sufficient gas production in nos of tubes of BGLB +Indole test in kovac's reagent (red ring upper layer) of tryptone water.

↓

MPN chart Faecal Coliform/gm of the sample.

4.4 Determination of Salmonella

Procedure:

4.5gm of Buffered Peptone Water [BPW] was suspended in 225 ml distilled water & slightly heated to dissolve completely and sterilized by autoclaving at 15 1bs pressure (121^0C) for 15 minutes. Rappaport vassiliadis: 4.92gm of Rappaport Vassiliadis was dissolved in 100ml of distilled water and then was stir slowly until completely dissolution and transferred it into test tubes (10ml each). Sterilized by autoclaving at 15 1bs pressure at (121^0C) for 15 minutes.

Selenite Cystine Broth: 1.9gm of part A and 0.4gm of part B of selenite broth was suspended in 100 ml distilled water. It was mixed well & was dispensed & sterilized in a boiling water bath or in free flowing steam for 10 mints. It was not done in autoclave because excessive heating in deter mental.

Xylose Lysine Deoxycholate (XLD) agar: 2.83gm of XLD agar was suspended in 50ml distilled water and was heated with frequent agitation until the medium boiled and transferred immediately to a water bath at 50^0C for 15 mints. After cooling, the medium was roused into sterile petridishes.

Bismuth Sulphite Agar (BSA): 2.61gm of BSA was suspended in 50ml of distilled water and boiled to dissolve the medium completely. The sensitivity of the medium depends largely upon uniform dispersion of precipitated bismuth sulphate in the Ginal gel, which should be dispersed before pouring into the sterile Petri plates.

Hektoen Enteric Agar (HEA): 3.83 gms. of HEA was suspended in 50 ml of distilled water and boiled to dissolve the medium completely. Then the medium was roused into sterile petridishes.

25.0 gm of sample was weighed and taken into a blender flask. 225 ml of sterile Buffered Peptone Water (BPW) was added into the sample & then Blender for 2 min. The sample was incubated at 35^0C or 37^0C for 18-24 hours. The selective Enrichment culture was mixed each to 0.1 ml of pre-enrichment culture to 10 ml Rappaport Vassiliadis (sterile RV

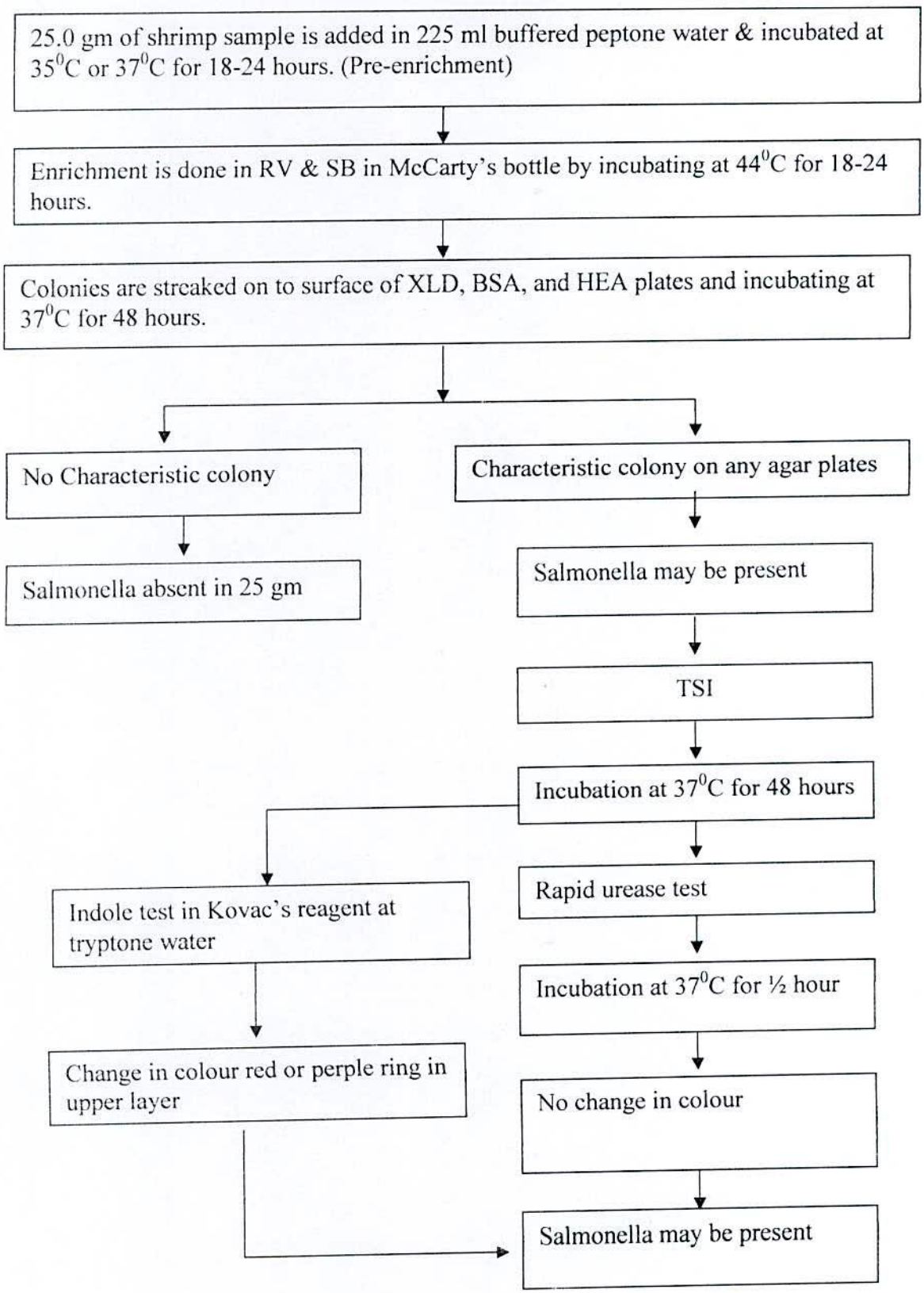
mixed each to 0.1 ml of pre-enrichment culture to 10 ml Rappaport Vassiliadis (sterile RV medium) and was incubated at 44^0C for 18-24 hours. and was added each to 1 ml of pre-enrichment culture to 10 ml Selinite Cystine Broth (Sterile) and was incubated at 35^0C or 37^0C for 18-24 hours.

After incubation, a loopful of each of the two selective enrichment broth were transferred to the surface of the agar media, i.e. Xylose Lysine Deoxycholate (XLD) agar, Bismuth Sulphite Agar (BSA), Hektoen Enteric Agar (HEA). The plates were then incubated at 37^0C for 48 hours and observed for characteristics colonies for Salmonella Sp. After 24-48 hours, Salmonella Sp in XLD agar shows pink (Red) colonies with or without black centre of H_2S on XLD, in HEA shows blue green to blue with or without black center and BSA shows black with metallic shin..

Suspected colonies of Salmonella Sp. were selected from agar plates for the biochemical tests.

Determination of Salmonella is shown in flow chart 4

Flow Chart 4



4.5 Determination of Vibrio Cholerae

Procedure :

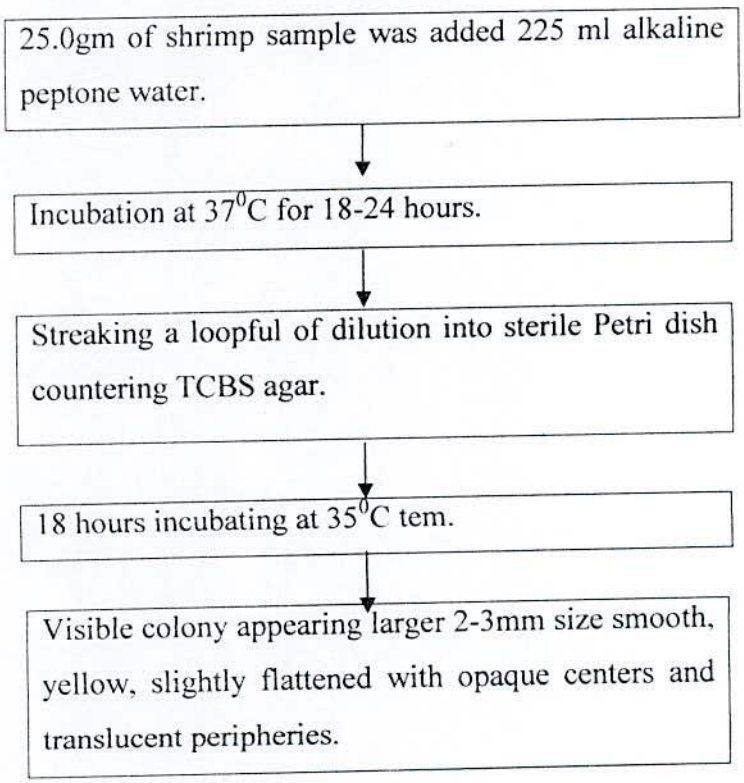
4.5gm of Alkaline Peptone Water [APW] was suspended in 225ml distilled water and slightly heated to dissolve completely and sterilized by autoclaving at 15 1bs pressure (121^0C) for 15minutes.

4.45 gm of Thio-sulphate Citrate Bile Salts Sucrose (TCBS) agar was first boiled 50 ml of distilled water to dissolve and boiled for 1 minute. 25gm of shrimp sample was added with 225ml of alkaline peptone water in a blender flask and blender for 2mins.The sample was in incubated at 37^0C $\pm 1^0$C for 6-8hours.

At the end of the incubation period a sterile loopful of the alkaline peptone water was streaked onto Tthio-sulphate Citrate Bile Salts Sucrose(TCBS) agar plates .The plates were then incubated at 37^0C $\pm 1^0$C for 24hours.At the end of the incubation plates were explored for the characteristic colony of vibrio cholerae. On TCBS agar colonies appeared as large (2-3mm), smooth and yellow, slightly flattened with opaque center and translucent peripheries

Detection of Vibrio cholerae is shown in flow chart 5

Flow Chart 5



4.6 INSTRUMENTATION AND MATERIALS

4.6.1 Equipments and Apparatus

(1).Work area, level table with sample surface in room that is clean, well-lighted (1oo foot –candles at working surface) and well –ventilated, and reasonably free of dust and drafts. The microbial density of air in working area, measured in fallout pour plates taken during plating, should not exceed 15 colonies /plate during 15 min. exposure.

(2).Storage space, free of dust and insects and adequate for protection of equipment and supplies.

(3). Petridishes, glass or plastic (at least 15 × 90mm)

(4). Pipettes with pipettes aids (no mouth pipe ting) or pipette's, 1,5 and 10 ml ,graduated in 0.1 ml unites.

(5).Dilution bottles, 6 oz (160 ml), borosilicate –resistant glass, with rubber stoppers or plastic screw caps.

(6). Pipette and Petri dishes containers, adequate for protection.

(7). Circulating water bath, for tempering agar, thermostatically controlled to 45^0C.

(8). Incubator, 370^o C

(9). Colony counter, dark field, with suitable light source and grid plate.

(10). Tally register.

(11). Sterile poly bags.

12. Volumetric flasks or bottles (250 ml)

13. Bunsen burner.

14. Lab blender /Stomacher blender.

15. Balance (120 gm capacity and 0.005 gm sensitivity)

16. Pipette feller

17. Durham's tube (10 ×75 mm)

18. Loof, Platinum/Nickel/Chromium (3 mm)

19. P^H meter

20. Sterile Bottles

21. Sterile Tubes

22. Water Baths

23. Sterilizer

4.6.2 Chemicals and materials

1. 0.1% peptone water
2. Plate Count Agar (PCA)
3. Laurel Sulphate Tryptose Broth (LSTB)
4. Brilliant Green Lactose Bile Broth (BGLBB)
5. Tryptone water +Indole test in Kovac's reagent
6. Hydrochoric Acid (HCl)
7. Rappaport Vassiliades (RV).
8. Selenite Cystene Broth
9. Xylose Lysine De-Oxycholate (XLD)
10. Bismuth Sulphite Agar (BSA)
11. Hektoen Enteric Agar (HEA)
12. Triple Sugar Iron agar (TSI)
13. Thio-sulphate Citrate Bile Salt Sucrose (TCBS)

RESULTS AND DISCUSSION

5.1 Experimental Values of Standard Plate Count (SPC), Total Coliform (T.Coli), Faecal Coliform (F.Coli), Salmonella and Vibrio Cholerae of Shrimps at Different Ponds (Ghers) of Khulna District.

The Experimental values of SPC, T.Coli, F.Coli, Salmonella and Vibrio Cholerae of shrimps at different ponds (ghers) of Koyra, Dumuria and Paikgacha of Khulna District are shown in Table 5.1

Table 5.1 Experimental Values of SPC, T.Coli, F.Coli, Salmonella and Vibrio Cholerae of Shrimps at Different Ponds (Ghers) of Khulna District.

Locations	SPC 10^5cfu/gm	T.Coli MPN/gm	F.Coli MPN/gm	Salmonella	Vibrio Cholera
Koyra					
Sample of					
Pond No-1	10.4	23	9	No	No
Pond No-2	2.75	240	15	No	No
Pond No-3	270.5	93	23	No	No
Domuria					
Pond No-4	3.6	460	93	Yes	No
Pond No-5	1.75	9	9	No	No
Pond No-6	15.6	15	4	No	No
Paikgacha					
Pond No-7	2.65	240	43	Yes	No
Pond No-8	3.41	150	20	No	No
PondNo-9	21.3	93	93	No	No

NB: For shrimps:

Standared limit of SPC = 10^6cfu/gm at 37^0c
Standared limit of T.Coli = 10 to100 MPN/gm at 37^0c
Standared limit of F.Coli = 10 MPN/gm at $44.5\pm.5^0$c

5.2 Standard Plate Count (SPC) of Shrimps at Different Ponds (Ghers) of Koyra, Dumuria and Paikgacha of Khulna District.

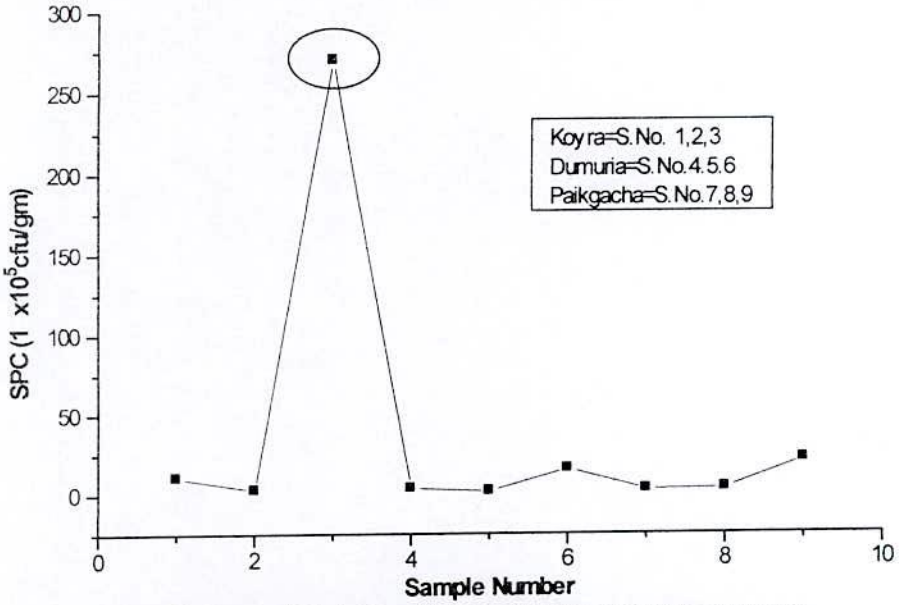


Figure 5.1 Standard Plate Count (SPC) of Shrimps at Different Ponds of Koyra, Dumuria and Paikgacha of Khulna District.

5.3 Total Coliform (TC) of Shrimps at Different Ponds (Ghers) of Koyra, Dumuria and Paikgacha of Khulna District.

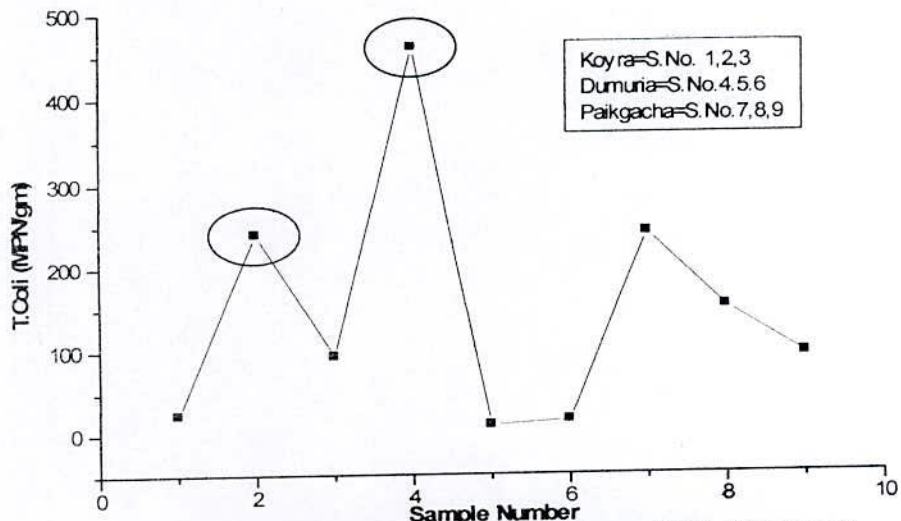


Figure 5.2 Total Coliform (T.Coli) of Shrimps at Differnt Ponds of Koyra, Dumuria and Paikgacha of Khulna District

It is observed from the figure 5.1 that the value of Standard Plate Count (SPC) of shrimps at different ponds (ghers) of Koyra, Dumuria and Paikgacha of Khulna District are found within standard limit in ponds nos. 1,2,4,5,7 and 8 and in ponds nos. 3,6 and 9 are higher than standared limit. But only in the pond no. 3 is more higher because cowdung was used into shrimp culture water.

The value of Total Coliform (TC) of Shrimps at different ponds (ghers) of Koyra, Dumuria and Paikgacha of Khulna District are found in ponds nos. 1,3,5,6 and 9 from figure 5.2, within standard limit and in ponds nos. 2,4,7 and 8 are higher than standared limit.But in ponds nos. 2 and 4 are more higher because cowdung, poultry liter were used into shrimp culture water.

5.4 Faecal Coliform (FC) of Shrimps at Different Ponds (Ghers) of Koyra, Dumuria and Paikgacha of Khulna District.

The value of Faecal Coliform (F.Coli) of shrimps at different ponds (ghers) of Koyra, Dumuria and Paikgacha of Khulna District are found in ponds nos. 1, 5 and 6 from figure 5.3, within standard limit and in ponds nos. 2, 3,4,7,8 and 9 are higher than standared limit.But in ponds nos. 4 and 9 are more higher because cowdung was used into shrimp culture water. It is found from table 5.1 that Salmonella were found at Dumuria in pond No.4 and Paikgacha in pond No.7 but Vibrio Cholerae were not detected any where.

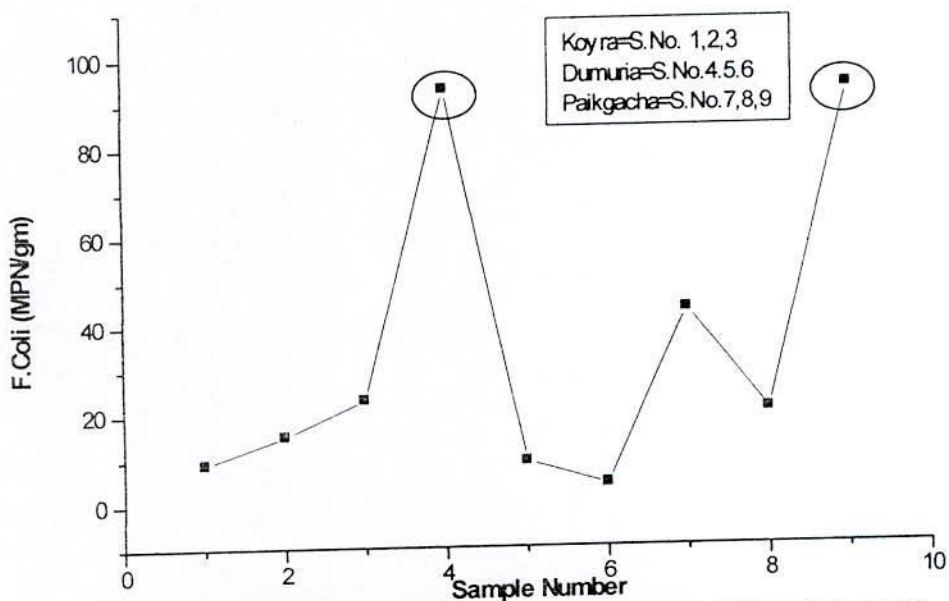


Figure 5.3 Faecal Coliform (F.Coli) of Shrimps at Different Ponds of Koyra, Dumuria and Paikgacha of Khulna District.

5.5 Experimental Values of Standard Plate Count (SPC), Total Coliform (T.Coli), Faecal Coliform (F.Coli), Salmonella and Vibrio Cholerae of Shrimps at Different Ponds (Ghers) of Bagerhat District.

The Experimental values of SPC, T.Coli, F.Coli, Salmonella and Vibrio Cholerae of shrimps at different ponds (ghers) of Rampal, Mongla and Bagerhat city area of Bagerhat District are shown in Table 5.2

Table 5.2 Experimental Values of SPC, T.Coli, F.Coli, Salmonella and Vibrio Cholerae of Shrimps at Different Ponds (Ghers) of Bagerhat District.

Locations	SPC 10^5cfu/gm	T.coli MPN/gm	F.coli MPN/gm	Salmonella	Vibrio cholera
Rampal					
Pond No-1	0.445	150	75	Yes	No
Pond No -2	12.4	210	20	Yes	No
Pond No-3	5.75	460	43	No	No
Mongla					
Pond No-4	1.7	21	9	No	No
Pond No-5	70.5	43	23	No	No
Pond No-6	0.31	7	4	Yes	No
Bagerhat City Area					
Pond No -7	187.5	21	9	No	No
Pond No-8	187.5	43	23	No	No
Pond No-9	0.42	7	4	Yes	No

NB: For shrimps:

Standared limit of SPC = 10^6cfu/gm at 37^0c
Standared limit of T.Coli = 10 to 100 MPN/gm at 37^0c
Standared limit of F.Coli = 10 MPN/gm at $44.5 \pm .5^0$c

5.6 Standard Plate Count (SPC) of Shrimps at Different Ponds (Ghers) of Rampal, Mongla and Bagerhat City Area of Bagerhat District.

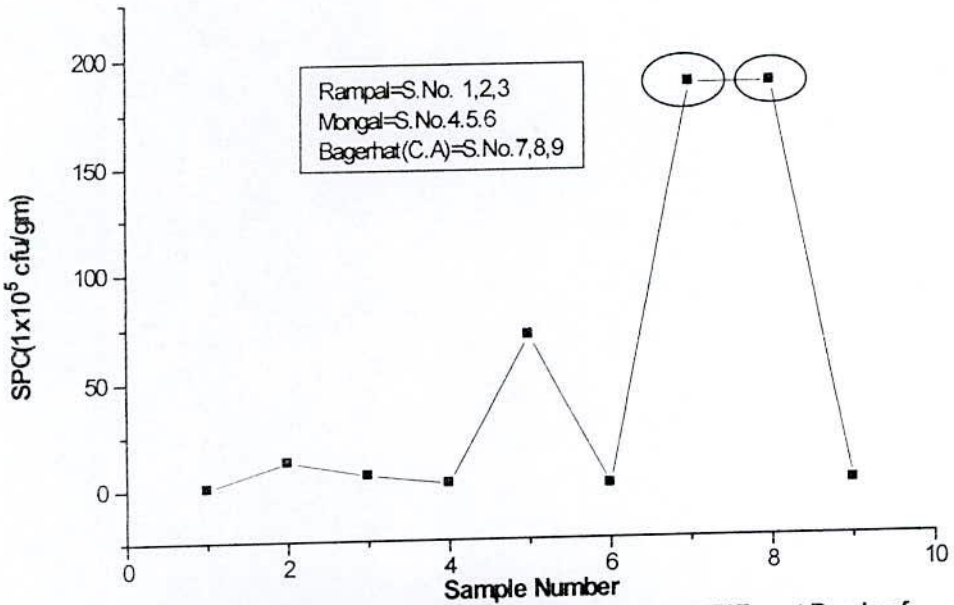


Figure 5.4 Standard Plate Count (SPC) of Shrimps at Different Ponds of Rampal, Mongal and Bagerhat City Area of Bagerhat District.

5.7 Total Coliform (TC) of Shrimps at Different Ponds (Ghers) of Rampal, Mongla and Bagerhat City Area of Bagerhat District.

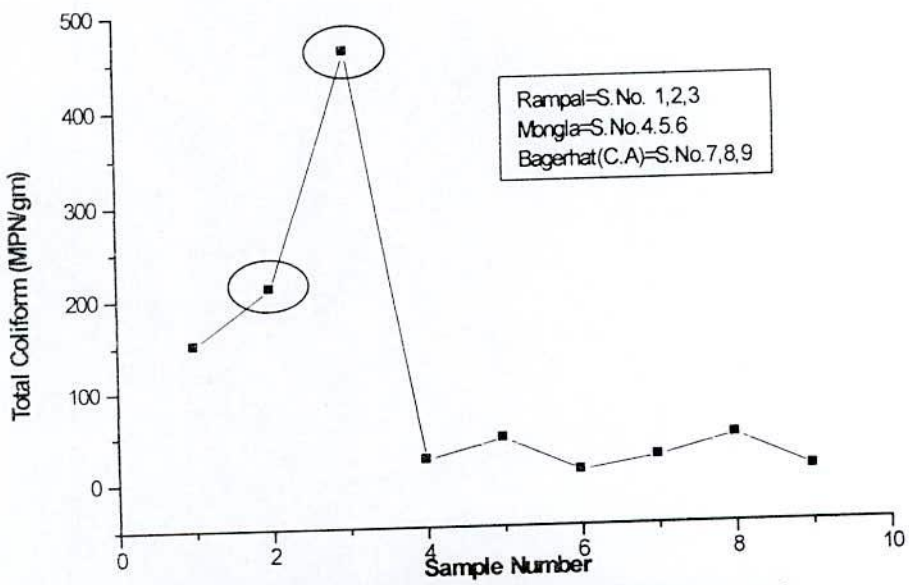


Figure 5.5 Total Coliform (T.Coli) of Shrimps at Different Ponds of Rampal, Mongla and Bagerhat City Area of Bagerhat District.

It is observed from the figure 5.4 that the value of Standard Plate Count (SPC) of shrimps at different ponds (ghers) of Rampal, Mongla and Bagerhat city area of Bagerhat District are found within standard limit in ponds nos. 1,3,4,6 and 9 and in ponds nos.2,5, 7 and 8 are higher than standard limit. But in ponds nos.7 and 8 are more higher because cowdung was used into shrimp culture water.

The value of Total Coliform (TC) of shrimps at different ponds (ghers) of Rampal, Mongla and Bagerhat city area of Bagerhat District are found in ponds nos.4,5,6,7,8 and 9 from figure 5.5, within standard limit and in ponds nos. 1,2, and 3 exceed the standard limit. But in ponds nos. 2 and 4 are more higher because cowdung, poultry liter were used into shrimp culture water.

5.8 Faecal Coliform (FC) of Shrimps at Different Ponds (Ghers) of Rampal, Mongla and Bagerhat City Area of Bagerhat District.

The value of Faecal Coliform (F.Coli) of shrimps at different ponds (ghers) of Rampal, Mongla and Bagerhat city area of Bagerhat District are found in ponds nos. 4, 6,7 and 9 from figure 5.6, within standard limit and in ponds nos.1,2,3, 5 and 8 exceed the standard limit. But in ponds nos. 1 and 3 are more higher because cowdung was used into shrimp culture water. It is observed from table 5.2 that salmonella were found at Rampal in ponds No.1,2, at Mongla in pond No 6 and at Bagerhat city area in pond No.9 but Vibrio Cholerae were not detected any where.

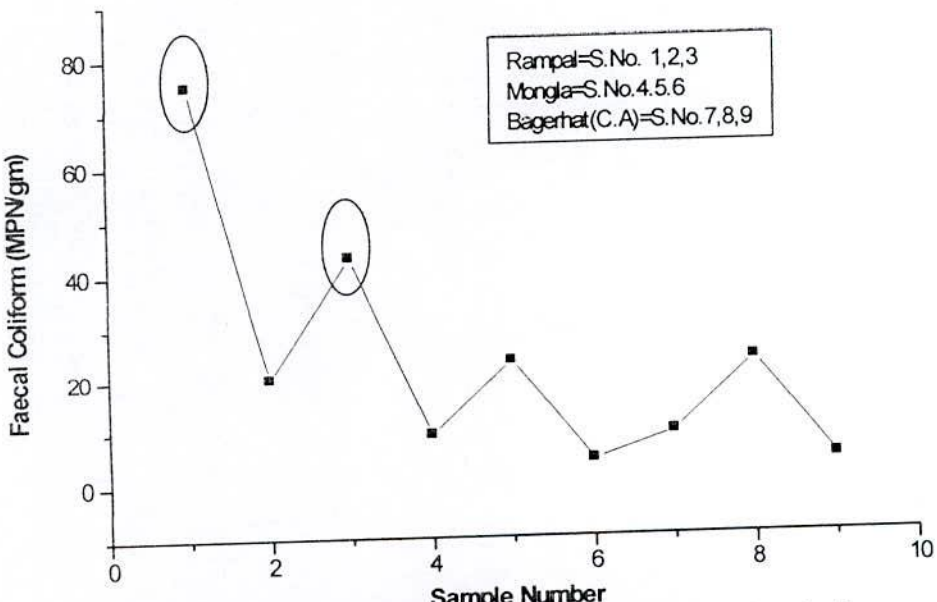


Figure 5.6 Faecal Coliform (F.Coli) of Shrimps at Different Ponds of Rampal, Mongla and Bagerhat City Area of Bagerhat District.

5.9 Experimental Values of Standard Plate Count (SPC), Total Coliform (T.Coli), Faecal Coliform (F.Coli), Salmonella and Vibrio Cholerae of Shrimps at Different Ponds (Ghers) of Satkhira District.

The Experimental values of SPC, T.Coli, F.Coli, Salmonella and Vibrio Cholerae of shrimps at different ponds (ghers) of Shamnagor, Ashasoni and Satkhira city area of Satkhira District are shown in Table 5.3

Table 5.3 Experimental Values of SPC, T.Coli, F.Coli, Salmonella and Vibrio Cholerae of Shrimps at Different Ponds (Ghers) of Satkhira District.

Locations	SPC 10^5cfu/gm	T.Coli MPN/gm	F.Coli MPN/gm	Salmonella	Vibrio Cholera
Shamnagor					
Pond No-1	3.7	460	15	No	No
Pond No-2	0.50	43	7	No	No
Pond No-3	1.45	93	15	No	No
Ashasuni					
Pond No-4	32.12	1100	39	Yes	No
Pond No-5	3.1	21	7	No	No
Pond No -6	79.5	1100	75	No	No
Satkhira City Area					
Pond No-7	15.95	93	21	No	No
Pond No-8	4.1	23	7	No	No
Pond No-9	206.5	43	23	No	No

NB: For shrimps:

Standared limit of SPC = 10^6cfu/gm at 37^0c
Standared limit of T.Coli = 10 to 100 MPN/gm at 37^0c
Standared limit of F.Coli = 10 MPN/gm at $44.5 \pm .5^0$c

5.10 Standard Plate Count (SPC) of Srimps at Different Ponds (Ghers) of Shamnagor, Ashasuni and Satkhira City Area of Satkhira District.

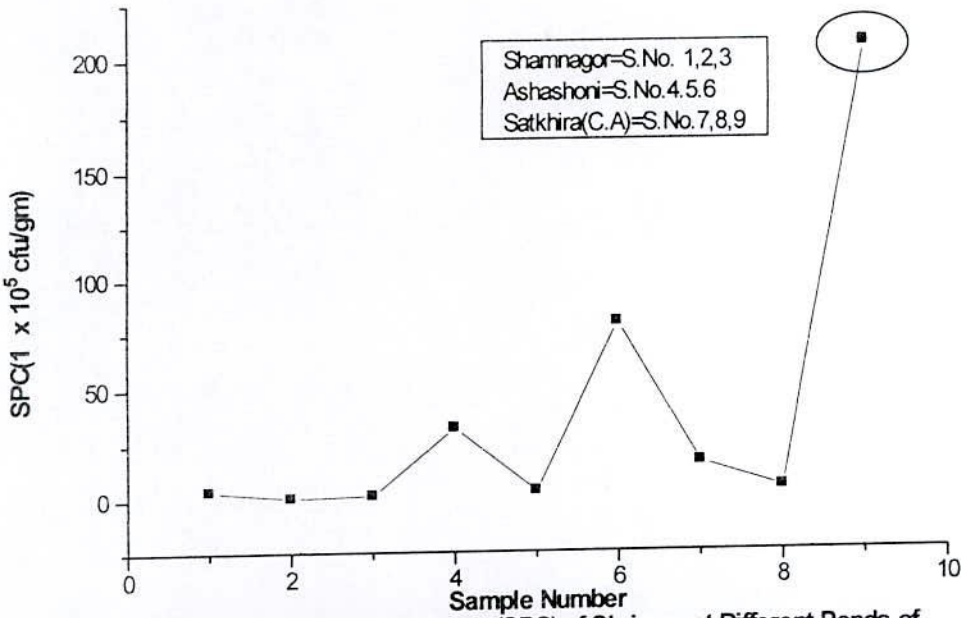


Figure 5.7 Standard Plate Count (SPC) of Shrimps at Different Ponds of Shamnagor, Ashashoni and Satkhira City Area of Satkhira District.

5.11 Total Coliform (TC) of Shrimps at Different Ponds (Ghers) of Shamnagor, Ashasuni and Satkhira City Area of Satkhira District.

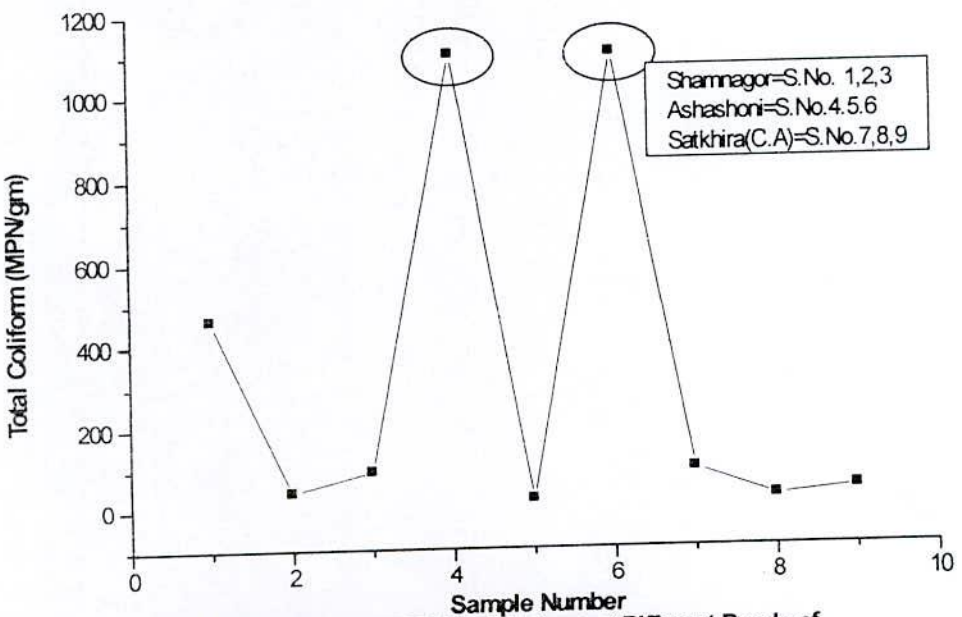


Figure 5.8 Total Coliform (T.Coli) of Shrimps at Different Ponds of Shamnagor, Ashashoni and Satkhira City Area of Satkhira District.

It is observed from the figure 5.7 that the value of Standard Plate Count (SPC) of shrimps at different ponds (ghers) of Shamnagor, Ashasuni and Satkhira city area of Satkhira District are found within standard limit in ponds nos. 1,2,3,5 and 8 and in ponds nos.4,6, 7 and 9 are higher than standard limit . But only in the pond no. 9 is more higher because cowdung was used into shrimp culture.

The value of Total Coliform (TC) of shrimps at different ponds (ghers) of Shamnagor Ashasuni and Satkhira city area of Satkhira District are found in ponds nos.2,3,5,7,8 and 9 from figure 5.8, within standard limit and in ponds nos. 1,4,6 exceed the standard limit. But in ponds nos. 4 and 6 are more higher because cowdung, poultry liter were used into shrimp culture water.

5.12 Faecal Coliform (FC) of Shrimps at Different Ponds (Ghers) of Shamnagor, Ashasuni and Satkhira City Area of Satkhira District.

The value of Faecal Coliform (F.Coli) of shrimps at different ponds (ghers) of Shamnagor Ashasuni and Satkhira city area of Satkhira District are found in ponds nos.2,5 and 8 from figure 5.9, within standard limit and in ponds nos. 1,3,4,6,7 and 9 exceed the standard limit. But in ponds nos. 4 and 6 are more higher because cowdung, poultry liter were used into shrimp culture water. It is found from table 5.3 that Salmonella were found at Ashasuni in pond No.4 but Vibrio Cholerae were not found anywhere.

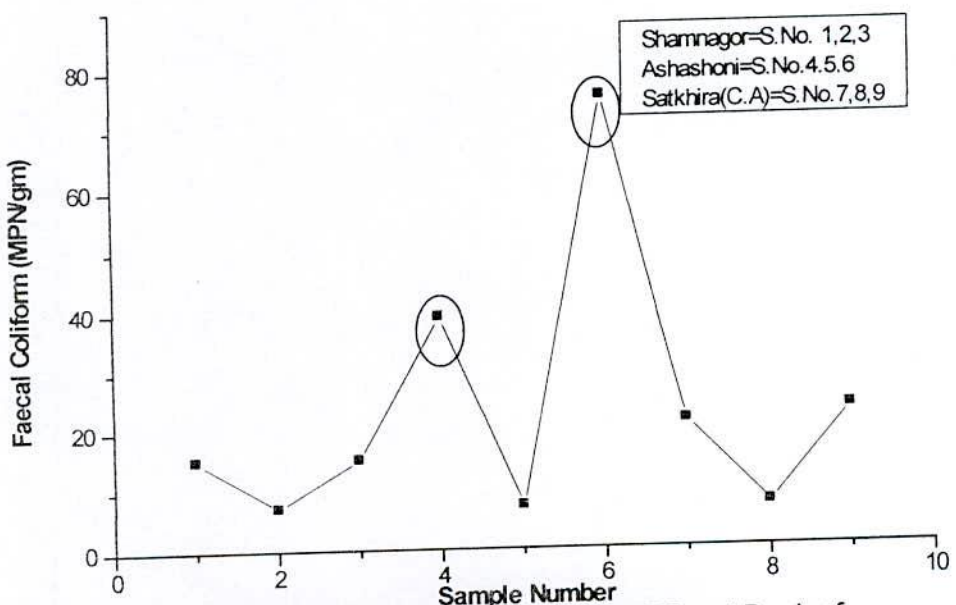


Figure 5.9 Faecal Coliform (F.Coli) of Shrimps at Different Ponds of Shamnagor, Ashashoni and Satkhira City Area of Satkhira District.

5.13 Experimental Values of Standard Plate Count (SPC), Total Coliform (T.Coli), Faecal Coliform (F.Coli), Salmonella and Vibrio Cholerae of Water at Different Ponds (Ghers) of Khulna District.

The Experimental values of SPC, T.Coli, F.Coli, Salmonella and Vibrio Cholerae of water at different ponds (ghers) of Koyra, Dumuria and Paikgacha of Khulna District are shown in Table 5.4

Table 5.4 Experimental Values of SPC, T.Coli, F.Coli, Salmonella and Vibrio Cholerae of Water at Different Ponds (Ghers) of Khulna District.

Locations	SPC 10^3cfu/ml	T.Coli MPN/100ml	F.Coli MPN/100ml	Salmonella	Vibrio Cholera
Koyra					
Sample of					
Pond No-1	1.94	90	90	No	No
Pond No-2	131	160	20	No	No
Pond No-3	1.53	25	08	No	No
Domuria					
Pond No-4	10.65	35	17	No	No
Pond No-5	43.05	35	5	No	No
Pond No-6	10	35	17	No	No
Paikgacha					
Pond No-7	7.12	180	180	No	No
Pond No-8	5.10	180	180	No	No
PondNo-9	6.95	160	35	No	No

NB: For water:

Standared limit of SPC = 10^3cfu/ml at 37^0c
Standared limit of T.Coli = 100 MPN/100ml at 37^0c
Standared limit of F.Coli = 0 MPN/100ml at $44.5 \pm .5^0$c

5.14 Standard Plate Count (SPC) of Water at Different Ponds (Ghers) of Koyra, Dumuria and Paikgacha of Khulna District.

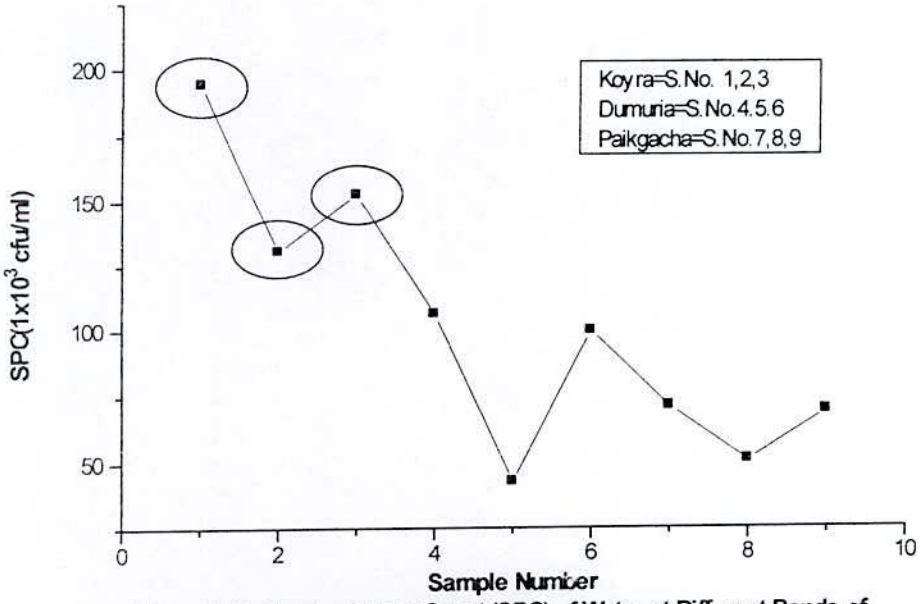


Figure 5.10 Standard Plate Count (SPC) of Water at Different Ponds of Koyra, Dumuria and Paikgacha of Khulna District.

5.15 Total Coliform (TC) of Water at Different Ponds (Ghers) of Koyra, Dumuria and Paikgacha of Khulna District.

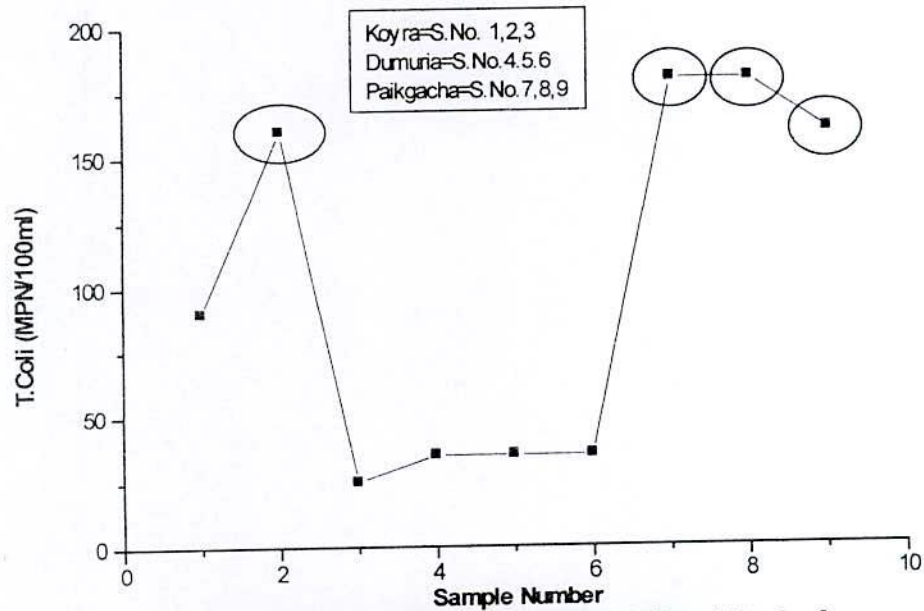


Figure 5.11 Total Coliform (T.Coli) of Water at Different Ponds of Koyra, Dumuria and Paikgacha of Khulna District.

It is observed from the figure 5.10 that the value of Standard Plate Count (SPC) of water at different ponds (ghers) of Koyra, Dumuria and paikgacha of Khulna District are found in ponds nos.1- 9 exceed the standard limit. But in ponds nos. 1, 2 and 3 are more higher because cowdung, poultry liter were used into shrimp culture water.

The value of Total Coliform (TC) of water at different ponds (ghers) of Koyra, Dumuria and Paikgacha of Khulna District are found in ponds nos.1,3,4,5 and 6 from figure 5.11, within standard limit and in ponds nos. 2,7,8 and 9 exceed the standard limit. But in ponds nos. 2,7,8 and 9 are more higher because cowdung, poultry liter were used into shrimp culture water.

5.16 Faecal Coliform (FC) of Water at Different Ponds (Ghers) of Koyra, Dumuria and Paikgacha of Khulna District.

The value of Faecal Coliform (FC) of water at different ponds (ghers) of Koyra, Dumuria and Paikgacha of Khulna District are found in ponds nos.1-9 from figure 5.12, exceed the standard limit. But in ponds nos. 7 and 8 are more higher because cowdung, poultry liter, urea were used into shrimp culture water. Salmonella and Vibrio Cholerae were not detected any where.

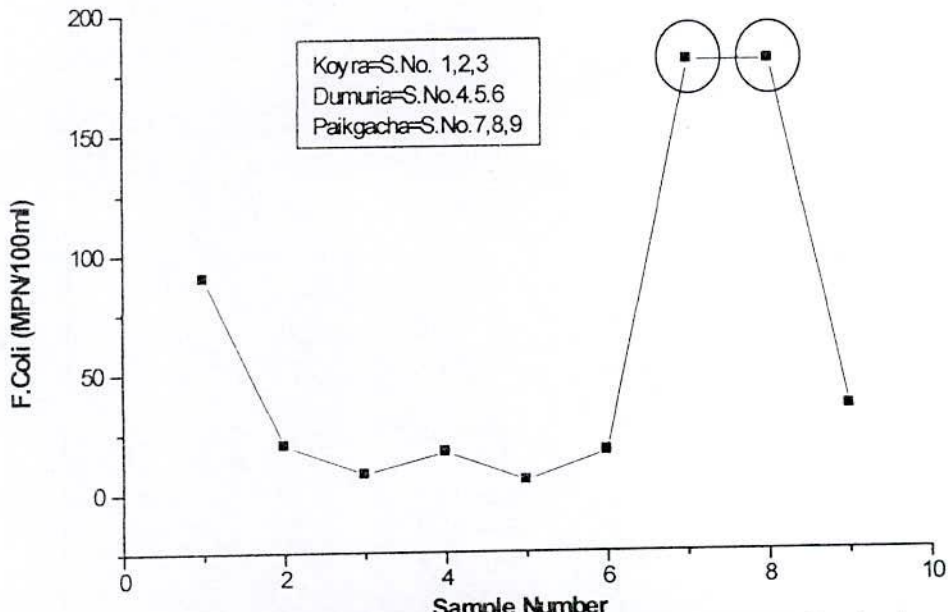


Figure 5.12 Faecal Coliform (F.Coli) of Water at Different Ponds of Koyra, Dumuria and Paikgacha of Khulna District.

5.17 Experimental Values of Standard Plate Count (SPC), Total Coliform (T.Coli), Faecal Coliform (F.Coli), Salmonella and Vibrio Cholerae of Water at Different Ponds (Ghers) of Bagerhat District.

The Experimental values of SPC, T.Coli, F.Coli, Salmonella Vibrio Cholerae of water at different ponds (ghers) of Rampal, Mongla and Bagerhat city area of Bagerhat District are shown in Table 5.5

Table 5.5 Experimental Values of SPC, T.Coli, F.Coli, Salmonella and Vibrio Cholerae of Water at Different Ponds (Ghers) of Bagerhat District.

Locations	SPC 10^3cfu/ml	T.Coli MPN/100ml	F.Coli MPN/100ml	Salmonella	Vibrio Cholera
Rampal					
Sample of					
Pond No-1	63.1	180	7	Yes	No
Pond No-2	233	160	12	No	No
Pond No-3	3.65	90	18	No	No
Mongla					
Pond No-4	14.55	50	25	No	No
Pond No-5	60.8	50	11	No	No
Pond No-6	2.40	17	11	No	No
Bagerhat City					
Area					
Pond No-7	8.5	160	14	No	No
Pond No-8	3.6	18	16	No	No
Pond No-9	5.9	160	14	No	No

NB: For water:

Standared limit of SPC = 10^3cfu/ml at 37^0c
Standared limit of T.Coli = 100 MPN/100ml at 37^0c
Standared limit of F.Coli = 0 MPN/100ml at $44.5 \pm .5^0$c

5.18 Sandard Plate Count (SPC) of Water at Different Ponds (Ghers) of Rampal, Mongla and Bagerhat City Area of Bagerhat District.

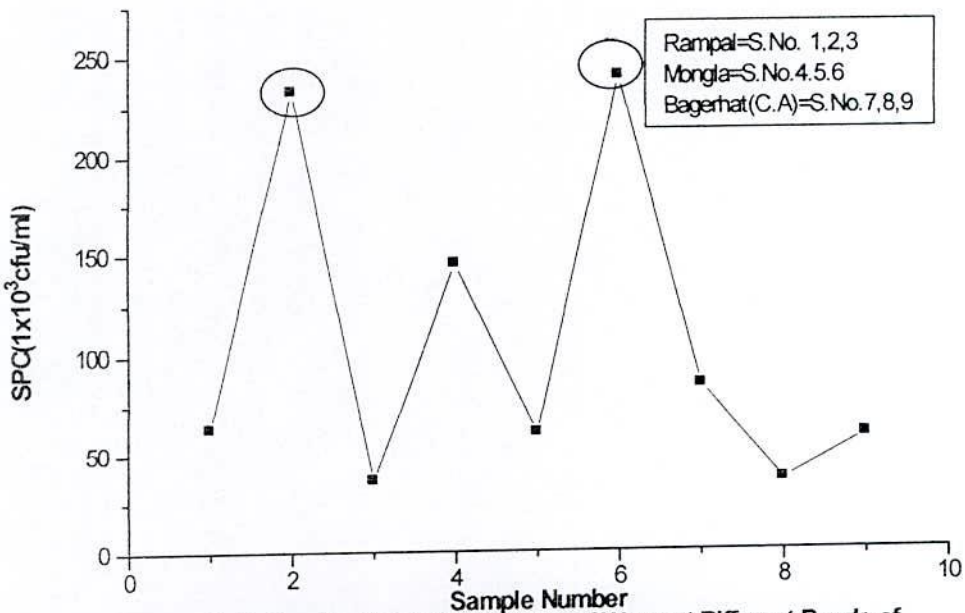


Figure 5.13 Standard Plate Count (SPC) of Water at Different Ponds of Rampal, Mongla and Bagerhat City Area of Bagerhat District.

5.19 Total Coliform (TC) of Water at Different Ponds (Ghers) of Rampal, Mongla and Bagerhat City Area of Bagerhat District.

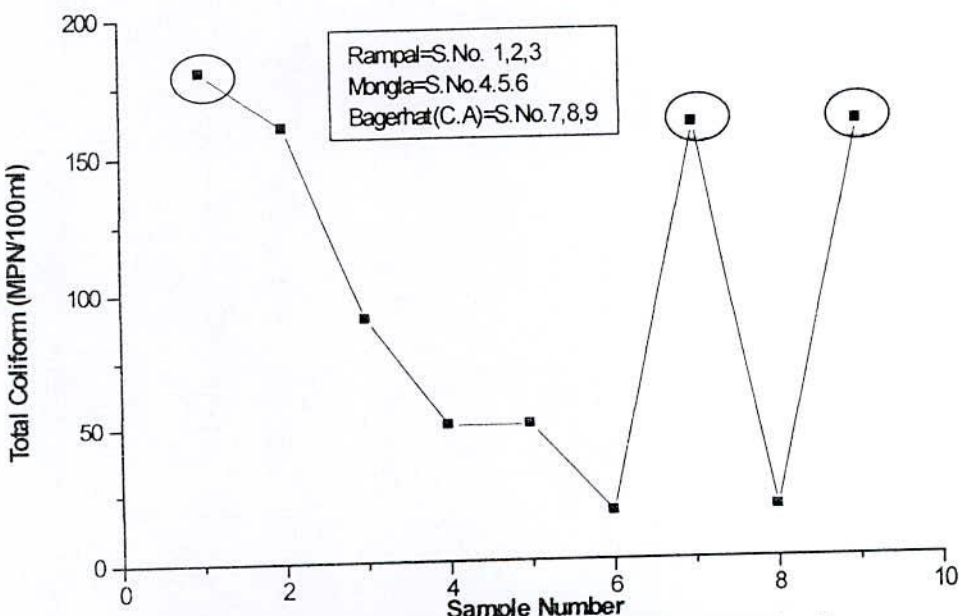


Figure 5.14 Total Coliform (T.Coli) of Water at Different Ponds of Rampal, Mongla and Bagerhat City Area of Bagerhat District.

It is observed from the figure 5.13 that the value of Standard Plate Count (SPC) of water at different ponds (ghers) of Rampal, Mongla and Bagerhat city area of Bagerhat District are found in ponds nos.1-9 exceed the standard limit. But in ponds nos. 2 and 6 are more higher because cowdung, poultry liter, phosphate were used into shrimp culture water.

The value of Total Coliform (TC) of water at different ponds (ghers) of Rampal, Mongla and Bagerhat city area of Bagerhat District are found in ponds nos.3,4,5, and 6 from figure 5.14, within standard limit and in ponds nos.1,2,7,8 and 9 exceed the standard limit. But in ponds nos.1,7 and 9 are more higher because cowdung, poultry liter, phosphate were used into shrimp culture water.

5.20 Faecal Coliform (FC) of Water at Different Ponds (Ghers) of Rampal, Mongla and Bagerhat City Area of Bagerhat District.

The value of Faecal Coliform (FC) of water at different ponds (ghers) of Rampal, Mongla and Bagerhat city area of Bagerhat District are found in ponds nos.1-9 from figure 5.15, exceed the standard limit. But in ponds nos. 3, 4 and 8 are more higher because cowdung, poultry liter, phosphate were used into shrimp culture water.It is found from table 5.5 that Salmonella were found at Rampal in pond No.1 and Vibrio Cholerae were not detected any where.

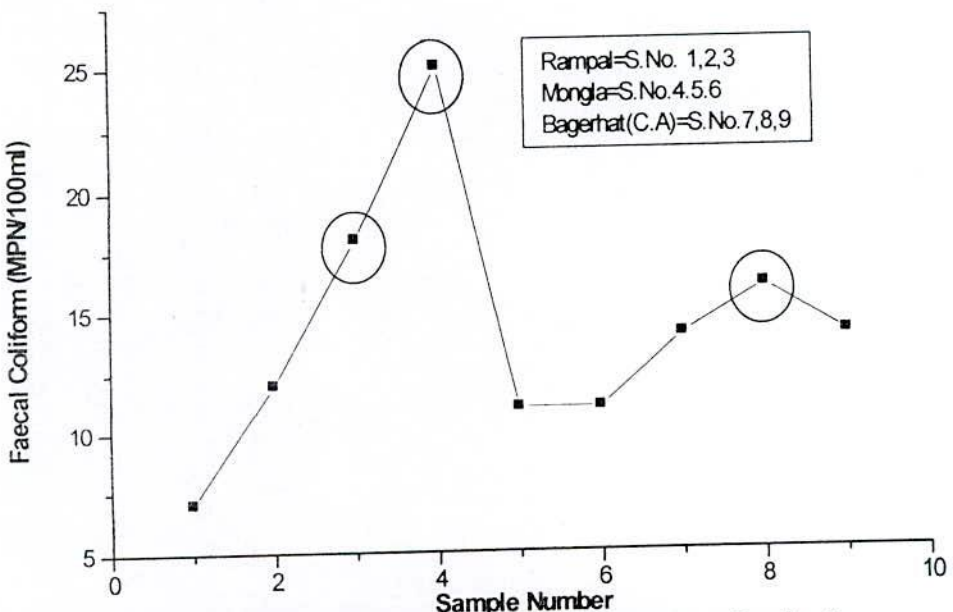


Figure 5.15 Faecal Coliform (F.Coli) of Water at Different Ponds of Rampal, Mongla and Bagerhat City Area of Bagerhat District.

5.21 Experimental Values of Standard Plate Count (SPC), Total Coliform (T.Coli), Faecal Coliform (F.Coli), Salmonella and Vibrio Cholerae of Water at Different Ponds (Ghers) of Satkhira District.

The Experimental values of SPC, T.Coli, F.Coli, Salmonella and Vibrio Cholerae of water at different ponds (ghers) of Shamnagor, Ashasuni and Satkhira city area of Satkhira District are shown in Table 5.6

Table 5.6 Experimental Values of SPC, T.Coli, F.Coli, Salmonella and Vibrio Cholerae of Water at Different Ponds (Ghers) of Satkhira District.

Locations	SPC 10^3cfu/ml	T.Coli MPN/100ml	F.Coli MPN/100ml	Salmonella	Vibrio Cholera
Shamnagor					
Sample of					
Pond No-1	1.06	160	35	No	No
Pond No-2	47	180	180	No	No
Pond No-3	58	180	160	No	No
Ashasuni					
Pond No-4	3.9	25	13	No	No
Pond No-5	44.5	10	7	No	No
Pond No-6	14	1	1	No	No
Satkhira					
City Area					
Pond No-7	278	180	180	No	No
Pond No-8	48	160	30	No	No
PondNo-9	62.1	180	180	No	No

NB: For water:

Standared limit of SPC = 10^3cfu/ml at 37^0c
Standared limit of T.Coli = 100 MPN/100ml at 37^0c
Standared limit of F.Coli = 0 MPN/100ml at $44.5 \pm .5^0$c

5.22 Standard Plate Count (SPC) of Water at Different Ponds (Ghers) of Shamnagor, Ashasuni and Satkhira City Area of Satkhira District.

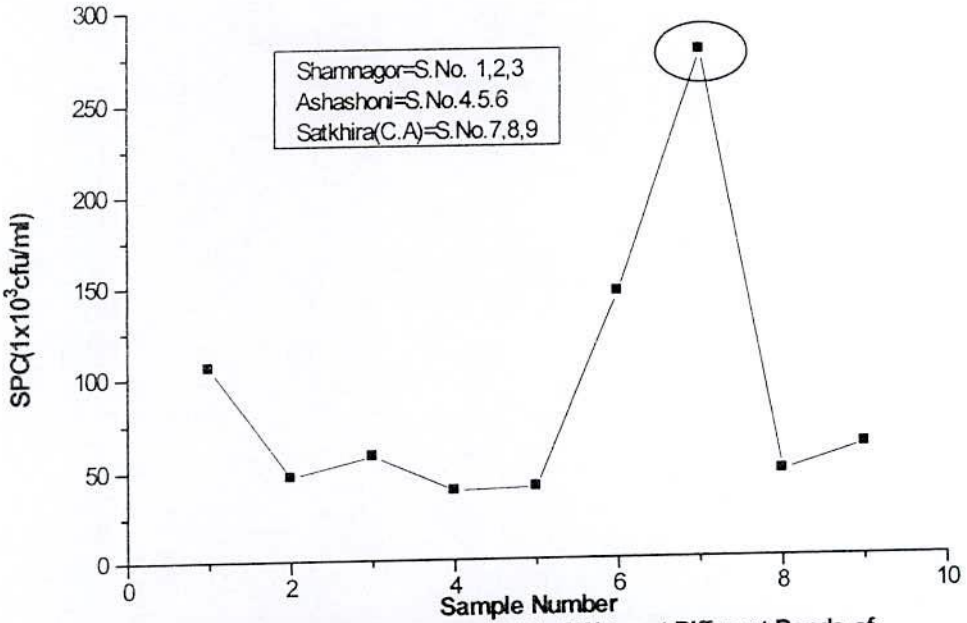


Figure 5.16 Standard Plate Count (SPC) of Water at Different Ponds of Shamnagor, Ashashoni and Satkhira City Area of Satkhira District.

5.23 Total Coliform (TC) of Water at Different Ponds (Ghers) of Shamnagor, Ashasuni and Satkhira City Area of Satkhira District.

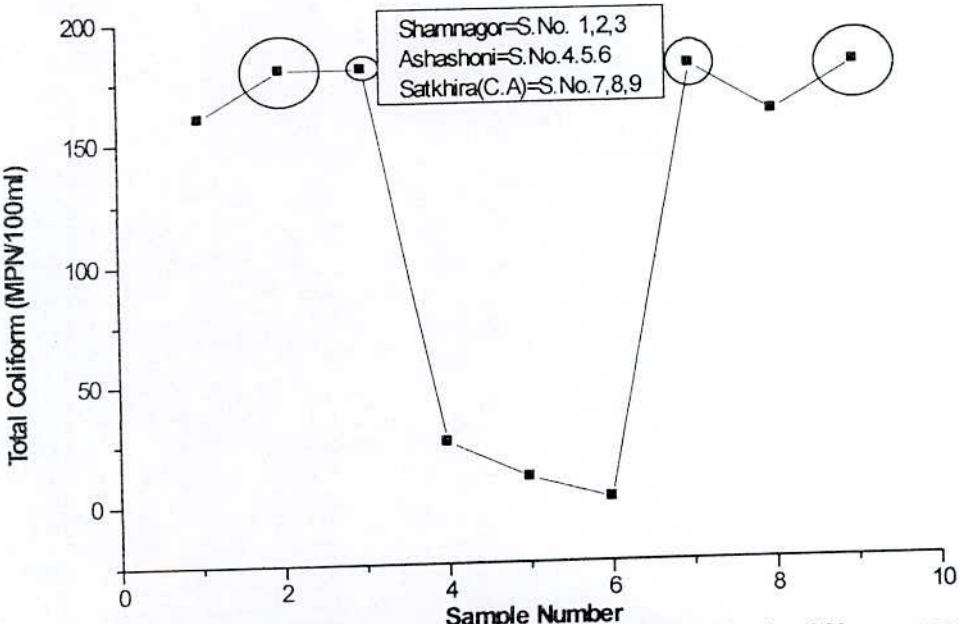


Figure 5.17 Total Coliform (T.Coli) of Water at Different Ponds of Shamnagor, Ashashuni and Satkhira City Area of Satkhira District.

It is observed from the figure 5.16 that the value of Standard Plate Count (SPC) of water at different ponds (ghers) of Shamnagor, Ashasuni and Satkhira city area of Satkhira District are found in ponds nos.1-9 exceed the standard limit. But in pond no. 7 is more higher because cowdung, poultry liter, urea were used into shrimp culture water.

The value of Total Coliform (TC) of water at different ponds (ghers) of Shamnagor, Ashasuni and Satkhira city area of Satkhira District are found in ponds nos.4,5 and 6 from figure 5.17, within standard limit and in ponds nos. 1,2,3,7,8 and 9 exceed the standard limit. But in ponds nos. 2,3,7 and 9 are more higher because cowdung, poultry liter, phosphate were used into shrimp culture water.

5.24 Faecal Coliform (FC) of Water at Different Ponds (Ghers) of Shamnagor, Ashasuni and Satkhira City Area of Satkhira District.

The value of Faecal Coliform (F.Coli) of water at different ponds (ghers) of Shamnagor, Ashasuni and Satkhira city area of Satkhira District are found in ponds nos.1-9 from figure 5.18, exceed the standard limit. But in ponds nos. 2,7 and 9 are more higher because cowdung, poultry liter, urea were used into shrimp culture water. It is observed from table 5.6 that Salmonella and Vibrio Cholerae were not detected any where.

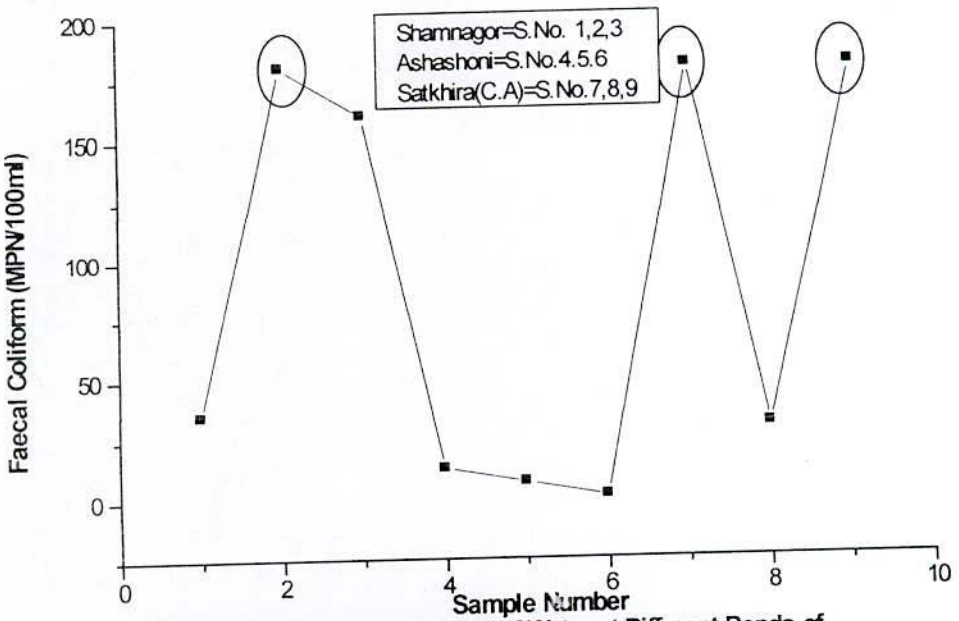


Figure 5.18 Faecal Coliform (F.Coli) of Water at Different Ponds of Shamnagor, Ashashuni and Satkhira City Area of Satkhira District.

CONCLUSION:

If a raw food is to be wholesome and safe for the consumer it will usually have lead to minimize the risk of disease and to stop the growth of harmful microbes. Harmful microbes such as Salmonella may arise without the agent having grown as the food because shrimps carrying the organisms from the Aquaculture farm.

The investigation involves the measurement of microbiological parameters in shrimps and also shrimps culture water from different locations of Bagerhat, Satkhira and Khulna to perform bacteriological analysis. It was shown from the investigation for shrimps that Standard Plate Count (SPC) was found within standard limit at Koyra (pond nos.1,2), Dumuria (pond nos.4,6), Paikgacha (pond nos.7,9), Rampal (pond nos.1,3), Mongla (pond nos.4,6), Bagerhat city area (pond nos.8,9), Shamnagor (pond nos.1,2,3), Ashasuni (pond no.5) and Satkhira city area (pond no.8) of Khulna region. It was also shown for shrimps that Total Coliform (T.Coli) was within Standard value at Koyra (pond nos.1,3), Dumuria (pond nos.5,6), Paikgacha (pond nos.7,8), Mongla (pond nos.4,5,6), Bagerhat city area (pond nos.7,8,9), Shamnagor (pond nos.2,3), Ashasuni (pond no.5) and Satkhira city area (pond nos.7,8,9). The Faecal Coliform (F.Coli) was found within standard value at Koyra (pond no.1), Dumuria (pond nos.5,6), Mongla (pond nos.4,6), Bagerhat city area (pond nos.7,9), Shamnagor (pond no.2), Ashasuni (pond no.5) and Satkhira city area (pond no.8). Salmonella were found at Dumuria (pond no.4), Paikgacha (pond no.7), Rampal (pond nos.1,2), Mongla (pond no.6), Bagerhat city area (pond no.9) and Ashasuni (pond no.4) but Vibrio Cholerae were not found anywhere. It was found that Standard Plate Count (SPC) and Faecal Coliform (F,Coli) for shrimp culture water were found above the standard value in all the area. The Total Coliform (T.Coli) were found within standard value at Koyra (pond nos.1,3), Dumuria (pond nos.4,5,6), Rampal (pond no.3), Mongla (pond nos.4,5,6), Bagerhat city area (pond no.8) and Ashasuni (pond nos.4.5,6). Salmonella was found at Rampal (pond no.1) but Vibrio Cholerae in shrimp culture water were not found in any pond. The microbiological parameters such as SPC, T.Coli, F.Coli, Salmonella and Vibrio Cholerae in shrimps were found to be satisfactory at Koyra (pond no.1), Dumuria (pond no.5), Mongla (pond no.4), Shamnagor (pond no.2), Ashasuni (pond no.5) and Satkhira city area (pond no.8) of

Khulna region. The result obtained from this investigation will immense to help the shrimps farmer's to produce improved quality of shrimps. The country will be able to export safe and quality products and earned more foreign currency. It will be helpful to develop our economy and the products will be made a room in the global market.

REFERENCE:

1. Hirono, Y. journal of the World Aquaculture Society 14, 1983, 451-457.
2. Clifford, H.C., III. 1985. Semi-intensive shrimp farming. Pages 13-40 in G.W. Chamberlain, MG. Haby and R.J. Miget, editors, Taxes shrimp farming manual, an update on current technology. Taxas Agricultural Extension Service, Taxas A & M University System, College Station, Taxas.
3. Taki,Y., J.H. Primavera, and J.A. Llobrera, Aquacop., Proceedings of the First International Conference on the Culture of Prawns/Shrimps. SEAFDEC, Iloilo City, Phillippines. 1985, Pages 3-10.
4. Sturmer, L.N., T.M. Samocha and A.L. Lawrence. 1992, Intensification of penaeid nursery systems. Pages 232-346 in A.W. Fast and L.G. Lester, editors. Culture of Marine Shrimp: Principles and Practices. Elsevier Scientific Publishing Company, Amsterdam, the Netherlands.
5. Samocha, T.M. and Lawrence, A.L., Shrimp nursery systems and their management. Paper presented at the World Aquaculture Society Annual Meeting, Orlando, and Florido. 1992.
6. Samocha, T.M., T. Blacher, and E.Estrada. 4th latin American Aquaculture Congress and Exhibition, October 25-28, 2000, ATLAPA Convention Center Panama City, Panama, 2000.
7. Samocha, T.M., T.Blacher, J.Cordova and A.De Wind, Advocate, 3 (6) 2000, 66-68.
8. Samocha, T.M., T. Blacher, J.Cordova and A. De Wind. 2001., Aquaculture, Annual Meeting of World Aquaculture Society, January 21-25, 2001, Orlando, FL.
9. Samocha, T.M., Benner, R. Industry Briers,. 7(1), 2001, 1-3.
10. Antony, M.M., G. Jeyasekaran, R.J. Shakija and S.a. Shanmugam, Asian Fisheries Science, 15, 2002, 33-41.
11. Sikorski, Z.E., and Sun Pan., 1994 preservation of seafood quality. In Shahidi f.and Botta J.r., ed. seafood: Chemistry, Processing Technology and quality. Blackie Academic and Professional, London, pp: 168-189.

12. Maches, J.R., 1982 Microbial changes in packages .In proceeding of the first National conference on seafood packaging and Shipping. (R.Martin,ed) .National Fisheries Inst Seattle,WA,pp:46-70.
13. Cheuk.W. L.,Finne,G., and Nikenson,11R.,1979.Stablity of adenosine deaminase and adenosine monphosphate deaminase during ice storage of pink and brown shrimp from Gulf of Mexico.J Food Sci.44:1625-1928.
14. Fatima,R.,Farooqui,B. and Qadri,R.B.,1981.Inosine monophosphate and hypoxanthine as indices of quality of shrimp (Penaeus Monodon). J.Food Sci .46, pp: 1125-1131.
15. Shamshad,S, I.,Kher-UN-Nisa,Roaz,R,and Qadri, R,B.,1990.Shelf life of shrimp (Penaeus Merguiensis) stored in different temperatures.J,Food Sci.,55(5),pp:1201-1242.
16. Yamagata, M and Low,L K.,1995.Banana shrimp, Penaeus Merguinsis, quality changes during iced and frozen storage .J. Food Sci.,60(4):pp:721-726.
17. Taar, h.L.A., 1954microbial deterioration of fish post mortem, its detection and control Bacteriol.Rev.18: pp: 1-15.
18. Wood, E.J.F., 1967 fish spoilage.In: microbiology of oceans and estuaries. Elsevier publishing co.,Amsterdam.Elsevier oceanography series.3:p.242.
19. Shewan, J . M .1961.The microbiology of sea water fish .Academic press, London pp: 487-560.
20. Shewan,J.M., and Hobbs, G. 1967. The bacteriology of fish spoilage and preservation. Prog.Indus. Microbiol. 6: pp: 169-208.
21. Ronsivalli,L.J.,and Charm,S.E.,1975.Spoilage and shelf life prediction of refrigerated fish.marine Fisheries Review37,pp:32-42.
22. Liston, J., 1982 Recent advances in the chemistry of iced fish spoilage .In chemistry and biochemistry of marine food products, (R.Martin, G,Flick, C.E. Hebardand D.R.Ward.eds). AVI publishing Co., Westport, Ct, pp: 27-36.
23. Botta, j, R., 1995, Evaluation of Seafood Freshness quality.VHC publishers, Inc, newYork.p.36.
24. Howgate, P, F., 1982.Quality assessment and quality control. In: Fish Handling and processing (2^{nd} ed) Aitken, A, et, al, eds.).Her Majesty's Stationary, Edinburgh, pp: 177-186.
25. Huss, H, H., 1988.Fresh Fish-Quality& Quality Cganges,FAO,Rome.

26. Love, R, M., 1988.The Food fishes:thrir intrinsic variation and practical Implications, Van Nostrand Reinhold Co.Inc.,New York,NY.

27 Botta,j,R,Bonnell,G and Squires,B.E.,1987.Effect of method of catching and time of seation on sensory quality of fresh raw Atlantic cod (Gadus morhua).J.,food.Sci,52(4):p.928.

28. Huss, H, H., 1988.Fresh Fish-Quality& Quality Changes, FAO, Rome.

29. Botta, j, R, Squires, B, E.,and Keates,S,L.,1989.Handling procedures and the quality of fresh raw snow crab. Can.Inst.Food Sci.Technol.j., 22(5):p.470.

30. Botta, j, R, 1994. Freshness quality of Seafood's review .In Shahidi,F,and Botta, J,R., ed. Seafood's Chemistry ,processing Technology and quality .Blackie Academic and professional ,London,pp:140- 162.

31. Shewan, J.M. &Ehrenberg, R.T., 1977.The bacteriology of fresh and spoilage fish &The Biochemical changes induced by Bacterial action .In handling, processing and marketing of Tropical Fish. Tropical products Institute, London pp: 51-66.

32. Botta, j, R Lauder, J, T., Jewer, M.A., 1984.Effect of methodology on total volatile basic nitrozen determination as an index of quality of shrimp Atlantic cod (Gadus morhua). .J. food.Sci, 48 pp: 734-736.

33. Fatima,R., Farooqui,B. and Qadri,R.B.,1981.Inosine monophosphate and hypoxanthine as indices of quality of shrimp (Penaeus Monodon). J.Food Sci .46, pp: 1125-1131.

34. Connell, J,J.,1976.COntrol of fish quality .Fishing New books Ltd .London.pp:127-129 Larmond, E.1986.Sensory evaluation can be objective.In:Kapsalis J.K.,ed .objective methods on food quality Assessment.CRC Press,pp:3-14.

35. Larmond, E.1986.Sensory evaluation can be objective.In:Kapsalis J.K.,ed . Objective methods on food quality Assessment.CRC Press, pp: 3-14.

36. Howgate, P,F.,1982.Quality assessment and quality control. In: Fish Handling and processing (2nd ed) Aitken, A, et, al, eds.).Her Majesty's Stationary, Edinburgh, pp: 177-186.

37. Shewan, J.M. &Ehrenberg, R.T., 1977.The bacteriology of fresh and spoilage fish &The Biochemical changes induced by Bacterial action .In handling, processing and marketing of Tropical Fish. Tropical products Institute, London pp: 51-66.

38. Harrison, J.M., &Lee J.S., 1969.Microbiological evaluation of pacific shrimp processing .Appl.Microbial. 18:p.188.

39. Cann, D, C., 1971.The bacteriologyof Scampi –ll.Detailed investigation on the bacterial flora of freshly caught sample .J.Fd.Technol .,6:p.153.

40. Novak,A.F.,1973. Microbiological considerations in handling and processing of crustacean shellfish, In: Microbial Safety of Fishery Products, ed.By C.O.chichester &H.D.Graham, London, Academic Press .p.59.

41. Lee,J.S. and Pfeifer,D.K,1977. Microbiological characteristics of pacific Shrimp (Pandalus jordani).Appl.Microbial.,33:pp:853.

42. Cann, D.C., 1977.Bacteriology of shellfish with reference to international trade .In: proc.Trop..prod.Inst.Conf.Handl.Process Market Fish .,p.511.

43. Zuberi,R.,Qadri,R.B.,and Siddiqui,P.M.A.,1983.Influence of processing on the bacterial quality of frozen shrimp .J.Food.Protect.46:p.572.

44. Surendran, P.K.,Mahadeva lyer ,K.and Gopakumar ,K.,1985.Succession of bacterial genera during ice storage of 3 species of tropical prawns p.indicus,Metapenaeus dobsoni and M.affinis.Fish Technol .,22.pp:117-120.

45. Reilly,A., Dangla, E., and Dela Cruz, a.,1986.Post harvest spoilage of shrimp (Penaeus Monodon).In:Maclean ,Dizon and Hosillos,eds. Processing of first Asian fisheries Forum ,Manila ,pp:455-458.

46. Lobrerra ,A.T.,Bulalaco,M.L.,and Tau,A.,1990.Effect of farming phase and in- plat processing on the microbiological quality of shrimp (penaeus Monodon).In: Papers presented at the seventh session of the Indo-Pacific Commission Working party on fish Technology and Marketing, FAO Fisheries report .pp:14.

47. De Silva, S, L., 1985.Water quality and shrimp value .INFOFISH Marketing Digest.2: pp: 39-41.

48. Lisac, H., 1983.Wash me-but well. INFOFISH Marketing Digest.6: pp:22-23.

49. Rajaduraic, p.n., 1985.Improving the quality of shrimp through proper handling INFOFISH Marketing Digest.1: pp: 42-44.

50. Putro,S.,Anggawati ,A.M.,Fawzya,Y.N. and Ariyani ,F.,1990.Studies on microbiology of farmed shrimp,In:Papers presented at the seventh session of the Indo-Pacific Commission Working party on fish Technology and Marketing,FAO Fisheries Report .pp:6-8.

51. Peranginangin,R.,Budhyatni,S.and Nasran,S.,1982.The Microbiology of fresh shrimps .j.Fish tech.res.19:pp:27-35.

52. Liston, J., 1980.Microbiology in fishery science.In Connell,J.J.et.al.,eds. Advances in Fish Science and Technology .Torry .Jubliee Conference, pp: 138-157.

53. Cann, D.C., 1974.Bacteriological aspects of tropical shrimp .In:Kreuzer, R.,ed. Fishery Products,West Byfleet,Fishery News .pp:338-344.

54 Vanderzant,C.,Mroz,E.,and Nickelson,R, 1970.Micribial flora of gulf of Maxico and pond shrimp .J.Milk food Techno.33:pp:346-350.

55. Fonseka, T.S.G., 1990.Microbial flora of pond cultured shrimp (Penaeus Monodon).In: papers presented at the seventh session of the Indo-Pacific Commission Working Party on Fish Technology and Marketing,FAO Fisheries Report.pp:24-27.

56. Rattagool, P.,Wongehinda,N.,and Sanghtong, N., 1990. Salmonella Contamination in Thai shrimp .In:papers presented at the seventh session of the Indo-pacific Commission Working Party on Fish Technology and Marketing in 1988.FAO Fisheries Report .pp:18-19.

57. Karunasagar, Sushella,M.,and Malathi,G.R.,1990.Incidence of Human Pathogenic Vibrios in Seafoods Harvested along the coast of Karnataka (India),In:Papers presented at the seventh session of the Indo-Pacific Commission Working party on fish technology and marketing in 1988.FAO Fisheries report.pp:53-54.

58. Jayaweera, V., and Subasinghe ,S.,1990.Some chemical and micribiological changes during chilled storage,.of shrimps(Penaeus Monodon) ,In:Papers presented at the seventh session of the Indo-Pacific Commission Working party on fish technology and marketing in 1988.FAO Fisheries report.pp: 68-70.

59. Rose, A., Cruz, G.D., Santos, L.M., Agudo, F., and Dangla, E., 1990. Microbiology of shrimp processing. In: Papers presented at the seventh session of the Indo-Pacific Commission Working party on fish technology and marketing in 1988.FAO Fisheries report.pp:86-98.

60. Nguyen Le Ha, 1997.Changes in Freshness of shrimp (Panaeus semisulcatus) during storage at various temperatures. M.Sc. Asian Institute of technology, Bangkok, Thailand.

61. Gunderson,M.F.,and K.D.,Rose,1984.Survival of bacteria in a precooked, freshfrozen food research 13,p.254.

62. Kereluk,K,and Gurderson,M,F.,1959.Studies on the bacteriological quality of frozen meat pies.IV.Longevity studies on the coliform bacteria and enterococci at low temperature .Appl.Microbial,7,p.327.

63. Kereluk,K,A.C.,peterson and M.F.,Gunderson .1961.Effect of different temperatures on various bacteria from frozen meat pies.j food .Sci.,26,p.21.

64. Luyet,B,J.,and P.M.,Gehenio,1940.The mechanism of injury and death by low temperatures ,Biodynamica 3,p.33.

65. Record,B.A.Taylor, R.J, 1963.gen microbial .9.p.475.

66. Hess, E.1934.The effects of freezing on marine bacteria. I. Quantitative studies j. Biol. Board Canada1, p.9.

67. keith,S,C,1913.Factores influencing the survival of bacteria at temperatures in the vicinity of the freezing point of water .Science 37,p.877.

68. Bryan,F.L.,1973.Activities of the center for disease control in public health problems related to the consumption of fish and fishery products.In:Microbial safety of fishery products .(Chichester,C.O.,and,H.D.,ed)pp:227-278.

69. Hess, E. 1934. The effects of freezing on marine bacteria. I. Quantitative studies. J.Biol.Board.Canada 1, p. 95.

70. Dangla, E., 1984.Storage stability of brackish water shrimps during processing for export .Food technology in Australia.Vol.36 (6).

71. Summer,j.l.,Sammaraweera,I.,Jayaweera,V.,and Fonseka,G.,1982.Asurvey of process Hygiene in the Sri Lanka shrimp industry, J.Sci.Food.Agric .33:pp: 802-808.

72. De Silva,S,L.,1985.Water quality and shrimp value .INFOFISH Marketing Digest.2:pp:39-41.

73. Shewan,J.M.,1962.The bacteriology of fresh and spoilage fish and some related chemical changes .Report adv. food sci.1:pp:167-193.

74. Cobb,B.F.,Vanderzant,C.,Hanna,M.O.,and yeh,C,P,S.,1976.Effect of iced storage on microbial and chemical changes in shrimp and melting ice in a model system j.food Sci.41:p.29.

75. Valenkar,N.K.,et.al.,1961. spoilage of shrimps at 0^0C and assessment of chemical and bacteriological tests.Indian J. Fisheries,8(1):pp:241-151.

76. Dempsey,H.V.1971.The organization of fish inspection system.FAO technical conference on fish inspection and quality control. Canada .pp:16-18.

77. Sunarya, 1996.Improvements in fish handling processing and quality control.Workshop on Strengthening Marine Fisheries Development in Indonesia.Jakarta/Apr.1996.p.12.

78. Smole Mozina,S.,and raspor ,p.,1997.Rapid methods of microbiological control in food processing,Ljubljana Univ.(Solvenia).pp:115-126.

79. Howgate,p.,1985.Bibliograpy of storage lives of wet and frozen Fish.pp:389-402.In:Storage lives of chilled and frozen fish products.Aberdeen ,Uk.Intronational institute of Refrigeration.

80. Pokern,J.,1990.Microbiologyin food processing .Ljublina(Yugoslavia).p.120.

81. Rankle,B. (1959). Microbiology in Fishery Science. Arch. Fish Criwissen 1,p. 117.

82. Cannon-Bonventure, K.,1992 Marketing Seafood : You can still come out on top. Saefood Business. 11(3): pp: 32-36.

83. Szczsnick,A,S.,1990.Texture:is it still an over looked food attribute?Food Technology 44,pp:88-95.

84. Gill,T.A.,Keith,R.A.,and smith,L.B.,1979.Textural deterioration of red hake and haddock muscle in frozen storage as related to chemical parameters and changes in the myofibrillar proteins,J.Food Sci.44,pp:661-667.

85. Poulter,R,G.,1981.Quality changes in three Sri Lankan fish species stored in ice Tropical Science,23(2):pp:155-168.

86. Lima dos Santos,C.A.M.,1981.The storage of tropical fish in ice a review. Tr0p.Sci.23:pp:97-127.

87. Stansby, M.E., and Olcott, H. S. 1963. Industrial Fisheries Technology, Composition of Fish. Reinhold Publishing Corporation. pp: 339-349.

88. Shewan,J.M.,1951.The chemistry and Metabolism of the Nitrogenous Extractives in fish Biochem.Soc.Symp.6:pp:28-48.

89. Reay,G.A.,and Shewan J.M.,1949.The spoilage of fish and its preservation by chilling .Adv.Food Res.2.pp:343-398.

90. Farber, L.1965.freshness tests .In Fish as food George Borgstrom (Editor). Academic Press,New York.Vol.4.pp:65-126.

91. Tanikawa,E.,1935.Spoilage pattern of some selected fresh and marine fish J.soc.Ind.Fish (Japan).3.pp:267-297.

92. Paryan, D.R.,and Pilgrim,F,I.,1957.Food technol champaign11.In studies on radiation preservation of catla catla (Hamilton –Buchannan,1822). M.SC. Thesis (Anon)Dearment of zoology,University of Dhaka.pp:9-14.

93. Stansby,M,E.,Vancleve,M,E,R.,and stern 1957.Scaitte Contract republic V,S,D,I., Bur. Comm. Fish. In.Spoilage of tropical fish and product development, edited by reilly,A.food and agricultural organization of the United nations (Rome,p.221.

94. Hilling,F.R.,Shelton,R,L.,Loghriy,H,J.,and fisner,J.1958.J.Ass.off Agrical. Chem.41.pp:763-776.

95. Jenson,M,H.,Peterson,A.,Roge,E.H.,and Connell,j.j.1979.Chilled and frozen storage. Advantages in fish science and technology,vol.I.fishing news Rooks. pp:294-299.

96. Lillard, H.S.et.al.,1984.Comparisil of brands of media for isolating bacteria form poultry, beef and shrint. J.Food. Prot.,47(5): PP: 394-397.

97. Matches, J.R., and Layrisse,M.E., 1985, Controlled atmosphere storage of spotted shrimp (pandalus platyceros) . J.Food.Prot., 48(8): PP:709-711.

98. Lannelongue, M. et.al., 1982. Storage characteristics of brown shrimp(penacus aztecus) stored in retail packaged containing CO_2 enriched atmospheres. J .Food.Sci., 47(3) : PP:911-913,P.923.

99. Varma,P.R.G.,Mathen, C. and Matthew, A.,1982. Bacteriological quality of frozen seafoods for export with special reference to Salmonella. Sym. Harvest/post harvest Fish Technol., India, PP: 665-666.

100. Azam, k., 2004, Quality of Shrimp in the Distribution Channel: An Approach in Improving the Livelihood of Fisher Community. UGC, DFID-SUFFER Project report, Fisheries and Marine Resource Technology Discipline, Khulna University, khulna. Bangladesh. PP: 1-50.

101.Paul, A.K.,1994. Bangladesher raptaniyouga Mathsya sampad (Exportable Fisheries Resource in Bangladesh), Tuli-Mili Prokashani, Khulna,Bangladesh.

102.Lyer,T.S.G.,Chaudhuri,D.R.,and Pilliai, V. K., 1969.Bacteriological aspects of frozen prawn and their significance in quality evaluation. Technical conference on fish inspection and quality control. Halifax,Canada.FAO Fish. Technol (India) 7(1):pp:86-90.

103. Williams, O.B.,Rees,H.B., and Campbell, L.L.,1952. The bacteriology of Gulf coast shrimp.I and II. Experimental procedures and quantitative results. Texas Journal Sci.4:49-52.

104. Green, M.,1949.Bacteriology of shrinp,quantitative studies of freshly caught and iced shrimp. Food Research .14:pp:372-383.

105. Castell,C.H.and Triggs, R.E.1955.Spoilage of Haddock in the Trawler at sea: The measurement of spoilage and Standards of Quality. J.Fish Res.Bd. Can.12(3):pp:329-341.

106. Georgala, D.L., 1957. Changes in the skin flora of cod after washing and icing J. Appl.Bact. 20.p.23.

107. Surendran, P.K. and Gopakumar, K., 1981 Selection of bacterial flora in the chlorocetracycline treated oil sardine (Sardinella longiceps), Indin mackereal and prawn during ice storage Fish Technol., 18(2):pp:133-141.

108. Pillai, 1969, Rat of survival of Organism of significance During Freezing and Subsequent Storage. Freezing and Irradiation of fish. Rudolf Kreuzer. Fishing News(Books) limited, Ludgate House, Flect street, London, EC4, England.

109. Christopher, F.M.et.al., 1978. Micro flora of pond reared shrimp (penaeus, Stylirostris, penaeus vennamei and penaeus setiferus).J.Fd.Prot.,41:pp:20-23.

110. Vanderzant, C., Nickelson, R., and Judkins, P.W., 1971. Micro flora of pond reared brown shrimp (penaeus aztecus). Applied Microbiol., 21(5):pp:96-920.

111. Durarraj, S., Chinnasamy, G., and Syed Mohamed, M., 1983. A bacteriological study of the natural flora of edible oyster Crassostrea madrasensis. Fshery Technology 20(2):pp:111-125.

112. Simidu, U., Kaneka, E., and Aiso,K., 1969, Microflora of fresh and stored flat fish (Kareius bicoloratus). Bull,Jpn. Soc.Fish.,35:pp:77-78.

113. Surendran, P.K. and Gopakumar, K., 1981 Selection of bacterial flora on the chlorocetracycline treated oil sardine. Indin mackereal and prawn during ice storage Fish Technol., 18(2):pp:133-141.

114. Reilly, A., Bernarte, M. A., and Dangla, E., 1984. Storage stability of brackish water prawns during processing for export. *Food* Tech. Aust. 36(6):pp:286-293.

115. Blake, P.A., Merson, M.H., Weaver, R. E., Hollis, D.G., and Heublein, 1979. Disease caused by marine vibrios. Naw England. J.Med.300:pp: 1-5.

116. Iyer, T.S.G., Varma, P.R.G., and Gopalkumar, K., 1990. Report on the FAO project on shrimp Hygiene and Quality Control. In: papers Presented at the seventh session of the Indo- pacific Commission Working party on Fish Technology and marketing in 1988. FAO Fisheries Report. pp:103-104.

117. Iyer,T.S.G.,and Chouduri,D,R.,1966.Influence of ice on the bacteriological quality of processed fishery products.Fish.Technol.3:p.113.